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Potassium Channels and Neurovascular Coupling

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Abstract

Neuronal activity is communicated to the cerebral vasculature so that adequate perfusion of brain tissue is maintained at all levels of neuronal metabolism. An increase in neuronal activity is accompanied by vasodilation and an increase in local cerebral blood flow. This process, known as neurovascular coupling (NVC) or functional hyperemia, is essential for cerebral homeostasis and survival. Neuronal activity is encoded in astrocytic Ca^{2+} signals that travel to astrocytic processes (‘endfeet’) encasing parenchymal arterioles within the brain. Astrocytic Ca^{2+} signals cause the release of vasoactive substances to cause relaxation, and in some circumstances contraction, of the smooth muscle cells (SMCs) of parenchymal arterioles to modulate local cerebral blood flow. Activation of potassium channels in the SMCs has been proposed to mediate NVC. Here, the current state of knowledge of NVC and potassium channels in parenchymal arterioles is reviewed.

Keywords

Arterial smooth muscle; Astrocytes; Neurovascular coupling; Parenchymal arterioles; Potassium channels

Maintenance of cerebral homeostasis requires dynamic regulation of oxygen and glucose supply so as to match nutrient delivery to metabolic demand.[1] This is achieved through extensive and precise regulation of blood flow within the brain. In the normally functioning brain, increases in neuronal activity are accompanied by rapid, spatially localized increases in blood flow that serve to avoid the development of ischemic conditions in active regions of the brain.[2–6] This coupling of increased neuronal activity to increased local cerebral blood flow (CBF), a process known as functional hyperemia or neurovascular coupling (NVC), occurs as a result of vasodilation at the level of the cerebral microcirculation and appears to depend on the generation of vasoactive substances.[1] Abnormalities associated with cerebral microcirculatory function have been associated with a number of disorders, including Parkinson’s disease, stroke, and migraine.[7] In addition, chronically insufficient CBF is an end result of most risk factors associated with neurodegenerative diseases such as Alzheimer’s disease and early onset dementia.[8] Thus, elucidating the mechanisms by which neuronal activity regulates intracerebral arterioles is critical to the development of new targets and effective therapies for pathological conditions associated with cerebral microcirculatory dysfunction. Potassium (K^+) channels play a central role in NVC and

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represent one such target for future therapeutics. The purpose of this review is to examine putative mechanisms of NVC and the role of K^+ channels in this process.

Blood flow to the brain is first conducted through a series of pial arteries on the surface of the brain, then through intracerebral arteries and arterioles within the brain tissue. Intracerebral (parenchymal) arterioles arise from branches of pial arteries that penetrate from the surface of the brain into the parenchyma (Figure 1).[9] Parenchymal arterioles are structurally and functionally distinct from pial arteries. Within the cerebral cortex, these arterioles begin immediately distal to the Virchow-Robin space, are encased in astrocytic processes called 'endfeet', and terminate as an extensive capillary network. Parenchymal arterioles lack the extrinsic innervation of larger pial arteries, with primary regulation arising from astrocytes and neurons of central origin.[2,10,11]

Recent evidence suggests that astrocytes play a significant role in the regulation of local CBF.[2,7,12–15] An individual astrocyte has numerous processes that surround multiple synapses, and thus forms a tripartite synapse with pre-and post-synaptic neurons.[16] Astrocytes also possess additional processes that target the cerebral microcirculation. These astrocytic processes terminate as perivascular endfeet that encase smooth muscle cells (SMCs) of parenchymal arterioles and capillaries (Figure 1).[2,12–15,17] This astrocytic architecture suggests that an individual astrocyte might be capable of integrating the activity of multiple neurons and translating this information into physiological signals, including those that regulate CBF by altering the vascular tone of parenchymal arterioles. Indeed, recent evidence points to a central role for astrocytes in functional hyperemia in the brain. Neurons themselves also release a number of important vasoactive substances, including nitric oxide, adenosine, and neurotransmitters; however, the potential role of these neuronal mediators in NVC is beyond the scope of the current review.[7,18]

Astrocytes act as vital regulators of neuronal function, serving to modulate extracellular potassium (K^+) concentration ($[K^+]_o$) and extracellular volume, and remove neurotransmitters from synapses.[19–21] Astrocytes possess numerous receptors that could be activated by neurotransmitters.[22–24] Activation of metabotropic glutamate receptors (mGluRs) located on astrocytic projections that surround synapses of glutamatergic neurons results in an increase in cytosolic calcium concentration ($[Ca^{2+}]_c$) in the soma, which then propagates through astrocytic processes, ultimately resulting in a $[Ca^{2+}]_c$ increase in the endfoot (Figure 2).[25,26] These $[Ca^{2+}]_c$ increases likely occur through activation of the phospholipase C/inositol trisphosphate ($InsP_3$) cascade, and involve release of endoplasmic reticulum Ca^{2+} through $InsP_3$ receptors and possibly ryanodine receptors.[26,27] Zonta et al elegantly showed that the dilation of cortical arterioles is dependent on astrocytic $[Ca^{2+}]_c$ increases induced by glutamate activation of mGluRs.[14] Thus, the release of vasoactive substances from astrocytes in response to neuronal activation requires elevation of endfoot $[Ca^{2+}]$, although the mechanisms underlying the generation of these signals and the nature of these mediators are poorly understood.[12–14]

Astrocytic Signals and NVC

Several signals based on arachidonic acid (AA) metabolism have been proposed to modulate vessel diameter in response to neuronal activity (Figure 2).[12,14,28,29] Glutamate activation of astrocytic mGluRs leads to the Ca^{2+}-dependent stimulation of phospholiase A_2 (PLA_2) and production of AA, which is metabolized to epoxyeicosatrienoic acid (EETs) by the action of cytochrome P450 (CYP) epoxygenases, with 11,12-EET and 14,15-EET being major vasoactive components.[30–32] EETs, which are potent vasodilators, have recently been implicated in suppressing vasomotion and maintaining vasodilation of cortical arterioles following astrocytic AMPA receptor stimulation.[30,33–35]

Based on the effects of cyclooxygenase (COX) inhibitors in neonatal brain slices, Zonta et al suggested that elevation of astrocytic $[Ca^{2+}]_c$ following glutamate activation of mGluRs leads to the generation of a COX product that activates receptors on vascular SMCs to generate vasodilation.[14] Consistent with the speculation that this COX product is PGE_2, Zonta and colleagues demonstrated that cultured astrocytes produce PGE_2 in a pulsatile manner and PGE_2 (20 μmol/L) dilates arterioles in brain slices.[14,36] Importantly, the vasodilatory responses observed in these studies exhibited a significant delay (1–3 min) following neuronal activation by electrical field stimulation (EFS), activation of astrocytic mGluRs (t-ACPD [{±}-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid]), patch seal-evoked stimulation, or by PGE_2 application. This delayed response is a phenomenon that is not observed in vivo in adult animals. The slow response in brain slices might reflect the use of neonatal animals in which the neurons and vasculature are not fully developed. Alternatively, the time course of PGE_2 action itself might be slow, which might suggest that this pathway does not mediate the rapid coupling of neuronal activity to vascular dilation but contributes instead to the regulation of blood flow on a longer time scale. Additionally, the observation that COX inhibition only partially suppresses vasodilation is consistent with the view that multiple mechanisms contribute to the neuronal regulation of arteriolar diameter.

In addition to inducing vasodilation, astrocyte-derived AA has been implicated in generating vasoconstriction. Mulligan and MacVicar have proposed that a rise in astrocytic endfoot $[Ca^{2+}]$ stimulates PLA_2, which produces AA that then diffuses to the parenchymal arteriolar SMCs, where it is converted to 20-HETE by CYP ω-hydroxylase.[12] The suggestion is that 20-HETE constricts arterioles by inhibiting SMC large-conductance calcium-sensitive K^+ (BK) channels, an inference based on the inhibitory effects of the PLA_2 inhibitor MAFP (100 μmol/L) and the 20-HETE synthesis-inhibitor HET0016 (100 μmol/L) on the observed constriction. However, these experiments examined non-preconstricted arterioles in brain slices, and the contribution of BK channels is expected to be negligible in the absence of tone.[37] Accordingly, it is difficult to reconcile the observed constrictor effect of 20-HETE with a mechanism involving inhibition of vascular SMC BK channels. In addition, these observations must be interpreted with caution as the concentration of HET0016 used was very high (100 μmol/L), and likely to non-selectively inhibit the formation of other AA metabolites that might be involved in NVC. In human renal microsomes, the IC_{50} of HET0016 on CYP epoxygenase and COX activities is 2.8 μmol/L and 2.3 μmol/L, respectively.[38] Also, 1 μmol/L HET0016 is sufficient to block 20-HETE production in intact

cerebral arteries by 90%.[39] Thus, the actual role of 20-HETE in NVC and its direct functional effects on parenchymal arterioles remains uncertain.

One astrocytic signal that clearly has the potential to generate both vasodilation and vasoconstriction is K^+.[40] The regulation of $[K^+]_o$ by astrocytes has been extensively studied in the retina, where astrocytes are suggested to function as a K^+ siphon, taking up K^+ from regions of high concentration (perisynaptic regions) and releasing K^+ to regions of lower extracellular concentration (perivascular regions).[21] Consistent with this K^+ buffering role, astrocytes display a large K^+ conductance; a hyperpolarized V_m (approximately −80 mV) that is positive to the K^+ equilibrium potential, E_K (−102 mV); and exhibit polarized expression of K^+ channels.[41,42] Specifically, BK channels are abundantly expressed in perivascular endfeet.[43] In brain slices, neuronal stimulation by EFS produces Ca^{2+}-sensitive K^+ (BK channel) currents in astrocytic endfeet that are activated by the rise in endfoot $[Ca^{2+}]$ and result in vasodilation.[44] Activation of endfoot BK channels releases K^+ into the perivascular space, producing a localized elevation in $[K^+]_o$ in the spatially restricted microenvironment between the endfoot and the arteriole that is sensed by the arteriolar smooth muscle. By altering the $[K^+]_o$ in the perivascular space through endfoot BK channel-mediated K^+ release, the astrocyte can generate a signal that is capable of producing vasodilation or vasoconstriction. Strong evidence indicates that the vasodilatory response to K^+ released from the endfoot is mediated by inward-rectifier K^+ (K_{ir}) channels in parenchymal arteriole smooth muscle. Barium, a selective inhibitor of K_{ir} channels (at concentrations <100 μmol/L), blocks vasodilation elicited by neuronal stimulation with EFS in brain slices, but does not affect the increase in $[Ca^{2+}]_c$ in astrocytic endfeet.[44]

K^+ Channels in the Control of Parenchymal Arteriolar Tone

The membrane potential of arterial SMCs is a key determinant of vascular tone and is regulated by K^+ channels. Opening of K^+ channels in vascular SMC membranes allows K^+ to flow out of the cell, resulting in membrane hyperpolarization, whereas inhibition of K^+ channels results in membrane depolarization. K^+ channel-mediated membrane hyperpolarization closes voltage-dependent Ca^{2+} channels (VDCCs), which decreases Ca^{2+} entry and leads to vasodilation.[45,46] VDCCs are very sensitive to membrane potential, such that modest fluctuations in membrane potential can dramatically change Ca^{2+} entry.[47] Consequently, minor changes in membrane potential can have a significant effect on arterial diameter.[48] The membrane potential of SMCs in parenchymal arterioles pressurized to physiological levels (40 mmHg) is approximately −45 mV, and the arterioles are about 30% constricted.[44] In cerebrospinal fluid, E_K is about −102 mV. Membrane potential hyperpolarization to −60 mV causes a maximum dilation of cerebral arteries, and depolarization to approximately −30 mV causes maximal constriction.[48] Therefore, even modest activation of SMC K^+ channels can cause significant vasodilation.

The 4 different subtypes of K^+ channels identified in arterial SMCs are inward rectifier K^+ (K_{ir}) channels, Ca^{2+}-sensitive K^+ (K_{Ca}) channels, voltage-dependent K^+ (K_v) channels, and ATP-sensitive K^+ (K_{ATP}) channels.[49] The expression profile and functional contribution of each subtype can vary according to tissue bed and caliber of the arterial segment.[50] There is

functional evidence for all 4 K^+ channel subtypes in SMCs of parenchymal arterioles, but their relative roles have not been as well characterized as in pial artery SMCs.[51–54]

K_{ir} Channels

A number of arteries, including cerebral and coronary arteries, dilate to a modest elevation in extracellular K^+. K^+-induced dilations are caused by activation of strong inward rectifier K^+ channels in the SMCs.[49] In pial arteries, disruption of the gene for the strong inward rectifier K^+ channel, $K_{ir}2.1$, abolished K^+-induced dilations.[55,56] The property of inward rectification is likely conferred to K_{ir} channels through the drawing out of positively charged polyamines plugging the inside of the pore by membrane potential hyperpolarization.[57–59] The midpoint-of the-activation curve for K_{ir} channels corresponds to E_K, such that elevation of external K^+ shifts the activation curve to more positive potentials.[60] The only experimentally useful blocker of K_{ir} channels is external barium, which blocks the pore in a voltage-dependent manner with an apparent half block constant of 10 μmol/L at −40 Mv.[61] At concentrations below 100 μmol/L, barium ions do not cause significant block of smooth muscle K_V, BK, or K_{ATP} channels.[62] There are no selective activators of K_{ir} channels, except external K^+ and membrane potential hyperpolarization.

The activity of K_{ir} channels in arteriolar myocytes is highly dependent on membrane potential and $[K^+]_o$. In parenchymal arteriolar SMCs, where the density of barium-sensitive K_{ir} currents is greater than that observed in pial artery myocytes, elevation of $[K^+]_o$ shifts the K_{ir} activation curve to more positive potentials, and an increase in $[K^+]_o$ from 3 mmol/L to 10 mmol/L increases the K_{ir} current density 6.3-fold.[44] The increase in K_{ir} current density with elevated $[K^+]_o$ is associated with suppression of SMC Ca^{2+} oscillations and vasodilation. Increasing $[K^+]_o$ activates K_{ir}, resulting in membrane potential hyperpolarization and vasodilation only up to a concentration of ~20 mmol/L – the $[K^+]_o$ at which E_K is similar to the SMC resting membrane potential. Further increases in $[K^+]_o$ depolarize the arteriolar SMC membrane potential and cause vasoconstriction. Treatment with barium prevents the dilation of parenchymal arterioles to modest elevations in $[K^+]_o$, but does not affect the constriction to greater than 20 mmol/L $[K^+]_o$, indicating that K_{ir} mediates K^+-induced dilation but not K^+-induced constriction in parenchymal arterioles. Exposure of parenchymal arterioles to barium alone does not affect vascular diameter, suggesting that SMC K_{ir} channels are not tonically active and contribute minimally to tone in these vessels.

BK Channels

BK channels have been identified in virtually every type of smooth muscle. The characteristic smooth muscle BK channel complex is composed of an α pore-forming subunit and an auxillary β1 subunit.[63–65] The β1 subunit acts to increase the apparent voltage- and Ca^{2+}-sensitivity of the channel. Targeted disruption of the β1 subunit gene leads to hypertension and left ventricular hypertrophy.[66] A number of recent animal studies have shown that the β1 subunit, and hence BK channel function, is downregulated in hypertension and diabetes.[67–71] Two polymorphisms of the BK channel β1 subunit – KCNMB1-E65K and KCNMB1-V110L – are common in multiple ethnic and racial groups; an additional polymorphism (R140 W) is found in individuals of African descent. The first

non-synonymous coding polymorphism was described by Fernandez-Fernandez et al, who identified the E65K polymorphism by direct sequence analysis of the 4 *Kcnmb1* exons in a Spanish cohort.[72] Heterologous expression studies in HEK293 cells established that the E65K polymorphism was a gain-of-function variant that increased BK-channel sensitivity to activation by Ca^{2+}. Genetic association studies in the Spanish cohort demonstrated that carriers of E65K, which was present in 21% of the population examined, were less likely to have diastolic hypertension.[72] In follow-up studies, the association of E65K with blood pressure appeared to be strongest among post-menopausal women.[73]

BK channels are selectively blocked by the scorpion toxins iberiotoxin and charybdotoxin, as well as the alkaloid paxilline.[74–76] There are a number of synthetic openers of BK channels.[77–79] However, the majority of these openers lack selectivity in intact tissues, and therefore their usefulness has been limited. An exception is NS11021, which has recently been shown to decrease smooth muscle excitability through activation of BK channels.[80] BK channels are directly and indirectly activated by vasodilators that elevate cGMP and cAMP concentration.[49,81,82] BK channels have also been shown to be modulated by AA metabolites. BK channel activity is inhibited by 20-HETE through protein kinase C (PKC) activation and increased by EETs.[83–87]

BK currents have been identified in parenchymal arteriolar SMCs; however, these currents have been suggested to be quite small in the physiologically relevant voltage range of −50 to −10 mV.[88] In addition, whereas treatment of pressurized pial arteries with BK channel inhibitors causes pronounced vasoconstriction, parenchymal arteriolar tone does not appear to be substantially affected by BK channel inhibition, as BK channel block by subdural superfusion of paxilline (1 μmol/L) does not significantly affect resting cortical CBF in vivo.[51] These observations suggest that, unlike pial arteries, where BK channels appear to play a major role in regulating tone, the functional significance of BK channel activity in parenchymal arterioles might not be substantial.[37] Reduced BK channel-mediated opposition of myogenic tone could potentially explain the greater relative tone observed in parenchymal arterioles compared to pial arteries.[89] The mechanism and physiological significance of diminished basal BK channel activity in pressurized parenchymal arterioles compared to pial arteries is unclear. However, BK channels in parenchymal arteriolar SMCs are involved in modulating vascular diameter in response to vasoactive chemical stimuli. For example, parenchymal arteriolar dilation to glutamate in newborn piglets is mediated by activation of SMC BK channels.[90]

Voltage-Dependent Potassium (K_V) Channels

K_V channels are present in all types of vascular smooth muscle, and serve to oppose depolarizing, contractile influences by virtue of their voltage-dependence.[49] In vascular smooth muscle, the consensus is that these channels are composed of heteromers of $K_V1.2/1.5$.[91,92] K_V channels can be inhibited by vasoconstrictors through PKC activation.[93–95] Recent evidence indicates modest elevations of glucose can also inhibit K_V channels through activation of PKC.[53,96] The 4-aminopyridine (4-AP) is a potent inhibitor of these channels.[62] Selective openers of these channels have not been identified. As in pial arteries, K_V channels play a significant role in determining the level of myogenic tone in

pressurized parenchymal arterioles. K_V channel currents activated by membrane depolarization are observed in parenchymal arteriolar SMCs.[53] The biophysical properties of these currents along with reverse transcription-polymerase chain reaction results indicate that K_V channels in parenchymal arterioles are heterotetrameric channels composed of $K_V1.2$ and $K_V1.5$ subtypes. This is the same type of K_V channel assembly present in pial artery SMCs. K_V current in isolated parenchymal arteriolar SMCs is significantly reduced by application of 4-AP, which also constricts pressurized arterioles and arterioles in brain slices by roughly 40%. Hence, at physiological intravascular pressures K_V channels actively oppose myogenic constriction in parenchymal arterioles. There is some evidence that K_V channels also mediate changes in membrane potential and vascular tone in parenchymal arterioles in response to vasoactive agents, although this has not been well studied. However, elevated extracellular glucose concentration diminishes K_V current in arteriolar SMCs and promotes vasoconstriction of parenchymal arterioles through activation of PKC.[53]

K_{ATP} Channels

K_{ATP} channels have been identified in a wide variety of smooth muscle types, and are likely an octomer of 4 sulfonylurea receptors (SUR2B) and 4 inward rectifier ($K_{ir}6.1$) subunits.[60,97] K_{ATP} channels lack voltage-dependence, and exhibit very weak inward rectification. They are also inhibited by intracellular ATP, and may open when ATP/ADP ratio changes during hypoxic or ischemic conditions.[60] Under physiological conditions, SMC K_{ATP} channels are potently activated by vasodilators that activate cAMP-dependent protein kinase and are inhibited by vasoconstrictors that activate PKC.[49,60] K_{ATP} channels in vascular smooth muscle are potently inhibited by sulfonylurea and hypoglycemic drugs, such as glibenclamide, and are selectively activated by a wide variety of synthetic compounds, such as pinacidil and cromakalim.[60,98] There is no direct electrophysiological evidence for K_{ATP} channels in SMCs of parenchymal arterioles. However, preconstricted parenchymal arterioles dilate in response to levcromakalim, a K_{ATP} channel agonist.[54] Additionally, dilation of parenchymal arterioles to mild hypercapnia is blocked by glibenclamide (5 μmol/L), a selective inhibitor of K_{ATP} channels.[99] It has also been reported that K_{ATP} channel activation mediates parenchymal arteriolar membrane hyperpolarization in response to basic fibroblast growth factor.[100] Glibenclamide-sensitive K_{ATP} currents measured in pial artery SMCs are activated by synthetic vasodilators such as pinacidil, as well as by endogenous vasodilators such as calcitonin gene-related peptide and VIP.[101,102] While there is evidence that supports the presence of K_{ATP} channels in parenchymal arteriolar SMCs, the biophysical properties of K_{ATP} channels in these vessels are not known, and the functional role of K_{ATP} channels in regulating parenchymal arteriolar tone requires further characterization.

Parenchymal Arteriolar Smooth Muscle K^+ Channels and NVC

Multiple parallel pathways are activated by the increase in $[Ca^{2+}]_c$ in astrocytes in response to neuronal activation. This is evidenced by the fact that pharmacological inhibition or genetic manipulation of any of the putative mediators of NVC never fully blocks the vascular response. Also, a rise in astrocytic endfoot $[Ca^{2+}]_c$ potentially activates a wide

range of Ca^{2+}-sensitive proteins. These Ca^{2+}-sensitive proteins include BK channels, which release K^+ ions as a vasoactive signal, as well as PLA_2, which provides the substrate (AA) for the generation of eicosanoid and prostanoid vasoactive mediators. Because functional hyperemia in the brain is a prerequisite for survival, it is not surprising that redundant mechanisms have evolved to maintain it. However, redundant parallel mechanisms that ensure functional NVC might ultimately converge on parenchymal arteriolar SMC K^+ channels to elicit their vascular response.

Vasodilation in Response to Neuronal Activity

Activation of parenchymal arteriolar smooth muscle K^+ channels during NVC would cause vasodilation and thereby increase local CBF. Here, we consider evidence that putative astrocytic mediators of NVC – external K^+, PGE_2, and EETs – dilate parenchymal arterioles through activation of smooth muscle K^+ channels.

External K^+

As noted above, isolated SMCs from parenchymal arterioles have a relatively high density of strong inward rectifier K^+ channels that are activated by external K^+ and membrane hyperpolarization, consistent with the properties of $K_{ir}2$ family members. Elevation of $[K^+]_o$ from 3 mmol/L to 8 mmol/L hyperpolarizes parenchymal arteriolar membranes from −45 to −80 mV, and causes a rapid and profound dilation of isolated pressurized parenchymal arterioles as well as arterioles in brain slices.[44] These effects are blocked by low concentrations of barium ions, which also block NVC responses to neuronal stimulation or Ca^{2+} uncaging in astrocytic endfeet in brain slices (by approximately 70%) and reduce hyperemic responses to whisker stimulation in vivo (by about 50%). Taken together, these results provide compelling evidence that parenchymal arterioles can dilate to external K^+ through activation of K_{ir} channels.

PGE_2

Based on the effects of COX inhibitors, several groups have proposed that PGE_2 released from astrocytic processes is responsible for NVC.[14,103–105] PGE_2 (20 μmol/L) has been shown to dilate arterioles in brain slices from neonatal rats.[14] PGE_2 might act through EP2 or EP4 prostanoid receptors in SMCs to elevate cAMP, which could lead to vasodilation by activating BK channels or K_{ATP} channels. This type of K^+ channel-dependent mechanism of PGE_2 dilation has been documented in renal arteries but has not been explored in parenchymal arterioles.[106] PGE_2 dilates pial arteries from cat and rat, and reportedly dilates human middle cerebral arteries through EP4 receptor activation.[107,108] However, there is limited direct evidence of PGE_2-mediated dilation in parenchymal arterioles. COX inhibition with indomethacin dilates pressurized parenchymal arterioles by 15%, but the mechanism of this effect is not known.[109] We have observed that in pressurized mesenteric arteries, PGE_2 only dilates the vessel in the presence of functional nitric oxide synthase, suggesting an endothelial effect rather than a SMC effect (unpublished observations). PGE_2 has also been reported to elicit vasoconstriction in a variety of arterial segments, including cerebral arteries.[110–112] The direct effect of PGE_2 on parenchymal arteriolar tone and its mechanism of action requires further investigation.

EETs

The release of EETs has been proposed to be involved in NVC.[113–116] Subdural superfusion of epoxygenase inhibitors reportedly reduces functional hyperemia in the rat somatosensory cortex by 28–69%.[115] EETs hyperpolarize cerebral artery SM membrane potential and potently dilate pial arteries through direct activation of SM BK channels and through indirect activation of SM BK channels by increasing Ca^{2+} sparks.[33,117–121] EET-mediated functional hyperemia in the brain very likely occurs through activation of K^+ channels in parenchymal arteriolar SMCs. However, the direct effect of EETs on K^+ channel activity, SMC membrane potential, and parenchymal arteriolar tone has not been investigated.

Vasoconstriction in Response to Neuronal Activity

Several groups have observed vasoconstriction of parenchymal arterioles in response to neuronal activation or Ca^{2+} uncaging in astrocytic endfeet.[12,51,113] The function of vasoconstriction in NVC is unknown, and this phenomenon might be more pathological than physiological. Inhibition of parenchymal arteriolar smooth muscle K^+ channels would depolarize the membrane and cause vasoconstriction, and there is evidence that the primary putative vasoconstrictor mediator of NVC, 20-HETE, acts through this mechanism.

As discussed above, inhibition of 20-HETE formation prevents vasoconstriction of parenchymal arterioles in response to neuronal stimulation in brain slices.[12] The 20-HETE inhibition also prevents light-evoked vasoconstrictions in mammalian retina.[113] This evidence suggests that 20-HETE constricts parenchymal arteriolar SMCs. Direct evidence in parenchymal arterioles is lacking, but 20-HETE potently constricts pial arteries.[122–124] The 20-HETE constricts arteries by inhibiting SMC BK channels through PKC.[84,85] The physiological significance of this mechanism is unclear, not only because of the questionable function of neuronal activity-induced vasoconstriction, but also because basal BK channel activity in parenchymal arteriole SMCs might be quite low. BK channel blockers have little effect on the diameter of arterioles in brain slices and on resting cortical CBF.[51]

External K^+ can produce parenchymal arteriolar constriction in addition to dilation. While modest elevation of $[K^+]_o$ dilates parenchymal arterioles through activation of K_{ir}, elevation of $[K^+]_o$ above 20 mmol/L evokes constriction through SMC membrane potential depolarization.[51,125] In brain slices, raising $[K^+]_o$ in the bath also converts evoked parenchymal arteriolar dilations with neuronal stimulation or endfoot Ca^{2+} uncaging into constrictions.[51] In NVC, the astrocyte can tune the polarity of the arteriolar diameter response by altering the magnitude of K^+ release from the endfoot.

PGE_2 also has the potential to mediate neuronal activity-induced constriction. Human pial artery dilation in response to PGE_2 occurs through EP4 receptors.[108] In some types of cerebral arteries, PGE_2 has been shown to cause constricton through EP1 or EP3 receptors.[112] The specificity of the cerebral vascular response to PGE_2 (dilation vs constriction) appears to be conferred by the prostanoid receptors stimulated. Which prostanoid receptor subtypes are expressed and their relative expression in SMCs of

parenchymal arterioles is not known. Also, as stated above, the direct effect of PGE_2 on parenchymal arterioles has not been studied.

In studies observing vasoconstriction with a rise in astrocytic endfoot Ca^{2+} or neuronal activation, a 20-HETE inhibitor, albeit at a high concentration, largely abolished the constrictor response. However, if there is a role for astrocyte-mediated vasoconstriction in normal physiology or in pathology, external K^+, PGE_2, and 20-HETE could potentially be mediators.

Indirect Role of Parenchymal Arteriolar SMC K^+ Channels in NVC

Smooth muscle K^+ channels also indirectly influence NVC by setting the resting level of tone in parenchymal arterioles prior to neuronal activation. In pial arterioles, the pre-existing level of tone in the vessel determines the magnitude and polarity (constriction vs vasodilation) of the vascular response to vasoactive substances.[126,127] Similarly, the response of parenchymal arterioles to putative signals involved in NVC is dictated by the resting tone of the vessel.[128] Varying the degree of preconstriction of parenchymal arterioles in brain slices with increasing concentrations of the thromboxane receptor agonist U-46619 alters the response of the arteriole to K^+ such that the greater the initial level of preconstriction, the greater the magnitude of the vasodilator response to 10 mmol/L $[K^+]_o$. Stimulation of mGluR to elicit a rise in astrocytic $[Ca^{2+}]_c$ evokes constriction in modestly preconstricted arterioles (70–100% of baseline) and dilation in arterioles with greater initial tone. The switch in polarity of vascular response appears to occur at a level of tone corresponding to approximately 50–70% of baseline diameter.

As key regulators of membrane potential, and therefore Ca^{2+} influx through VDCCs, SMC K^+ channels are primary determinants of parenchymal arteriolar tone. Therefore, arteriolar SMC K^+ channels likely play a role in determining the magnitude and polarity of the vascular response to neuronal activation by setting the initial level of tone of the arteriole.

Concluding Remarks

Precise regulation of CBF is essential for homeostasis in the brain. Smooth muscle K^+ channels are critical regulators of vascular tone and blood flow in cerebral arteries and arterioles. Many mechanisms of chemical, mechanical, and humoral regulation of CBF involve modulation of SMC K^+ channel activity. Parenchymal arteriolar SMC K^+ channels are important in the process of NVC and functional hyperemia in the brain, and have potential as therapeutic targets in pathological conditions in which NVC is compromised, including Alzheimer's disease, dementia, diabetes, and hypertension.

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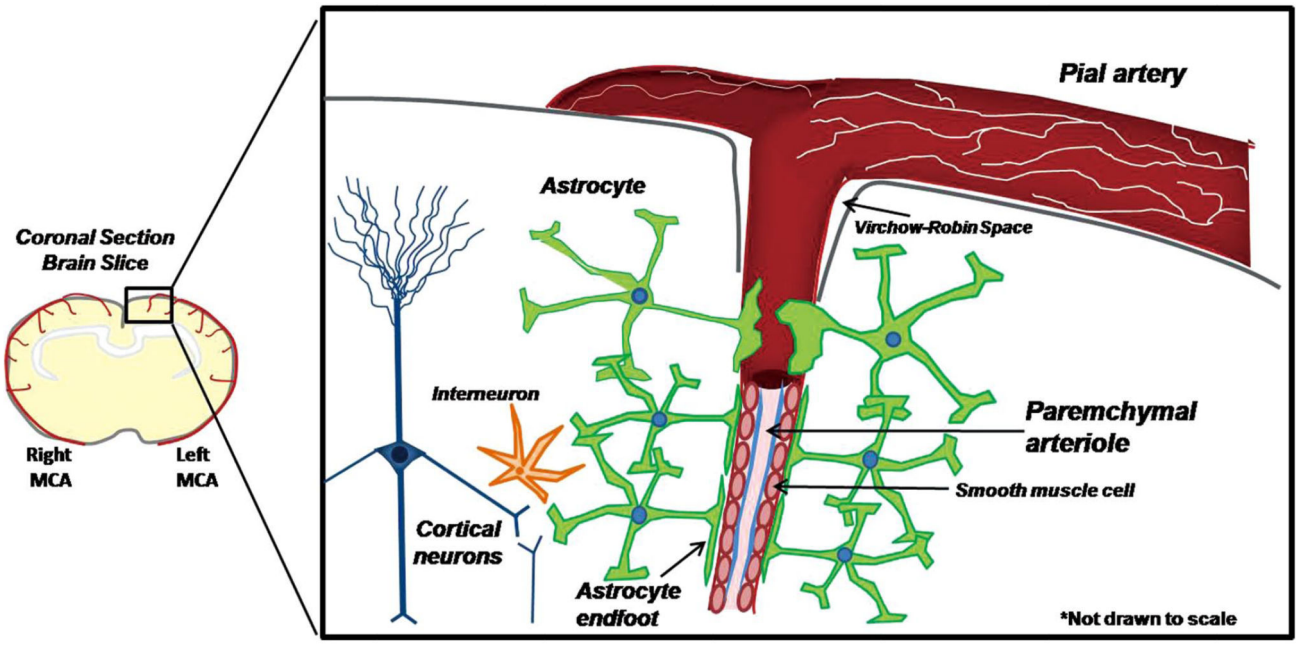

Figure 1.

Pial arteries on the surface of the brain penetrate into the cortical parenchyma to give rise to parenchymal arterioles. Pial arteries are extrinsically innervated by sympathetic nerves (shown in white). As the arteriole penetrates into the brain parenchyma beyond the Virchow-Robin space, extrinsic innervation is lost and the arteriole becomes completely encased in astrocytic terminal processes called ‘endfeet’. Astrocytes integrate information from neurons and other cell types (ie, interneurons) and translate that information into dynamic astrocytic Ca^{2+} signals that propagate to the endfoot and regulate parenchymal arteriolar tone in order to regulate local cerebral blood flow according to the metabolic needs of the surrounding tissue.

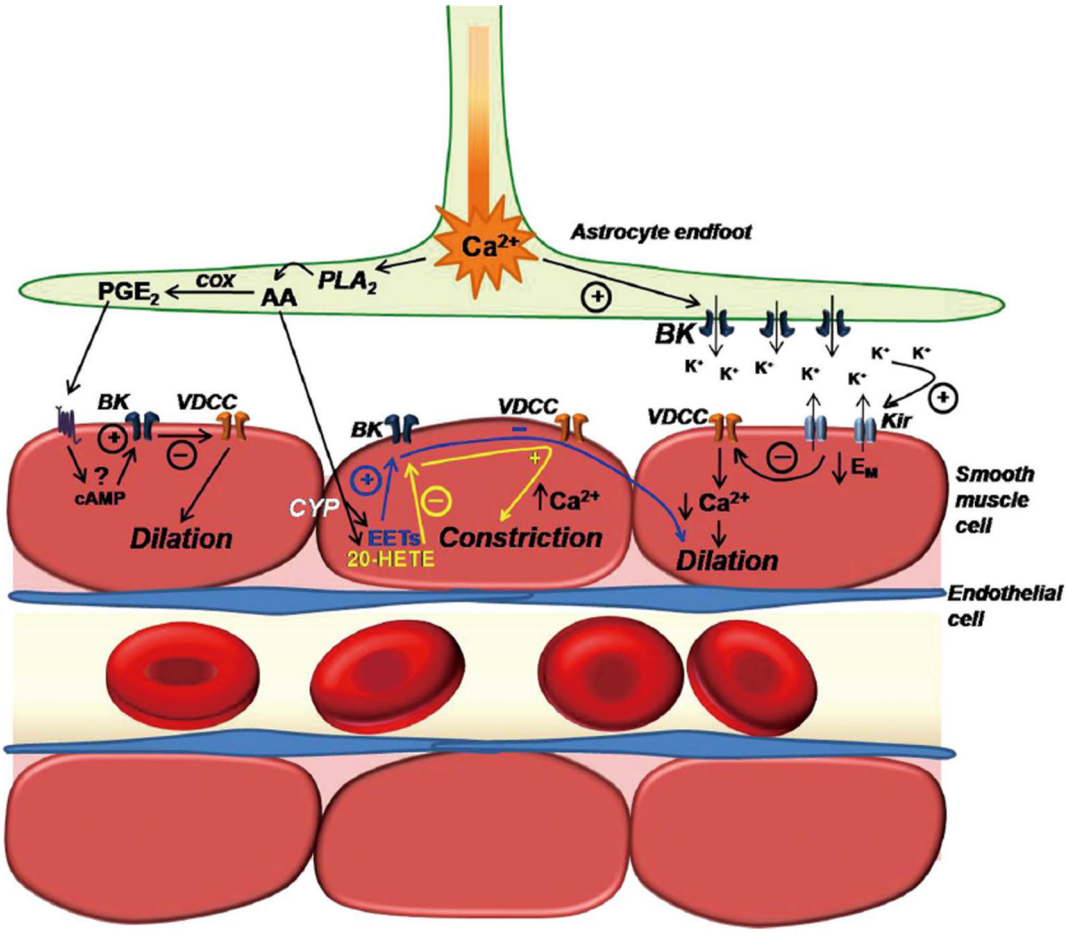

Figure 2.

Illustration depicting the involvement of parenchymal arteriolar smooth muscle cell (SMC) K^+ channels in putative mechanisms of neurovascular coupling. Neuronal activity stimulates astrocytic metabotropic glutamate receptors (not shown) to produce a propagating rise in $[Ca^{2+}]_i$ that terminates in perivascular endfeet. Increased astrocytic endfoot $[Ca^{2+}]$ activates BK channels to release K^+ into the perivascular cleft. Moderate elevations in $[K^+]_o$ in the perivascular cleft activate Kir channels in parenchymal arteriole SMCs, resulting in SMC membrane potential hyperpolarization, decreased Ca^{2+} entry through VDCCs, decreased $[Ca^{2+}]_i$, and vasodilation. Increased astrocytic endfoot $[Ca^{2+}]$ also activates the Ca^{2+} sensitive enzyme cytosolic phoshpolipase A2 (PLA2). PLA2 hydrolyzes membrane phospholipids to release the fatty acid arachidonic acid (AA). AA is then metabolized by cyclooxygenase (COX) and prostaglandin synthases to yield PGE2, or is believed to diffuse to the parenchymal arteriole SMC, where it is metabolized by cytochrome P-450 enzymes to generate EETs and/or 20-HETE. The mechanism of action of PGE2 on parenchymal arteriole SMCs is unknown, but based on studies done in pial arteries, it might involve cAMP-dependent activation of SMC BK channels, and subsequent SMC hyperpolarization and vasodilation. EETs formed in arteriolar SMCs by the metabolism of astrocytederived AA are proposed to activate SMC BK channels to elicit membrane hyperpolarization and

vasodilation. Conversely, 20-HETE is believed to inhibit parenchymal arteriolar SMC BK channels resulting in membrane depolarization, activation of VDCCs, elevation of $[Ca^{2+}]_i$, and vasoconstriction.