BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Spring Meeting

Friday, April 22th 2016

PROGRAMME

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ABSTRACTBOOK

Venue

Palace of the Academies
Royal Academy of Medicine of Belgium
“Espace Roi Baudouin - Atrium”
Rue Ducale / Hertogsstraat 1
1000 Brussels

Local host

Prof. Dr. David GALL
Laboratoire d’Enseignement de la Physique (CP613/3)
Laboratoire de Neurophysiologie (CP601)
ULB Neuroscience Institute
Université Libre de Bruxelles
route de Lennik 808
B-1070 Bruxelles

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Royal Flemish Academy of Belgium for Science and the Arts

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Palace of the Academies
"Royal Academy of Medicine of Belgium
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Main Lecture

10.00-11.00 Ca^{2+}-binding proteins as modulators of Ca^{2+}-dependent cellular function: implications from cell physiology to behaviour".

Prof. Dr. Beat SCHWALLER, Abteiling Anatomie – Departement Medizin, Universitaet Fribourg, CH-Fribourg (Switzerland).

Oral Communications

11.00-11.15 H. IVANOVA, A. RITANE, L. WAGNER, T. LUYTEN, G. SHAPOVALOV, K. WELKENHUYZEN, G. MONACO, H. DE SMEDT, N. PREVARSKAYA, D.I. YULE, J. B. PARYS, G. BULTYNCK (KULeuven, Inserm U-1003, France, Univ. of Rochester, New York USA)
Trans-membrane domain of Bcl-2α as an unexpected and critical determinant for efficient IP_3R inhibition.

11.15-11.30 T. VERVLOESSEM, H. AKL, R. LA ROVERE, T. TOUSSEYN, L. MISSIAEN, H. DE SMEDT, J.B. PARYS, G. BULTYNCK (KULeuven)
Reciprocal sensitivity of B-cell cancer cells towards BH3 mimetics versus BH4-domain-targeting peptides.

11.30-11.45 F. SEGHERS, X. YERNA, P. GAILLY (UCLouvain)
Role of TRPV4 in pressure-induced inhibition of renin secretion by juxta-glomerular cells.

11.45-12.00 C. BOYDENS, B. PAUWELS, L. VANDEN DAELE, J. VAN DE VOORDE (UGent)
Protective effect of resveratrol and quercetin on in vitro-induced diabetic mouse corpus cavernosum.

12.00-12.15 A. KURDI, G.R.Y. DE MEYER, W. MARTINET (UAntwerpen)
The rapamycin derivative everolimus attenuates growth of pre-existing atherosclerotic plaques and improves survival in ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice.

12.15-13.45 **Lunch – Guided Poster Session**

Posters

(height 120 cm – width 100 cm)

1. C. DRESSEN, B. SCHWALLER, G. VEGH, F. LELEUX, P. LEBRUN, P. LYBAERT (ULBruxelles, Universitaet Fribourg, Switzerland)
Calcium-binding proteins and mouse sperm motility.

2. K. HELD, S. PHILIPP, T. VOETS, J. VRIENS (KULeuven, Univ. des Saarlandes, Homburg Germany)
A 13 amino acid difference in two TRPM3 splice variants results in tremendous pharmacological variations.

3. G. DESTREEL, V. SEUTIN, D. ENGEL (ULiège)
Properties of excitatory synaptic inputs onto dopaminergic neurons in the substantia nigra.

4. C. HMAIED, J. SCUVÉE-MOREAU, A. SWIJSEN, V. SEUTIN (ULiège)
Mechanism of the fast afterhyperpolarization in rat serotonergic neurons.

5. S. LEPANNETIER, O. SCHAKMAN, F. SEGHERS, P. GAILLY (UCLouvain)
Role of TRPC1 ion channel in hippocampal neurons.

6. V. JORIS, L. MENCHI, I. LOBYSHEVA, G.L. CONDORELLI, J.-L. BALLIGAND, D. CATALUCCI, C. DESSY (UCLouvain, Instituto Humanitas, Milan Italy)
Implication of the microRNAs 199a-3p and 199a-5p in the vascular function: modulation of the NOS/NO pathway.

7. T. METZINGER, G. RATH, L. VANHOUTTE, S. HORMAN, C. DESSY (UCLouvain)
Modification of vascular tone in conductance and resistance arteries in response to cardiac hypertrophy.

8. M. FALTOT, S. SEBAA, C. HAUET, L. MALASHKINA, Z. BOUCHERIT-OTMANI, P. COURTOIS (ULBruxelles, Univ. Abou Bekr Belkaïd, Tlemcen Algeria, Haute Ecole Francisco Ferrer, Brussels)
Candidacide effect of hypohalous solution.

9. R. CRUTZEN, M. VIRREIRA, W.J. MALAISSE, R. BEAUWENS, A. BOOM, P.E. GOLSTEIN (ULBruxelles)
Anoctamin 1 (Ano1) is required for glucose-induced membrane potential oscillations and insulin secretion by murine β-cells.

10. A. HANTHAZI, P. JESPERS, JY. SPRINGAEL, L. DEWACHTER, K. MC ENTEE (IRIBHM – ULBruxelles)
Chemerin added to endothelin-1 induces pulmonary artery smooth muscle cells proliferation.

11. L. VANGEEL, S. LIEVENS, J. TAVERNIER, T. VOETS (KULeuven, VIB, UGent)
The role of interacting proteins in TRPV4 channelopathies.

Oral Communications

13.45-14.00. T. MARICHAL, N. GAUDENZIO, L.L. REBER, P. STARKL, A. ROERS, F. BUREAU, S.Y. TAM, M. TSAI, S.J. GALLI (supported by V. SEUTIN) (ULiège, Stanford University, USA, University Dresden Germany)
Keratinocyte-restricted Rabgef1 gene deletion results in the development of lesions resembling atopic dermatitis, as well as systemic inflammation, in vivo.

14.00-14.15 C. MESNIL, S. RAULIER, G. PAULISSEN, D. PIROTTIN, T. JANSS, M. BIRRELL, M. BELVISI, X. XIAO, L. GILLET, C. DESMET, F. BUREAU, T. MARICHAL (supported by V. SEUTIN) (ULiège, Imperial College London UK)
Lung resident eosinophils represent a distinct cell subset with homeostatic functions.

14.15-14.30 J.I. STAS, E. BOCKSTEINS, C.S. JENSEN, N. SCHMITT, D.J. SNYDERS (UAntwerpen, University of Copenhagen)
Neuronal Kv2-mediated current suppression by the anticonvulsant retigabine.

14.30-14.45 J. ALVAREZ-COLLAZO, A. LÓPEZ-REQUENA, A. TALAVERA, Y.A. ALPIZAR, J.L. ALVAREZ, K. TALAVERA (KULeuven, VUBrussel, La Habana Cuba)
Cinnamaldehyde exhibits local anesthetic-like block on human $Na_v1.5$ channels.

14.45-15.00 C. MINSART, C. LIEFFERINCKX, S. RORIVE, A. LEMMERS, E. QUERTINMONT, E. TREPO, J. DEVIERE, C. MORENO, I. LECLERCQ, R. MOREAU, T. GUSTOT (supported by D. GALL) (ULBruxelles, C.U.B. Hôpital Erasme, CMMI Gosselies, UCLouvain , Inserm Unité 1149, Paris, France, Hôpital Beaujon Clichy, France)
HMGB1-driven feedforward hepatocytes necroptosis circuit in lethal acetaminophen-induced liver injury.

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Coffee - Tea

ABSTRACTS

Legend
O = Oral communication
P = Poster

O-01 (11.00-11.15)

THE TRANS-MEMBRANE DOMAIN OF BCL-2α AS AN UNEXPECTED AND CRITICAL DETERMINANT FOR EFFICIENT IP_3R INHIBITION

H. Ivanova[1], A. Ritane[2], L. Wagner[3], T. Luyten[1], G. Shapovalov[2], K. Welkenhuyzen[1], G. Monaco[1, 4], H. De Smedt[1], N. Prevarskaya[2], D. I. Yule[3], J. B. Parys[1], G. Bultynck[1]

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Bcl-2 is a well-known anti-apoptotic protein, which regulates cell-fate decisions by inhibiting Bax/Bak oligomerization at the mitochondria and IP_3R-mediated Ca^{2+} signals at the endoplasmic reticulum. Here, we aimed to identify the molecular determinants in Bcl-2, which in addition to the BH4 domain, were responsible for the efficient IP_3R. Using mutation (Bcl-$2^{GR/AA}$) or pharmacological inhibition (ABT-199) to antagonize Bcl-2's hydrophobic cleft, we excluded this functional domain as responsible for Bcl-2-mediated IP_3Rs inhibition. In contrast, the deletion of the C-terminus, containing the trans-membrane domain, which is only present in Bcl-2α, but not in Bcl-2β, led to impaired inhibition of IP_3R-mediated Ca^{2+} release. Strikingly, the trans-membrane domain was sufficient for IP_3R binding and inhibition. We therefore propose a novel model, in which the Bcl-2's C-terminus serves as a functional anchor, which beyond mere ER-membrane targeting, underlies efficient IP_3R inhibition by (i) positioning the BH4 domain in the close proximity of its binding site on IP_3R, thus facilitating their interaction; (ii) inhibiting IP_3R-channel openings through a direct interaction with the C-terminal region of the channel downstream of the channel-pore. Finally, since the hydrophobic cleft of Bcl-2 was not involved in IP_3R regulation, our findings indicate that ABT-199 does not interfere with IP_3R regulation by Bcl-2 and its mechanism of action as a cell-death therapeutic in cancer cells likely does not involve Ca^{2+} signaling.

O-02 (11.15-11.30)

RECIPROCAL SENSITIVITY OF B-CELL CANCER CELLS TOWARDS BH3 MIMETICS VERSUS BH4-DOMAIN-TARGETING PEPTIDES

T. Vervloessem[1], H. Akl[1], R. La Rovere[1], T. Tousseyn[2], L. Missiaen[1], H. De Smedt[1], J.B. Parys[1], G. Bultynck[1]

[1]KU Leuven, Laboratory of Molecular and Cellular Signalling, Department of Cellular and Molecular Medicine & Leuven Kanker Instituut, Leuven, Belgium, [2]KU Leuven, Translational Cell & Tissue Research, Department of Imaging & Pathology, Leuven Belgium

Bcl-2 is often upregulated in B-cell malignancies like chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DL-BCL) to neutralize the BH3-only protein Bim at the mitochondria. BH3 mimetics (like ABT-199) that disrupt this interaction have entered cancer therapy. However, some cancers display poor responses towards BH3 mimetics despite high Bcl-2 levels indicating an additional function for anti-apoptotic Bcl-2 in these cancers. Indeed, it is proposed that Bcl-2 interacts, via its BH4 domain, with and inhibits the inositol 1,4,5-trisphosphate receptor (IP_3R), the main intracellular Ca^{2+} channel on the endoplasmic reticulum (ER) thereby preventing pro-apoptotic Ca^{2+} signals. Recently, a peptide, the **B**cl-2/**I**P_3R **D**isrupter-2 (**BIRD-2**), was developed, killing Bcl-2 dependent CLL and DL-BCL by targeting Bcl-2's BH4 domain. Eight "primed to death" DL-BCL cell lines were compared for their apoptotic sensitivity toward BIRD-2 and ABT-199 by determing the IC_{50} value using flow cytometric cell-death analysis. A reciprocal sensitivity of DL-BCL cells towards ABT-199 versus BIRD-2 was found. Using immunoblotting, the IP_3R2 and Bim-expression levels were measured in DL-BCL cell lysates. We found that the sensitivity towards BIRD-2 positively correlated with the expression of IP_3R2, the isoform with the highest sensitivity towards its endogenous ligand IP_3, but negatively correlated with Bim. Hence, our findings indicate a dual addiction of cancer cells towards Bcl-2 either at the mitochondria or at the ER rendering them selectively sensitive to BH3 mimetics versus BH4-domain-targeting compounds. This is important since the chemotherapeutic response appears to correlate with their sensitivity to BH3 mimetics, but our data suggest that BH3 mimetics "resistant" cancers might be targeted using BH4-domain-targeting tools like BIRD-2.

O-03 (11.30-11.45)

ROLE OF TRPV4 IN PRESSURE-INDUCED INHIBITION OF RENIN SECRETION BY JUXTAGLOMERULAR CELLS

F. Seghers, X. Yerna, P. Gailly

Université catholique de Louvain (UCL), Laboratory of Cell Physiology, 1200 Brussels, Belgium

The renin – angiotensin system is a crucial blood pressure regulation system. It consists of a hormonal cascade where the rate-limiting enzyme is renin, which is secreted into circulation by renal juxtaglomerular (JG) cells in response to low pressure in the renal afferent arteriole. In contrast, an increase in blood pressure in the afferent arteriole results in a decrease in renin secretion. This is accompanied by a transitory increase in cytosolic calcium concentration ($[Ca^{2+}]_i$) of JG cells. The inverse relationship between $[Ca^{2+}]_i$ and renin secretion has been called the “calcium paradox” of renin release and is due to a Ca^{2+} -dependent inhibition of the adenylate cyclase (AC5 and AC6) that produces cAMP, which activates PKA and triggers renin vesicles release. How increase in pressure induces a $[Ca^{2+}]_i$ transient in these cells is however unknown. We observed that $[Ca^{2+}]_i$ transients induced by mechanical stimuli in JG As4.1 cells were inhibited by Gd^{3+} and ruthenium red, two non specific inhibitors of mechano-sensitive channels. More specifically, the response was reduced by siRNA-mediated repression of TRPV4 expression, but not after repression of TRPV2 or Piezo1 ion channels that are also expressed in As4.1 cells. As expected, Ca^{2+} response was inhibited by HC067047 and RN1734, two inhibitors of TRPV4. Interestingly, the stimulation of renin secretion by the AC activator forskolin was blunted by GSK1016790A and 4α−PDD, two activators of TRPV4. Moreover, in isolated perfused kidneys from *Trpv4*$^{-/-}$ mice, the pressure - renin relationship was significantly altered. Acute blocking of TRPV4 by HC067047 perfusion in wild type kidney triggered an increase in renin secretion. Altogether, our results suggest that TRPV4 is involved in the pressure-induced entry of Ca^{2+} in JG cells, which inhibits renin release and allows the negative feedback regulation on blood pressure.

O-04 (11.45-12.00)

PROTECTIVE EFFECT OF RESVERATROL AND QUERCETIN ON IN VITRO-INDUCED DIABETIC MOUSE CORPUS CAVERNOSUM

C. Boydens, B. Pauwels, L.Vanden Daele, J. Van de Voorde

Department of Pharmacology, Ghent University, Ghent, 9000, Belgium

Hyperglycemia and increased levels of methylglyoxal (MGO) can trigger the development of vascular complications in diabetes. Resveratrol and quercetin are red wine polyphenols with known beneficial cardiovascular properties, including an antioxidant capacity. This study evaluated whether resveratrol and/or quercetin could prevent in vitro-induced diabetic changes in neurogenic and vascular relaxant responses of mouse arteries and corpora cavernosa. Isometric tension of isolated aorta, mesenteric arteries and corpora cavernosa was measured using organ bath systems. Diabetic conditions were mimicked in vitro by co-incubating the tissues for 2 h with high glucose (HG, 30 mM) and MGO (120 µM). The presence of HG and MGO significantly blunted acetylcholine (Ach)-induced relaxations in corpora cavernosa and mesenteric arteries but not in aorta. Electrical field stimulated (EFS) responses of corpora cavernosa were also significantly inhibited by these diabetic conditions. In corpora cavernosa 2 h co-incubation with resveratrol (30 µM) or quercetin (30 µM) significantly attenuated HG and MGO-induced deficits in Ach- and EFS-responses. Our study demonstrates that in mouse arteries, HG and MGO rather affect endothelium derived hyperpolarizing factor-mediated than nitric oxide (NO)-mediated relaxations. In corpora cavernosa HG and MGO interfere with NO release. Resveratrol and quercetin protect mouse corpora cavernosa from diabetic-induced damage to NO-mediated relaxant responses. This might rely on their antioxidant capacity.

O-05 (12.00-12.15)

THE RAPAMYCIN DERIVATIVE EVEROLIMUS ATTENUATES GROWTH OF PRE-EXISTING ATHEROSCLEROTIC PLAQUES AND IMPROVES SURVIVAL IN APOE$^{-/-}$ FBN1$^{C1039G+/-}$ MICE

G. Kurdi, G.R.Y. De Meyer, W. Martinet

Laboratory of Physiopharmacology, University of Antwerp, Wilrijk, 2610, Belgium

Everolimus is an inhibitor of the mechanistic target of rapamycin and is currently under investigation for its ability to counter atherosclerosis. While multiple reports seem to consolidate these positive outcomes in preventive experimental settings, little or no information exists regarding the curative effect of everolimus on pre-existing atherosclerosis. In this study, we used the recently described ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ atherosclerotic mouse model, which closely resembles human atherosclerosis both in plaque complexity and complications, to administer everolimus (1.5 mg/kg/day) subcutaneously via osmotic minipumps after 12 weeks of western type diet. Mice continued to receive both drug and diet for another 12 weeks after which they were sacrificed. With plasma levels as high as 501 ± 58 nM, everolimus was able to attenuate plaque growth in the proximal ascending aorta ($p = 0.033$) while enhancing plaque stability markers. Moreover, everolimus blocked intraplaque microvessel formation and hemorrhages. Treatment with everolimus also attenuated cardiac dysfunction by reducing (perivascular) fibrosis, myocardial infarctions and coronary plaque formation. Importantly, everolimus decreased the number of circulating B-cells and neutrophils and reduced pro-atherosclerotic ly6C^{high} monocytes. These effects led to improved survival of treated ApoE$^{-/-}$ Fbn1$^{C1039G+/-}$ mice despite increased total blood cholesterol and LDL fraction. Taken together, our results point to a positive effect of everolimus in pre-existing atherosclerotic plaques that is largely mediated by its anti-inflammatory properties.

O-06 (13.45-14.00)

KERATINOCYTE-RESTRICTED RABGEF1 GENE DELETION RESULTS IN THE DEVELOPMENT OF LESIONS RESEMBLING ATOPIC DERMATITIS, AS WELL AS SYSTEMIC INFLAMMATION, IN VIVO

T. Marichal[1,2,*], N. Gaudenzio[2,*], L. Reber[2], P. Starkl[2], A. Roers[3], F. Bureau[1], Tam SY.[2,$], Tsai M. [2,$], Galli SJ[2,$]

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[*]These authors contributed equally and are co-first authors, [$]These authors jointly directed the work and are co-last authors.

RabGEF1, a guanine nucleotide-exchange factor for Rab5 GTPase, can negatively regulate mast cell activation in vitro. In addition, RabGEF1-deficient mice develop morbidity and severe skin inflammation associated with marked increases in skin mast cell numbers. These findings suggest that over-reactive skin mast cells may contribute to the observed skin pathology in vivo. In order to identify the cell type(s) which contribute to skin inflammation when RabGEF1 is absent, we attempted to delete Rabgef1 gene specifically in three major skin cell types, namely mast cells, myeloid cells and keratinocytes; thus, Rabgef1 floxed ($^{fl/fl}$) mice were crossed with mice expressing the Cre-recombinase under the influence of the promoter of Mcpt5, LysZ or K14, respectively, and the efficiency and specificity of Cre-mediated Rabgef1 gene deletion were assessed by single-cell genomic PCR. Unexpectedly, Mcpt5-Cre;Rabgef1$^{fl/fl}$ and Lysz-Cre;Rabgef1$^{fl/fl}$ mice appeared normal without phenotypic abnormalities. However, K14-Cre;Rabgef1$^{fl/fl}$ mice were normal at birth but rapidly developed morbidity and skin inflammation after 2-3 days, and died between 1 and 8 weeks of age. Skin pathology was characterized by epidermal hyperplasia, keratinocyte differentiation defects, and death of keratinocytes, followed by dermal infiltration of eosinophilic granulocytes, increases in mast cell numbers, and increased pro-inflammatory cytokine expression. Mice surviving 6-8 weeks also displayed signs of systemic inflammation, such as lymphadenopathy and splenomegaly, associated with increased granulocyte infiltration, blood eosinophilia and neutrophilia, and elevated levels of serum IgE. Further experiments will attempt to determine the mechanism by which keratinocyte-restricted RabGEF1 expression plays an essential role in maintaining skin immune homeostasis in vivo.

O-07 (14.00-14.15)

LUNG RESIDENT EOSINOPHILS REPRESENT A DISTINCT CELL SUBSET WITH HOMEOSTATIC FUNCTIONS

C. Mesnil[1,*], S. Raulier[1,*], G. Paulissen[1], D. Pirottin[1], T. Janss[1], M. Birrell[2], M. Belvisi[2], X. Xiao[3], L. Gillet[3], C. Desmet[1], F. Bureau[1,$], T. Marichal[1,$]

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Eosinophils are well known effector cells in the context of parasite infections or allergic disorders, where they can contribute to the inflammatory responses. However, under normal conditions, eosinophils are present in several tissues where they can regulate a variety of biological functions. Lung resident eosinophils (rEos) constitute one of the most abundant populations of tissue eosinophils, yet they have been little-studied so far. Here we combined flow cytometry phenotyping and sorting, immunohistochemistry, microscopy and microarray analyses to identify and characterize lung eosinophil populations in naive and allergic mice. We then used eosinophil-deficient mice as well as models of allergic airway sensitization to assess their functions. We show that mouse lung rEos display unique morphological and phenotypical features that unambiguously distinguish them from the inflammatory eosinophils (iEos) recruited to the lung during allergen-induced airway inflammation. Parenchymal rEos were not affected at all during the effector phase of airway allergy and cohabited with peribronchial iEos. In addition, we provide evidence supporting that rEos and iEos are released from the bone marrow as differentiated cells, as two phenotypically distinct subsets of rEos-like and iEos-like eosinophils were present in the blood of allergic mice. In addition, our data indicate that lung rEos, unlike iEos, do not depend on IL-5 (i.e., the most potent cytokine for the eosinophil lineage) for their presence in the lung. Functionally, transcriptomic analyses revealed a more regulatory profile for rEos than for iEos. In agreement with this finding, we further show that lung rEos are able to prevent the development of pulmonary type 2 immunity against inhaled allergens, thereby contributing to the maintenance of immune homeostasis in the airways. Finally, we show the existence of lung parenchymal rEOS in healthy human subjects, suggesting that our findings in mice are relevant to humans. This study identifies a novel regulatory mechanisms involved in lung homeostasis.

O-08 (14.15-14.30)

NEURONAL Kv2-MEDIATED CURRENT SUPPRESSION BY THE ANTICONVULSANT RETIGABINE

J.I. Stas[1,2], E. Bocksteins[1], C.S. Jensen[2], N. Schmitt[2], D.J. Snyders[1]

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Retigabine (RTG) is a recently developed and approved anticonvulsant drug for the treatment of partial/focal seizures. RTG enhances the activity of the neuronal M-current, encoded by voltage-gated K+ (Kv) 7 channels (i.e. Kv7.2 – Kv7.5) which results in suppression of neuronal excitability. Recent clinical reports have shown that RTG induces toxic side-effects upon chronic exposure in patients. On the other hand, RTG also has neuroprotective properties. In both cases, the mechanisms underlying this toxicity and neuroprotective effects are not fully understood. We found that the auxiliary KCNE2 subunit reduced the retigabine sensitivity of heterotetrameric Kv7.2-Kv7.3 channels. In addition, clinically relevant retigabine concentrations inhibited Kv2.1 channel function both in HEK cells and cultured hippocampal neurons. Suppression of the Kv2.1 conductance was poorly reversible. Our results indicate that auxiliary subunits, like KCNE2, as well as off-target effects on Kv2.1 channels might be involved in some of retigabine's clinical features.

O-09 (14.30-14.45)

CINNAMALDEHYDE EXHIBITS LOCAL ANESTHETIC-LIKE BLOCK ON HUMAN $NA_V1.5$ CHANNELS

J. Alvarez-Collazo[1], A. López-Requena[1], A. Talavera[2], Y.A. Alpizar[1], J.L. Alvarez[3], K. Talavera[1]

[1]Laboratory for Ion Channel Research, Department of Cellular and Molecular Medicine, KU Leuven, Belgium; [2]Molecular Recognition Unit, Department of Structural Biology, VUB, Brussels, Belgium; [3]Laboratory of Electrophysiology, Institute of Cardiology and Cardiovascular Surgery, La Habana, Cuba.

Cinnamon is commonly known as a flavorant and has been widely used in traditional medicine as anti-diabetic and anti-hypertensive agent. Its major component, cinnamaldehyde (CA), is a well-known activator of TRPA1 channels. However, we have recently shown that CA blocks L-type Ca^{2+} channels in cardiac and vascular smooth muscle cells and exhibits local anaesthetic (LA)-like actions in sensory neurons. CA shares some structural similarities with lidocaine, which seem to be important for neuronal Na^+ channel inhibition. The aim of the present work was to study whether CA possesses LA-like actions on the human voltage-gated Na^+ 1.5 channel isoform ($hNa_v1.5$), widely expressed in heart and in the gastrointestinal tract, and to analyse if it shares the LA-binding site. Whole-cell patch-clamp experiments were performed in HEK293T cells transiently transfected with the $hNa_v1.5$ or the single point F1760A mutant. CA blocked Wild Type (WT) Na^+ currents (I_{Na}) in an essentially tonic manner with an IC_{50} = 1.04 ± 0.08 mM. The activation curve was not altered but the inactivation curve was shifted by ~12 mV to more negative potentials. In the F1760A mutant, CA was less potent (IC_{50} = 2.36 ± 0.05 mM) and exhibited much less voltage-dependent action. Docking simulations using a $hNa_v1.5$ channel model indicate that CA and lidocaine bind in close proximity to the residue F1760. Our results demonstrate that CA inhibits $hNa_v1.5$, and suggest that CA could be used to treat pain and motility disorders in gastrointestinal diseases like irritable bowel syndrome.

O-10 (14.45-15.00)

HMGB1-DRIVEN FEEDFORWARD HEPATOCYTE NECROPTOSIS CIRCUIT IN LETHAL ACETAMINOPHEN-INDUCED LIVER INJURY

C. Minsart[1], C. Liefferinckx[1], S. Rorive[2], A. Lemmers[1,3], E. Quertinmont[1], E. Trepo[1,3], J. Deviere[1,3], C. Moreno[1,3], I. Leclercq[4], R. Moreau[5], T. Gustot[1,3,5]

[1]Laboratory of Experimental Gastroenterology, ULBruxelles, [2]DIAPATH-center for microscopy and molecular imaging (CMMI), Gosselies, Belgium, [3]Department of Gastroenterology, HepatoPancreatology and Digestive Oncology, C.U.B. Hôpital Erasme, [4]Laboratory of Hepato-Gastroenterology, UCLouvain, [5]Inserm Unité 1149, Centre de Recherche sur l'inflammation (CRI), Paris, France

Release of damage-associated molecular patterns, in particular High-mobility group box (HMGB) 1, contributes to acetaminophen (APAP)-induced liver injury but the mechanisms involved are currently incompletely understood. The aim of the study is to investigate the contribution of HMGB1 in vivo and in vitro at early time points of the APAP-induced liver injury and its role in the propagation of necrosis process. APAP hepatotoxicity was induced in vivo by intraperitoneal injection in C57Bl/6 mice and in vitro on cultured HepaRG cells. HMGB1 was quantified by ELISA or immuno-staining. Cell death was determined by MTT, ALT, LDH and caspase-3 assays. Glycyrrhizin (GL) and ethyly pyruvate (EP) was used to inhibit HMGB1. Liposomal clodronate was administrated to mice to deplete Kupffer cells (KC). Expression of HMGB1 receptors was assessed by RT-PCR and flow cytometry. Dabrafenib and necrostatin-1was used to inhibit receptor-interacting protein (RIP)3 and RIP1 respectively. In APAP-challenged mice, GL inhibited the HMGB1 release (decrease of serum levels and increase in hepatocellular retention in centrolobular area) with improved survival. Depletion of KC in mice exacerbated APAP- induced hepatocyte necrosis and HMGB1 release suggesting that HMGB1 did not act through KC activation. Addition of APAP on cultured HepaRG induced cell necrosis characterized by LDH release without caspase-3 activation, and HMGB1 release. Moreover, HepaRG were exposed to APAP for 6 hours and the so-conditioned medium induced cell death of unexposed HepaRG. Inhibition of HMGB1 by GL or EP reduced APAP- and conditioned medium-induced HepaRG necrosis and further HMGB1 release. Exposure of HepaRG and primary human hepatocytes to rhHMGB1 resulted in their death, underlining that HMGB1 acts directly on hepatocytes. HepaRG expressed previously described HMGB1 receptors (TLR2, TLR4 and TLR9) at mRNA and protein level. Pre-treatment of HepaRG by dabrafenib, a specific RIP3 inhibitor, and not by necrostatin-1prevented this HMGB1-induced cell death. It is concluded that HMGB1 contributes to APAP-induced liver injury through a RIP3-dependent hepatocyte necroptosis using a feedforward mechanism. Inhibition of HMGB1 at the early phase of APAP-induced liver injury improved animal survival by reducing the propagation of this regulated hepatocyte necrosis.

P1

CALCIUM-BINDING PROTEINS AND MOUSE SPERM MOTILITY

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Motility is one of the most important calcium-dependent processes regulating sperm fertility. Calcium channels and calcium stores have been extensively studied in sperm cells, although the involvement of EF-hand calcium-binding proteins remains poorly described in spermatozoa. The aim of the present study was to analyse the potential role of EF-hand calcium-binding proteins in murine sperm motility. Our investigations compared sperm motility from Wild Type (WT), Calretinin knock-out ($CR^{-/-}$) and Calretinin/Calbindin D-28k/Parvalbumin knock-out ($CR^{-/-}CB^{-/-}PV^{-/-}$) mice. The presence of Calretinin, Calbindin D-28k and Parvalbumin was detected by immunofluorescence and Western blotting in WT sperm cells as well as in the control tissue (cerebellum). Sperm motility parameters were measured using a Computer Assisted Sperm Analysis (C.A.S.A.) system under basal and stimulated conditions (NH_4Cl 25mM). Under basal experimental conditions, the curvilinear velocity was faster and the lateral head movement of higher magnitude in WT than in $CR^{-/-}$ sperm. By contrast, the beat cross frequency was lower in WT than in $CR^{-/-}CB^{-/-}PV^{-/-}$ sperm. An increase in the curvilinear velocity and in the amplitude of the lateral head movement, as well as a decrease in the beat cross frequency, were observed following the incubation of WT and knock-out spermatozoa with NH_4Cl. All these modifications appeared to be less marked in $CR^{-/-}$ mice. The NH_4Cl-induced decrease in beat cross frequency was significantly less pronouced in the absence of expression of the three proteins ($CR^{-/-}CB^{-/-}PV^{-/-}$ sperm). Moreover, *in vivo* studies revealed a reduction in the number of offsprings in $CR^{-/-}$ mice compared to the WT mice. In conclusion, our study documents, for the first time, the potential role of Calretinin, Calbindin D-28k and Parvalbumin on mouse sperm motility. Further experiments will be performed to confirm the impact of EF-hand calcium-binding proteins on sperm fertility.

P2

A 13 AMINO ACID DIFFERENCE IN TWO TRPM3 SPLICE VARIANTS RESULTS IN TREMENDOUS PHARMACOLOGICAL VARIATIONS

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TRPM3 is a cation conducting ion channel that belongs to the superfamily of transient receptor potential (TRP) channels. It is polymodally regulated by stimuli including heat, hypoosmolarity, the endogenous agonist pregnenolone sulphate (PS) as well as the synthetic agonist nifedipine (Nif). Furthermore, we have recently shown that combined application of PS and the antifungal drug clotrimazole (Clt) leads to a huge potentiation of the PS-induced TRPM3 currents. At the moment, a vast amount of TRPM3 splice variants have been described. In this study, we have selected one splice variant, TRPM3α1, that differs from the commonly used TRPM3α2 isoform only in the presumed pore region. More precise, TRPM3α1 shows a difference of only 13 amino acids, including an insertion of 12 amino acids within the presumed pore loop and an alanine instead of a proline following that insertion. Remarkably, the TRPM3α1 isoform did not show any increase in channel activity during stimulation by PS or Nif. However, HEK293 cells transfected with TRPM3α1 showed a robust increase in current amplitudes during stimulation by Clt alone. Here, we conducted a comparative study of the TRPM3α1 and α2 isoforms. This study aims to elucidate the isoform-specific pharmacological and biophysical characteristics of the TRPM3 channel.

P3

PROPERTIES OF EXCITATORY SYNAPTIC INPUTS ONTO DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA

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Dopamine (DA) neurons of the substantia nigra pars compacta (SNc) exhibit two main firing patterns, single action potential (AP) firing and bursting. The level of DA released by these neurons in postsynaptic areas depends on the presynaptic AP firing pattern and is essential to modulate several aspects of behaviour such as the control of movement and the coding of reward. Bursting activity is mediated by glutamatergic afferents and specifically by the activation of NMDA receptors (NMDARs). However, the link between the activation of NMDARs and the generation of bursts is poorly understood. In addition, it is not clear how information from glutamatergic afferents is integrated and where NMDARs are localized at the subcellular level. We addressed these questions by studying the contribution of NMDA currents to excitatory postsynaptic currents (EPSCs) in SNc DA neurons in brain slices from juvenile rats. We carried out our experiments at +40 and -70 mV to calculate the NMDA/AMPA ratio (expressed as the maximal NMDA current amplitude at +40 mV/AMPA current amplitude at -70 mV). A clear NMDA component could be observed at +40 mV. The mean charge transfer through NMDARs was in control conditions 249.7 fC and was potentiated by the application of 300 µM glycine to 438.6 fC. In addition, the NMDA/AMPA ratio was significantly higher with the coagonist (0.65) than in control conditions (0.47). In conclusion, these results show that NMDARs are present at glutamatergic synapses of SNc DA neurons and that the NMDAR glycine site is not saturated at these synapses in our slice preparation. In further experiments, we will investigate the effect of D-serine and blockers of the glycine transporters on excitatory synaptic transmission in DA neurons.

P4

MECHANISM OF THE FAST AFTERHYPERPOLARIZATION IN RAT SEROTONERGIC NEURONS

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The dorsal raphe nucleus is a serotonergic brainstem nucleus which has been strongly associated with brain dysfunction, especially mood disorders. Electrophysiological studies have established that putative serotonergic neurons have a high input resistance (150-400 MΩ), long duration action potentials (~1.8 ms at mid-height), and a prominent medium duration afterhyperpolarization (mAHP) (200-800 ms). This mAHP is blocked by apamin, indicating that it is mediated by small-conductance Ca^{2+}-activated K^+ channels (SK) channels. Intracellular recordings have shown that blockade of this mAHP uncovers another AHP, peaking shortly after the action potential and decaying with a τ of ~30 ms. The mechanism of this faster AHP (fAHP), which may be at least as important as the mAHP in regulating firing frequency, is unknown. The purpose of this study was to characterize the currents mediating this fAHP. Intracellular recordings of DR neurons were performed in rat (3-6 week-old) brain slices. The role of large conductance Ca^{2+}-activated K^+ channels (BK) was first ruled out using the selective blocker paxilline. We next examined the effect of other classical K^+ channel blockers, such as TEA and 4-AP. The fAHP was not modified by these drugs either. We next investigated the possible contribution of Na^+-activated K^+ channels (K_{Na} channels, which are encoded by the Slick and Slack genes), by replacing external Na^+ by Li^+. This manipulation markedly reduced the fAHP but induced non-specific effects on the shape of the action potentials, thus making the interpretation of this experiment difficult. To better investigate the nature of this current, we started voltage clamp experiments using patch clamp electrodes. In preliminary experiments, depolarizing pulses (from -60 mV to +20 mV) induced an outward K^+ current, which appeared to be reduced by the Na^+ channel blocker tetrodotoxin, suggesting that K_{Na} channels are expressed in these neurons.

P5

ROLE OF TRPC1 ION CHANNEL IN HIPPOCAMPAL NEURONS

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Transient receptor potential-canonical 1 (TRPC1) is a non selective cation channel (PCa/PNa ~ 1). It is involved in store-operated Ca^{2+} entry in cooperation with Orai1 channel and activated by STIM1, a sensor of Ca^{2+} contents in the endoplasmic reticulum. However, several pieces of evidence suggest that TRPC1 can be activated independently of store depletion in response to agonists. Using a LacZ reporter mouse, we found that TRPC1 is abundantly expressed in neurons from the CA1-CA3 regions and to a lesser extent from the dentate gyrus region of the hippocampus. We compared cellular culture of hippocampal neurons from *Trpc1*$^{-/-}$ mice to wild type mice. Interestingly, stimulation with dihydroxyphenylglycine (DHPG), an agonist of group I metabotropic glutamate receptors (consisting of mGluR1 and mGluR5) induced a store-independent entry of Ca^{2+} that was very much reduced in hippocampal neurons from *Trpc1*$^{-/-}$ mice. Studying brains slices on microelectrode arrays (MEA, Multi Channel Systems), we observed that LTP in Schaffer collaterals-CA1 synapses was diminished in *Trpc1*$^{-/-}$ mice when induced with a mild stimulus (theta burst consisting of 4 stimuli at 100 Hz repeated at 5 Hz during 1s). Cognition tests such as Y-shape modified maze and contextual fear-conditioning, realized on 4-8 months old mice revealed a deficit of memory. Moreover, we noted that the specific hippocampal over-expression of the immediate early gene zif268 related to new environment learning was blunted in *Trpc1*$^{-/-}$ mice. We conclude that TRPC1 channels are activated by mGluR stimulation and play a role in neuronal plasticity.

P6

IMPLICATION OF THE MICRORNAS 199a-3p and 199a-5P IN THE VASCULAR FUNCTION: MODULATION OF THE NOS/NO PATHWAY

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MicroRNAs (miR) 199a3p and 199a5p have been first identified as modulators of cell proliferation and survival in the context of cancerogenesis. However recent data suggest a role in cardiovascular functions. In this context, we seek to evaluate how miR199a3p and miR199a5p modulate endothelial function and to identify their molecular targets. In this aim, mice were treated with miR199a3p/5p inhibitors, the contractile profile and endothelial function were evaluated ex vivo in the aorta. Circulating HbNO was evaluated by EPR in venous blood. Cultured endothelial cells were transfected with miR199a3p/5p inhibitors or a scramble sequence. Nitric oxide (NO) production, and the expression and activity of eNOS and its major regulators were measured by electro-paramagnetic resonance (EPR) and Western blot respectively. Angiogenesis was investigated in 2D-culture in matrigel support. Results showed that in vessels from mice treated with antagomirs against miR199a3p or 5p showed a larger NO-dependent relaxation compared to controls. Circulating HbNO measured in venous blood collected from these mice was significantly increased compared to controls suggesting a role of both miRs in the control of the NOS/ NO pathway.In endothelial cells, repression of miR 199a3p/5p improved eNOS activity through an increased eNOS phosphorylation at serine 1177 and a decreased phosphorylation status of threonine 495. Accordingly, both treatments promoted Akt activation and calcineurin expression. Furthermore, addition of LY294002, an Akt inhibitor, inhibited the increase of eNOS phosphorylation at serine 1177 induced by LNA treatment. The eNOS allosteric regulator, Cav-1, was not modulated by LNA treatments. In addition, inhibition of miR199a5p upregulated VEGF production and promoted tubes formation in endothelial cells. In conclusion, our results demonstrate that miR199a3p/5p modulate the NOS/NO pathway in the endothelium by promoting NO production and repressing NO degradation. By pointing at calcineurin and Akt as targets of miR199a3p/5p, our works confirm that miR 199a3p/5p are important regulators of endothelial function. These results also suggest a strong implication of the Akt pathway in this modulation. Interestingly, data obtained by inhibiting miR199a5p demonstrated an additional control of angiogenesis.

P7

MODIFICATION OF VASCULAR TONE IN CONDUCTANCE AND RESISTANCE ARTERIES IN RESPONSE TO CARDIAC HYPERTROPHY

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Left ventricular hypertrophy (LVH) is a pathophysiological adaptive response associated with coronary endothelial dysfunction. This work aimed to evaluate if LVH causes vascular modifications outside of coronary circulation. Vascular tone was assessed by wire myography in aorta and left carotid artery (LCA), and by pressure myography in small mesenteric resistance arteries (SMRA). Pathological LVH was evoked by transverse aortic constriction model (TAC) or induction of β1-adrenoceptors- activating –autoantibodies (ADRB1 model). These models were compared to physiological cardiac hypertrophy associated with voluntary wheel running (VWR). NO-mediated relaxation was reduced in LCA of both hypertrophic models (Two-Way ANOVA: ADRB1: $p<0.0001$, treated n=10, control n=10; TAC: $p<0.0002$, Sham n=8, TAC n=6), as well as in aorta from TAC animals (Two-Way ANOVA: $p=0.0208$, TAC n=7, Sham n=8). In LCA from ADRB1 treated mice, total endothelial relaxation decreased proportionally to the degree of LVH (Pearson; $p<0.0001$, $R^2=0.98$, n=7). Contractility of LCA was significantly increased in TAC animals in response to depolarization (t-test; 1.67±0.10vs1.18±0.11mN/mm Sham treated, $p=0.087$, n=6/5) and to phenylephrine (t-test; 1.27±0.10vs1.02±0.05mN/mm Sham, $p=0.0404$, n=6/5). Reduced EC50 to phenylephrine were observed in SMRA in the TAC model (1.04e-6±0.08 vs 2.09e-6±0.08M Sham, $p=0.0076$, n=9/9) and LCA of ADRB1 (2.35e-8±0.10vs5.81e-8±0.12M control, $p=0.0018$, n=4/5). These vascular alterations were not recapitulated in vessels from VWR animals. Pathological LVH is associated with endothelial dysfunction and increased developed force to phenylephrine as a sensitization to this drug, suggesting that LVH does not only affect heart function and circulation but also induces modification in the entire vascular tree.

P8

CANDIDACIDE EFFECT OF HYPOHALOUS SOLUTION

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Candida albicans is a yeast that colonizes removable dentures through the formation of biofilm which may initiate stomatitis. Copying salivary antimicrobial mechanisms (especially peroxidase systems) enables the design of hygiene products able to reduce yeast denture contamination and consequently to prevent mucosal infection. This study aims to evaluate the antifungal effect of hypothiocyanite ($OSCN^-$) and hypoiodite (OI^-) compounds produced in vitro by a lactoperoxidase system and tested ex vivo on dentures colonized by Candida. Hypohalous solution was obtained by diving lactoperoxidase adsorbed on beads in a pH 7.40 phosphate buffer solution containing hydrogen peroxide (H_2O_2), thiocyanate (SCN^-) and iodide (I^-). This oxidant solution has been shown to eliminate in vitro 2 x 10^8 yeast cells after a 5 min incubation at room temperature. Candida survival rates in phosphate buffer and in water have been shown similar. Dentures (n = 46) were investigated ex vivo by swabbing before and after diving in the oxidant solution (N = 12) or in sterile water (N = 15). The screening on ChromAgar Petri dishes showed Candida colonization on prostheses in 58.7% of individuals. The most frequent species isolated were C. albicans (74.1 % of the positive cultures), C. glabrata (18.5 %) and C. tropicalis (3.7 %). Carriage of more than one yeast species was found in 14.8 % of the contaminated dentures. A single diving in the oxidant solution during 5 min produced a yeast-count decrease down to 10% of the initial value in 7 prostheses out of 12 and control solution never decreased Candida colonization (Chi-square test: p = 0.0006; Fisher's exact test: p = 0.0009). The data suggest that hypohalous compounds can play the role of an ecological determinant reducing denture load of Candida, already after a single immersion.

P9

ANOCTAMIN 1 (Ano1) IS REQUIRED FOR GLUCOSE-INDUCED MEMBRANE POTENTIAL OSCILLATIONS AND INSULIN SECRETION BY MURINE B-CELLS

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Anions have been known for long to play a role in glucose-induced insulin secretion (GSIS). The purpose of this study was to establish whether anion exit is mediated through the recently identified calcium sensitive chloride channel, Ano1in β cells and the importance of the latter efflux for GSIS. By RT-PCR, western blotting and immunohistochemistry, Ano1 was found to be expressed in murine islets. Its gating switches on/off the oscillating electrical activity, which are critical for GSIS. The Ano1 blockers2-(5-ethyl-4-hydroxy-6-methylpyrimidin-2-ylthio)-N-(4-(4-methoxyphenyl)thiazol-2-yl)acetamide (T-AO1) and tannic acid (TA) reduce action potential (AP) rate and repolarize residual AP peak on islets and dispersed murine β-cells. In the latter case, the reduction of AP rate is larger than 87 %. With T-AO1, the average membrane potential is repolarized by at least 13 mV. These inhibitors effects on membrane potential are associated with inhibition of GSIS (8.3 mM glucose, 100 % with T-AO1 and 94 % with TA; 16.7 mM glucose, 100 % with T-AO1 and 67 % with TA). Blocking Ano1 antibodies also abolish the 16.7-mM GSIS increment.Single-channel Cl- currents from rat β-cells are activated by 1 μM Ca^{2+}. Current-voltage relationship is linear and exhibits an unique unitary slope-conductance of 8.37 pS, matching the Ano1 described conductance. T-AO1 and TA respectively inhibit by 100 and 86 % the open probability while they had no effect on Ba^{2+} currents. Lowering intracellular anion concentration (low Cl^- and HCO_3^- media with bumetanide and acetazolamide) provokes a 65% reduction in AP amplitude and a 15 mV AP peak repolarization. Taken together, these data demonstrate that Ano1 is required to sustain membrane potential oscillations and insulin secretion.

P-10

CHEMERIN ADDED TO ENDOTHELIN-1 INDUCES PULMONARY ARTERY SMOOTH MUSCLE CELLS PROLIFERATION

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The adipokine chemerin has been recently reported to regulate proliferation of myoblasts. In pulmonary arterial hypertension (PAH), proliferation of pulmonary artery smooth muscle cells contributes mostly to the deleterious vascular remodeling process. In the present study, we hypothesized that chemerin alone or added to endothelin-1, a major mediator implicated in the pathogenesis of PAH, induces proliferation of pulmonary artery smooth muscle cells. We realized primary cultures of rat pulmonary artery and thoracic aorta smooth muscle cells. Proliferation was tested by bromodeoxyuridine incorporation after 24h incubation with increasing concentrations of chemerin ($0.5.10^{-8}$, 10^{-8}, $0.5.10^{-7}$ and 10^{-7} mol/L) with or without endothelin-1 (10^{-7} mol/L). In aorta smooth muscle cells, chemerin alone with or without endothelin-1 did not induce any proliferation. In pulmonary artery smooth muscle cells, chemerin at 10^{-8}, $0.5.10^{-7}$ and 10^{-7} mol/L added to endothelin-1 induced pulmonary artery smooth muscle cells proliferation, while chemerin or endothelin-1 alone did not induce any proliferation. It is concluded that chemerin added to endothelin-1 induces the proliferation of pulmonary artery smooth muscle cells but not of thoracic aorta smooth muscle cells.

P11

THE ROLE OF INTERACTING PROTEINS IN TRPV4 CHANNELOPATHIES

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Members of the superfamily of transient receptor potential (TRP) channels are crucial players in various physiological systems and are involved in the development of numerous channelopathies. TRPV4 is a non-selective Ca^{2+} channel activated by multiple physical and chemical stimuli and is widely expressed in various cell types including keratinocytes, osteoclast, sensory neurons and endothelial cells. Interestingly, mutations in TRPV4 cause a broad phenotypic spectrum of diseases, classified in skeletal dysplasias (SD), arthropathies and distal motor neuropathies. The majority of the disease causing mutations is located in the N-terminal ankyrin repeat domain of TRPV4, essential in protein-protein interaction. This designates a potential role for cell-type specific interacting proteins in the development of the diverse TRPV4 diseases. To identify potential binding partners of the human TRPV4 protein, we performed a MAmmalian Protein-Protein Interaction Trap (MAPPIT) screening using wild type or mutant N-terminus of hTRPV4 as bait. Among the newly identified interacting partners we focused on the zinc-finger domain containing protein ZC4H2. Mutations in the gene encoding for ZC4H2 cause Arthrogryposis Multiplex Congenita (AMC) and Wieacker-wolff syndrome, characterized by congenital joint contractions and developmental defects of the musculoskeletal and nervous system, reminiscent of features observed in TRPV4 channelopathies. Quantitative real time PCR experiments showed expression of ZC4H2 in relevant TRPV4-expressing cell types including primary osteoclasts, osteoblasts and spinal motor neurons. Fura 2-mediated Ca^{2+}-imaging experiments indicate that ZC4H2 increases basal and agonist-evoked activity of hTRPV4 in HEK293-T cells. We further use patch-clamp recordings, TIRF microscopy and biochemical analysis to elucidate the mode of interaction between TRPV4 and ZC4H2, which may enlighten the etiology of TRPV4-dependent channelopathies.