Abstracts

Experiments on animals and animal tissues

It is a requirement of The Society that all vertebrates (and Octopus vulgaris) used in experiments are humanely treated and, where relevant, humanely killed.

To this end authors must tick the appropriate box to confirm that:

For work conducted in the UK, all procedures accorded with current UK legislation.

For work conducted elsewhere, all procedures accorded with current national legislation/guidelines or, in their absence, with current local guidelines.

Experiments on humans or human tissue

Authors must tick the appropriate box to confirm that:

All procedures accorded with the ethical standards of the relevant national, institutional or other body responsible for human research and experimentation, and with the principles of the World Medical Association's Declaration of Helsinki.

Guidelines on the Submission and Presentation of Abstracts

Please note, to constitute an acceptable abstract, The Society requires the following ethical criteria to be met. To be acceptable for publication, experiments on living vertebrates and Octopus vulgaris must conform with the ethical requirements of The Society regarding relevant authorisation, as indicated in Step 2 of submission.

Abstracts of Communications or Demonstrations must state the type of animal used (common name or genus, including man. Where applicable, abstracts must specify the anaesthetics used, and their doses and route of administration, for all experimental procedures (including preparative surgery, e.g. ovariectomy, decerebration, etc.).

For experiments involving neuromuscular blockade, the abstract must give the type and dose, plus the methods used to monitor the adequacy of anaesthesia during blockade (or refer to a paper with these details). For the preparation of isolated tissues, including primary cultures and brain slices, the method of killing (e.g. terminal anaesthesia) is required only if scientifically relevant. In experiments where genes are expressed in Xenopus oocytes, full details of the oocyte collection are not necessary. All procedures on human subjects or human tissue must accord with the ethical requirements of The Society regarding relevant authorisation, as indicated in Step 2 of submission; authors must tick the appropriate box to indicate compliance

SA01

Blood pressure regulation by endothelial cell TMEM16A channels

Jonathan Jaggar[1]

[1]*University of Tennessee Health Science Center, Memphis, United States*

Endothelial cells line all blood vessels and regulate the contractility of smooth muscle cells to tune regional organ blood flow and systemic pressure. Chloride (Cl^-) is the most abundant anion in endothelial cells, yet the molecular identities and physiological functions of Cl^- channels in endothelial cells are poorly understood. I will describe our recent work where we generated tamoxifen-inducible, endothelial cell-specific TMEM16A channel knockout mice to investigate signaling mechanisms and physiological functions of this Cl^- channel in this cell type. Our data indicate that TMEM16A channels generate Ca^{2+}-activated Cl^- currents in endothelial cells. Vasodilators stimulate TMEM16A channels in endothelial cells, leading to arterial hyperpolarization, vasodilation, and a reduction in blood pressure. I will also provide evidence that TMEM16A channel activation reduces intracellular Cl^- concentration, which stimulates With-No-Lysine (WNK), a Cl^--sensitive kinase, in endothelial cells. WNK kinase signaling then activates transient receptor potential V4 channels in endothelial cells to induce vasodilation.

SA02

Interstitial cells, drivers of smooth muscle function

Kenton Sanders[1,3], Sal Baker[1,4], Sean Ward[1,5]

[1]Department of Physiology and Cell Biology, University of Nevada, Reno School of Medicine, Reno, United States, [2]Department of Physiology and Cell Biology, University of Nevada, Reno School of Medicine, Reno, United States, [3]University of Nevada, Reno School of Medicine, Reno, United States, [4]University of Nevada, Reno School of Medicine, Reno, United States, [5]University of Nevada, Reno School of Medicine, Reno, United States

Interstitial cells, drivers of smooth muscle function

Kenton M. Sanders, Sal A. Baker and Sean M. Ward

Department of Physiology and Cell Biology

University of Nevada, Reno School of Medicine

Reno, NV 89511

Traditional reviews and courses on the physiology of gastrointestinal (GI) motility have stressed 'myogenic' and 'neurogenic' mechanisms of regulation. However, now it is known that features long-considered part of myogenic regulation are more complex. In fact, smooth muscle cells (SMCs) in the gastrointestinal (GI) tract do not function in isolation. Populations of interstitial cells (interstitial cells of Cajal (ICC) and PDGFRa$^+$ cells) make gap junctions with SMCs, forming a super-syncytium known as the SIP syncytium. ICC and PDGFRa$^+$cells display spontaneous Ca^{2+} release events that activate signature conductances expressed by these cells, ANO1 in ICC and SK3 in PDGFRa$^+$ cells. Due to the electrical coupling, conductances activated in ICC and PDGFRa$^+$ cells conduct to SMCs and affect the excitability and excitation-contraction coupling of the musculature. A specific property of some ICC is generation of pacemaker activity that causes periodic depolarizations to conduct through the SIP syncytium. These events, known as slow waves, have a complex mechanism and conductances not present in SMCs. Thus, slow waves conduct passively to SMCs and cause depolarizations superimposed upon resting potentials. Depolarization of SMCs activates voltage-dependent L-type Ca^{2+} channels, Ca^{2+} entry and excitation-contraction coupling. This mechanism is responsible for development and propagation of rhythmic, phasic contractions that constitute peristalsis and segmentation in GI organs. Thus, ICC are responsible for the patterned contractions of GI motility. Neural inputs are largely transduced by ICC and PDGFRa$^+$ cells and not directly by SMCs. ICC express specific receptors and signaling mechanisms to facilitate motor neurotransmission. Dominant motor neurotransmitters, acetylcholine (ACh) or nitric oxide (NO), enhance or depress Ca^{2+} release and activation of ANO1 channels, respectively. PDGFRa$^+$ cells express receptors and signaling pathways to transduce purinergic and peptidergic inhibitory neurotransmission. Recent evidence also suggests inputs from multiple GI hormones are also transduced by interstitial cells. Together, inputs from ICC and PDGFRa$^+$ cells tune the excitability of GI SMCs to enhance or depress

propagating phasic contractions or modulate the tonic contractions of sphincters. GI motor patterns, originate in and are regulated by the integrated behaviors of the SIP syncytium.

SA03

M2 receptors and ionic mechanisms in airway smooth muscle contraction: folklore and dogma.

Keith Thornbury[1], Gerard Sergeant[1], Mark Hollywood[1], Tuleen Alkawadri[1], Srijit Ghosh[1]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland, [2]Dundalk Institute of Technology, Dundalk, Ireland, [3]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland

It is generally agreed that increased cholinergic tone is an important factor in bronchoconstriction in chronic obstructive airways disease (COPD). Despite this, the mechanisms underlying acetylcholine-induced contractions are incompletely understood. Cholinergic contractions are widely thought to be mediated by postjunctional M3 muscarinic receptors (M3R) on airway smooth muscle (ASM), even though they are outnumbered 4:1 by postjunctional M2 receptors (M2R) in most species. M2R are also expressed prejunctionally on parasympathetic nerves, where they inhibit acetylcholinerelease[1]. Pharma companies have therefore sought to develop M3R-selective anticholinergic drugs to treat COPD because of the risk that blocking prejunctional M2R could increase acetylcholine release and exacerbate bronchoconstriction[1].

Recently, we have discovered that M2R can contribute more to cholinergic contractions than previously thought[4]. When mouse bronchial rings were subjected to electrical field stimulation (EFS) at 100s intervals (2Hz, 1s trains), phasic contractions were evoked that were entirely blocked by the M3R blocker, 4-DAMP and were unaffected by M2R blockers. Surprisingly, when the stimulus interval was shortened to 10s, the contraction amplitude increased by 2-3-fold. Again, the contraction was completely blocked by 4-DAMP, but this time M2R blockers (methoctramine, AFDX-116), selectively blocked all of the increase in amplitude at 10s stimulus intervals (Fig.1). Taken together, this suggested that shortening the stimulus interval resulted in M2R sensitisation of M3R-mediated contractions. When similar experiments were carried out in M2R knockout mice, shortening the stimulus interval failed to enhance contraction amplitude. Interestingly, when a sub-threshold concentration of carbachol was applied to tissues receiving the stimulus trains at 100s intervals, there was a methoctramine-sensitive increase in the amplitude of the phasic contractions in wild-type mice, but not in M2R knockout mice. This is consistent with carbachol stimulating extra-junctional M2R.

ASM cells possess L-type voltage-dependent Ca^{2+} channels (L-VDCC) and the means to regulate membrane potential via TMEM16A channels and various K^+ channels. However, there is a lack of consensus as to the roles played by these channels in ASM contraction[2,3]. We further explored the mechanisms underlying the M2R-mediated enhancement of EFS-evoked contractions using blockers of either L-VDCC (nifedipine, verapamil) or TMEM16A (Ani9, $CaCC_{inh}A01$). These drugs all failed to affect responses evoked at 100 s intervals, but completely reversed the enhancement seen on switching to 10s stimulation. Thapsigargin, a SERCA pump blocker, enhanced EFS responses at 100s intervals in a similar manner to switching to 10s intervals, and this effect was reversed by nifedipine or Ani9.

We propose a model whereby cholinergic stimulation results in L-VDCC activation by TMEM16A-mediated membrane depolarisation. In M3R-exclusive responses, the incoming Ca^{2+} is immediately taken up into sub-membrane sarcoplasmic reticulum via SERCA (the 'superficial buffer barrier' mechanism). When M2Rs (possibly located extra-junctionally) are co-activated, Gi protein-mediated suppression of cAMP production results in SERCA pump inactivation, which allows the incoming Ca^{2+} to reach the contractile proteins. Since extra-neuronal production of acetylcholine has been proposed to contribute to increased cholinergic tone in COPD[5], we suggest that this may stimulate M2R on ASM and contribute to the bronchoconstriction typical of this disorder.

All work was carried out in compliance with EU legislation.

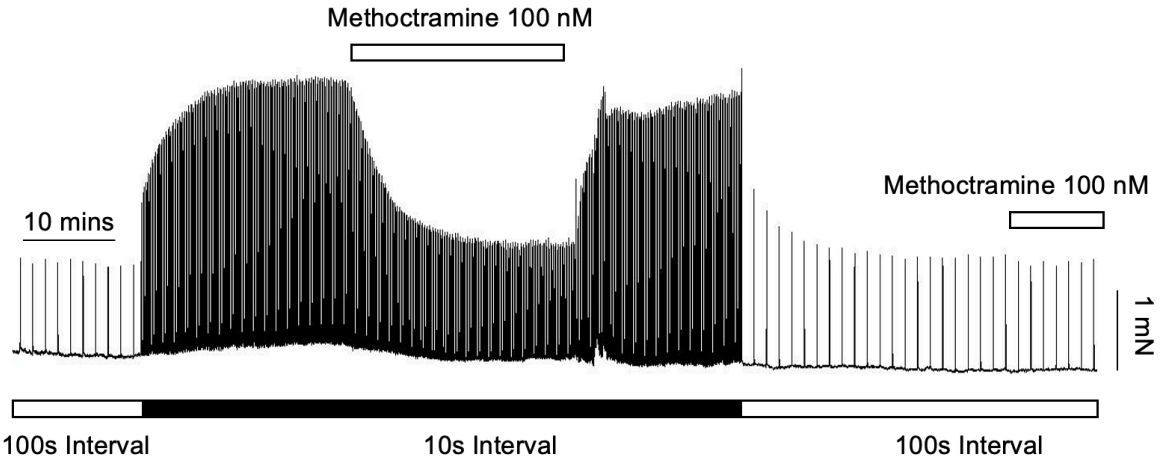

Figure 1. M2 muscarinic receptors augment nerve-evoked contractions of airway smooth muscle. A) Electric field stimulation (EFS, 1 second trains at a frequency of 2 Hz) evoked contractions of airway smooth muscle. Reducing stimulus interval from 100s to 10s enhanced the amplitude of neurogenic cholinergic contractions three-fold. This effect was reversed by the M2 receptor antagonist methoctramine, but it did not inhibit contractions evoked at 100s intervals.

1. Moulton BC, Fryer AD. (2011). Muscarinic receptor antagonists, from folklore to pharmacology; finding drugs that actually work in asthma and COPD. Br J Pharmacol. 163(1):44-52. doi: 10.1111/j.1476-5381.2010.01190.x. 2. Janssen LJ. (2002). Ionic mechanisms and Ca(2+) regulation in airway smooth muscle contraction: do the data contradict dogma? Am J Physiol Lung Cell Mol Physiol. 2002 Jun;282(6):L1161-78. doi: 10.1152/ajplung.00452.2001. 3. Brueggemann LI, Kakad PP, Love RB, Solway J, Dowell ML, Cribbs LL, Byron KL. (2012). Kv7 potassium channels in airway smooth muscle cells: signal transduction intermediates and pharmacological targets for bronchodilator therapy. Am J Physiol Lung Cell Mol Physiol. 302(1):L120-32. doi: 10.1152/ajplung.00194.2011. 4. Alkawadri T, McGarvey LP, Mullins ND, Hollywood MA, Thornbury KD, Sergeant GP. (2021). Contribution of Postjunctional M2 Muscarinic Receptors to Cholinergic Nerve-Mediated Contractions of Murine Airway Smooth Muscle. Function (Oxf). 20;3(1):zqab053. 5. Pieper MP. (2012). The non-neuronal cholinergic system as novel drug target in the airways. Life Sci. 91(21-22):1113-8. doi: 10.1016/j.lfs.2012.08.030.

SA04

Reshaping vascular smooth muscle GPCR signaling in essential hypertension

M. Teresa Perez-Garcia[1], Nuria Dagbouche-Rubio[1], Jorge Rojo-Mencía[1], Manuel Navedo[2], Madeline Nieves-Cintrón[2], Pilar Cidad[1], José R Lopez-Lopez[1]

[1]Departamento de Bioquímica y Biología Molecular y Fisiología, Universidad de Valladolid, and Unidad de Excelencia, Instituto de Biología y Genética Molecular (IBGM), CSIC,, Valladolid, Spain, [2]Department of Pharmacology, University of California Davis, Davis, CA, United States

Introduction and objectives: 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. Here we explored the changes in G protein-coupled receptors (GPCR) contractile responses of mesenteric arteries in a mice model of essential hypertension.

Methods: We have used a mice model of essential HT (BPN, blood pressure normal, and BPH, blood pressure high) to explore changes in expression and functional contribution of several GPCR expressed in vascular smooth muscle cells (VSMCs). Mice were anesthetized by isoflurane inhalation (5% O2 at 2.5 Lmin-1) and sacrificed by cervical dislocation, following the EC guiding principles regarding the care and use of animals (Directive 2010/63/UE). Microarrays, qPCR and immunocytochemical techniques in isolated VSMCs were used to explore expression, whereas the contractile responses of endothelial-denuded mesenteric arteries in response to agonists and/or modulators of the receptors using wire and pressure myography provide the functional correlate.

Results: Microarrays of vascular smooth muscle cells (VSMCs) from BPN and BPH mesenteric arteries provided differential expression of several elements in GPCR signaling pathways. 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 treatment with the ATR1 blocker losartan, 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.

Conclusions: 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.

SA05

The TMEM16A anion channel as a key regulator of microvascular blood flow

Paolo Tammaro[1]

[1]*University of Oxford, Oxford, United Kingdom*

The TMEM16A Ca^{2+}-gated Cl^- channel couples intracellular Ca^{2+} handling with cell electrical activity in contractile vascular cells. The channel is a potential therapeutic target for diseases of impaired (micro)vascular blood flow, including cerebral. In the brain, increased neuronal activity is coupled with a rise in regional metabolic activity which leads to a concomitant increase in cerebral blood flow (CBF). Contractile pericytes, cells that surround capillaries, are important determinants of CBF by controlling the diameter of the capillary and microvascular resistance to blood flow. In ischaemia, pericytes contract and then die in rigor, hindering CBF. Here we show that TMEM16A is expressed in pericytes and constitutes a depolarising force in response to a rise in intracellular Ca^{2+} or ischaemia. We perform a systematic analysis of the selectivity and potency of several structurally unrelated synthetic modulators of the TMEM16A channel, including a range of test compounds, the FDA approved drug niclosamide, and a recently identified activator, termed PAM_16A in this study. Selective pharmacological inhibition or activation of TMEM16A, respectively reduced or increased pericyte Ca^{2+} rise and capillary constriction in response to GqPCR agonists, with no effect on the electrical activity of cortical neurons. Exposure of cortical slices to oxygen-glucose deprivation (OGD), to simulate ischaemia, led to pronounced pericyte death. This was reduced or further enhanced by pharmacological inhibition or activation of the TMEM16A channel, respectively. In a rodent stroke model, TMEM16A inhibition reduced the ischaemia-evoked Ca^{2+} rise, capillary constriction, and pericyte death. These pharmacological agents similarly modulated the tone of isolated rat aorta and mesenteric arteries, which also express the TMEM16A channel. In summary, capillary diameter, as well as the tone of a range of artery types, can be finely controlled with TMEM16A modulators. This highlights TMEM16A as a possible target in a range of disorders involving impaired vascular tone including stroke, vascular dementia and hypertension.

SA06

Decoding pleiotropic G protein-coupled receptor activity in human pregnancy and labour

Aylin Hanyaloglu[1]

[1]*Imperial College London, London, United Kingdom*

The G protein-coupled receptor (GPCR) superfamily plays central and diverse physiological via activation of a limited set of signal pathways. Thus, our understanding of GPCR signalling have evolved to provide insight into the molecular mechanisms mediating signal diversity, and how such complex signalling may be decoded by a cell into specific responses. The pregnant human uterus undergoes dramatic reprogramming during human labour to drive both contractions and inflammation via the action of oxytocin and prostaglandins PGE2 and PGF2alpha. Our studies on the oxytocin receptor (OTR), PGE2 receptor, EP2, and PGF2alpha receptor, FP, in term pregnancy highlight that GPCR action is exquisitely complex. This talk will discuss our evolving models of GPCR action in the term pregnant human myometrium, whereby GPCRs are pleiotropically coupled to diversify their cellular signalling. Furthermore, that during human labour, oxytocin activated OTR selectively 'rewires' distinct GPCRs to promote pro-labour responses via receptor crosstalk. This highlights a central role of diversification of GPCR activity in dynamic biological systems such the pregnant myometrium, and new avenues to harness this knowledge to generate pathway selective modulators as potential novel therapeutic strategies in management of pre-term or overdue pregnancies.

SA07

Not just an affair of the heart: ERG channels in smooth muscles

Iain Greenwood[1]

[1]*Cardiovascular & Genomics Institute, St George's University of London, London, United Kingdom*

Kv11.1-11.3 are potassium channels encoded by Ether-a-go-go related genes (ERG1-3 also known as KCNH2, KCNH6 and KCNH7). Kv11.1 encoded by KCNH2 are key components of the cardiac action potential whereas KCNH6 /7 expression is mainly in neurones. These channels exhibit many fascinating structural and biophysical features including a prominent C-type inactivation that make Kv11 channels amenable to blockade by many structurally different agents. Kv11.1 blockade is responsible for the majority of acquired cardiac arrhythmias and so called 'ERG screens' feature prominently in most drug development programmes. However, ERG expression is not restricted to the heart and brain and in many visceral smooth muscles ERG channels especially Kv11.1 have a key functional role in suppressing contractility. This talk will provide an overview of ERG channels in smooth muscles highlighting the isoform expression in different tissues including portal vein, stomach, jejunum, uterus and bladder. The functional impact of these channels derived from work with pharmacological blockade will also be highlighted. Work will also be presented on ERG channel expression and function in mouse and human myometrial smooth muscle where functional impact of these channels is lost in pregnancy. The molecular mechanisms behind this functional switch will be highlighted. Overall, this presentation will provide an insight into the role of potassium channels usually associated with the heart in smooth muscle excitability.

SA08

Unravelling the role of post-junctional M2Rs in ASM contractions and their regulation by β-AR agonists.

Tuleen Alkawadri[1], Mark Hollywood[1], Keith Thornbury[1], Gerard Sergeant[1]

[1]*Dundalk Institute of Technology, Dundalk, Ireland*

β2-adrenoceptor (β2-AR) agonists are the primary bronchodilator treatments used to treat obstructive lung conditions such as COPD and asthma [1]. These conditions are associated with elevated cholinergic nerve activity [2] and it is recognised that β2-AR agonists inhibit cholinergic contractions of airway smooth muscle (ASM), however the cellular mechanisms underlying their effects are still unclear. Contractions of ASM induced by release of acetylcholine (ACh) from cholinergic nerves are thought to result from activation of postjunctional M3 muscarinic receptors (M3Rs) [3]. ASM also has an abundance of post-junctional M2Rs, which outnumber the M3Rs by a ratio of 4:1 in some species [3], yet their contribution to cholinergic nerve-induced contractions of ASM is poorly understood. β-ARs couple to Gs-proteins which activate adenylate cyclase and elevate cytosolic cAMP levels, while activation of M2Rs leads to a reduction inadenylate cyclase activity. Therefore, it was generally considered that the role of M2Rs on ASM was confined to offsetting relaxations induced by activation of β2-ARs. Recently, Alkawadri*et al.* (2021) reported a profound M2R-mediated hypersensitisation of M3R-dependent contractions of murine ASM, indicating that activation of postjunctional M2Rs made a greater contribution to cholinergic nerve-mediated contractions of ASM than previously realised [4]. This raised the possibility that the bronchodilator effects of β-AR agonists could involve inhibition of M2R-dependent contractions of ASM. Studies on murine ASM revealed that M2R-dependent contractions, induced by electric field stimulation (EFS) or the cholinergic agonist carbachol, were inhibited by the β-AR agonist denopamine, and that these effects were reduced in bronchial ring preparations taken from M2R KO mice [5]. Our data fit with the model that postjunctional M2Rs are involved in the bronchoconstrictor effects of ACh and that the therapeutic effects of b-AR agonists in the treatment of asthma and COPD may involve inhibition of M2R-dependent contractions of ASM.

1. Matera MG, Page CP, Calzetta L, Rogliani P, Cazzola M. (2020). Pharmacology and Therapeutics of Bronchodilators Revisited. Pharmacol Rev. Jan;72(1):218-252. doi: 10.1124/pr.119.018150 2. Kume H. (2021). Role of airway smooth muscle in inflammation related to asthma and COPD. Adv Exp Med Biol. 1303:139–172. doi: 10.1007/978-3-030-63046-1_9 3. Moulton BC, Fryer AD. (2011). Muscarinic receptor antagonists, from folklore to pharmacology; finding drugs that actually work in asthma and COPD. Br J Pharmacol. 163(1):44-52. doi: 10.1111/j.1476-5381.2010.01190.x. doi: 10.1111/j.1476-5381.2010.01190.x 4. Alkawadri T, McGarvey LP, Mullins ND, Hollywood MA, Thornbury KD, Sergeant GP. (2021). Contribution of Postjunctional M2 Muscarinic Receptors to Cholinergic Nerve-Mediated Contractions of Murine Airway Smooth Muscle. Function (Oxf). 20;3(1):zqab053. doi: 10.1093/function/zqab053 5. Alkawadri T, Wong PY, Fong Z, Lundy FT, McGarvey LP, Hollywood MA, Thornbury KD, Sergeant GP. (2022). M2 Muscarinic Receptor-Dependent Contractions of Airway Smooth Muscle are Inhibited by Activation of β-Adrenoceptors. Function (Oxf). 3(6):zqac050. doi: 10.1093/function/zqac050

SA09

PIEZO1 force sensor in health, disease and therapeutics

David Beech[1]

[1]*University of Leed, Leeds, United Kingdom*

PIEZO1 and PIEZO2 proteins form trimeric calcium-permeable non-selective cation channels that are activated by mechanical forces. We found significance of PIEZO1 in cardiovascular biology, showing its sensing of physiological fluid shear stress and role in embryonic vascular maturation [1]. By generating conditional genetic deletion in the adult mouse we found that in endothelium it is required for elevated blood pressure during physical activity, capillary density in skeletal muscle, physical exercise performance and lipid homeostasis via signalling to parenchymal regulatory genes in liver and small intestine [2-4]. We found evidence for it as a master mediator of force sensing, conferring force sensitivity on many other mechanisms: calcium-regulated proteases (CAPN2, ADAM10), nitric oxide production via NOS3, cell interaction via NOTCH1, cell apoptosis via thrombospondin-2 and (in cardiac fibroblasts) inflammation and fibrosis via p38, interleukin-6 and tenascin c. We found that it locates to endothelial cell-cell junctions where it interacts with cell adhesion molecules, PECAM1 and CDH5, to regulate junctional remodelling. With a clinical genetics team we identified natural variants that associate with lymphedema and disrupt the channel's ability to sense force [5]. To understand how it operates at the molecular level, we worked with a computation molecular dynamics team in develop models of the channel in endothelial membrane, predicting and testing structural rearrangements and lipid interactions. To explore potential therapeutic implications, we worked with medicinal chemists to develop pharmacology that activates or inhibits the channels. Overall, we suggest that PIEZO1 forms an exceptional mechanical detector of the cardiovascular system with importance spanning lymphatic drainage, skeletal muscle function and lipid homeostasis.

[1] Li, J. et al. Piezo1 integration of vascular architecture with physiological force. Nature doi: 10.1038/nature13701 (2014). [2] Rode, B. et al. Piezo1 channels sense whole body physical activity to reset cardiovascular homeostasis and enhance performance. Nat Comm doi: 10.1038/s41467-017-00429-3 (2017). [3] Bartoli, F. et al. Endothelial Piezo1 sustains muscle capillary density and contributes to physical activity. J Clin Invest doi:10.1172/JCI141775 (2022). [4] Lichtenstein, L. et al. PIEZO1 force sensing controls global lipid homeostasis. bioRxiv preprint https://doi.org/10.1101/2023.07.23.550198 (2023). [5] Ludlow, M.J. et al. Small-molecule functional rescue of PIEZO1 channel variants associated with generalised lymphatic dysplasia. medRxiv preprint doi.org/10.1101/2023.08.01.23292554 (2023).

SA10

Spooky intercellular signaling in the vascular endothelium

Charlotte Beelzebub Buckley[1], Matthew Lycanthropic Lee[1], Xun Grave Dancing Zhang[1], Calum 666 Wilson, John Ghoulie Chopper McCarron[1]

[1]*University of Strathclyde, Glasgow, United Kingdom*

The single layer of cells lining all blood vessels, the endothelium, is a sophisticated coordination center that controls a wide range of vascular functions which include the regulation of blood pressure and blood flow via changes in vascular contractility. To coordinate vascular function, cell communication and interactions are required for tissue level responses to emerge. A significant form of cell-cell communication occurs by the propagation of Ca^{2+} signals between cells ('Ca^{2+} waves'). We find that multiple mechanisms maintain communication so that Ca^{2+} wave propagation occurs irrespective of the status of cell connectivity. Between adjoining cells, g-protein coupled, regenerative IP_3-induced IP_3 production transmits Ca^{2+} signals and explains the propagated vasodilation that underlies the increased blood flow accompanying tissue activity. By controlling the production of IP_3, the inositide is itself sufficient to evoke a regenerative phospholipase C-dependent Ca^{2+} wave across coupled cells. None of gap junctions, Ca^{2+} diffusion or the release of extracellular messengers are required to support this type of intercellular Ca^{2+} signaling. In contrast, when a discontinuity between cells exists, ATP released as a diffusible extracellular messenger transmits Ca^{2+} signals and drives propagated vasodilation. These results show that self-reinforcing IP_3-induced IP_3 production drives intercellular signalling and reveal how communication is maintained in the face of endothelial damage. The findings provide a new framework for understanding Ca^{2+} wave propagation and cell-cell signaling in the endothelium

C01

Contribution of TMEM16A chloride channels to the complex action potential and contraction in human myometrium during normal and dysfunctional labour

Helena Parkington[2], Ranga Siriwardhana[2], Mary Tonta[2], Shaun Brennecke[3], Penelope Sheehan[1], Marianne Tare[2], Harold Coleman[2]

[1]Royal Women's Hospital, Parkville, Australia, [2]Monash University, Clayton, Australia, [3]University of Melbourne, Parkville, Australia

Introduction: The calcium essential for uterine contractions in human myometrium occurs mainly via influx through calcium channels embedded in the cell membrane. The calcium influx is recorded as a sharp spike of depolarization, followed by sustained plateau of depolarization (~ 1-2 minutes), the action potential (AP). The open state of the spike channels is primarily regulated by membrane voltage via voltage-gated calcium channels (VGCCs). The mechanism(s) underpinning the plateau remains elusive.

Aims: The plateau, –20 to –30mV, is at the equilibrium potential for chloride in smooth muscle, and here we probed the potential involvement of the calcium-activated chloride channel TMEM16A.

Methods: Human myometrium was obtained following caesarean delivery at term not in labour (NIL) and during labour (IL) (n=7 women for both). Tension was recorded simultaneously with membrane potential, using small myometrial strips (3×1×0.5mm) and intracellular microelectrodes.

Results: In NIL tissues resting membrane potential was –61±1 mV. TMEM16A blocker $CaCC_{inhib}$ (5μM) reduced AP plateau amplitude by 9±1mV ($p<0.0001$) and duration to 37±7% ($p=0.0026$). This was associated with weaker contractions (to 11±3%). $CaCC_{inhib}$ was more effective in IL samples. Resting membrane potential was –56±1mV, and $CaCC_{inhib}$ completely abolished APs (spike and plateau) within 8 minutes.

We probed the possible source of calcium responsible for the plateau (influx via VGCCs, or release from ER store). VGCC blockade (verapamil) entirely abolished APs (spike and plateau, n=4 NIL). Solution containing 30mM K (Na replacement) induced a plateau to the same level and duration of spontaneous AP plateaux but was not accompanied by contraction (verapamil still present, n=4 tissues). Blocking filling of the ER store using CPA increased plateau amplitude and duration. Then, application of $CaCC_{inhib}$ reversed the effect of CPA on the plateau level and duration.

Oxytocin (OT 50nM) significantly prolonged the plateau. It also induced a period of after-hyperpolarization between APs, critical for successful labour. In NIL (n=4) and IL (n=2), the OT plateau and after-hyperpolarization were suppressed by $CaCC_{inhib}$, reducing the OT response to simple spike APs and contractions to 46±3%.

Single-cell patch clamp studies demonstrated a $CaCC_{inhib}$-sensitive current in ~50% of NIL cells (32 cells, n=7 women). $CaCC_{inhib}$-sensitive current was 0.9±0.1 pA/pF in positive cells. All 15 cells from n=6 IL women had $CaCC_{inhib}$-sensitive current (1.7±0.3 pA/pF (NIL/IL $p=0.0207$). Myometrial cells

from one additional IL woman with failure-to-progress-in-labour (FPL) had $CaCC_{inhib}$-sensitive current of 0.4 pA/pF (NIL similar).

Using Western blotting, TMEM16A channel protein expression was increased IL (118±1 n=6) versus NIL (51±8 n=6, p=0.005). This increase in expression failed to occur in tissues from 4 FPL women (51±10). Immunohistochemistry confirmed smooth muscle cell colocalization of TMEM16A and SM actin. TMEM16A area was greater IL (2.7±0.4 n=5) compared with NIL (1.2±0.2 n=6).

Conclusions: We identified a role for TMEM16A in the plateau component of the AP in human myometrium. TMEM16A facilitates large, long-duration contractions by “clamping” the membrane at a depolarized plateau level, and is implicated in the OT response. When TMEM16A expression fails, labour fails and emergency CS IL is required.

C02

Potent vasodilatory effects of clinically-relevant concentrations of niclosamide, an anthelmintic drug and TMEM16A channel modulator, on isolated rodent arteries and capillaries.

Rachel Kaye[1], Claire Pearson[1], Rumaitha Al Hosni[1], Paolo Tammaro[1]

[1]*University of Oxford, Oxford, United Kingdom*

Background: Ca^{2+}-activated Cl^- channels encoded by the *TMEM16A* gene are highly expressed in arterial smooth muscle cells (aSMCs) and in contractile cerebral pericytes. In these cells, TMEM16A activation induces membrane depolarisation and vessel constriction. The FDA-approved drug niclosamide has been shown to modulate the TMEM16A channel (1-3) and thus may constitute a new therapeutic agent for diseases of altered vessel tone including stroke, hypertension and vascular dementia (4,5). Here, we investigate the effects of niclosamide, at concentrations comparable to those observed in plasma of patients treated with niclosamide, on (i) TMEM16A currents and (ii) the tone of isolated arteries and capillaries.

Methods: Whole-cell patch-clamp recordings of native and cloned TMEM16A currents were used to investigate how niclosamide modulates the TMEM16A channel. Isometric tension recordings of isolated rat aortae, pulmonary and mesenteric arteries, as well as Differential Interference Contrast (DIC) imagining of acute rat brain slices, were used to investigate how channel regulation by niclosamide affects vessel tone.

Results: Clinically-relevant niclosamide concentrations (≤1 μM) were examined on heterologous TMEM16A currents in HEK293T cells under various membrane potential (V_m) and intracellular free Ca^{2+} ($[Ca^{2+}]_i$) conditions. At 0.3 μM $[Ca^{2+}]_i$, niclosamide induced a biphasic effect by inhibiting at positive V_m (by 97±1.6% (n=8) at +100 mV) and potentiating at negative V_m (by 20±4.3 (n=10) fold at -100 mV) . These effects diminished as the $[Ca^{2+}]_i$ was raised to levels that cause half-maximal or maximal TMEM16A activation.

Niclosamide (1 μM) reduced responses to the phenylephrine, contractile G_qPCR agonist, in isolated rat aortae (by 81.0±3.5%, N = 8), pulmonary (52.0±7%, N = 10) and mesenteric arteries (76±4%, N=11). Niclosamide also impaired rat cortical pericyte constriction by 40.9±9.5% (N = 8) after exposure to endothelin-1 (10 nM). Unlike Ani9, a selective TMEM16A inhibitor, niclosamide reduced isolated artery responses to increased extracellular K^+. Patch-clamp recordings of isolated smooth muscle cells demonstrated that niclosamide inhibits Ca_V currents and activates a hyperpolarising current, mediated at least in part by K^+ ions.

Conclusions: Niclosamide is a potent vasodilator of a range of artery types and cortical cerebral capillaries. The underlying mechanism involves modulation of multiple membrane currents including Ca_v and hyperpolarising K^+ current in addition to TMEM16A inhibition.

1. Braga, L et al. (2021). Nature, 594(7861):88-93. 2. Cabrita, I et al. (2019). JCI Insight, 4(15):e128414. 3. Miner K et al. (2019). Front Pharmacol 10:51. 4. Korte N et al. (2022). J Clin Invest 132(9):e154118. 5. Al-Hosni R et al. (2022). Trends Pharmacol Sci 43: 712–725.

C03

Mutation of phenylalanine residues in LINGO1 and LINGO2 transmembrane domain perturb BK gating and inactivation.

Pei Xin Boon[1], Srikanth Dudem[1], Gerard Sergeant[1], Keith Thornbury[1], Mark Hollywood[1]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland

Large conductance Ca^{2+} and voltage-activated potassium (BK) channels regulate cell excitability and their gating can be modulated by the auxiliary b, γ and LINGO subunits[1,2]. BK:gamma1 channels activate at resting potentials in 0 Ca^{2+} and this shift in activation $V_{1/2}$ ($V_{1/2ACT}$) was reduced by mutation of F273 in the g1 transmembrane domain[3]. Interestingly, the novel regulatory BK subunit LINGO2 also caused a negative shift in $V_{1/2ACT}$ when co-expressed with BK channels and induced rapid inactivation[4], rather like the effects of LINGO1[2]. Given that gamma and LINGO proteins appear similar in structure[5], we performed an alanine scan of phenylalanine residues in the transmembrane region of LINGO1 and LINGO2 and co-transfected these with BK cDNA, to ascertain their effect on BK channel activity.

HEK cells were transfected with BK:LINGO:eGFP cDNA (100:500:150ng ratio) and currents were studied under voltage clamp. Experiments were carried out using the inside-out configuration of the patch clamp technique with equimolar 140 mM K^+ solutions, at 37℃. The cytosolic surface of the patch was exposed to the same solutions and the $[Ca^{2+}]_i$ ranged from 100nM-10μM Ca^{2+}. Deletion of the last four residues of LINGO2 (BK:$LINGO2_{DMKMI}$) abolished inactivation and significantly shifted $V_{1/2ACT}$ more negatively to 104±1 mV (n=6) in 100nM Ca^{2+} compared to 130±2 mV (n=8) in full length BK:LINGO2 ($p<0.05$, paired t-test). Two TM mutants, $LINGO2_{F558A}$ and $LINGO2_{F560A}$ shifted $V_{1/2ACT}$ positively to 162±2 and 160±2 mV respectively, in 100 nM Ca^{2+}, compared to BK:LINGO2 (n=5, $p<0.05$, ANOVA) and these values were practically identical to the $V_{1/2ACT}$ recorded in BK alone. In five experiments with BK:$LINGO2_{F550A}$ the mean $V_{1/2ACT}$ was 152±2 (n=5) but this shift failed to reach statistical significance compared to BK:LINGO2. Similarly, the $V_{1/2ACT}$ obtained with the BK:$LINGO2_{F564A}$ mutant 134±4 mV, (n=7, $p>0.05$) was indistinguishable from BK:LINGO2. Although the $V_{1/2ACT}$ of BK:$LINGO2_{F552A}$ mutant channels (139±1 mV, n=6) was not significantly different to BK:LINGO2, currents recorded with this mutant failed to show inactivation at any voltage in 100nM, 1μM or 10μM Ca^{2+}. Co-expression of BK:LINGO1 produced rapidly inactivating currents under voltage clamp and these had a $V_{1/2ACT}$ of 113±3mV (n=6), in agreement with previous studies[2]. Mutation of the equivalent residue to $LINGO2_{F552}$ in LINGO1 (F568A) was next performed, to establish if it also failed to inactivate when co-transfected with BK cDNA and recorded under the same conditions. In 5 experiments the $V_{1/2ACT}$ of the BK:$LINGO1_{F568A}$ mutant was shifted significantly positive to 140±2 mV compared to BK:LINGO1 ($p<0.05$) and inactivation was completely abolished.

In conclusion, the data suggest that $LINGO2_{F550}$, $LINGO2_{F552}$, $LINGO2_{F564}$ do not contribute significantly to the shift in BK $V_{1/2ACT}$ induced by LINGO2, whereas $LINGO2_{F558}$ and $LINGO2_{F560}$ contributed equally to the negative shift. However, mutations of the residue equivalent to $gamma1_{F273}$ in either LINGO1 (F568) or LINGO2 (F552) abolished inactivation of BK channels at all voltages and $[Ca^{2+}]_i$ tested. These data suggest that this conserved residue in LINGO plays a critical

role in allowing the LINGO proteins to associate with BK channels or that its mutation modified the structure or alignment of the LINGO tail to prevent inactivation.

1. Latorre R, Castillo K, Carrasquel-Ursulaez W, Sepulveda RV, Gonzalez-Nilo F, Gonzalez C, Alvarez O (2017). Molecular Determinants of BK Channel Functional Diversity and Functioning. Physiol Rev 97: 39–87. 2. Dudem S., Sergeant G.P., Thornbury K.D., Hollywood M.A. (2021) Calcium-Activated K+ Channels (KCa) and Therapeutic Implications. In: Handbook of Experimental Pharmacology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/164_2021_459 3. Li, Q., Guan, X., Yen, K., Zhang, J., & Yan, J. (2016). The single transmembrane segment determines the modulatory function of the BK channel auxiliary γ subunit. The Journal of General Physiology, 147(4), 337–351. https://doi.org/10.1085/jgp.201511551 4. Dudem, S., Boon, P. X., Mullins, N., McClafferty, H., Shipston, M. J., Wilkinson, R. D. A., Lobb, I., Sergeant, G. P., Thornbury, K. D., Tikhonova, I. G., & Hollywood, M. A. (2023). Oxidation modulates LINGO2-induced inactivation of large conductance, Ca2+-activated potassium channels. The Journal of Biological Chemistry, 102975. https://doi.org/10.1016/j.jbc.2023.102975 5. Yamanouchi, D., Kasuya, G., Nakajo, K., Kise, Y., & Nureki, O. (2023). Dual allosteric modulation of voltage and calcium sensitivities of the Slo1-LRRC channel complex. Molecular cell, 83(24), 4555–4569.e4. https://doi.org/10.1016/j.molcel.2023.11.005

C04

Reduced cerebral blood flow due to damaged vascular smooth muscle cell Ca2+ handling in a mouse model of AD.

Katy Walsh[1], Thea Danby[1], Alison Gurney[1], Adam Greenstein[1], Harry Pritchard[1]

[1]*Universityof Manchester, Manchester, United Kingdom*

One of the earliest biomarker abnormalities in Alzheimer's Disease is a reduction in cerebral blood flow (CBF). Blood flow to the brain is regulated by the intrinsic properties of arteries to constrict and relax in response to different stimuli, such as intraluminal pressure. Our recent work (Taylor et al., 2022 - PMID: 37549299) has shown that Ca2+ release events from the sarcoplasmic reticulum (SR) and subsequent large-conductance Ca2+-activated K+ (BK) channel activation are disrupted in 18-month male APP23 mice compared to wild-type (Wt) controls. BK channels promote a more hyperpolarised smooth muscle that prevent excessive vasoconstriction in response to increases in intraluminal pressure. Therefore, a reduced BK channel activity results in a more contractile artery that contributes to reduced CBF. We also showed that acute exposure to Aβ(1-40) disrupts intracellular vascular smooth muscle cell (VSMC) Ca2+ signalling in healthy cerebral arteries, characterised as an increase in pathogenic Ca2+ waves. This project looked to determine if there was a reduction in CBF in male and female APP23 mice, at 9 months of age, to determine if this age represented an intermediate phenotype between 18 month APP23 mice and acute Aβ(1-40) exposure.

All experiments were covered by the UK Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986 and approved by a local committee (PPL: PP9466981). 9-month APP23 mice display reduced CBF compared to Wt littermates (1082 ± 24.3 vs. 999 ± 25.03, $p < 0.0001$, $n = 10$ each group) shown by laser speckle imaging. Spontaneous transient outward currents (STOCs) were recorded to measure BK channel activity on freshly isolated pail artery SMCs over a range of membrane potentials. Pial artery SMCs isolated from APP23 mice showed a decreased STOC frequency ($p < 0.05$, $n = 15$ each group) and amplitude ($p < 0.05$, $n = 15$ each group) compared to cells isolated from Wt littermates. Ex-vivo pressurised pial arteries from APP23 mice showed dysregulated Ca2+ signalling. APP23 arteries loaded with the Ca2+ indicator (Cal-520) showed a decreased Ca2+ spark frequency (15.72 ± 2.736 vs. 9.872 ± 1.192 Hz, $p < 0.05$, $n = 18$ each group) and an increased Ca2+ wave frequency (0.02582 ± 0.004478 vs. 0.07144 ± 0.009742 waves/cell/s, $p < 0.0001$) compared to Wt littermates. To determine SR store load, a caffeine bolus was applied to APP23 arteries and showed a ~40% reduction in the Ca2+ transient (3.025 ± 0.5442 vs. 1.70 0± 0.2785 AUC, $p < 0.05$, $n = 18$ each group) compared to Wt littermates.

9-month APP23 mice have reduced Ca2+ in the intracellular store, that disrupts correct Ca2+ signalling. This reduces BK channel activity and contributes to a more contractile artery and reduced CBF. Future experiments will determine the mechanisms reducing store load and if this can be restored following treatment.

C05

KATP channel-Dependent Electrical Signaling Links Capillary Pericytes to Arteriolar Smooth Muscle in the Brain

Thomas Longden[1], Dominic Isaacs[1], Liuruimin Xiang[1], Ashwini Hariharan[1]

[1]*University of Maryland Baltimore, Baltimore, United States*

The brain has evolved mechanisms to dynamically modify blood flow, enabling the timely delivery of energy substrates in response to highly fluctuating local metabolic demands. Several such neurovascular coupling mechanisms have been identified, but the vascular signal transduction and transmission mechanisms that enable dilation of penetrating arterioles remote from sites of increased neuronal activity are unclear. Given the exponential relationship between vessel diameter and blood flow, tight control of arteriole smooth muscle membrane potential and diameter is a crucial aspect of neurovascular coupling. Recent evidence suggests that that capillaries play a major role in sensing neural activity and transmitting signals to modify the diameter of upstream vessels. Thin-strand pericyte processes cover around 90% of the capillary bed but their contributions to blood flow control are not understood. We show that thin-strand pericytes play a central role in neurovascular coupling by sensing neural activity and generating and relaying electrical signals to arteriolar smooth muscle. We identify a K_{ATP} channel-dependent neurovascular signaling pathway that is explained by the recruitment of capillary pericytes, and deploy vascular optogenetics to show that currents generated in individual thin-strand pericytes are sent over long distances to upstream arterioles in vivo to cause dilations. Genetic disruption of vascular K_{ATP} channels reduces the arteriole diameter response to neural activity and laser ablation of thin-strand pericytes eliminates the K_{ATP}-dependent component of neurovascular coupling. Our work indicates that thin-strand pericytes actively sense neural activity and transform this into K_{ATP} channel-dependent electrometabolic signals that inform upstream arteriolar smooth muscle of local energy needs, promoting spatiotemporally precise energy distribution.

C06

Characterization of interstitial cells within the mouse gastroesophageal junction.

Emer Ni Bhraonain[1], Karen Hannigan[1], Jack Turner[1], Kenton Sanders[1], Caroline Cobine[1]

[1]University of Nevada, Reno, Reno, United States, [2]University of Nevada, Reno, Reno, United States

Background/Objectives: The esophagus connects the oral cavity to the stomach and terminates at the gastroesophageal junction (GEJ) where the lower esophageal sphincter (LES), a thickening of the circular smooth muscle is located. The mouse esophagus has been described to consist entirely of skeletal muscle, despite this, phasic contractile activity is present in the distal portion. In other gastrointestinal (GI) muscles phasic activity is associated with slow waves generated by interstitial cells of Cajal (ICC*)*. **S**mooth muscle cells (SMCs) form an electrical syncytium with **I**CC and a second type of interstitial cell, **P**DGFRa$^+$ cells (**SIP** syncytium; Sanders *et al*., 2014). ICC are present throughout the mouse esophagus (Rumessen *et al.*, 2001) while PDGFRa$^+$ cells have been observed in the human esophagus (Chen *et al.*, 2013). The aims of our study were to characterize the distribution of SIP cells, and evaluate the functional role of ICC within the mouse esophagus and LES.

Methods: Dual labeling immunohistochemistry was performed on tissues from wildtype (C57Bl/6) and smooth muscle eGFP (smMHC$^{-eGFP/+}$) mice. ICC were labeled with antibodies against Kit or ANO1. PDGFRa$^+$ cells were labeled with antibodies against PDGFRa or SK3. Enteric motor neurons and glia were labeled with antibodies against nNOS, VIP (inhibitory), vChAT, TH (excitatory) and GFAP (glia) respectively. Images were acquired with a Leica Stellaris 5 confocal microscope and processed using LasX software. Contractile activity was recorded from LES clasp and distal esophagus muscle strips. ICC Ca^{2+} transients were imaged from the Kit-GCaMP6f mouse GEJ using confocal spinning disc microscopy. The contributions of ANO1 and Cav_L channels were determined with Ani9 and nifedipine or pinacidil respectively.

Results: SMCs were present throughout the esophagus though their density declined proximally. In contrast to other GI regions, only intramuscular ICC (ICC-IM) were present; these cells expressed ANO1 and declined in density proximally. Submucosal and intramuscular PDGFRa$^+$ cells were distributed throughout the esophagus and LES. Only intramuscular PDGFRa$^+$ cells expressed SK3 though this expression declined proximally. SIP cells were closely associated with one another as well as with enteric neurons and glia. The LES clasp generated tone whereas the distal esophagus exhibited phasic contractions. Contractile activity in both muscles was abolished by inhibiting ANO1 or Cav_L. As observed previously, ICC-IM in the LES exhibited asynchronous Ca^{2+} activity (Type I ICC-IM) (Drumm *et al.*, 2022). In contrast, in the distal esophagus, two Ca^{2+} signaling behaviors were present with a second ICC-IM population exhibiting whole-cell Ca^{2+} transients (Type II ICC-IM). Whole-cell Ca^{2+} transients in Type II ICC-IM were abolished by inhibiting ANO1 or Cav_L revealing underlying Type I-like asynchronous Ca^{2+} transients. This suggests that these cells likely regulate motility within this region.

Discussion/Conclusions: Our studies revealed that SIP cells are distributed throughout the mouse GEJ and that they are closely associated with one another as well as with enteric neurons and glia. Functional studies suggest that ICC-IM play an important role in regulating motility within

the GEJ. Future studies will utilize mice to evaluate the role of SIP cells in neuromuscular transmission within the GEJ.

Chen JH, Wang XY, Liu LW, Yu W, Yu Y, Zhao L & Huizinga JD. (2013). On the origin of rhythmic contractile activity of the esophagus in early achalasia, a clinical case study. Front Neurosci 7, 77. Drumm BT, Hannigan KI, Lee JY, Rembetski BE, Baker SA, Koh SD, Cobine CA & Sanders KM. (2022). Ca2+ signalling in interstitial cells of cajal contributes to generation and maintenance of tone in the mouse and monkey lower oesophageal sphincters. J Physiol 600, 2613-2636. Rumessen JJ, de Kerchove d'Exaerde A, Mignon S, Bernex F, Timmermans JP, Schiffmann SN, Panthier JJ & Vanderwinden JM. (2001). Interstitial cells of Cajal in the striated musculature of the mouse esophagus. Cell Tissue Res 306, 1-14. Sanders KM, Ward SM & Koh SD. (2014). Interstitial cells: regulators of smooth muscle function. Physiol Rev 94, 859-907.

C07

Calcium waves and spatio-temporal mapping reveals myosalpinx excitability and propagation along the murine oviduct.

Sean Ward[1], Sung Jin Hwang[1,2]

[1]University of Nevada, Reno, Reno, United States, [2]University of Nevada, Reno, Reno, United States

Introduction: Oviducts, or fallopian tubes (in primates), are smooth muscle-lined tubular organs that facilitate several essential physiological processes including gamete transport, ovum fertilization and early embryo development. Contractions of the oviduct smooth muscle (myosalpinx) and the wafting motion of the ciliated epithelium that lines these tubes, facilitate bi-directional transport of gametes so that the newly released ovum(s) is transported in one direction (pro-uterus) while spermatozoa are transported in the opposite direction (pro-ovary). For successful fertilization to occur, these transport processes must be temporally coordinated so that the ovum and spermatozoa meet each other in the ampulla; the site of fertilization. Once the ovum is fertilized, the early embryo then begins another precisely timed journey toward the uterus for implantation. Myosalpinx contractions also facilitate this journey while luminal secretions from secretory epithelial cells aid early embryo maturation and influence gamete viability.

Aims/Objectives: To develop a better understanding of the excitable nature of the oviduct myosalpinx, we utilized a mouse model with a genetically engineered calcium (Ca^{2+}) indicator (PDGFRa/cre-Gcamp6f/loxp) that was expressed in the myosalpinx. Specific questions to be addressed were: (i) are all regions of the oviduct excitable? (ii) how does propagation occur along the different segments of the oviduct? (iii) did activity that originated in the oviduct propagate to the adjacent uterus? (iv) what were the cellular mechanisms responsible for the generation and propagation of Ca^{2+}calcium waves in the oviduct?

Methods: All animals were maintained, and the experiments performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Ca^{2+} imaging and spatiotemporal mapping was performed at different sites along the oviduct. Simultaneous intracellular microelectrode recordings and Ca^{2+} imaging was also performed. Confocal imaging was used to determine cellular expression of Gcamp6.

Results: PDGFRa/cre-Gcamp6f/loxp mice displayed robust spontaneous Ca^{2+} waves throughout all regions of the oviduct. Ca^{2+} waves propagated from ovary to uterus, but also from uterus to oviduct, often colliding in a specialized region within the Ampulla/isthmus, the possible site of fertilization. Ca^{2+} waves that originated in the isthmus often propagated into the uterus activating an excitable wavefront that spread along this organ. Electrical slow waves were responsible for the generation of Ca^{2+} waves. Ca^{2+} waves were sensitive to removal of extracellular Ca^{2+}, inhibition of intracellular Ca^{2+} stores with cyclopiazonic acid (CPA) and caffeine and were reduced by the Ano1 Ca^{2+}-activated chloride channel (CaCC) inhibitor $CaCC_{inh}$-A01.

Conclusions: For the first time, we are able to track the generation and propagation of Ca^{2+} waves as an indicator of oviduct myosalpinx excitability. Propagation of Ca^{2+} waves that occurred in both

directions along the oviduct suggests that myosalpinx activity contributes to the transport of both gametes. These events relied on the release of intracellular Ca^{2+} and were likely generated by the CaCC Ano1.

C08

Interaction of Anoctamin-1-encoded Ca2+-activated Cl- and voltage-dependent Kv2.1 channels in mouse pulmonary artery smooth muscle and HEK-293 cells

Connor L. Jimenez[1], Elizabeth J. Akin[1], Normand Leblanc[1]

[1]*University of Nevada, Reno, Reno, NV 89557, United States*

Ca^{2+}-activated Cl^- channels (CaCC) encoded by the *Anoctamin-1* (ANO1) or *Tmem16a* gene, Cav1.2 voltage-dependent Ca^{2+} channels and inositol-tris-phosphate receptors (IP_3R) were recently shown to be colocalized in super clusters in the plasma membrane of mouse pulmonary artery smooth muscle cells (PASMC), and suggested to play an important role in triggering Ca^{2+} waves mediated by Ca^{2+} release from IP_3R.[1] Because of the similarity of these super clusters with clusters formed by the voltage-dependent K^+ channel Kv2.1 in both mammalian neurons[2,3] and vascular smooth muscle cells,[4] we tested the hypothesis that surface expression of Kv2.1, which is known to be expressed in PASMCs, is confined to the same microdomains as those formed by ANO1/Cav1.2/IP_3R in mouse PASMCs. Immunolabeling and confocal imaging using specific antibodies to Kv2.1 and ANO1 showed that the two ion channels displayed both punctate staining and larger super clusters; interestingly, the two channels only colocalized in the larger microdomain structures. Numerous studies have shown that the membrane clustering ability of Kv2.1 could be recapitulated by expression of this K^+ channel in mammalian cell lines. GFP-tagged mouse Kv2.1 expressed in HEK-293 cells exhibited prominent clustering at the plasma membrane in agreement with previous findings by other groups. Whole-cell patch clamp experiments were next carried out to determine if co-expression of ANO1-mCherry alters the properties of Kv2.1. These experiments were carried out in the presence of normal solutions containing K^+, with 5 mM EGTA in the pipette solution to minimize the activity of ANO1. While the expression of ANO1-*ac* had no effect on the I-V relationship of peak outward current, steady-state activation curve, or voltage-dependence of activation kinetics of Kv2.1, it decelerated deactivation kinetics as denoted by a -26 mV hyperpolarizing shift in the voltage-dependence of deactivation (Kv2.1: $V_{0.5}$ = -32.4 ± 0.7, n=7; Kv2.1 + ANO1: -55.5 ± 3.1 mV, n=8, $P < 0.05$). Moreover, block of Kv2.1 at +80 mV by the highly specific Kv2.1 blocker and gating modifier Guanxitoxin-1E (GxTx; 20 nM) was significantly attenuated by co-expression of ANO1-*ac* (Kv2.1: 69 ± 8.6%, n=6; Kv2.1 + ANO1: 37 ± 10.5%, n=4, $P < 0.05$). Finally, in Cs- and TEA-based solutions, and 500 nM free internal Ca^{2+} to activate ANO1-*a* current, 100 nM GxTx surprisingly enhanced ~ 30% (+29 ± 13%, n=5; $P < 0.001$) when ANO1 was co-expressed with Kv2.1, while it inhibited the current when ANO1 was expressed alone (-52.2 ± 7.7%, n=3; $P < 0.001$). Although we have to remain cautious about interpreting these preliminary results, our findings suggest that when co-expressed together, ANO1 and Kv2.1 may physically and functionally interact, an observation that may bear important implications for compartmentalized Ca^{2+}signaling and contractility in PASMCs, and perhaps other cell types expressing these two ion channels. All procedures pertaining to housing conditions and animal handling were approved by the University of Nevada IACUC (protocol #20-06-1016-1) in accordance with the Guide for the Care and Use of Laboratory Animals by the National Research Council of the National Academies (8^{th} edition, 2011).

1. Akin et al. (2023). J Gen Physiol 155. 2. Antonucci et al. (2001). Neuroscience 108, 69-81. 3. O'Connell et al. (2010). Proc Natl Acad Sci U S A 107,12351-12356. 4. Matsumoto et al. (2023). Commun Biol 6,1165. 5. Hawn et al. (2021). Channels (Austin) 15, 569-603.

C09

Does microtubule depolymerization affect spontaneous or agonist-induced contraction and relaxation in the myometrium?

Sarah Arrowsmith[1]

[1]*Manchester Metropolitan University, Manchester, United Kingdom*

In order to develop better therapeutics to prevent or treat problems in labour, a greater understanding of the mechanisms that generate and regulate myometrial contraction is urgently needed. Microtubules are filamentous intracellular structures and integral components of the cytoskeleton. They mediate several cellular functions, including cell division and intracellular protein transport, as well as modulating signal transduction arising from extracellular ligand binding. Microtubule stability has been shown to affect signalling from several G protein-coupled receptors (GPCRs) in several cell types. Their disruption using depolymerization agents such as colchicine and nocodazole enhances signalling by cAMP-linked GPCRs such as β-adrenoreceptors (β-AR) in some cells. In renal and mesenteric arteries, microtubule disruption increases vasorelaxation via the β-AR agonist, isoprenaline, which is brought about by increased microtubule delivery and insertion of Kv7 channels in the membrane, facilitating hyperpolarisation (Lindman, 2018). In cerebral artery myocytes, microtubules have also shown to be important in supporting coupling between the SR and the plasma membrane, with roles in facilitating contraction and vascular tone (Pritchard, 2017). However, little is known about the contribution of microtubules towards ion channel and membrane receptor dynamics, and hence excitation, in other smooth muscles, including myometrium.

Here, we investigated the effect of microtubule depolymerization on spontaneous, β2-AR-mediated relaxation and PGE_2-mediated contraction in pregnant and non-pregnant rodent uterus.

Isometric tension recordings were made on strips of longitudinal myometrium (8 mm x 5mm x 2mm) from time-mated pregnant (d18) c57/BL6 mice (n=5) or non-pregnant Wistar rats (n=5) following humane sacrifice. Firstly, the effect of increasing concentrations of colchicine (1μM-500μM) was investigated on force of spontaneous contraction (mN) and integral of force (area under the curve, AUC) (n=4). Next, the effect of increasing concentrations (1nM-1μM) of ritodrine (a β2-AR-selective agonist) or PGE_2 (1nM-100μM) was investigated on paired strips with and without pre-treatment (40 mins) with 500μM colchicine (n=5). Significance was taken as $P<0.05$ by one-way ANOVA and Dunnett's post-hoc test. IC50s and EC50s were compared by F test.

Application of increasing concentrations of colchicine alone (up to 500μM) had no significant effect on force of spontaneous contraction ($P=0.2247$) or force integral ($P=0.8464$). Pre-incubation with colchicine however, enhanced the relaxatory effect of ritodrine in pregnant myometrium: Following colchicine pre-treatment, IC50s for ritodrine on force and AUC reduced from 570nM (95% CI 51.5nM–6.0μM) to 67.5nM (95% CI 29.9nM–153.0nM) and from 63.9nM (95% CI 45.3nM–90.2nM) to 37.8nM (95% CI 26.8nM–53.0nM) respectfully, ($P=0.0295$), but did not change in non-pregnant myometrium ($P=0.0693$ and $P=0.21$). Colchicine pre-treatment also tended to increase the potency of PGE_2 in non-pregnant myometrium: the EC50s for PGE_2 on AUC reduced from 2.09μM (95% CI 122.0nM–42.8μM) to 85.5nM (332pM–22μM) but were not significantly different ($P=0.460$).

Like other smooth muscles, data suggest that microtubule integrity plays an important role in mediating cellular signalling from some GPCRs in myometrium. Differences observed between pregnant and non-pregnant tissues indicate that there may be gestation-dependent effects on the activity of the microtubule network and warrants further investigation, as does investigating the effect of microtubule disruptors on agonist-mediated contraction and relaxation in human myometrium.

References: Lindman J et al., (2018) Hypertension. 71(2):336-345 Pritchard HAT et al. (2017) Sci Signal. 10 (497)

C10

The role of membrane potential in corpus cavernosum smooth muscle contractility

Mitchell Mercer[1], Gerard P. Sergeant[1], Mark A. Hollywood[1], Keith D. Thornbury[1]

[1]Dundalk Institute of Technology, Dundalk, Ireland

Erectile dysfunction (ED) affects 30% of men >40 years old worldwide [1] and is often treated with phosphodiesterase-5 inhibitors (PDE5Is, e.g. Viagra™) which prevent degradation of cyclic guanosine monophosphate (cGMP). cGMP promotes relaxation of the corpus cavernosum smooth muscle (CCSM) of the penis to form an erection. However, >50% of diabetic men are resistant to treatment with PDE5Is [2]. Therefore, there is a need to develop new therapies for ED. Approaches include exploration of the pathways downstream of formation of cGMP, or alternative pathways that cause relaxation. KCNQ-encoded voltage-dependent potassium (K_v7) channels are present on CCSM cells [3,4]. Here we examine the role of K_v7 channels in the control of contractility of the mouse corpus cavernosum smooth muscle.

Isometric tension recordings were made from CCSM crura dissected from C57BL/6 mice euthanised via pentobarbital injection. Intracellular Ca^{2+} was imaged from single CCSM cells incubated with the Ca^{2+} indicator, Fluo-4-AM.

PCR revealed the expression of KCNQ1,3,4 and 5 within cDNA isolated from mouse corpus cavernosum (n=3). Furthermore, qPCR results indicated dominant expression of KCNQ5 transcripts (n=3). Isometric tension recording showed that addition of a_1-agonist, phenylephrine (PE), to CCSM crura generates phasic contractions, whilst displaying large tonic contractions only at higher concentrations. The K_v7 channel blocker, XE-991 (10μM), increased the amplitude of the phasic contractions (from 0.9±0.2mN to 1.3±0.3mN; P=0.0205, paired t-test) and their frequency (from 4.0±0.6min^{-1} to 10.1±1.0min^{-1}; P<0.001, paired t-test) when CCSM crura were precontracted with 300 nM PE (n=6). In contrast, the K_v7 channel activator, retigabine (10μM), abolished the phasic contractions (control frequency: 6.2±1.2min^{-1}; P=0.0312, Wilcoxon test; control amplitude: 0.9±0.3mN; P=0.0312, Wilcoxon test; n=6) induced by 300 nM PE . Furthermore, both retigabine (n=8) and an L-type Ca^{2+} channel blocker, nifedipine (1μM; n=6), abolished only the phasic activity, whilst the tonic responses remained at higher concentrations (3μM, 10μM and 30μM) of PE.

Ca^{2+} imaging experiments revealed that isolated CCSM cells developed spontaneous phasic Ca^{2+} oscillations. XE-991 increased the frequency of these oscillations (from 1.0±0.6min^{-1} to 17.0±3.3min^{-1}; P= 0.0018, Friedman test; n=6). In contrast, the oscillations were abolished by retigabine (control frequency: 13.5±3.3min^{-1}; P=0.0030, Friedman test; n=6) or nifedipine (control frequency: 10.7±1.4min^{-1}; P=0.0187, Friedman test; n=6). Furthermore, 100 nM PE caused Ca^{2+} oscillations, which were abolished by both retigabine (n=6) and nifedipine (n=6). Application of XE-991 increased the amplitude and frequency of the PE-induced Ca^{2+} oscillations (n=6).

Taken together with the effects found in isometric tension recording, these data suggest that alterations in membrane potential are the basis for the phasic activity in tension recording and Ca^{2+} oscillations in single cells. Therefore, K_v7 channels could be utilised as potential therapeutic target for ED.

[1] Corona, G. et al. (2010). J. Sex. Med., 7(4pt1), 1362–1380. [2] de Tejada I. S. (2004). Int. J. Imp. Res., 16 Suppl 1, S40–S42. [3] Jepps, T. A., et al. (2016). Br. J. Pharmacol., 173(9), 1478–1490. [4] Lee, J. H., et al. (2020). Pflugers Archiv, 472(1), 89–102.

C11

Interstitial cell of Cajal-like cells (ICC-LC) exhibit dynamic spontaneous activity but are not functionally innervated in mouse urethra

Neha Gupta[1], Salah A. Baker[2], Kenton M. Sanders[2], Caoimhin S. Griffin[1], Gerard P. Sergeant[1], Mark A. Hollywood[1], Keith D. Thornbury[1], Bernard T. Drumm[1]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland, [2]Department of Physiology & Cell Biology, University of Nevada, Reno, United States

Urethral smooth muscle cells (USMC) sustain tonic contractions to occlude the internal urethral sphincter during bladder filling. Interstitial cells also exist in urethral tissues and are hypothesized to influence USMC behaviours and neural responses. These cells are similar to Kit^+ interstitial cells of Cajal (ICC), gastrointestinal pacemakers and neuroeffectors. Isolated cell studies of urethral ICC-like cells (ICC-LC) exhibit spontaneous intracellular Ca^{2+} signalling behaviours linked to proposed roles as USMC pacemakers and neuromodulators similar to ICC in the gut, although observation and direct stimulation of ICC-LC within intact urethral tissues is lacking. Using a mouse line with cell-specific expression of the Ca^{2+} indicator GCaMP6f, driven off the Kit promoter (Kit-GCaMP6f mice), we unequivocally identified ICC-LC within *in situ* urethra preparations and characterized their activity. Across 54 cells imaged from 13 animals, ICC-LC fired spontaneous Ca^{2+} waves that propagated on average 40.1 ± 0.7 mm, with varying amplitudes (0.08 - 1.96 DF/F_0), originating from multiple firing sites per cell (average 3.1 ± 0.2). ICC-LC imaged from Kit-GCaMP6f urethra did not form interconnected networks. ICC-LC activity was uncoordinated across multiple cells with no obvious entrainment of ICC-LC. ICC-LC Ca^{2+} event frequency was unaffected by the L-type Ca^{2+} channel inhibitor nifedipine (P=0.25, n=5) but was abolished by cyclopiazonic acid (P=0.0005, n=6) and decreased by an inhibitor of store-operated Orai Ca^{2+} channels (GSK-7975A, P<0.0001, n=6). While the alpha-adrenoreceptor agonist phenylephrine increased Ca^{2+} wave frequency (P=0.0007), amplitude (P=0.04) and spread (P=0.14) (n=6), the nitric oxide (NO) donor DEA-NONate had no effect (n=7). Electrical field stimulation (EFS, 10 Hz) of intrinsic nerves under excitatory (n=6) or inhibitory conditions (n=7) failed to elicit responses in ICC-LC. In contrast, EFS of ICC from Kit-GCaMP6f colon yielded consistent excitatory (cholinergic, n=3) and inhibitory (nitrergic, n=5) postjunctional responses. We conclude urethral ICC-LC are spontaneously active but are not functionally innervated.

C12

Contribution of renal TRPM3 channels to blood flow control and blood pressure regulation

Jose R Lopez-Lopez[1], Jorge Rojo-Mencia[1], Lucía Alonso-Carbajo[2], Karel Talavera[2], Pilar Cidad[1], M.Teresa Pérez-Garcia[1]

[1]Departamento de Bioquímica y Biología Molecular y Fisiología, Universidad de Valladolid, and Unidad de Excelencia, Instituto de Biología y Genética Molecular (IBGM), CSIC, Valladolid, Spain, [2]Department of Cellular and Molecular Medicine, Laboratory of Ion Channel Research, KU Leuven, Leuven, Belgium

Introduction: 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 preclude an explanation of the role of TRPM3 channels in the control of blood pressure (BP), hindering their potential role as novel therapeutical targets against hypertension.

Aims: Here we explored the vascular phenotype of a TRPM3-KO mice and we analyzed the potential contribution of TRPM3 channels to renal control of BP, exploring their expression in kidneys and their role in renal blood flow regulation.

Methods: TRPM3-KO mice and their corresponding WT littermates were used for this study. BP measurements were carried out in awake animals with tail-cuff CODA® system. Mice were anesthetized by isoflurane inhalation (5% O2 at 2.5 Lmin-1) and sacrificed by cervical dislocation, following the EC guiding principles regarding the care and use of animals (Directive 2010/63/UE). Renal TRPM3 expression was measured with qPCR, and channel location throughout the nephron was studied combining RNAscope™ with immunohistochemistry. The functional contribution of TRPM3 in renal vasculature was analyzed by exploring the effect of PS in renal flow at constant pressure in isolated kidneys in organ bath.

Results : BP measurements showed that TRPM3-KO mice were hypotensive and resistant to angiotensin II-induced hypertension, which could be explained by renal modulation of the RAAS system by TRPM3. In support of this, qPCR showed a 2-fold increase in TRPM3 mRNA expression in kidneys from hypertensive BPH mice. TRPM3 mRNA expression was detected in the glomeruli, the distal convoluted tubule (DCT) and the collecting ducts (CD), but not in renal vessels. 10μM PS-induced vasodilation was observed in basal conditions and in the presence of Phenylephrine. The effect of PS was fully abolished by BIBN-4096 (a CGRP antagonist) or the NOS blocker L-NAME, but was unaffected by COX inhibition with Indomethacin.

Conclusions: We suggest that TRPM3 at perivascular nerve endings contributes to renal flow regulation through an CGRP- and NO-dependent pathway, while TRPM3 at the nephron could be involved in BP regulation via tubule-glomerular feedback, contributing to the hypotensive phenotype of TRPM3-KO.

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).

C13

Secretin Modulate Interstitial Cells of Cajal and Inhibits Small Intestinal Motility

Sal Baker[1], Allison Bartlett[1], Kenton Sanders[1]

[1]Department of Physiology and Cell Biology, University of Nevada, Reno, Reno, United States

Abstract

Background: Secretin is a member of the secretin-glucagon-vasoactive intestinal peptide hormone superfamily and is a multifunctional gastrointestinal- (GI) and neuro- peptide hormone. Secretin is primarily secreted postprandially from the crypts of Lieberkühn of duodenal enteroendocrine S cells into circulation where it reaches secretin receptor targets in the central nervous system and periphery. This dynamic hormone has been shown to act as a key signaling molecule in the regulation of digestion, metabolism and energy expenditure, water retention, reproduction, thermogenesis in adipose tissue, and in gastric and intestinal motility. Secretin's canonical role in the GI tract is to stimulate the secretion of bicarbonate and bile from pancreatic ducts and bile ducts to neutralize acidic chyme exiting the stomach. Secretin has also been shown to slow intestinal motility, but its targets and mechanism of action is poorly understood.

Aims: Several studies have proposed that secretin acts to slow intestinal motility primarily through secretin receptors present on vagal afferents in the GI tract. In fact secretin receptors (SCTR) are also highly expressed by interstitial cells of Cajal (ICC) in the small intestine, suggesting an alternate and complementary signaling pathway. We hypothesized that ICC act as a liaison to facilitate a reduction in contractile force through binding of SCRT and stimulation of the second messenger, cyclic adenosine monophosphate (cAMP) in ICC, and this mediates reduced contractile force generated by intestinal muscles.

Methods: Spinning-disk confocal microscopy was used monitor Ca^{2+} signaling in ICC from small intestinal muscles of GCaMP6f x KitiCre mice. Additionally, cAMP levels were evaluated using CAMPER mice. Intestinal muscle contractions were assessed using in vitro myography techniques.

Results: Secretin reduces small intestinal force of contraction in the presence of tetrodotoxin (TTX) and dampens effects of cholinergic neurotransmission. SCTR is expressed by ICC within the deep muscular plexus (ICC-DMP) in the small intestine and Ca^{2+} imaging confirmed the effects are primarily localized within ICC-DMP. Secretin reduced carbachol induced contractions and reduced Ca^{2+} transients in ICC-DMP in response to electrical field stimulation (EFS) in the presence of L-NNA (NO synthase inhibitor) and MRS2500 (P2Y1 antagonist). Secretin caused an increase in cAMP in ICC-DMP in muscles from Kit-iCre-CAMPER mice. PKA inhibitors rescued some of the inhibitory effects of secretin on ICC-DMP Ca^{2+} signaling. All experiments were conducted with a minimum sample size of n=6.

Conclusions: Secretin inhibits small intestinal motility by binding to SCTR on ICC-DMP and a cAMP-mediated mechanism. Intestinal ICC are a novel target for secretin that appears to mediate part of the inhibitory effects of this hormone on intestinal motility.

Funding: This project was supported by R01 DK-120759 from the National Institute of Diabetes and digestive and Kidney (NIDDK).

C14

Unravelling the molecular mechanism of inactivation of BK channels by the novel regulatory subunit - LINGO1

Yaly Al Kawadri[1], Heather McClafferty[2], Irina Tikhonova[3], Keith. D Thornbury[5], Gerard. P Sergeant[6], Michael Shipston[7], Mark. A Hollywood[8]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland, [2]Centre for Discovery Brain Sciences, University of Edinburgh, Scotland, Edinburgh, United Kingdom, [3]Department of Pharmacy, Queen's University of Belfast, Northern Ireland, Belfast, United Kingdom, [4]Centre for Discovery Brain Sciences, University of Edinburgh, Scotland, Edinburgh, United Kingdom, [5]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland., Dundalk, Ireland, [6]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland., Dundalk, Ireland, [7]Centre for Discovery Brain Sciences, University of Edinburgh, Scotland, Edinburgh, United Kingdom, [8]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland., Dundalk, Ireland

BK channels are ubiquitously expressed and alterations in their expression was associated with motor disorders[1-3]. Their biophysical properties are modulated by splice variants as well as auxiliary β, γ, and LINGO subunits[4]. LINGO proteins are Leucine Rich Repeat proteins with an extracellular (ED), transmembrane (TM) and cytosolic tail (TD) domain[3-5]. BK co-expression with LINGO1-2 resulted in inactivating BK currents and shifted their half maximal activation voltage ($V_{1/2ACT}$) negatively (~-50mV and ~-30mV, respectively)[3,5]. However, unlike LINGO2, BK co-expression with LINGO1 reduced their plasmalemmal expression[3,5]. We exploited the differences between LINGO1&2 via using six chimeras to identify the domains linked with the reduction in BK expression and the negative shift in $V_{1/2ACT}$. Chimeras were named based on the origin of their ED, TM or TD.

The inside-out configuration of the patch clamp technique was used on HEK cells co-transfected with BK cDNA with either LINGO1 or the chimeras (300:300 ng) or with BK and LINGO2 cDNA (100:500 ng) using equimolar 140 mM K^+ solutions, at 37°C, with 100 nM-10 µM Ca^{2+} applied to the patch cytosolic surface. Currents were evoked by a step to +160 mV for 40 ms, with 5 MΩ pipettes in 100 nM Ca^{2+}. Steps from -100 mV to +200 mV in 20 mV increments were applied and the currents utilised to generate GV curves. Data is presented as mean ± SEM and statistical significance tested using one-way ANOVA.

Inactivation was observed with all chimeras co-expressed with BK (Figure 1) and the mean current amplitude for BK was 5650±600 pA (n=57). Co-expression with LINGO1 caused a ~93% reduction in current amplitude (401±105 pA, n=77, $p<0.0001$). A significant reduction in current amplitude was noted for BK:121 (888±136 pA, n=50), BK:112 (1398±260 pA, n=51) and BK:122 (1107±150 pA, n=65), when compared to BK ($p<0.0001$). In contrast, no significant difference was observed for BK:LINGO2 (4538±471 pA, n=56) compared to BK as shown previously[5]. Currents from BK:221 patches had smaller current amplitudes (2614±479 pA, $p<0.05$) than BK:LINGO2.

The $V_{1/2ACT}$ of all chimeras with a LINGO1 TD (BK:211, 75±2 mV, n=12; BK:221, 71±2 mV, n=6; BK:121, 69±3 mV, n=9) were similar to BK:LINGO1 in 1 µM Ca^{2+} (76±3 mV, n=11, $p\geq0.6938$). The

$V_{1/2ACT}$ for BK:LINGO2, 51±2 mV (n=6) and all chimeras with a LINGO2 TD in 1 µM Ca^{2+} were all similar (BK:122, 44±4 mV, n=7; BK:112, 40±4 mV, n=6; BK:212, 48±3 mV, n=8; p≥0.8249).

Deleting the last four C-terminus residues (BK:211_{DMKMI}) abolished inactivation and the $V_{1/2ACT}$ was 90±3 mV in 100 nM Ca^{2+} (n=6). Similarly the $V_{1/2ACT}$ of BK:$211_{DRKFNMKMI}$, was 113±2 mV in 100 nM Ca^{2+} (n=8), suggesting that the negative shift in activation was retained in the absence of the last eight residues.

These data suggest that the extracellular domain of LINGO1 is responsible for the reduction in plasmalemmal BK expression. Although the TD of LINGO appears responsible for setting $V_{1/2ACT}$ in 1 µM Ca^{2+}, the last eight residues of this region were not necessary for this.

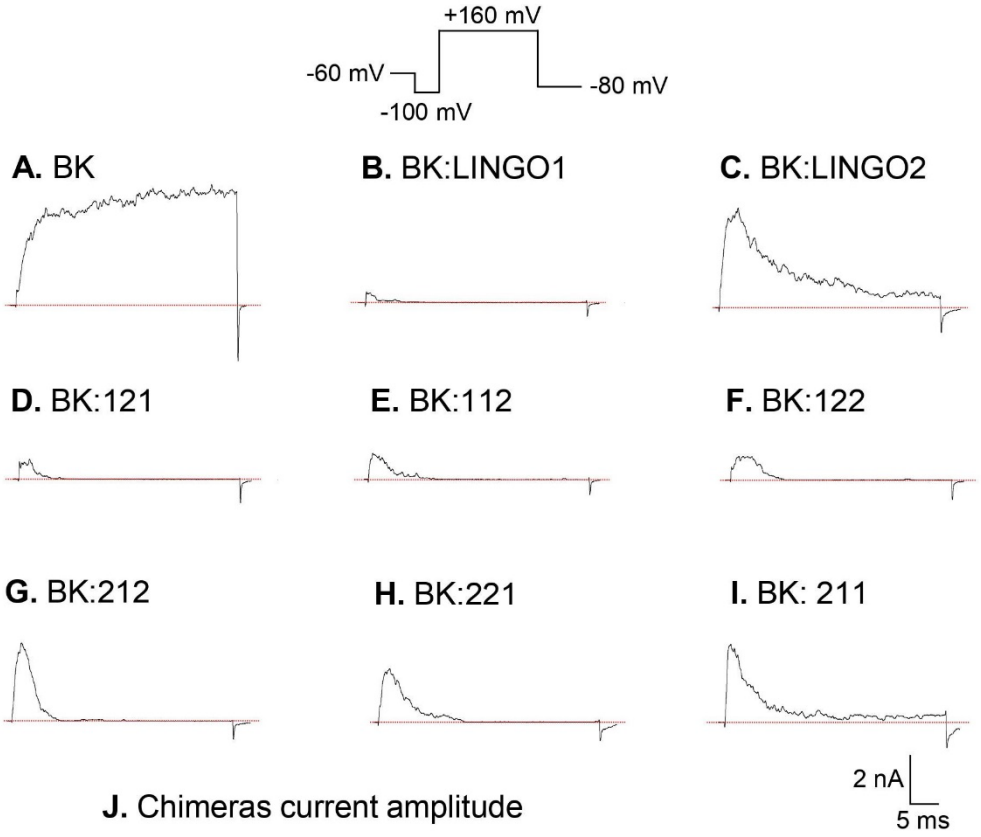

Figure 1. A-I Show typical currents from LINGO chimeras and **J** shows mean current amplitudes.

1. Shruti, S., Urban-Ciecko, J., Fitzpatrick, J.A., Brenner, R., Bruchez, M.P., Barth, A.L., (2012). The Brain-Specific Beta4 Subunit Downregulates BK Channel Cell Surface Expression. PLoS One 7, e33429. 2. Cheron, G., Márquez-Ruiz, J., Cheron, J., Prigogine, C., Ammann, C., Lukowski, R., Ruth, P., Dan, B., (2018). Purkinje cell BK channel ablation induces abnormal rhythm in deep cerebellar nuclei and prevents LTD. Sci Rep, 8, 4220. 3. Dudem, S., Large, R. J., Kulkarni, S., McClafferty, H., Tikhonova, I. G., Sergeant, G. P., Thornbury, K. D., Shipston, M. J., Perrino, B. A., & Hollywood, M. A. (2020). LINGO1 is a regulatory subunit of large conductance, Ca2+-activated potassium channels. Proceedings of the National Academy of Sciences of the United States of America, 117(4), 2194–2200 4. Dudem S., Sergeant G.P., Thornbury K.D., Hollywood M.A. (2021) Calcium-Activated K+ Channels (KCa) and Therapeutic Implications. In: Handbook of Experimental Pharmacology. Springer, Berlin, Heidelberg. 5. Dudem, S., Boon, P.X., Mullins, N., McClafferty, H., Shipston, M.J., Wilkinson, R.D.A., Lobb, I., Sergeant, G.P., Thornbury, K.D., Tikhonova, I.G., Hollywood, M.A., (2023). Oxidation modulates LINGO2-induced inactivation of large conductance, Ca2+-activated potassium channels. J. Biol. Chem. 299, 102975.

C15

The role of Orai channels in regulating cholinergic contractions of the detrusor

Alexandru Mircea[1], Bernard Drumm[1], Gerard Sergeant[1], Mark Hollywood[1], Keith Thornburry[1], Caoimhin Griffin[1]

[1]Dundalk Institute of Technology, Dundalk, Ireland, [2]Dundalk Institute of Technology, Dundalk, Ireland

Store-operated Ca^{2+} entry (SOCE) is a well-established cellular mechanism involved in the replenishment of sarcoplasmic reticulum (SR) calcium (Ca^{2+}) content in smooth muscle cells (SMCs). Reduction of [Ca^{2+}] within the SR is sensed by the luminal domain of Stromal interaction molecule 1 (STIM1) which leads to activation of plasmalemmal Calcium release-activated calcium channels (ORai). Ca^{2+} influx into the cytosol via ORai channels can then be sequestered into the SR – restoring Ca^{2+} levels. Many studies have shown that cholinergic-mediated contractions of bladder smooth muscle (Detrusor) rely on Ca^{2+} influx from the extracellular space (~70%) and Ca^{2+} release from the SR (~30%), however, identification of the molecular candidates that facilitate SOCE and SR refilling in the bladder are poorly understood. This purpose of this study was to investigate the contribution of Orai channels to cholinergic-mediated responses of murine detrusor muscle.

Firstly, transcriptional expression of Orai subtypes was performed on RNA extracted from murine detrusor muscle strips. qRT-PCR experiments reveal that Orai 1, 2 & 3 subtypes are expressed in detrusor muscle, with Orai 3 mRNA levels two-fold higher than Orai1 & 2 (n=4). Isometric tension experiments were utilized to assess the contractility of detrusor muscle strips. SOCE was induced in detrusor muscle via blockade of SR refilling with thapsigargin. SOCE-mediated responses were significantly reduced, in a concentration-dependent manner by the selective Orai channel inhibitor, GSK7975A (ANOVA, $p<0.0001$, n=6). Next, cholinergic-mediated responses were investigated using the Muscarinic receptor agonist, Carbachol (CCh). In the presence of GSK7975A, CCh-induced contractions of the detrusor were reduced from 8493 ± 616.6 mN to 5065 ± 602.3 mN (40% reduction, $p<0.0001$, n=6), and CCh-induced Ca^{2+} transients in isolated detrusor SMCs, were ablated in the presence of GSK7975A (n=5). Collectively, these results indicate that ORai channels play a significant role in mediating cholinergic-mediated responses of detrusor smooth muscle.

C16

Inactivation of Anoctamin-1 Channels by CaMKII Phosphorylation is Hampered by the Presence of Alternatively Spliced Variant d

Connor L. Jimenez[1], Robert E. del Carlo[1], Matthew B. Hawn[1], Elizabeth J. Akin[1], Normand Leblanc[1]

[1]*University of Nevada, Reno, Reno, NV 89557, United States*

Opening of Ca^{2+}-activated Cl^- channels encoded by the *Tmem16a* or *Anoctamin-1* (ANO1) gene is thought to play a key role in the depolarization, Ca^{2+} signaling and contraction of vascular smooth muscle cells (VSMC) triggered by vasoconstricting agonists linked to G_q-protein-coupled receptors.[1] Ca^{2+}-activated Cl^- currents ($I_{Cl(Ca)}$) in VSMC display rundown after seal rupture in whole-cell patch clamp experiments, with a significant portion of this rundown involving at least one phosphorylation step mediated by CaMKII.[2] Interestingly, the rundown of $I_{Cl(Ca)}$ recorded under similar conditions in pulmonary arterial smooth muscle cells (PASMC) from different species is variable, with $I_{Cl(Ca)}$ in rabbit and rat PASMC running down by ~ 80%[3] and 20%[4] over 5-10 min, respectively. Alternative splicing is known to regulate the biophysical properties of $I_{Cl(Ca)}$ resulting from ANO1 expression.[2] $I_{Cl(Ca)}$ resulting from the expression of the *a* variant of mouse ANO1 in HEK-293 cells displayed similar rundown that was partially attenuated by blocking CaMKII with KN-93 or ARIP, or by mutating Serine 528 to Alanine (S528A), suggesting that the rundown of $I_{Cl(Ca)}$ is in part due to direct CaMKII-induced phosphorylation of ANO1 protein.[5] In this study, we found that the rundown of ANO1 was greatly attenuated when splice variant *d* (ANO1-*ad*), a 26 amino acid segment located in the first intracellular loop of ANO1, was expressed with splice variant *a* in HEK-293 cells (ANO1-*a*: 60 ± 5%, n=10; ANO1-*ad*: 16 ± 10%, n=7; $P < 0.05$). Since splice variant *d* is very near S528, and the rundown of ANO1-*ad* was similar to that of ANO1-*a* in the presence of CaMKII inhibitors, or the ANO1-*a* S528A mutant, we hypothesized that the presence of the *d*variant could limit access of CaMKII to phosphorylate Serine 528, or alternatively eliminate the impact of the phosphorylation on channel activity. Consistent with this hypothesis, blocking CaMKII with ARIP (5 mM) had no effect on the rundown of ANO1-*ad* (ANO1-*ad* + ARIP: 17 ± 10%, n=6; $P > 0.05$). We next modeled the impact of incorporating splice variant *d* on stable interaction energies in the vicinity of S528 using the AlphaFold Protein Structure Database. Adding splice variant *d* increased the ΔG of S528 from -27.98 to -55.11 kj/mol. Incorporating splice variant *d* to phosphoserine 528 led to a similar but more profound stabilization of local interactions with the ΔG of this residue increasing from -55.85 to -113.23 kj/mol. These simulations indicated that the stabilization of energies by splice variant *d* was likely the product of the residue being locked in a more rigid alpha helical structure, which may hamper the ability of CaMKII to phosphorylate this site, or prevent phosphorylation from exerting its down-regulating effect on channel activity. These results show that splice variant *d* has a profound influence on the regulation of ANO1 by CaMKII, an observation that may partially explain why ANO1-induced $I_{Cl(Ca)}$ recorded in different cell types or in the same cell type in different species, exhibit variable stability in patch clamp recordings.

1. Leblanc et al. (2015) Pulm Circ 5, 244-268. 2. Hawn et al. (2021) Channels (Austin) 15, 569-603. 3. Angermann et al. (2006) J Gen Physiol 128, 73-87. 4. Forrest et al. (2012) Am J Physiol Cell Physiol 303, C1229-C1243. 5. Ayon et al. (2019) Am J Physiol Cell Physiol 317, C1093-C1106.

C17

Molecular determinants underlying the shift in activation V1/2 by LINGO2 subunits.

Zainab Baig[1], Kaneez E Rabab[1], Sai Neeraj Palakurthy[1], Gerard P Sergeant[1], Keith D Thornbury[1], Mark A Hollywood[1]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland

Large conductance Ca^{2+} and voltage-activated (BK) channels are pore-forming proteins studded in the membrane of excitable and non-excitable cells. They are ubiquitously expressed and can modify functions as diverse as smooth muscle contraction, neurotransmission and epithelial surface volume. The biophysical properties of BK channels are modulated by regulatory β1-4[1,2] and γ1-4[3] subunits in a tissue-specific manner. We have recently discovered a new family of BK channel regulatory subunits called the LINGO proteins[4-5] which can alter BK plasmalemmal expression to varying degrees, induce inactivation and shift the voltage at which the BK channels activate[4-5].

The focus of this study was to investigate the molecular mechanisms underlying the negative shift in the activation $V_{1/2}$ ($V_{1/2ACT}$) observed when $LINGO_{1/2}$ proteins are co-expressed with BK channels[4]. Recently, cryo-EM studies[5] have suggested that γ subunits shift BK channel $V_{1/2ACT}$ by a salt-bridge interaction between D370 in the BK channel and positively-charged residues in the juxta-membrane region of γ subunits. Given that γ and LINGO proteins appear similar in structure, we examined if a similar interaction occurred between BK and LINGO2.

We hypothesised that LINGO2 induced its negative shift in BK $V_{1/2ACT}$ via an interaction between K574 located intracellularly at the juxta membrane region of LINGO2 and D370 in the RCK1 domain of BK. The effects of disrupting this putative interaction were examined using mutagenesis and electrophysiology.

BKα, LINGO2 and eGFP cDNA (100:500:150ng ratio) were transiently co-transfected into HEK cells. Currents were recorded under voltage clamp at 37˚C from inside out patches from HEK cells that expressed BKα and LINGO2. K^+ solutions containing 140mM KCl, 10mM glucose, 10mM HEPES, and either 1mM EGTA or HEDTA were used as pipette and internal solutions. Current voltage relationships were determined in patches held at -60mV and stepped from -100mV to +200mV in 20mV increments for 50ms. GV curves were constructed from these and fitted with a Boltzmann equation to determine BK $V_{1/2ACT}$. Summary data is presented as mean ± SEM and Student's unpaired t-test or ANOVA were used to assess statistical significance, as appropriate.

In wildtype BK channels, currents activated in 100 nM Ca^{2+} with a $V_{1/2ACT}$ of 151±1 mV (n=6) compared to 129±2 mV in BK:LINGO2 channels (n=6, p=0.0085). The $V_{1/2ACT}$ was unaltered in the BK_{D370A} mutant (152±2 mV, n=6) compared to wildtype BK channels. In contrast, the $V_{1/2ACT}$ derived from BK_{D370A}:LINGO2 currents (163±2 mV) was positively shifted in comparison to BK:LINGO2 (n=6, p=0.0046). Similarly the $V_{1/2ACT}$ of BK:$LINGO2_{K574A}$ was shifted positively to 166±2 mV (n=6, p=0.0017) and to 151±2 mV (n=6) in the double BK_{D370A}:$LINGO2_{K574A}$ mutant.

In conclusion the shift in $V_{1/2ACT}$ induced by LINGO2 appears to be due to an interaction between K574 in the LINGO2 tail and D370 in the BK RCK1 domain, possibly due to the formation of a salt bridge between charged residues in the juxta membrane region of LINGO2 and the N lobe of RCK1 in BK. These data suggest that γ and LINGO subunits may utilize similar mechanisms to shift activation V1/2 of BK channels.

1. Uebele et al. (2000). Journal of Biological Chemistry 275(30):23211-8 2. Brenner et al. (2000). Nature. 407(6806):870-6 3. Dudem et al. (2020). Proc. Natl. Acad. Sci. 117, 2194–2200 4. Dudem et al. (2022). Acta Physiologica, B06-10 (#617) 5. Yamanouchi et al. (2023). Molecular Cell 83, 4555-4569.e4

C18

A systemic analysis of the effects of TMEM16A/Anoctamin 1 channel modulators on artery tone

Rumaitha Al Hosni[13], Emilio Agostinelli[12], Zeki Ilkan[11], Lara Scofano[10], Rachel Kaye[9], Ria L. Dinsdale[8], Kathryn Acheson[7], Andrew MacDonald[6], Dean Rivers[5], Alice Biosa[4], Martin J. Gunthorpe[3], Frances Platt[2], Paolo Tammaro[1]

[1]Department of Pharmacology, University of Oxford, Oxford, United Kingdom, [2]Department of Pharmacology, University of Oxford, Oxford, United Kingdom, [3]Autifony Therapeutics Ltd., Stevenage, United Kingdom, [4]Autifony Srl, Istituto di Ricerca Pediatrica Citta' della Speranza, Padua, Italy, [5]Autifony Therapeutics Ltd., Stevenage, United Kingdom, [6]Autifony Therapeutics Ltd., Stevenage, United Kingdom, [7]Department of Pharmacology, University of Oxford, Oxford, United Kingdom, [8]Department of Pharmacology, University of Oxford, Oxford, United Kingdom, [9]Department of Pharmacology, University of Oxford, Oxford, United Kingdom, [10]Department of Pharmacology, University of Oxford, Oxford, United Kingdom, [11]Department of Pharmacology, University of Oxford, Oxford, United Kingdom, [12]Department of Pharmacology, University of Oxford, Oxford, United Kingdom, [13]Department of Pharmacology, University of Oxford, Oxford, United Kingdom

Background: The TMEM16A chloride channel serves as depolarising mechanism in arterial smooth muscle cells (SMCs). The channel is a suggested target for diseases of impaired vascular tone including stroke, vascular dementia, and (systemic, pulmonary) hypertension [1, 2]; however, the selectivity and mode of action of available TMEM16A modulators are incompletely defined. We define the mode of action of a recently disclosed positive allosteric modulator (Patent WO2021/014168, compound 1) [3] of the TMEM16A channel (PAM_16A) and examine its selectivity alongside that of a series of established TMEM16A inhibitors (Ani9, MONNA and CaCCinhA01).

Methods: Patch-clamp electrophysiology, isometric tension recordings of isolated rat arteries were employed to study the effects of selective pharmacological control of TMEM16A on vascular function. Data are given as mean±SEM alongside the number of independent experiments (n) and number of animals (N).

Results: In the presence of sub-maximal (300 nM) intracellular free Ca^{2+} concentration $[Ca^{2+}]_i$, PAM_16A activated the heterologously expressed TMEM16A channels at positive and negative potentials (EC_{50}≈3.6±0.5 nM), while producing a minimal effect on the highly homologous TMEM16B channel. PAM_16A did not activate the TMEM16A currents in the absence of intracellular Ca^{2+} or in the presence of saturating $[Ca^{2+}]_i$ (12 μM). Non-stationary noise analysis revealed that PAM_16A caused an increase in channel open probability. Consistently, mutant TMEM16A channels with the intracellular gate constitutively open were much less sensitive to PAM, suggesting that PAM may act as a modifier of TMEM16A channel gating. PAM_16A selectively activated TMEM16A currents in isolated SMCs by 1.3±0.1 fold at +100 mV and 4.4±0.6 fold at -100 mV (N=5, n=17) ($p<0.05$) and promoted aortic smooth muscle contraction by 1.6±0.8 fold (N=7, n=9) ($p<0.05$). Unlike PAM_16A and Ani9, a range of other available modulators were found to interfere with endogenous cationic currents in SMCs.

Conclusion: Arterial tone can be finely controlled with TMEM16A modulators higlighting the channel as a posisble novel therapeutic target in a range of disorders involving impaired vascular tone, including stroke, and hypertension.

[1] R. Al-Hosni, Z. Ilkan, E. Agostinelli, P. Tammaro, The pharmacology of the TMEM16A channel: therapeutic opportunities, Trends Pharmacol Sci, 43 (2022) 712-725. [2] R. Al-Hosni, R. Kaye, C.S. Choi, P. Tammaro, The TMEM16A channel as a potential therapeutic target in vascular disease, Curr Opin Nephrol Hypertens, (2024). [3] Mass, J., Sharp E. . (2021). SOLID FORMS OF 4-[[2-(5-CHLORO-2-HYDROXY-PHENYL)ACETYL]AMINO]-N-(1,1-DIMETHYLPROP-2-YNYL)PYRIDINE-2-CARBOXAMIDE In I. B. World Intellectual Property Organisation (Ed.).

C19

Mechanisms underlying potentiation of nerve-evoked contractions of airway smooth muscle by activation of postjunctional M2 muscarinic receptors

SRIJIT GHOSH[1], TULEEN ALKAWADRI[1], KEITH THORNBURY[1], MARK HOLLYWOOD[1], GERARD SERGEANT[1]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland

Postjunctional M2 muscarinic receptors (M2Rs) on airway smooth muscle (ASM) outnumber M3 receptors (M3Rs) by a ratio of 4:1 in most species, yet it is the M3Rs that are thought to mediate the bronchoconstrictor effects of acetylcholine. However, it is now known that stimulation of M2Rs causes a profound enhancement of M3R-mediated ASM contractions induced by low frequency stimulation (2 Hz) and when the stimulus interval was decreased from 100 to 10 s [1]. The purpose of the present study was to investigate the cellular mechanisms responsible for M2R-dependent potentiation of nerve-evoked contractions of murine ASM. Contractions of murine bronchial rings were induced by electric field stimulation (EFS) and recorded using isometric tension recording. Reduction in stimulus interval from 100 to 10 s increased mean contraction amplitude from 0.8 ± 0.1 to 1.6 ± 0.2mN (N=6, $p<0.0001$). Subsequent addition of the L-type Ca^{2+} channel (LTCC) blocker, nifedipine (1 μM) reduced mean contraction amplitude to 0.83 ± 0.14mN (N=6, $p<0.0001$). In contrast, contractions evoked at 100 s intervals, that are exclusively mediated by M3Rs, were unaffected by nifedipine. Previous studies on canine ASM found that Ca^{2+} influx via LTCC was buffered by the activity of superficial sarcoplasmic/endoplasmic reticulum ATPase (SERCA) [2]. We hypothesised that M2R-dependent contractions of ASM resulted from reduced buffering of Ca^{2+} entry via LTCC by SERCA. Quantitative polymerase chain reaction (qPCR) analyses revealed that SERCA2b exhibited the highest expression among SERCA isoforms in murine bronchi tissues and staining of isolated ASM cells with SERCA2 antibodies using immunocytochemistry showed that SERCA2 was located at the periphery of ASM cells, adjacent to the plasma membrane. Inhibition of SERCA with thapsigargin (1 μM) increased the mean amplitude of nerve-evoked contractions of ASM, at 100 s intervals, from 1.4 ± 0.1 to 3.2 ± 0.3 mN (N=6, $p<0.001$) and these effects were also reversed by nifedipine which lowered the amplitude to 0.7 ± 0.1mN (N=6, $p<0.001$). Thapsigargin also increased the area of ASM contractions induced by the LTCC activator FPL64176 (1 μM), from 776 ± 221 to 2686 ± 643 mN.s (N=6, $p<0.05$). Similar to the effects of nifedipine, the Ano1 channel blocker Ani9 (1 μM) abolished M2R-dependent enhancement of EFS-evoked contractions at 10 s intervals (from 1.4 ± 0.1 to 0.44 ± 0.1 mN (N=6, $p<0.01$), but did not affect responses at 100 s intervals. Ani9 also reversed the stimulatory effects of thapsigargin on EFS-evoked contractions at 100 s. Thapsigargin increased mean contraction amplitude from 0.9 ± 0.1 to 1.9 ± 0.1mN (N=6, $p<0.01$) and Ani9 reduced contraction amplitude to 0.7 ± 0.1 mN (N=6, $p<0.001$). These data indicate that M2R-mediated potentiation of cholinergic-nerve mediated contractions of ASM relies on activation of Ano1 channels and Ca^{2+} influx via LTCC by a mechanism involving inhibition of buffering of Ca^{2+} influx by SERCA.

[1] Alkawadri T. et al. (2021). Function (Oxf) 3(1):zqab067 [2] Janssen L.J. et al. (1999). Am J Physiol 276(5):L744-L753

C20

Anoctamin 1 expressed by interstitial cells of Cajal controls rhythmic contractility of human left colon

Thomas Jawad[1], Jonathan Sutcliffe[2], Bernard Drumm[3], Nikita Gamper[1], Jillian Daniel[1], Hanan El-Kuwalia[1], Andrew Smith[1], Jim Deuchars[1]

[1]University of Leeds, Leeds, United Kingdom, [2]Leeds General Infirmary, Leeds, United Kingdom, [3]Dundalk Institute of Technology, Dundalk, Ireland

Introduction

Interstitial cells of Cajal (ICC) are Kit positive cells found throughout the gastrointestinal tract. ICC generate rhythmic intracellular Ca2+ signals that drive phasic contractions of the gut and maintain key digestive processes, like peristalsis and maintenance of tone (Koh et al., 2022). Studies in rodents identified the Ca2+-activated Cl- channel, Anoctamin (Ano1), as a key driver for the rhythmic ICC signalling responsible for gut pacemaking (Hwang et al., 2009). However, functional significance of Ano1 in human gastrointestinal tract is yet to be revealed. Gut motility disorders affect millions worldwide, therefore understanding the molecular mechanisms controlling gut motility in humans, in comparison to other mammals, may drastically improve clinical outcomes for sufferers of these conditions (Wetherill & Sutcliffe, 2014).

Aims

This research aims to investigate the role of ICC and Ano1 in human gut through comparisons of expression and functional activity of Ano1 in human left colon and rat proximal colon.

Methods

Samples of smooth muscle from left colon of 3 paediatric patients were obtained from stoma closure surgeries. Proximal colon samples from 9 Wistar rats of both genders were also taken. Circular sections with dimensions of 2x5mm were cut and placed into a 50ml organ bath at 37ºC for isometric contraction measurements and the effects of voltage-gated Na2+ channel blocker, tetrodotoxin (TTX; 1μM) and a selective Ano1 inhibitor, Ani9 (≤10μM) were recorded. Sections of smooth muscle from the same areas in both human and rat were fixed onto slides, and immunostained using anti-Kit and anti-Ano1 antibodies. Ano1 transcript levels in human and rat proximal colon were analysed using reverse-transcription polymerase chain reaction (RT-PCR). Human tissue experiments are covered under REC reference 14/NS/0018 and IRAS number 92423.

Results

In rats, application of TTX removed enteric modulation and significantly increased ($p<0.05$) contractile intensity. The addition of Ani9 in increasing concentrations (0.1-3μM) led to a concentration-dependent decrease in the intensity and frequency of contractions, until contractions stopped completely. In human tissue, adding Ani9 also caused a significant ($p<0.02$) decrease in the frequency of contractions, however the effects did not manifest until higher

concentrations of Ani9 (3μM) and there was no significant change in the intensity of contractions. The blocking effect by Ani9 in human tissue was incomplete even at 10μM, unlike in rodents. The contraction frequency was reduced from 4.3±1.0mHz (control) to 1.8±0.1mHz (10μM Ani9; n=3; $p<0.02$).

Imaging studies revealed similar patterns of Kit positive ICC in human and rodent tissue with a strong colocalization between Kit and Ano1 immunoreactivity. Presence of Ano1 in the colon of either species was confirmed by RT-PCR.

Conclusion

This study reports functional importance of Ano1 for rhythmic contractility of human colon. Immunofluorescence imaging revealed strong similarities between localisation and expression patterns of ICC and Ano1 in human and rodent tissue. The contractility measurements uncovered strong contributions of Ano1 to human colon rhythmicity, albeit it not as strong as in rat. This study paves way towards better understanding of molecular mechanisms of human gut contractility.

Hwang SJ, Blair PJ, Britton FC, O'Driscoll KE, Hennig G, Bayguinov YR, Rock JR, Harfe BD, Sanders KM, Ward SM. Expression of anoctamin 1/TMEM16A by interstitial cells of Cajal is fundamental for slow wave activity in gastrointestinal muscles. J Physiol. 2009 Oct 15;587(Pt 20):4887-904. doi: 10.1113/jphysiol.2009.176198. Epub 2009 Aug 17. PMID: 19687122; PMCID: PMC2770154. Koh, S.D., Drumm, B.T., Lu, H., Kim, H.J., Ryoo, S.-B., Kim, H.-U., Lee, J.Y., Rhee, P.-L., Wang, Q., Gould, T.W., Heredia, D., Perrino, B.A., Hwang, S.J., Ward, S.M. and Sanders, K.M. 2022. Propulsive colonic contractions are mediated by inhibition-driven poststimulus responses that originate in interstitial cells of Cajal. Proceedings of the National Academy of Sciences. 119(18). Wetherill, C. and Sutcliffe, J. 2014. Hirschsprung disease and anorectal malformation. Early Human Development. 90(12), pp.927–932.

C21

TRPML Channels Regulate Proximal Colon Contractility

Caoimhin Griffin[1], Bernard Drumm[1], Cathal O'Gradaigh[1]

[1]Dundalk Institute of Technology, Dundalk, Ireland

TRPML (transient receptor potential mucolipin) are a group of calcium (Ca^{2+})-permeable, nonselective cation channels primarily found in the membranes of late endosomes and lysosomes. The release of Ca^{2+} through TRPML is believed to be crucial for maintaining the acidic pH inside vesicles, as well as for the maturation, fusion, and trafficking of lysosomes (Venkatachalam *et al*., 2015). Recently, TRPML channels subtype 1 (TRPML1) have been shown to regulate vascular, bladder and urethral smooth muscle contractility and excitability (Thakore *et al*., 2019; Griffin *et al*., 2020). TRPML1 activity is thought to exert its regulatory effect on these tissues, by initiating Ca^{2+} sparks from ryanodine receptors (RyR), via a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism. TRPML1-mediated Ca^{2+} spark activity leads to BK channel activation, membrane potential hyperpolarization, and smooth muscle cell (SMC) relaxation. In the proximal colon, Ca^{2+} signaling is pivotal for the regulation of smooth muscle contractility, however the role of TRPML channels in mediating these Ca^{2+} signaling pathways and/or muscle contractility has yet to be explored. Therefore, our aim was to investigate the role of TRPML channels in regulating proximal colon contractility.

Using isometric tension of murine proximal colon circular muscle strips, we found that the proximal colon displayed robust spontaneous contractile activity, similar to previous studies (Drumm *et al*,. 2019; Koh *et al*., 2022). Application of a selective TRPML agonist, ML-SA1, increased both the frequency (contractions min^{-1}) and tension (Area; mN.s) of proximal colon spontaneous contractions in a concentration dependent manner (100nM to 10μM). For example, 10μM of MLSA1 increased contraction frequency by 475% and contraction tension by 151% compared to control activity (n=2). Interestingly, application of a selective TRPML antagonist, ML-SI3, mimicked the effect of the agonist, ML-SA1, by increasing both contraction frequency and contraction tension (n=2). Tonic release of Nitric oxide (NO) from inhibitory motor neurons has been shown to suppress proximal colon contractility. The influence of tonically released NO on contractile activity can be removed by inhibiting nerves with tetrodotoxin (TTX; Drumm et al., 2019). We found that application of TTX (300 nM) increased spontaneous contractile activity of the proximal colon. However, when we applied the TRPML agonist, ML-SA1, in the presence of TTX, spontaneous contractile activity decreased in a concentration dependent manner (100nM to 10μM). For example, application of 10μM of MLSA1 decreased contraction tension by 67% in comparison to control (n=2). The TRPML antagonist, ML-SI3, also reduced contraction tension in the presence of TTX, however to a lesser extent (32%; n=2).

Taken together, our data suggest that TRPML channels regulate proximal colon activity, however it appears that a complex relationship exists between TRPML channels, NO-releasing inhibitory nerves and the excitability of the smooth muscle tissue. In the proximal colon, other cell types such as interstitial cells of cajal (ICC) and platelet derived growth factor receptor alpha (PDGFRα) cells also influence the excitability of the muscle and may express TRPML channels. Therefore,

future studies should elucidate the precise cell type (s) and signaling pathway (s) that TRPML is exerting its effect on in the proximal colon.

Drumm BT, Rembetski BE, Baker SA, Sanders KM. (2019). Tonic inhibition of murine proximal colon is due to nitrergic suppression of Ca2+ signaling in interstitial cells of Cajal. Sci Rep. Mar 13;9(1) Griffin CS, Alvarado MG, Yamasaki E, Drumm BT, Krishnan V, Ali S, Nagle EM, Sanders KM, Earley S. (2020). The intracellular Ca2+ release channel TRPML1 regulates lower urinary tract smooth muscle contractility. Proc Natl Acad Sci U S A. 117(48):30775-30786. Koh SD, Drumm BT, Lu H, Kim HJ, Ryoo SB, Kim HU, Lee JY, Rhee PL, Wang Q, Gould TW, Heredia D, Perrino BA, Hwang SJ, Ward SM, Sanders KM. (2022). Propulsive colonic contractions are mediated by inhibition-driven poststimulus responses that originate in interstitial cells of Cajal. Proc Natl Acad Sci U S A. 119(18):e2123020119. Thakore P, Pritchard HAT, Griffin CS, Yamasaki E, Drumm BT, Lane C, Sanders KM, Feng Earley Y, Earley S. (2020). TRPML1 channels initiate Ca2+ sparks in vascular smooth muscle cells. Sci Signal. 13(637): eaba1015 Venkatachalam K, Wong CO, Zhu MX. (2015). The role of TRPMLs in endolysosomal trafficking and function. Cell Calcium. 58(1):48-56

C22

Lauric acid enhances endothelium-independent relaxation in the corpus cavernosum of streptozotocin-induced diabetic male Wistar rats

Makinde Olubiyi[1,2], Mohammed Magaji[2], Rabiu Magaji[2], Hussein Salahdeen[3], Mohammed Kawu[2]

[1]University of Rwanda, Huye, Rwanda, [2]Ahmadu Bello University, Zaria, Nigeria, [3]Lagos state University, Lagos, Nigeria

Introduction: Diabetes mellitus is a risk factor for erectile dysfunction. Patient dissatisfaction with conventional medications has prompted exploration into alternative therapies, particularly those derived from therapeutic plants. Therapeutic properties of coconut oil have been reported and lauric acid, the predominant constituent is believed to be responsible for its health benefits. The corpus cavernosum smooth muscle plays an important role in erectile function with its relaxation facilitating penile erection. Hence this study sought to investigate the potential relaxant effects and underlying mechanisms of lauric acid on the corpus cavernosum of diabetic male Wistar rats.

Methods: Ethical approval was obtained from the Ahmadu Bello University Animal Care and Use Committee and the rats were handled in compliance with the World Medical Association Declaration of Helsinki. Twenty-five male Wistar rats were divided into five groups (n=5). Group 1: control; Group 2: diabetic untreated; Groups 3, 4, and 5: diabetic rats treated with varying doses of lauric acid (90 mg/Kg, 180 mg/Kg, and 360 mg/Kg, respectively). Diabetes was induced through an intraperitoneal injection of streptozotocin. Lauric acid was administered orally once daily for a duration of 4 weeks. Upon completion of the experiment, the rats were euthanized and then corpus cavernosum tissues from the penis were extracted and placed in an organ bath filled with Krebs solution. Following phenylephrine or potassium chloride (KCl)-induced contraction, the relaxation responses to a cumulative concentration of acetylcholine (10^{-9} – 10^{-5} M) and sodium nitroprusside (SNP) (10^{-9} – 10^{-5} M) were then assessed to evaluate endothelium-dependent and nitric oxide-mediated relaxation respectively. Results were presented as mean ± S.E.M. Data was analysed using ANOVA and Turkey post-hoc tests. Values with $p < 0.05$ were considered significant.

Results: Percentage relaxation of phenylephrine-contracted tissues to acetylcholine was significantly lower ($p < 0.05$): in Group 2 (at 10^{-7} M, 10^{-6} M and 10^{-5} M); in Group 3 (at 10^{-7} M, 10^{-6} M and 10^{-5} M); in Group 4 (at 10^{-7} M, 10^{-6} M and 10^{-5} M); and Group 5 (at 10^{-7} M and 10^{-5} M), respectively compared to Group 1. Percentage relaxation of KCl-contracted tissues to acetylcholine was significantly lower ($p < 0.05$): in Group 2 (at 10^{-6} M and 10^{-5} M); in Group 4 (at 10^{-6} M and 10^{-5} M); and Group 5 (at 10^{-6} M and 10^{-5} M); respectively compared to Group 1. Percentage relaxation of phenylephrine-contracted tissues to SNP was significantly lower ($p < 0.05$): in Group 2 (at 10^{-5} M); and Group 5 (at 10^{-7} M, 10^{-6} M, and 10^{-5} M); respectively compared to Group 1. Percentage relaxation of KCl-contracted tissues to SNP was significantly lower ($p < 0.05$) in Group 2 (at 10^{-8} M, 10^{-7} M) compared to Group 1.

Conclusion: Lauric acid improved the depressed relaxation of corpus cavernosum in diabetic rats by mechanisms independent of the endothelium; by augmenting the nitric oxide-mediated relaxing effect of sodium nitroprusside and possibly inhibiting KCl-induced contraction.

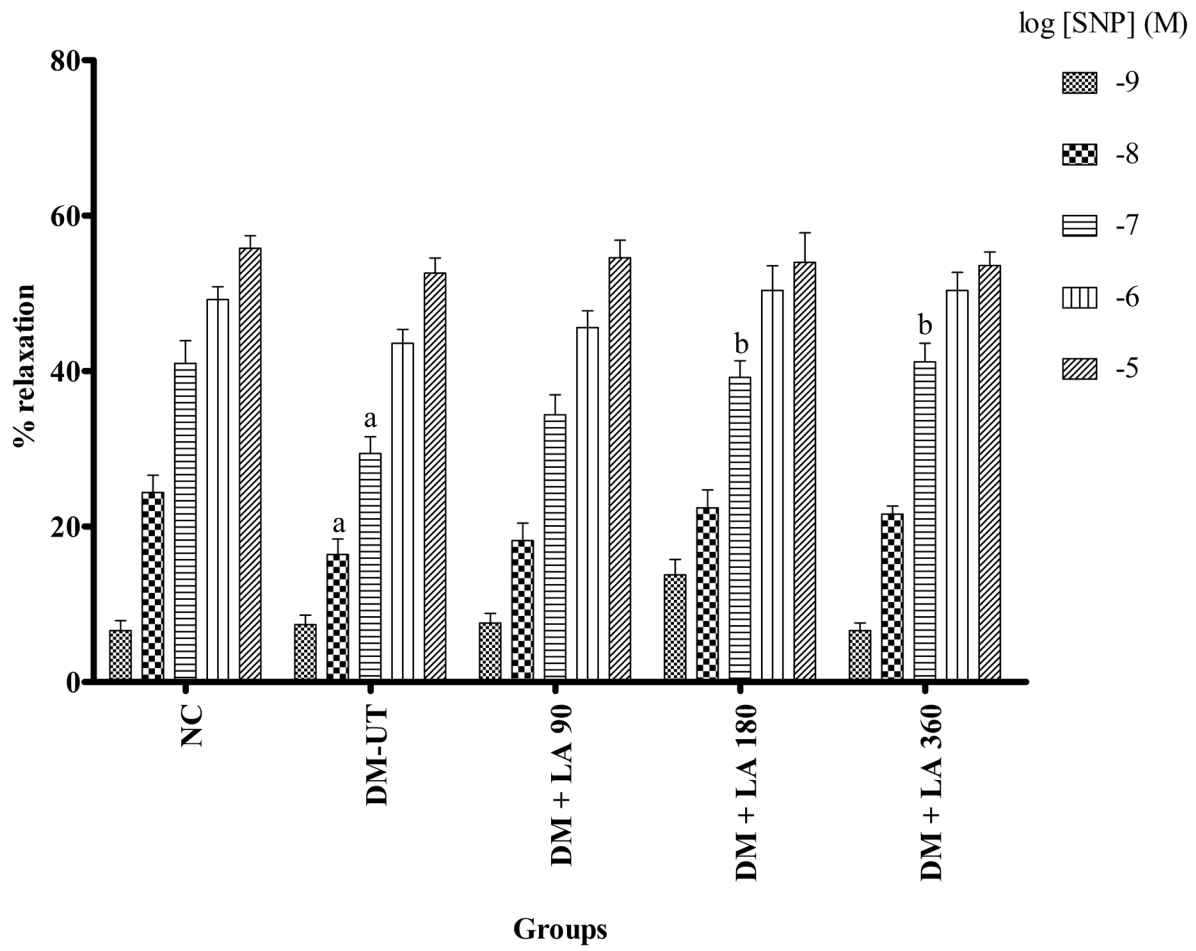

Figure 4: **Relaxation response of KCl pre-contracted corpus cavernosum to cumulative concentrations of sodium nitroprusside**

NC: normal control, DM-UT: diabetic untreated, DM + LA 90 diabetic treated with lauric acid (90 mg/kg), DM + LA 180 diabetic treated with lauric acid (180 mg/kg), DM + LA 360 diabetic treated with lauric acid (360 mg/kg). a - Significant compared to NC ($p < 0.05$). b - Significant compared to DM-UT

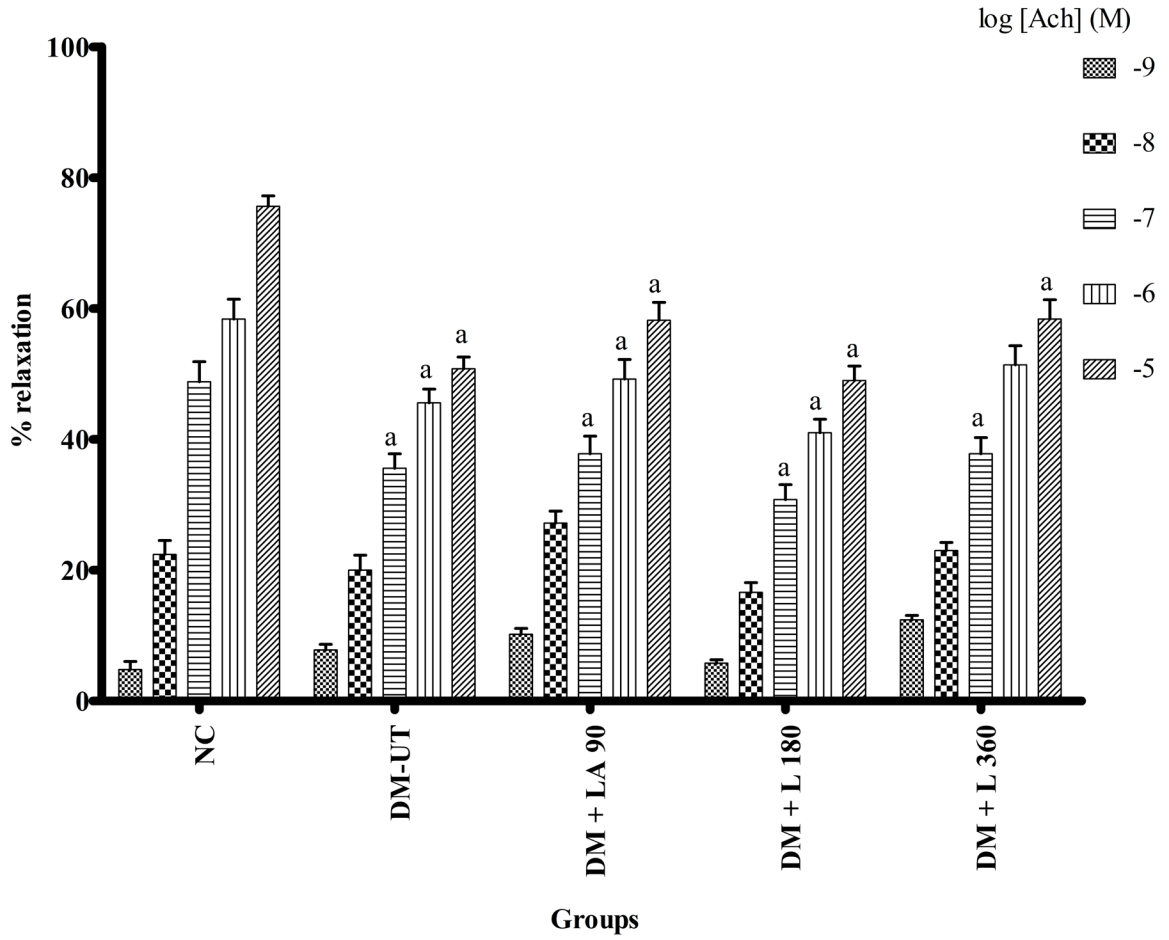

Figure 1: **Relaxation response of phenylephrine pre-contracted corpus cavernosum to cumulative concentrations of acetylcholine.**

NC: normal control, DM-UT: diabetic untreated, DM + LA 90 diabetic treated with lauric acid (90 mg/kg), DM + LA 180 diabetic treated with lauric acid (180 mg/kg), DM + LA 360 diabetic treated with lauric acid (360 mg/kg). a - Significant compared to NC ($p < 0.05$)

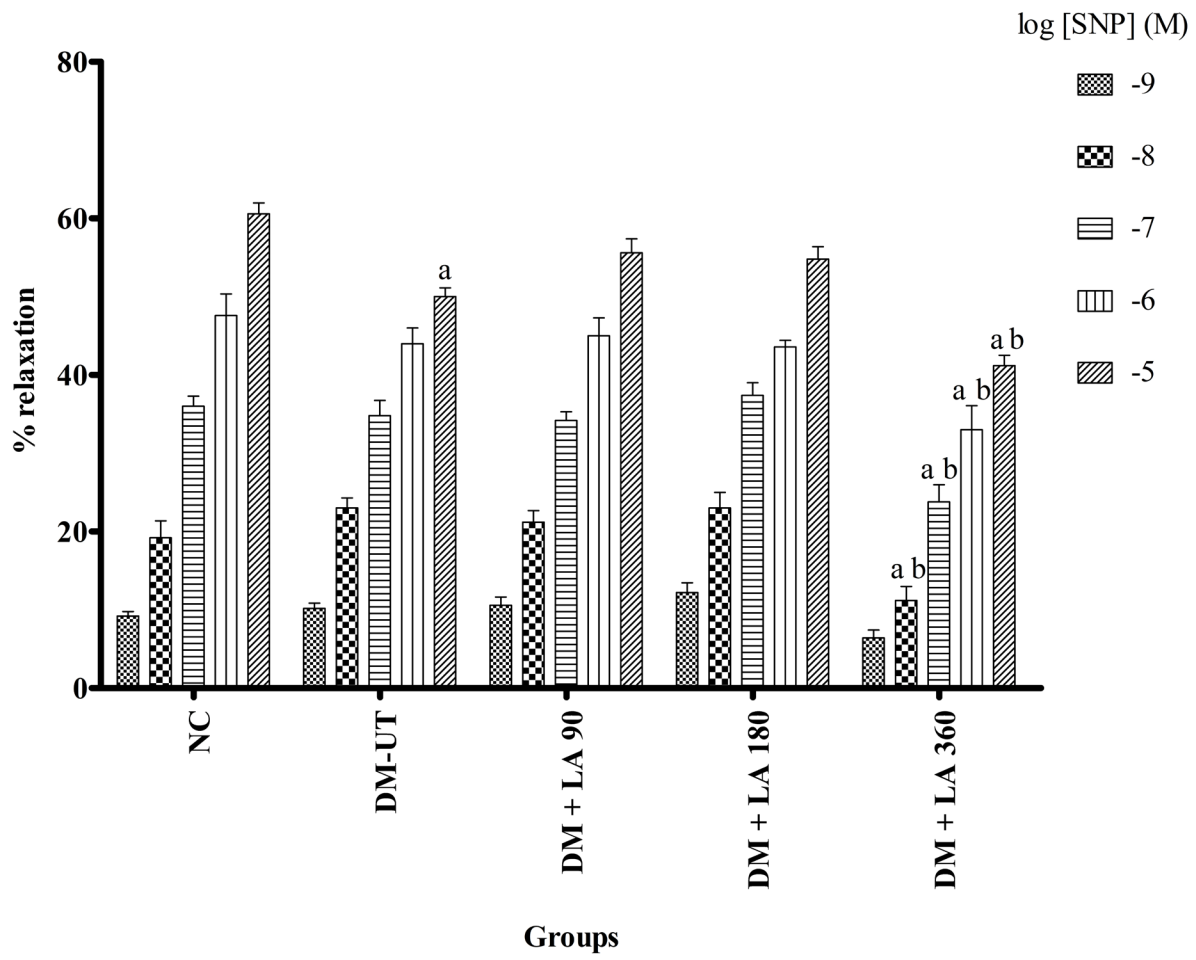

Figure 3: **Relaxation response of phenylephrine pre-contracted corpus cavernosum to cumulative concentrations of sodium nitroprusside**

NC: normal control, DM-UT: diabetic untreated, DM + LA 90 diabetic treated with lauric acid (90 mg/kg), DM + LA 180 diabetic treated with lauric acid (180 mg/kg), DM + LA 360 diabetic treated with lauric acid (360 mg/kg). a - Significant compared to NC ($p < 0.05$). b - Significant compared to DM-UT

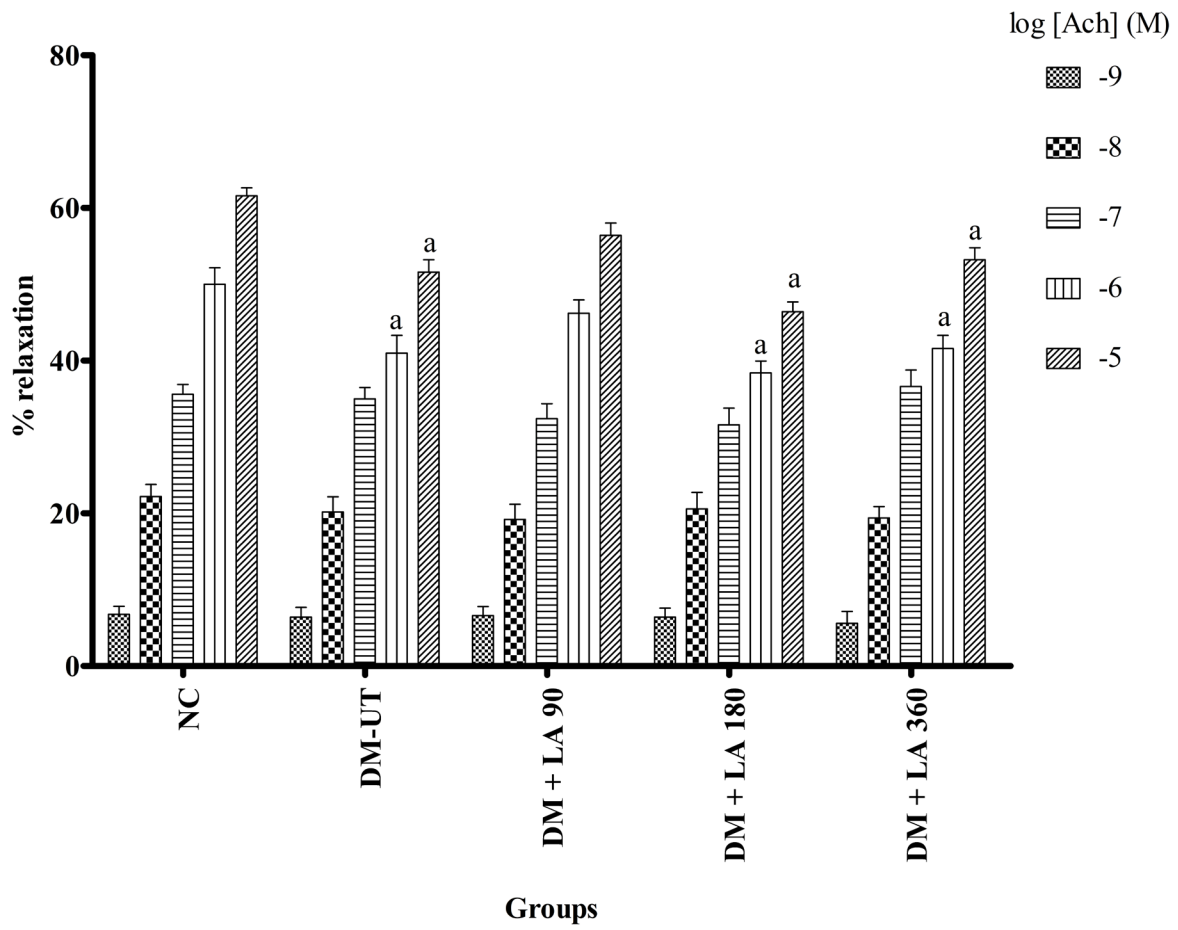

Figure 2: **Relaxation response of KCl pre-contracted corpus cavernosum to cumulative concentrations of acetylcholine.**

NC: normal control, DM-UT: diabetic untreated, DM + LA 90 diabetic treated with lauric acid (90 mg/kg), DM + LA 180 diabetic treated with lauric acid (180 mg/kg), DM + LA 360 diabetic treated with lauric acid (360 mg/kg). a - Significant compared to NC ($p < 0.05$)

1. Hearon CM, Richards JC, Racine ML, Luckasen GJ, Larson DG, Dinenno FA, Schultz H, Santana F (2019) Amplification of endothelium-dependent vasodilatation in contracting human skeletal muscle: role of K IR channels. J Physiol 597:1321–1335. https://doi.org/10.1113/JP276998 2. Dayrit FM (2015) The properties of lauric acid and their significance in coconut oil. J Am Oil Chem Soc 92:1–15. https://doi.org/10.1007/s11746-014-2562-7 3. Iranloye B, Oludare G, Olubiyi M (2013) Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. J Diabetes 3:221–226. https://doi.org/10.4236/jdm.2013.34034 4. Alves NFB, de Queiroz TM, de Almeida TR, Magnani M, de Andrade BV (2017) Acute treatment with lauric acid reduces blood pressure and oxidative stress in spontaneously hypertensive rats. Basic Clin Pharmacol Toxicol 120:348–353. https://doi.org/10.1111/bcpt.12700 5. Salahdeen HM, Idowu GO, Yemitan OK, Murtala BA, Alada ARA (2015) The relaxant actions of ethanolic extract of Tridax procumbens (Linn.) on rat corpus cavernosum smooth muscle contraction. J Basic Clin Physiol Pharmacol 26:211–216. https://doi.org/10.1515/jbcpp-2013-0032