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Ion channel networks in the control of cerebral blood flow

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Abstract

One hundred and twenty five years ago, Roy and Sherrington made the seminal observation that neuronal stimulation evokes an increase in cerebral blood flow.[1] Since this discovery, researchers have attempted to uncover how the cells of the neurovascular unit—neurons, astrocytes, vascular smooth muscle cells, vascular endothelial cells and pericytes—coordinate their activity to control this phenomenon. Recent work has revealed that ionic fluxes through a diverse array of ion channel species allow the cells of the neurovascular unit to engage in multicellular signaling processes that dictate local hemodynamics.

In this review we center our discussion on two major themes: (1) the roles of ion channels in the dynamic modulation of parenchymal arteriole smooth muscle membrane potential, which is central to the control of arteriolar diameter and therefore must be harnessed to permit changes in downstream cerebral blood flow, and (2) the striking similarities in the ion channel complements employed in astrocytic endfeet and endothelial cells, enabling dual control of smooth muscle from either side of the blood–brain barrier. We conclude with a discussion of the emerging roles of pericyte and capillary endothelial cell ion channels in neurovascular coupling, which will provide fertile ground for future breakthroughs in the field.

Keywords

Ion channels, calcium signaling, calcium channels, potassium channels, transient receptor potential channels, neurovascular coupling, functional hyperemia, cerebral blood flow, neurovascular unit, parenchymal arteriole, pial artery, smooth muscle, endothelium, astrocytic endfoot, cerebrovascular resistance

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Introduction

To support ongoing function, neurons must extract oxygen and glucose from the cerebral circulation on an as-needed basis. The continuous supply of nutrients to match local demand is made possible by neurovascular coupling (NVC). The NVC signaling cascade recruits multiple cell types to link neuronal activity to a rise in local blood flow—a phenomenon termed functional hyperemia—thus providing the energy substrates required to satisfy the enhanced metabolic requirements of active neurons. A number of mechanisms have evolved in parallel to ensure the fidelity of neurovascular communication, and many of these are dependent on ion channel signaling. Ion channels are also important contributors to the control of basal cerebral arteriolar tone, which sets the resting level of brain perfusion and permits dynamic changes in blood flow to match variations in local neuronal activity.

If the NVC cascade is impaired or cerebrovascular tone is pathologically altered, cerebral blood flow is compromised and neuronal dysfunction ensues. Cerebral blood flow is disturbed in a range of disorders, including Alzheimer's disease,[2] hypertension,[3] stroke,[4] diabetes,[5] and CADASIL (cerebral autosomal

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dominant arteriopathy with subcortical infarcts and leukoencephalopathy).[6] Thus, a fuller understanding of how cerebrovascular tone and functional hyperemia are controlled may unearth new treatment options for brain disorders with a vascular component.

In this review, our primary focus is on neurovascular ion channel signaling at the level of the parenchymal arteriole, where the influence of signaling molecules released from astrocytic endfeet on arteriolar diameter and blood flow is well established. We explore two major themes. First, we examine the ion channels that facilitate tight control of smooth muscle (SM) membrane potential (V_m) and thus the contractile state (tone) of these cells. Second, we focus on the marked similarity in ion channel expression and signaling between astrocytic endfeet and endothelial cells (ECs), which exert dual control of SM tone from parenchymal and luminal sides of the vessel wall, respectively. Our discussion centers on studies examining ion channel signaling *in vivo* and in acutely isolated, intact *ex vivo* preparations. Where relevant, we compare parenchymal arterioles with vessels from other circulatory beds. We conclude with an exploration of a growing frontier of research: the control of cerebral blood flow at the capillary level. Here we highlight the known roles of ion channels in pericytes and capillary ECs, which likely have an important role in conducting signals from deep within the capillary bed upstream to parenchymal arterioles. The major ion channels reviewed are summarized in Table 1.

The neurovascular unit

The neurovascular unit (NVU) consists of neurons, astrocytes and the cells of parenchymal arterioles, which consist of a single layer of SM cells (SMCs) surrounding the endothelium (Figure 1a). Parenchymal arterioles originate from pial (surface) vessels and penetrate into the brain, where they become almost completely encased by astrocytic endfeet.[30,31] This positions endfeet to act as intermediates between neurons and the vasculature. Similarly, endothelial membrane extensions project through fenestrations in the internal elastic lamina and basement membrane of arterioles to directly contact SMCs (Figure 1b). These structures—termed myoendothelial projections (MEPs)—not only provide direct contact between these two cell types through gap junctions but also offer a unique intracellular and extracellular microdomain signaling environment for controlling vascular tone. In contrast, direct cell–cell contact has not been observed between astrocytic endfeet and SMCs, although the membranes of these cells are closely opposed. Each parenchymal arteriole supplies a large territory of downstream capillaries, an anatomical organization that positions parenchymal arterioles as bottlenecks to the entry of blood into the brain.[32] Therefore, control of parenchymal arteriole diameter by NVC mechanisms is of vital importance to the regulation of downstream blood flow.

The ionic composition in the NVU establishes the basal conditions for controlling cerebral blood flow

The choroid plexuses produce cerebrospinal fluid (CSF), which fills the ventricles and the subarachnoid space and circulates from the latter into the brain parenchyma via the Virchow-Robin space, taking a paravascular route through the 'glymphatic' system.[34] The composition of CSF is distinct from that of plasma (see Table 2, reproduced from Brown et al.[35]). Thus, with the luminal surface of ECs exposed to plasma and the parenchymal surface of SMCs bathed in CSF, SMCs and ECs experience extracellular milieus with different ionic compositions. The concentrations of ions in these extracellular compartments dictate their equilibrium potentials and, by extension, both ion channel activity and V_m. This, in turn, influences the level of resting SM tone and tissue perfusion. Variations in local ion concentrations during neuronal activity modulate the SM V_m, thereby exerting a powerful effect on parenchymal arteriolar diameter; this is the central relationship underlying the dynamic regulation of cerebral blood flow.

Part I: control of the smooth muscle membrane potential is central to the control of cerebral blood flow

The key role of the voltage-dependent Ca^{2+} channel as a membrane potential biosensor

The V_m of SM exerts profound control over cerebral artery diameter.[9,36] In isolated parenchymal arterioles, a graded increase in intravascular pressure, for example from 5 mm Hg to 40 mm Hg, causes graded V_m depolarization from approximately –60 mV to –40 mV, an elevation in intracellular calcium ($[Ca^{2+}]_i$) from ~120 nM to ~250 nM, and approximately 50% constriction[9] (Figure 2). In contrast, V_m hyperpolarization to –60 mV causes near-maximal vasodilation (i.e. almost equivalent to the passive diameter of the vessel).[9,36] The depolarization/vasoconstriction to increasing pressure is termed the 'myogenic response' and is an intrinsic property of small resistance arterioles from a range of vascular beds.[37] In brain arterioles, the myogenic response is particularly important for cerebral autoregulation, in which vessels constrict or relax to maintain a constant level of brain perfusion despite varying peripheral blood pressure.[30]

Table 1. Expression and function of key ion channels in astrocytic endfeet, and ECs and SMCs of parenchymal arterioles.

	Astrocytic Endfeet	Notes, References	Parenchymal Endothelium	Notes, References	Parenchymal Smooth muscle	Notes, References
Membrane potential (mV)	−87	*In vivo,* cell soma.[7]	−35 to −40?	Not measured. Inferred from observations that EC-SMC Vm is nearly equivalent in hamster feed arteries.[8]	−35 to −40	*In situ,* 40 mm Hg.[9,10]
Ca^{2+} channels						
IP_3R	+	Ca^{2+} wave generation.[11]	+?	Not directly tested. Mediates Ca^{2+} signaling in other arteries.[12]	+?	Not directly tested. UTP evokes Ca^{2+} waves in pial arteries.[13]
RyR	–	Not present.[11]	–?	Not directly tested. Not present in other arterioles.	+	Ca^{2+} waves *in situ* at 40 mm Hg. Ca^{2+} sparks in response to H^+.[14]
VDCC	–?	Nifedipine inhibition of Ca^{2+} waves.[15]	–?	Not directly tested. Not present in other arterioles.	+	Depolarization-evoked Ca^{2+} entry leading to constriction.[9]
TRPV4	+	Ca^{2+} entry and wave propagation.[16]	+?	Not directly tested. Present in pial endothelium.[17,18]	+	Ca^{2+} influx.[19]
TRPC6/M4	n.d.		–	No effect of endothelium removal on TRPM4 responses.[20]	+	TRPM4 contributes to myogenic tone.[20] Both C6 and M4 contribute to tone in pial arteries.[21]
K^+ channels						
BK	+	Vasoactive K^+ release.[22,23]	–	Not present in acutely isolated cells.[10]	+	Present *in situ*. Low basal activity at 40 mm Hg.[10]
SK	–	Not detected.[24]	+	Present in acutely isolated cells.[10]	–	No effect of NS309 after endothelial removal.[10]
IK	+	Detected in processes and endfeet. Engaged in NVC.[24]	+	Present in acutely isolated cells.[10]	–	No effect of NS309 after endothelial removal.[10]
Kv	–?	Not directly tested. Membrane depolarization sufficient to engage Kv is unlikely to occur.	–	No voltage-activated currents after SK/IK blockade.[10]	+	Tonically active *in situ* at 40 mm Hg. Resists constriction.[25]
$K_{IR}2$	+?	Not directly observed in endfeet, but expressed by astrocytes.[26]	+	Present in other arterioles.[27] Preliminary data indicates present in parenchymal endothelium.[26]	+	Activated by extracellular K^+.[22,23,28,29]

Summary of the expression of ion channels involved in neurovascular coupling (NVC) in native preparations of astrocytic endfeet, parenchymal arteriole ECs and SMCs. Studies in culture systems were not considered. Question marks are used to highlight inferences drawn in cases where channels have not been directly observed in a particular cell type. EC-SMC Vm: endothelial cell/smooth muscle cell membrane potential; VDCC: voltage-dependent calcium channel; TRP: Transient receptor potential; BK: large-conductance calcium-activated potassium channel; SK: small-conductance calcium-activated potassium channel; IK: intermediate-conductance calcium-activated potassium channel; Kv: Voltage-dependent potassium channel; n.d.: no data.

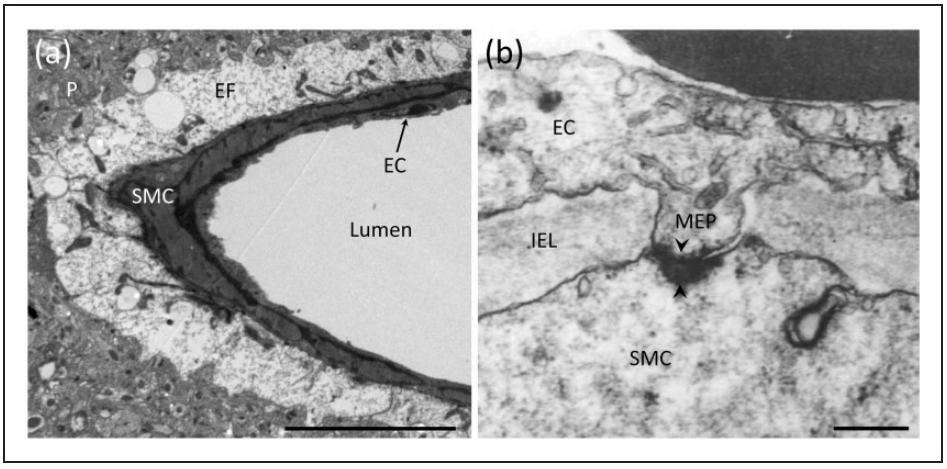

Figure 1. Anatomical features of the NVU and MEPs. (a) Electron micrograph depicting astrocytic endfeet (EF) enveloping a parenchymal arteriole with a single layer of SMCs and underlying ECs. Adjacent to the endfeet is the brain parenchyma (P) containing neuronal and astrocytic processes. Scale bar: 10 μm. (b) A MEP site through a fenestration in the internal elastic lamina (IEL) between an EC and SMC in a human parenchymal arteriole. Black arrowheads indicate a myoendothelial gap junction. Scale bar: 250 nm. Reproduced with permission from Aydin et al.[33]

Table 2. The composition of plasma and cerebrospinal fluid.

	Plasma	CSF
Na^+ (mM)	155	151
K^+ (mM)	4.6	3.0
Mg^{2+} (mM)	0.7	1.0
Ca^{2+} (mM)	2.9	1.4
Cl^- (mM)	121	133
HCO_3^- (mM)	26.2	25.8
Glucose (mM)	6.3	4.2
Amino acids (mM)	2.3	0.8
pH	7.4	7.4
Osmolality (mosmol.Kg H_2O^{-1})	300	305
Protein[a] (mg $100\,g^{-1}$)	6500	25

[a]Rabbit CSF; all other values are for dog CSF. Reproduced from Brown et al.,[35] with permission.

Therefore, myogenic regulation sets the level of resting perfusion, while also providing a baseline tone from which arterioles can dilate or constrict in response to factors released during neuronal activity. As such, myogenic mechanisms can be viewed as the foundation upon which NVC mechanisms are constructed.

Pial arteries on the surface of the brain develop less myogenic tone in response to pressure compared with parenchymal arterioles, despite the fact that the relationship between SM $[Ca^{2+}]_i$ and constriction is identical in both of these types of vessel (see Figure 2d). This is because parenchymal arteriole SM is more depolarized than pial SM at lower pressures (Figure 2b) and thus exhibits a greater influx of Ca^{2+} (Figure 2c) and greater constriction (Figure 2a). The molecular arrangement that leads to the higher sensitivity of parenchymal arterioles to pressure has not yet been elucidated but likely involves a loss of negative feedback control of the SM V_m by Ca^{2+} spark-BK channel interactions (see 'Ryanodine Receptors in Smooth Muscle', below).

L-type VDCCs are the key voltage sensors in vascular SM.[38] These channels translate changes in the V_m into alterations in $[Ca^{2+}]_i$ and thereby adjust the contractile state of the cell. Their central importance is evidenced by the fact that V_m changes induced by pressure or other agents have no effect on vascular tone in the presence of VDCC inhibitors,[36] highlighting the vital role of Ca^{2+} entry through L-type VDCCs in vasoconstriction. Parenchymal arteriolar myocytes express L-type VDCCs composed of pore-forming $Ca_V1.2$ α_{1C}-subunits and associated $\alpha_2\delta$-, β- and γ-subunits.[9,39] Recent studies have also presented evidence for the expression and function of T-type VDCCs in SMCs of pial arteries.[40,41] However, no contribution of T-type channels to either $[Ca^{2+}]_i$ or arteriolar tone has been shown at physiological pressures in isolated parenchymal arterioles.[9]

Neurovascular signaling mediators that converge on the SM utilize a variety of mechanisms to drive changes in V_m. Critically, this leads to either (1) closure of VDCCs (in the case of membrane hyperpolarization), a fall in global $[Ca^{2+}]_i$ and vasodilation or (2) depolarization and an increase in VDCC open probability (Po), causing an increase in $[Ca^{2+}]_i$ and vasoconstriction.[38,42] Thus, tuning the SM V_m to modulate Ca^{2+} entry through VDCCs is the key mechanism for controlling the diameter of parenchymal arterioles and rapidly influencing cerebral blood flow.

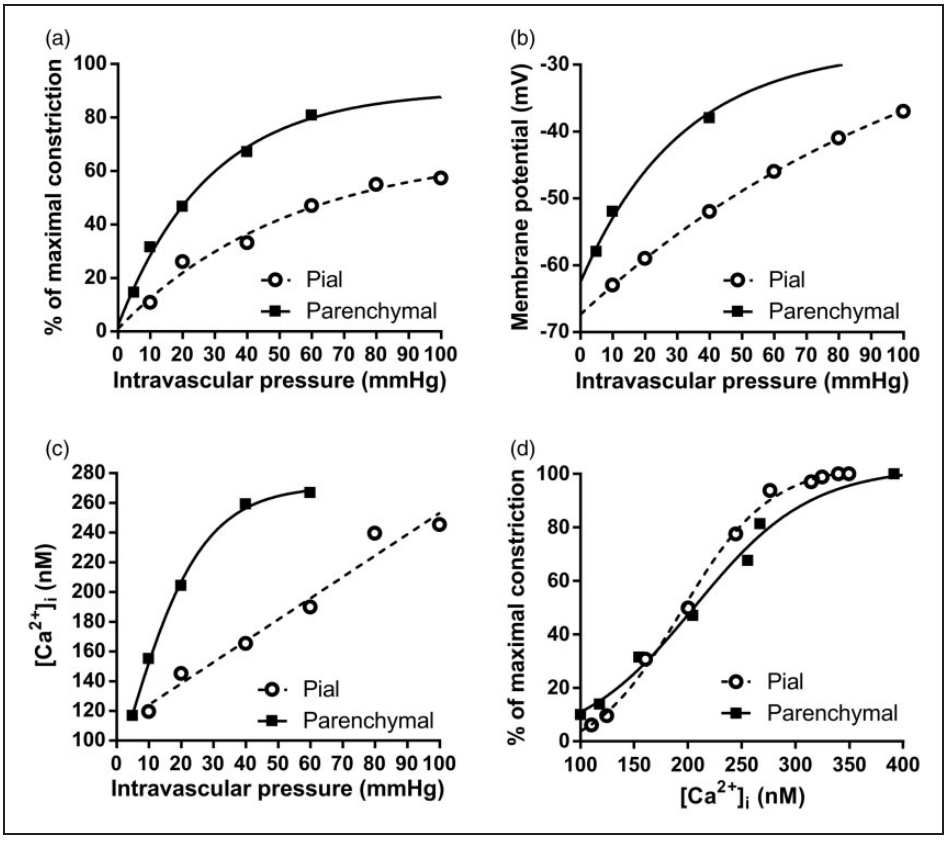

Figure 2. Relationships between intravascular pressure, vessel diameter, $[Ca^{2+}]_i$ and V_m in pial arteries and parenchymal arterioles. (a) One fundamental difference between vessels of the cerebral circulation is that parenchymal arterioles develop more tone in response to lower intravascular pressure compared to pial arteries. (b) The phenomenon in *A* is linked to the parenchymal arteriole SM V_m, which is more depolarized in response to lower pressure compared to pial arteries. (c) Increases in $[Ca^{2+}]_i$ in response to increasing intravascular pressures are greater in parenchymal arterioles than in pial arteries owing to higher voltage-dependent calcium channel (VDCC) activity caused by the greater degree of SMC cell depolarization, as illustrated in (b). (d) There is no difference in the sensitivity of the SM contractile apparatus to Ca^{2+} between pial arteries and parenchymal arterioles, indicating that the difference in the pressure-constriction relationship between these two types of vessels is due to the difference in SM V_m in response to pressure. Data were re-plotted from ref.[9] Parenchymal arteriole V_m data were obtained from F. Dabertrand (personal communication) and from Nystoriak et al.[9] and Hannah et al.[10]

A role for smooth muscle transient receptor potential channels in myogenic depolarization: evidence from pial and parenchymal arterioles

Transient receptor potential (TRP) channels are a diverse family of cation-permeable channels activated by a wide range of stimuli.[43] Members of the TRP family share an overall structural similarity, forming tetrameric homomeric or heteromeric channels from subunits containing six transmembrane domains. TRP channels are broadly classified on the basis of sequence homology into six subfamilies in mammals: TRPA (ankyrin), TRPC (classic or canonical), TRPM (melastatin), TRPML (mucoliptin), TRPP (polycystin) and TRPV (vanilloid).[44] Most of these channels act as routes for the entry of Ca^{2+}, although some, such as a subset of TRPM channels, primarily mediate Na^+ entry. For a comprehensive overview of TRP channels in the vasculature, see Earley and Brayden.[45]

Mounting evidence suggests that intravascular pressure or vasoconstrictor agonists can cause V_m depolarization through the activation of TRP channels in pial artery myocytes; this, in turn, increases the activity of VDCCs and promotes vasoconstriction. TRPV4, TRPC3, TRPC6 and TRPM4 channels have all been identified in pial SM,[46–49] and recent studies indicate that pressure causes depolarization through activation of TRPM4 and TRPC6 channels. Monovalent cation-permeable TRPM4 channels, though impermeable to Ca^{2+} ions,[44] are activated by sarcoplasmic reticulum (SR) 1,4,5-trisphosphate receptor (IP_3R)-mediated Ca^{2+} release events in pial artery myocytes.[50] This results in an influx of Na^+ through TRPM4, which can be measured as a transient inward cation current (TICC).[50] Pressure-induced constriction of *ex vivo*

cerebral arteries requires functional TRPM4 channels,[46] and blocking TRPM4 causes almost maximal vasodilation of pressurized arteries,[51] suggesting that these channels are vitally important for the myogenic response. Similarly, activation of TRPC6 channels, which have a Ca^{2+}:Na^{+} permeability ratio of ~6:1,[52] is another contributor to pressure-induced constriction of isolated pial arteries, as evidenced by the fact that knockdown of TRPC6 using antisense oligonucleotides attenuates the myogenic response.[47] A recent study,[21] conducted in isolated myocytes and pressurized pial arteries, unifies the contributions of TRPC6 and TRPM4 into a single mechanism for myogenic constriction. Here, pressure-induced activation of phospholipase C (PLC) γ1 liberates IP_3 and diacyl glycerol (DAG). Subsequently, DAG activates TRPC6 channels directly and the resulting Ca^{2+} influx across the sarcolemma acts synergistically with IP_3 to activate IP_3Rs, releasing Ca^{2+} from the SR that then activates TRPM4 channels to depolarize the membrane.[21]

Despite these major advances in our understanding, the mechanism by which pressure activates TRP channels has not been firmly established. In addition to the possibility that TRP channels directly sense membrane deformation,[53] they may be attached to other cellular structures that respond to pressure, such as the cytoskeleton.[54] Alternatively, upstream mechanosensitive signaling elements may transduce physical forces into second messenger signaling (such as PLC activation),[21] which is then detected by TRP channels.[55] These possibilities, which are not mutually exclusive, have recently been reviewed in detail.[56]

In contrast to the vasoconstriction associated with TRPC6 and TRPM4 activation, SM TRPV4 channels, which are non-selective but possess a high permeability to Ca^{2+}, have been implicated in vasodilatory mechanisms in pial arteries.[49] According to this mechanism, reported by Earley et al., epoxyeicosatrienoic acids (EETs) activate TRPV4 channels, which conduct Ca^{2+} into the cell and enhance ryanodine receptor (RyR)-mediated Ca^{2+} spark activity (see below),[49] resulting in vasodilation.[57]

The studies described above were conducted using pial arteries. Recently, Li et al.[20] pioneered the exploration of SM TRP channel contributions to myogenic tone in parenchymal arterioles. In keeping with their myogenic role in pial arteries, TRPM4 channels were found to be vitally important for the development of tone in isolated arterioles from this vascular bed. Here, TRPM4 activation and membrane depolarization lies downstream of direct mechanoactivation of purinergic $P2Y_4$ and $P2Y_6$ G_q-type G-protein coupled receptors (G_qPCRs) and consequent stimulation of PLC activity by intravascular pressure,[20,58,59] suggesting that mechanisms similar to those reported for pial arteries may also be at play in parenchymal arterioles (Figure 3).

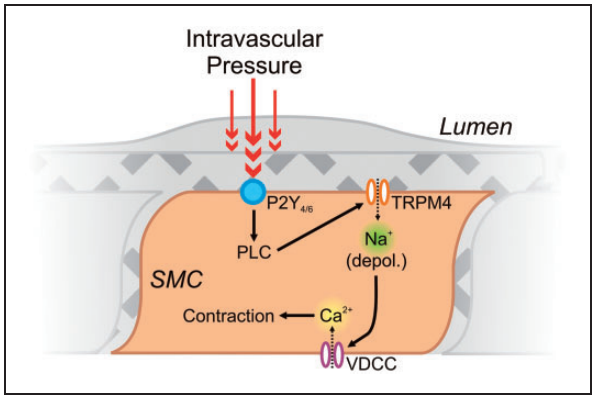

Figure 3. The central roles of SM TRPM4 channels and VDCCs in myogenic constriction of parenchymal arterioles. Intravascular pressure (~40 mm Hg[60]) activates G_q-coupled P2Y receptors on the SM, leading to PLC activation. Through an as-yet-undefined pathway in parenchymal arterioles (see text for insights from pial arteries[21]), PLC activation leads to a depolarizing Na^{+} influx through TRPM4, triggering Ca^{2+} influx through VDCCs and leading to myocyte contraction.[20]

Ryanodine receptors in smooth muscle: Ca^{2+}-release channels that oppose myogenic constriction

Although extracellular Ca^{2+} entry through VDCCs has a central role in the delivery of Ca^{2+} for SM contraction, Ca^{2+} release from the SR through RyRs has the opposite effect.[61] Thus, Ca^{2+} in SM has a dichotomous role, with distinct Ca^{2+} signaling modalities either promoting or opposing vasoconstriction.

RyRs are homotetrameric assemblies located on the sarco-/endoplasmic reticulum (SR/ER) in many cell types. They are gated by Ca^{2+} and modulated by Mg^{2+}, ATP, calmodulin and FK506-binding proteins as well as by phosphorylation by protein kinases such as protein kinase A (PKA), Ca^{2+}/calmodulin-dependent kinase II and cGMP-dependent protein kinase (PKG).[62] Ca^{2+} release through RyRs can lead to two types of signal: waves and sparks. Ca^{2+} waves are propagating events with considerable spatial spread that result from Ca^{2+}-induced Ca^{2+} release (CICR), a process whereby the presence of a sufficiently high concentration of local Ca^{2+} evokes Ca^{2+} release from nearby RyRs and/or IP_3Rs in the SR membrane. This released Ca^{2+} then evokes further release from adjacent channels; the process repeats, leading to a regenerative Ca^{2+} wave that propagates along the SR. The role of Ca^{2+} waves in vascular SM is not fully understood, although waves in pial arteries may contribute to myogenic tone development, particularly at lower pressures.[63] In contrast, Ca^{2+} sparks are highly localized, brief events

mediated by the simultaneous opening of several clustered RyRs that cause local Ca^{2+} to reach micromolar concentrations.[61,64,65] In pial artery SM, sparks occur at physiological pressures (60 mm Hg).[66] In these vessels, and a range of other vascular beds, large-conductance Ca^{2+}-activated K^+ (BK) channels are localized in the sarcolemma adjacent to SR RyR Ca^{2+} spark sites. These channels are activated by sparks, releasing K^+ and resulting in a spontaneous transient outward current (STOC) that causes a brief hyperpolarization.[61] In the pial circulation, this process acts as a negative feedback on membrane depolarization and Ca^{2+} entry through VDCCs[61,67] and thus is an important determinant of arteriolar tone.

In contrast to pressurized pial arteries, where Ca^{2+} spark activity is a prominent feature, spark activity is absent in the SM of *ex vivo* pressurized (40 mm Hg) parenchymal arterioles; instead, asynchronous ryanodine-sensitive Ca^{2+} waves are the predominant form of Ca^{2+} signal here.[14] However, the RyR Ca^{2+} spark-BK channel STOC signaling architecture is still present and can be engaged under certain circumstances,[14] making this a potential target for neurovascular signaling mediators. Dabertrand and co-workers[14] demonstrated this principle by acidifying artificial CSF from pH 7.4 to pH 7.0, which converted SM RyR-sensitive Ca^{2+} waves to sparks in parenchymal arterioles. The resultant increase in spark activity opposed pressure-induced vasoconstriction and led to a rapid and sustained vasodilation that was sensitive to both ryanodine and the BK channel blocker paxilline in a non-additive manner.[14] It is presently unclear why Ca^{2+} sparks do not occur under basal (experimental) conditions in isolated parenchymal arterioles, but this observation may help to explain why these arterioles develop higher tone at lower pressures compared with pial arteries. Indeed, one possibility is that RyRs in parenchymal arterioles have a higher basal open probability than their counterparts in pial arteries.[68] Such higher basal sensitivity to Ca^{2+} could lead to the preferential generation of waves rather than sparks in parenchymal arterioles, leaving an absence of the hyperpolarizing influence of spark-STOC signaling and higher tone. This contrasts with pial arteries, where basal spark-STOC signaling activity acts as a negative feedback that resists SM membrane depolarization, resulting in lower tone at the same pressure.

IP_3 receptors

IP_3Rs are ubiquitous ligand-gated Ca^{2+}-permeable channels located in the membrane of the SR/ER.[69] To date, IP_3R signaling in parenchymal arteriole SM has not been examined, but in pial arteries, UTP-induced PLC activation has been linked to IP_3R-dependent Ca^{2+} waves that lead to vasoconstriction.[13] Moreover, Xi and co-workers identified a possible physical interaction of IP_3Rs with TRPC3 in pial SM that was independent of SR Ca^{2+} release. The resultant cation current was demonstrated to promote vasoconstriction in response to endothelin-1.[48] Since TRPC3 channels do not participate in pressure-induced constriction,[70] this interaction might be important for receptor activation-induced constriction of cerebral arteries. As previously noted, pial SM IP_3R activation leads to depolarizing TRPM4 currents.[21] Since TRPM4 channels have a similar role in parenchymal arterioles,[20] IP_3R-mediated Ca^{2+} signaling is a likely candidate for mediating activation of TRPM4 channels here as well. IP_3Rs are also involved in activation of the Ca^{2+}-dependent transcription factor NFATc3 and subsequent regulation of pial SM gene expression.[71]

K^+ channels in smooth muscle

The resting V_m of SM (−35 to −45 mV) indicates that the ratio of K^+ permeability (pK) to the permeability of other ionic species (assuming a collective reversal potential of 0 mV) is around 0.5–0.8:1.[26] Thus, the SM V_m is quite positive to the K^+ equilibrium potential (E_K), which is approximately −103 mV in CSF (assuming 3 mM extracellular K^+ and 140 mM intracellular K^+). These basal conditions mean that the activation of K^+ channels can impart a major hyperpolarizing influence on cerebral SM, and thereby exert rapid and robust control over vascular diameter.

Smooth muscle inwardly rectifying K^+ channels: Targets of endfoot K^+ release

A modest elevation of external K^+ is a rapid and powerful vasodilator of pial arteries and parenchymal arterioles.[22,23] For example, raising K^+ from 3 mM to 8 mM hyperpolarizes the SM in isolated pressurized parenchymal arterioles to near the new E_K of −76 mV, and causes maximum vasodilation.[22] For this to occur, K^+ permeability has to increase more than 50-fold; this is achieved through activation of SM inwardly rectifying K^+ (K_{IR}) channels.[26]

K_{IR} channels, which form tetramers composed of two-transmembrane α-subunits, can be distinguished by their rectification properties.[72] The K_{IR} subtypes expressed in parenchymal arterioles have yet to be definitively established, but the pharmacological sensitivity of the parenchymal SM K_{IR} current to Ba^{2+} ions *in situ*[22,28] is consistent with the presence of functional $K_{IR}2$-containing channels. In addition, mRNA data indicate the expression of $K_{IR}2.1$ and $K_{IR}2.2$ isoforms in rat parenchymal arterioles.[28] In pial arteries, knockout of $K_{IR}2.1$, but not $K_{IR}2.2$, completely ablates the

dilatory response of vessels to small increases in extracellular K^+,[29] suggesting a principal role for the $K_{IR}2.1$ subtype. Furthermore, downregulation of SM $K_{IR}2.1$ expression leads to impairment of K^+-mediated dilation of rat parenchymal arterioles,[28] strongly suggesting that $K_{IR}2.1$ is of similar central importance in these smaller vessels. $K_{IR}2$ family members are strongly rectifying and are activated by hyperpolarization and external K^+, with their half-activation voltage corresponding to E_K.[73] At potentials positive to E_K, outward K^+ current through K_{IR} channels progressively declines with depolarization as a result of intracellular polyamine block.[74] As described previously, at basal extracellular K^+ (3 mM), E_K is approximately −103 mV and the SM V_m is about −35 to −40 mV.[9,10] Under these conditions, K_{IR} channel activity is very low.[26] K^+ is released from neurons and from endfoot BK channels during NVC (see below), and raising K^+ by a small amount (e.g., to 8 mM) leads to rapid unblock of K_{IR} channels and an enormous increase in K_{IR} conductance.[26] The ensuing increase in K^+ permeability drives the SM V_m to the new E_K (−76 mV with 8 mM $[K^+]_o$), in the process promoting even greater K_{IR} channel activation by virtue of their steep voltage dependence.[26] This ~40 mV hyperpolarization closes VDCCs and leads to maximal vasodilation, making K_{IR} activation by K^+ released during neuronal activity a powerful mechanism for increasing local cerebral blood flow. The stark hyperpolarizing effect of K^+ on parenchymal SM V_m likely also involves K_{IR} channels located on the underlying endothelium, for which there exists preliminary evidence.[26] Here, K^+ may diffuse through the SM, or hyperpolarizing signals could be conducted upstream from capillaries (see below), to activate EC K_{IR} channels. This would promote EC hyperpolarization which can be transmitted via gap junctions to reinforce SM hyperpolarization, further amplifying K_{IR} channel activation and locking V_m at E_K (Figure 4). For an extended discussion of K^+-induced activation of parenchymal arteriole K_{IR} channels in the control of cerebral blood flow, see Longden et al.[26]

K_{IR} subunits are also components of ATP-sensitive K^+ (K_{ATP}) channels, which are found in many peripheral vascular beds. K_{ATP} channels, composed of $K_{IR}6.1$ or 6.2 with one of four types of auxiliary sulfonylurea (SUR) receptors, are weakly rectifying and their gating is determined by local concentrations of intracellular nucleotides.[72] K_{ATP} channels composed of $K_{IR}6.1$ and $K_{IR}6.2$ plus SUR2B subunits are expressed in basilar and middle cerebral pial arteries[75] and may play a role in the dilatory responses of large surface arterioles to hypoxia[76] and acidosis.[77] However, these channels appear to be absent from the SM of parenchymal arterioles, as evidenced by a lack of vasodilatory response to the K_{ATP} channel opener cromakalim.[14]

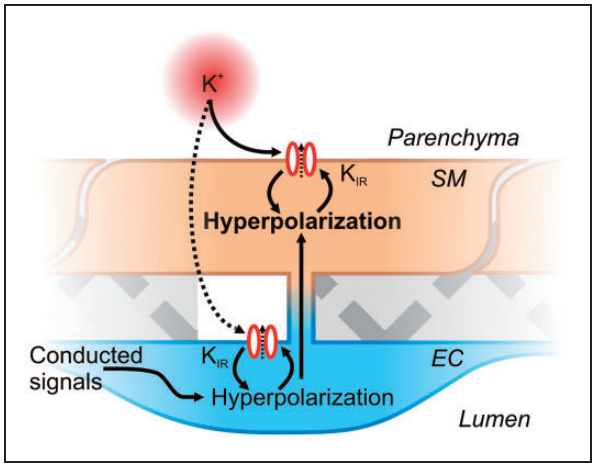

Figure 4. Feed-forward hyperpolarization of SMCs by K_{IR} channel activation by K^+ and V_m. A small amount of K^+ released from neurons or astrocytes during neuronal activity may activate K_{IR} channels on the SM and possibly also diffuse to K_{IR} channels on ECs. Activation of K_{IR} channels initiates membrane hyperpolarization, in the process promoting further K_{IR} channel activation to amplify hyperpolarization and lead to V_m reaching E_K. EC hyperpolarization, either due to K^+ activation of K_{IR} or resulting from conducted signaling from downstream capillaries, could also be transmitted to the SMs by gap junctions, further amplifying SMC hyperpolarization.

Ca^{2+}-activated K^+ channels: the hyperpolarizing influence of smooth muscle BK channel activity can be exploited by NVC mediators

The Ca^{2+}-activated potassium (K_{Ca}) channel family is divided into three subgroups: small-conductance (SK) channels consisting of $K_{Ca}2.1$ (SK1), 2.2 (SK2) and 2.3 (SK3) isoforms, the intermediate-conductance ($K_{Ca}3.1$; IK) channel and the large conductance ($K_{Ca}1.1$; BK) channel.[78] BK channel α-subunits possess six transmembrane domains with an extended intracellular C-terminus. These subunits gain their Ca^{2+}-sensitivity from C-terminal regions, termed the Ca^{2+}-bowl and the RCK (regulator of conductance for K^+) domain. Four α-subunits associate with four β-subunits, the latter of which modulate the overall gating properties of the channel. α-subunits also possess a functional voltage sensor in their S4 region whose sensitivity is modified by local Ca^{2+} as well as by phosphorylation by a number of intracellular enzymes, making these channels responsive to a diverse range of intracellular stimuli.[79]

In line with observations in other vascular beds, parenchymal myocytes express functional BK channels, but do not express SK or IK channels.[10] In contrast to their important role in opposing pressure-induced

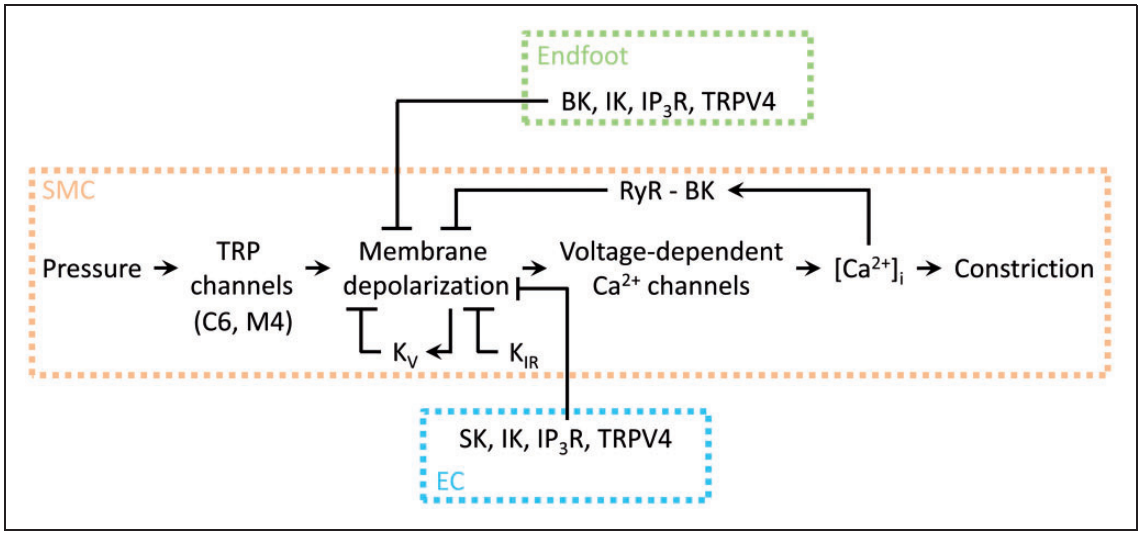

Figure 5. Schematic illustration of the central role of SM V_m in cerebrovascular constriction. Arrowheads indicate a stimulatory effect, whereas flat lines indicate a negative influence. TRP channel activity is engaged by pressure, which depolarizes the membrane and increases VDCC activity, leading to an increase in $[Ca^{2+}]_i$ and constriction. Membrane depolarization directly stimulates K_V channels, which mediate hyperpolarizing currents that, in turn, exert a negative feedback on depolarization; smooth muscle K_{IR} channel activation also counteracts depolarization. Under certain conditions, elevated $[Ca^{2+}]_i$ can induce RyR-mediated Ca^{2+} sparks, which couple to BK channels to hyperpolarize the membrane and limit depolarization. Astrocytic endfoot and endothelial signaling acts to hyperpolarize the SM membrane. The balance of these signaling elements controls Ca^{2+} entry into the myocyte and thus the contractile state of the cell. Adapted from Dabertrand et al.[83]

constriction in pial artery SM,[67] BK channels contribute only modestly to parenchymal arteriolar tone.[10] However, these channels represent a key target for many putative NVC mediators (e.g. EETs, nitric oxide and prostaglandins), which may harness BK channel activity to bring about membrane hyperpolarization.

K_V channels exert a tonic hyperpolarizing influence on the smooth muscle membrane potential

Voltage-dependent potassium (K_V) channels constitute the largest subfamily of the K^+ channel superfamily.[80] Functional channels are formed from four six-transmembrane α-subunits, either homo- or heteromeric, plus additional β-subunits.[81] Rat parenchymal arterioles express mRNA for $K_V1.2$ and $K_V1.5$ α-subunits.[25] Inhibiting K_V channels in parenchymal arterioles causes a significant constriction, indicating that these channels provide a hyperpolarizing K^+ efflux that opposes vasoconstriction at physiological pressures.[25] Similarly, pial arteries express protein for $K_V1.2$ and $K_V1.5$ subtypes, which are thought to assemble into heterotetramers; these channels are tonically active in isolated pressurized pial arteries.[82] Figure 5 provides an overview of the ion channel influences on SM V_m, the balance of which determines arteriolar tone at any given point.

Part II: dual control of arteriolar smooth muscle by the endothelium and astrocytic endfeet

Both the endothelium and astrocytic endfeet share a number of common features that allow them to control V_m and the contractile state of the SM sandwiched between them. Each cell type exhibits local modulated Ca^{2+} signals, which lead to the release of a similar complement of vasoactive substances onto the SM. In keeping with this theme, astrocytes and ECs also express a highly similar repertoire of ion channel species.

The astrocyte cell membrane possesses a large passive K^+ conductance and a negative resting potential, typically measured in the −70 to −80 mV range in brain slice electrophysiological recordings.[84–86] One recent study using intracellular microelectrodes to record at the level of the astrocytic soma *in vivo* determined that the resting V_m was approximately −87 mV.[7,8] Although parenchymal endothelial V_m has yet to be directly measured, dual microelectrode recordings from ECs and SM in hamster feed arteries showed that the V_m of these two cell types were very nearly equivalent.[8] Moreover, measurements performed on peripheral arterioles using a range of preparations and techniques[8,12,87–89] have established that endothelial resting V_m is between approximately −30 and −45 mV. Thus, it is likely that parenchymal endothelial

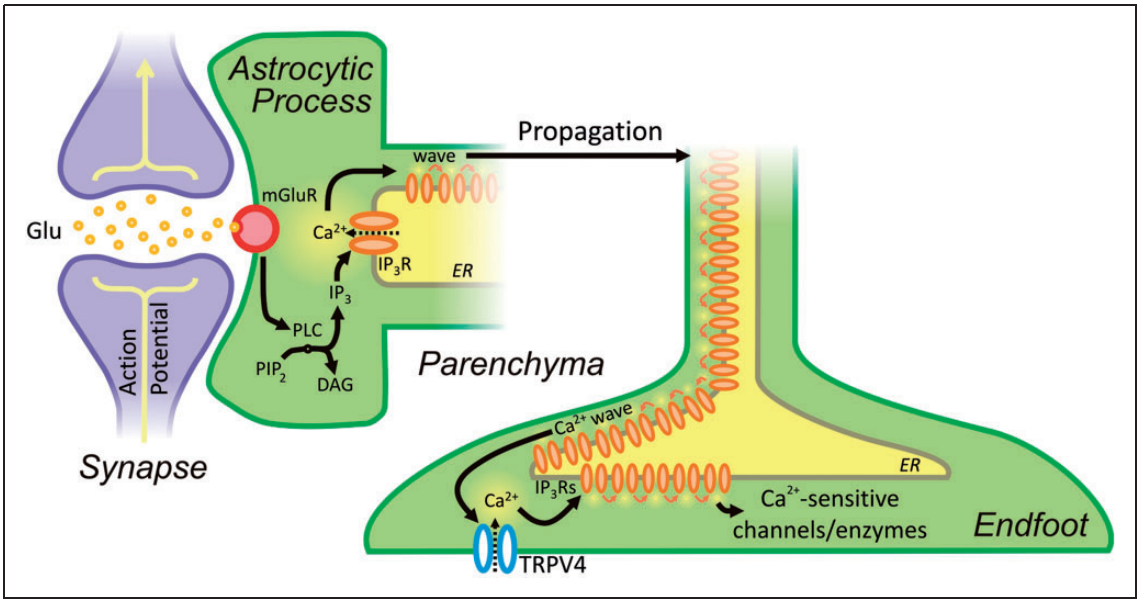

Figure 6. Neurovascular communication is initiated by neuronal activity, which drives the production of IP_3 by PLC, in turn initiating a propagating Ca^{2+} wave. Ca^{2+} waves arriving at the endfoot activate TRPV4 channels in a positive-feedback loop that further enhances their spread, ultimately leading to the stimulation of Ca^{2+}-sensitive ion channels and enzymes.

resting V_m lies within this range, closely matching that of the electrically connected overlying SM.

IP_3Rs and TRPV4 play central roles in Ca^{2+} signaling in the endothelium and endfoot

In contrast to the RyR-mediated Ca^{2+} signaling that predominates in SM, Ca^{2+} release through IP_3Rs has a key role in communication to the SM by both the parenchymal endothelium and astrocytic endfeet. Although the mechanisms by which extracellular Ca^{2+} ions enter the endothelium and endfoot remain unsettled, it is unlikely that VDCCs contribute to Ca^{2+} influx in either cell type. Instead, local Ca^{2+} entry through TRPV4 channels has been shown to be important in both ECs[90] and astrocytes.[16]

IP_3Rs

Astrocytes are equipped with a range of neurotransmitter receptors,[91] enabling them to respond to a diverse array of neuronal signaling molecules. Glutamate is the predominant excitatory neurotransmitter in the brain, and the prevailing view is that astrocytes detect glutamatergic neuronal activity through G_q-coupled metabotropic glutamate receptors (mGluRs) expressed on processes that contact synapses.[92] According to this model, activation of these G_qPCRs causes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by PLC, resulting in the production of IP_3 and DAG.[93] IP_3, in turn, acts on IP_3Rs to increase their sensitivity to $[Ca^{2+}]_i$ and increase Ca^{2+} efflux from the ER (Figure 6). The IP_3R2 isoform is thought to be primarily responsible for the release of Ca^{2+} from astrocytic intracellular stores in hippocampal and cortical astrocytes, as some studies have observed that ablation of the gene encoding this channel attenuates G_qPCR-stimulated and neuronal activity-evoked astrocytic Ca^{2+} signaling.[94–96] Further studies, using IP_3R2-knockout mice, have raised questions about the role of IP_3R-mediated astrocytic Ca^{2+} signaling in the control of cerebral blood flow, reporting that functional hyperemia is unaffected in these mice.[97,98] While these studies raise the intriguing possibility of IP_3R2-independent NVC, their broader conclusion—that astrocytic Ca^{2+} signaling is dispensable for NVC and functional hyperemia—is premature and directly contradicts a substantial body of evidence to the contrary. For example, several laboratories have firmly established that uncaging Ca^{2+} or IP_3 in astrocytic endfeet leads to diameter changes in the adjacent arteriole in brain slices and *in vivo* in both rats and mice.[23,11,99–101] It should also be noted that retention of NVC upon IP_3R2 knockout is not a universal finding[95]; a recent study of IP_3R2-knockout mice using brain slices and *in vivo* preparations identified a diverse range of persistent astrocytic Ca^{2+} signaling events, localized mainly to fine processes and endfeet rather than somata.[102] Further recent studies have confirmed that astrocytic, fast Ca^{2+} transients that occur in the cell soma, fine processes, and endfeet reliably precede functional hyperemia in adult mice *in vivo*.[103,104] Thus, it appears that astrocytes from

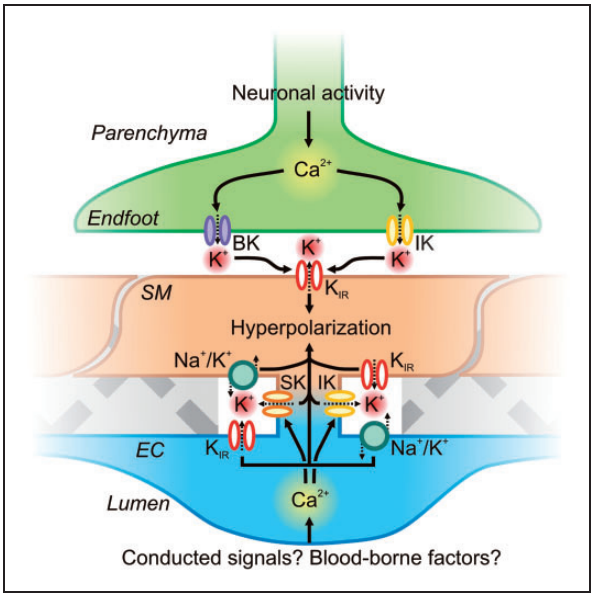

Figure 7. Astrocytic endfoot and EC K_{Ca} channel influences on SM V_m. Astrocytic Ca^{2+} waves initiated by neuronal activity can activate BK and IK channels directly on the endfoot. K^+ accumulates in the restricted extracellular space, activating SM K_{IR} channels. Subsequent K^+ efflux through these channels hyperpolarizes the SM membrane, which decreases VDCC open probability and thereby lowers global $[Ca^{2+}]_i$ to promote vasorelaxation. On the luminal side of the SM, the endothelium may be engaged during neurovascular coupling (for example by conducted signaling from the capillary bed or by luminal factors such as altered shear), leading to an increase in EC Ca^{2+} that could engage IK and/or SK channels in MEP microdomains to drive EC membrane hyperpolarization and also raise local K^+. EC membrane hyperpolarization could then be transmitted via gap junctions to the overlying SM. EC and SM K_{IR} channels and Na^+/K^+ ATPase pumps could also respond to released K^+ to amplify hyperpolarization.

IP_3R2-knockout mice do continue to engage in Ca^{2+} signaling, perhaps due to compensatory upregulation of other IP_3R isoforms, likely on a spatial and temporal scale that previous studies were unable to detect.

The astrocytic ER extends into the endfoot, as evidenced by the fact that both electrical field stimulation and direct photolysis of caged-IP_3 evoke Ca^{2+} release in endfoot processes adjacent to arterioles.[11] Thus, it is thought that the presence of sufficiently high concentrations of IP_3 generated in response to neuronal activity evoke regenerative CICR, leading to a Ca^{2+} wave that propagates into the astrocytic endfoot.[11] Arriving Ca^{2+} waves stimulate the production and/or release of numerous vasoactive molecules, which cause vasodilation and a resultant increase in local cerebral blood flow. Consistent with this, astrocytes in brain slices respond to electrical and pharmacological mGluR stimulation with an elevation in Ca^{2+}.[11,105–107] These findings, which were obtained in juvenile animals, are compatible with an interesting recent study reporting that expression of G_q-coupled mGluRs is prominent in cortical and hippocampal astrocytes of young mice but is lost in adulthood.[107] However, the implication of this latter study—that expression of these receptors is a transient, developmental phenomenon—must be weighed against functional evidence that mGluR-mediated Ca^{2+} signaling in astrocytes is known to occur in adult animals *in vivo*.[99,108] These seemingly irreconcilable observations suggest that the signaling cascade leading to astrocytic Ca^{2+} waves may be more complex than currently conceived—particularly in mature animals[107]—and indicate that further work is needed to fully understand this phenomenon.

Given that the overlying SM acts as a barrier to immediate neuronal or astrocytic communication, it is unlikely that parenchymal arteriolar endothelium is *directly* stimulated during NVC. However, it is plausible that conducted signaling from capillary ECs (see below) could lead to parenchymal arteriole EC engagement.[109] In ECs of small arteries of the periphery, Ca^{2+} release through IP_3Rs is well-established as an important signaling step leading to vasodilation. In particular, brief, high-amplitude, IP_3R-mediated Ca^{2+} signals localized specifically to MEP microdomains—termed Ca^{2+} pulsars—activate local IK channels, causing K^+ efflux and hyperpolarization.[12] This hyperpolarization can then be transmitted via gap junctions directly to the underlying SM; the released K^+ may also amplify hyperpolarization by activating local K_{IR} channels and/or Na^+/K^+ ATPase pumps on the endothelium and/or SM[110] (Figure 7). Although currently unexplored, it is highly likely that this same signaling modality exists in parenchymal arteriole ECs, and if so, this could conceivably be engaged by conducted or blood-borne signals to further sculpt blood flow responses.

TRPV4 channels

In addition to the well-studied IP_3-mediated waves described above, recent evidence indicates that spatially restricted astrocytic Ca^{2+} signals are also vital for the astrocyte to carry out its diverse roles, including the control of cerebral blood flow. Several studies have identified TRPV4 channels in astrocytes.[16,111] TRPV4 channels are enriched in astrocytic processes and endfeet adjacent to blood vessels and the glia limitans.[111] Dunn et al.[16] recently studied the contribution of TRPV4 signaling to endfoot Ca^{2+} handling during NVC. This study identified spatially restricted TRPV4-mediated Ca^{2+}-entry events that couple to endfoot IP_3Rs and then propagate throughout the endfoot as a Ca^{2+} wave[16] (Figure 6). Inhibition of TRPV4 prior to neuronal stimulation was shown to substantially reduce the magnitude of the evoked endfoot Ca^{2+}

transient, suggesting that TRPV4 is an important contributor to endfoot Ca^{2+} entry during neurovascular signaling. Although these channels can be activated by EETs, Dunn and co-workers found no evidence for EET-mediated activation of TRPV4 during neuronal activity. Instead, the authors proposed that IP_3R-mediated Ca^{2+} release engages Ca^{2+}-sensitive TRPV4 channels in the endfoot membrane, stimulating further Ca^{2+} entry into the cytosol to help propagate the wave. These data also raise the intriguing possibility of TRPV4 activation by local physical forces.[55,112] For example, mechanical activation of TRPV4 by diameter changes in blood vessels could lead to endfoot Ca^{2+} increases that then either feedback to modulate vessel diameter and blood flow[113] or propagate in retrograde fashion towards the astrocyte soma, where they might influence signaling to neurons.[16,114]

In ECs of mesenteric arteries, Ca^{2+}-entry events mediated by the gating of individual TRPV4 channels—termed 'TRPV4 sparklets'—act as powerful regulators of vascular tone.[90] Here, endothelial Ca^{2+} entry though TRPV4 evoked by synthetic agonists primarily activates IK channels leading to vasodilation.[90] In the cerebral circulation, TRPV4 channels have been identified in the endothelium of the isolated middle cerebral (pial) arteries, where they contribute to Ca^{2+} entry in response to stimulation of purinergic G_qPCRs with UTP.[17] Similarly, Hamel and co-workers[18] recently confirmed the presence of functional TRPV4 channels in the endothelium of pressurized posterior cerebral (pial) arteries, showing that these channels link muscarinic receptor stimulation (with acetylcholine) to IK and SK channel activation. In the mesenteric endothelium, TRPV4 is co-localized with other signaling molecules at MEP sites,[90] where cooperative gating among TRPV4 channels in a four-channel cluster is enhanced by localized concentrations of A-kinase anchoring protein (AKAP) 150.[115] Similarly, TRPV4 is also found at MEPs in cremaster arterioles.[116] This same clustered organization may also occur at MEPs in cerebral arteries/arterioles, a hypothesis supported by preliminary evidence.[117] To date, TRPV4 expression and function have not been explored in the parenchymal endothelium, but given its importance in mesenteric and pial ECs, this channel is a likely candidate for mediating Ca^{2+} entry into ECs in parenchymal arterioles as well.

Expression of Ca^{2+} targets in astrocytic endfeet and the endothelium

K_{Ca} channels and K^+ signaling: a mechanism for bi-directional control of parenchymal arteriole diameter

Among the key targets of endfoot Ca^{2+} increases are K_{Ca} channels. There is good evidence for the functional expression of BK channels in the endfoot membrane adjacent to underlying parenchymal arterioles.[22,118] Astrocytes also express IK channels on processes and endfeet, whereas evidence for the functional expression of SK channels in astrocytes is currently lacking.[24] During neurovascular communication, IP_3-mediated Ca^{2+} waves arriving at the endfoot stimulate the opening of BK,[22,23] and possibly IK,[24] channels. K^+ ions then accumulate in the extracellular nano-space between the endfoot and SM.[22,23] This increase in local K^+ concentration induces hyperpolarization and relaxation of the SM through activation of myocyte (and possibly endothelial[26]) K_{IR} channels[22] (Figure 7). In addition to being supported by *in situ* and *in vivo* experimental data,[22,23] a number of the features of this mechanism have been confirmed using computational modeling.[119–111]

The magnitude of the neuronally evoked endfoot Ca^{2+} increase dictates relative BK channel activity and thus the concentration of K^+ released into the nano-space between endfeet and arterioles. Notably, K^+ can promote vasodilation or vasoconstriction depending on its concentration, inducing dilation at <20 mM through activation of K_{IR} channels, and constriction at higher concentrations through depolarization of the SM and activation of VDCCs.[23] Accordingly, the degree of Ca^{2+} elevation in the astrocytic endfoot is a vital determinant of the polarity of the vascular response. This was demonstrated in experiments showing that endfoot Ca^{2+} levels could be manipulated by varying the intensity of electrical field stimulation or by carefully controlling uncaging of Ca^{2+} directly in the endfoot. Ca^{2+} levels less than $\sim$500 nM consistently caused dilation, whereas higher concentrations induced constriction. Both dilations and constrictions were sensitive to block by paxilline, supporting a model in which K^+ release through endfoot BK channels mediates both vasodilation and vasoconstriction, depending on the intensity of the stimulus.[24]

In contrast to astrocytic endfeet, the parenchymal endothelium expresses IK and SK channels, which contribute to basal parenchymal arteriole tone.[10] SK and IK channels share a common topology, consisting of homomeric tetramers composed of four six-transmembrane α-subunits associated with intracellular Ca^{2+}-binding calmodulin subunits at their C-terminal domains.[79] This renders these channels exquisitely sensitive to changes in $[Ca^{2+}]_i$.[122] Their vestigial voltage sensor, which contains fewer charged amino acids in the S4 transmembrane domain than that of K_V channels, means that SK and IK channels are voltage-insensitive.[123] Hannah et al.[10] reported that blocking IK and SK channels in isolated pressurized parenchymal arterioles caused substantial vasoconstriction, and this same manipulation decreased resting cortical cerebral blood

flow by 15% *in vivo*. Conversely, activation of these channels with NS309 maximally dilated isolated arterioles and greatly enhanced cortical cerebral blood flow, highlighting the role of these channels as powerful controllers of parenchymal arteriolar tone.[10] In contrast, isolated, pressurized middle cerebral arteries do not constrict to IK and SK channel inhibition, suggesting that these channels are not tonically active under basal conditions in larger surface arteries.[124] In mesenteric arteries, the SK3 subtype has been identified at MEPs and EC-EC borders.[125] The loss of apamin-sensitive K_{Ca} currents in mesenteric ECs from an SK3 gene suppression mouse confirms that this is the primary SK isoform for the regulation of vascular tone.[126] SK2 is also present in ECs, but appears to be restricted to peri-nuclear regions of the cell, whereas SK1 is not present.[127]

Engagement of SK and IK channels is dependent on $[Ca^{2+}]_i$; thus, the above observations suggest a level of tonic endothelial Ca^{2+} signaling in parenchymal arterioles *in situ* and *in vivo*. Extrapolating from mesenteric arteries, where IP_3-mediated Ca^{2+} pulsars activate IK channels within MEPs[12] and thereby contribute to the control of vascular tone, it is possible to suggest that this same signaling architecture is present in the parenchymal circulation. If further studies establish that this is the case, K^+ released through IK and SK channel activation by Ca^{2+} pulsars would be predicted to couple to local K_{IR} channel and Na^+/K^+ ATPase activation, which could act as an amplification mechanism to hyperpolarize the membrane and drive vasorelaxation[110] (Figure 7).

Arachidonic acid metabolites

One early proposal[128] was that neuronally evoked Ca^{2+} waves activate Ca^{2+}-sensitive cytosolic phospholipase A_2 in astrocytes, mobilizing arachidonic acid (AA) from membrane phospholipid pools and leading to subsequent metabolism of AA to EETs by cytochrome P450 2C11 enzymes. The four possible EET regioisomers—5,6-, 8,9-, 11,12- and 14,15-EET—act as short-range signaling hormones, dilating cerebral arteries *in situ*[129] and contributing to the control of cerebral blood flow *in vivo*.[130]

EETs are synthesized by astrocytes in culture,[131] and cytochrome P450 2C11 mRNA has been detected in perivascular astrocytes.[130] Astrocyte-derived EETs could potentially act in either an autocrine or paracrine manner to open endfoot[132] or SM[133] BK channels, respectively. In the case of endfoot BK channels, this would cause the release of K^+, which could then stimulate SM K_{IR} channel activity.[132] If EETs diffuse to the SM, they could open BK channels to hyperpolarize the membrane directly.[133] The mechanism of EET action at BK channels involves ADP-ribosylation of $G_s\alpha$ subunits,[134] resulting in an increase in channel activity.[134] Astrocyte-derived EETs could also promote vasodilation through actions on TRPV4 channels in the endothelium,[17,18] myocytes,[19,49] and/or endfeet.[16] Despite these possibilities, recent *in vivo* data argue against a direct contribution of endfoot-derived EETs to SM relaxation during NVC, as vasodilation evoked by uncaging Ca^{2+} directly in astrocytic endfeet *in vivo* was insensitive to cytochrome P450 blockade.[99] These observations suggest that the mechanism underlying the contribution of astrocyte-derived EETs to NVC is more complex and indirect than was first envisioned.

AA liberated by PLA_2 can also serve as a substrate for the production of vasoactive prostaglandins (PGs). In this reaction, the dual peroxidase-cyclooxygenase actions of cyclooxygenase (COX) enzymes initially metabolize AA to PGG_2 followed by the rapid conversion of this intermediate to PGH_2. PGH_2 is metabolized by cytochrome P450 enzymes to a range of PG products. Of these, PGE_2 has been proposed to play a role in NVC.[105] In this model, PGE_2 is liberated from the endfoot to act upon E-prostanoid (EP) receptors on SMCs. Notably, EP_4 receptors are G_s-coupled GPCRs, and stimulation of these receptors leads to activation of PKA, which can produce vasodilation.[135] However, recent studies have raised questions about the astrocytic involvement in the vasodilatory response to COX products. One group reported that COX-1 and -2 are expressed in astrocytes in juvenile rat hippocampal-neocortical slices and showed that pharmacological blockade of COX activity inhibited *in situ* vasodilations evoked by mGluR agonists and astrocyte Ca^{2+} uncaging.[136] However, another group found that COX-1 mRNA was expressed in only 10% of rat cortical astrocytes, whereas COX-2 was absent.[137] This same group recently identified a sub-population of COX-2 expressing pyramidal neurons as the primary cell type able to synthesize PGE_2, which reportedly acts through G_s-coupled EP_2 and EP_4 receptors to produce hyperemia.[137,138] However, a fundamental issue is raised by a recent study showing that application of PGE_2 directly to isolated, pressurized parenchymal arterioles from both rat and mouse evoked constriction rather than dilation, through activation of EP_1 receptors, suggesting that PGE_2 is unlikely to act as a direct mediator of NVC on parenchymal arteriole smooth muscle.[139] However, this does not rule out PGE_2-mediated vasodilatory effects through indirect actions on neurons[139] or on the capillary endothelium.

Liberation of AA from plasma membrane phospholipids by PLA_2 also occurs in the endothelium. In contrast to astrocytes, the endothelium has been clearly demonstrated to possess abundant COX-1 and PGI_2 synthase enzymes.[140] PGI_2 derived from the activities of these enzymes is the primary vasodilatory PG liberated from the endothelium in response to a variety of stimuli, including agonists of muscarinic, bradykinin

and purinergic receptors.[141,142] Similar to the actions of PGE_2 on EP receptors, PGI_2 activates G_s-coupled I prostanoid receptors on SMCs, evoking PKA-mediated vasodilation.[140] Although, PGI_2 and its analogues do relax cerebral arteries and arterioles,[143] preliminary evidence from mouse brain slices suggests that PGI_2 is not involved in signaling during NVC.[144]

The parenchymal endothelium is also a likely source of EETs, as ECs produce these molecules in a range of vascular beds.[145,146] Moreover, cerebral arteries and arterioles dilate in response to direct EET application.[129,147] However, to our knowledge, the endothelial production of these molecules has yet to be directly investigated in parenchymal arterioles. Nonetheless, it is reasonable to speculate that endothelial EETs could influence blood flow by interacting with a range of ion channels in the parenchymal NVU, such as myocyte[10] or astrocytic[22,132] BK channels or endothelial,[17,18] SM[49,19] and astrocytic[16] TRPV4 channels. EET-mediated Ca^{2+} influx through endothelial TRP channels[148] could also contribute to endothelial SK and IK channel activation and membrane hyperpolarization.

Nitric oxide: NVC mediator or modulator?

Another commonly touted Ca^{2+}-dependent NVC mediator is nitric oxide (NO). However, the potential contribution of NO is complex and its actual role remains uncertain. NO derived from both neuronal and endothelial sources has been implicated in the control of cerebral blood flow,[149–151] whereas it is generally accepted that astrocytes do not generate NO under normal conditions. NO activates guanylate cyclase, leading to the production of cyclic guanosine monophosphate (cGMP).[152] Cyclic GMP, in turn, is a substrate for PKG, which interacts with multiple downstream effectors. Considering ion channel targets alone, PKG activity can modulate the phosphorylation status of BK channels, leading to an increase in their activity,[153,154] and may act through similar mechanisms to inhibit VDCCs.[155] Cyclic GMP can increase RyR-mediated Ca^{2+} spark frequency,[57] and NO may also decrease IP_3-mediated Ca^{2+} release through the actions of cGMP and PKG.[156] In the parenchymal NVU, NO from either eNOS or nNOS could interact with guanylate cyclase in both astrocytes[157] and SM[158] to increase the activity of BK channels in these respective cell types. Signaling to SM VDCCs or RyRs would also be predicted to promote vasodilation and increased blood flow. Moreover, because NO-cGMP-PKG signaling has been reported to inhibit TRPV4 channels (alone[159] or when complexed with TRPC1[160] or TRPP2[161]), endothelial and endfoot Ca^{2+} signaling could also be affected.

Recent work has identified a contribution of eNOS signaling to resting tone in isolated pressurized middle cerebral arteries and parenchymal arterioles,[124] but whether NO is actively recruited during NVC is controversial.[149,162,163] However, emerging evidence supports the concept of endothelial engagement during NVC through the release of parenchymal factors that increase eNOS activity.[164,165] A study by LeMaistre-Stobart and co-workers[165] reported that astrocytic release of D-serine, in combination with glutamate, may activate endothelial *N*-methyl D-aspartate (NMDA) receptors, which conduct Na^+ and Ca^{2+} into the cell. This could then stimulate eNOS, increasing the production of NO and causing vasodilation of arterioles through the actions of NO on the adjacent SMCs.[165] In support of this model, this same group previously demonstrated that endothelial NMDA-receptor activation dilates isolated middle cerebral arteries and brain slice parenchymal arterioles through an eNOS-dependent mechanism.[164] However, the functional expression of NMDA receptors in native parenchymal endothelium is yet to be confirmed.

The next frontier: what are the roles of capillary EC and pericyte ion channels in the control of blood flow in the brain microcirculation?

Capillaries vastly outnumber parenchymal arterioles and are intimately associated with neurons and astrocytes, and are also covered by pericytes. Approximately 90% of capillaries have pericytes,[166] which cover an estimated 37% of the endothelial surface area.[167] This angioarchitecture suggests that capillary ECs and pericytes play an important role in communicating neuronal activity from the capillary bed to upstream arterioles to modulate blood flow into the deep microcirculation. Indeed, recent work from the Attwell laboratory, conducted in brain slices and *in* vivo, suggested that hyperemia is initiated at the level of pericytes before being conducted upstream to arterioles.[168] These authors demonstrated that brain capillary pericytes are capable of both contraction (in response to noradrenaline) and relaxation in response to exogenous agonists and—importantly—neuronal activity.[168,169] Of particular note, glutamate, NMDA or neuronal stimulation evoked a ~30 pA hyperpolarizing current in pericytes patch-clamped *in situ*, thought to underlie pericyte relaxation. The ion channel mediating this hyperpolarization was not firmly identified, although the authors suggest it is highly likely to conduct K^+.[168] Interestingly, only 25–30% of brain pericytes respond to contractile stimuli,[169] suggesting a subpopulation of non-contractile pericytes.

To date ion channel expression and function have not been well explored in brain pericytes, but more is known about retinal pericytes. Relevant in this context,

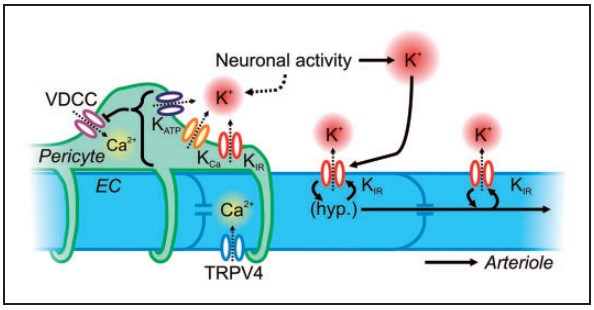

Figure 8. Neurovascular communication at the capillary level. Capillary ECs possess K_{IR} and TRPV4 channels, which can hyperpolarize the membrane and allow Ca^{2+} into the cytosol, respectively. K_{IR} channels may endow capillary ECs with the ability to sense K^+ released by neuronal activity and to conduct a regenerative, retrograde hyperpolarization by virtue of the voltage-dependence of K_{IR} channels. This will lead to rapid upstream dilation of parenchymal arterioles and pial arteries. Pericytes cover more than one-third of the capillary surface area and express VDCCs as a major pathway for the delivery of Ca^{2+} for contraction. They also express K_{ATP}, K_{Ca} and K_{IR} channels, which could be recruited through various mechanisms triggered by neuronal activity to drive membrane hyperpolarization and induce pericyte relaxation. Collectively, ion channels in both ECs and pericytes may provide the means to finely control blood flow deep within the vascular tree.

retinal pericytes are known to possess VDCCs,[170,171] endowing them with an avenue through which V_m changes can modulate intracellular Ca^{2+} and adjust the contractile state of the cell. Retinal pericytes also possess K_{IR} channels,[172] K_{Ca} (BK and SK) channels[173,174] and, in contrast to parenchymal SM, K_{ATP} channels.[175] If this complement of K^+ channels is also present in brain pericytes, they could be harnessed by a variety of signaling pathways – for example external K^+ activation of K_{IR} channels – to produce membrane hyperpolarization and relaxation in response to neuronal activity, thereby modulating capillary blood flow (Figure 8). Notably, the ion channel expression profiles of contractile and non-contractile pericytes are likely to differ substantially, reflecting different roles for each subclass of pericytes—an area that requires further exploration. Readers are directed to Hamilton et al.[176] for a more comprehensive review of the literature on pericyte ion channel expression and function.

Less still is known about native ion channels functionally expressed in brain capillary ECs, but preliminary work indicates that these cells possess both K_{IR} and TRPV4 channels, but unexpectedly lack SK and IK channels and K_{ATP} channels.[177] This repertoire, although incompletely characterized, equips capillaries with a molecular toolkit for detecting local changes in K^+ concentration during neuronal activity and responding with robust hyperpolarization; it also provides a pathway for the elevation of intracellular Ca^{2+}. Membrane hyperpolarization and propagating intercellular Ca^{2+} waves are prominent features of conducted signaling along arterioles,[178] which is primarily enabled by the endothelial lining.[179] Therefore, capillary K_{IR} and TRPV4 channels may endow capillaries with the ability to rapidly communicate to upstream arterioles and pericytes—indeed the endothelium has been suggested as an efficient electrical pathway linking pericytes in the retina[180]—to finely tune blood flow in the deep microcirculation (Figure 8).

Conclusion

The cells of the NVU express a diverse array of ion channels that engage in intra- and intercellular signaling to control the diameter of parenchymal arterioles and modulate cerebral blood flow. There are close similarities in ion channel expression and function between astrocytic endfeet and ECs, leading to parallels in the signaling mechanisms employed by both cell types to control SM V_m, vascular tone and blood flow. Any defect—for example the accumulation of perivascular deposits such as occurs in Alzheimer's disease or CADASIL[6]—could impair ion channel signaling during NVC. As the field progresses and the roles of ion channel signaling in the control of cerebral blood flow are more clearly defined, future studies will likely establish NVU ion channel dysregulation and NVC impairment as important mechanisms in a broad range of cerebrovascular disease states.

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