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SA01

The role of vasodilatory nanodomains in health and dementia

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Dementia is now the leading cause of death in the UK. Most cases are caused by Alzheimer's disease (AD), vascular dementia (VaD), or a combination of both. Both AD and VaD are associated with reduced cerebral blood flow, which is thought to contribute to disease progression. Therefore, understanding the mechanisms that regulate cerebral blood flow has become an exciting therapeutic opportunity.

The volume of blood the brain receives is in part determined by the arterial diameter of the small arteries and arteriole that run along the surface of the brain. Intraluminal pressure drives vascular smooth muscle depolarisations which drives voltage gated Ca^{2+} channel activity, and therefore vasoconstriction. To prevent over-constriction, this depolarisation is buffered by the activation of large conductance Ca^{2+} activated K^+ (BK) channels ensuring correct cerebral perfusion.

We have found in mouse model of Alzheimer's disease (APP23) that there is a reduction in BK channel activation, which is also present in a mouse model of hypertension induced vascular dementia (BPH/2). Although both models showed impaired BK channel activation, this is via different mechanisms. BK channels are activated by high amplitude spatially and temporally restricted events know as Ca^{2+} sparks. In AD, there is a reduction in Ca^{2+} spark frequency which impairs BK channel function, whereas in VaD this is a physical separation of Ca^{2+} sparks and BK channels.

Because these distinct mechanisms require different strategies to restore normal function and cerebral blood flow, this talk will outline the current approaches we are pursuing.

SA02

Ion Channel Signaling in Brain Capillary Endothelial Cells and Pericytes

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The brain capillary network represents an active signaling interface that dynamically regulates cerebral blood flow (CBF). Capillary endothelial cells (cECs) and pericytes form the functional core of this network, integrating neuronal and metabolic cues through specialized ion channel signaling pathways that govern membrane potential (VM) and electrical communication. However, the molecular mechanisms underlying ion channel-mediated signaling within the capillary network remain incompletely understood. Using complementary electrophysiological, imaging, and in vivo approaches — including transgenic mouse models with cell-specific manipulation of ion channel function — we characterized the ion channel repertoire of brain cECs and pericytes. Our studies demonstrate that KATP channels are functionally expressed in both cECs and pericytes, where they mediate adenosine-induced hyperpolarization and increases in CBF through an A2A receptor-Gαs/cAMP/PKA signaling pathway, identifying capillary KATP channels as key regulators of CBF. We further show that pericytes express a diverse repertoire of K^+ channels, including Kir2, KV1 and BKCa channels, providing complementary mechanisms for setting pericyte VM, modulating capillary hemodynamics, and regulating CBF. Finally, we identified functional hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels in brain endothelial cells, revealing a previously unrecognized cAMP-sensitive conductance. Rather than acting as signal-generating pacemaker channels, as in cardiomyocytes and neurons, endothelial HCN channels may function as terminators of propagating hyperpolarizing signals— a conceptual departure from their canonical role in excitable tissues.

SA03

Endothelial PIEZO mechanosensors in health and disease

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PIEZO proteins are multi-pass membrane proteins forming calcium permeable nonselective cation channels. They are remarkable for their mechanical force sensing properties and structural attributes that optimise them for force sensing. We found the PIEZO1 channel is pivotal for vascular endothelium to feel fluid shear stress and enables flow dependent vascular maturation in embryonic development. At the adult stage, we found endothelial PIEZO1 regulates blood pressure and skeletal muscle microvascular density for physical performance, muscle growth for strength and hepatobiliary gene expression for whole body lipid homeostasis. We found endothelial PIEZO1 delivers downstream effects through calpain protease activation, altered phosphorylation and abundance of NOS3 nitric oxide synthase, ADAM10-dependent NOTCH1 cleavage and intercellular communication to pericytes. As integrated ion channels with unusual blade structures deep in the membrane, PIEZOs are increasingly recognised to depend on lipid interactions. We found, for example, the ceramide lipid disables intrinsic inactivation in endothelial PIEZO1 channels to promote their activity. To generate new ideas about how the channels work as lipid-integrated machines in endothelial membrane, we established computational molecular dynamics simulations of PIEZO1 channels in membranes of endothelial composition and applied forces to observe responses, testing the models by electrophysiology and intracellular calcium measurement in cells. We found anionic lipids such as phosphatidylinositol phosphates have critical roles, controlling PIEZO1 force sensitivity via inter-blade handshaking. Lipid dysregulation is common in disease and obesity, so we are working to understand and predict how lipids interact with PIEZO force sensing and influence health.

SA04

Mechanosensitive ion channels as mediators of vascular function

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Endothelial cells form the innermost layer of blood vessels and are continuously exposed to haemodynamic forces generated by blood flow. This unique position enables the vascular endothelium to sense and respond to mechanical stimuli, thereby regulating essential vascular functions. A key adaptive mechanism is the dynamic modulation of endothelial surface mechanics. Changes in endothelial stiffness directly affect the bioavailability of vasodilators such as nitric oxide (NO), with softer endothelial cells releasing more NO than stiffer cells. Thus, endothelial stiffness serves as an important indicator of cellular function and vascular health.

Our work has demonstrated that chronic stiffening of the endothelial surface contributes to endothelial dysfunction and vascular inflammation, two hallmarks of many cardiovascular diseases. We focus on the mechanical properties of the endothelial surface and their interaction with mechanosensitive ion channels. In particular, we investigate two mechanically distinct compartments: the endothelial glycocalyx (eGC), which forms the outermost surface layer, and the underlying actin-rich endothelial cortex located approximately 50–150 nm beneath the plasma membrane. Using atomic force microscopy (AFM)-based nanoindentation, we quantify the mechanical properties of these structures and assess their alterations under inflammatory conditions.

Beyond serving as structural elements, the endothelial glycocalyx and cortex are integral components of cellular mechanotransduction. Their mechanical properties are tightly linked to cytoskeletal dynamics and influence the activity of mechanosensitive proteins and ion channels. Since mechanosensitive ion channels are expressed in both endothelial and vascular smooth muscle cells, coordinated signalling between these cell layers is essential for the precise regulation of vascular tone and function. During the last years, we have focused on the endothelial ENaC and Piezo 1. We could show that the activity of these mechanosensitive ion channels determines endothelial mechanics and thus vascular function.

Our work aims to elucidate how endothelial mechanics and mechanosensitive ion channels interact to regulate vascular homeostasis and how their dysfunction contributes to cardiovascular disease. Particular emphasis is placed on the emerging concept that the endothelial surface represents a highly specialized mechanosensitive compartment and a potential therapeutic target in vascular pathologies. Understanding these mechanisms may open new avenues in the rapidly evolving fields of channelopathies and mechanomedicine.

SA05

Role of ANO1 in propagating spontaneous activity of capillary pericytes

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In microvascular of visceral organs, such as the bladder and gut, α-smooth muscle actin negative, non-contractile pericytes in capillaries periodically generate spontaneous Ca^{2+} transients. The spontaneous Ca^{2+} transients are not only synchronously developed amongst a network of capillary pericytes but also capable of propagating upstream to contractile pericytes in precapillary arterioles and thereby regulate capillary perfusion. The capillary pericytes express ANO1 Ca^{2+}-activated chloride channels and generate ANO1-dependent spontaneous transient inward currents and corresponding spontaneous transient depolarisations (STDs). Both spontaneous Ca^{2+} transients and STDs primarily arise from a cycle of spontaneous Ca^{2+} release and uptake through sarcoplasmic reticulum (SR) Ca^{2+} stores that is maintained by Ca^{2+} influx via Orai channels, while neither L- nor T-type voltage-dependent Ca^{2+} channels play a fundamental role. Thus, STDs arising from spontaneous Ca^{2+} transients serve as a means of intercellular communication where STDs appear to gain a regenerative nature depending on depolarisation-induced SR Ca^{2+} release, presumably via InsP3 production.

SA06

Mitochondrial acute oxygen sensing and signaling to membrane calcium channels in vascular smooth muscle cells.

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Blood oxygen tension (PO2) is an important local factor in the regulation of circulation. Arteries and arterioles are able to sense changes in PO2 in the blood or surrounding tissues and acutely respond with a vasomotor response (constriction or dilation) that depends on the vascular territory. Resistance pulmonary arteries constrict in response to a decrease in alveolar PO2 (alveolar hypoxia), thereby diverting blood flow from poorly ventilated lung regions towards areas with a higher PO2, optimizing the ventilation/perfusion ratio. Under conditions of persistent hypoxemia (low blood PO2), hypoxic pulmonary vasoconstriction (HPV) contributes to the pathogenesis of pulmonary hypertension. Unlike resistance pulmonary arteries, conduit pulmonary vessels and most systemic arteries dilate in response to hypoxia. Hypoxic vasodilation (HVD) also has a relevant physiologic role as it favors the perfusion of O2-deprived tissues. Previous findings have suggested that voltage-gated L-type Ca^{2+} channels in vascular smooth muscle are O2-sensitive and may therefore critically contribute to acute HVD and HPV. However, how variations of O2 tension are detected and the mechanisms whereby these changes are conveyed to membrane ion channels have remained elusive. We have observed: i) an antagonistic modulation of Ca^{2+} channel activity in systemic (inhibition) vs pulmonary resistance (potentiation) myocytes, under acute hypoxia, accompanied by corresponding changes in intracellular calcium concentration; ii) a direct modulation of L-type Ca^{2+} channel currents by NADH and H2O2 in these cells; and iii), that the effect of hypoxia on Ca^{2+} currents and cytosolic Ca^{2+} levels is dependent on mitochondrial complex I (MCI) activity, as it is absent in myocytes from genetically modified MCI-SM mice (lacking the NDUFS2 subunit, a component of MCI catalytic core). These findings suggest that MCI plays a key role in acute O2 sensing by myocytes from systemic and pulmonary resistance arteries, likely by mediating the production of signaling molecules (NADH and H2O2) that regulate membrane Ca^{2+} channels. Furthermore, mice exposed to chronic hypoxia (10% O2 tension for 3-4 weeks) exhibit increases in medial wall thickness of pulmonary resistance arteries as well as the right ventricular area. The changes induced by chronic hypoxia were abolished or markedly reduced in MCI-SM mice. Overall, these results strongly suggest that mitochondria may not only play an essential role in acute vascular O2 sensing and signaling, but also in the (mal)adaptive responses of pulmonary vessels to chronic hypoxia.

SA07

Bicarbonate Transport and Sensing in the Regulation of Tissue Perfusion and Ischemia Susceptibility

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Local acid–base disturbances arise physiologically during increased metabolic activity, such as intense exercise or heightened neuronal firing. In pathological conditions, including ischemia and solid tumours, acidic metabolites accumulate when blood flow fails to meet metabolic demand. We have uncovered novel mechanisms through which acid–base changes regulate cardiovascular function and influence susceptibility to ischemic injury.

Extracellular acid–base disturbances contribute to the metabolic regulation of cardiovascular function, but the underlying sensors and signalling pathways have remained elusive. Using out-of-equilibrium CO_2/HCO_3^- solutions, generated by rapidly mixing precursors with different CO_2, HCO_3^-, and pH compositions, we can independently control extracellular pH, HCO_3^-, and CO_2. Selective reductions in extracellular HCO_3^- at constant pH 7.4 and 5% CO_2 enhance cerebral artery contractions through a mechanism requiring receptor protein tyrosine phosphatase (RPTP)γ. Our findings reveal that RPTPγ, a transmembrane protein with homology to carbonic anhydrases, promotes vasodilation by augmenting endothelial Ca^{2+} signalling, endothelium-dependent hyperpolarization, and nitric oxide synthesis.

Cellular HCO_3^- uptake also contributes to intracellular pH regulation. In the vascular wall and cardiac atria, HCO_3^- uptake is mediated by the electroneutral Na^+,HCO_3^--cotransporter NBCn1 (SLC4A7). NBCn1 supports endothelial function by promoting nitric oxide synthesis. Loss of NBCn1 causes arterial hypertension and secondary cardiac hypertrophy, underscoring the importance of HCO_3^- transport in cardiovascular homeostasis.

Genetic analyses of UK Biobank data identify PTPRG, the gene encoding RPTPγ, as a risk locus for ischemic disease in the heart and brain. Consistent with this association, RPTPγ-deficient mice show increased vulnerability to hypoxia, greater susceptibility to stroke, and exacerbated cardiac ischemic injury following reduced organ perfusion. These mice also exhibit diminished exercise capacity during both forced and voluntary running. RNA sequencing reveals attenuated exercise-induced transcriptional adaptation and a markedly enhanced inflammatory response to ischemia.

In this presentation, I will discuss the multifaceted roles of HCO_3^- in cardiovascular physiology: as a substrate for membrane transporters that regulate intracellular pH, as a signal that modulates smooth-muscle and endothelial function, and as a determinant of vascular responses to acid–base disturbances arising from increased metabolic demand.

SA08

Developing strategies to protect the coronary microcirculation – focus on DAPTs and IL-36

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Despite timely reperfusion of an occluded coronary artery by primary percutaneous coronary intervention (PCI), many patients with myocardial infarction (MI) continue to develop myocardial injury and progress to heart failure. This damage is increasingly attributed to impaired perfusion at the level of the coronary microcirculation. Using real-time intravital imaging of the mouse beating heart in vivo, we have previously described multiple microcirculatory disturbances following myocardial ischaemia-reperfusion (IR) injury, with platelet-rich microthrombi emerging as key contributors to reduced functional capillary density [1]. This presentation will outline the intravital and laser speckle contrast imaging (LSCI) methodologies used to enable high-resolution imaging of the anaesthetised mouse beating heart (anaesthesia: ketamine hydrochloride and medetomidine hydrochloride) and describe the spectrum of thromboinflammatory and perfusion related microvascular perturbations observed during myocardial IR injury [2]. All procedures described received local approval from the Animal Welfare and Ethical Review Body (AWERB) and were conducted in accordance with the Animals (Scientific Procedures) Act of 1986 (HO Project licence P552D4447). The presentation will then address whether contemporary dual antiplatelet therapy (DAPT), widely prescribed post-MI to prevent recurrent ischaemic events and prevent stent thrombosis, confers benefit at the level of the coronary microcirculation. Comparative data following pre-treatment with aspirin in combination with the P2Y12 receptor inhibitors clopidogrel, ticagrelor, or prasugrel will be presented [3]. The findings indicate that, despite partial reductions in capillary microthrombus formation (with variable efficacy between agents), DAPT does not restore ventricular perfusion to pre-injury levels, as assessed by novel application of LSCI to the beating heart. Notably, all DAPT regimens are associated with increased coronary neutrophil recruitment, above that noted with IR injury alone, a response that may exacerbate tissue injury in sterile inflammatory settings and limit salvage of viable myocardium. To address this, data will be presented exploring inhibition of the pro-inflammatory cytokine interleukin-36 (IL-36), a new member of the IL-1 cytokine family, as a strategy to limit neutrophil infiltration and mitigate microvascular damage [4]. Finally, given the clinical relevance of comorbidities, the presentation will highlight marked differences in basal and post-IR microvascular dysfunction between normoglycaemic and chronically hyperglycaemic mice, and evaluate how DAPT and IL-36R antagonism as monotherapies differentially impact these groups [3,5].

SA09

The role of transient receptor potential vanilloid 4 channels in the development of dementia

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Failure of cerebral arteries to regulate oxygen and nutrient delivery to the brain parenchyma can be catastrophic. In patients with hypertension, this cerebrovascular insufficiency is largely caused by cerebral small vessel disease (CSVD). Importantly, CSVD has been causally linked to the development and progression of all dementias and is the most common pathology underlying the development of vascular cognitive impairment and dementia. Strong evidence links the development of midlife hypertension to cognitive impairment with aging. We utilized pressure myography to assess the effects of hypertension on the function of cerebral parenchymal arterioles from stroke-prone spontaneously hypertensive rats (SHRSP), a model of essential hypertension and CSVD. Through this, we showed that TRPV4-mediated vasodilation is impaired in cerebral arterioles during hypertension. Follow-up studies using TRPV4 knockout rats and endothelial cell-specific TRPV4 knockout mice directly connected the loss of TRPV4 function to cognitive decline. These studies suggested that improving TRPV4 function could be an effective treatment for VCI/VaD. The extreme hemodynamic effects of global TRPV4 activation preclude the use of small-molecule activators as dementia therapies. Therefore, we sought to harness the effects of endogenous TRPV4 agonists, such as epoxyeicosatrienoic acids (EETs), by inhibiting their metabolism by soluble epoxide hydrolase (sEH). We tested the hypothesis that pharmacological inhibition of sEH with trifluoromethoxyphenyl-3- (1-propionylpiperidin-4-yl)urea (TPPU) in adult (SHRSP) would restore endothelial function in hippocampal parenchymal arterioles (HPAs) and improve cognition. 20-week-old SHRSP were treated with vehicle (n=4-8) or TPPU (3mg/kg/day, n=4-8) for 8 weeks. HPA function was assessed using pressure myography. Data are presented as means ± SEM. In male SHRSP, TPPU improved endothelium-dependent dilation mediated by the TRPV4 agonist GSK1016790A (Vehicle: 22.2±6.1; TPPU: 70.2±6.5; p=.0019) and the nonselective activator of small- and intermediate-Ca^{2+}-activated K^+ channels (SKCa and IKCa) NS309 (Vehicle: 30.5±6.8; TPPU: 55.9±5.2; p=.028). In female SHRSP, TPPU improved NS309-mediated dilation (Vehicle: 34.5±4.2; TPPU: 59.0±7.5; p=.045) but not dilation induced by TRPV4 activation (Vehicle: 59.6±3.4; TPPU: 64.2±5.1; p=.99). IKCa- and SKCa- specific antagonists, TRAM-34 and apamin, respectively, were used to determine the individual ion channel contributions to vasodilation. IKCa channels are the major contributor to NS309-mediated dilation in HPAs. TPPU treatment increased IKCa-mediated vasodilation in male (Vehicle: 4.7%±19.0k TPPU: 63.8%±5.7: p < .01) and female SHRSP (Vehicle: 31.2%±10.2; TPPU: 76.7%±1.2: p=.04). sEH inhibition did not affect dilation induced by SKCa activation in either sex (data not shown). TPPU enhanced cerebral perfusion assessed by laser speckle contrast imaging in males (Vehicle: 209.8±3.4; TPPU: 227.4±6.5; p=.035), but not females (Vehicle: 300.1±13.8; TPPU: 302.5±10.8; p=.89). Novel object testing was used to assess memory function. TPPU improved nonspatial memory in female (Vehicle: 42.3±5.8; TPPU: 64.4±6.3 % novel object exploration; p=.02) and male (Vehicle: 43.3±7.5%; TPPU: 65.1±5.4% % novel object exploration; p=.03) SHRSP. These data suggest that sEH inhibition could be a promising treatment for hypertension-associated cognitive impairment, however the mechanism of this impairment differs between the sexes.

SA10

Smooth operators - microcirculatory control at the molecular, micro & macro scales, & all at high throughput: where will the marriage of automated patch clamp & organ-on-a-chip take translatable science?

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Microcirculatory control is fundamentally governed by ion channel activity, yet its physiological consequences emerge across multiple biological scales. Modulation at the single-channel level can alter cellular excitability and calcium handling, with downstream effects on vascular tone and perfusion. Translating these effects into meaningful, tissue-relevant insight remains a key challenge.

Here, we focus on the use of automated patch clamp (APC) as a scalable platform for high-throughput ion channel profiling. APC enables both single-channel and whole-cell recordings with improved efficiency and reproducibility, supporting the rapid interrogation of compound libraries whilst retaining mechanistic resolution. This is particularly relevant in microcirculatory systems, where multiple ion channels contribute to the regulation of vascular smooth muscle and endothelial function.

However, ion channel data alone are not sufficient to predict functional outcomes. The current direction of our work is therefore to corroborate APC-derived findings with more physiologically relevant models. Initial efforts are centred on cardiac muscle systems, where contractile responses provide a direct functional readout of ion channel modulation. These studies aim to establish clearer links between electrophysiological effects and changes in tissue behaviour.

We aim to expand this from cardiac ion channel to cardiomyocyte contractility model for improved translation in cardiac safety to smooth muscle for microvascular studies. Thus, organ-on-a-chip approaches are being developed to extend this framework towards more complex smooth muscle microvascular models. While still in development, these systems offer the potential to capture additional layers of microcirculatory regulation that are not accessible through electrophysiology alone. Future work will explore their application across a broader range of smooth muscle and microcirculatory processes.

Taken together, this evolving workflow reflects a pragmatic progression: from high-throughput ion channel screening towards increasingly integrated functional models. By anchoring discovery in robust electrophysiological data and incrementally incorporating tissue-level validation, this approach aims to improve the translational relevance of ion channel research in microcirculatory control.

C01

Regulation of arterial response by sodium-myo-inositol transporter1 / Kv7 channel complexes.

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Introduction: As the sodium/ myoinositol transporter (SMIT1) is a positive regulator of Kv7.4/7.5 channels in arterial smooth muscle we postulated that altering SMIT1 expression could have a major impact upon vascular reactivity. Consequently, this study aimed to characterise the effects of changes of SMIT1 membrane abundance on vascular tone and the molecular mechanisms involved.

Methods: Isometric tension recordings on 2nd order mesenteric arteries and left anterior coronary arteries from male and female Wistar Han rats were performed with morpholino based protein knockdown of SMIT1. All studies involved > 5 biological repeats. Whole artery membrane potential recording alongside electrophysiology patch-clamp recordings of Kv7.4 channels. Proximity ligation assay and whole artery antibody-based imaging.

Results: Morpholino-mediated knockdown of SMIT1 enhanced U46619- and methoxamine-mediated contractions, while attenuating relaxations to the Kv7 activator ML213, isoprenaline and CGRP in mesenteric arteries. Incubating HEK cells expressing Kv7.4 with raffinose for 1 h increased current amplitude and left shifted voltage-dependence by ~8mV when cells were co-expressed with SMIT-1 only. Similar treatments of arteries increased SMIT1 membrane abundance in smooth muscle cells, impaired receptor-mediated contractions of mesenteric and coronary arteries, augmented relaxations to ML213 and adenosine and induced membrane potential hyperpolarisation. The SGK1 inhibitor EMD638683, involved in regulating SMIT1 membrane expression, prevented the acute raffinose-induced increase in SMIT1 and the associated anti-contractile effects. Proximity ligation assays revealed that raffinose incubation increased the association of SMIT-Kv7.4/7.5 as well as Kv7.4 and Gβγ subunits.

Conclusions: Altering the membrane abundance of SMIT1 had a dramatic effect on the arterial response to vasoconstrictors and vasodilators mediated by greater coordination of Kv7 channels and Gβγ subunits. This work identified SMT1-Kv7 channel complexes and SGK1 regulation as key modulators of arterial responsiveness.

C02

The vascular smooth muscle Ca^{2+} spark - BKCa channel axis is altered in maternal microvascular arteries in women with preeclampsia.

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Background

Pre-eclampsia (PE) is a hypertensive disorder of pregnancy, and a significant clinical problem worldwide. Despite decades of research, this disorder remains without an effective intervention, and the mechanism(s) underlying maternal microvascular dysfunction have not been fully defined.

Within vascular smooth muscle cells (VSMCs), the principal pressure-induced vasodilatory pathway relies upon activation of large-conductance Ca^{2+}-activated potassium channels (BKCa). Activation is dependent on the release of localised Ca^{2+}-release events from sarcoplasmic reticulum (SR) ryanodine receptors, termed 'Ca^{2+} sparks', which stimulate BKCa channels to cause spontaneous transient outward currents (STOCs) and vasorelaxation. Investigation of BKCa channels has been proven effective in advancing treatment of hypertension outside of pregnancy; here, the Ca^{2+} spark – BKCa axis is assessed in preeclampsia for the first time.

Methods

Omental biopsies were obtained with informed consent during caesarean section from normotensive women, and women with a clinical diagnosis of preeclampsia, at St Mary's Hospital, Manchester in accordance with ethics committee approval (National Research Committee North-West – Haydock Board 15/NW/0829).

Pressurised omental resistance arteries were imaged by high-speed spinning-disk laser confocal microscopy to assess Ca^{2+} spark frequency at increasing pressures (20-110 mmHg). Caffeine-evoked Ca^{2+} release was measured in a subset of arteries (10 mmol/L; 80 mmHg), and area under curve (AUC) and transient amplitude calculated. A liquid chromatography–selected reaction monitoring–mass spectrometry (LC-SRM-MS) assay was developed to measure Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} ATPase-2 (SERCA2) and unphosphorylated/phosphorylated proteoforms of regulatory phospholamban. Electrophysiology was used to assess spontaneous transient outward currents (STOCs; -60 to 0 mV) in isolated VSMCs.

Results

In microvascular arteries from women with preeclampsia there was reduced Ca^{2+} spark frequency compared to normal pregnancy (0.53 events/second and 1.90 events/second respectively at 80 mmHg, $p=7.3x10^{-4}$; N=13-15), which persisted across the range of pressures measured ($p=1.1x10^{-10}$, N=6-15). There was also reduced SR Ca^{2+} release in response to caffeine (AUC 3.42 vs 7.70, $p=5.1x10^{-4}$; N=12-13) and decreased ratio of SERCA2 to inhibitory unphosphorylated phospholamban (0.94 vs 2.11,

p=0.030; N=5-7), implicating increased SERCA2 inhibition. In VSMCs, STOC frequency was reduced in the preeclampsia cohort (0.11 vs 0.49 events/ second at 10 mV, $p=7.1x10^{-15}$; N=5-6).

Conclusions

This data has identified fundamental differences in Ca^{2+} - mediated mechanisms of vascular control in preeclampsia. In microvascular arteries from women with preeclampsia, there is a reduction in pressure-induced Ca^{2+} spark frequency and subsequent BKCa STOCs. Additionally, there is a reduction in total SR Ca^{2+} store and the ratio of SERCA2 to its inhibitor, unphosphorylated phospholamban, implicating SR Ca^{2+} loading as the cause of reduced Ca^{2+} sparks. These identified VSMC defects may provide novel therapeutic targets to restore physiological control of the vasculature in preeclampsia.

C03

Kv1.3 potassium channel in uremia-induced vascular dysfunction: role in early remodeling and stiffness

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Introduction

Chronic kidney disease (CKD) is associated with a high cardiovascular risk and the development of vascular calcification (VC), an active process characterized by the phenotypic transition of vascular smooth muscle cells (VSMCs) toward an osteogenic phenotype, together with alterations in the extracellular matrix (ECM).[1,2] The potassium channel Kv1.3 has been implicated in VSMC proliferation, migration, and phenotypic modulation (PM).[3] We previously demonstrated that uremic serum increases its expression and that its inhibition partially attenuates calcification in vitro.[4] In this study, we investigated its role in early uremia-induced vascular remodeling using an ex vivo model.

Materials and methods

Aortic rings from C57BL/6 wild-type (WT) and Kv1.3−/− mice were used and cultured ex vivo and incubated with either control serum (CS) or 10% human uremic serum (US), in the presence or absence of the selective Kv1.3 inhibitors PAP-1 and margatoxin. Human samples were obtained with informed consent and institutional ethical approval, and all animal procedures were performed in accordance with European Directive 2010/63/EU and the regulations of the University of Valladolid.

Results

US induced a loss of the contractile VSMC phenotype, with decreased calponin (CNN1) and increased expression of osteogenic markers, including osteopontin (OPN) ($n=6$ mice; $p<0.01$) and alkaline phosphatase (ALPL) ($n=6$ mice; $p<0.001$). The increase in ALPL was attenuated by PAP-1 ($p<0.001$), suggesting the involvement of Kv1.3 in early osteogenic activation.

Functionally, WT aortic rings showed a significant increase in stiffness after exposure to uremic serum ($n=7$; $p<0.01$), an effect that was reduced by Kv1.3 inhibition. In contrast, Kv1.3−/− vessels did not show significant changes in elasticity between conditions.

ECM analysis revealed increased disorganization of elastin fibers in vessels treated with uremic serum, an effect reversed by pharmacological Kv1.3 inhibition and absent in Kv1.3−/− vessels. Ongoing studies using atomic force microscopy are further characterizing the local biomechanical alterations associated with these structural changes.

Conclusion

Our findings support the concept that Kv1.3 acts as a key regulator of early uremia-induced vascular remodeling. Its inhibition or absence attenuates VSMC osteogenic transition, preserves ECM

organization, and prevents increased arterial stiffness. These results support the role of Kv1.3 in early stages of CKD-associated vascular calcification and its potential as a therapeutic target.

C04

Disrupted ion channel gene expression in heart failure endothelial cells revealed by meta-analysis of single-nucleus RNA-sequencing data

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Introduction
Endothelial cell ion channels play critical roles in vascular mechanosensing, volume regulation, and the control of membrane potential and vascular tone. These functions are disrupted in heart failure, contributing to impaired cardiac perfusion and disease progression. Single-cell transcriptomics offers an unparalleled opportunity to characterise these changes at cell-type resolution, but individual studies are underpowered to identify consistent transcriptional signatures. Here we apply KOSMIC - an open-source platform we developed for standardised processing and patient-level meta-analysis of single-cell transcriptomic data - to identify robust ion channel transcriptional changes in cardiac endothelial cells across independent heart failure cohorts..

Methods
We systematically searched the Gene Expression Omnibus to identify single-cell transcriptomic datasets from failing and non-failing human hearts. Five single-nucleus RNA-sequencing studies met inclusion criteria, providing 241,043 endothelial cells from 156 donors (85 failing, 71 non-failing) after standardised quality control, clustering, and cell-type annotation using KOSMIC. Patient-level differential expression analysis of ion channel expression was performed on endothelial cells. To ensure reliable quantification, meta-analysis was restricted to ion channel genes detected in ≥5% of cells in all five cohorts, reducing a curated reference set of 303 ion channel genes to 46 high-confidence targets, which were then pooled using Restricted Maximum Likelihood (REML) random-effects models.

Results
Meta-analysis identified a consistent and selective pattern of ion channel dysregulation in endothelial cells of the failing heart. Of 46 high-confidence ion channel genes taken forward for analysis, 16 were significantly differentially expressed after correction for multiple testing (FDR<0.05). Importantly, 9 of these (56%), including the volume-regulated anion channel subunit LRRC8B and the polycystin PKD2, would have been missed by the analysis of any single study and were only detectable by pooling evidence across cohorts. At the family level, 8 of 35 ion channel families were significantly dysregulated (FDR<0.05). Chloride intracellular channels were the most robustly downregulated family (logFC −0.36, FDR=2×10^{-17}; I^2=0%), driven by CLIC5 (logFC −0.50, FDR=4×10^{-15}, 5/5 studies concordant) and CLIC1/3/4. Aquaporins were also markedly reduced (logFC −0.54, FDR=5×10^{-12}; I^2=0%), with AQP3 showing the most consistent and pronounced downregulation of any individual gene (logFC −2.45, FDR=5×10^{-28}). In contrast, mechanosensitive channels were consistently upregulated: PIEZO channels (logFC +0.20, FDR=5×10^{-3}, 5/5 studies directionally concordant) and calcium-activated chloride channels (Anoctamins, logFC +0.14, FDR=0.023), driven by ANO10 and ANO8.

Conclusions

Meta-analysis of single-nucleus RNA-sequencing data reveals a consistent and selective pattern of ion channel dysregulation in heart failure endothelium: mechanosensing machinery is upregulated while volume regulation, chloride transport, and KATP-mediated metabolic sensing are suppressed. These findings identify endothelial ion channel remodelling as a consistent feature of heart failure and highlight specific channel families as potential therapeutic targets in cardiovascular disease.

C05

Loss of TMEM16A function as a potential mechanism underlying moyamoya disease: biophysical analysis of a novel disease-associated variant

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Introduction

TMEM16A Ca^{2+}-gated Cl^- channels (CaCCs) provide a key depolarising conductance in in contractile vascular cells such as arterial smooth muscle cells and contractile pericytes (Al-Hosni et al., 2024). Single-point mutations in TMEM16A (ANO1) have recently been linked to moyamoya disease (MMD), a cerebrovascular disorder characterised by progressive carotid artery occlusion and an increased risk of ischaemic stroke (Pinard et al., 2023). Several MMD-associated TMEM16A variants have been identified, most exhibiting gain-of-function effects through enhanced Ca^{2+} sensitivity. In contrast, a variant with substitution of arginine (R) 890 to glutamine (Q) appears to cause channel loss of function. However, the mechanisms linking altered TMEM16A activity to MMD remain poorly understood.

Aims:

We aim to investigate the functional consequences of the MMD-associated hTMEM16A-R890Q mutation and assess its role in the pathological neovascularisation observed in the patient carrying this variant (Pinard et al., 2023). Here, we study the effects of this mutation on TMEM16A channel function.

Methods

Whole-cell and inside-out patch-clamp recordings of heterologously expressed wild-type and mutant TMEM16A currents were used to investigate how TMEM16A currents are affected by the R890Q mutation. Site-directed mutagenesis and stationary noise analysis were used to assess the impact of the mutation on channel gating, open probability (P_o), and single-channel current amplitude (i). Immunocytochemistry was employed to evaluate surface expression of HA-tagged hTMEM16A and hTMEM16A-R890Q channels.

Results

Heterologous hTMEM16A-R890Q currents were reduced compared to wild-type in a range of intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) (e.g. by 84±20% at 100 mV in the presence of 100 nM $Ca^{2+}I$, n=13 in each case, $p<0.001$). Inside-out patch-clamp recordings revealed a decrease in maximal current amplitude from 0.90±0.13 nA for hTMEM16A-WT channels to 0.32±0.06 nA for hTMEM16A-R890Q channels (n=6 in each case, $p<0.01$). This reduction in channel activity was accompanied by a significant rightward shift in the Ca^{2+} concentration–response relationship, with the concentration causing half maximal activation (EC_{50}) for intracellular Ca^{2+} increasing from 386 ± 12 nM in hTMEM16A-WT channels to 528 ± 26 nM in hTMEM16A-R890Q channels (n = 6 for each group; $p < 0.001$). Stationary noise analysis demonstrated that hTMEM16A-R890Q has reduced plasmalemmal expression by 72±26% (n=6, $p<0.01$), and altered channel gating. These findings were further supported by immunocytochemical analyses, which demonstrated reduced plasma membrane expression of hTMEM16A-R890Q channels. In addition, the R890Q mutation altered both anion selectivity and conductance across a range of permeant anions.

Conclusions

Collectively, our findings reveal a complex biophysical alteration of hTMEM16A-R890Q function, involving impaired plasma membrane trafficking and altered channel gating that together reduce macroscopic currents. This provides new insights into MMD pathogenesis and into how TMEM16A channel dysfunction may contribute to cerebrovascular disease.

C06

Oxidative phosphorylation is the primary source of endothelial ATP and undergoes progressive decline in hypertension and heart failure

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Introduction: Arteries and veins are lined by endothelial cells that play a critical role in regulating blood flow. Despite being exposed to oxygen-rich blood, endothelial cells are characterised as predominantly glycolytic, with estimates suggesting that 85% of their ATP derives from glycolysis rather than mitochondrial oxidative phosphorylation. This paradigm has shaped vascular research for decades, with mitochondrial contributions interpreted through non-energetic roles - such as calcium buffering and reactive oxygen species signalling - rather than ATP production. However, we previously demonstrated that mitochondrial ATP production is required for endothelial calcium signalling and vasodilation in intact mesenteric arteries (Wilson et al., Function, 2022). If mitochondria contribute only ~15% of endothelial ATP, why does mitochondrial dysfunction have such catastrophic consequences for endothelial function? We aimed to resolve this paradox by systematically evaluating the contribution of OXPHOS to endothelial ATP production and determining its relevance to cardiovascular disease.

Methods: We performed a systematic review and meta-analysis of studies using the Seahorse Real-Time ATP Rate Assay to simultaneously measure glycolytic and oxidative ATP production rates in endothelial cells. Quantitative meta-analysis used random-effects models with the DerSimonian-Laird method to pool estimates of the ratio of oxidative to glycolytic ATP production across 39 datasets from 31 studies spanning 17 endothelial cell models. Patient-level differential expression analysis of single-nucleus RNA-seq data (GSE183852; 37,895 cardiac endothelial cells from 13 failing and 25 non-failing hearts) was performed to determine whether endothelial metabolic pathways are disrupted in heart failure.

Results: Meta-analysis revealed that OXPHOS contributes approximately 59% of basal endothelial ATP versus 41% from glycolysis (pooled ln ratio = 0.36, 95% CI: 0.21–0.52, $P<0.0001$; ratio = 1.43, 95% CI: 1.23–1.68) - a four-fold increase over the prevailing ~15% estimate. This finding was robust across species, vascular beds, and endothelial cell models, confirmed by leave-one-out sensitivity analysis (OXPHOS contribution 57–60% across all iterations; $I^2 = 98.9\%$). In human heart failure, patient-level differential expression analysis revealed comprehensive energetic remodelling in cardiac endothelial cells: 8 of 12 core metabolic pathways were significantly downregulated, including all ATP-generating pathways — glycolysis (−41%), mitochondrial transport (−43%), TCA cycle (−28%), OXPHOS (−26%), and fatty acid oxidation (−26%). Strikingly, glucose transport was unchanged despite profound suppression of all downstream glucose-dependent ATP production pathways. Analysis of electron transport chain gene expression (92 genes; per-donor log-normalised mean) revealed a progressive monotonic decline across normotensive controls (0.367 ± 0.09, n=12), hypertensive controls (0.33 ± 0.08, n=13; −13.5% vs normotensive), and hypertensive heart failure patients (0.25 ± 0.08, n=5; −37.6% vs normotensive, p=0.02, Welch's t-test).

Conclusions: These findings overturn the prevailing model of endothelial glycolytic predominance and resolve a long-standing paradox in vascular biology. Oxidative phosphorylation, and not glycolysis, is the primary source of endothelial ATP and is functionally required for endothelial calcium signalling and vasodilation. In cardiovascular disease, coordinated collapse of all ATP-generating pathways - without glycolytic compensation - characterises endothelial energetic failure in heart failure. Strikingly, mitochondrial bioenergetic deterioration begins at the hypertensive stage, before overt heart failure,

identifying endothelial mitochondrial metabolism as an early and progressive therapeutic target in cardiovascular disease.

C07

TMEM16A Inhibition Impairs Mitochondrial Function in Vascular Endothelial Cells

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Introduction: Endothelial cell (EC) ion channels regulate the dynamic changes in blood vessel diameter through the release of various chemical mediators, for example, in the case of calcium-dependent release of nitric oxide (NO) to adjacent smooth muscle, as well as through hyperpolarisation of the plasma membrane. Mitochondria play a critical role in endothelium-dependent vascular function; loss of OXPHOS in ECs has been shown to inhibit calcium signalling [1]. Some calcium-permeable cation channels, such as the calcium-sensitive channel TRPV4, have had their role well characterized[2], yet the contributions of anion channels to control of blood vessel diameter is much less understood. The calcium-activated chloride channel, TMEM16A (otherwise known as anoctamin-1) has been suggested as a potential regulator of calcium signalling [3] as well as mitochondrial function, however, it's mechanisms still remain unclear. This study was designed with the aim of investigating the role of TMEM16A in regulating EC vasodilator signalling pathways.

Methods: All experiments included used isolated blood vessels from healthy male Sprague-Dawley rats euthanized by Schedule 1 Procedures (Animals {Scientific Procedures] Act 1986, UK). Calcium activity was studied using mesenteric artery preparations, flat-mounted and loaded with the calcium-sensitive fluorophore, Cal520-AM. Freshly isolated aortic endothelial cell sheets were utilized to study mitochondrial membrane potential, loaded with the mitochondrial membrane potential sensitive fluorophore, TMRE.

Results: Antagonism of the calcium-activated chloride channel, TMEM16A with the specific blocker Ani9, did not inhibit the calcium response induced by acetylcholine in the intact arteries (n=5). However, the 3 other TMEM16A blockers (MONNA, CaCCinh-A01 and Benzbromarone, n=5 each) caused a significant decrease in calcium activity in the flat-mounted tissue. Similar activity was seen in the isolated aortic ECs with MONNA, CaCCinh-A01 and Benzbromarone all causing a rapid loss of the mitochondrial membrane potential (n=5). In contrast, the mitochondrial membrane potential was unaffected by a ten-minute treatment with Ani9 (n=5).

Conclusion: In this study, we show that TMEM16A channels are essential for the propagation of the calcium signalling pathways that underpin the control of vascular tone. Treatment with 3 of the well-studied TMEM16A blockers caused inhibition of calcium signalling and lead to the depolarisation of the mitochondrial membrane potential. With regards to Ani9, both EC calcium signalling and the mitochondrial membrane potential remained unaffected. Loss of the mitochondrial membrane potential will cause the reduced availability of mitochondrial ATP, accounting for the loss of EC calcium signalling. Our findings indicate a role for TMEM16A in the endothelium's control of blood vessel diameter.

C08

Orai1-Mediated Ca^{2+} Signaling Promotes Post-Infarction Angiogenesis Through VEGF and Notch Pathways

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Orai1-Mediated Ca^{2+} Signaling Promotes Post-Infarction Angiogenesis Through VEGF and Notch Pathways

Introduction: Neovascularization is crucial for cardiac repair after myocardial infarction (MI), and Ca2+ signaling plays a critical role in endothelial cell (EC) activation.

Aims: Although Orai1-dependent store-operated calcium entry (SOCE) is known to promote angiogenesis, its specific contribution to post-MI neovascularization remains poorly understood.

Methods: To investigate pro-angiogenic mechanisms and mimic the systemic post-infarction environment, we stimulated human umbilical vein endothelial cells with serum from patients with ST-segment elevation myocardial infarction (STEMI). Integrative analyses were performed using transcriptomics, proteomics, and single-cell RNA sequencing (scRNA-seq).

Results: STEMI serum enhanced angiogenic responses in ECs by activating VEGF, Notch, and Ca^{2+} signaling pathways. Notably, ischemic serum increased Orai1 expression and exacerbated SOCE activity, which was required for EC migration and proliferation. In a mouse model for MI, scRNA-seq revealed augmented Orai1 expression, particularly in tip and proliferating EC clusters. Orai1 expression was further confirmed in in ECs located in peri-infarct regions of the mouse heart. Proteomics analysis demonstrated that Orai1 silencing dysregulated the expression of VEGF and Notch1-related pro-angiogenic proteins. Furthermore, interleukin-17A (IL-17A) mimicked the effects of patients serum by inducing Orai1-mediated SOCE and promoting ECs migration.

Conclusions: These findings identify a novel role for Orai1-dependent mechanism in post-MI angiogenesis, highlighting Orai1 as a promising therapeutic target for cardiac repair.

C09

Visualisation of vasodilatory nanodomain structure in vascular smooth muscle cells

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Vascular smooth muscle cells (VSMCs) regulate blood flow through dynamic control of vessel diameter. This is achieved through a balance of vasoconstrictive and vasodilatory signalling pathways, where the latter is focussed upon in this research. Central to vasodilation is the sarcoplasmic reticulum (SR)–plasma membrane (PM) nanodomain, a specialised junction in which Ca^{2+} released from clusters of type-2 ryanodine receptors (RyR2) activates large-conductance Ca^{2+}-activated K^+ (BK) channels at the PM, driving membrane hyperpolarisation and promoting vasodilation. Previous modelling has estimated that SR-PM distances must be <20 nm for local [Ca^{2+}] released from RyRs, known as a Ca^{2+} spark, to reach concentrations sufficient for BK channel activation, highlighting the critical importance of nanodomain geometry for functional coupling. We have recently demonstrated that BK channel dysfunction is associated with impaired cerebral blood flow in both Alzheimer's disease and hypertension (Taylor et al., 2022; 2023), yet the nanoscale structural organisation of the SR-PM nanodomain in VSMCs remains poorly characterised, with the field largely relying on assumptions drawn from cardiac and other non-vascular cell types.

To address this, we employed volume electron microscopy (vEM) with 3D reconstruction, and the super-resolution microscopy technique of DNA-points accumulation for imaging in nanoscale topography (DNA-PAINT), to characterise the SR-PM nanodomain ultrastructure in VSMCs, using both isolated cells and fixed cerebral arteries. vEM enabled direct visualisation of SR-PM proximity and precise junctional geometry within cerebral arteries from C57BL/6J mice, while DNA-PAINT utilised a GFP-tagged RyR mouse model (RyR2D4365-GFP), provided RyR2 cluster architecture and spatial organisation, all at nanometre resolution.

vEM analysis reveals that SR-PM junctions in VSMCs have a modal junctional distance of approximately 10 nm, well within the suggested distance required for efficient Ca^{2+} spark–BK channel coupling. The SR appears to adopt a linear arrangement running parallel to the cell, as well as isolated contact sites, suggesting two spatially orientated nanodomains that were previously unknown. DNA-PAINT further reveals that RyR2 organise into small discrete clusters of 1–8 RyRs in VSMCs, in stark contrast to the larger clusters and 'super-clusters' of up to and exceeding 100 RyRs identified within cardiac muscle, indicating a VSMC-specific organisation. These findings provide the first direct nanoscale characterisation of the SR-PM nanodomain in cerebral artery SMCs, establishing a structural framework for the vasodilatory signalling axis and offering new insight into how disruption of nanodomain architecture may contribute to cerebrovascular dysfunction in disease.

C10

Kv1.3–Extracellular Matrix Interactions as an Early Switch for Vascular Smooth Muscle Cell Phenotypic Modulation

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Introduction

Vascular smooth muscle cell (VSMC) phenotypic modulation (PM) is a central process in vascular remodeling, involving dedifferentiation from a contractile to a synthetic/migratory state. While ion channels have been increasingly recognized as regulators of cell behavior beyond membrane excitability, their role in extracellular matrix (ECM) interactions remains poorly understood. The voltage-gated potassium channel Kv1.3 has been reported to physically and functionally associate with β1 integrins in immune and cancer cells, forming mechanochemical hubs that link ion fluxes to cytoskeletal remodeling, adhesion, and migration[1,2]. We hypothesized that Kv1.3–ECM interactions represent a key mechanism driving early VSMC dedifferentiation and that Kv1.3 plays a general role in VSMC PM through its interaction with the ECM.

Material and Methods

The institutional ethics committee approved all animal protocols, which comply with Directive 2010/63/EU. Vascular remodeling was explored in an endoluminal lesion model[3]of femoral arteries from WT and Kv1.3 knockout ($Kv1.3^{-/-}$) mice with immune-histochemical techniques. Kv1.3–ECM interactions were studied in HEK293 cells overexpressing Kv1.3 or Kv1.5 or a poreless Kv1.3, and primary femoral VSMCs from WT and $Kv1.3^{-/-}$mice. Adhesion assays were performed on fibronectin (FN)- or poly-L-lysine (PLL)-coated surfaces, and cell motility was evaluated using single-cell tracking assays for up to 48h. Integrin β1 and Kv1.3 channel blockers were used to explore mechanisms, and AAV-Kv1.3 transduction of $Kv1.3^{-/-}$ VSMC to rescue KO phenotype.

Results

$Kv1.3^{-/-}$ femoral arteries showed increased wall thickness and reduced cell number at baseline, consistent with a dedifferentiated phenotype, and developed less intimal hyperplasia after injury. At the cellular level, $Kv1.3^{-/-}$ VSMCs were larger and showed a transcriptional profile consistent with dedifferentiation, with upregulation of OPN and downregulation of CNN1and KCNA5 genes.

Kv1.3 (but not Kv1.5) expression significantly enhanced HEK cell adhesion to FN and increased cell motility independently of ion flux. In native VSMCs, $Kv1.3^{-/-}$ showed impaired adhesion to FN. Adhesion was dependent on the Kv1.3–integrin β1 interaction and sensitive to channel blockers, indicating a functional role of Kv1.3 channels. $Kv1.3^{-/-}$ VSMCs exhibited changes in motility that were partially normalized with AAV-mediated Kv1.3 re-expression.

Conclusions

Kv1.3 actively contributes to VSMC adhesion and migration through its interaction with integrin β1. The vascular phenotype of Kv1.3$^{-/-}$mice supports the conclusion that Kv1.3–ECM interactions are functionally relevant for vascular remodeling following PM. These findings position Kv1.3 as an early molecular switch in VSMC dedifferentiation, with potential implications for understanding and targeting pathological vascular remodeling.

C11

Towards understanding how dietary fatty acids modulate TMEM16A channels and contribute to vasorelaxation

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Introduction:
Docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid (PUFA), exerts beneficial cardiovascular effects, including vasodilation and blood pressure reduction, although the underlying mechanisms remain incompletely understood. In vascular smooth muscle, TMEM16A chloride channels contribute to membrane depolarisation and contraction in response to vasoconstrictor stimuli. DHA has previously been reported to inhibit cloned TMEM16A channels REF[PT2] .

Aim:
To determine whether inhibition of TMEM16A contributes to the vasorelaxant actions of DHA and to investigate the underlying mechanisms.

Methods:
Whole-cell patch-clamp electrophysiology was used to assess the effects of DHA on TMEM16A currents in HEK293T cells expressing TMEM16A alone or together with α1-adrenoceptors or Danio rerio voltage-sensitive phosphatase (DrVSP). Vascular effects were examined by wire myography in mouse aortic and carotid artery rings. Intracellular Ca^{2+} responses were measured using GCaMP6-based calcium imaging.

Results:
DHA inhibited heterologously expressed TMEM16A currents with an IC_{50} of 15.4 ± 1.5 μM and a Hill coefficient of 1.1 ± 0.1 (n = 10). In cells co-expressing TMEM16A and α1-adrenoceptors, DHA (70 μM) markedly suppressed phenylephrine-induced current activation, reducing current amplitude at +95 mV from 371.3 ± 66.9 (n = 7) to 37.3 ± 14.0 (n = 10; $P < 0.005$).

DHA inhibited TMEM16A with similar potency in cells expressing either inactive DrVSP(C302S) (IC_{50} = 15.9 ± 3.9 μM, n = 7) or wild-type DrVSP (IC_{50} = 16.7 ± 1.0 μM, n = 6), indicating that inhibition is independent of membrane PIP_2 depletion. Calcium imaging demonstrated that DHA did not alter phenylephrine-induced intracellular Ca^{2+} release, with peak fluorescence increases of approximately 2.5-fold ($\Delta F/F_0$) observed under both control and DHA-treated conditions.

In carotid artery rings, DHA (70 μM) reduced U46619-induced contraction by ~40% (n = 10), whereas the selective TMEM16A inhibitor Ani9 (2 μM) reduced contraction by ~20% (n = 12). Combined treatment produced ~70% inhibition (n = 9; $P < 0.005$). In aortic rings, DHA reduced contraction by ~90% (n = 12; $P < 0.005$), whereas Ani9 alone produced only ~10% inhibition (n = 12). The combination of DHA and Ani9 was not more effective than DHA alone.

DHA also modulated additional ion channels involved in vascular excitability. At 70 μM, DHA inhibited CaV1.2 and Kv2.1 currents while enhancing KATP channel activity, effects expected to favour vasorelaxation.

Conclusions:
TMEM16A inhibition contributes significantly to the vasorelaxant actions of DHA but does not fully

account for them. DHA acts through coordinated modulation of multiple ion channels, including TMEM16A, CaV1.2, Kv2.1 and KATP channels, with the relative contribution of each pathway varying between vascular beds. These findings identify TMEM16A as a novel vasorelaxant target and provide new mechanistic insight into the cardiovascular benefits of omega-3 PUFAs.

C12

TRPM8 and TRPA1 channels mediate intrinsic cold-induced vasoconstriction in cutaneous arteries

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Introduction

The response of cutaneous vasculature to thermal stimuli is a fundamental homeostatic function critical for mammalian survival. In the skin, local cold exposure typically triggers rapid vasoconstriction to minimize heat loss. Although this response is generally attributed to centrally coordinated sympathetic reflexes, local neurovascular mechanisms may also contribute to cold-induced vascular control. In this context the transient receptor potential (TRP) channels, TRPA1 and TRPM8, have been identified as key molecular sensors in this complex vascular response, acting through both perivascular sensory and sympathetic nerve fibers (1, 2). Here, we tested the hypothesis that TRPM8 and TRPA1 mediate an intrinsic cold response in peripheral cutaneous arteries, independently of central nervous system reflexes.

Material and Methods

All animal procedures complied with European Directive 2010/63/EU and were approved by the relevant institutional ethics committees. We performed pressure myography experiments in isolated plantar arteries from C57BL/6J, Trpa1 and Trpm8 KO mice to determine changes in diameter upon exposure to low temperature. Pharmacological experiments included TRPA1 blockade (HC030031), CGRP receptor inhibition (BIBN 4096) and sympathetic blockade (Guanethidine). TRPA1 and TRPM8 mRNA expression was assessed by qPCR in vascular and sympathetic preparations, and confocal microscopy was used to examine TRPM8 localization in perivascular fibers labelled for sensory (anti-CGRP) and sympathetic (anti-Tyrosine Hydroxylase) markers.

Results

Our findings reveal that cold (15°C) induces a significant intrinsic contraction in isolated plantar arteries (28 ± 3.5%, n=11), whereas mesenteric arteries showed no reaction. This vasoconstriction was significantly attenuated in plantar arteries from Trpa1 KO (11.04 ± 2.5%, n=9) and Trpm8 KO mice (10.9 ± 1.9%, n=6) and fully abolished by simultaneous blockade of both channels. The response was unchanged in plantar arteries from Tpv4 KO and Trpm3 KO mice. In the presence BIBN (blockade of sensory fibers), the cold-induced vasoconstriction response was potentiated (39.2 $\pm$ 5.5%, n=6) while sympathetic fiber blockade led to a cold-induced vasodilation (13.6 $\pm$ 4.3%, n=5). TRPA1 and TRPM8 channels mRNA were detected in sympathetic ganglia, and confocal images of plantar arteries confirmed Trpm8 presence in perivascular sympathetic fibers.

Conclusions

Our results support that Isolated plantar arteries display an intrinsic vasomotor response to local cooling, mediated by TRPM8 and TRPA1 channels present in both sympathetic and sensory perivascular nerves. The net vasoconstrictor response observed is predominantly driven by activation of sympathetic perivascular fibers. These channels may represent promising targets for cold-dependent peripheral vascular disorders, such as Raynaud's disease.

C13

Cold-sensitive TRP channels and vascular dysfunction in Complex Regional Pain Syndrome

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Introduction

Complex Regional Pain Syndrome type I (CRPS-I) is a chronic pain condition marked by persistent pain disproportionate to the initial injury, with vasomotor, sudomotor, motor, and trophic disturbances, but no major nerve damage1. Although CRPS-I mechanisms remain unclear, vascular dysfunction likely contributes to its pathophysiology.

The Chronic Post-Ischemia Pain (CPIP) model replicates key CRPS-I features—mechanical hypersensitivity, cold allodynia, and autonomic dysfunction—making it useful to study links between vascular function and sensory changes2. Given the role of cold-sensitive TRP channels in vascular tone and sensory signaling, we evaluated whether CPIP alters vascular responses mediated by these channels.

Material and Methods

All experimental procedures were approved by the Animal Ethics Committee of the University of Valladolid and the Regional Government. CPIP was induced in male C57BL/6 mice aged 9–16 weeks using a 3-hour hind paw ischemia followed by reperfusion. Mechanical, cold and heat sensitivity were assessed using the von Frey, acetone and hot plate tests, respectively. Vascular function in isolated plantar arteries was assessed by pressure myography, one day after injury, comparing phenylephrine- and cold-induced constriction in CPIP and sham animals. TRP mRNA was analyzed by qPCR.

Results

Behavioral testing showed marked mechanical hypersensitivity in the ipsilateral paw of CPIP animals (n=10) vs. sham controls (n=9) from 3 hours to 90 days post-reperfusion ($p < 0.001$; von Frey test). Cold allodynia was present from 3 hours to 3 days post-induction in CPIP animals ($p < 0.001$; acetone test), but not at later time points. Thermal sensitivity (hot plate test) did not differ between groups.

In pressure myography, CPIP arteries showed increased sensitivity to phenylephrine than sham arteries (EC50 0.37 vs 1.02 µM; n=5) and a reduced vasoconstrictor response to cold. Accordingly, the cold-to-phenylephrine response ratio was lower in CPIP mice than in sham mice (0.86 ± 0.16 vs 1.41 ± 0.09; n=3). The contribution of TRPM8 and TRPA1 to cold-induced vasoconstriction in CPIP was analyzed with selective agonist and blockers of the channels.

Conclusions

The CPIP model reproduces key CRPS-I sensory features and causes early plantar artery changes, including enhanced phenylephrine sensitivity (increased adrenergic responsiveness) and reduced cold-induced vasoconstriction. These findings suggest that vascular dysfunction and sensory abnormalities interact to contribute to post-ischemic pain, pointing to potential vascular targets for treating CRPS-I and related chronic pain disorders.

C14

Comparative regulation of smooth Muscle Contractility: Distinct mechanisms of Kv7 and BKCa channel interactions control L-type Ca2+ channels in urethral vs. vascular smooth muscle

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Introduction

Myogenic tone in vascular smooth muscle cells (VSMC) relies on Ca^{2+} influx via voltage-dependent L-type Ca^{2+} channels (LTCC), and LTCC activity is tightly regulated by concurrent hyperpolarizing braking mechanisms of voltage-gated Kv7 channels and large-conductance Ca^{2+}-activated K^+ (BKCa) channels activated by sarcoplasmic reticulum (SR) Ca^{2+} sparks (Ma et al., 2020; Dopico et a., 2018). Whether this negative feedback signal is a universal feature shared by visceral smooth muscle remains poorly understood. In murine urethral smooth muscle cells (USMC), LTCCs are expressed but surprisingly fail to contribute to basal tone or contractile responses (Gupta et al., 2023). Drawing a direct comparative parallel to VSMC dynamics, we hypothesized that robust basal activation of Kv7 and/or BKCa channels in the mouse urethra exerts a powerful hyperpolarizing brake that completely silences LTCC contribution to contractility, hiding a conserved mechanism between urinary and vascular systems.

Results

All experimental procedures were approved by the DkIT Ethics Committee and conformed to EU guidelines on the care and use of animals in research. In isometric tension experiments, when urethral rings were pre-contracted by phenylephrine (PE, 1 μM), or by electrical field stimulation (EFS), neither XE991 (10 μM, Kv7 inhibitor) nor iberiotoxin (300 nM, BKCa inhibitor) had any effect when applied in isolation (n=6, $P>0.05$). However, joint application of iberiotoxin and XE991 induced robust phasic contractions superimposed on PE responses (n=12, $P<0.001$) and enhanced EFS-evoked contractions (n=7, $P<0.01$), both of which were fully reversed by the LTCC inhibitor nifedipine. During in situ Ca^{2+} imaging of USMC, combined application of XE991 and iberiotoxin converted asynchronous, localized intracellular Ca^{2+} signals into coordinated, propagating intercellular waves abolished by nifedipine (n=5). Crucially, defining a key divergence from classic vascular mechanisms, pre-contracted urethral rings were relaxed by the Orai channel inhibitors GSK-7975A (n=6) or Synta 66 (n=5). Under Orai inhibition, XE991 alone successfully evoked nifedipine-sensitive phasic activity, whereas subsequent iberiotoxin addition had no additional effect (n=6, $P>0.05$), indicating that BKCa channel activation in the urethra depends, at least in part, on upstream Orai-mediated Ca^{2+} influx.

Conclusion

These data demonstrate a fundamental similarity between vascular and visceral tissues: both utilize a tandem Kv7/BKCa hyperpolarizing brake to dampen LTCC activity. However, while vascular BKCa activation typically relies on SR mediated Ca^{2+} sparks, mouse USMC relies on Ca^{2+} influx via Orai channels to drive some element of BKCa activity. This comparative insight reveals that while the downstream regulators of contractility control is conserved across smooth muscle types, the architecture of coordination between these players is tissue-specific.

C15

Ca^{2+}Tracker: open-source software for accessible, standardised analysis of calcium imaging data

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Intracellular Ca^{2+} imaging is a mainstay of vascular physiology and is central to studying vascular ion channel function. Ca^{2+} acts as the convergence point for signalling through a wide range of ion channels and receptors (IP_3 receptors, TRP channels, store-operated entry, ryanodine receptors), and the resulting spatial and temporal patterns of Ca^{2+} activity report directly on how these pathways are engaged. Subcellular Ca^{2+} events, whole-cell oscillations, and coordinated activity across a vessel each carry distinct mechanistic information about channel function in health and in disease.

For most researchers, the core requirement is simple: to delineate the cells or regions of interest and quantify how their Ca^{2+} signals evolve over time. Yet the available tools are frequently prohibitive. Some are commercial, with licensing costs beyond the reach of many laboratories; others are free but inflexible, or powerful yet built around a single imaging modality that does not generalise to vascular preparations. Lacking a common platform, each laboratory develops its own approach, undermining the cross-study comparability and reproducibility that funders and publishers increasingly demand.

To address this, we developed Ca^{2+}Tracker: a free, open-source application for the interactive analysis of Ca^{2+} imaging data, designed to be fully usable without programming experience. A functional prototype is already operational, with continued development underway (www.calciumimaging.com). It reads the image formats produced by common confocal and widefield systems (TIFF stacks, Bio-Formats) and runs on both Windows and macOS. Regions of interest can be defined manually, semi-automatically across large fields, or generated directly from patterns of spontaneous Ca^{2+} activity, allowing active cells to be identified without prior anatomical segmentation. From these regions, Ca^{2+}Tracker extracts $\Delta F/F_0$ traces using flexible baseline-selection strategies that accommodate drifting backgrounds, photobleaching, and variation in resting fluorescence. Ca^{2+} traces can be inspected interactively, and Ca^{2+}Tracker quantifies event amplitude, frequency, and rise/fall kinetics directly, with results exported in standard formats for any further analysis.

Critically, Ca^{2+}Tracker is designed to support automated comparative analysis across multiple datasets, a capability absent from existing tools and increasingly essential as experiments grow in scale. By standardising core workflows, it offers a common analytical framework where the field currently has none. Ca^{2+}Tracker is deliberately general, handling the full range of vascular Ca^{2+} preparations: isolated cells, intact en face preparations, and whole pressurised vessels. Because it is open-source and modular, users can extend it with their own analytical methods and share these with the community as plugins, allowing the tool to grow as the needs of the field evolve.

Ca^{2+}Tracker builds on our experience developing VasoTracker, an open-source pressure myography platform now used by vascular laboratories internationally, with its successor (VasoTracker2 [1]) recently published in The Journal of Physiology. Ca^{2+}Tracker applies the same iterative, user-centred, openly developed approach. By removing cost and expertise barriers to a core technique and providing a standardised framework for analysis, Ca^{2+}Tracker aims to improve reproducibility and broaden participation in vascular ion channel research.

C16

Renal TRPM3 channels regulate blood pressure through tubuloglomerular feedback and plasma volume control

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Introduction

Transient receptor potential melastatin 3 (TRPM3) is a calcium-permeable non-selective cation channel with broad sensory and metabolic functions, yet its role in renal and cardiovascular physiology remains poorly defined. Given the central role of the kidney in long-term blood pressure (BP) regulation, this study examined whether TRPM3 contributes to systemic haemodynamic control through renal tubular and vascular mechanisms.

Material and Methods

All animal procedures were in accordance with the EC guiding principles regarding the care and use of animals (Directive 2010/63/UE), with protocols approved by the institutional ethics committee. BP was measured in conscious wild-type (WT) and Trpm3 knockout (KO) mice under basal conditions and following angiotensin II (AngII) infusion or losartan treatment. Renal TRPM3 expression was assessed by RNAscope and reporter labelling, and renal function was evaluated in isolated perfused kidneys and by in vivo glomerular filtration rate (GFR) measurements.

Results

Trpm3-KO mice exhibited a mild hypotensive phenotype relative to WT animals (WT n=66 male, 31 female; KO n=61 male, 19 female; $P<0.0001$, Aligned Rank Transform-ANOVA), accompanied by increased urinary Na^+ excretion, reduced plasma volume, and unchanged haematocrit, suggesting that enhanced natriuresis underlies hypotension. AngII infusion raised BP in WT (from 84±3 mmHg to 99±8 mmHg; P=0.00017) but not in Trpm3-KO mice (from 81±3 mmHg to 81±6 mmHg; P=0.95 ANOVA). By contrast, losartan lowered BP similarly in both genotypes, arguing against altered AT1 receptor responsiveness as the primary cause of the phenotype.

Within the kidney, TRPM3 was detected in the juxtaglomerular apparatus (macula densa), distal convoluted tubule, and collecting duct, but absent from proximal tubule, VSMCs, and renin-positive granular cells, consistent with a role in tubular sensing and distal Na^+/water handling. Dapagliflozin, which increases Na^+ delivery to the macula densa, reduced renal flow and GFR in WT, and both responses were significantly attenuated in Trpm3-KO mice, indicating impaired tubuloglomerular feedback (TGF) in the absence of TRPM3. Accordingly, the TRPM3 blocker Primidone similarly attenuated the dapagliflozin-evoked vasoconstrictor response in WT kidneys (WT n=6 vs. KO n=5, P=0.005; WT+primidone n=5, P=0.02; ANOVA), further supporting a functional role for TRPM3 in TGF.

TRPM3 activation by pregnenolone sulfate induced vasodilation in pre-constricted renal vessels. This response was abolished by calcitonin gene-related peptide receptor (CGRP) blockade or nitric oxide synthase inhibition and was absent in Trpm3-KO kidneys. Immunolabelling of renal arteries showed TRPM3 reporter signal colocalised with CGRP in perivascular sensory nerve fibres, with no detectable

signal in VSMCs or endothelium. This sensory neurovascular component indicates that renal TRPM3 can also modulate vascular tone through CGRP- and nitric oxide-dependent signalling.

Conclusions

These findings identify renal TRPM3 as a regulator of Na^+ and water balance that contributes to long-term BP homeostasis through TGF and distal nephron function, while also engaging a perivascular sensory pathway that shapes renal haemodynamics.

C17

Physioxia enhances ion channel activities in brain microvascular endothelial cells and neurons

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The majority of cell-based studies are performed under atmospheric oxygen (~18kPa), even though most tissues in vivo, including the brain, experience only ~4–8kPa O2, defined as physioxia (Keeley & Mann, 2019). This mismatch has influenced experimental design and our understanding of cellular physiology, with emerging evidence confirming cellular responses, proteome and metabolic profiles differ significantly in cells adapted to in vivo-like O2 conditions (~4–8kPa, physioxia) compared to hyperoxic atmospheric O2 environments.

To characterise the effects of physiological O2 on neuronal and brain endothelial physiology, we assessed ion channel function in differentiated mouse neuroblastoma N2a cells, primary embryonic mouse cortical neurons and human brain microvascular endothelial cells (hCMEC/D3) following long-term (4-5 days) adaptation to physiological (5kPa) and hyperoxic (18kPa) environments.

N2a cells were cultured in DMEM+GlutaMAX (Gibco) and differentiated (1% foetal bovine serum (FBS, Sigma Aldrich), 20µM retinoic acid (RA) for 7-10 days) and subjected to reduced serum and RA for 7-10 days prior to experimentation. Primary embryonic cultures were derived from brain cortices of C57BL/6J mice embryos (E15-17, Charles River UK; Envigo) following Schedule-1 procedure and cultured in DMEM+GlutaMAX supplemented with 10% FBS. hCMEC/D3 cells were cultured in 1% rat-tail collagen I coated-substrates (Sigma) in EBM phenol-red free basal cell media (Lonza, Switzerland) supplemented with EGM-2MV growth factors.

Electrophysiological recordings were made using a Nanion Port-a-Patch (Nanion Technologies) or MultiClamp 700B amplifier and HEKA Patchmaster. Immunocytochemistry and proliferation assays were performed at various timepoints. Physioxia and was achieved by culturing cells for 4-5 days at 5kPa O2. Unpaired Student's t-test and ANOVA were used where appropriate with $P<0.05$ considered significant.

Under physioxic conditions, all three cell types showed altered electrophysiological phenotypes with markedly enhanced outward K^+ currents which were sensitive to the Kv3/BK channel blocker TEA (3mM). Following partial current inhibition in hCMEC/D3 cells by TEA (@50 mV, 5kPa and 18kPa: TEA: 62±5 % P=0.0381 versus 79±8 % P=0.3723), the remaining K^+ currents showed differential sensitivity to the SK and IK channel blockers 50µM TRAM-34 and 100nM apamin, respectively, under both O2 adaptations. At 5kPa adaptations, N2a cells showed comparable TEA sensitivities (@50 mV, 12% versus 18%), whereas primary neurons showed larger TEA sensitivity (@50 mV, 31% versus 14%), Both cell types exhibited larger voltage-activated Na^+ currents under physioxia and activation voltages for both, voltage-gated Kv and Nav currents in N2a and primary neurons displayed left-shifted values.

Current-evoked neuronal excitability and action potential (AP) amplitudes were unaffected under physioxia, however, AP half-width were strongly reduced in N2a cells mirroring the enhanced activity of

Kv currents under physioxia. Passive membrane properties were partially altered in physioxia, resulting in lower membrane time-constants (tm) and membrane resistances (Rm) but unchanged cell capacitance (Cm). Furthermore, N2a cells showed an increased proportion of NeuN-positive cells and enhanced axonal outgrowth following physiological oxygen adaptations without effects on cell proliferation.

Our data provide new evidence that adaptation of cells to physioxia alters neuronal and endothelial ion channel characteristics, reinforcing the need for investigating the effects of physiologically oxygen levels on the redox signalling in cell models to improve clinical translation.

C18

iLifeSci: an open-source, browser-based simulation platform for ion channel and vascular physiology education

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Introduction
Interactive Life Science (iLifeSci) is an open-source, browser-based platform combining interactive simulations with guided learning pathways for physiology education. The platform is accessible to any student, anywhere, for free, and without installation, licensing, or specialist hardware. It was built around a simple observation: many physiological concepts involve dynamic interactions across biological scales that static teaching resources cannot fully convey. Simulation addresses this by shifting the focus away from the performance of an experiment and towards experimental design, analysis, and interpretation — allowing students to explore physiological systems, test hypotheses, and visualise how molecular events translate into tissue/organ function. Integrated vascular physiology is a particular challenge: connecting ion channel activity to calcium signalling, membrane potential, and arterial tone across multiple scales, cell types, and organs. Developing a scaffolded simulation pathway that teaches this chain, from ion channel biophysics to integrative vascular function, is the central aim of the current phase of iLifeSci development.

Methods
iLifeSci is built using modern web technologies (React, TypeScript, Vite), runs in any web browser without installation or licensing, and is fully open-source. Simulations are self-contained and quantitatively realistic — multiple drugs and concentrations can be applied, interactions between drugs modelled, and experiments repeated or redesigned in real time. Content is organised as scaffolded pathways — guided narratives with interactive simulations embedded at every conceptual step, taking students from first principles to integrative function. Development is supported by a Physiological Society Education and Teaching Award.

Results
More than ten simulations are currently live at https://www.ilifesci.com. A neurophysiology pathway begins at the molecular level — ion diffusion and the Nernst equation, a particle-based ion channel visualiser driven by real gating variables, and saltatory conduction in myelinated axons including demyelination — and extends to a Hodgkin-Huxley neuron model with 7 antiepileptic drugs and 3D ion channel visualisation. The cardiovascular pathway scaffolds from first principles to integrative function: a guided receptor theory journey covering receptor occupancy, affinity, efficacy, and competitive antagonism leads into VasoSim — a virtual pressure myograph modelling arterial diameter responses to receptor pharmacology, IP3/calcium signalling, and myogenic pressure responses — and from there to a web version of RatCVS (1), a whole-animal cardiovascular simulation modelling blood pressure and heart rate responses to autonomic drugs.

Conclusions
iLifeSci demonstrates that rigorous physiology and pharmacology teaching simulations can be delivered as free, open-source, browser-based tools — from ion channel biophysics to integrative cardiovascular function. The cardiovascular simulation suite, including VasoSim and RatCVS, is in active development. We welcome input from the vascular physiology community to help shape this next stage of this free and open-source resource.