frontiers in Physiology

REVIEW
published: 08 February 2022
doi: 10.3389/fphys.2022.805149

Endothelial Ion Channels and Cell-Cell Communication in the Microcirculation

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Endothelial cells in resistance arteries, arterioles, and capillaries express a diverse array of ion channels that contribute to Cell-Cell communication in the microcirculation. Endothelial cells are tightly electrically coupled to their neighboring endothelial cells by gap junctions allowing ion channel-induced changes in membrane potential to be conducted for considerable distances along the endothelial cell tube that lines arterioles and forms capillaries. In addition, endothelial cells may be electrically coupled to overlying smooth muscle cells in arterioles and to pericytes in capillaries *via* heterocellular gap junctions allowing electrical signals generated by endothelial cell ion channels to be transmitted to overlying mural cells to affect smooth muscle or pericyte contractile activity. Arteriolar endothelial cells express inositol 1,4,5 trisphosphate receptors (IP_3Rs) and transient receptor vanilloid family member 4 (TRPV4) channels that contribute to agonist-induced endothelial Ca^{2+} signals. These Ca^{2+} signals then activate intermediate and small conductance Ca^{2+}-activated K^+ (IK_{Ca} and SK_{Ca}) channels causing vasodilator-induced endothelial hyperpolarization. This hyperpolarization can be conducted along the endothelium *via* homocellular gap junctions and transmitted to overlying smooth muscle cells through heterocellular gap junctions to control the activity of voltage-gated Ca^{2+} channels and smooth muscle or pericyte contraction. The IK_{Ca}- and SK_{Ca}-induced hyperpolarization may be amplified by activation of inward rectifier K^+ (K_{IR}) channels. Endothelial cell IP_3R- and TRPV4-mediated Ca^{2+} signals also control the production of endothelial cell vasodilator autacoids, such as NO, PGI_2, and epoxides of arachidonic acid contributing to control of overlying vascular smooth muscle contractile activity. Cerebral capillary endothelial cells lack IK_{Ca} and SK_{Ca} but express K_{IR} channels, IP_3R, TRPV4, and other Ca^{2+} permeable channels allowing capillary-to-arteriole signaling *via* hyperpolarization and Ca^{2+}. This allows parenchymal cell signals to be detected in capillaries and signaled to upstream arterioles to control blood flow to capillaries by active parenchymal cells. Thus, endothelial cell ion channels importantly participate in several forms of Cell-Cell communication in the microcirculation that contribute to microcirculatory function and homeostasis.

Keywords: endothelial ion channels and cell-cell communication ion channels, endothelial cells, arterioles, vascular smooth muscle cells, pericytes, capillaries, blood flow, functional hyperemia

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Specialty section:
This article was submitted to Vascular Physiology, a section of the journal Frontiers in Physiology

Received: *29 October 2021*
Accepted: *05 January 2022*
Published: *08 February 2022*

Citation:
Jackson WF (2022) Endothelial Ion Channels and Cell-Cell Communication in the Microcirculation. Front. Physiol. 13:805149. doi: 10.3389/fphys.2022.805149

INTRODUCTION

The microcirculation is the business-end of the cardiovascular system. It is here that oxygen, substrates, hormones, etc. are supplied to the parenchymal cells to meet their metabolic and other physiological demands (Durán et al., 2011). In most tissues and organs, blood flow to the microcirculation is regulated to exactly match the metabolic demands of the perfused parenchyma such that there is a linear relationship between tissue oxygen consumption (metabolic demand) and tissue blood flow (supply of oxygen and other substrates; Laughlin et al., 2011). This tight coupling between blood flow and tissue metabolism strongly depends on cell-cell communication among the cells that comprise the blood vessels that make up the microcirculation as well as the surrounding parenchymal cells. This perspective will focus on the function of resistance artery, arteriolar, and capillary endothelial cell ion channels and how they contribute to cell-cell communication involved in control of blood flow in the microcirculation.

Endothelial cells express a diverse array of ion channels that importantly contribute to their function and especially cell-cell communication (Jackson, 2016; Garcia and Longden, 2020). These vascular cells are tightly coupled by homocellular (EC-EC) gap junctions such that they function as an electrical syncytium allowing changes in endothelial cell membrane potential to be conducted long distances (millimeters) along capillaries and arterioles in the microcirculation (de Wit and Griffith, 2010; Socha et al., 2012a). Endothelial cells also are electrically coupled to overlying smooth muscle cells (in arterioles and resistance arteries) or pericytes (in capillaries) by heterocellular gap junctions, modulating the membrane potential and contraction of these mural cells to control microvascular blood flow (de Wit and Griffith, 2010; Socha et al., 2012a; Molica et al., 2018). Ion channels in endothelial cell plasma and smooth endoplasmic reticulum membranes are responsible for generating ionic currents that either produce or modulate membrane potential, an important signal for cell-cell communication in the microcirculation (de Wit and Griffith, 2010; Socha et al., 2012a). In addition, membrane potential affects the electrochemical gradient for diffusion of ions through all ion channels (Nilius and Droogmans, 2001). Thus, membrane hyperpolarization, for example, will augment Ca^{2+} influx through Ca^{2+} permeable channels, whereas membrane depolarization will have the opposite effect.

Intracellular Ca^{2+} is another signal for cell-cell communication that is generated by ion channels (de Wit and Griffith, 2010; Edwards et al., 2010; Socha et al., 2012a; Jackson, 2016). Calcium signals can be transmitted through gap junctions to neighboring cells (de Wit and Griffith, 2010; Socha et al., 2012a,b) and, in endothelial cells, intracellular Ca^{2+} controls the production of endothelial cell autacoids (NO, PGI_2, epoxides of arachidonic acid, H_2O_2, etc.) that themselves are important signals for cell-cell communication in the wall of microvessels (Edwards et al., 2010; Socha et al., 2012a; Jackson, 2016). Thus, endothelial cell ion channels play a central role in cell-cell communication and regulation of blood flow in the microcirculation.

The functional "unit" of the microcirculation consists of a terminal arteriole, that branches from a network of feed arterioles, and the capillaries perfused by the terminal arteriole (**Figure 1**). The terminal arteriole is invested with a single layer of contractile vascular smooth muscle cells surrounding an endothelial cell tube (**Figure 1**). The terminal arteriole transitions and branches into 10–20 capillaries that form the capillary bed (**Figure 1**). In the cerebral microcirculation, the initial capillary segment consists of an endothelial cell tube coated with contractile pericytes as shown in **Figure 1** (Gonzales et al., 2020; Longden et al., 2021; Thakore et al., 2021). Blood flow to the capillaries is controlled both by smooth muscle-induced changes in the diameter of the terminal arterioles (Delashaw and Duling, 1988) and changes in the contractile activity of the pericytes in the initial segment (Longden et al., 2017, 2021; Thakore et al., 2021). Contractile pericytes located at branch points allow fine tuning of blood flow to capillaries adjacent to metabolically active parenchymal cells (neurons and glial cells in the case of the brain microcirculation; Gonzales et al., 2020; Longden et al., 2021). More distal portions of capillaries lose their coat of contractile pericytes and instead are associated with occasional pericytes that are non-contractile (not shown in **Figure 1**; Gonzales et al., 2020; Longden et al., 2021; Thakore et al., 2021). Capillaries then coalesce into venules that are invested with pericytic smooth muscle cells, rather than the circumferentially oriented arteriolar smooth muscle cells (Jackson, 2012). The remainder of this perspective will focus on endothelial cell ion channels and cell-cell signaling between adjacent endothelial cells and overlying smooth muscle cells in arterioles (**Figure 2**) and between capillary endothelial cells, arteriolar endothelial cells, and overlying pericytes or smooth muscle cells (**Figures 3–5**).

ARTERIOLAR ENDOTHELIAL CELL ION CHANNELS AND CELL-CELL COMMUNICATION

Excellent examples of cell-cell communication in the arteriolar wall that depend on endothelial ion channels are endothelium-dependent, agonist-induced vasodilation, myoendothelial feedback, and shear-stress-dependent vasodilation. The primary ion channels involved in these three processes are displayed in **Figure 2**.

Endothelium-dependent, agonist-induced vasodilation is initiated when vasodilator agonists bind to and activate $G\alpha_q$-coupled receptors, which, in turn, activate phospholipase C β (PLCβ), leading to formation of inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG) from membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) stores (Berridge, 1993; **Figure 2**). The formed IP_3 binds to IP_3 receptors (IP_3R) in the endoplasmic reticulum membrane, sensitizing them to activation by cytosolic Ca^{2+} to cause Ca^{2+}-induced Ca^{2+}-release (CICR; Foskett et al., 2007; Mak and Foskett, 2015; **Figure 2**).

Local subplasmalemmal increases in Ca^{2+} from this process, along with DAG- and Ca^{2+}-activated protein kinase C (PKC; Sonkusare et al., 2012) and reduced membrane PIP_2 levels (Harraz et al., 2018) activate clusters of transient receptor

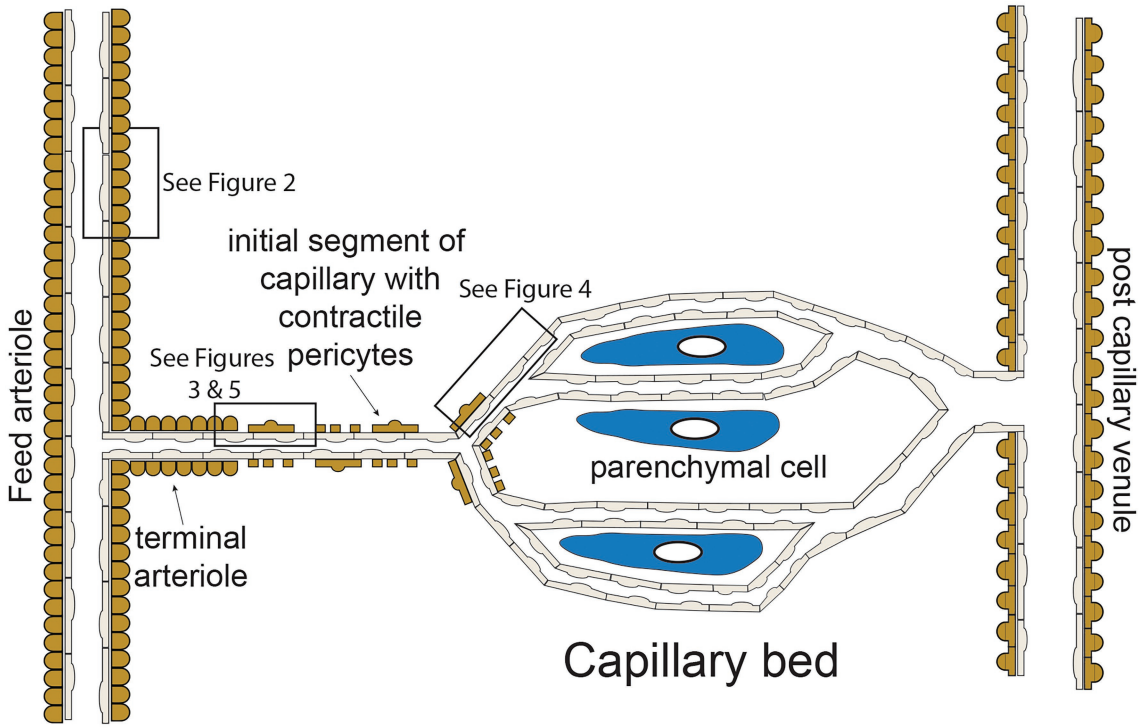

FIGURE 1 | Microvascular unit—schematic representation of hypothetical microvascular unit that consists of a feed arteriole that branches into a terminal arteriole. The terminal arteriole transitions into an initial capillary segment that is coated with contractile pericytes including at the initial branch points, as shown. As the capillaries progress, they lose coverage by contractile pericytes which are replaces by non-contractile pericytes (not shown in the figure for clarity). As shown, the capillaries are in close association with parenchymal cells. The capillaries then converge and eventually drain into venules, which are coated in pericytic smooth muscle cells that are morphologically distinct from arteriolar smooth muscle cells. Boxes in the figure depict regions corresponding to **Figures 2–5** as shown. See text for details and references.

potential vanilloid family member 4 (TRPV4) channels located in myoendothelial projections (MEPs), the site of myoendothelial gap junctions (MEGJs; Earley, 2011; Sonkusare et al., 2012, 2014). In cerebral arterioles, TRPA1 channels also are located in this microdomain (Earley et al., 2009; Qian et al., 2013; Sullivan et al., 2015). Calcium influx through TRPV4 (or TRPA1) channels, visualized as Ca^{2+} sparklets (Sullivan and Earley, 2013), is amplified by CICR through IP_3R resulting in MEP-localized increases in $[Ca^{2+}]_{in}$ termed Ca^{2+} pulsars (Ledoux et al., 2008), which can propagate into larger IP_3R-mediated Ca^{2+} wavelets (Tran et al., 2012) and even larger whole-cell-based Ca^{2+} waves (Duza and Sarelius, 2004; Kansui et al., 2008; Socha et al., 2012b).

Release of Ca^{2+} through IP_3R from the endoplasmic reticulum stimulates Ca^{2+} influx through several plasma membrane cation channels that conduct Ca^{2+} (Jackson, 2016). The loss of Ca^{2+} from the endoplasmic reticulum is sensed by stromal interacting molecule 1 (STIM1) resulting in activation of plasma membrane channels composed of ORAI1 channels (Abdullaev et al., 2008; Sundivakkam et al., 2012; Zhou et al., 2014) likely in combination with transient receptor potential C family member 1 (TRPC1; Mehta et al., 2003; Ahmmed et al., 2004; Kwiatek et al., 2006; Sundivakkam et al., 2009, 2012) and/or TRPC4 channels (Freichel et al., 2001; Tiruppathi et al., 2002; Sundivakkam et al., 2012) providing a source of $[Ca^{2+}]_{in}$ to help refill endoplasmic reticulum Ca^{2+} stores *via* smooth endoplasmic reticulum Ca^{2+} transporter (SERCA). The agonist-induced DAG production also can activate plasma membrane TRPC3 (Senadheera et al., 2012; Kochukov et al., 2014) and TRPC6 channels (Loga et al., 2013) that can contribute to agonist-induced increases in $[Ca^{2+}]_{in}$.

The agonist-induced increases in $[Ca^{2+}]_{in}$ mediated by TRPV4 channels (or TRPA1 channels), IP_3R, and other ion channels activate IK_{Ca} which tend to cluster at MEPs in macromolecular signaling domains (Sonkusare et al., 2012), and SK_{Ca} channels that are more broadly distributed around the periphery of endothelial cells, resulting in K^+ efflux from the cells and membrane hyperpolarization (Jackson, 2016). Agonist-induced activation of TRPC3 channels may also, in some endothelial cells, specifically activate SK_{Ca} channels that likewise are found in signaling domains in the cell membrane (Kochukov et al., 2014).

The SK_{Ca}- and IK_{Ca}-initiated hyperpolarization then can be transmitted to overlying smooth muscle cells through MEGJs, deactivating voltage-gated Ca^{2+} channels, resulting in vasodilation (Earley, 2011). Endothelial cell (and vascular smooth muscle cell) inwardly rectifying K^+ (K_{IR}) channels are activated by the SK_{Ca}- and IK_{Ca}-initiated hyperpolarization, amplifying the hyperpolarization (Sonkusare et al., 2016; Jackson, 2017).

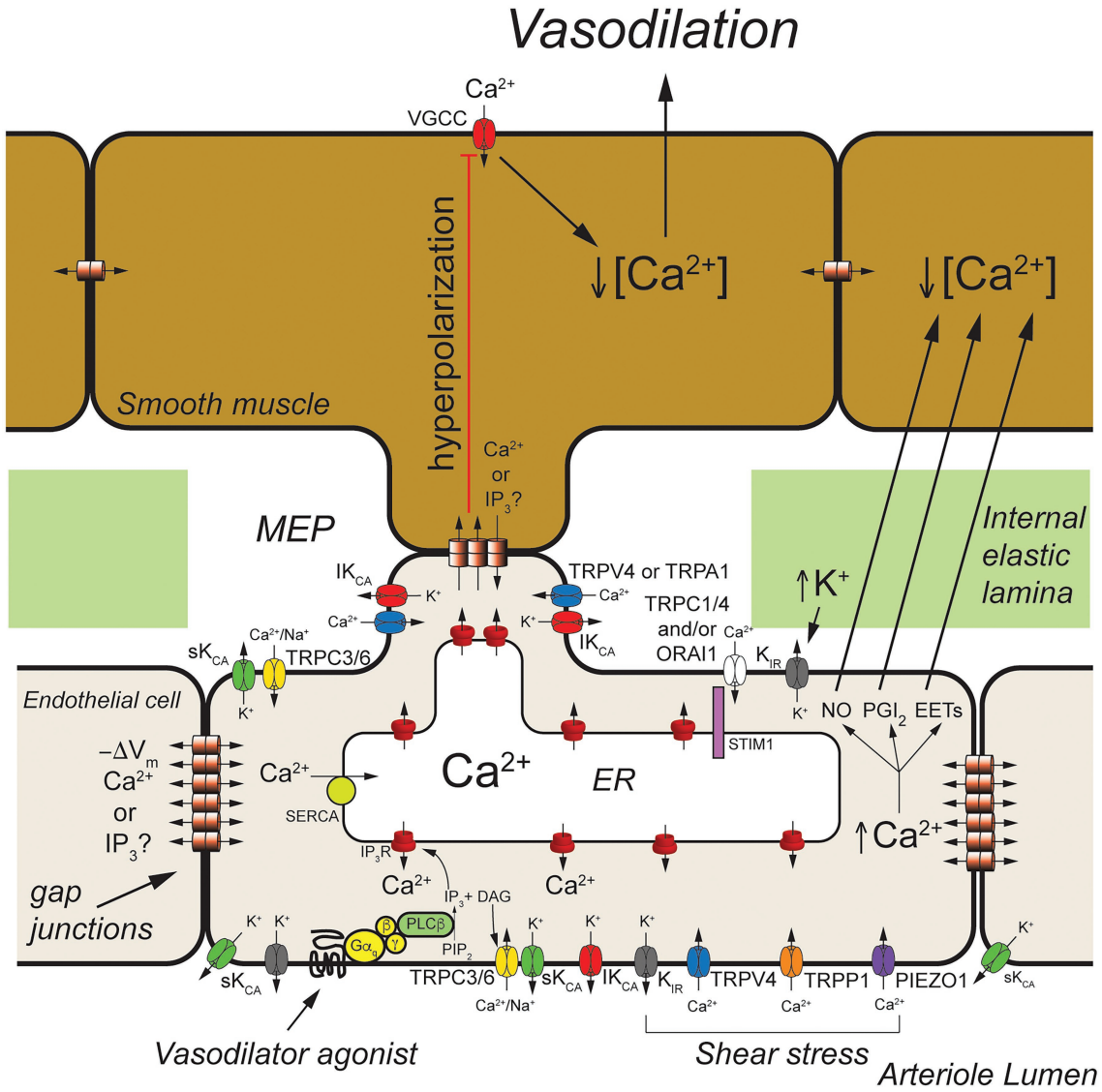

FIGURE 2 | Arteriolar endothelial ion channels and cell-cell communication. Shown is a schematic representation of a longitudinal cross-section through one wall of an arteriole showing cross-sections of endothelial cells and overlying smooth muscle cells. Endothelial cells communicate with overlying smooth muscle cells at myoendothelial projections (MEPs) that pass through the internal elastic lamina to make contact with overlying smooth muscle cells, as shown. Gap junctions may form at MEPs to yield myoendothelial gap junctions (MEGJs) allowing endothelial cell hyperpolarization to be conducted to the smooth muscle cells, closing smooth muscle voltage-gated Ca^{2+} channels (VGCCs), decreasing $[Ca^{2+}]_{in}$, and leading to vasodilation. At MEPs transient receptor potential vanilloid family member 4 (TRPV4), intermediate conductance Ca^{2+}-activated K^+ (IK_{Ca}) channels and inositol 1,4,5 trisphosphate receptors (IP_3R) are clustered forming signaling complexes to direct the endothelial cell responses to vasodilator agonists. In cerebral arteries/arterioles, transient receptor potential ankyrin family member 1 (TRPA1) is also in these complexes. Other ion channels, such as transient receptor potential C family member 3 (TRPC3) channels and small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels, may cluster elsewhere to form other signaling complexes. Endothelium-dependent vasodilators, such as acetyl choline, act on $G\alpha_q$-coupled receptors to activate phospholipase C-β (PLCβ) which hydrolyses membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) forming IP_3 and diacylglycerol (DAG). The IP_3 activates IP_3Rs to release Ca^{2+} from the ER increasing $[Ca^{2+}]_{in}$. The DAG activates membrane TRPC3 and TRPC6 channels which serve as receptor-operate channels conducting Na^+ and Ca^{2+} into the cells. DAG also activates protein kinase C (PKC; not shown) which phosphorylates and activates TRPV4 channels. Calcium influx through TRPV4 channels acts on IP_3-sensitized IP_3R, amplifying Ca^{2+} influx through the TRPV4 channels. At MEP, the increase $[Ca^{2+}]_{in}$ results in activation of IK_{Ca} channels causing membrane hyperpolarization ($-\Delta V_m$) that is transmitted through MEGJs to hyperpolarize overlying smooth muscle causing vasodilation. Transient receptor potential C family members 1 and 4 (TRPC1 and TRPC4), and ORAI1 channels are activated upon release of Ca^{2+} from the endoplasmic reticulum (ER) *via* IP_3Rs that is sensed by stromal interaction molecule 1 (STIM1) in the ER membrane. Inward rectifier K^+ (K_{IR}) channels (likely $K_{IR}2.1$) are expressed and can be activated by membrane hyperpolarization to facilitate hyperpolarization induced by SK_{Ca} and IK_{Ca} channels. They also are activated by increases in extracellular K^+ and involved with sensing shear stress. Shear stress also appears to activate TRPV4 channels, transient receptor potential polycystin family member 1 (TRPP1) channels, and PIEZO1 channels leading to increased endothelial $[Ca^{2+}]_{in}$ and vasodilation. In addition to activating SK_{Ca} and IK_{Ca} channels to produce hyperpolarization-induced vasodilation, increased endothelial cell $[Ca^{2+}]_{in}$ also results in production of endothelial cell vasodilator autacoids, such as nitric oxide (NO), prostacyclin (PGI_2), and epoxides of arachidonic acid (EETs) that cause arteriolar vasodilation. See text for additional information and references.

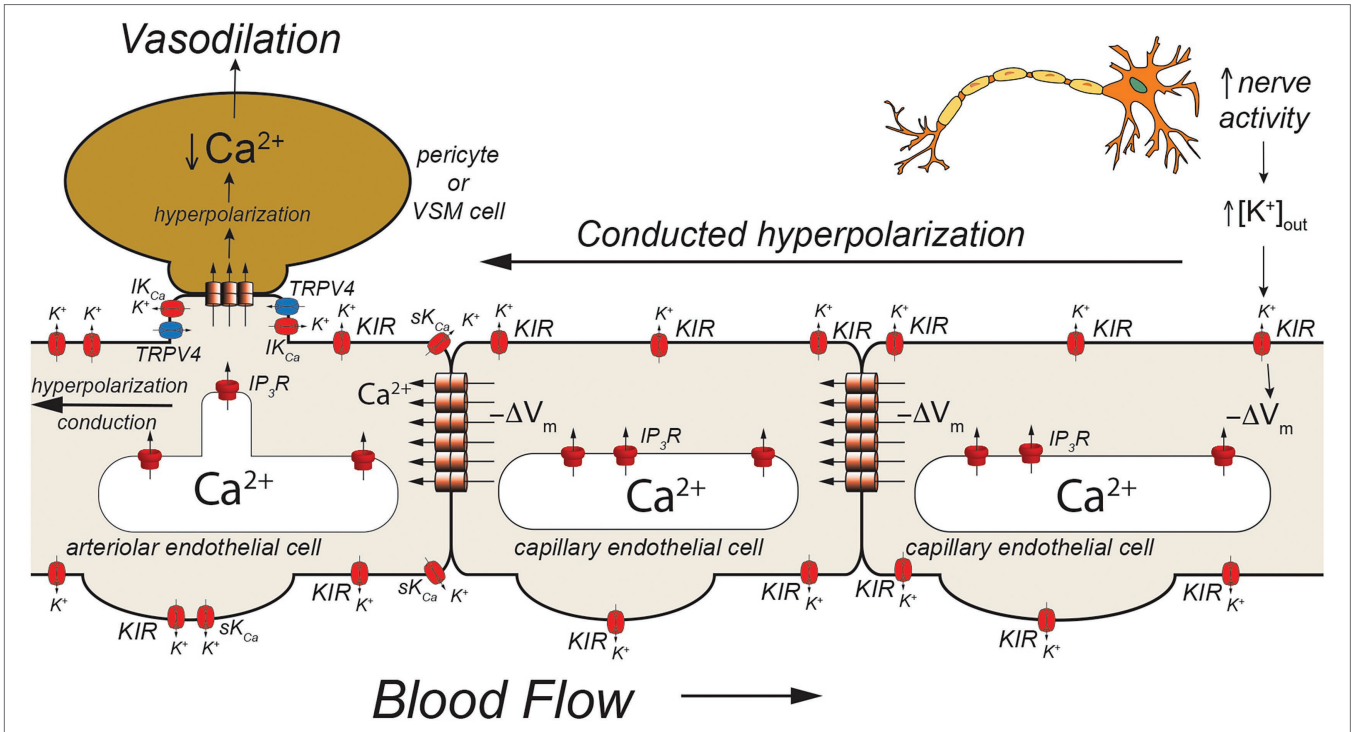

FIGURE 3 | Capillaries as sensors of $[K^+]_{out}$. Schematic of a capillary segment that transitions to a vessel invested with contractile pericytes (initial capillary segment) or smooth muscle cells (terminal arteriole). Active neurons release K^+ during action potential repolarization. This increases $[K^+]_{out}$ which is sensed by $K_{IR}2.1$ channels (KIR). Movement of K^+ out of activated $K_{IR}2.1$ channels results in membrane hyperpolarization ($-\Delta V_m$). The local membrane hyperpolarization activates adjacent $K_{IR}2.1$ channels allowing this hyperpolarization to be conducted from the site of initiation through homocellular gap junctions from endothelial cell-to-endothelial cell back toward the initial capillary segment (invested with contractile pericytes) and the terminal arteriole (with smooth muscle cells). These contractile mural cells are coupled to underlying endothelial cells by heterocellular gap junctions which allow transmission of the hyperpolarization to the contractile cells. In smooth muscle cells (and presumably contractile pericytes), the hyperpolarization will deactivate voltage-gated Ca^{2+} channels, decreasing $[Ca^{2+}]_{in}$ and leading to smooth muscle (or pericyte) relaxation and vasodilation. Dilation of the terminal arteriole (or the initial capillary segment) will result in an increase in blood flow to the microvascular unit, directing blood flow to the site of increased neural activity.

The SK_{Ca}- and IK_{Ca}-initiated hyperpolarization also increases the electrochemical gradient driving Ca^+ influx through TRPV4 (and all cation channels) another means of amplifying this signaling pathway (Qian et al., 2014).

Because endothelial cells are tightly coupled by homocellular gap junctions (de Wit and Griffith, 2010; Bagher and Segal, 2011), and because of the amplifying effect of K_{IR} channels (Jackson, 2017), endothelial cell hyperpolarization initiated at one end of an arteriole, for example, can be conducted for mm distances along the arteriole which is the electrical basis of the rapid conducted vasodilation that is observed in the microcirculation (de Wit and Griffith, 2010; Bagher and Segal, 2011; Socha et al., 2012a). The arteriolar endothelial cell Ca^{2+} signal also appears to be able to be transmitted from endothelial cell-to-endothelial cell but with a much slower time course (Domeier and Segal, 2007; Uhrenholt et al., 2007; Socha et al., 2012b). The signal (Ca^{2+} and/or IP_3) that moves through endothelial cell homocellular gap junctions to transmit the Ca^{2+} signal from cell-to-cell has not been established.

In addition to activation of SK_{Ca}- and IK_{Ca}-initiated hyperpolarization, increased $[Ca^{2+}]_{in}$ will activate production and release of endothelial cell autacoids including nitric oxide (NO), prostaglandin I_2 (prostacyclin; PGI_2), epoxides of arachidonic acid (EETs), and hydrogen peroxide (H_2O_2; Edwards et al., 2010). These can diffuse to overlying smooth muscle cells and induce smooth muscle relaxation and vasodilation through mechanisms that include activation of smooth muscle K^+ channels (Tykocki et al., 2017). It also should be noted that K^+-efflux from endothelial cells *via* SK_{Ca} and IK_{Ca} can raise extracellular $[K^+]$ and activate smooth muscle K_{IR} channels (Edwards et al., 1998) and the Na^+/K^+ ATPase (Weston et al., 2002) contributing to endothelium-dependent smooth muscle hyperpolarization and vasodilation in some arteries.

Ex vivo studies of isolated resistance arteries and arterioles suggest that the SK_{Ca}- and IK_{Ca}-initiated endothelial cell hyperpolarization and MEGJ-mediated smooth muscle relaxation and vasodilation are the major form of cell-cell communication during endothelium-dependent vasodilation in these vessels (Edwards et al., 2010). *In vitro* studies of isolated vessels in which endothelial cell and vascular smooth muscle cell membrane potential were measured simultaneously have clearly shown electrical coupling between endothelial cells and smooth muscle cells (Emerson and Segal, 2000, 2001; Yamamoto et al., 2001). However, *in vivo* studies of arteriolar endothelium-dependent vasodilation have suggested that this myoendothelial electrical coupling may not be as prevalent (de Wit et al., 2008;

Schmidt and de Wit, 2020). For example, in mouse cremaster muscle arterioles studied by intravital microscopy, acetylcholine-induced hyperpolarization of endothelial cells was inhibited by apamin, but unaffected by iberiotoxin, whereas hyperpolarization of smooth muscle cells was inhibited by apamin (although to a lesser extent than the endothelium) and iberiotoxin (Siegl et al., 2005). If endothelial cells and smooth muscle cells were tightly electrically coupled, apamin should have equally blocked hyperpolarization of both cell types. That iberiotoxin inhibited smooth muscle hyperpolarization suggests that some signal other than hyperpolarization is being transmitted to the smooth muscle cells to activate BK_{Ca} channels. Other studies refute these findings and argue that endothelial cells and vascular smooth muscle cells are well electrically coupled (Wolfle et al., 2011). Additional experiments will be required to resolve these conflicting findings.

Endothelial ion channels and cell-cell communication also are involved in myoendothelial feedback. In arterioles and resistance arteries, increases in smooth muscle tone induced by vasoconstrictors result in an increase in endothelial cell $[Ca^{2+}]_{in}$, activation of IK_{Ca} and SK_{Ca} channels to hyperpolarize the endothelial cells, and activation of production of NO and likely other endothelial cell Ca^{2+}-dependent vasodilator autacoids [see Lemmey et al. (2020) for a detailed review of this topic]. The hyperpolarization is transmitted back to the smooth muscle through MEGJs, deactivating smooth muscle voltage-gated Ca^{2+} channels, and blunting the vasoconstrictor-induced smooth muscle tone. Endothelium-derived NO (or other vasodilator autacoids) likewise feedback to the smooth muscle limiting vasoconstrictor-induced smooth muscle tone. Studies in mouse mesenteric arteries have shown that vasoconstrictors activate endothelial TRPV4 channels located in MEPs producing TRPV4 Ca^{2+} sparklets that activate MEP-localized IK_{Ca} channels to hyperpolarize the endothelial cells. Calcium release through IP_3Rs (also located in MEPs) was implicated in the activation of TRPV4 channels during this process (Hong et al., 2018).

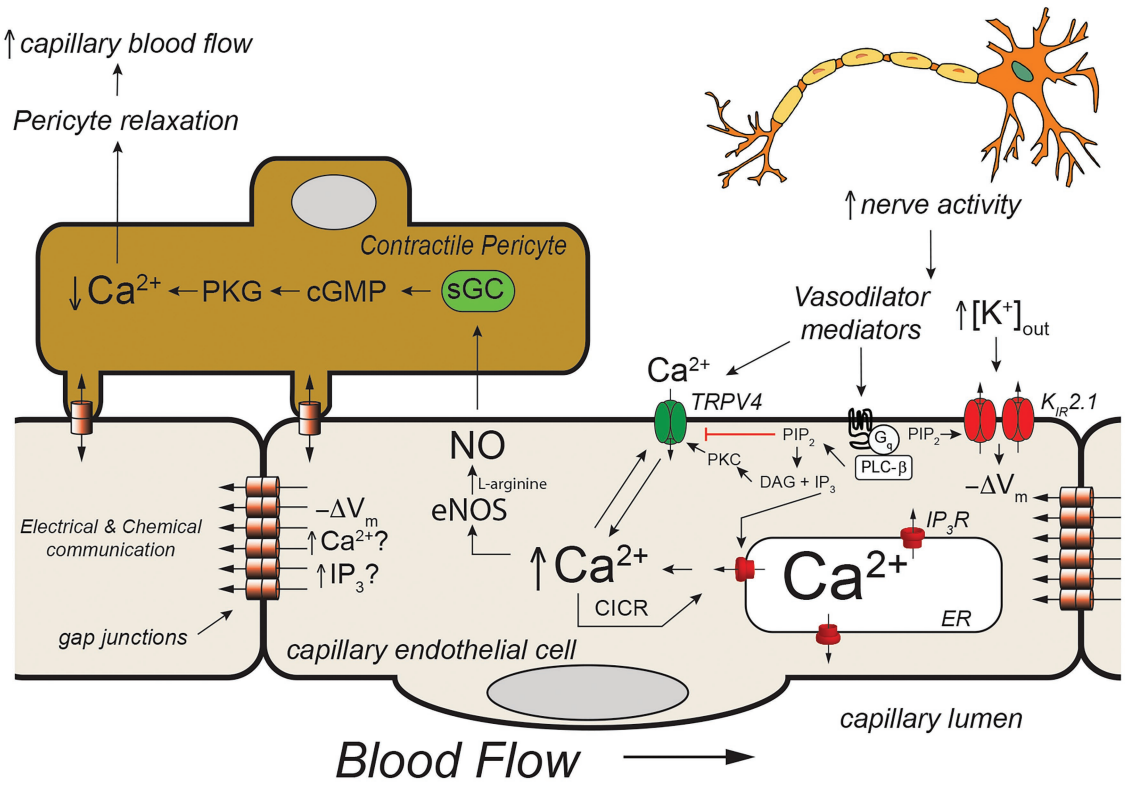

FIGURE 4 | Capillary Ca^{2+} signaling to control pericyte contraction. Schematic of a capillary segment invested with a contractile pericyte. Increased nerve activity will result in accumulation of extracellular K^+ (as in **Figure 2**) and a yet unidentified mediator (like PGE_2, for example). The increase in $[K^+]_{out}$ will hyperpolarize the membrane ($-\Delta V_m$) as in **Figure 2**. The mediator will activate $G\alpha_q$-coupled receptors, leading to hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) to form IP_3 and diacylglycerol (DAG). The IP_3 will activate IP_3R in the endoplasmic reticulum resulting in Ca^{2+} release. The increase in $[Ca^{2+}]_{in}$, along with reduced membrane PIP_2, and DAG- and Ca^{2+} activation of protein kinase C (PKC) will activate membrane transient receptor potential vanilloid family member 4 (TRPV4) channels resulting in Ca^{2+} influx which will be bolstered by K_{IR}-mediated hyperpolarization. This will further increase local $[Ca^{2+}]_{in}$ resulting in additional Ca^{2+} release through IP_3R (Ca^{2+}-induced-Ca^{2+} release; CICR). The resultant increase in $[Ca^{2+}]_{in}$ will activate eNOS, increasing production of NO. Nitric oxide will diffuse to the overlying contractile pericyte, activating guanylyl cyclase (GC) to form cGMP, which, in turn, will activate protein kinase G (PKG). Protein kinase G will phosphorylate a number of target proteins resulting decreased $[Ca^{2+}]_{in}$ in the pericyte and relaxation. Pericyte relaxation will cause local dilation of the capillary resulting in an increase in blood flow (red cell flux) to this active capillary segment. While the membrane hyperpolarization clearly can be transmitted from endothelial cell-to-endothelial cell as in **Figure 2**, it is not clear if Ca^{2+} and/or IP_3 can be transmitted through endothelial cell gap junctions in capillaries to promote cell-to-cell conduction of Ca^{2+} signals. See text for additional information and references.

Endothelial TRPV4 channels in MEPs also have been shown to be activated at low intravascular pressure resulting in TRPV4 Ca^{2+} sparklets, activation of IK_{Ca} channels, and a reduction in myogenic tone (Bagher et al., 2012). The signal that is transmitted from vascular smooth muscle cells to endothelial cells *via* MEGJs to initiate this TRPV4 channel/IP_3R/IK_{Ca} channel-mediated myoendothelial feedback remains unclear with evidence for both transmission of Ca^{2+} (Garland et al., 2017) and IP_3 (Tran et al., 2012; Hong et al., 2018) from smooth muscle to endothelial cells to activate MEP-localized TRPV4 channels and/or IP_3Rs. Additional research will be required to define the signaling molecule responsible for myoendothelial feedback.

Shear-induced dilation of resistance arteries and arterioles also involves cell-cell communication and several endothelial cell ion channels including K_{IR} channels (Ahn et al., 2017; Fancher and Levitan, 2020), TRPV4 channels (Mendoza et al., 2010), PIEZO1 channels (Li et al., 2014; Ranade et al., 2014; Rode et al., 2017), transient receptor potential polycystin family member 1 (TRPP1) channels (MacKay et al., 2020), IP_3R, SK_{Ca} channels, and IK_{Ca} channels (MacKay et al., 2020; **Figure 2**).

Barium-induced block of K_{IR} channels or use of $K_{IR}2.1$ heterozygotes were shown to inhibit a significant portion of shear-stress-induced vasodilation in mouse mesenteric arteries that involved phosphorylation of AKT and eNOS and increased NO production, but not activation of SK_{Ca} channels (Ahn et al., 2017). The precise role played by $K_{IR}2.1$ channels in this process remains to be established.

Shear-induced activation of TRPV4 (Mendoza et al., 2010) and/or PIEZO1 (Li et al., 2014; Ranade et al., 2014; Rode et al., 2017) also has been implicated in shear-induced dilation of resistance arteries. It has been postulated that Ca^{2+} influx through these channels increases $[Ca^{2+}]_{in}$ to cause both Ca^{2+}-dependent production of NO and other endothelial cell vasodilator autacoids and activation of SK_{Ca} and IK_{Ca} channels to produce endothelial cell hyperpolarization and transmission of this hyperpolarization to overlying smooth muscle cells through MEGJs (Fancher and Levitan, 2020).

Shear-induced activation of endothelial cell TRPP1 channels has also been shown to contribute to shear-stress-induced vasodilation (MacKay et al., 2020). Conditional knockout of TRPP1 from endothelial cells was shown to attenuate

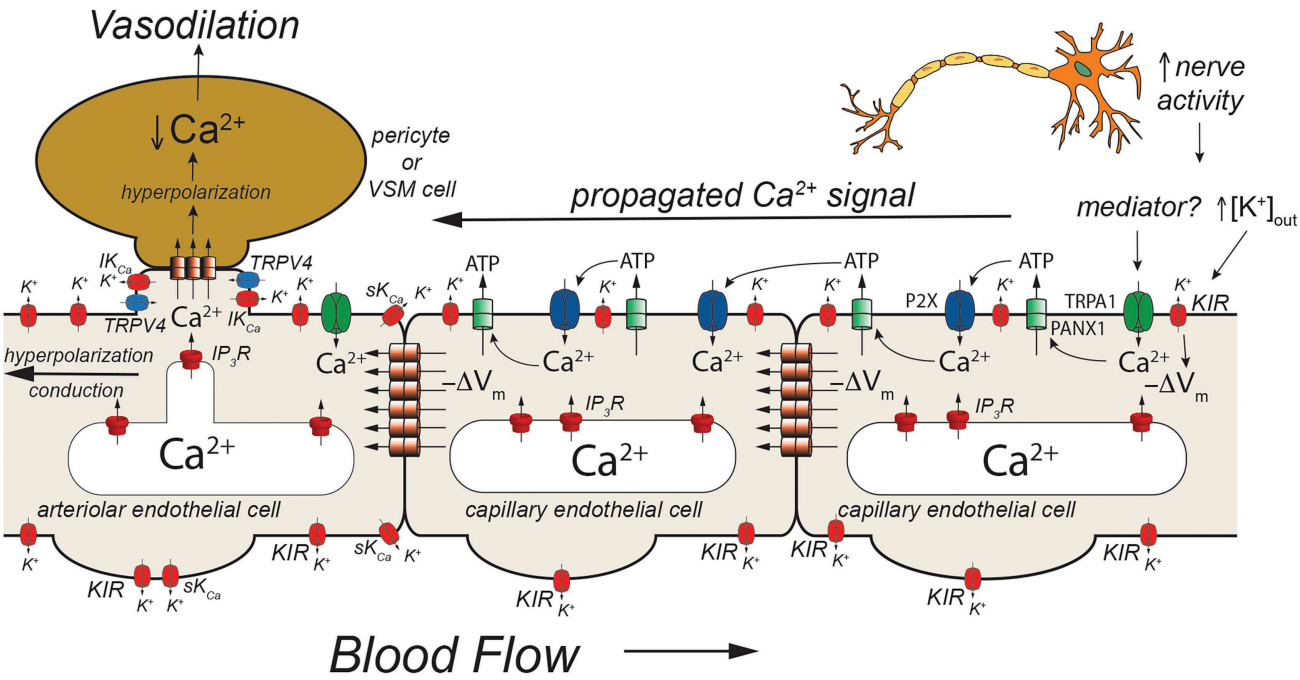

FIGURE 5 | TRPA1- and pannexin-mediated signaling in capillaries. Schematic of a capillary segment that transitions into the initial segment of the capillary coated with contractile pericytes or the terminal arteriole invested with smooth muscle cells. Increased neuron activity leads to increased accumulation of extracellular K^+ (as in **Figures 2, 3**) and a yet unidentified mediator. The mediator activates TRPA1 channels in the membrane leading to an increase in $[Ca^{2+}]_{in}$ that then activates adjacent pannexin 1 (PANX1) channels which release ATP into the extracellular space. ATP binds to and activates P_2X receptors resulting in additional Ca^{2+} influx. The additional increase in $[Ca^{2+}]_{in}$ activates more PANX1 channels, propagating the Ca^{2+} signal from cell-to-cell. As shown in **Figure 2**, increased $[K^+]_{out}$ activates K_{IR} channels causing membrane hyperpolarization ($-\Delta V_m$). This likely helps to maintain the electrochemical gradient for Ca^{2+} influx through TRPA1 and P_2X receptors. The hyperpolarization also will be conducted cell-to-cell *via* homocellular gap junctions. When the Ca^{2+} signal reaches endothelial cells in the initial segment of the capillary (coated with contractile pericytes) and also the endothelium in the terminal arteriole, SK_{Ca} and IK_{Ca} channels will be activated resulting in membrane hyperpolarization that is supported by hyperpolarization-induced activation of K_{IR} channels. The hyperpolarization will then be transmitted to overlying contractile mural cells *via* heterocellular gap junctions. In smooth muscle cells (and presumably contractile pericytes), the hyperpolarization will deactivate voltage-gated Ca^{2+} channels, decreasing $[Ca^{2+}]_{in}$ and leading to smooth muscle (or pericyte) relaxation and vasodilation. Dilation of the terminal arteriole (or the initial capillary segment) will result in an increase in blood flow to the microvascular unit, directing blood flow to the site of increased neural activity. See text for more information and references.

shear-induced activation of endothelial SK_{Ca} and IK_{Ca} channels as well as smooth muscle hyperpolarization induced by intralumenal flow (MacKay et al., 2020). The eNOS/NO component of shear-stress-induced vasodilation in mouse mesenteric arteries also was reduced by conditional knockout of endothelial cell TRPP1 (MacKay et al., 2020). What remains to be established is how these ion channels ($K_{IR}2.1$, TRPV4, PIEZO1, and TRPP1) integrate into a consolidated view of shear-stress-mediated vasodilation in arterioles and resistance arteries (Fancher and Levitan, 2020).

CAPILLARY ENDOTHELIAL CELL ION CHANNELS AND CELL-CELL COMMUNICATION

Capillaries are the primary site of exchange of respiratory gasses (O_2 and CO_2) and substrates (glucose, amino acids, fatty acids, nucleotides, etc.) that support parenchymal cell metabolism (Durán et al., 2011), and recent evidence suggests that these exchange microvessels serve as important sensors of parenchymal cell metabolism which then communicate upstream to segments of the capillary with contractile pericytes and/or to endothelial cells and overlying smooth muscle cells in terminal arterioles to control local vascular resistance such that blood flow is directed to capillaries supplying active parenchymal cells (Murrant and Sarelius, 2015; Longden et al., 2017, 2021; Garcia and Longden, 2020; Zhao et al., 2020). Data from the cerebral microcirculation are particularly compelling. As in arteriolar endothelial cells, cerebral capillary endothelial cells express a diverse array of ion channels involved in their homeostatic functions including: IP_3R, TRPV4 channels, and $K_{IR}2.1$ channels (Garcia and Longden, 2020; Longden et al., 2021; **Figures 3, 4**) as well as transient receptor potential ankyrin family member 1 (TRPA1), pannexin 1 (PANX1), and P_2X purinergic receptors (Thakore et al., 2021; **Figure 5**). The notable exception is the apparent lack of SK_{Ca} and IK_{Ca} channels from the bulk of capillary endothelial cells (Longden et al., 2017, 2021), although recent evidence suggests that SK_{Ca} and IK_{Ca} channels are expressed in the endothelial cells in the initial segment of capillaries that are covered by contractile pericytes (Thakore et al., 2021).

Using a novel *ex vivo* preparation consisting of a cerebral parenchymal arteriole along with a terminal arteriole and capillaries that arise from these vessels, Longden et al. (2017) showed that application of elevated extracellular K^+ ($[K^+]_{out} = 10$ mM) to the capillaries in this preparation resulted in dilation of the parenchymal arteriole and that this $[K^+]_{out}$-induced dilation was mediated by endothelial cell $K_{IR}2.1$ channels, confirmed both by sensitivity in Ba^{2+} and also by endothelial cell knockout of $K_{IR}2.1$. They further showed that application of elevated $[K^+]_{out}$ to cerebral capillaries, *in vivo*, resulted in arteriolar dilation and an increase in capillary red cell flux that could be blocked by Ba^{2+}. Importantly, they also demonstrated that functional hyperemia in the whisker barrel cortex (neurovascular coupling) was diminished in endothelial cell $K_{IR}2.1$ knockouts and substantially inhibited by application of Ba^{2+}. Together, these data support a model where increases in neural activity result in an elevation in $[K^+]_{out}$ in the vicinity of the active neurons (**Figure 3**). The elevated $[K^+]_{out}$ activates capillary endothelial cell $K_{IR}2.1$ channels, hyperpolarizing the endothelial cells. This hyperpolarization is conducted along the capillary through endothelial cell homocellular gap junctions and facilitated by hyperpolarization-induced activation of upstream $K_{IR}2.1$ channels. Upon reaching the arteriolar endothelium, the $K_{IR}2.1$-mediated hyperpolarization is transmitted to overlying smooth muscle cells *via* MEGJs, hyperpolarizing the smooth muscle cells, and deactivating smooth muscle voltage-gated Ca^{2+} channels, resulting in vasodilation. While elevated $[K^+]_{out}$ has long been posited as a potential mediator of functional hyperemia (Davis et al., 2011), this was the first study to define exactly where the elevated $[K^+]_{out}$ is sensed. Whether this model also applies to skeletal muscle where K^+ also is implicated in functional hyperemia (Armstrong et al., 2007; Crecelius et al., 2014) remains to be established.

In vivo studies in mice expressing the genetically encoded Ca^{2+} sensor, GCaMP8 in their endothelial cells has shown that neural activity is associated with endothelial cell $[Ca^{2+}]_{in}$ signals that are triggered by neuronal activity and mediated by Ca^{2+} release through IP_3R. Longden et al. (2021) went on to show that these Ca^{2+} signals could be inhibited by tetrodotoxin (to inhibit neural activity) and reduced by blocking $G\alpha_q$ signaling, $K_{IR}2.1$ channels, and TRPV4 channels. Furthermore, they showed that these capillary Ca^{2+} signals resulted in formation of NO in capillaries covered by contractile pericytes which relaxed the pericytes to direct red cell flux (a surrogate for blood flow) to the capillaries with active Ca^{2+} signals. Their observations suggest a model (**Figure 4**) in which active neurons release both K^+ (necessary for repolarization of each nerve action potential) and a small molecule mediator (they suggested PGE_2) that activates $G\alpha_q$ signaling to produce IP_3. The $G\alpha_q$ signaling involves PLCβ which hydrolyzes PIP_2 to form IP_3 and DAG. This process reduces PIP_2 levels in the membrane and results in increased TRPV4 channel activity and, at the same time, restrains $K_{IR}2.1$ channel activity (Harraz et al., 2018). The formed IP_3 sensitizes IP_3R to CICR which is facilitated by Ca^{2+} entry through the TRPV4 channels. The $[K^+]_{out}$-induced K_{IR}-mediated endothelial cell hyperpolarization enhances the Ca^{2+} influx through TRPV4 channels by increasing the electrochemical gradient for Ca^{2+} influx. The elevated endothelial cell $[Ca^{2+}]_{in}$ activates endothelial cell nitric oxide synthase (eNOS), increasing the formation of NO that diffuses to the overlying pericytes, relaxing them to increase capillary diameter and direct blood flow to the actively signaling capillary segment. What remains unclear is how far these Ca^{2+} signals are transmitted along the capillaries and the exact nature of the neuron (or glial cell)-produced "mediator" that activates a capillary $G\alpha_q$ receptor in this scheme. Longden et al. (2021) suggested that the Ca^{2+} events are restricted to single cells and do not conduct between endothelial cells. Given how well endothelial cells are coupled by homocellular gap junctions, this finding is unexpected, because studies in resistance arteries have shown conduction of Ca^{2+} signals between many endothelial cells (Domeier and Segal,

2007; Uhrenholt et al., 2007). Additional research will be required to resolve this issue and determine how distal portions of the capillary which are devoid of contractile mural cells signal to more proximal sections endowed with contractile pericytes if these Ca^{2+} signals indeed do not conduct from cell-to-cell.

The mediator responsible for the Ca^{2+} signals observed by Longden et al. (2021) also remains to be established. They suggested PGE_2 as a candidate and showed that PGE_2 could elicit Ca^{2+} signals in capillary endothelial cells. However, subsequent studies using the parenchymal arteriole with attached capillary preparation suggest that PGE_2 may not be the mediator (Rosehart et al., 2021). In these studies, while PGE_2 applied to the capillaries elicited signals conducted from the capillaries to the arteriole to produce vasodilation, the authors found that this response was not attenuated by blocking TRPV4 or TRPA1 channels. These data are inconsistent with PGE_2 being the mediator responsible for the *in vivo* capillary Ca^{2+} signals recorded by Longden et al. (2021) and shown in **Figure 4**. Additional research will be required to identify the mediator linking neural activity with capillary Ca^{2+} signaling.

An additional model has developed from studies that suggest a role for TRPA1, PANX1, and P_2X purinergic receptors in cerebral functional hyperemia (Thakore et al., 2021; **Figure 5**). Thakore and colleagues (Thakore et al., 2021) showed that application of agonists for TRPA1 channels to capillaries in the novel arteriole-capillary preparation noted above, resulted in a slowly conducting signal that could be inhibited by blockers or genetic deletion of PANX1 channels, destruction of extracellular ATP, or block of P_2X receptors. They went on to show that functional hyperemia in the whisker barrel cortex was inhibited by either a TRPA1 antagonist or genetic knockout of endothelial cell TRPA1 channels. In their model (**Figure 5**), neural activity produces a mediator that activates TRPA1 channels. The Ca^{2+} influx through TRPA1 channels activates adjacent PANX1 channels, releasing ATP into the extracellular space. The "cloud" of extracellular ATP activates P_2X receptors increasing intracellular Ca^{2+} and activating additional PANX1 channels producing a slowly propagating Ca^{2+} signal that travels from cell-to-cell to the initial segment of the capillary where SK_{Ca} and IK_{Ca} channels are expressed in the endothelial cells. The increased $[Ca^{2+}]_{in}$ activates the SK_{Ca} and IK_{Ca} channels producing a rapidly conducting electrical signal that travels to and through the arteriolar endothelium *via* endothelial cell homocellular gap junctions. This endothelial cell hyperpolarization is then transmitted to overlying smooth muscle cells *via* MEGJs to deactivate voltage-gated Ca^{2+} channels and produce vasodilation. Capillary-to-arteriole signaling in skeletal muscle also has been proposed (Murrant and Sarelius, 2015; Lamb et al., 2021) and may involve pannexins and ATP (Lamb et al., 2021). The exact mediator that triggers activation of TRPA1 channels remains to be found.

In the heart, a novel signaling mechanism involving heterocellular gap junctions between cardiac myocytes and capillary endothelial cells has been proposed to mediate functional hyperemia (Zhao et al., 2020). Zhoa and colleagues propose that increases in cardiac metabolism (increased heart rate and/or contractility) result in activation of cardiac ATP-sensitive K^+ channels and membrane hyperpolarization. The cardiac myocyte hyperpolarization is transmitted to capillary endothelial cells *via* heterocellular gap junctions. The hyperpolarization is then transmitted along the capillary endothelium *via* homocellular gap junctions to the endothelium in terminal arterioles, which is facilitated by hyperpolarization- and $[K^+]_{out}$-induced activation of endothelial K_{IR} channels. Once in the arteriolar endothelium, the hyperpolarization is transmitted by MEGJs to overlying smooth muscle cells to cause deactivation of voltage-gated Ca^{2+} channels and vasodilation. Whether other forms of capillary-to-arteriole signaling function in the heart remain to be established.

CONCLUSION AND FINAL PERSPECTIVE

Endothelial cell ion channels importantly contribute to cell-cell communication between cells in the wall of arterioles and between parenchymal cells, capillary endothelial cells, arteriolar endothelial cells, and contractile mural cells (smooth muscle or pericytes). An important question remaining is: how do all of these ion channels fit into a consolidated scheme? For example, are the TRPV4/IP_3R model (**Figure 4**) and the TRPA1/PANX1 model (**Figure 5**) really separate pathways, or are they integrated into a more complicated single scheme of cell-cell communication in the microcirculation? Also, how general are the models presented? Can the pathways identified in the cerebral microcirculation (**Figures 3–5**) be extended to microvascular beds in other tissues (such as skeletal muscle and the heart) or are separate signaling pathways, such as that described in the heart involving gap junction signaling between cardiac myocytes and capillary endothelial cells, functional? Capillary-to-arteriole signaling has been proposed in skeletal muscle (Murrant and Sarelius, 2015) but critical tests of this hypothesis remain to be performed. While local superfusion experiments have provided evidence for conducted signals and capillary-to-arteriole signaling in skeletal muscle [see Twynstra et al. (2012), for example], interpretation of the findings of such experiments is difficult because skeletal muscle fibers are longer than the scale of microvascular units such that effects of the applied superfusate on skeletal muscle fibers cannot be excluded, for example. Answering the questions outlined above will require additional research and development of new and novel approaches to study cell-cell communication in the microcirculation of organs and tissues around the body.

The effects of disease states on ion channels and cell-cell communication in the microcirculation also is an area that is developing. In the brain, for example, K_{IR} signaling in capillaries (as per **Figure 3**) is crippled in a murine model of Alzheimer's disease, a defect that can be improved by application of exogenous PIP_2 (Mughal et al., 2021). How are the other pathways of capillary-to-arteriole signaling affected in this disease? We already know that capillary TRPV4 channels are activated by PIP_2 loss (Harraz et al., 2018), does this mean that TRPV4 channel signaling will be increased in cerebral capillaries in Alzheimer's disease? Is a PIP_2-related increase in TRPV4 channel activity compensatory to the loss of K_{IR} channel function, or part of the vascular pathology in this neurodegenerative disease? Obviously, additional research will be required to answer these

additional questions about endothelial cell ion channels and cell-cell communication in the microcirculation.

AUTHOR CONTRIBUTIONS

WJ conceived, wrote, and edited this manuscript. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

FUNDING

This work was supported by National Heart, Lung and Blood Institute grants HL-137694 and PO1-HL-070687.

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