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Brain Capillary Ion Channels: Physiology and Channelopathies

Osama F. Harraz[*], **Ahmed M. Hashad**

Department of Pharmacology, Larner College of Medicine, Vermont Center for Cardiovascular and Brain Health, University of Vermont, Burlington, Vermont

Abstract

The brain relies on an intricate vascular network to deliver oxygen and nutrients through functional hyperemia, a process critical for matching blood flow to neuronal activity. This review explores the roles of ion channels in brain capillary endothelial cells and pericytes, focusing on their contributions to neurovascular coupling. Key endothelial ion channels, including Kir2.1, K_{ATP}, TRPV4, TRPA1, and Piezo1, regulate membrane potential and calcium dynamics, facilitating rapid electrical and chemical signaling that modulates blood flow. Pericytes, categorized as ensheathing and thin-strand, express ion channels such as K_{ATP}, voltage-gated calcium channels, TRPC, and TMEM16A, which govern contractility and orchestrate blood flow responses. Additionally, we discuss channelopathies in conditions like Alzheimer's disease, cerebral small vessel diseases, hypertension, and ischemic stroke, where ion channel dysfunction impairs brain blood flow regulation. Emerging evidence underscores the therapeutic potential of targeting capillary ion channels to restore neurovascular function in these disorders.

1. INTRODUCTION

Despite being a metabolically demanding organ, the brain has limited energy reservoirs. Therefore, the brain relies on its vascular networks to supply active regions with oxygen and nutrients. This on-demand delivery is essential to supply active neurons with their needs and to clear their metabolic waste (1, 2). Functional hyperemia (FH) is the phenomenon by which local and rapid increases in blood flow (i.e., *hyperemia*) match the elevated demands of *functional* neurons. The vascular network of the mammalian brain includes pial arteries on the surface from which penetrating arterioles orthogonally dive into the brain parenchyma. Penetrating arterioles branch at regular intervals in the parenchyma forming capillary networks (Fig. 1). These extensive networks lie in close proximity to all neurons, enabling them to sense and respond to neural activity (3, 4).

Capillaries consist of endothelial cells (ECs), that are electrically coupled via endothelial gap junctions, and overlying pericytes embedded in the capillary basement membrane (4–6). Interactions and communications between ECs and pericytes are arguably crucial for the regulation of cerebral blood flow (CBF) and the maintenance of barrier function (7,

[*]**Corresponding Author**: Osama F. Harraz, PhD, Firestone Medical Research Building, FMRB-458, 149 Beaumont Ave, Burlington, VT 05405, Osama.Harraz@uvm.edu.

DECLARATION OF INTERESTS

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8). Noteworthy, pericyte characteristics and morphology change with the capillary branch order (9). This review will primarily focus on two types: ensheathing and thin-strand pericytes (Fig. 1). *Ensheathing* pericytes typically cover the initial ~3-4 capillary branch orders (~1st to 3rd/4th order) originating from penetrating arterioles. They express α smooth muscle actin (α-SMA, encoded by *Acta2*), allowing them to rapidly contract and modify downstream blood flow (9, 10). *Thin-strand* pericytes, on the other hand, predominate in deep, higher-order (~5th order or higher) capillaries, and have very low expression levels of α-SMA. As the name implies, thin-strand pericytes have long-extending processes (250-300 μm) covering the capillary length and allowing each pericyte to be in close contact with similar pericytes (5, 7, 10). They also form peg-socket junctions with the underlying ECs, presumably facilitating charge and/or molecule transfer between the two cell types (11, 12). Unlike ensheathing pericytes, thin-strand pericytes only retain partial ability to contract and regulate CBF on a much slower time scale (13–15).

To properly sense and respond to nearby neurons so that adequate tissue perfusion is maintained, capillaries are equipped with the necessary machinery, in ECs and pericytes, to do so. These molecular machineries include polymodal ion channels and receptors that sense neuronal firing and translate different stimuli into membrane potential (V_M) shifts or intracellular $[Ca^{2+}]_i$ changes in ECs and pericytes (4, 5). Capillary ECs and pericytes encode a long list of ion channel genes. However, considering the relative recent development of our understanding of capillary ion channels and that transcriptome (mRNA) is not necessarily translated into functional proteins, this work will focus on brain capillary ion channels shown to be functionally expressed, and where there is evidence—at least for some of them—that they affect CBF.

2. ION CHANNELS IN THE CAPILLARY ENDOTHELIUM

2.1. Potassium channels

The first ion channel identified in brain capillaries was a potassium (K^+) channel (16). We now know that several K^+ channels are functionally expressed in capillary ECs. The strong inwardly rectifying K^+ (Kir2.1) channel and the ATP-sensitive K^+ (K_{ATP}) channel are expressed, and they play a crucial role in CBF regulation (16, 17) (Fig. 2).

2.1.1. Kir2.1: Brain capillary ECs express Kir2.1 channels as demonstrated by the *Kcnj2* gene expression and electrophysiological recordings using freshly isolated ECs (16, 18–20). The Kir2.1 channel has a unique current-voltage relationship with a large inward current and a much smaller, yet physiologically critical, outward component. The strong inward rectification is attributed to the channel pore blockade by intracellular Mg^{2+} and polyamines (21, 22). The rise in extracellular K^+ concentration ($[K^+]_{External}$) will alleviate the channel block, resulting in the efflux of K^+ ions and EC hyperpolarization (21, 23). This is particularly important considering the modest increases in $[K^+]_{External}$ that derive from neurons—as a byproduct of neuronal repolarization—and astrocytes in close proximity to capillaries (24). The increase in capillary Kir2.1 conductance hyperpolarizes ECs; retrograde propagation of hyperpolarization is facilitated through endothelial gap junctions till it reaches upstream arterioles (Fig. 2). The transmission of hyperpolarization to the smooth

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muscle layer, via myoendothelial gap junctions, inhibits smooth muscle voltage gated Ca^{2+} channels and dilates arterioles to ultimately increase blood flow and red blood cell (RBC) flux in the downstream capillary (16). This capillary EC Kir2.1-dependent mechanism was tested *in vivo* and *ex vivo* where external capillary stimulation with K^+ triggered upstream arteriolar hyperpolarization and vasodilation, effects that were absent when Kir2.1 channel was genetically deleted or pharmacologically blocked (16).

The capillary Kir2.1-initiated electrical communication from capillaries to upstream arterioles ensures a rapid boost in capillary blood flow during neuronal firing. A major determinant of the gain of capillary Kir2.1-mediated electrical signaling is the availability of the cofactor phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) (20). Earlier structural and functional studies used heterologous expression systems and demonstrated that inwardly rectifying Kir2 channels are strongly bound to PIP_2, which is essential for channel activation (25–27). In brain capillaries, we showed that PIP_2 levels determine Kir2.1 activity and capillary-to-arteriole electrical signaling. Disruption of the dynamic PIP_2 synthesis inhibited capillary Kir2.1 function (20). Gq protein-coupled receptor (GqPCR) activation depletes PIP_2 via downstream activation of phospholipase C (PLC) and PIP_2 hydrolysis into inositol-4,5- triphosphate (IP_3) and diacyl glycerol (DAG) (28). Consistent with this, we showed that brain EC GqPCR signaling inhibits Kir2.1 activity and electrical signaling in a PIP_2-depednent manner (20). Our work confirmed that GqPCR activation decreased Kir2.1 function in capillary ECs due to PIP_2 depletion, rather than DAG or IP_3 generation (20). This is important considering that—in addition to the rapid electrical component of neurovascular coupling—slower chemical signaling is triggered via receptor agonist release and GqPCR activation. Activated neuron and astrocyte release chemical mediators that activate GqPCR on nearby capillary ECs with downstream PIP_2 hydrolysis.

2.1.2. K_{ATP}: ATP-sensitive K^+ (K_{ATP}) channels are sensitive to intracellular adenosine triphosphate (ATP) and adenosine diphosphate (ADP) levels (ATP/ADP ratio). Lower ATP concentrations and/or higher ADP concentrations enhance K_{ATP} channel opening, K^+ efflux and ultimately hyperpolarization (29). Since intracellular ATP/ADP ratio is a major determinant of K_{ATP} activation, the channel function is often a reflection of cellular metabolism and its translation into an electrical signal (29, 30). Using patch clamp electrophysiology and membrane potential measurements, recent work has confirmed the functional expression of K_{ATP} channels in capillary ECs, as well as pericytes (*see* section 3.5.1.) (17). Intriguingly, previous studies in arterial smooth muscle cells suggested that the primary trigger to K_{ATP} channels was Gs protein-coupled receptor (GsPCR) signaling and downstream cyclic adenosine monophosphate (cAMP) production and protein kinase A (PKA) activation (31, 32). Observations in capillary ECs similarly demonstrated K_{ATP} activation downstream of adenosinergic GsPCR stimulation by adenosine—a vasodilatory neurotransmitter released during hypoxic conditions (Fig. 2). Importantly, capillary EC K_{ATP} channel activation hyperpolarizes ECs and enhances CBF, effects that were absent in mice expressing non-functional K_{ATP} channels or when cAMP/PKA signaling was inhibited (17). Neurovascular coupling studies in awake mice pointed to a similar conclusion, with systemic pharmacological inhibition and genetic manipulation of K_{ATP} markedly disrupting

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functional hyperemia (33). Collectively, there is convincing evidence that EC K_{ATP} channel contributes to the regulation of capillary endothelial V_M and therefore CBF.

The predominant hyperpolarizing ion channels identified in brain capillary ECs are Kir2.1 and K_{ATP} channels (Fig. 2). Hyperpolarization of capillaries not only initiates rapidly propagating electrical signals that dilate upstream arterioles and enhance CBF but can also influence Ca^{2+} dynamic in ECs. A recent study revealed that this capillary hyperpolarization increases the driving force for Ca^{2+} influx—effectively linking electrical signaling with Ca^{2+} signaling through "*electrocalcium coupling*" and integrating different signaling modalities in brain capillaries (34). It remains unknown whether and how this coupling could impact CBF control in physiological and pathophysiological contexts.

2.2. Transient Receptor Potential Channels

The transient receptor potential (TRP) channel family is comprised of 28 members with varying structures and functions (35). Here, we focus on two members that are functionally expressed in brain capillary ECs: TRP vanilloid 4 (TRPV4) channel and TRP ankyrin 1 (TRPA1) channel.

2.2.1. TRPV4: The first cation influx channel characterized in the brain capillary endothelium was TRPV4. The *Trpv4* gene is expressed in mouse brain capillaries (18), and TRPV4 function has been characterized using patch clamp electrophysiology in capillary ECs (19). TRPV4 channel is permeable to Ca^{2+} and Na^{+} and has a conductance of 45-60 pS under physiological conditions (36). Brain capillary TRPV4 channels exhibit extremely low activity (19), especially when compared with peripheral endothelial TRPV4 (37). We discovered that low TRPV4 activity was attributed to the tonic inhibition of the channel by PIP_2; depletion of PIP_2 downstream of GqPCR signaling increases capillary TRPV4 channel activity several folds (19). While the exact mechanism underlying PIP_2-mediated regulation of capillary TRPV4 is unclear, experiments in overexpression systems showed that PIP_2 directly interacts with the ankyrin repeat domain of the TRPV4 N-terminus (38). There is also some evidence that PIP_2 facilitates channel structural rearrangements and therefore affects function (39). Additional studies are warranted to ascertain the exact mechanism by which PIP_2 modulates capillary TRPV4 function in the brain.

The activation of TRPV4 in brain capillaries could lead to different functional consequences. TRPV4-mediated Ca^{2+}/Na^{+} influx depolarizes ECs and can therefore short circuit hyperpolarization signals (Fig. 2). Noteworthy, the ability of capillary TRPV4 to mediate depolarization is distinct from the well-established peripheral arterial EC hyperpolarization that involves TRPV4-mediated Ca^{2+} influx and subsequent activation of Ca^{2+}-activated K^{+} (K_{Ca}) channels (37). This distinction is presumably attributed to the lack of functional K_{Ca} channels in the brain capillary endothelium (16). Capillary TRPV4 activation can also activate endothelial nitric oxide synthase (eNOS), NO production, adjacent proximal pericyte relaxation and ultimately a local increase in capillary CBF (40). Longden and colleagues showed that neural activation evokes Ca^{2+} transients in nearby capillary ECs via the activation of the prostanoid GqPCR (EP1) by prostaglandin E_2 (PGE_2) (40) (Fig. 2). These endothelial Ca^{2+} events were attributed to TRPV4-mediated Ca^{2+} influx, due to

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channel disinhibition by PIP_2 depletion, and IP_3-mediated Ca^{2+} release from the intracellular stores (40) (Fig 2). However, *ex vivo* experiments revealed that PGE_2 application onto capillaries evokes upstream arteriolar vasodilation that is not TRPV4-dependent (41). The latter suggests that TRPV4 might not be necessary for PGE_2/EP1-mediated increases in CBF. Another important observation is that only a small fraction of capillary Ca^{2+} events translate into NO-induced CBF increases (40). The EC Ca^{2+}/CBF increase was evident in proximal capillaries covered with contractile pericytes, but this link was between Ca^{2+} signals and blood flow was absent in deep capillaries.

2.2.2. TRPA1: TRPA1 is a nonselective Ca^{2+}/Na^{+}-permeable channel that is functionally expressed in brain capillary ECs (42). Capillary TRPA1 activation during neural activity has been shown to generate biphasic retrograde signals that ultimately dilate upstream arterioles. TRPA1 activation initiates slowly propagating Ca^{2+} signals, where TRPA1-mediated Ca^{2+} influx activates pannexin-1 channel leading to ATP release and subsequent activation of purinergic P2X receptors to facilitate further Ca^{2+} influx into ECs. Increased intracellular [Ca^{2+}] further activates pannexin-1 and mediates Ca^{2+} waves that propagate to upstream *transitional* segments, where Ca^{2+} signals are converted to rapidly propagating electrical signals. The conversion of the slow phase into a rapid phase in the transitional segment, often referred to as 1st order capillary, is mediated through the activation of small- and intermediate-conductance K_{Ca} channels to hyperpolarize ECs and initiate a Kir2.1-dependent fast electrical signal (42) (Fig. 2). The resulting hyperpolarization dilates upstream arterioles and increases blood flow. Importantly, pharmacological inhibition or genetic deletion of endothelial TRPA1 attenuated the functional hyperemic response *in vivo* (42). Reactive oxygen species (ROS) are likely the physiological activator of capillary TRPA1 and subsequent hyperemia, as shown in cerebral arteries (43, 44). One possibility is that neural activity engages capillary TRPA1 channels via ROS generated by metabolically active neurons or astrocytes, an area warranting additional exploration.

2.3 IP_3 receptors

IP_3 receptor (IP_3R) is the predominant Ca^{2+} release channel expressed in capillary ECs (18). IP_3Rs are located on the endoplasmic reticulum membrane and their activation is dependent on cytosolic [Ca^{2+}] and IP_3 binding (45). IP_3 is a byproduct of PIP_2 hydrolysis and is therefore generated downstream of GqPCR activation. Longden and colleagues elegantly showed capillary EC GqPCR activation during neural activation and further observed intracellular [Ca^{2+}] elevation due to IP_3R-mediated Ca^{2+} release and TRPV4-mediated Ca^{2+} influx (Fig. 2). These Ca^{2+} signals facilitate NO production and proximal capillary dilation (40). Importantly, endothelial hyperpolarization—resulting from Kir2.1 or K_{ATP} channel activation—intersects with IP_3R function through electrocalcium coupling. Hyperpolarization increases the driving force for Ca^{2+} entry via TRPV4 channel, which in turn promotes Ca^{2+}-induced Ca^{2+} release through IP_3Rs (34).

2.4. Piezo1

Piezo1 is a mechanosensitive ion channel expressed in brain capillary ECs. Our group has shown that mechanical or pharmacological Piezo1 activation yields inward Ca^{2+}/Na^{+} currents and Ca^{2+} transients in capillary ECs; responses that were absent upon genetic

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deletion of Piezo1 from ECs (46). Piezo1 activity in peripheral arteries has been linked to NO generation and flow-mediated vasodilation (47, 48). Such a mechanism (Piezo1/NO) could be functional in the brain, where Piezo1 activation could generate NO and increase proximal capillary blood flow. This is an active area of research in which we aim to ascertain whether local Piezo1-mediated Ca^{2+} signals facilitate focal blood flow increases within capillary networks.

Flow in brain arteries and arterioles has been linked to vasoconstriction (rather than vasodilation) (49), suggesting that flow-mediated vasodilation mechanisms only occur under specific circumstances. Endothelial Piezo1 activity has been shown to depolarize ECs in peripheral arteries (50). In line with these scenarios, we recently demonstrated that Piezo1-mediated activation depolarizes the brain endothelium and resets the membrane potential (51) (Fig. 2). In the context of functional hyperemia in the brain, active neurons increase extracellular $[K^+]$, which in turn activates capillary Kir2.1 channels and hyperpolarizes ECs (16). Much like an inverted action potential (52), endothelial hyperpolarization enhances blood flow—a mechanical stimulus that engages Piezo1 channels to depolarize ECs and bring the endothelial V_M to baseline. In other words, hyperemia triggers Piezo1 opening and a depolarizing current that counteracts Kir2.1-mediated hyperpolarization and helps bring CBF back to baseline levels—a "*mechano-feedback*" mechanism that ensures blood flow returns to baseline at the cessation of neural activity. Consistent with a mechano-feedback depolarizing role for endothelial Piezo1, genetic or pharmacologic augmentation of Piezo1 activity reduced functional hyperemia in the somatosensory cortex (51). Whether Piezo1-mediated mechano-feedback mechanism intersects with focal Piezo1-mediated vasodilation awaits further testing.

Little is known about endothelial Piezo1 regulation in the brain. There is, however, emerging evidence of Piezo1 modulation in the peripheral vasculature and heterologous expression systems. For instance, a recent study suggested that PKA and PKC phosphorylate Piezo1 Serine-1612, leading to enhanced channel activity and slowed inactivation (53). This regulation is involved in blood pressure regulation, since mutant Piezo1-S1612A knock-in mice suffered elevated blood pressure and compromised exercise endurance, similar to EC-specific Piezo1 knockout mice. Considering that PKA and PKC are activated downstream of GsPCR and GqPCR, respectively, it would be intriguing to investigate if similar regulation exists in brain capillaries. This could provide a means by which GPCR activation during functional hyperemia could alter Piezo1 activity in brain ECs. Not only do protein kinases modulate Piezo1 activity, but also membrane lipids have been shown to influence endothelial Piezo1 activity via direct lipid-channel interaction or by altering the plasma membrane fluidity (54, 55). For example, sphingomyelinase function in native endothelial cells dictates Piezo1 function. Sphingomyelin, the sphingomyelinase substrate, alters Piezo1 mechanosensitivity. Ceramide, a key product of sphingomyelinase activity, disables Piezo1 inactivation (56). Another study showed that fatty acids influence Piezo1 activity in cultured ECs: margaric acid, a saturated fatty acid, inhibits Piezo1, whereas polyunsaturated fatty acids regulate Piezo1 inactivation (57). Given the critical role of PIP_2 in modulating brain capillary ion channels (58) and the evidence that PIP_2 can modulate non-vascular Piezo1 function (54, 59–61), it will be important to explore whether PIP_2

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affects capillary Piezo1 function. More studies are warranted to better understand how brain capillary Piezo1 channel is regulated in health and disease.

3. ION CHANNELS IN BRAIN CAPILLARY PERICYTES

A surge in brain pericyte research has evolved over the past decade. Emerging evidence of ion channels in brain pericytes exists, and it remains an active area of research. It is important to reemphasize that brain pericytes represent a diverse population of cells with heterogenous contractility, morphology and Ca^{2+} signaling characteristics. Here, we cover ion channels in brain pericyte and their contribution to CBF control. For simplicity, we divided capillary pericytes into two groups: rapid-contracting (*ensheathing*) that are found on the first few capillary branches from penetrating arterioles (1^{st}-4^{th}-order), and the *thin-strand* pericytes found in deep/distal capillaries. Please, note that while we did not dedicate a section for pericyte Ca^{2+} release channels (IP_3R and ryanodine receptors), they are briefly covered under other channels functioning with them as signaling complexes.

3.1. Voltage-gated Ca^{2+} channels

Voltage-gated Ca^{2+} channels (VGCCs) are activated when the plasma membrane depolarizes. Genes encoding the L-type Cav1.2 (*Cacna1c*) and the T-type Cav3.2 (*Cacna1h*) VGCCs are expressed in mouse brain capillary pericytes as shown in transcriptomic studies (18, 62). *Ex vivo* and *in vivo* studies to date have demonstrated a functional role of L-type Ca^{2+} channel in capillary pericytes (Fig. 3). Pharmacological inhibition of Cav1.2 or the removal of extracellular Ca^{2+} abolished depolarization-induced elevation of intracellular $[Ca^{2+}]_i$ in rat CNS pericytes (63). Further, Cav1.2 blockers attenuate the rise in pericyte $[Ca^{2+}]_i$ and contraction in response to intravascular pressure elevation (15). It is important to note that VGCC's contribution to pericyte contraction varies widely depending on the capillary order. This differential VGCC contribution to contraction is intriguing, considering that the kinetics of pericyte $[Ca^{2+}]_i$ rise, and subsequent contraction were much slower in thin-strand distal pericytes compared with proximal ensheathing pericytes (9, 10, 15). In the brain and the retina, Ca^{2+} influx and contraction of ensheathing pericyte are mediated by VGCCs. Ca^{2+} signals in deep brain capillary pericytes were, however, not affected by L- and T-type VGCC blockers, and the slower contraction was not VGCC-mediated, suggesting the involvement of an additional Ca^{2+} influx component (15, 64). Using *in vivo* and *ex vivo* imaging, Korte and colleagues showed that the GqPCR agonist endothelin-1 increases ensheathing pericyte $[Ca^{2+}]_i$ leading to contraction in a VGCC-dependent manner, further confirming that VGCCs are the main Ca^{2+} influx pathway in brain ensheathing pericytes (65). These data collectively support a crucial role for L-type Cav1.2 channel in not only depolarization- but also agonist-induced pericyte contraction. We note that the L-type channel blockers used to dissect the role of VGCC in pericyte Ca^{2+} handling and contraction could alter T-type channel function. More studies are needed to dissect whether T-type Ca^{2+} channels are functional in brain pericytes, as they are in peripheral pericytes (66).

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3.2. TRPC

VGCC blockers minimally lowered Ca^{2+} signaling in deep capillaries in cortical brain slices (64). This aligns with observations in the pressurized mouse retina preparation demonstrating that VGCC inhibition does not affect [Ca^{2+}] in higher-order capillary pericytes (15). These data suggest that Ca^{2+} influx in distal pericytes is driven by a non-VGCC mechanism. Several canonical TRP (TRPC) isoforms are expressed in pericytes with TRPC3 showing the highest expression. TRPC channels are Na^{+}/Ca^{2+}-permeable and are activated downstream of GqPCR stimulation (35). In mouse cortical slices and pressurized retina preparations, TRPC channel block by SKF96365 suppressed baseline and pressure-induced Ca^{2+} signaling in distal pericytes, suggesting a possible role for TRPC channels in the coordination of pericyte Ca^{2+} transients (15, 67). It should be noted however that more selective TRPC3 and TRPC6 blockers failed in other studies to alter deep pericyte Ca^{2+} transients suggesting an off-target effect for SKF96365 on other Ca^{2+} entry mechanisms (e.g., store-operated Ca^{2+} entry) (64). The discrepancies regarding TRPC involvement, or lack of, in pericyte Ca^{2+} signaling and contraction could be attributed to the different pericyte types being explored. It was recently demonstrated that TRPC3 is critical for the contraction of 1^{st}-4^{th} order pericytes (68). Intravascular pressure elevation or pharmacological TRPC3 activation depolarized pericytes, increased intracellular $[Ca^{2+}]_i$ and led to contraction—effects that were abolished upon genetic TRPC3 deletion (Fig. 3). In this scenario, TRPC3 is not the primary mechanism of Ca^{2+} entry, TRPC3-mediated cation influx rather depolarizes pericytes, facilitating VGCC activation and ultimately contraction (68). We believe that exploring functional currents that can be attributed to different TRPC isoforms with selective pharmacology will be a necessary step towards disentangling which isoforms are the most relevant for physiology.

3.3. Orai

Store-operated Ca^{2+} entry (SOCE) was proposed as a non-VGCC mechanism underlying Ca^{2+} entry in deep capillary pericytes (64). The sarcoplasmic reticulum-Ca^{2+} sensor protein stromal interaction molecule 1 (STIM1) and Orai channels at the plasma membrane are critically involved in SOCE. Capillary pericytes express STIM1, Orai1 and Orai3 (18). STIM1/Orai signaling is initiated when the intracellular Ca^{2+} stores are depleted—due to Ca^{2+} release via IP_3Rs and RyRs—which in turn is sensed by STIM1, ultimately leading to Orai channel activation and Ca^{2+} influx. In brain slices, basal Ca^{2+} signaling in deep capillary pericytes was sensitive to Orai channel blockers or to extracellular Ca^{2+} removal (64). Further, it was suggested that SOCE augments and maintains GqPCR-mediated Ca^{2+} signals in pericytes (64) (Fig. 3). Whether, and if so how, SOCE in pericytes affects CBF is unknown.

3.4. TMEM16A

TMEM16A, also known as Anoctamin-1 (ANO1), is a Ca^{2+}-activated Cl^{-} channel protein encoded by the *ANO1* gene. It is expressed in various cell types, including brain pericytes and smooth muscle cells (18). Since the Cl^{-} equilibrium potential (E_{Cl}) in vascular cells is notably depolarized—unlike neuronal E_{Cl} (~-90 mV)—vascular TMEM16A activation depolarizes vascular cells. In response to elevated intracellular $[Ca^{2+}]_i$, TMEM16A

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undergoes a conformational change, allowing Cl^- efflux across the plasma membrane and membrane depolarization (69, 70). In pericytes, TMEM16A regulates Ca^{2+} signaling and vascular tone, influencing capillary contraction. Research studies have demonstrated that TMEM16A channels are functionally expressed in brain pericytes (65). These studies show that TMEM16A plays a significant role in regulating pericyte tone and capillary function. For instance, activation of the endothelin-1 GqPCR in pericyte evokes agonist-induced contraction via mechanisms involving TMEM16A. Through a series of *ex vivo* and *in vivo* experiments, GqPCR activation and downstream Ca^{2+} release have been convincingly shown to activate TMEM16A, leading to Cl^- efflux, pericyte depolarization, Cav1.2 opening and ultimately contraction (65). While TMEM16A's involvement in agonist-induced contraction was evident, depolarization-induced contraction was not affected when TMEM16A channels were absent or blocked, suggesting that different cascades underlie pericyte contraction in response to different stimuli. This is also distinct from the reported role for TRPC3 in pericyte depolarization-induced contraction. Taken together, a model underlying agonist-induced pericyte contraction likely involves a functional complex comprised of GqPCR, TMEM16A and Cav1.2 (Fig. 3).

3.5. K^+ Channels

Several K^+ channels are expressed in brain capillary pericytes. To date, expression and functional studies have confirmed the presence of K_{ATP}, Kir, voltage gated K^+ channel (K_v) and the large conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) in brain pericytes (71). It should be noted that the electrophysiological evidence for several K^+ channels could not discern the specific pericyte subtype (i.e., ensheathing or thin-strand) in which currents were recorded (71).

3.5.1. K_{ATP}: K_{ATP} channels, composed of inwardly rectifying Kir6.1 (encoded by *Kcnj8*) and sulfonylurea receptor type 2 (SUR2) subunits, are the most abundantly expressed ion channels in brain capillary pericytes (18). High intracellular $[ATP]_i$ (elevated ATP/ADP ratio) inhibits K_{ATP} channel and lowers its open probability, positioning the channel as an ideal sensor for cellular metabolism (29). Not only are K_{ATP} channels present in pericytes, but they are also functionally expressed in capillary ECs (71), as discussed earlier. Intriguing recent observations suggest that K_{ATP} channels in thin-strand pericytes play a key role in neurovascular coupling. In this context, K_{ATP} activation hyperpolarizes thin-strand pericytes and initiates retrograde transmission of hyperpolarization via the endothelium and Kir2.1 channels, and ultimately upstream arteriolar dilation hundreds of microns away (72). A proposed model involves thin-strand pericyte K_{ATP} channel-mediated hyperpolarization as an initial and crucial input to neurovascular coupling, whereby it engages underlying endothelial Kir2.1 channel to facilitate ascending hyperpolarization and upstream vasodilation (72). This paradigm is intriguing as it helps reimagine thin-strand pericytes as active players in CBF regulation.

Two scenarios for physiological activation of brain pericyte K_{ATP} channel are proposed. The first is a metabolic change where a decline in extracellular [glucose] activates K_{ATP} channel and rapidly hyperpolarizes pericytes. In this context, pericytes are sensitive to glucose levels fluctuations especially when glucose levels fall below a threshold leading to K_{ATP}-mediated

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hyperpolarization. Selective inhibition of the glucose transporter GLUT1 prevents glucose entry, hyperpolarizes pericyte V_M in a K_{ATP}-dependent manner and increases upstream arteriolar vasodilation (73). Alternatively, pericyte K_{ATP} channel opening can be achieved in response to GsPCR signaling via the $G\alpha_s$/cAMP/PKA cascade (17) (Fig. 3). Adenosine, an astrocyte-derived nucleotide that is released when there is a mismatch between energy supply and demand, activates K_{ATP} channel and hyperpolarizes pericytes via adenosine A_{2A} receptor signaling and the downstream generation of cAMP and PKA activation. Importantly, genetic inactivation of pericyte K_{ATP} channel compromised adenosine-induced CBF increase at the capillary and arteriolar level (17). Collectively, recent studies have established an important role for pericyte K_{ATP} channel function in brain blood flow control.

3.5.2. Kir2: There is electrophysiological evidence of an inwardly rectifying current in brain pericytes that is sensitive to micromolar barium—a pore blocker that is selective for strongly inwardly rectifying Kir2 channels (71) (Fig. 3). Further, imaging data suggest that inhibiting Kir2 channel with barium suppressed pericyte Ca^{2+} signaling (67). How pericyte Kir2 function could affect CBF remains however unknown. As mentioned earlier, pericyte K_{ATP}-induced hyperpolarization engages EC Kir2.1 channel to elicit upstream vasodilation (72). It would be interesting to examine the possible contribution of pericyte Kir2 to this process. One challenge to be considered is that most studies using Ba^{2+} cannot discriminate between endothelial and pericyte Kir2 channels. Transcriptomic analyses interestingly showed profound expression of Kir2.2 (encoded by *Kcnj12*), rather than Kir2.1 (*Kcnj2*), in mouse brain pericytes (18). This warrants further studies to dissect the specific Kir2 subtypes and the corresponding pericyte types to determine their physiological role in CBF regulation.

3.5.3. Kv and BK_{Ca}: Messenger RNA encoding several voltage gated K^+ (Kv) channels has been found in capillary pericytes (18). Recent electrophysiological evidence has confirmed that Kv channels are functional in brain pericytes (71) (Fig. 3). Measured currents from freshly isolated pericytes suggest that Kv1 is the predominant subtype, based on the characteristic kinetics and their sensitivity to 4-aminopyridine, a Kv1.2/1.5 channel blocker (71). Activation kinetics in pericytes were however distinct from those in arterial smooth muscle cells. Exact subtypes as well as their potential role in brain blood flow regulation are not yet characterized. The large conductance BK_{Ca} current was also measured in capillary pericytes. Pharmacological and biophysical characteristics (large conductance, Ca^{2+}/voltage-dependent gating) in pericytes are comparable to vascular smooth muscle cells, but the current density was particularly smaller (71). Considering the high input resistance of pericytes, even a small BK_{Ca} current could significantly shift pericyte V_M. The activation mechanisms of pericyte BK_{Ca} have not been fully elucidated. At a theoretical level, and similar to smooth muscle, BK_{Ca} channel could be activated by VGCC-mediated Ca^{2+} influx and subsequent Ca^{2+} release from the sarcoplasmic reticulum. BK_{Ca} activation and K^+ efflux will likely induce a hyperpolarization that counteracts initial depolarizing signals or transfers to ECs (Fig. 3). Additional studies are needed to uncover several unknown aspects about pericyte K^+ channels and their roles in CBF regulation.

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4. CHANNELOPATHIES IN BRAIN CAPILLARIES

It has been only over the past decade that brain capillary ion channel research has advanced. Yet, several ion channel pathologies—referred to as *channelopathies*—have been described. In this section, we discuss these channelopathies in brain capillary ECs and pericytes.

4.1. Alzheimer's disease

Brain capillary constriction likely contributes to diminished CBF observed during Alzheimer's disease (AD). Excessive pericyte contraction and associated capillary constriction have been reported in brain biopsies obtained from AD patients and in AD mouse models (74, 75). The accumulation of amyloid beta (Aβ) peptides in AD brains presumably constrict capillaries by accelerating ROS generation, which in turn facilitate endothelin-1 (ET-1) production and ultimately pericyte contraction (74). We discussed earlier that pericyte contraction relies on a rise in intracellular $[Ca^{2+}]_i$ due to Ca^{2+} influx, by plasma membrane ion channels, or Ca^{2+} release from internal stores downstream of GqPCR activation. The GqPCR agonist ET-1 activates several ion channels in pericytes including VGCC, TMEM16A and Orai, which suggests that targeting Ca^{2+} influx or release pathways could enhance CBF in AD by reversing pericyte contraction. Consistently, VGCC inhibition attenuated Aβ-induced capillary pericyte contraction in human cortical tissues (Fig. 3). Furthermore, VGCC blockade in early AD decreased capillary stalling and enhanced brain blood flow (75). Given the ability of TMEM16A to depolarize pericytes and activate VGCCs, the suppression of TMEM16A is expected to have a similar beneficial effect on CBF in AD (65) (Fig. 3). Whether targeting other Ca^{2+} influx or release pathways has similar effects remains to be examined.

In addition to ion channel dysregulation in pericytes during AD, capillary endothelial Kir2.1 dysfunction has been implicated in the defective neurovascular coupling and reduced FH response in AD (Fig. 2). The Kir2.1 current was significantly lower in capillaries from AD mice compared with control mice (76). Kir2.1-mediated cortical CBF increase during whisker stimulation was similarly compromised. Interestingly, PIP_2 application onto capillary ECs restored channel function and PIP_2 systemic injection corrected FH in AD mice (76). This could be clinically relevant considering that Aβ can decrease PIP_2 levels by increasing its hydrolysis and that decreased PIP_2 levels have been reported in the brains of AD patients (77–79). Moreover, PLC—which hydrolyses PIP_2 into DAG and IP_3—is elevated in AD brains (80), which could be implicated in reduced PIP_2 levels and subsequent failure of capillary endothelial Kir2.1 signaling. Beyond endothelial Kir2.1, we have recently showed that Aβ also affects capillary EC Piezo1 function (81). The Aβ isoform that is heavily deposited in the brain circulation, $Aβ_{1-40}$, increased Piezo1 activity and enhanced its mechanosensitivity via a mechanism that involved ROS generation (81). These observations could unravel a *double-hit* scenario in which a rise in depolarizing Piezo1 function in AD aggravates the decline in hyperpolarizing Kir2.1 function, but this remains to be tested along with the possible functional consequences on CBF.

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4.2. Cerebral small vessel diseases

Cerebral small vessel diseases (cSVDs) account for 25% of ischemic strokes and more than 40% of dementia cases (82, 83). The most common form of hereditary SVD is the cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), that is caused by mutations of the *NOTCH3* gene (83, 84). Both patients and CADASIL mouse models suffer defects in neurovascular coupling (85–87). Mutations in the genes encoding collagen type IV (*COL4A1* and *COL4A2*) cause another inherited form of cSVD as part of the Gould syndrome. *Col4a1* mutant mice demonstrate disrupted FH, like CADASIL mice (88). Importantly, studies using CADASIL or *Col4a1* mutant mice have shown profound reductions in capillary EC Kir2.1 function, leading to compromised capillary-to-arteriole electrical signaling and FH (87, 88) (Fig. 2). The molecular mechanisms of CADASIL and Gould syndrome are distinct, yet PIP_2 was able to correct Kir2.1 function and endothelial electrical signaling in these diseases—as it did in AD animals. Capillary Kir2.1 channelopathy in CADASIL was attributed to diminished PIP_2 synthesis, due to disrupted mitochondrial metabolism and reduced ATP levels (87). PIP_2 depletion in *Col4a1* mutant mice was distinctively a result of elevated phosphatidylinositol-3-kinase (PI3K) activity, which diminishes PIP_2 bioavailability by phosphorylation modification (88). Despite the pathological differences of the two cSVDs and the mechanisms affecting PIP_2 availability, elevating PIP_2 levels by systemic injection of a PIP_2 derivative or a PI3K inhibitor successfully restored the hyperemic response and enhanced blood flow increase in response to whisker stimulation (87, 88). These findings collectively demonstrate a therapeutic potential for PIP_2 in correcting Kir2.1 channelopathies and improving CBF.

4.3. Hypertension

Hypertension disrupts the structure and function of the cerebral circulation, with hypertensive patients exhibiting significant impairment in FH (89–93). Hypertension animal models display a progressive decline in hyperemic responses with age (93–95). Capillary constriction in the CNS during hypertension suggests that pericyte and/or endothelial functions might be compromised. In a polygenic hypertension mouse mode, this decline in FH was largely driven by the downregulation of capillary EC Kir2.1 function, as evident by decreased Kir2.1 currents in electrophysiology recordings and diminished capillary-to-arteriole signaling in response to capillary stimulation with external K^+ (Fig. 2) (95). Downregulation of endothelial Kir2.1 was observed in AD and cSVDs. It awaits further investigation to determine the mechanisms underlying Kir2.1 dysfunction in hypertension, and whether such dysregulation persists in other models of hypertension.

4.4. Ischemic stroke

Capillary constriction is implicated in the pathogenesis of ischemic stroke (96). Pericyte contraction during ischemia and the following restoration of blood flow “no reflow phenomenon” leads to capillary constriction and ensuing brain under-perfusion (96, 97). This contraction is attributed to increased pericyte intracellular $[Ca^{2+}]_i$, resulting from decreased ATP levels and ion pumping failure. Pericyte contraction could also be driven by the production of vasoconstrictor mediators such as ET-1 and thromboxane during

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ischemia (98–100). These vasoconstrictors elevate $[Ca^{2+}]_i$ by enhancing Ca^{2+} influx or Ca^{2+} release from the intracellular stores. For example, ET-1, released during ischemia, augments TMEM16A activation in capillary pericytes which in turn amplifies depolarization and Ca^{2+} influx via VGCCs (65) (Fig. 3). TMEM16A activation during ischemia is involved in the long-lasting capillary pericyte contraction and reduced CBF observed after ischemic injury. Further, there is a strong link between increased TMEM16A expression and poor recovery after ischemia (65). The inhibition of pericyte contraction could therefore provide a means to improve stroke outcomes. The pharmacological blockade of VGCCs ameliorate pericyte contraction, and TMEM16A inhibition is also linked to significant benefits after ischemia such as reduced capillary stalling by neutrophils and enhanced CBF and reduced infarct size (65). Since GqPCR signaling could be altered during ischemia, it is possible that downstream PLC activation and PIP_2 depletion could affect ion channels not only in pericytes, but also in capillary ECs.

5. CHANNELOPATHIES AS TRIGGERS FOR NEUROVASCULAR DYSFUNCTION

The high metabolic demands of neurons place them within a critical ATP energetic range, where channelopathies in brain capillaries could dramatically exacerbate neurovascular dysfunction. Optical ATP imaging demonstrates that presynaptic nerve terminals maintain a narrow energetic ATP margin of ~1–2 mM, sufficient for only a minute or two of activity without synthesis (101). This limited reserve underscores the brain's reliance on rapid FH to deliver glucose and oxygen to sustain synaptic transmission (101). More recent evidence even suggests that ATP concentration in postsynaptic spines is in the sub-millimolar range (102). This establishes the simple fact that the narrow ATP margin in neurons makes them susceptible to energetic perturbations. Therefore, precisely tuned blood flow during FH is indispensable to meet on-demand ATP synthesis. Further, recent cardiac studies have elegantly shown that resting ATP levels in cardiac myocytes are much lower than canonically thought and further show that ATP fluctuations are synchronized with heart beats (103, 104)—similar to neuronal ATP fluctuations during synaptic transmission. These findings suggest that hyperemia is the only way to support ongoing ATP synthesis in the heart and the brain. Channelopathies such as Kir2.1 dysfunction in brain capillary ECs, observed in AD, cSVDs, and hypertension, disrupt hyperpolarizing signals critical for FH, reducing CBF and risking ATP depletion below the threshold required for neuronal function (76, 87, 88, 95). This tight energetic margin necessitates rapid hyperemia to prevent synaptic failure, highlighting channelopathies as initiators of neurovascular dysfunction.

Channelopathies in brain capillaries not only acutely disrupt capillary blood flow but could also drive long-term functional and anatomical capillary rarefaction, compromising perfusion capacity and metabolic support. Dysregulation of channels like Kir2.1, VGCC, and TMEM16A, as seen in AD, cSVDs, hypertension, and ischemic stroke, leads to sustained capillary constriction and impaired FH, mediated by mechanisms such as PIP_2 depletion, Aβ-induced ROS generation, and ET-1-driven Ca^{2+} influx (65, 74, 76, 87, 88). Chronic activation of these pathways promotes oxidative stress and inflammation, with ROS and inflammatory cytokines inducing endothelial and pericyte apoptosis, resulting in

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functional rarefaction (reduced capillary perfusion) and anatomical rarefaction (physical loss of capillaries) (105)(74). This microvascular loss diminishes CBF, exacerbating the brain's narrow energetic margin and limiting substrate delivery for neuronal activity. Consequently, channelopathies create a vicious cycle where acute perfusion deficits, driven by impaired capillary ion channel activities, evolve into chronic neurovascular dysfunction, worsening metabolic stress and accelerating neurodegenerative and cerebrovascular pathologies.

6. CONCLUDING REMARKS AND FUTURE DIRECTIONS

This review underscores the pivotal role of ion channels in brain capillary ECs and pericytes in orchestrating neurovascular coupling and maintaining CBF. The intricate interplay between endothelial and pericyte ion channels facilitates rapid and precise responses to neuronal activity, ensuring adequate brain perfusion. However, channelopathies in diseases such as Alzheimer's, cerebral small vessel diseases, hypertension, and ischemic stroke highlight the vulnerability of these mechanisms, leading to impaired CBF and exacerbating neuropathology. The therapeutic potential of targeting ion channels offers promising avenues for improving neurovascular outcomes. Future research should focus on elucidating the precise molecular mechanisms governing ion channel regulation, such as the role of PIP_2 in pericyte Kir2 and EC Piezo1 and TRPV4 function, and the interplay between pericyte and endothelial channels in deep capillary networks. Additionally, exploring the subtype-specific roles of pericyte ion channels (e.g., Kir2, Kv, BK_{Ca}) and their contributions to CBF regulation will enhance our understanding of neurovascular dynamics. Developing targeted therapies that modulate ion channel activity with high specificity, alongside advanced imaging techniques to monitor real-time channel function *in vivo*, will be critical for translating these findings into interventions for cerebrovascular and neurodegenerative disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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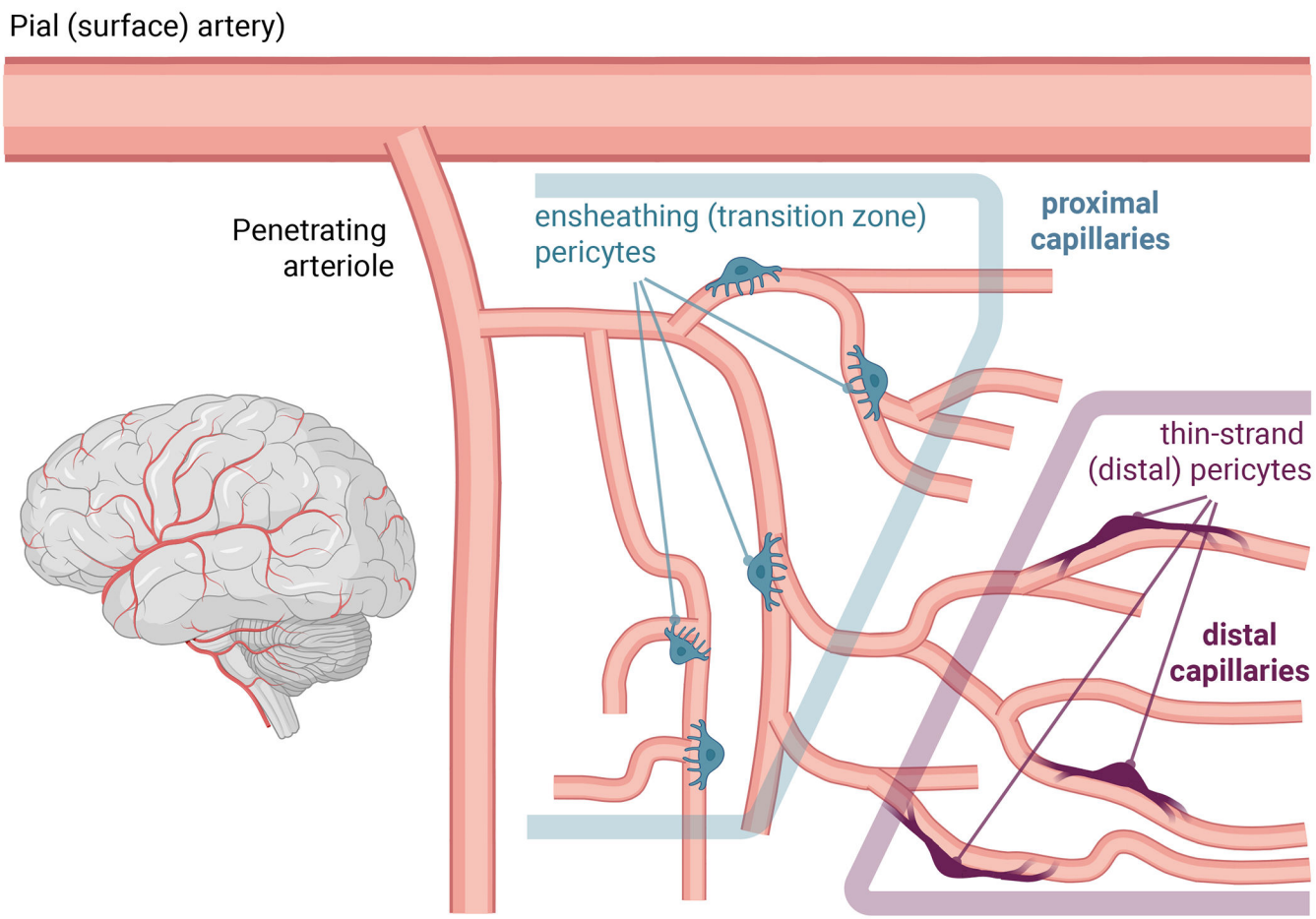


Figure 1. Simplified anatomy of the brain vasculature.

Pial arteries run on the brain surface, from which penetrating arterioles orthogonally dive into the brain parenchyma. Penetrating arterioles branch at regular intervals forming capillary networks that lie near all neurons (not shown). Capillary networks are primarily comprised of endothelial cells (ECs) throughout the network, and pericytes that are embedded in the capillary basement membrane. Pericyte characteristics and morphology change with the capillary branch order: proximal capillaries (~1st-4th order) are covered with ensheathing (transition zone) pericytes, and deeper capillaries are covered with thin-strand (distal) pericytes.

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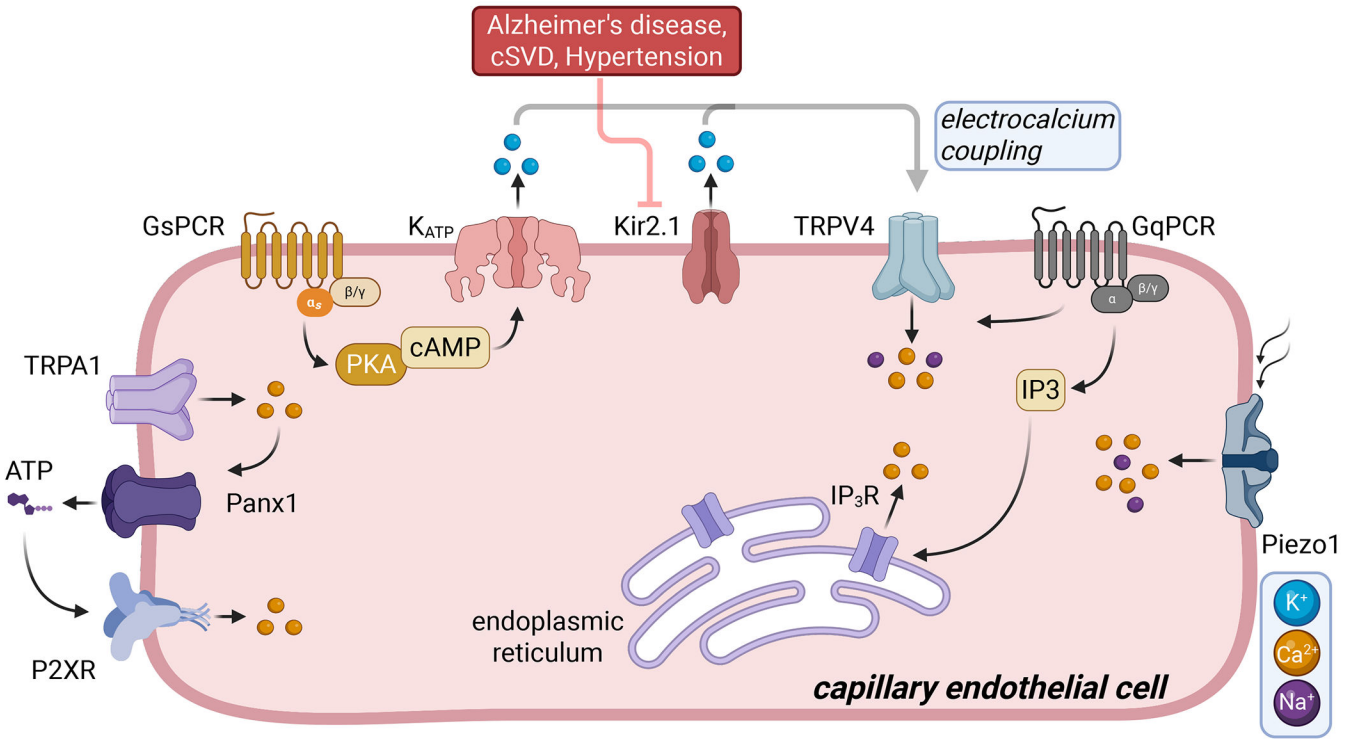


Figure 2. Capillary endothelial ion channels.

Kir2.1 channels are expressed in capillary ECs and are activated by neuronal derived extracellular K^+. K_{ATP} channels are also expressed but are rather activated downstream of GsPCR stimulation. K^+ efflux—through Kir2.1 and/or K_{ATP} channels—hyperpolarizes ECs, and the electrical signal is transmitted upstream through gap junctions to dilate arterioles and increase blood flow. The Ca^{2+}-permeable TRPV4 channel is activated downstream to GqPCR stimulation. Endothelial Ca^{2+} events are also mediated by IP_3-mediated Ca^{2+} release from the intracellular stores. Hyperpolarization, by Kir2.1 and K_{ATP} channels, influence endothelial Ca^{2+} dynamics by increasing the driving force of Ca^{2+} entry (electrocalcium coupling). TRPA1 channel is also present in capillary ECs, where neuronally mediated TRPA1 activation leads to Ca^{2+} influx to activate Pannexin-1 channel and then purinergic receptors to facilitate additional Ca^{2+} influx. The resulting Ca^{2+} wave propagates to upstream segments to hyperpolarize them and relax smooth muscle cells. Capillary ECs expresses the mechanosensor Piezo1. During functional hyperemia, Piezo1 activation could depolarize ECs, counteract endothelial hyperpolarization and therefore help repolarize ECs. Several pathologies are associated with Kir2.1 dysfunction in brain capillaries, which in turn is involved in the associated CBF defects.

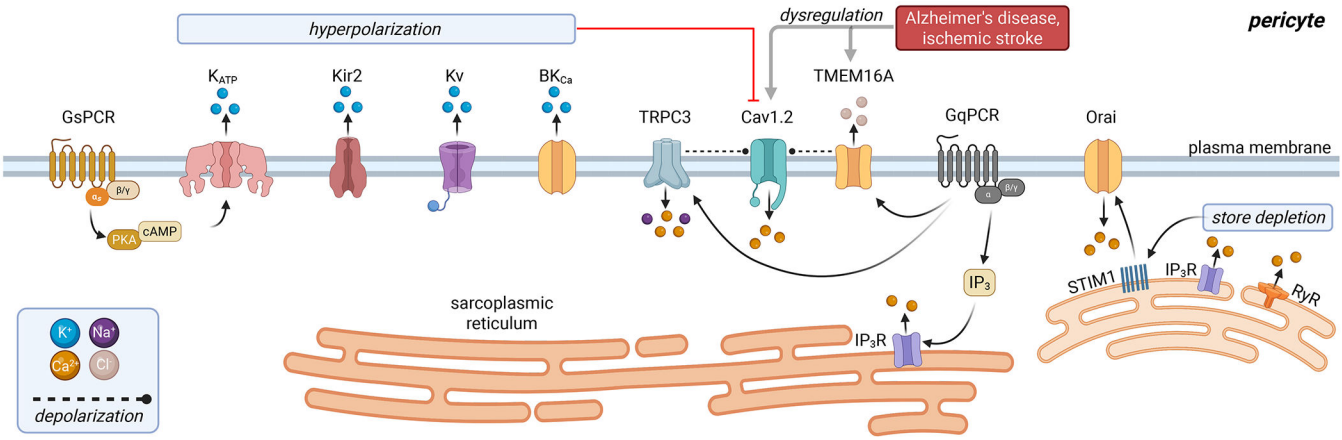


Figure 3. Ion channels in brain capillary pericytes.

Several K^+ channels have been reported in capillary pericytes, among which K_{ATP} is highly expressed. GsPCR activation in distal pericytes activates K_{ATP} channels and hyperpolarizes the cell. Pericyte hyperpolarization is transmitted to adjacent ECs to activate Kir2.1 channels; retrograde hyperpolarization subsequently dilates feeding arterioles. Pericytes also express Kir2, Kv and BK_{Ca} channels, but their exact roles in CBF regulation are not clear. Pericyte GqPCR activation is associated with increased cytosolic [Ca^{2+}], not only through downstream IP_3R activation but also via several other ion channels (Cav1.2, TMEM16A, TRPC3, Orai). VGCCs are the main Ca^{2+} influx pathway in ensheathing pericytes. TMEM16A and TRPC3 potentiate VGCC function, by depolarizing pericytes. TMEM16A is activated via IP_3-mediated Ca^{2+} release from the sarcoplasmic reticulum stores, and TRPC3 is likely activated downstream of mechanosensitive GqPCR. The role of Cav1.2 in Ca^{2+} influx is minimal in distal thin-strand pericytes, a function that is likely mediated via store-operated Ca^{2+} entry, where STIM1/Orai signaling transduces when Ca^{2+} stores are depleted. Pericyte contraction leading to capillary constriction has been observed in AD and ischemic stroke. The excessive contraction involves TMEM16A upregulation, and excessive Ca^{2+} influx via VGCCs. Cav1.2 blockers decrease capillary stalling and enhance CBF in AD models, highlighting their involvement in AD pathogenesis.

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