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Vascular Inward Rectifier K^+ Channels as External K^+ Sensors in the Control of Cerebral Blood Flow

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Abstract

For decades it has been known that external potassium (K^+) ions are rapid and potent vasodilators that increase cerebral blood flow (CBF). Recent studies have implicated the local release of K^+ from astrocytic endfeet—which encase the entirety of the parenchymal vasculature—in the dynamic regulation of local CBF during neurovascular coupling (NVC). It has been proposed that the activation of strong inward rectifier K^+ (K_{IR}) channels in the vascular wall by external K^+ is a central component of these hyperemic responses; however, a number of significant gaps in our knowledge remain. Here, we explore the concept that vascular K_{IR} channels are the major extracellular K^+ sensors in the control of CBF. We propose that K^+ is an ideal mediator of NVC, and discuss K_{IR} channels as effectors that produce rapid hyperpolarization and robust vasodilation of cerebral arterioles. We provide evidence that K_{IR} channels, of the $K_{IR}2$ subtype in particular, are present in both the endothelial and smooth muscle cells of parenchymal arterioles and propose that this dual positioning of $K_{IR}2$ channels increases the robustness of the vasodilation to external K^+, enables the endothelium to be actively engaged in neurovascular coupling, and permits electrical signaling through the endothelial syncytium to promote upstream vasodilation to modulate CBF.

Keywords

K_{IR} channels; neurovascular coupling; cerebral blood flow; functional hyperemia; parenchymal arteriole; capillary; smooth muscle; endothelium; astrocytic endfoot

INTRODUCTION

Brain function is dependent on the ability to match regional neuronal metabolic requirements with an appropriate level of local blood flow. When neurons become active, signaling cascades are initiated that result in hyperemia—a surge of local blood which delivers the energy substrates needed to support ongoing neuronal function. The term ‘functional hyperemia’ arose to describe these neuronally-driven blood flow responses, and the discovery of this phenomenon is attributed to Roy and Sherrington in 1890 [105].

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'Neurovascular coupling' (NVC) describes the molecular mechanisms underpinning neuron-to-vasculature communication, and this involves coordinated intra- and intercellular signaling between the cells of the neurovascular unit—neurons, astrocytes, vascular smooth muscle (SM), and likely also endothelial cells (ECs) and pericytes. Work throughout the last 125 years has elucidated some of the mechanisms underlying NVC. However, there exists controversy over the identities of the key mediators of this process, and many significant gaps in our knowledge still remain.

NVC demands the rapid coupling of neuronal activity to blood flow responses; therefore, an ideal mediator of this process would possess the following properties:

1) Rapid release and onset of action.

2) The ability to evoke robust and sustained vasodilation for the duration of its presence.

3) Rapid off-kinetics and clearance.

One factor that fits this profile is potassium (K^+) ions. It has been appreciated for many years that computationally active neurons release K^+ during the repolarization phase of the action potential, which leads to fluctuations in the K^+ concentration of the brain's external milieu. Moreover, it is well known that small elevations in K^+ can dilate cerebral arteries and arterioles and increase brain blood flow *in vivo* [26,27,51,58]. Using this information as a takeoff point, our laboratory has developed a working model of K^+-mediated dilation in NVC that satisfies the three criteria outlined above (Figure 1). Although our data, based on *ex vivo* models, provide strong experimental support for the proposed model and results obtained to date *in vivo* are generally consistent with this, the precise mechanisms through which K^+ induces hyperemia *in vivo* remain poorly defined. It is also not currently clear how the various proposed vasodilatory mechanisms are integrated to achieve a coordinated hyperemic response. This latter issue is the subject of a number of excellent recent reviews and will not be addressed here [1,10,52,95].

In this article, we explore the concept of K^+ as an ideal mediator of NVC and argue that strong inward-rectifier K^+ (K_{IR}) channels in the vascular wall are the key sensors of external K^+ in the brain. We also place our proposed model—and extensions to it—in the context of the biophysical and electrophysiological properties of the K_{IR} channel to demonstrate how these channels are capable of converting local K^+ signals into profound smooth muscle (SM) membrane hyperpolarization to relax arterioles and increase CBF.

REGULATION OF CEREBRAL ARTERY DIAMETER BY EXTERNAL K^+: THE VASCULAR SMOOTH MUSCLE AS A K^+ ELECTRODE

The molecular mechanisms that control arteriolar diameter differ between distinct segments of the cerebral vasculature and, more broadly, throughout the vascular tree as a whole. Here, our focus is on penetrating cerebral parenchymal arterioles (PAs). PA vascular SM exhibits a steep relationship between membrane potential (V_m) and arteriolar diameter, making control of V_m central to the control of cerebral blood flow. Elevation of intravascular pressure to physiological levels—40 mm Hg for PAs—sets the SM V_m to between -35 and -40 mV [37,89]. The basis of this resting V_m lies in the balance between depolarizing ion

conductances that are incrementally activated in response to increasing pressure and hyperpolarizing conductances that counteract them. Depolarization increases the activity of voltage-dependent calcium (Ca^{2+}) channels (VDCCs), leading to an elevation of intracellular Ca^{2+}, which engages the SM contractile machinery and thereby causes constriction. In cerebral arteries, membrane depolarization also activates voltage-dependent K^+ (K_V) channels [49] and large-conductance Ca^{2+}- and voltage-sensitive K^+ (BK) channels [6], which conduct K^+ out of the cell and thereby provide a hyperpolarizing influence, acting as a brake on constriction. The result of the balance between depolarizing-contractile and hyperpolarizing-relaxing conductances is the constriction to pressure known as the 'myogenic response', and the level of 'myogenic tone' sets basal cerebral vessel diameter and resting CBF, which can then be modulated during NVC.

Under experimental conditions with low intravascular pressure (e.g., 5 mm Hg), at which PAs display little myogenic tone, the PA SM V_m is approximately -60 mV which is 43 mV positive to the K^+ equilibrium potential (E_K) of -103 mV [89], assuming 3 mM extracellular K^+ [7] and 140 mM intracellular K^+. According to a parallel conductance model [84,85] used to calculate the relative conductance for K^+ versus other major ions (i.e., Na^+, Ca^{2+}, Cl^-) under these conditions, these values indicate that the K^+ conductance of the membrane dominates over other conductances by about 1.4:1 (see Box 1). If intravascular pressure is then increased to physiological levels (e.g., 40 mm Hg), the SM V_m is depolarized (to between -35 and -40 mV) [37,89], and this ratio decreases to about 0.6:1, reflecting an increase in the conductance of Na^+/Ca^{2+}-permeable channels relative to K^+ conductances. This pressure-induced depolarization is likely mediated by the activation of transient receptor potential (TRP) channels (e.g., TRPC6 and TRPM4) and VDCCs [28,64], promoting the influx of cations. In pial arteries, the increase in VDCC conductance is offset by activation of K_V and BK channels [6,49]. In contrast, in PAs, where there is little BK channel activity at the resting potential of these arterioles [37], the increase in depolarizing conductances is offset by negative-feedback elevation of K_V channel activity [116].

Elevation of external K^+ depolarizes most cells. Counter-intuitively, however, small elevations of external K^+ cause a striking hyperpolarization of SM cells in cerebral arteries [51] and PAs [26]. For example, elevation of external K^+ from physiological cerebrospinal fluid levels (3 mM) [7] to 8 mM causes a rapid and near maximal dilation of PAs (Figure 2A) by causing a hyperpolarization from between -35 and -40 mV to close to the new E_K of -76 mV (Figure 2B) [26]. To come within even 2 mV of E_K under these conditions, the ratio of K^+ to $Na^+/Cl^-/Ca^{2+}$ conductances would have to increase at least 58-fold—from 0.6:1 to 37:1.

This increase in K^+ conductance is enabled by the activation of K_{IR} channels within the vascular wall (the term 'vascular wall' is used where the precise cellular localization of the channel—i.e., smooth muscle and/or endothelium—is not yet known). The K_{IR} channel family consists of seven subfamilies (K_{IR}1-7) comprising a total of 15 subunit isoforms (K_{IR}1.1, K_{IR}2.1-4, K_{IR}3.1-4, K_{IR}4.1-2, K_{IR}5.1, K_{IR}6.1-2, and K_{IR}7.1). Each subunit is a two-transmembrane protein connected by a pore-forming loop with intracellular C- and N-terminal domains. Functional K_{IR} channels are tetramers formed from homo- or heteromeric assemblies of individual subunits. K_{IR} channels can be further separated into four groups on

the basis of their functional characteristics: classical, G-protein gated, ATP-sensitive and K^+ transport channels [39]. Members of this family are expressed in cells throughout the neurovascular unit (Table 1). Notably, the classical $K_{IR}2$ channels are expressed in the vascular wall of PAs and cerebral arteries, and exert a strong influence over SM V_m and contractile state [5,26,51,67,99,134], with the $K_{IR}2.1$ isoform apparently having a prominent role [5,134].

$K_{IR}2$ channels display negative slope conductance—meaning that their activity increases upon membrane hyperpolarization [111]—and are also activated by increases in external K^+ (Figure 3A). Outward current through these channels decreases with membrane potential depolarization positive to E_K. This "inward rectification" occurs because of voltage-dependent intracellular channel blockade by polyvalent cations, such as magnesium (Mg^{2+}), and polyamines, such as spermine, spermidine and putrescine [69,72] (Figure 2C and D). The electrical potential across the membrane appears to be a key driver of this block, forcing one or more Mg^{2+} ions and/or polyamines into the pore of the channel, where they ultimately bind to the multiple anionic regions therein [13,69,72,101]. It has been suggested that, when external K^+ is elevated, blocking particles may be driven out of the pore by electrostatic forces imparted by the increased concentration of extracellular K^+ ions, which appear to interact directly with a 'K^+ coordination site' on the outer mouth of the channel [113].

The external K^+- and voltage-dependence of $K_{IR}2$ channel activity have been known for quite some time [63]. Channel conductance is half-maximal at E_K [98]; therefore, at a given V_m, elevation of external K^+ will increase channel conductance with the rightward shift in E_K (Figure 3A). Also, membrane hyperpolarization increases channel conductance *e*-fold for every 7.4 mV [98], presumably reflecting voltage-dependent removal of polyamine block. These two facets of K_{IR} behavior synergize with an increase in external K^+, leading to substantial increases in K_{IR} channel conductance that can be estimated using the equation,

$$gK_{IR}/gK_{IR}\quad max=\left\{1+e^{[(V-V_{0.5})/k]}\right\}^{-1}, \quad \text{Eq 1}$$

where gK_{IR} is K_{IR} channel conductance, $gK_{IR}max$ is the maximal slope conductance for a given concentration of external K^+ (proportional to $\{[K^+]_o\}^{0.5}$), V is V_m, $V_{0.5}$ is the half-maximal activation potential for K_{IR} channels (equal to E_K) and k is the steepness factor (equal to 7.4 mV) [98]. According to this equation, when external K^+ is raised from 3 mM to 8 mM (causing PA membrane hyperpolarization from about -40 mV to -76 mV), K_{IR} channel conductance increases approximately 4,000-fold. This enormous increase in K_{IR} channel conductance accounts for the hyperpolarization to E_K despite the simultaneous loss of K_V channel conductance [116]. Increasing K^+ beyond 8 mM causes a much smaller relative increase in K_{IR} channel conductance (see Figure 3B).

Thus, the biophysical properties of the $K_{IR}2$ channel explain how small increases in external K^+ can act as a powerful vasodilatory force, directly generating rapid and profound hyperpolarization and dilation of the arterioles that participate in NVC (see Figure 1). Accordingly, when K^+ is raised, the SM membrane acts as a K^+ electrode, tracking E_K (Figure 2B). Gradual increases in K^+ eventually lead to membrane depolarization, which

also activates voltage-sensitive K_V and BK channels, further increasing K^+ permeability to keep V_m clamped at E_K. Pressurized cerebral arteries and arterioles begin to develop detectable constriction at about -60 mV [50]. At this potential, the open probability of VDCCs is sufficient to deliver Ca^{2+} to cause constriction [50,106] (see Figure 2A, K^+ concentrations above 20 mM). For example, when external K^+ is elevated to 25 mM, V_m/E_K (approximately -46 mV) is close to the resting V_m measured with 3 mM external K^+ (approximately -40 mV). As expected, the degree of constriction under each of these conditions is approximately the same (Figure 2A) [27,67]. Increasing K^+ further (e.g., to 30 and 60 mM) depolarizes V_m and promotes more substantial constriction [27,67]. Overall, this leads to the characteristic biphasic relationship between external K^+ and arteriolar diameter, with elevations of external K^+ to lower concentrations (<20 mM) causing vasodilation and higher concentrations causing constriction. Therefore, elevation of K^+ can provide a unified mechanism to account for both PA vasodilations and vasoconstrictions in response to neuronal activity [27,54] (see Figure 1).

In contrast to the hyperpolarization induced by optimal increases in K^+, at lower concentrations of K^+ that are insufficient for maximal K_{IR} channel activation it is likely that a ‘tug of war’ between hyperpolarizing and depolarizing forces ensues. Accordingly, as the K_{IR} channel activates in response to K^+, the membrane would begin to hyperpolarize, shutting off K_V and BK channels, and thereby counteracting the increase in K^+ permeability to K_{IR} channel activation. Provided that depolarizing influences remain relatively constant, deactivation of K_V and BK channels would tend to depolarize V_m. This repolarization would, in turn, reactivate K_V and BK channels, and this process would repeat, manifesting as a bi-stable SM V_m and diameter oscillations. (For example, see Figure 1, panel A in [76], where raising K^+ from 6 to 7 mM results in diameter oscillations in rat cerebral arteries.) This phenomenon indicates that there is a critical level of external K^+ required for stable and sustained V_m hyperpolarization and vasodilation.

The Na^+/K^+ ATPase ‘pump’ has also been suggested as a sensor for extracellular K^+ in some peripheral vessels, such as the hamster cremaster, and rat mesenteric and tail arteries [8,128,129]. However, considering the comparatively small increase in pump activity in response to K^+, and the modest and transient nature of K^+-induced vasodilation that is attributable to pump activation in cerebral arteries (see Box 2), it is unlikely that Na^+/K^+ ATPases are major contributors to K^+-induced dilation during NVC. However, it is possible that the transient hyperpolarization provided by the pump could act in synergy with K_{IR} channel activation to ‘kick-start’ robust K^+-induced vasodilations. Na^+/K^+ ATPases likely also aid in rapidly clearing K^+ from the perivascular space after NVC (Figure 1).

WHAT ARE THE SITES OF K^+ RELEASE IN THE BRAIN?

To exert an effect on the vascular wall K_{IR} channel, K^+ ions must be concentrated within an appropriate range in the restricted extracellular space between the SM and the overlying astrocytic endfeet. These endfeet completely encase the cerebral microcirculation [71], and thus represent an anatomical barrier that limits the direct diffusion of K^+ ions from their site of release (neurons) to their target of action (cerebral microvessels). However, there is now mounting evidence that astrocytes act as intermediaries in this process [91,119], with the

release of K^+ from the endfeet in response to intracellular signaling cascades evoked by neuronal activity serving as a key mechanism of NVC (Figure 1).

In the 1980s, the concept of astrocytic K^+ spatial buffering/siphoning gained traction as a plausible mechanism for the redistribution of K^+ from areas of high concentration, such as around the synapse after neuronal activity, to areas of lower concentration, such as the perivascular space. According to this conceptually appealing idea, K^+ was thought to be absorbed by perisynaptic astrocytic processes and then exit the cell via specialized foot processes adjacent to the brain surface and the cerebral microcirculation [56,86,87,90]. Several functions were ascribed to this phenomenon, including the maintenance of ion homeostasis around the synapse to ensure continued normal neuronal function [86,87], and a potential role in neuronal activity-evoked increases in CBF [93]. Follow-on experiments in retina and brain suggested that the molecular entity underlying glial K^+ siphoning was the intermediate inward rectifier channel $K_{IR}4.1$ [42,53,83,120]. However, subsequent work provided strong evidence that efflux of K^+ via astrocytic $K_{IR}4.1$ channels does not contribute to NVC. These experiments, performed in retina, showed that injections of depolarizing current into patch-clamped perivascular astrocytes (to drive K^+ efflux through open K_{IR} channels) had no effect on arteriole diameter, and further demonstrated that light-evoked vasodilation was unchanged in $K_{IR}4.1$-knockout mice [79].

Despite this negative result, recent evidence supports the concept that functional hyperemia does indeed involve the release of K^+ from astrocytic endfeet [26,27,67]. However, instead of release via $K_{IR}4.1$ channels, K^+ release from the endfoot is driven by astrocytic inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} waves initiated by local neuronal activity [115], which engage BK channels on the endfoot plasma membrane [26,27,97]. This increases the concentration of K^+ in the extracellular nanospace between the endfoot and SM to activate SM $K_{IR}2$ channels (see Figure 1). Theoretically, during NVC, a single endfoot BK channel need open for just 200 ms to release sufficient K^+ ions to raise K^+ in the perivascular space to 10 mM—a concentration high enough to rapidly hyperpolarize the SM V_m to -70 mV and cause substantial vasodilation [26]. In addition to BK channels, intermediate-conductance Ca^{2+}-activated K^+ (IK) channels are also expressed in astrocyte endfeet [68], providing an additional Ca^{2+}-dependent pathway through which K^+ might contribute to NVC (Box 3).

VASCULAR K^{IR} CHANNELS AS THE EXTERNAL K^+ SENSORS IN CEREBRAL BLOOD FLOW CONTROL: CURRENT EVIDENCE AND QUESTIONS ARISING

It has long been known that elevation of external K^+ increases pial artery diameter *in vivo* [58], and that cerebral arteries [24] (and more recently PAs [26]) possess SM inward rectifier currents. Thus, a potential mechanism for increases in CBF is activation of K_{IR} channels in the vascular wall by external K^+ released into the perivascular space by astrocytic endfeet in response to neuronal activity, as described in Figure 1. In support of this hypothesis, the K_{IR} channel blocker barium (Ba^{2+}) at 100 μM, a concentration that is selective for $K_{IR}2$ isoforms (see Table 1), prevents increases in local CBF to whisker stimulation or the metabotropic glutamate receptor agonist trans-ACPD [27]. Ba^{2+} at this

concentration also inhibits pial artery dilation *in vivo* in response to sciatic nerve stimulation [124]. Further support for the involvement of this K^+-to-K_{IR} mechanism is demonstrated by the ability of Ba^{2+} to inhibit vasodilation evoked by neuronal stimulation in brain slice preparations (by 50-70%) [26,67]. Notably, the salient features of this mechanism have been successfully recapitulated by computer models [131,132]. These studies suggest that K_{IR} channel activity in response to endfoot-derived K^+ is indeed a vitally important mechanism for NVC, although the possibility that K^+ could act indirectly through Ba^{2+}-sensitive K_{IR} channels in another cell type, such as neurons or astrocytes (Table 1), cannot currently be discounted. A fuller understanding of the molecular details underlying this mechanism awaits an answer to this and a number of other important questions.

One key question with important implications for the operation of the K_{IR} channel-dependent vasodilatory circuitry is whether this channel is localized to the PA SM, endothelium or both. In other vascular beds, K_{IR} channels have been localized to ECs alone (such as in the mesenteric artery [15]), or have been found in both SM and ECs (such as in hamster cremaster arterioles [8,44]). The existence of K_{IR} channels in both SM and ECs may provide a mutually reinforcing amplifier for the hyperpolarization evoked by small elevations in external K^+. This reinforcing amplification would, in turn, be predicted to dampen the tug of war between K_{IR} and K_V/BK channels that ensues following K_{IR} channel activation. Although K_{IR} channel expression and function in PA endothelium have not been reported in the literature, we have obtained recordings of Ba^{2+} (100 μM)-sensitive K_{IR} currents in freshly isolated ECs from PAs (Figure 3C), suggesting that functional $K_{IR}2$ channels are present in this cell type as well as SM cells. While this is not yet definitive evidence (as it is possible that the ion channel properties of acutely isolated cells may differ to those of intact arterioles), it nonetheless sets the stage for a situation in which K^+ released from the endfoot could also recruit endothelial K_{IR} channels in addition to activating SM K_{IR} channels, thereby helping to stabilize the hyperpolarized SM V_m and eliminate diameter oscillations, especially at lower levels of elevated K^+. In arteries of the peripheral microcirculation, endothelial K_{IR} channels have also been implicated in conducted vasodilatory signaling within and along the vessel wall [31,44]. In this setting, K_{IR} channels transmit EC hyperpolarization to the SM via myo-endothelial gap junctions and along the length of the vessel through endothelial-endothelial gap junctions. Extending this communication paradigm to PAs, it is possible to hypothesize that K^+ released during NVC engages both the SM and endothelium through $K_{IR}2$ channels expressed in these two cellular compartments, leading to a regenerative hyperpolarization along the endothelial lining of PAs up to pial arteries on the brain surface that would simultaneously also be transmitted to the SM through gap junctions. If this hypothesis were confirmed, it would support a major functional contribution of the endothelium to NVC. In support of this concept, Hillman and colleagues recently demonstrated that light-dye disruption of endothelial function halts the propagation of pial artery vasodilation *in vivo* in response to electrical hindpaw stimulation, thereby blunting increases in surface CBF [11]. Additionally, if brain capillary ECs are found to express K_{IR} channels and exhibit electrical coupling, it would argue that these channels also play a role in conducted signaling from the capillary bed upstream to PAs, as has been suggested to occur in other capillary beds [20], providing

a unified mechanism for vasoconduction throughout the entire vascular network of the brain. This overall concept is illustrated in Figure 4.

Which K_{IR} channel isoforms are functionally expressed in the SM and endothelium of the PA wall? We have detected transcripts for both $K_{IR}2.1$ and 2.2 in PA homogenates [67], but to our knowledge, the potential expression of $K_{IR}2.3$ and 2.4 isoforms in these vessels has yet to be explored. Functional data indicate that the Ba^{2+} sensitivity of SM inward-rectifier currents match those carried by $K_{IR}2.1$-2.3 channels [26,67] (Table 1). Further, a study of pial arteries showed that global knockout of $K_{IR}2.1$ resulted in a complete loss of K_{IR} currents in isolated SM cells and K^+-mediated dilations. In contrast, knockout of $K_{IR}2.2$ had no effect on K^+-mediated vasodilation in this vascular bed [134]. These results suggest that the $K_{IR}2.1$ subunit is a vital component of K_{IR} channels in pial arteries. However, the $K_{IR}2.2$ subunit does appear to be expressed at the mRNA level in both pial arteries and PAs where, interestingly, transcripts for this subunit are more abundant than those for $K_{IR}2.1$ [67,111,133]. Accordingly, it has been suggested that SM K_{IR} channels in cerebral vessels may be composed of heteromers of $K_{IR}2.1$ and 2.2 subunits under normal conditions [133]. In any case, it appears that the $K_{IR}2.1$ subunit is essential for the formation of functional channels. In pial arteries, Wu *et al.* [133] observed $K_{IR}2.1$, 2.2 and 2.4 subunit transcripts in endothelium-intact homogenates, whereas $K_{IR}2.4$ expression was absent in SM-only homogenates. Whether expression of $K_{IR}2.4$ mRNA in the endothelium, as implied by these results, translates to a functional role for this channel in these cells was not established by these studies. However, the Ba^{2+} (100 μM) sensitivity of the currents we observed in ECs from PAs would at least indicate that functional $K_{IR}2.4$ homomers (Ba^{2+} IC_{50} = 390 μM [123]) do not likely contribute to the EC currents recorded from these arterioles.

The presence of K_{IR} channels with identical pharmacological profiles in both SM cells and ECs of the cerebral microcirculation presents a technical challenge for accurately dissecting the relative contribution of SM and EC K_{IR} channels to NVC. Furthermore, $K_{IR}2$ family members are expressed in neurons and astrocytes in the CNS (Table 1) [17,40,47,107,108]. Global K_{IR}-knockout mice are thus not the answer. Even if they were, germline ablation of the gene for $K_{IR}2.1$—the predominant vascular isoform—leads to the development of a cleft palate; as a result, newborn mice fail to suckle and die shortly after birth [134]. To circumvent this problem, we have generated a floxed $K_{IR}2.1$ mouse that we have crossed with mice expressing Cre recombinase under the control of EC- or SM cell-specific promoters. This cell-specific knockout approach will ultimately enable us to discern the specific role of the K_{IR} channel in each vascular cell type. These conditional mouse models are also less likely to be influenced by the development of compensatory changes, which are a concern with germline knockouts.

DISRUPTION OF NEUROVASCULAR K^+ COMMUNICATION IN DISEASE: LESSONS FROM A CHRONIC STRESS MODEL

Too little or too much K^+ signaling is a plausible mechanism for the loss of control of CBF in a range of brain disorders. An interesting recent study demonstrates this principal in glioma tissue. Here, the elevation of perivascular K^+ caused by release from cancerous glial cells, which migrate along the vascular wall and displace healthy astrocytic endfeet, results

in a loss of control of vascular tone to the invading tumor [127]. Similarly, pathological conditions such as cortical spreading depression are accompanied by an extracellular K^+ wave that increases K^+ from 3 mM to as high as 60 mM [112], which might contribute to increases or decreases in CBF depending on the concentration of external K^+ [2,60,61,74].

Pathologies are also capable of disrupting $K_{IR}2$ channel expression and function in many different tissues and vascular beds, making this channel a potential therapeutic target in a broad range of diseases. Prominent among these is Andersen-Tawil syndrome, caused by a mutation in $K_{IR}2.1$ and characterized by cardiac arrhythmias, dysmorphic features and periodic paralysis [96]. Additionally, K_{IR} channel function is impaired in mesenteric arteries [130] and posterior cerebral arteries [76] in hypertension, and K_{IR} channel activity in pial arteries is suppressed by protein kinase C overactivity in streptozotocin-induced diabetes [124]. Very recent evidence from our laboratory suggests that K^+ signaling during NVC can be disrupted in PAs at the level of the SM K_{IR} channel in the context of a rodent model of chronic stress [67].

A substantial body of evidence has demonstrated that long-term exposure to stressful stimuli has a negative impact on human health, with links to both psychopathologies and cardiovascular disease [14,18,21,57,66,78,100]. ‘Stress’ is a vague term that has been used in a wide variety of contexts, but here stress refers to any real or psychologically perceived threat to an organism's homeostasis that prompts a stereotyped ‘fight or flight’ response.

The amygdala is one of several brain regions that is engaged during stressful events [62,103], and stressor processing here ultimately leads to an increase in sympathetic nervous system tone and hypothalamic-pituitary adrenal (HPA) axis output, resulting in a “fight or flight response”. This response includes an immediate increase in circulating catecholamines —which act rapidly to stimulate an increase in heart and breathing rate, shut down digestion, increase blood pressure and shunt blood to major muscle groups—and a slower increase in glucocorticoid hormones, principally corticosterone in rodents and cortisol in humans [18]. The neuronal effects of repeated exposure to stressors have been well explored [18]. In contrast, comparatively little work has been devoted to investigating the effects of stress on other cells of the brain, such as astrocytes, and the SM and ECs and of PAs have received even less attention.

With this in mind, we examined the possible impact of stress on cells of the neurovascular unit and on NVC by applying a chronic stress model to male rats. Subjects were exposed to one of five heterotypical stressors per day for a total of seven days. Rats that were exposed to this paradigm developed an anxious behavioral phenotype, in line with previous observations [34,102]. In brain slices prepared from stressed rats, we observed a marked impairment of NVC in the amygdala. Intriguingly, neuronal activity-evoked vasodilation was substantially blunted after stress, whereas the preceding astrocytic endfoot Ca^{2+} wave was elevated. This enhanced Ca^{2+} wave is likely the result of stress-induced amygdalar neuron hyperactivity [33,104], a phenomenon in which electrical stimulation may elicit more intense network activity that is transduced into larger Ca^{2+} signals in local astrocytes. It is also possible that stress-related signaling may alter astrocytic Ca^{2+} handling, for

example by increasing the sensitivity or number of IP_3 receptors in the endoplasmic reticulum.

As discussed above (Figure 1), astrocytic Ca^{2+} waves lead to the release of K^+ from the endfoot, which then activates K_{IR} channels on the juxtaposed SM to cause membrane hyperpolarization, leading to relaxation and vasodilation [26]. After stress, not only were isolated arterioles substantially less sensitive to K^+, but also Ba^{2+} no longer inhibited NVC in slices, strongly suggesting a loss of K_{IR} channel function. This was confirmed by experiments examining K_{IR} channel current density in isolated PA SM cells, which indicated fewer functional channels in the membrane after stress, and by molecular experiments, which suggested that the $K_{IR}2.1$ isoform was downregulated in PAs by stress [67].

How might K_{IR} channel expression/function be reduced by stress? SM cells and ECs of PAs possess abundant glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs) [29,30,77,88]. Because it readily crosses the blood-brain barrier, corticosterone, which in rats is elevated for prolonged periods after stressor exposure, could plausibly act at these vascular receptors. At basal corticosterone concentrations, MRs are completely occupied, whereas the lower-affinity GRs are available for binding elevated corticosterone after stressor exposure [18]. By inhibiting GRs or exogenously applying corticosterone in the absence of stressors, we were able to protect against or mimic NVC impairment by stress, respectively. These findings support a model in which stress-induced corticosterone-GR signaling causes down-regulation of K_{IR} channel gene expression in the SM, leading to fewer functional channels in the membrane. As a consequence, when a neuronally evoked Ca^{2+} wave arrives at the astrocytic endfoot and stimulates the release of K^+, the SM is no longer equipped to robustly respond, leading to a blunted vasodilatory response during NVC [67]. As we did not examine EC function in this study, we cannot directly rule out a role for EC K_{IR} channels in the stressed phenotype. However, as a blunted K^+ induced dilation remains in PAs after stress [67], this is consistent with the concept that that EC K_{IR} channels are present and possibly suggests that they are even protected from stress, although further work is required to examine this possibility.

The consequences of this impairment remain to be elucidated, although it is reasonable to speculate that the loss of SM K_{IR} channels could have a negative impact on neuronal function if it were to remain chronic. Although we did not directly test amygdalar blood flow during NVC in our model, our results would predict a substantial decrease with stress. Thus, the resulting limited availability of oxygen and glucose could contribute to neuronal injury over time.

Because stress is a factor in many brain disorders, including dementia [46], depression [35], schizophrenia [125], anxiety disorders [109] and multiple sclerosis [82], the loss of SM K_{IR} channels and NVC impairment may also be a contributory factor in such pathologies. The loss of K_{IR} channel function may also occur in other disorders through glucocorticoid-independent mechanisms, such as altered levels of intracellular polyamines, suppression by membrane cholesterol [36] or changes in channel phosphorylation by protein kinase C [124], leading to the disruption of NVC and CBF.

CONCLUSIONS

Our understanding of K^+-induced cerebral hyperemia has advanced greatly over the past four decades. Recent work has established K^+-to-K_{IR} signaling as a rapid and robust mechanism for eliciting vasodilation and increasing blood flow in response to neuronal activity *in vivo*. Central to this mechanism are the $K_{IR}2$ channels positioned in the vascular wall (SM and ECs) adjacent to astrocytic endfeet—key sites of K^+ release during periods of increased neuronal activity. Cerebrovascular K_{IR} 2 channels respond to small increases in extracellular K^+ with a profound increase in conductance, effectively clamping the SM V_m at the new hyperpolarized E_K. This hyperpolarization drives substantial vasodilation, leading to a rapid increase in blood flow. The K_{IR} channel is also sensitive to disruption. Indeed, channel expression and function in the vascular wall can be dramatically altered by stress, and may also be disrupted in a broad range of brain pathologies. Thus, targeting $K_{IR}2$ channels in the vascular wall with the aim of restoring normal hemodynamic function may be a future therapeutic option for such disorders.

Cell-specific genetic ablation models will enable further advances in our understanding of the role of vascular K_{IR} channels in brain in the control of CBF. In the past, the sole pharmacological agent available for testing K_{IR} channel functionality was its pore-blocker, Ba^{2+}; but the recently developed, selective inhibitor ML133 [126] now adds to the pharmacological armamentarium available for functional studies of $K_{IR}2$ channels. Little is known of the nature and roles of ion channels—in particular K_{IR} channels—in native brain capillary ECs or native brain pericytes (although K_{IR} currents have been observed in retinal pericytes [73]); yet, this information may prove to be critical to a full understanding of the regulation of CBF by K^+. The outcome of future studies along these lines may reveal a robust K_{IR} channel-dependent mechanism that allows membrane hyperpolarization to be transmitted from the capillary bed all the way to the brain surface, providing an effective mechanism for engaging the entire vascular tree during NVC.

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Abbreviations used

Ba^{2+}	barium ions
BK	large-conductance calcium-activated potassium channel
Ca^{2+}	calcium ions
CBF	cerebral blood flow
Cl^-	chloride ions
EC	endothelial cell

E_K	potassium equilibrium potential
GR	glucocorticoid receptor
HPA	hypothalamic pituitary adrenal
IK	intermediate-conductance calcium-activated potassium channel
IP_3	inositol 1,4,5-trisphosphate
K^+	potassium ions
K_{IR}	inward rectifier potassium channel
K_V	voltage-dependent potassium channel
Mg^{2+}	magnesium ions
MR	mineralocorticoid receptor
Na^+	sodium ions
NVC	neurovascular coupling
PA	parenchymal arteriole
SM	smooth muscle
VDCC	voltage-dependent calcium channel
V_m	membrane potential

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Box 1. A simple parallel conductance model for the estimation of K^+ conductance versus depolarizing ion conductances

To estimate the K^+ conductance of SM relative to other major ionic conductances (i.e., Na^+, Ca^{2+} and Cl^-), we used the following equation [84,85]:

$$V_m = \frac{(G_K E_K) + (G_C E_C)}{(G_K + G_C)}$$

where G_K is the K^+ conductance of the membrane and G_C represents the combination of other major ionic conductances, with an assumed reversal potential of 0 mV. G_K:G_C was solved for V_m values derived from experimental data under a range of conditions, as noted in the text.

Box 2. Potential contribution of the Na^+/K^+ ATPase to K^+-induced dilations during NVC

Functional Na^+/K^+ ATPase pumps are complexes composed of one of four α-subunit isoforms plus one of three ß-subunits, with the possible addition of an FXYD-domain-containing γ-subunit. The molecular composition of the pump dictates its sensitivity to external K^+ (along with $[Na^+]_i$ and ATP), with α_2-containing ($K_{0.5}$ = 3.6-4.8 mM) and α_3-containing (~5.3-6.2 mM) pumps possessing a lower affinity for K^+ than α1-containing pumps (~2 mM) [4]. With 3 mM basal $[K^+]_o$, Na^+/K^+ ATPases containing any α isoform could theoretically be recruited by K^+ increases. However, in contrast to the robust K_{IR} channel activation that would occur under these circumstances, K^+ evokes only a transient activation of the Na^+/K^+ ATPase, because the rate of pumping is limited by $[Na^+]_i$. Thus, increasing $[K^+]_o$ will increase the rate of pumping, providing a hyperpolarizing current, while $[Na^+]_i$ will simultaneously fall, thereby curtailing the pump current [25]. Accordingly, a plausible role for the Na^+/K^+ ATPase during NVC may be to provide a brief accompanying hyperpolarizing current at the beginning of a K^+-induced dilation that might ensure the robustness and fidelity of the response; however, sustained pump activation will not occur. Consistent with this idea, in rat cerebral arteries, small, transient dilations attributable to Na^+/K^+ ATPase activity occur when stepping $[K^+]_o$ in 1-mM increments from 0 to 4 mM. However, subsequent elevations do not have this effect (but do engage K_{IR} channel activity), suggesting that the native Na^+/K^+ ATPase is saturated at concentrations above 4 mM [75]. Although the molecular identity of Na^+/K^+ ATPase subunit isoforms has not been investigated here, these data suggest that α_1- containing pumps ($K_{0.5}$ = ~2 mM [4] and therefore saturated at 4 mM) are the predominant isoform in these arteries. Whether this is also the case for PAs and the brain capillary endothelium awaits investigation.

Box 3. Roles for IK channels in NVC

Of the members of the Ca^{2+}-activated K^+ channel family, IK channels are the most Ca^{2+}-sensitive, responding to small increases in intracellular Ca^{2+} [43] on the order of those that evoke vasodilation in the neurovascular unit [27]. Thus, these channels may also be activated by Ca^{2+} waves entering the endfoot and could conceivably contribute to K^+ release during NVC. Indeed, blockers of this channel inhibit arteriolar dilation in brain slices and functional hyperemia *in vivo* by 40-50%, suggesting input from IK channels during NVC [68]. While the most straightforward interpretation of these results is that astrocytic IK channels contribute to the neurovascular signaling cascade, this has not yet been unequivocally demonstrated. Thus, it remains possible that IK channels in other cell types may also play a role in NVC. The other major site of IK channel expression in the neurovascular unit is the vascular endothelium, which recent evidence suggests may actively participate in NVC [11]. Indeed, inhibiting IK channels in isolated PAs causes a tonic constriction, suggesting a degree of channel activity under basal conditions. Conversely activation of these channels evokes maximal vasodilation [37], highlighting the fact that endothelial IK channel activity can exert powerful control over vascular diameter. Therefore, if the endothelium were 7 indeed actively engaged during NVC, recruitment of IK channel signaling would be a robust mechanism for influencing the contractile state of the SM.

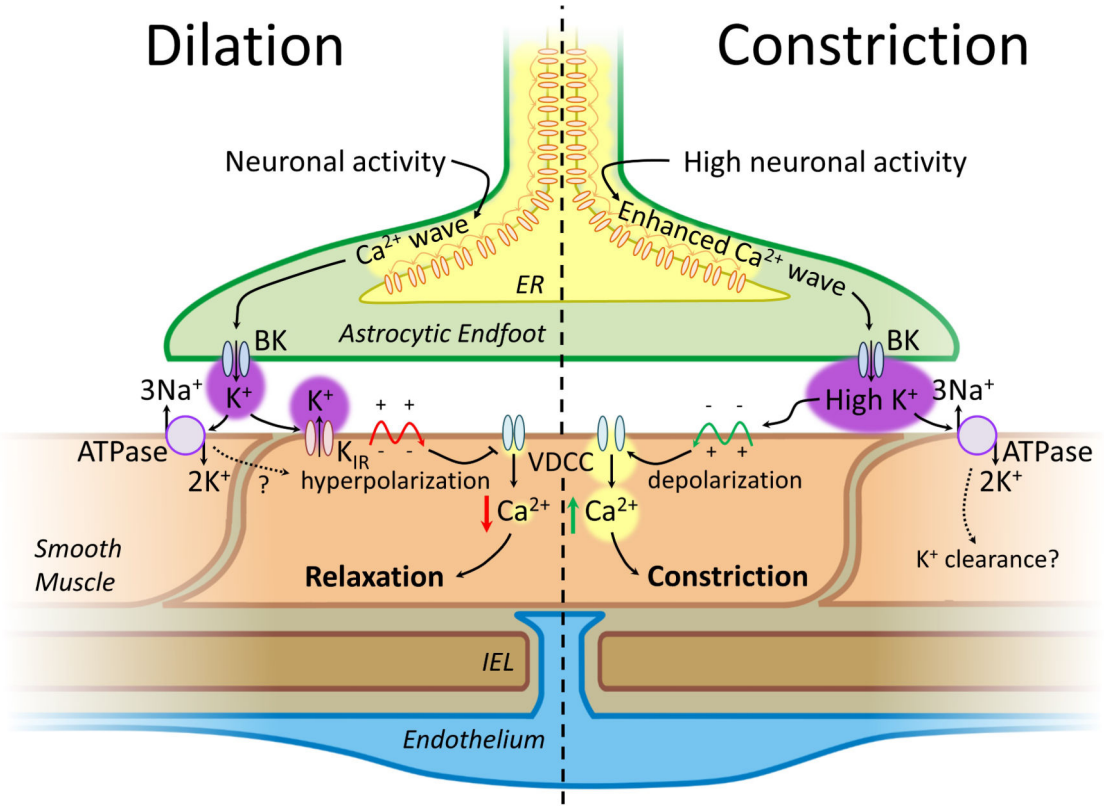

Figure 1.

K^+ signaling mechanisms in NVC. K^+-mediated dilation (left) begins with neuronal activity which is detected by astrocytic processes adjacent to synapses leading to phospholipase C (PLC)-mediated liberation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) from membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) pools, ultimately resulting in an IP_3-mediated Ca^{2+} wave which propagates into astrocytic endfeet enwrapping the cerebral microcirculation [115]. This engages large-conductance calcium (Ca^{2+})-activated K^+ (BK) channels on the endfoot plasma membrane [26,27,97], and their activation leads to an increase in the concentration of K^+ in the extracellular nanospace between the endfoot and SM. The rise in external K^+ activates strong inward-rectifier K^+ (K_{IR}) channels on the smooth muscle, leading to membrane hyperpolarization, closure of voltage-dependent Ca^{2+} channels, vasorelaxation and increased blood flow [26]. During more intense neuronal activity (right), larger Ca^{2+} waves promote the release of higher concentrations of K^+ from the endfoot, leading to membrane depolarization, VDCC activation and constriction [27]. Under both conditions, the Na^+/K^+ ATPase likely contributes to K^+ clearance and may provide a brief accompanying hyperpolarizing current. Adapted from [67] and [27].

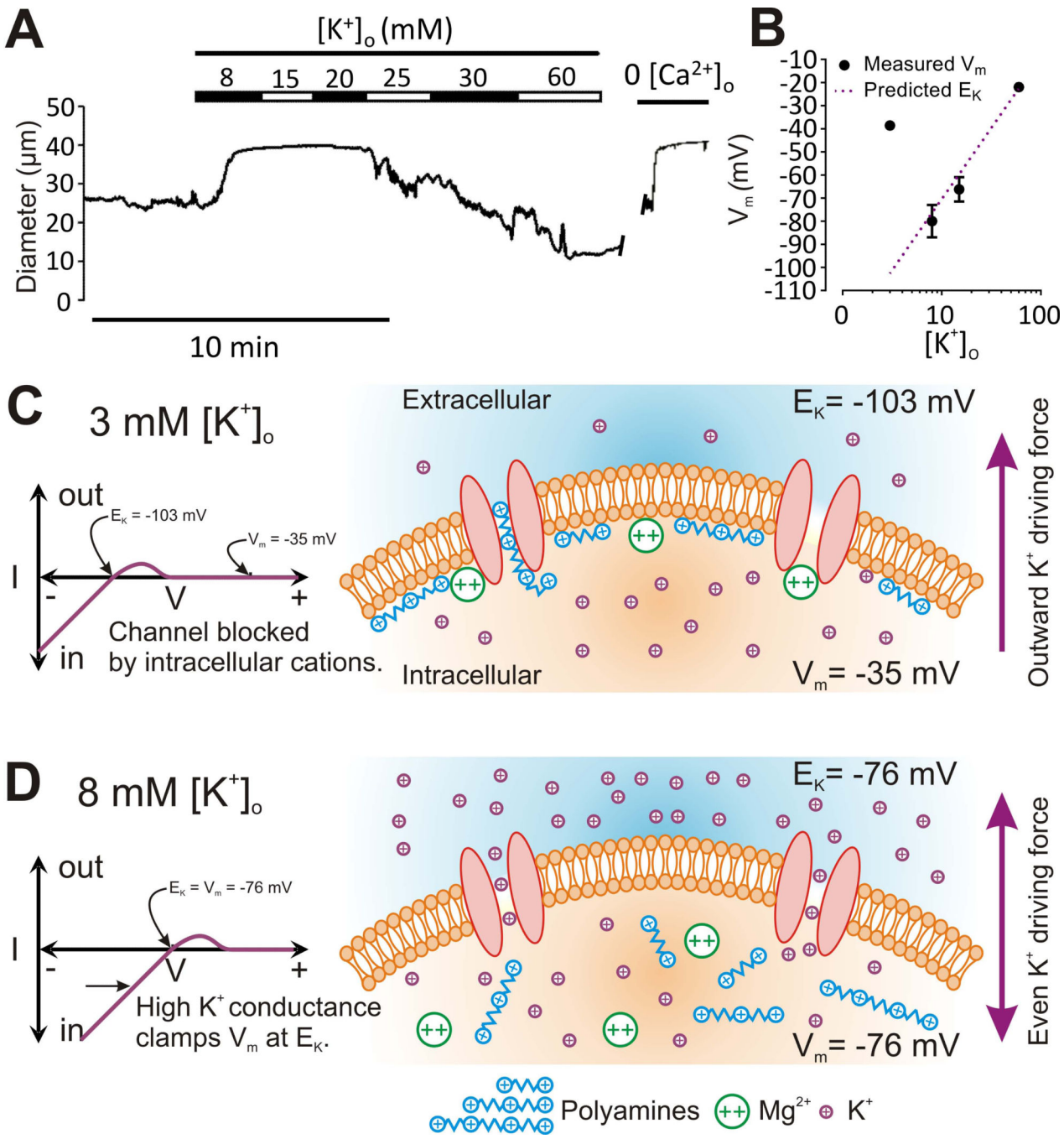

Figure 2.

Inward-rectifier K^+ channels are activated by increases in external K^+. (**A**) *In situ*, raising K^+ to <20 mM causes rapid and substantial vasodilation of pressurized (40 mm Hg) PAs due to K_{IR} channel activation; further increases in K^+ drive membrane depolarization and constriction. Trace from [67]. (**B**) The SM of PAs behaves as a K^+ electrode with increasing concentrations of extracellular K^+. Experimentally observed membrane potential (V_m) data (from [26,37,89]) are shown versus the potassium equilibrium potential (E_K) predicted by the Nernst equation. At 3 mM K^+, V_m is depolarized relative to E_K due to myogenic inward

cation currents. Raising K^+ activates K_{IR} channels, and the resultant K^+ efflux effectively locks V_m at E_K. (**C**) Hypothetical K_{IR} current-voltage relationship (left) and illustration (right) showing that at basal extracellular K^+ (3 mM) the pore of the K_{IR} channel is blocked by Mg^{2+} or large cationic polyamines. Under these conditions (assuming 140 mM $[K^+]_i$), E_K is -103, which is highly negative compared to the resting V_m of SM (approximately -35 to -40 mV) at the physiological intravascular pressure of 40 mmHg. The strong driving force for cation efflux under these conditions leads to blockade of the channel pore by the larger cations, resulting in very little channel activity. (**D**) When $[K^+]_o$ is elevated to 8 mM (e.g., when released from the astrocytic endfoot during NVC), intracellular blockade of the channel is relieved. Channel unblock allows K^+ to exit the cell, driving V_m to the new E_K—where it will remain until the extracellular K^+ is cleared—and leading to substantial vasodilation.

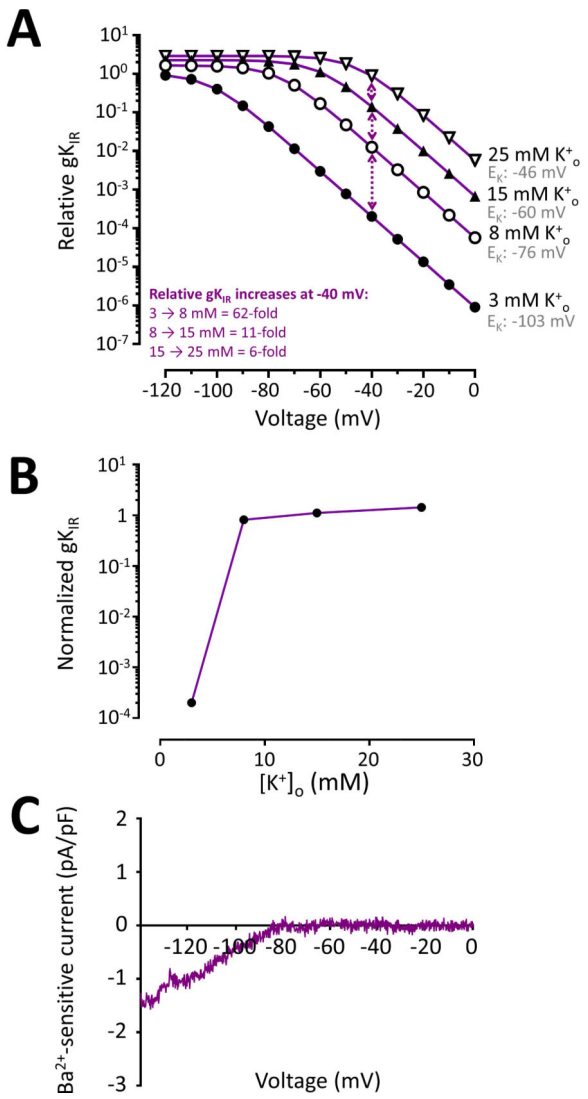

Figure 3.

(**A**) K_{IR} channel conductance over a range of V_m values with increasing concentrations of K^+. With 3 mM $[K^+]_o$ at -40 mV (the approximate resting V_m; dotted arrows), K_{IR} channel conductance is very low. Raising K^+ greatly increases K_{IR} channel activity at a given V_m; channel activity is also increased by membrane hyperpolarization. Data were plotted according to the eq. 1 in the text. (**B**) The relationship between external K^+ concentration and K_{IR} channel conductance based on the K^+- and voltage-dependence of $K_{IR}2$ channels, calculated using eq. 1 (see text), and measured effects of external K^+ on PA SM V_m [26,37]. Elevation of $[K^+]_o$ from 3 mM to 8 mM causes an enormous, near-maximal increase in K_{IR} channel conductance, with further elevations to 15 and 25 mM causing only small subsequent increases in conductance. (**C**) Subtracted 100-μM Ba^{2+}-sensitive currents from a freshly dissociated EC from a rat PA in response to a voltage ramp from -140 to 0 mV indicating the presence of functional $K_{IR}2$ channels in these cells. External K^+ was 6 mM in this experiment.

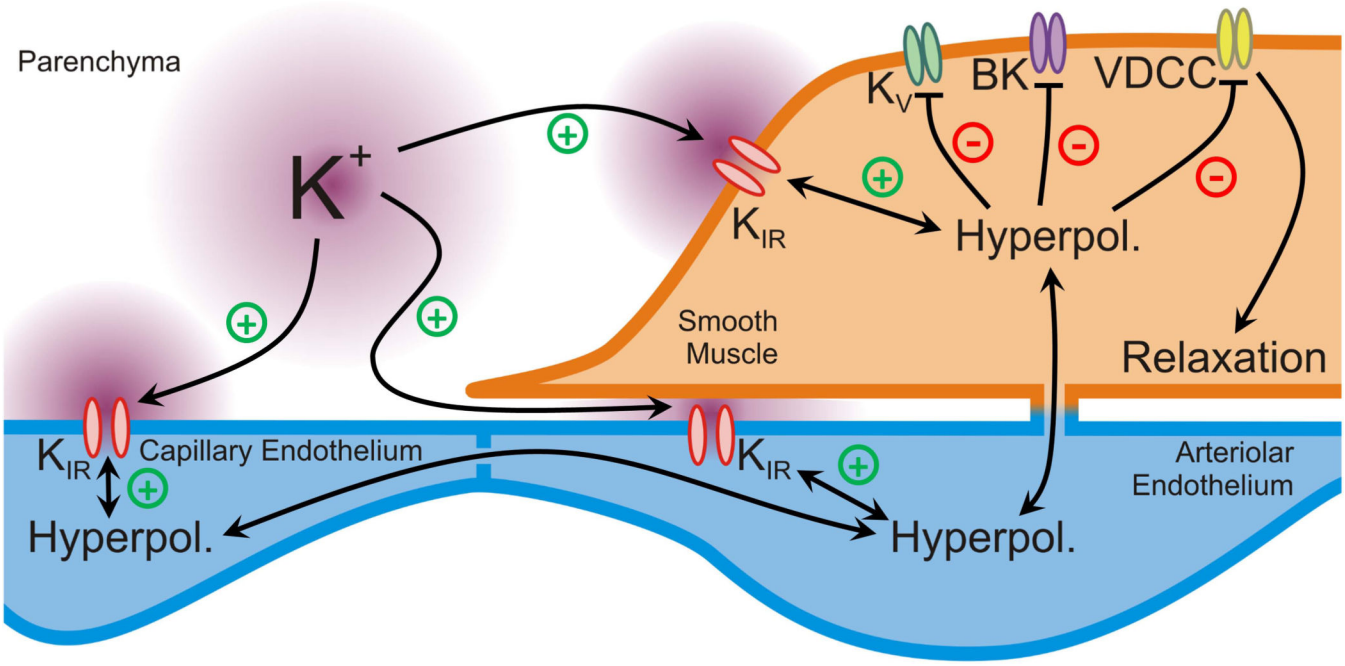

Figure 4.

Proposed scheme for K_{IR} channels as K^+ sensors to control cerebral blood flow. K^+ ions released from astrocytic endfeet and neurons activates $K_{IR}2$ channels present on SM cells and ECs in PAs and (possibly) on capillary ECs. Hyperpolarization caused by K_{IR} engagement may then spread bi-directionally throughout the vascular syncytium, causing further K_{IR} channel activation while at the same time deactivating K_V and BK channels and VDCCs in the SM. This locks the membrane potential at E_K until K^+ is cleared and causes near maximal vasodilation and a substantial increase in cerebral blood flow.

Table 1

K_{IR} channel Ba^{2+} sensitivity, K^+ activation and subunit distribution throughout the neurovascular unit. This table is not exhaustive, as some channels are formed from heteromeric subunit assemblies. Molecular studies for many subunits have not been performed for PAs, although pharmacological data are consistent with the expression of K_{IR} channels that are activated by external K^+ and blocked by <100 μM Ba^{2+} (i.e., $K_{IR}2$ channels). Ba^{2+} blockade is voltage-sensitive, and where data are available we have listed IC_{50} values for negative potentials (note that the highly positive voltages listed for $K_{IR}1.1$ and 6.1 mean that IC_{50} values at more negative potentials will be lower).

Isoform	Rectification	Ba^{2+} IC_{50} (nM)	Voltage (mV)	$[K^+]_o$ (mM)	K^+-activated?*	NVU expression*			Refs
$K_{IR}1.1$	Weak	4300	0	100	Yes	**Neurons**	**Astrocytes**	**Parenchymal arteriole**	[3,12,23,48,70,110]
						✓	n.d.	n.d	
$K_{IR}2.1$	Strong	3.2	−100	60	Yes	✓	✓	✓	[17,26,39,47,65,67,107,108]
$K_{IR}2.2$	Strong	0.5	−100	60	Yes	✓	✓	✓	[17,26,39,47,65,67,107,108]
$K_{IR}2.3$	Strong	10.3	−100	60	Yes	✓	✓	n.d.	[17,39,47,65,107,108]
$K_{IR}2.4$	Strong	390	−80	96	n.d	✓ - cranial nerve nuclei	**x**	n.d.	[17,65,123]
$K_{IR}3.1$	Strong	14 *(+3.4)* [a]	−130	90	No	✓	✓	n.d.	[9,47,59,80]
$K_{IR}3.2$	Strong		n.d. [b]		No	✓	**x**	n.d.	[9,47,80]
$K_{IR}3.3$	Strong		n.d.		No	✓	**x**	n.d.	[9,47,80]
K|r3.4	Strong	92	−60	100	No	✓	**x**	n.d.	[9,45,47,80]
K|r4.1	Intermediate	7.1	−130	50	No	**x**	✓	n.d.	[9,22,38,108]
$K_{IR}4.2$	Intermediate		n.d. [b]		Yes	**x**	**x**	n.d.	[38,94]
$K_{IR}5.1$	Strong		n.d. [b]		n.d	✓ - cell culture	✓	n.d.	[38,108,121]
$K_{IR}6.1$	Weak	89.3	+60	5.4	No	**x**	✓	**x**	[16,41,122]
$K_{IR}6.2$	Weak	29.3 *(+SUR1)*	−102	5.6	No	✓	**x**	**x**	[16,32,81,117,118,122]
K|r7.1	Weak	1100-1900	−110	150	No	✓	✓	n.d	[19,55,92]

✓ = expression confirmed, x = lack of expression observed. n.d. = not investigated to date, to the authors' knowledge.

*K^+-activation is defined as an increase in channel conductance in the presence of elevated $[K^+]_o$ at a constant electrical driving force.

#All brain regions considered, where it is known that expression is highly restricted this is noted. No distinction is made between the smooth muscle and endothelial expression of K_{IR} subunits in parenchymal arterioles due to the current lack of evidence.

[a] $K_{IR}3.1$ homomers are non-functional, association with other $K_{IR}3.x$ subunits is required for functional channels [39].

[b] $K_{IR}3.2$ [114], 4.2 [94] and 5.1 [121] homomers are all inhibited by Ba^{2+} ions, but IC_{50} data are currently lacking.