

Hyperpolarization and the endothelium

Christopher J Garland^{*} and Kim A Dora

The ability of vascular endothelial cells to generate and conduct membrane hyperpolarization is a critical integrative mechanism controlling local blood flow and systemic blood pressure. This mechanism is particularly apparent in the microcirculation. Hyperpolarization initiated in the endothelium by receptor activation or local influences such as K^+ stimulates vasodilation by passive, radial current spread via heterocellular myoendothelial gap junctions (MEJs) and/or the release of a diffusible factor(s). In addition, the endothelium has high-input resistance and serves as an effective conduit, conducting hyperpolarization bidirectionally through microvascular networks. This not only coordinates vasomotor responses but also causes ascending vasodilation, both of which reduce resistance sufficiently to allow an increase in tissue blood flow. These processes will be disrupted by the endothelial dysfunction in disease, helping explain why enhanced vasoreactivity and vasospasm develops in resistance arteries, limiting blood flow into the microcirculation.

Address

University Department of Pharmacology, Mansfield Road, Oxford OX1 3QT, UK

Corresponding author: Dora, Kim A (kim.dora@pharm.ox.ac.uk)

^{*} Twitter account: @ChrisVascpharm

Current Opinion in Physiology 2023, 34:100674

This review comes from a themed issue on **Endothelium**

Edited by **Jeremy Pearson** and **Paul C Evans**

For complete overview of the section, please refer to the article collection, "**Endothelium**"

Available online 25 April 2023

<https://doi.org/10.1016/j.cophys.2023.100674>

2468–8673/© 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

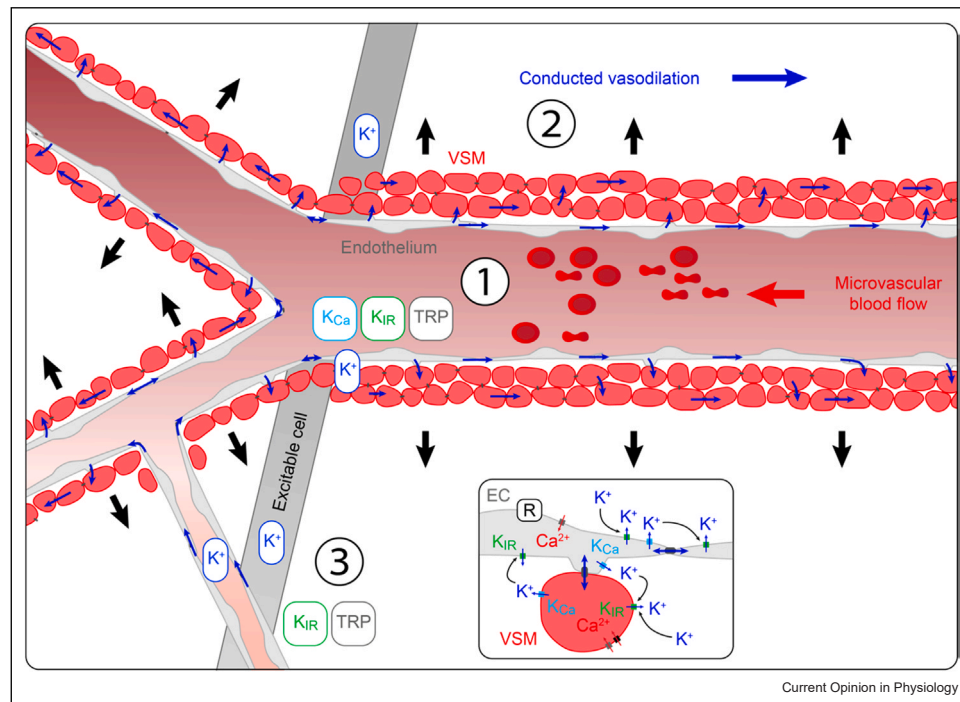
Vasoreactivity of arterial smooth muscle cells (VSM) is strongly influenced by the endothelium. The long (~50–100 μm), thin endothelial cells (ECs) lining the inner surface of blood vessels do not contain voltage-gated Ca^{2+} channels (VGCC, $\text{Ca}_v1.2$ α -subunit), and suppress vasoreactivity by Ca^{2+} -dependent synthesis of nitric oxide (NO) and activation of Ca^{2+} -activated potassium channels (K_{Ca} channels) to generate hyperpolarization, which then spreads between ECs, passing into

the muscle layers to reduce VGCC open probability and as a consequence initiate vasodilation.

Historically, the inhibitory influence of the endothelium is usually ascribed to the synthesis and release of NO, which was discovered and initially named endothelium-dependent relaxant factor by Furchgott in 1980. However, the endothelium is also able to generate and conduct membrane hyperpolarization, which by spreading into the artery wall, causes vasodilation by influencing the open probability of VSM VGCCs. Physiologically, hyperpolarization underpins the conducted (*aka* ascending) vasodilation that is essential to coordinate changes in blood flow, ensuring increases in flow are sufficient to meet the metabolic demands of organs such as the heart and brain. Importantly, the vasodilation also helps determine blood pressure by influencing peripheral vascular resistance. As complex multicellular structures, arteries must respond effectively to numerous, often-opposing, extrinsic signals, such as changing sympathetic nerve activity, blood flow-dependent variation in shear stress, and local autacoids. The rapid spread of hyperpolarization between vascular cells therefore plays a crucial coordinating role, reviewed recently in Ref. [22].

As artery size decreases, the density of key ion channels, such as inwardly rectifying potassium channels and voltage-gated calcium channels, increases. The core of the work characterizing endothelium-dependent hyperpolarization (EDH but initially referred to as EDHF) was obtained in small resistance arteries, where muscarinic stimulation of the endothelium generates hyperpolarization that can completely relax precontracted arteries, independently of NO [23]. The key discovery that identified the K-channels involved in generating EDH was use of the toxins apamin and charybdotoxin, which blocked hyperpolarization and vasorelaxation but only if applied together [53]. Apamin specifically blocks $\text{K}_{\text{Ca}2.3}$ (SK_{Ca}) channels, and while charybdotoxin is not specific, it inhibits $\text{K}_{\text{Ca}3.1}$ (IK_{Ca}), $\text{K}_{\text{Ca}1.1}$ (BK_{Ca}), and K_{v} channels, selective $\text{K}_{\text{Ca}3.1}$ blockers, such as the clotrimazole derivative TRAM-34, and more recently the benzothiazinone NS6180 [6], have shown the second K-channel involved in generating EDH is $\text{K}_{\text{Ca}3.1}$. Thus, in most vascular beds studied, EDH involves activation of both small $\text{K}_{\text{Ca}2.3}$ and intermediate $\text{K}_{\text{Ca}3.1}$ conductance calcium-activated potassium channels. These channels were shown to be restricted to the endothelium, with EDH hyperpolarization generated by an increase in EC Ca^{2+} , rather than the channels providing a VSM target

Figure 1



Mechanisms leading to EC hyperpolarization and vasodilation. Cartoon depicting the microcirculation of any vascular bed. Pathway ① represents the generally utilized model of EDH in isolated arteries stimulated by an agonist such as acetylcholine, acting as a muscarinic receptor on ECs (ECs, R in inset). This activates EC K_{Ca} channels (blue) to hyperpolarize the endothelium, and this current can pass to adjacent VSM and ECs. The process can be amplified by K⁺ acting at nearby K_{IR} channels (green, inset). EC Ca²⁺ can also produce diffusible factors that activate VSM K_{Ca} channels. Pathway ② represents the movement of current in the endothelium, in this case upstream, to stimulate conducted vasodilation. Pathway ③ represents the activation of capillary ECs by extracellular K⁺ to open K_{IR} channels and, if K_{Ca} channels are expressed, by increases in EC Ca²⁺. There is evidence for mechanosensitive and TRP (Transient Receptor Potential) channel expression (gray, inset) in ECs and VSM, yet the balance between depolarizing and hyperpolarizing currents is not yet clear. Ultimately, if predominant, EC hyperpolarization passes to VSM to reduce the open probability of VGCC expressed in VSM (black, inset), leading to rapid, coordinated, and dynamic changes in diameter.

for activation by a diffusible ‘factor’ (EDHF). However, K⁺ efflux through these endothelial channels does act as a diffusible factor and contributes to EDH-mediated vasodilatation [15] (Figure 1). There are also other important examples where factors seem to play a role in initiating hyperpolarization in certain settings, such as hydrogen peroxide and lipid mediators acting at VSM K_{Ca}1.1 channels [19,31]. But regardless of the target K-channel, any agent able to increase EC Ca²⁺ can potentially generate EDH, that is, EC hyperpolarization and associated vasorelaxation.

Unlike K_{Ca}1.1 channels, both K_{Ca}2.3 and K_{Ca}3.1 channels are voltage-independent and share similar Ca²⁺ sensitivity, yet in many cases can be activated independently [8,20]. This reflects a differential distribution of K_{Ca} channel isoforms within ECs, revealed by immunolabeling. K_{Ca}3.1 channels cluster within fine myoendothelial projections (MEPs) that pass through perforations in the internal elastic lamina linking ECs to the adjacent VSM [12,42]. In contrast, the distribution of K_{Ca}2.3 channels is widespread across ECs and

particularly dense around the (relatively) large EC–EC gap junctions [12,42]. K⁺ ions passing out through EC K_{Ca}2.3 and K_{Ca}3.1 channels to accumulate in the restricted myoendothelial intercellular space stimulate smooth muscle hyperpolarization by activating strong inwardly rectifying (K_{IR}2.1) channels and the α -2 or -3 subunit of Na⁺/K⁺ ATPase [15,24]. These two important ion channels, K_{IR} and K_{Ca}, are therefore key players, or targets, in the spread of EDH.

MEPs can contain myoendothelial gap junctions (MEJs) that provide a bridge to the medial smooth muscle layers. Therefore, MEPs and MEJs together occupy a key strategic position that enables them to detect and modify information flowing between the different arterial cell layers. As such, they can operate as signaling hubs, as MEP K_{Ca}3.1 channels have been shown to align closely with other proteins to form a signaling microdomain, proteins such as Na⁺/K⁺-ATPase, Cx37, and Cx40 (integral to the MEJs), calcium-sensing receptors, and transient receptor potential channels such as TRPV4, TRPA1, and TRPC3 [1,12,14,33,42,45,48]. The

channels also closely align with SR IP₃-sensitive Ca²⁺ stores, which enables spontaneous fixed location Ca²⁺ pulsars to activate K_{Ca}3.1, generating sufficient current to hyperpolarize VSM by around 8 mV [29]. TRPV4 colocalization with K_{Ca}3.1 channels depends on A-kinase-anchoring protein (AKAP150), a scaffolding protein concentrated exclusively within the MEPs [2,49]. Individually, TRPV4 channels generate spontaneous elementary Ca²⁺ influx events, or TRPV4 sparklets, and the AKAP150 scaffold ensures these are amplified sufficiently to activate K_{Ca}3.1 and ensure an effective vasodilator signal [49].

It is worth noting that MEPs in small arteries also contain caveolae-associated NO synthase, the caveolae enabling a structural complex with the hemoglobin α -chain and cytochrome B5 reductase 3. It is suggested that Hb α reduction promotes NO scavenging, limiting local NO bioavailability and helping explain the predominance of EDH signaling in small arteries, alongside the increased density of VGCCs. This NO microdomain appears absent from the sparse MEPs of conduit arteries [38,46,5,50].

Myoendothelial gap junctions help transfer endothelial cell hyperpolarization

When MEJs are present, they enable hyperpolarization to pass from the endothelium with minimal detriment, but also in the opposite direction, from VSM to ECs [17,56]. MEJs become more numerous as artery size decreases, with formation regulated by plasminogen activator inhibitor-1, another protein enriched within MEPs in small resistance arteries [27,41,43].

Demonstrating directly that EC hyperpolarization spreading through MEJs is able to cause significant vasorelaxation is not easy, because gap junction uncouplers are nonselective in many ways. However, selective targeting of MEJs, without affecting the more numerous homocellular gap junctions between ECs and between VSM, was achieved by selective EC loading of antibodies directed against the intracellular domain of MEJ connexins in pressurized arteries (isobaric recording). These data suggest a central role for Cx40 (targeting the intracellular carboxy-terminal residues 340–358) as EDH vasodilatation was blocked by Cx-40 antibodies during near-maximal vasoconstriction to phenylephrine, conditions necessary to antagonize the effect of K⁺ ion released by the endothelium, which acts as a diffusible EDH [33]. These observations were supported by high-resolution immunolabeling showing that Cx40 is present within the MEJs [33].

EDH vasodilation is therefore the consequence of two components, a chemical factor acting directly on the VSM (such as K⁺ or hydrogen peroxide) and passive

spread of hyperpolarization to VSM via MEJs. The relative contribution of each varies in different arteries, reflecting artery structure, the complement of ion channels on the ECs and VSM, and the prevailing cause and intensity of background vasoconstriction.

Longitudinal spread of hyperpolarization via the endothelium

Hyperpolarizing current also passes bidirectionally along the length of the vessel (axial spread) (Figure 1). Furthermore, it relies on the endothelium and is independent of either nerves or changes in blood flow [11,17,39,44,55,9]. Studies in exteriorized microvascular beds *in situ* [11], and arterioles isolated from the same vascular beds, have used acetylcholine to activate endothelial muscarinic receptors selectively to evoke robust responses following micropipette application to the outside of arterioles.

As there is a high density of homocellular EC–EC gap junctions, hyperpolarizing current passes rapidly along the endothelium in both longitudinal directions, with movement upstream against the direction of blood flow able to stimulate ascending or conducted vasodilatation. Conducted vasodilatation has been most extensively studied in the microcirculation, and is activated by agonists that open VSM or EC K-channels [9]. The signal for conducted dilatation is thought simply to be current moving to and between adjacent cells that have not been stimulated directly by an agonist [11,17]. As the very restricted vasodilation caused locally by autacoids and metabolites is not effective in reducing resistance, which is a function of vessel length, conduction provides the drop in resistance necessary to increase blood flow downstream. The axial alignment of the long (~100 μ m) but thin ECs, means each one spans around 10–20 smooth muscle cells. Thus, with low-resistance coupling (effective passage of current), but high-resistance membranes (few open ion channels), hyperpolarization can pass along the endothelium and access many muscle cells via MEJs [17,51]. Current flow along the endothelium is facilitated by a circa 1000-fold higher resistance across MEJs compared to that between ECs (reviewed in Ref. [54]). The presence of voltage-gated and other K-channels in VSM means current can more readily leave these cells, thereby reducing intercellular conduction distances when the channels are open [4].

As conducted vasodilatation is effective over greater distance than predicted for passive current spread, EC current may also be actively sustained or regenerated. In small arteries, hyperpolarization during current injection decays more rapidly than EC hyperpolarization evoked by acetylcholine, even though the initial increase in potential is similar [16]. One possibility is that EC K_{IR} channels sustain current spread, and this is consistent

with the ability of the selective K_{IR} channel inhibitor barium to reduce markedly both local and conducted vasodilatation to various agonists in the porcine coronary microcirculation [40]. However, this may not always be the case, as barium did not affect the robust conducted dilation to bradykinin in $<300\text{-}\mu\text{m}$ -diameter human coronary intramuscular arteries [13].

Another suggestion in skeletal muscle arterioles is that $K_{Ca2.3}$ channels present at EC borders are activated by local Ca^{2+} entry, as the result of mechanical deformation during active hyperemia, leading to Cx40-dependent hyperpolarization for rapid conduction across ECs [32,35,36]. Whether regenerative or not, once EC hyperpolarization ascends to larger arteries, an increase in blood flow will follow. The resulting increase in shear stress releases NO to enhance vasodilatation in these feed arteries.

While evidence showing capillaries are electrically coupled to arterioles and responsive to agonist stimulation was derived in striated muscle decades ago [3,34], the concept that K^+ released from surrounding cells acts as an effective stimulus for K_{IR} channels in capillaries is more recent. During the repolarization phase in excitable cells, such as cardiac muscle and neurones, K^+ efflux through voltage- and ATP-sensitive K-channels can activate capillary K_{IR} channels with the resulting hyperpolarizing current passing upstream to contribute to the drive for conducted dilation *in situ* (Figure 1). This has been demonstrated both in the heart [57] and brain [18,32,52], and aligns very well with earlier reports in striated muscle [7,37]. Furthermore, recent evidence suggests vascular pericytes may also release K^+ via open K-channels, such as K_{ATP} channels, another important example of a metabolic sensor [25]. These pericytes may also directly couple to each other, to capillary ECs, and to VSM. Most recently, and somewhat intriguingly, the possibility that mechanical forces can control Ca^{2+} responses in capillaries warrants further investigation [26]. If K_{Ca} channels are expressed in these ECs, the associated hyperpolarization may also potentially initiate upstream vasodilation.

Overall, it is very clear that arterial hyperpolarization represents a major signaling mechanism, particularly in the microcirculation. However, in terms of vascular function, it must be considered alongside NO signaling. So far, we have focused on EC-to-VSM signaling, but the nature of gap junctions means bidirectional signaling is also possible. During myogenic or agonist-induced vasoconstriction, some cytoplasmic Ca^{2+} entering the VSM via L-type VGCCs passes, along with IP_3 , into EC MEPs, activating EDH and the release of NO to complete an inhibitory myoendothelial feedback loop (MEF). Functionally, feedback is sufficient to suppress

agonist and/or nerve-induced vasoconstriction and myogenic tone [20,30].

As the loss of NO bioavailability and compromised EDH are ubiquitous features of cardiovascular disease, this will not only disrupt vasodilation *per se*, as is generally reported, but also MEF and conducted responses. Furthermore, less-effective homo- and heterocellular electrical coupling between cells in the artery wall will disrupt the coordination of responses normally associated with the spread of hyperpolarization through microvascular networks (reviewed in Ref. [54]). Therefore, future research should probe how both MEF and the ability to pass current to distal sites are affected when the endothelium is damaged by disease; that is, consideration should be given to *both* radial and axial signaling mechanisms. As the endothelium is the most effective conduit for hyperpolarization, compromise by disease will mean hyperpolarization originating in the VSM will be ineffective in efforts to improve blood flow (reviewed in Ref. [21]). Emphasizing the need to consider both local *and* conducted vasodilation, one area of particular interest is the role in human coronary microvascular disease associated with various forms of angina and heart failure [13]. Importantly, ischemic damage is not restricted to the cardiac muscle cells, it also affects vascular cells. Remarkably, in $\sim 50\%$ of small intramyocardial arteries in patients undergoing voluntary open-heart surgery, the ability to contract (and hence dilate) is markedly compromised, which will significantly diminish coronary blood flow control [10]. Therefore, preventing and reversing this damage could provide an effective approach for therapy in many patients with heart disease.

Endothelium-dependent hyperpolarization and depolarizing spike potentials

When the endothelium is healthy, that is, functions normally by generating NO and EDH, certain vasoconstrictors, including the α_1 -adrenergic agonist phenylephrine, cause rhythmic oscillations in membrane potential and tension, termed vasomotion. This physiological response includes components driven by both NO and EDH and serves to optimize blood flow and, as a consequence, the delivery of oxygen and nutrients. Inhibiting NO synthase, to mimic the loss of NO bioavailability in cardiovascular disease, causes a dramatic change in the usually electrically quiescent VSM in resistance arteries. Without NO present, the muscle cells become electrically excitable and generate fast, Ca^{2+} -based depolarizing spikes, similar to action potentials, except that the amplitude can vary. If the endothelium is still able to generate EDH, these spike potentials fire in bursts separated by periodic hyperpolarization/vasorelaxation, brief periods where EDH activated by MEF can overcome depolarization. When the endothelium is

damaged, vasoconstriction is also augmented but now with continuous ~constant-amplitude spike potentials associated with vasospasm [47]. If EDH alone is blocked, but NO still available, vasomotion is blocked and vasoconstriction augmented. However, spikes do not usually appear even though the VSM depolarizes beyond the threshold necessary to initiate them [20,47]. The cause of the switch of VSM into an electrically excitable state appears to be recruitment of latent T-type VGCCs upon loss of NO. Once available, these T-type channels provide sufficient trigger current for action potential firing with associated vasoconstriction. Both of these responses are sensitive to either T- or L-type VGCC blockers [47]. As loss of NO bioavailability is responsible for these changes and is also a ubiquitous feature of disease, it seems reasonable to suggest, for example, that angina-linked vasospasm in patients with no apparent obstructive disease might reflect raised electrical activity in the small coronary arteries. Therapeutically, T-type VGCC block may then offer an effective way to counter increased vasoreactivity, without disrupting the myogenic tone that enables autoregulation of tissue blood flow, as myogenic tone depends on L-type VGCCs [28,47]. Defining just how NO normally suppresses the availability of VSM VGCCs therefore offers the potential to identify novel vascular targets to limit the impact of disease-related small artery vasospasm at an early stage.

In summary, we now appreciate the central role of the endothelium in generating and conducting vascular hyperpolarization across microvascular networks, effectively matching blood flow to metabolic demand. However, precisely how hyperpolarization is sustained over distance still needs to be defined, recognizing that the underlying mechanisms may vary, at least slightly, in different beds. It is also clear that NO does more than simply suppress vasoreactivity by providing vasodilator drive. It also influences the reactivity of the VSM by controlling ion channel availability in some way. Understanding how this occurs offers the opportunity to identify novel therapeutic targets to treat the increased vasoreactivity that is a ubiquitous feature of cardiovascular disease.

Funding

British Heart Foundation PG/20/10260; PG/19/36/34396; FS/13/16/30199.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

We have no conflicts of interest.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Bagher P, Beleznaï T, Kansui Y, Mitchell R, Garland CJ, Dora KA: **Low intravascular pressure activates endothelial cell TRPV4 channels, local Ca^{2+} events, and IK_{Ca} channels, reducing arteriolar tone.** *Proc Natl Acad Sci USA* 2012, **109**:18174-18179, <https://doi.org/10.1073/pnas.1211946109>
2. Bagher P, Garland CJ: **Scaffolding builds to reduce blood pressure.** *Sci Signal* 2014, **7**:pe16, <https://doi.org/10.1126/scisignal.2005527>
3. Beach JM, McGahren ED, Duling BR: **Capillaries and arterioles are electrically coupled in hamster cheek pouch.** *Am J Physiol Heart Circ Physiol* 1998, **275**:H1489-1496, <https://doi.org/10.1152/ajpheart.1998.275.4.H1489>
4. Beleznaï TZ, Yarova P, Yuill KH, Dora KA: **Smooth muscle Ca^{2+} -activated and voltage-gated K^{+} channels modulate conducted dilation in rat isolated small mesenteric arteries.** *Microcirculation* 2011, **18**:487-500, <https://doi.org/10.1111/j.1549-8719.2011.00109.x>
5. Billaud M, Lohman AW, Johnstone SR, Biber LA, Mutchler S, Isakson BE: **Regulation of cellular communication by signaling microdomains in the blood vessel wall.** *Pharmacol Rev* 2014, **66**:513-569, <https://doi.org/10.1124/pr.112.007351>
6. Brown BM, Shim H, Christophersen P, Wulff H: **Pharmacology of small- and intermediate-conductance calcium-activated potassium channels.** *Annu Rev Pharmacol Toxicol* 2020, **60**:219-240, <https://doi.org/10.1146/annurev-pharmtox-010919-023420>
7. Cohen KD, Sarelius IH: **Muscle contraction under capillaries in hamster muscle induces arteriolar dilatation via K_{ATP} channels and nitric oxide.** *J Physiol* 2002, **539**:547-555, <https://doi.org/10.1113/jphysiol.2001.013388>
- This study demonstrated the concept of muscle activity activating K_{ATP} channels to increase blood flow to the active tissue. While the location of the open K_{ATP} channels and a role for K_{IR} channels were not established, the parallels to excitable neurons and cardiac myocytes are clear.
8. Crane GJ, Gallagher N, Dora KA, Garland CJ: **Small- and intermediate-conductance calcium-activated K^{+} channels provide different facets of endothelium-dependent hyperpolarization in rat mesenteric artery.** *J Physiol* 2003, **553**:183-189, <https://doi.org/10.1113/jphysiol.2003.051896>
9. Delashaw JB, Duling BR: **Heterogeneity in conducted arteriolar vasomotor response is agonist dependent.** *Am J Physiol Heart Circ Physiol* 1991, **260**:H1276-1282, <https://doi.org/10.1152/ajpheart.1991.260.4.H1276>
10. Dora KA, Borysova L, Ye X, Powell C, Beleznaï TZ, Stanley CP, Bruno VD, Starborg T, Johnson E, Pielach A, Taggart M, Smart N, Ascione R: **Human coronary microvascular contractile dysfunction associates with viable synthetic smooth muscle cells.** *Cardiovasc Res* 2022, **118**:1978-1992, <https://doi.org/10.1093/cvr/cvab218>
11. Dora KA, Damon DN, Duling BR: **Microvascular dilation in response to occlusion: a coordinating role for conducted vasomotor responses.** *Am J Physiol Heart Circ Physiol* 2000, **279**:H279-284, <https://doi.org/10.1152/ajpheart.2000.279.1.H279>
12. Dora KA, Gallagher NT, McNeish A, Garland CJ: **Modulation of endothelial cell $\text{K}_{\text{Ca}}3.1$ channels during endothelium-derived**

- hyperpolarizing factor signaling in mesenteric resistance arteries. *Circ Res* 2008, **102**:1247-1255, <https://doi.org/10.1161/CIRCRESAHA.108.172379>**
13. Dora KA, Lin J, Borysova L, Beleznaï T, Taggart M, Ascione R, Garland C: **Signaling and structures underpinning conducted vasodilation in human and porcine intramyocardial coronary arteries.** *Front Cardiovasc Med* 2022, **9**:980628, <https://doi.org/10.3389/fcvm.2022.980628>
 14. Earley S, Gonzales AL, Crnich R: **Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca²⁺-activated K⁺ channels.** *Circ Res* 2009, **104**:987-994, <https://doi.org/10.1161/CIRCRESAHA.108.189530>
 15. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH: **K⁺ is an endothelium-derived hyperpolarizing factor in rat arteries.** *Nature* 1998, **396**:269-272, <https://doi.org/10.1038/24388>.
This study was the first to report the possibility that K⁺ released from ECs can activate VSM hyperpolarization by opening K_{IR} channels and activating the Na⁺/K⁺-ATPase. This concept relates to later work showing the importance of K⁺ in initiating and facilitating conducted dilation.
 16. Emerson GG, Neild TO, Segal SS: **Conduction of hyperpolarization along hamster feed arteries: augmentation by acetylcholine.** *Am J Physiol Heart Circ Physiol* 2002, **283**:H102-H109, <https://doi.org/10.1152/ajpheart.00038.2002>
 17. Emerson GG, Segal SS: **Endothelial cell pathway for conduction of hyperpolarization and vasodilation along hamster feed artery.** *Circ Res* 2000, **86**:94-100, <https://doi.org/10.1161/01.res.86.1.94>
 18. Filosa JA, Bonev AD, Straub SV, Meredith AL, Wilkerson MK, Aldrich RW, Nelson MT: **Local potassium signaling couples neuronal activity to vasodilation in the brain.** *Nat Neurosci* 2006, **9**:1397-1403, <https://doi.org/10.1038/nn1779>
 19. Fleming I: **The factor in EDHF: cytochrome P450 derived lipid mediators and vascular signaling.** *Vasc Pharmacol* 2016, **86**:31-40, <https://doi.org/10.1016/j.vph.2016.03.001>
 20. Garland CJ, Bagher P, Powell C, Ye X, Lemmey HAL, Borysova L, Dora KA: **Voltage-dependent Ca²⁺ entry into smooth muscle during contraction promotes endothelium-mediated feedback vasodilation in arterioles.** *Sci Signal* 2017, **10**:1-14, <https://doi.org/10.1126/scisignal.aal3806>
 21. Garland CJ, Dora KA: **EDH: endothelium-dependent hyperpolarization and microvascular signaling.** *Acta Physiol* 2017, **219**:152-161, <https://doi.org/10.1111/apha.12649>
 22. Garland CJ, Dora KA: **Endothelium-dependent hyperpolarization: the evolution of myoendothelial microdomains.** *J Cardiovasc Pharmacol* 2021, **78**:S3-S12, <https://doi.org/10.1097/FJC.0000000000001087>
 23. Garland CJ, McPherson GA: **Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery.** *Br J Pharmacol* 1992, **105**:429-435, <https://doi.org/10.1111/j.1476-5381.1992.tb14270.x>
 24. Garland CJ, Weston AH: **The vascular endothelium: still amazing us 30 years on.** *Br J Pharmacol* 2011, **164**:837-838, <https://doi.org/10.1111/j.1476-5381.2011.01611.x>
 25. Hariharan A, Robertson CD, Garcia DCG, Longden TA: **Brain capillary pericytes are metabolic sentinels that control blood flow through a K_{ATP} channel-dependent energy switch.** *Cell Rep* 2022, **41**:111872, <https://doi.org/10.1016/j.celrep.2022.111872>.
This study elegantly brings together many concepts to show how metabolic demand can open K_{ATP} channels in capillary pericytes to hyperpolarize capillary ECs. This link between low ATP levels and improving blood flow adds to the possible activation of K_{ATP} channels in vascular ECs and SMCs, and excitable cells near arterioles and capillaries. How the signal from pericytes reaches arterioles can be via many pathways, including coupling through ECs and the pericyte network.
 26. Harraz OF, Klug NR, Senatore AJ, Hill-Eubanks DC, Nelson MT: **Piezo1 is a mechanosensor channel in central nervous system capillaries.** *Circ Res* 2022, **130**:1531-1546, <https://doi.org/10.1161/CIRCRESAHA.122.320827>.
This study takes the concept of Piezo1 channels to capillary ECs. There is much to do to take this forward, including establishing the overall change in capillary EC membrane potential and how the subsequent signal manifests to change blood flow. If negative pressure activates cEC Piezo1, what turns it off and how do passing blood cells and the capillary glycocalyx influence this signal?
 27. Heberlein KR, Straub AC, Best AK, Greyson MA, Looft-Wilson RC, Sharma PR, Meher A, Leitinger N, Isakson BE: **Plasminogen activator inhibitor-1 regulates myoendothelial junction formation.** *Circ Res* 2010, **106**:1092-1102, <https://doi.org/10.1161/CIRCRESAHA.109.215723>
 28. Kaski JC, Crea F, Gersh BJ, Camici PG: **Reappraisal of ischemic heart disease.** *Circulation* 2018, **138**:1463-1480, <https://doi.org/10.1161/CIRCULATIONAHA.118.031373>
 29. Ledoux J, Taylor MS, Bonev AD, Hannah RM, Solodushko V, Shui B, Tallini Y, Kotlikoff ML, Nelson MT: **Functional architecture of inositol 1,4,5-trisphosphate signaling in restricted spaces of myoendothelial projections.** *Proc Natl Acad Sci USA* 2008, **105**:9627-9632, <https://doi.org/10.1073/pnas.0801963105>
 30. Lemmey HAL, Garland CJ, Dora KA: **Intrinsic regulation of microvascular tone by myoendothelial feedback circuits.** *Curr Top Membr* 2020, **85**:327-355, <https://doi.org/10.1016/bs.ctm.2020.01.004>
 31. Leurgans TM, Bloksgaard M, Brewer JR, Bagatolli LA, Fredgart MH, Rosenstand K, Hansen ML, Rasmussen LM, Irmukhamedov A, De Mey JG: **Endothelin-1 shifts the mediator of bradykinin-induced relaxation from NO to H₂O₂ in resistance arteries from patients with cardiovascular disease.** *Br J Pharmacol* 2016, **173**:1653-1664, <https://doi.org/10.1111/bph.13467>
 32. Longden TA, Dabertrand F, Koide M, Gonzales AL, Tykocki NR, Brayden JE, Hill-Eubanks D, Nelson MT: **Capillary K⁺-sensing initiates retrograde hyperpolarization to increase local cerebral blood flow.** *Nat Neurosci* 2017, **20**:717-726, <https://doi.org/10.1038/nn.4533>
 33. Mather S, Dora KA, Sandow SL, Winter P, Garland CJ: **Rapid endothelial cell-selective loading of connexin 40 antibody blocks endothelium-derived hyperpolarizing factor dilation in rat small mesenteric arteries.** *Circ Res* 2005, **97**:399-407, <https://doi.org/10.1161/01.RES.0000178008.46759.d0>.
This is the only study showing selective block of MEJs in arteries, achieved by pinocytic loading of connexin-specific antibodies into ECs of pressurized arteries. It also shows how K⁺ acting as an EDHF acts parallel to myoendothelial electrical coupling.
 34. McGahren ED, Beach JM, Duling BR: **Capillaries demonstrate changes in membrane potential in response to pharmacological stimuli.** *Am J Physiol* 1998, **274**:H60-H65, <https://doi.org/10.1152/ajpheart.1998.274.1.H60>
 35. Milkau M, Kohler R, de Wit C: **Crucial importance of the endothelial K⁺ channel SK3 and connexin40 in arteriolar dilations during skeletal muscle contraction.** *FASEB J* 2010, **24**:3572-3579, <https://doi.org/10.1096/fj.10-158956>
 36. Moshkforoush A, Ashenagar B, Harraz OF, Dabertrand F, Longden TA, Nelson MT, Tsoukias NM: **The capillary Kir channel as sensor and amplifier of neuronal signals: modeling insights on K⁺-mediated neurovascular communication.** *Proc Natl Acad Sci USA* 2020, **117**:16626-16637, <https://doi.org/10.1073/pnas.2000151117>
 37. Murrant CL, Sarelius IH: **Local and remote arteriolar dilations initiated by skeletal muscle contraction.** *Am J Physiol* 2000, **279**:H2285-H2294, <https://doi.org/10.1152/ajpheart.2000.279.5.H2285>
 38. Ottolini M, Daneva Z, Chen YL, Cope EL, Kasetti RB, Zode GS, Sonkusare SK: **Mechanisms underlying selective coupling of endothelial Ca²⁺ signals with eNOS vs. IK/SK channels in systemic and pulmonary arteries.** *J Physiol* 2020, **598**:3577-3596, <https://doi.org/10.1113/JP279570>
 39. Rivers R: **Conducted arteriolar dilations persist in the presence of nitroarginine.** *J Cardiovasc Pharmacol* 1997, **30**:309-312, <https://doi.org/10.1097/00005344-199709000-00006>
 40. Rivers RJ, Hein TW, Zhang C, Kuo L: **Activation of barium-sensitive inward rectifier potassium channels mediates remote dilation of coronary arterioles.** *Circulation* 2001, **104**:1749-1753, <https://doi.org/10.1161/hc4001.098053>

41. Sandow SL, Hill CE: **Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses.** *Circ Res* 2000, **86**:341-346, <https://doi.org/10.1161/01.res.86.3.341>
42. Sandow SL, Neylon CB, Chen MX, Garland CJ: **Spatial separation of endothelial small- and intermediate-conductance calcium-activated potassium channels (K_{Ca}) and connexins: possible relationship to vasodilator function?** *J Anat* 2006, **209**:689-698, <https://doi.org/10.1111/j.1469-7580.2006.00647.x>
43. Sandow SL, Tare M, Coleman HA, Hill CE, Parkington HC: **Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor.** *Circ Res* 2002, **90**:1108-1113, <https://doi.org/10.1161/01.res.0000019756.88731.83>
44. Segal SS: **Regulation of blood flow in the microcirculation.** *Microcirculation* 2005, **12**:33-45, <https://doi.org/10.1080/10739680590895028>
45. Senadheera S, Kim Y, Grayson TH, Toemoe S, Kochukov MY, Abramowitz J, Housley GD, Bertrand RL, Chadha PS, Bertrand PP, Murphy TV, Tare M, Birnbaumer L, Marrelli SP, Sandow SL: **Transient receptor potential canonical type 3 channels facilitate endothelium-derived hyperpolarization-mediated resistance artery vasodilator activity.** *Cardiovasc Res* 2012, **95**:439-447, <https://doi.org/10.1093/cvr/cvs208>
46. Shu X, Ruddiman CA, St Keller TC, Keller AS, Yang Y, Good ME, Best AK, Columbus L, Isakson BE: **Heterocellular contact can dictate arterial function.** *Circ Res* 2019, **124**:1473-1481, <https://doi.org/10.1161/CIRCRESAHA.118.313926>
47. Smith JF, Lemmey HAL, Borysova L, Hiley CR, Dora KA, Garland CJ: **Endothelial nitric oxide suppresses action-potential-like transient spikes and vasospasm in small resistance arteries.** *Hypertension* 2020, **76**:785-794, <https://doi.org/10.1161/HYPERTENSIONAHA.120.15491>
48. Sonkusare SK, Bonev AD, Ledoux J, Liedtke W, Kotlikoff MJ, Heppner TJ, Hill-Eubanks DC, Nelson MT: **Elementary Ca^{2+} signals through endothelial TRPV4 channels regulate vascular function.** *Science* 2012, **336**:597-601, <https://doi.org/10.1126/science.1216283>
49. Sonkusare SK, Dalsgaard T, Bonev AD, Hill-Eubanks DC, Kotlikoff MJ, Scott JD, Santana LF, Nelson MT: **AKAP150-dependent cooperative TRPV4 channel gating is central to endothelium-dependent vasodilation and is disrupted in hypertension.** *Sci Signal* 2014, **7**:ra66, <https://doi.org/10.1126/scisignal.2005052>
50. Straub AC, Lohman AW, Billaud M, Johnstone SR, Dwyer ST, Lee MY, Bortz PS, Best AK, Columbus L, Gaston B, Isakson BE: **Endothelial cell expression of haemoglobin alpha regulates nitric oxide signalling.** *Nature* 2012, **491**:473-477, <https://doi.org/10.1038/nature11626>
51. Takano H, Dora KA, Spitaler MM, Garland CJ: **Spreading dilatation in rat mesenteric arteries associated with calcium-independent endothelial cell hyperpolarization.** *J Physiol* 2004, **556**:887-903, <https://doi.org/10.1113/jphysiol.2003.060343>
52. Thakore P, Alvarado MG, Ali S, Mughal A, Pires PW, Yamasaki E, Pritchard HA, Isakson BE, Tran CHT, Earley S: **Brain endothelial cell TRPA1 channels initiate neurovascular coupling.** *Elife* 2021, **10**, <https://doi.org/10.7554/eLife.63040>
53. Waldron GJ, Garland CJ: **Effect of potassium channel blockers on L-NAME insensitive relaxations in rat small mesenteric arteries.** *Can J Physiol Pharmacol* 1994, **72**:115.
54. Welsh DG, Tran CHT, Hald BO, Sancho M: **The conducted vasomotor response: function, biophysical basis, and pharmacological control.** *Annu Rev Pharmacol Toxicol* 2018, **58**:391-410, <https://doi.org/10.1146/annurev-pharmtox-010617-052623>
55. Winter P, Dora KA: **Spreading dilatation to luminal perfusion of ATP and UTP in rat isolated small mesenteric arteries.** *J Physiol* 2007, **582**:335-347, <https://doi.org/10.1113/jphysiol.2007.135202>
56. Yamamoto Y, Imaeda K, Suzuki H: **Endothelium-dependent hyperpolarization and intercellular electrical coupling in guinea-pig mesenteric arterioles.** *J Physiol* 1999, **514**:505-513, <https://doi.org/10.1111/j.1469-7793.1999.505ae.x>
57. Zhao G, Joca HC, Nelson MT, Lederer WJ: **ATP- and voltage-dependent electro-metabolic signaling regulates blood flow in heart.** *Proc Natl Acad Sci USA* 2020, **117**:7461-7470, <https://doi.org/10.1073/pnas.1922095117>