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Boosting the signal: Endothelial inward rectifier K+ channels

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Abstract

Endothelial cells express a diverse array of ion channels including members of the strong inward rectifier family composed of $K_{IR}2$ subunits. These two-membrane-spanning-domain channels are modulated by their lipid environment, and exist in macromolecular signaling complexes with receptors, protein kinases and other ion channels. Inward rectifier K^+ channels (K_{IR}) currents display a region of negative slope conductance at membrane potentials positive to the K^+ equilibrium potential that allows outward current through the channels to be activated by membrane hyperpolarization, permitting K_{IR} to amplify hyperpolarization induced by other K^+ channels and ion transporters. Increases in extracellular K^+ concentration activate K_{IR} allowing them to sense extracellular K^+ concentration and transduce this change into membrane hyperpolarization. These properties position K_{IR} to participate in the mechanism of action of hyperpolarizing vasodilators and contribute to cell-cell conduction of hyperpolarization along the wall of microvessels. Expression of K_{IR} in capillaries in electrically active tissues may allow K_{IR} to sense extracellular K^+, contributing to functional hyperemia. Understanding the regulation of expression and function of microvascular endothelial K_{IR} will improve our understanding of the control of blood flow in the microcirculation in health and disease and may provide new targets for development of therapeutics in the future.

Keywords

Potassium channels; KIR2.1; KCNJ2; endothelial cells; microcirculation; arterioles; hyperpolarization; vasodilation; functional hyperemia

Introduction

Ion channels in the membranes of endothelial cells contribute to all aspects of the function of these cells [57]. Calcium influx and release through members of these membrane proteins determines intracellular Ca^{2+} concentration. This second messenger importantly regulates a number of endothelial cell processes related to the regulation of vascular function including endothelial cell production of autacoids such as NO, prostaglandins and epoxides of arachidonic acid (EETs) [31], as well as the activity of Ca^{2+}-dependent ion channels [31]. Intracellular Ca^{2+} also regulates endothelial cell barrier function [39,64], gene expression

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Conflicts:

None

[82,93], and proliferation [83,87]. Cell volume regulation also depends on the activity of plasma membrane ion channels [52]. Importantly, ion channels determine and regulate endothelial cell membrane potential. Modulation of this separation of charge, through changes in ion channel activity, functions as an important signal for endothelial cell-endothelial cell communication and endothelial cell-smooth muscle cell communication, because these cells are electrically coupled by gap junctions [23]. Membrane potential also affects the electrochemical gradient for movement of all ions across the plasma membrane, potentially modulating Ca^{2+} influx through endothelial cell Ca^{2+} channels [6,46,47,51,87], although this topic remains controversial [16,26,77,80,107]. Thus, the physiology and pathophysiology of endothelial cells depends heavily on the expression and function of ion channels.

As in all cells [50], K^+ channels play a central role in setting and modulating membrane potential of endothelial cells. At the resting membrane potential of microvascular endothelial cells in intact pressurized vessels (~30–40 mV [32,102,103,116]), with physiological ion gradients (3–5 mM K^+ in the extracellular fluid, ~140 mM K^+ in the cytosol), the electrochemical gradient for diffusion of K^+ across endothelial cell membranes is outward. Thus, K^+ will flow out of cells when K^+ channels open, producing membrane hyperpolarization. Closure of open K^+ channels will have the opposite effect, membrane depolarization.

Endothelial cells may express four or more classes of K^+ channels [57]. The expression and function of strong inward rectifier K^+ ($K_{IR}2.X$) channels will be the focus of this review. Because ion channel expression and function change dramatically during cell culture [7,8,14,87,99], emphasis will be placed on data originating from intact microvessels and freshly isolated endothelial cells. Earlier literature may be accessed from prior reviews [87–89].

Structure of K_{IR} channels

Inward rectifier K^+ channels in the $K_{IR}2.1 – 2.4$ family represent the products of four genes (loci: KCNJ2, KCNJ12, KCNJ4, KCNJ14, respectively) in the 15 member family of K_{IR} channels [49]. These channels consist of a tetramer of pore-forming α-subunits [49,86]. Each α-subunit has two membrane spanning domains (M1 and M2, Figure 1) with intracellular carboxy and amino termini [49,86]. A P-loop links the membrane spanning domains (Figure 1). The P-loop and M2 form the ion-conducting pore [49,86]. Conserved amino acids in the P-loop (T142-I143-G144-Y145-G146-F147-R148 in $K_{IR}2.1$) comprise the channels' K^+ ion selectivity filter [49,86,108] (Figure 1). Voltage-dependent block of outward K^+ currents by intracellular polyamines [34,37,73] and Mg^{2+} [78,112] causes the distinguishing K_{IR} channel current inward rectification (Figure 2) [75]. Magnesium ions and polyamines interact with basic residues in M2 (D172 in $K_{IR}2.1$, D173 in $K_{IR}2.2$) and in the carboxy terminus (E224, E299 and D255 in $K_{IR}2.1$; E225, E300 and D256 in $K_{IR}2.2$) to produce voltage-dependent block of outward current flow at membrane potentials positive to the K^+ equilibrium potential [75,108] (Figures 1 and 2). Based on studies of the crystal structure of chicken $K_{IR}2.2$ [108], K^+ ions also will interact with these same residues,

explaining why inward rectification shifts to more positive membrane potentials as extracellular K^+ concentration is elevated.

Negative-slope-conductance and the K_{IR} channel current-voltage relationship

Although K_{IR} channels are named for the inward rectification of current, it is the small outward "hump" in the current-voltage relationship (i.e., the region of negative slope conductance [29,98], Figure 2) that is present between the K^+ equilibrium potential and resting membrane potential (~−40 to −30 mV [32,102,103,116]) of endothelial cells that is important for the physiological function of K_{IR} channels. Membrane hyperpolarization from the resting potential will increase outward K_{IR} channel current, amplifying the original hyperpolarization [12,55,58,72,104,106] (Figure 2). Microvascular endothelial cells have high membrane resistance at resting membrane potential (2–100 GΩ; [63,106,114]). Thus, activation of only a few K_{IR} channels can effectively modulate endothelial cell membrane potential; in mouse mesenteric arteries, there are on the order of only 144 functional K_{IR} channels per endothelial cell that significantly contribute to regulation of vessel function [106]. In K_{IR} channels containing $K_{IR}2.2$ subunits, steady-state membrane hyperpolarization decreases the channel's open-state probability in a time-dependent fashion, a feature unique to this $K_{IR}2$ family member [49].

Membrane lipids modulate K_{IR} channels

The lipid environment around K_{IR} channels strongly regulates their function. Phosphatidylinositol 4,5-bisphosphate (PIP_2) activates $K_{IR}2$ channels by interacting with basic amino acids in M2 (K177, K178, R179 for $K_{IR}2.1$) and in the cytoplasmic tails of the channels (H53, R67, K187, K188, R189, K219, R228, and R312 for $K_{IR}2.1$) [49] (Figure 1). This interaction opens the potential for K_{IR} channel modulation by phospholipase-mediated hydrolysis of PIP_2 and its synthesis via kinases [49]. It is possible that activation of $G_{q/11}$-coupled receptors and the subsequent activation of phospholipase Cβ-mediated PIP_2 hydrolysis could inhibit $K_{IR}2.2$ and 2.3, because they have a relatively low PIP_2 affinity [28]. In contrast, $K_{IR}2.1$ affinity for PIP_2 binding is sufficiently high that phospholipase-induced PIP_2 hydrolysis may not remove activator PIP_2 from these channels [28]. Angiotensin II, vasopressin and GTPγS in the patch pipette inhibit K_{IR} currents in porcine cerebral capillary endothelial cells, in vitro [54]. These data suggest that microvascular endothelial cell K_{IR} channels may be physiologically modulated by activation of G-protein coupled receptors. However, it is not known if these effects were due to stimulation of PIP_2 hydrolysis.

Membrane cholesterol also modulates K_{IR} channel function. Increases in membrane cholesterol inhibit, while decreases in membrane cholesterol stimulate currents through $K_{IR}2$ channels in macrovascular endothelial cells [33,36,95–97] and in heterologous expression systems [94]. The hinge region of M1 (L85, V93, S95 in $K_{IR}2.1$) and M2 (I166, V167, I175, M183 in $K_{IR}2.1$) and the region between M1 and the cytosolic domains (L69, A70, V77 in $K_{IR}2.1$) are the sites where cholesterol interacts with the channels [96] (Figure 1).

K_{IR} channels reside in signaling complexes

$K_{IR}2$ channels reside in cholesterol-rich lipid rafts [94]. Loss of cholesterol results in channel movement out of these microdomains [109]. The caveolar protein, Caveolin-1, also inhibits $K_{IR}2$ channel function [45]. Inward rectifier K^+ channels exist in membrane signaling microdomains in cardiac myocytes [67,117], where they interact with receptors, other ion channels, protein kinases, etc. [9]. Heterologously expressed $K_{IR}2.1$ channels interact with AKAP79 that targets these channels to protein complexes, which include PKA, calcineurin and other signaling proteins [22]. The carboxy terminus of $K_{IR}2.1$ contains the PDZ domain recognition sequence, (E424-S425-E426-I427; Figure 1), that interacts with postsynaptic density protein (PSD) 95 [84]. This adaptor protein, which is present in endothelial cells [110], interacts with AKAPS, nitric oxide synthase and other signaling proteins [11,110]. Thus, $K_{IR}2.1$ channels are likely located in signaling complexes in endothelial cells, providing significant potential for modulation of channel function, although this has not been explored in microvascular endothelial cells.

Protein kinases modulate K_{IR} channels

Studies in other systems suggest that there is considerable potential for K_{IR} channel regulation by kinases. There is a protein kinase A (PKA) consensus sequence in the C-terminus of $K_{IR}2.1$ (S425 in the PDZ domain in $K_{IR}2.1$; Figure 1) [119]. However, the effects of phosphorylation of this residue on K_{IR} channel function is far from clear. Phosphorylation of S425 by PKA inhibits inward K_{IR} currents at potentials more negative than the K^+ equilibrium potential [113,118], but increases outward currents at more positive potentials [113] when the channels are expressed in COS cells. Activation of PKA inhibits native K_{IR} channels in the heart [113]. However, in contrast, inward $K_{IR}2.1$ channels expressed in Xenopus oocytes are activated by PKA [35], whereas native $K_{IR}2.1$ channels in cultured pulmonary endothelial cells are unaffected by activation of PKA [60]. Thus, how K_{IR} channels are modulated by PKA depends on the particular cell type in which the channels are expressed, and, most likely, also on the composition and configuration of the signaling complexes in which they are located. Regulation of microvascular endothelial cell K_{IR} channels by PKA has not been studied.

There is also a tyrosine kinase consensus sequence in $K_{IR}2.1$ at Y242 [118]. However, as with PKA, how phosphorylation of this site affects $K_{IR}2.1$ channel activity remains unclear with evidence for both decreased [118] and increased [123] activity reported. In addition, the tyrosine kinase Src inhibits currents through channels containing $K_{IR}2.2$ independent from protein kinase C (PKC), but does not inhibit homomeric $K_{IR}2.1$ channels [125]. In contrast, PKC mediates α_{1A}-adrenergic receptor-induced inhibition of $K_{IR}2.3$ channels [125]. Also, $G\beta\gamma$-subunits inhibit $K_{IR}2.3$-containing channels [17].

Channels containing $K_{IR}2.2$ or 2.3 are inhibited by PKC [28,126], with no effect on $K_{IR}2.1$ channels in mammalian expression systems [28,48], or in cultured endothelial cells [60]. However, activation of β_3-adrenoreceptors activates $K_{IR}2.1$ channels through a mechanism involving PKC in Xenopus oocytes [100]. In vascular smooth muscle cells that express

$K_{IR}2.1$ and $K_{IR}2.2$, hypoosmotic-induced cell swelling inhibits K_{IR} channels through a mechanism involving PKC [120].

There is also evidence for modulation of the expression and function of K_{IR} channels by other protein kinases. Calmodulin-dependent protein kinase II increases protein expression and activates currents through $K_{IR}2.1$ channels in cultured macrovascular endothelial cells [92]. Surface expression of $K_{IR}2.1$ is decreased via AMP kinase-dependent phosphorylation of the ubiquitin ligase, Nedd4-2 [3].

Other modulators of K_{IR} channels

Intracellular acidosis inhibits K_{IR} channels through effects on residues in the N-terminal domain of the channel [49]. In taste buds, acid-induced closure of $K_{IR}2.1$ contributes to acid-induced depolarization that is involved with the transduction of sour taste [121]. Whether pH modulates the function of endothelial K_{IR} channels has not been studied.

Fluid shear stress activates $K_{IR}2.1$ channels in macrovascular endothelial cells [53,68,90] and in heterologous expression systems through a mechanism that may involve a tyrosine kinase [53]. Whether a similar mechanism functions in microvascular endothelial cells remains to be established, but would be predicted to produce endothelial cell hyperpolarization and vasodilation. Shear stress also modulates transcription of K_{IR} channels in cultured human coronary endothelial cells, with $K_{IR}2.2$, 2.3 and 2.4 being upregulated by increased shear stress [61]. It is not known if altered shear stress produces similar changes in native microvascular endothelial cells. The impact of sheer stress-induced changes in endothelial K_{IR} channel expression on microvascular endothelial cell function also has not been established. However, such altered K_{IR} channel isoform expression would be predicted to modify the modulation of K_{IR} channel function by protein kinases, for example, potentially depressing or augmenting K_{IR} channel-mediated changes in endothelial cell membrane potential.

Increases in nitric oxide result in S-nitrosylation of a cysteine residue in the amino terminus of $K_{IR}2.1$ (C76) and an increase in K_{IR} channel activity in the heart and in heterologously expressed channels [40]. This may account for the increase in K_{IR} channel activity induced by NO in vascular smooth muscle K_{IR} channels [101].

K_{IR} channel pharmacology

Extracellular Ba^{2+} potently blocks K_{IR} channels in a voltage-dependent fashion [43]: at physiological membrane potentials (−30 to −40 mV) the K_d = 19–30 μM for $K_{IR}2.1$ [2,69]; for $K_{IR}2.2$, the K_d = 9 μM [69], and for $K_{IR}2.3$, the K_d = 70 μM [69]. Barium ions also block K_{ATP} channels (IC_{50} = 100 μM [10]). Barium block of $K_{IR}2.1$ channels involves interactions with two residues, one in the outer vestibule of the channel (E125) and one just before the selectivity filter (T141) (Figure 1) [2]. In addition to Ba^{2+}, extracellular Cs^+ ions block K_{IR} channels [49], as they do all K^+ channels.

Currents through $K_{IR}2$ channels are inhibited by ML133 (IC_{50} = 1.9 μM for K_{IR} 2.1; 2.3 μM for $K_{IR}2.2$ and 4 μM for $K_{IR}2.3$) [115]. This compound blocks K_{IR} channel currents in

endothelial cells from rat middle cerebral arteries [63], and in rat mesenteric artery endothelial cells [106]. Residues in M2 (D172 and I176 in $K_{IR}2.1$) are involved in the mechanism of action of ML133 [115]. This compound also blocks K_{ATP} channels composed of KIR6.2 subunits (IC_{50} = 7.7 μM) [115], channels that are likely expressed in some microvascular endothelial cells [38]. Inward rectifier K^+ channels are also non-specifically blocked by a number of drugs including: antihistamines such as diphenhydramine and mepyramine; the antimalarial, choloroquin; and the class Ia antiarrhythmic, quinidine [49].

Potassium ions are an important physiological agonist of K_{IR} channels [72], increasing K_{IR} channel conductance proportional to the square root of the extracellular K^+ concentration [44,66,72,74,76,98]. Extracellular K^+ interacts with a residue near the K^+ selectivity filter (R148 in $K_{IR}2.1$) [49]. Furthermore, increases in extracellular K^+ concentration, by moving the K^+ equilibrium potential to more positive potentials, will move the negative slope conductance region to more depolarized potentials (Figure 2). This results from interactions of K^+ ions with the negatively charged residues that bind Mg^{2+} and polyamines and which are responsible for inward rectification [108]. The K^+-induced shift in the current-voltage relationship will recruit outward current through the channels, also promoting membrane hyperpolarization (Figure 2). Thus, as with vascular smooth muscle K_{IR} channels, endothelial cell K_{IR} channels can serve as sensitive sensors of extracellular K^+ concentration, transducing small changes (e.g., 3–5 mM to 8–15 mM K^+) into membrane hyperpolarization [72].

The antiarrhythmic drug, flecainide activates cardiac $K_{IR}2.1$ channels by interacting with a cysteine residue in the carboxy terminus of the channel (C311 in $K_{IR}2.1$; Figure 1), a residue that is not found in $K_{IR}2.2$ or 2.3 [13]. This activation results from decreased affinity of the channels for polyamines, like spermine, through an allosteric mechanism [13]. Other drugs, such as the antiarrhythmic, propafenone and the β-adrenergic receptor antagonist, timolol may activate $K_{IR}2.1$ channels through a similar mechanism [41].

Expression of K_{IR} channels in microvascular endothelial cells

Early studies of cultured cells suggested that microvascular endothelial cells might not express K_{IR} channels [1,87]. However, more recent investigation of freshly isolated microvascular endothelial cells have clearly shown Ba^{2+}-sensitive K_{IR} channel currents [18,21,54,55,63,70,72,114] (Figure 2). Barium also blocks endothelial cell K_{IR} currents in rat superior mesenteric arteries, in situ [15].

Capillary endothelial cells reportedly express mRNA for $K_{IR}2.1$, 2.2 and possibly 2.3 [69,81]. Rat cremaster muscle arteriolar endothelial cells express protein for $K_{IR}2.1$, but not $K_{IR}2.2$ (Figure 3). Similar results were observed in mouse arteriolar endothelial cells [57]. Inward rectifier K^+ currents are blocked by the selective $K_{IR}2$ inhibitor, ML 133 [115], in rat middle cerebral artery [63] and mouse 3rd-order mesenteric artery [106] endothelial cells. Knockout of endothelial cell expression of $K_{IR}2.1$ suppresses Ba^{2+}-sensitive K_{IR} currents in mouse 3rd-order mesenteric artery [106] and brain capillary [70] endothelial cells. Taken together, these data indicate that $K_{IR}2.1$ is an essential subunit of the native endothelial K_{IR}

channels expressed in rats and mice. The presence and functions of other $K_{IR}2$ channel subunits in microvascular endothelial cells remains to be established.

Functions of endothelial K_{IR} channels

Vascular smooth muscle K_{IR} channels amplify hyperpolarization induced by the activation of other K^+ channels, and transduce small increases in extracellular K^+ into cell hyperpolarization [59,62,72,85,104,122]. However, the function of endothelial K_{IR} channels is not as clear, based solely on studies utilizing rat and mouse mesenteric arteries where functional K_{IR} channels are confined to the endothelium [18,25,27,42,104,106,107]. Barium attenuates endothelium-dependent hyperpolarization and conducted vasodilation in rat mesenteric arteries in normotensive rats [42]. In addition, Ba^{2+} inhibits K^+-induced hyperpolarization and vasodilation in these arteries [25,42]. Studies in mouse 3rd-order mesenteric arteries demonstrate that endothelial cell K_{IR} channels amplify the effects of drugs that act via endothelial cell hyperpolarization [106]. Endothelial K_{IR} channels can also mediate K^+-induced vasodilation in these vessels [106]. Importantly, Sonkusare et al. [106] showed that endothelial cell-selective knockout of $K_{IR}2.1$ abolished these effects, demonstrating the crucial role for $K_{IR}2.1$ in murine resistance artery endothelial cells as end-stage boosters of membrane hyperpolarization. These studies are consistent with a significant role for endothelial cell K_{IR} channels in the vasoreactivity of small mesenteric arteries.

In contrast, other studies in rat mesenteric arteries do not support a major role for endothelial cell K_{IR} channels. Takano, et al. [107] found that Ba^{2+} had no effect on conducted vasodilation induced by agents that hyperpolarized either endothelial cells or smooth muscle cells. Barium also had no effect on conducted dilation or K^+-induced vasodilation in mesenteric arteries, in contrast to cerebral and coronary arteries that show robust expression of smooth muscle K_{IR} channels [104]. Thus, there is evidence both for and against a significant role for endothelial cell K_{IR} channels.

The level of agonist-induced activation of the smooth muscle could be one possible explanation that might reconcile the opposing views of the role-played by endothelial K_{IR} channels. Increased agonist-induced activation of smooth muscle has been shown to inhibit Ba^{2+}-sensitive K^+-induced dilation of rat mesenteric arteries [25]. However, Goto et al. [42] (who observed a role for endothelial K_{IR} channels), Takano et al. [107] and Smith et al. [104] (who both found no role for endothelial K_{IR} channels) used similar concentrations of phenylephrine (~1μM) to pre-constrict their rat mesenteric arteries, and yet observed disparate results. Thus, there does not appear to be a simple reason that can reconcile these opposing views.

Nonetheless, the disparate findings outlined in the preceding paragraphs do suggest that endothelial cell K_{IR} channel function may be modulated, allowing fine-tuning of vascular function to maintain homeostasis, as well as dysregulation of these channels in disease states. Consistent with this latter proposition, endothelium-dependent hyperpolarization and conducted vasodilation are depressed in mesenteric arteries from spontaneously hypertensive rats, and Ba^{2+} is without effect in these arteries suggesting that hypertension decreases

endothelial cell K_{IR} channel function [42]. The mechanism responsible for the hypertension-induced K_{IR} channel dysfunction has not been determined.

Ischemia [91] and stress [71] also depress K_{IR} channel function in cerebral vascular smooth muscle cells. The stress-induced suppression of K_{IR} channel function results from glucocorticoid-mediated decreases in $K_{IR}2.1$ channel transcript levels and reduced expression of functional K_{IR} channels in the smooth muscle cells [71]. Diabetes alters retinal pericyte K_{IR} channel function through increased polyamine synthesis and increased inward rectification [79]. Mutations in *KCNJ2* result in a multi-system disorder (Andersen-Tawil syndrome) that includes cardiac arrhythmias, periodic paralysis and dysmorphogenesis related to decreased K_{IR} channel function [111]. These data support the hypothesis that K_{IR} channels are targets for modulation during disease states, and that mutations that lead to altered K_{IR} channel function produce significant pathologies. The impact of diseases and mutations on microvascular endothelial cell K_{IR} channel function remains to be established.

In arterioles, endothelial cell K_{IR} channels also may be positioned to sense changes in extracellular K^+ concentration in the restricted space between the endothelium and overlying smooth muscle cells, as has been hypothesized for smooth muscle K_{IR} channels [30] (Figure 4). As noted above, in mesenteric arteries, endothelial K_{IR} channels have been shown to mediate dilation induced by elevated extracellular K^+ [25,42]. Thus, local increases in K^+ produced by the activity of other endothelial cell or smooth muscle cell K^+ channels also may activate endothelial cell K_{IR} channels (Figure 4). This provides another mechanism (in addition to activation by hyperpolarization), to amplify the activity of other K^+ channels, in either the endothelium or smooth muscle layers, and could contribute to the regulation of endothelial cell membrane potential and other microvascular functions.

Capillary endothelial cell K_{IR} channels

Cells in excitable tissues like brain, heart and skeletal muscle rely on the opening of K^+ channels and efflux of K^+ ions to repolarize the cells during each action potential. During periods of increased activity, this results in accumulation of K^+ in the interstitium that can routinely be on the order of 8–10 mM, more than sufficient to activate K_{IR} channels [72]. Expression of K_{IR} channels in capillary endothelial cells opens the exciting possibility that capillaries may be ideally positioned to sense these increases in extracellular K^+, transducing this signal into hyperpolarization that could be transmitted, via gap junctions, to upstream arterioles to elicit vasodilation and increases in capillary blood flow to match the increased activity of the tissue (functional hyperemia) [72].

This hypothesis has recently been tested in mouse cerebral microcirculation. Using a novel ex vivo system composed of pressurized cerebral parenchymal arterioles with attached capillaries, Dabertrand and colleagues [21] have shown that microapplication of 10 mM K^+ onto the capillaries results in vasodilation of the upstream arterioles that can be blocked by Ba^{2+} and which is absent in vessels isolated from endothelial cell $K_{IR}2.1^{-/-}$ mice. Similarly, Longden et al, [70] demonstrated that application of 10 mM K^+ to brain capillaries, in vivo, results in upstream arteriolar dilation and increased red blood cell flux through the capillaries. Whether similar mechanisms operate in heart and skeletal muscle remains to be

established. Coronary capillary endothelial cells display robust K_{IR} channel currents and express mRNA for K_{IR} channels [69,114] suggesting a potential role in the heart. In skeletal muscle, K_{IR} channels have been implicated in both the rapid-onset of vasodilation and steady-state increases in blood flow associated with muscle contraction [4,19,20]. However, the location of the K_{IR} channels (endothelium, smooth muscle, skeletal muscle fibers, etc.) that mediate skeletal muscle functional hyperemia is not known. While, conduction of signals from capillaries to arterioles has long been proposed [5,24,65,105,124], and conduction of electrical activity from capillaries to arterioles has been observed [5], Dabertrand and colleagues' study is the first to define the ion channel that mediates such a response and to exclude other cell types for initiation of signal transmission from capillaries to arterioles.

Conclusions

While K_{IR} channel expression in endothelial cells has been known for some time, the physiological function of these ion channels is just beginning to be unraveled. Current evidence suggests that these K^+ channels serve to boost hyperpolarization induced by opening of other K^+ channels or transporters (such as the Na^+/K^+ ATPase), and to transduce changes in extracellular K^+ concentration into endothelial cell membrane hyperpolarization. The hyperpolarization booster function of K_{IR} channels will be particularly effective for small hyperpolarizations due to opening of only few K^+ channels, for example. Large hyperpolarizations, that drive the membrane potential close to the K^+ equilibrium potential on their own, will be little affected by the recruitment of current through K_{IR} channels, because of the shape of the K_{IR} channel current-voltage relationship (Figure 2).

These functions position endothelial cell K_{IR} channels (along with their smooth muscle counterparts) to be involved in the mechanism of action of all hyperpolarizing vasodilators, with modulation of K_{IR} channel function providing an additional mechanism to fine tune the reactivity of arterioles in the microcirculation. Endothelial K_{IR} channels also may contribute to conduction of hyperpolarization along the wall of microvessels, participating in the coordination of local blood flow regulation. The expression of K_{IR} channels in capillary endothelial cells positions these ion channels to play a major role in sensing extracellular K^+ and contributing to functional hyperemia in electrically active tissues such as the brain, heart and skeletal muscle.

How the expression and function of native microvascular endothelial cell K_{IR} channels is modulated during cell signaling processes in health and disease remains to be established. However, based on studies in other systems, it seems likely that microvascular endothelial cell K_{IR} channels also may be modulated in diseases like hypertension, diabetes, and stress. It also is likely that endothelial K_{IR} channels reside in macromolecular signaling complexes. However, knowledge of the protein partners with which these channels interact is simply lacking. At a more fundamental level, our knowledge and understanding of the expression and function of the full complement of ion channels in microvascular endothelial cells, smooth muscle cells and pericytes remains in its infancy. Molecular approaches need to be applied to define the complete ion channel transcriptome and proteome in native, not cultured microvascular cells, in multiple vascular beds. For example, we know much about

the function of endothelial K_{IR} channels from the study of mesenteric arteries. However, how these findings apply to other vascular beds, is simply not known. Functional assays of ion channel function using patch-clamp approaches need to be applied so that the actual currents, in the cells of interest, which are responsible for a physiological response, are identified and characterized to verify what is often inferred from the application of pharmacology to complex systems. This will mean developing new, or adapting old techniques to isolate microvascular cells from the particular vessels of interest, including capillaries. The use of cell specific and hopefully, conditional knockouts of K_{IR} channels should help delineate the function of K_{IR} channels in specific microvascular cells. However, given that these ion channels are likely part of much larger signaling complexes, such knockouts have the potential to disrupt more than just the function of a single ion channel. Thus, multiple approaches, including careful pharmacology, must be applied, rather than relying on any single strategy. Understanding the control of expression and function of microvascular endothelial cell K_{IR} channels will improve our understanding of local blood flow control in health and disease, and may, in the future, provide new targets for the development of therapeutics directed at these important ion channels.

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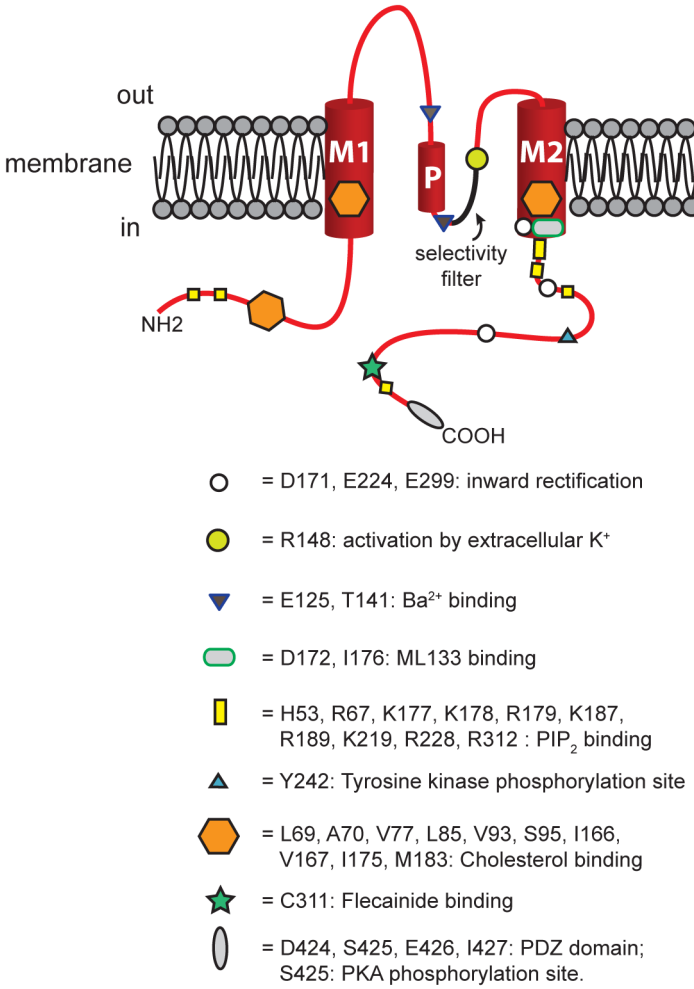

Figure 1.

Structure of $K_{IR}2$ channels. Shown is a schematic representation of one $K_{IR}2$ channel subunit positioned in the lipid bilayer of a cell membrane as shown. These channels have two membrane spanning helical domains, denoted M1 and M2 connected by a P-loop that contains a helical domain (P in the drawing). The channel's pore is formed by the P-loop and M2, with the selectivity filter sequence highlighted in the drawing. Cytosolic amino (NH_2) and carboxy (COOH) termini are also shown. Approximate locations of sites where regulatory molecules interact with the channel protein are shown as indicated. See text for references and more information.

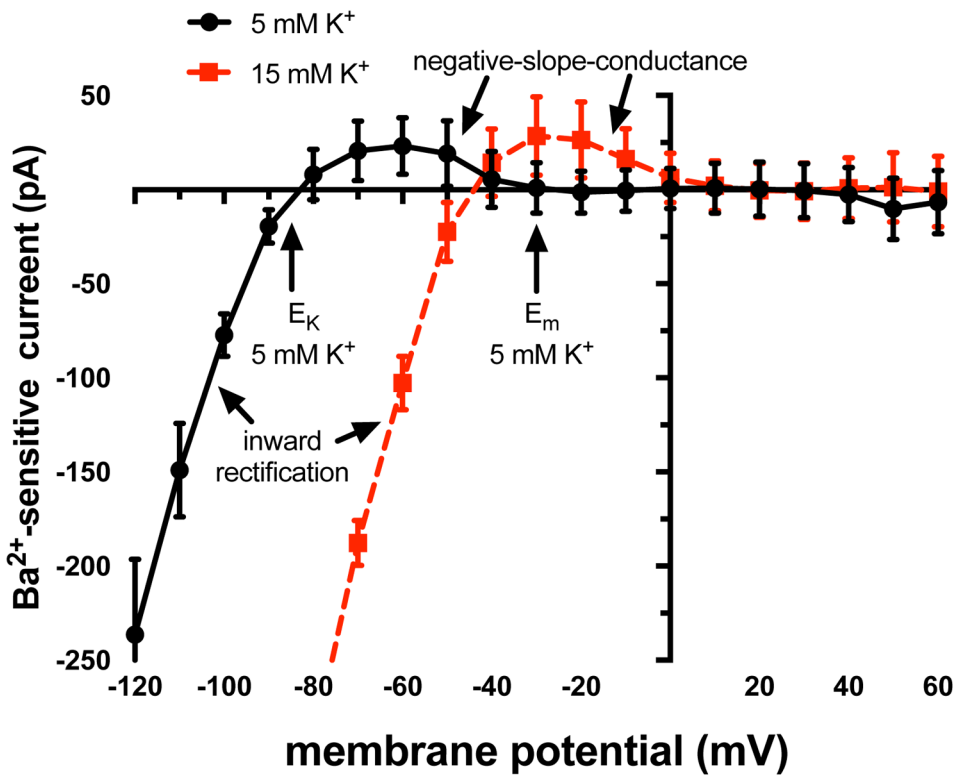

Figure 2.

K_{IR} currents in arteriolar endothelial cells. Shown are mean ± SE (n = 5) current-voltage (I–V) relationships for Ba^{2+} -sensitive currents recorded from enzymatically isolated endothelial cell tubes (see [55] for more information). At membrane potentials (E_ms) negative to the K^+ equilibrium potential (E_K), inward currents are carried by the channels (inward rectification). At membrane potentials positive to E_K, outward currents are carried by the channels, however, the outward currents diminish as the membrane becomes more positive producing a region of negative slope conductance, where hyperpolarization increases outward current flow through the channels. At normal resting E_m (5 mM K^+ outside and 140 mM K^+ inside, ~−30 mV), little current flows through these channels due to block by intracellular polyamines and Mg^{2+}. However, membrane hyperpolarization will recruit current through the channels and amplify the initial hyperpolarization. Also shown is the shift in the I–V relationship with an increase in extracellular K^+ (due to the shift in E_K). Note that at the resting level of E_m (−30 mV) with 15 mM K^+ outside, there is now outward current through K_{IR} channels that will tend to hyperpolarize the membrane toward the new E_K. Data from [55] and graph modified from the same publication, with permission.

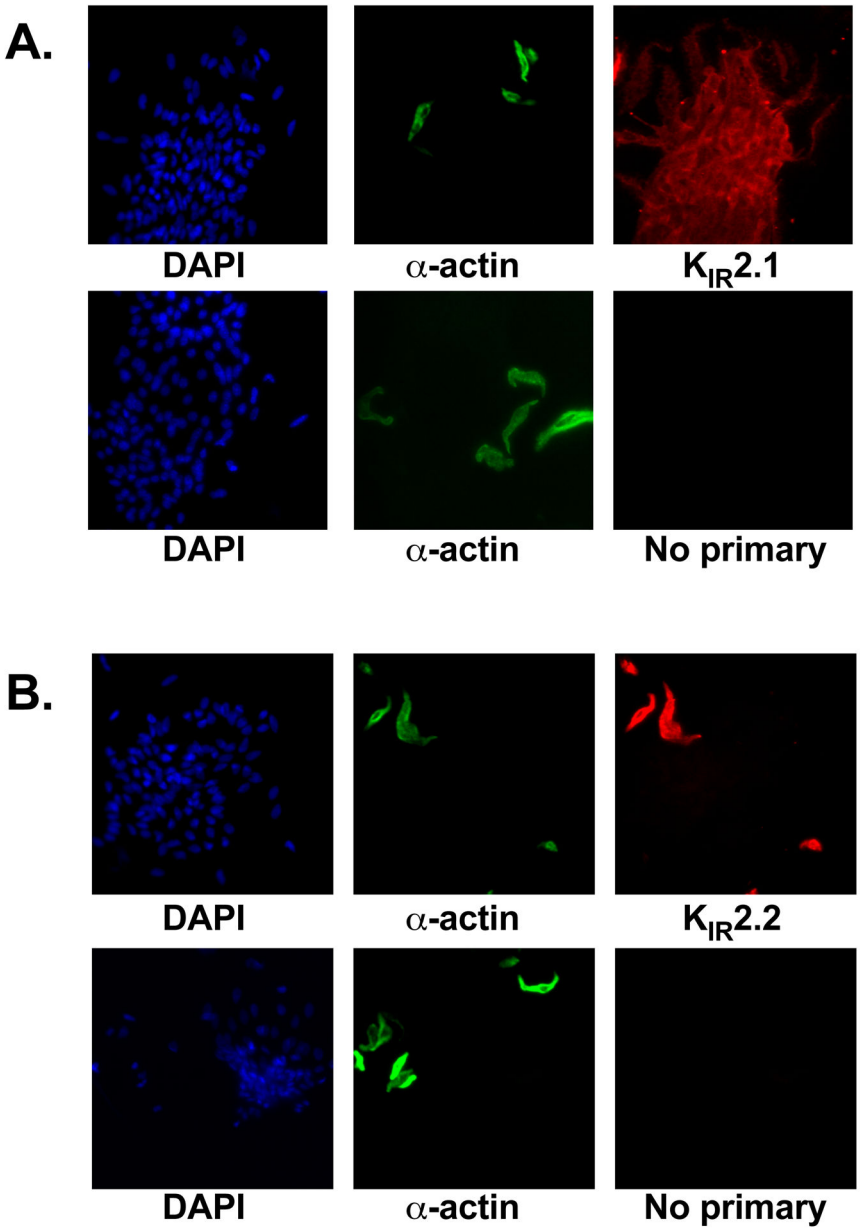

Figure 3.

Expression of $K_{IR}2.1$ in arteriolar endothelial cells – Shown are fluorescent micrographs of enzymatically isolated rat cremaster arteriolar smooth muscle and endothelial cells that were fixed in 4% paraformaldehyde, permeabilized with Triton X-100, and labeled with DAPI (all cell nuclei – Left panels), FITC-labeled primary antibody for α-smooth muscle actin to positively identify smooth muscle cells (1:1000 – Sigma, Middle Panels), and primary antibodies for (A) $K_{IR}2.1$ (1:200 - Alomone) and (B) $K_{IR}2.2$ (1:400 – Alomone) (Right Panels). The secondary antibodies for the K_{IR} channels were Texas-red-labeled donkey anti-rabbit (Jackson Immunoresearch). Bottom row of panels in A and B shows results in the absence of primary antibodies. Panel A shows that endothelial cells display $K_{IR}2.1$ immunoreactivity, consistent with expression of $K_{IR}2.1$, while staining of smooth muscle

cells in the same preparation was weak. Panel B shows that $K_{IR}2.2$ immunoreactivity is not present in endothelial cells, but is robustly expressed in smooth muscle cells as a positive control. Data are representative of 3 experiments. Similar results were obtained for mouse arteriolar endothelial cells [55].

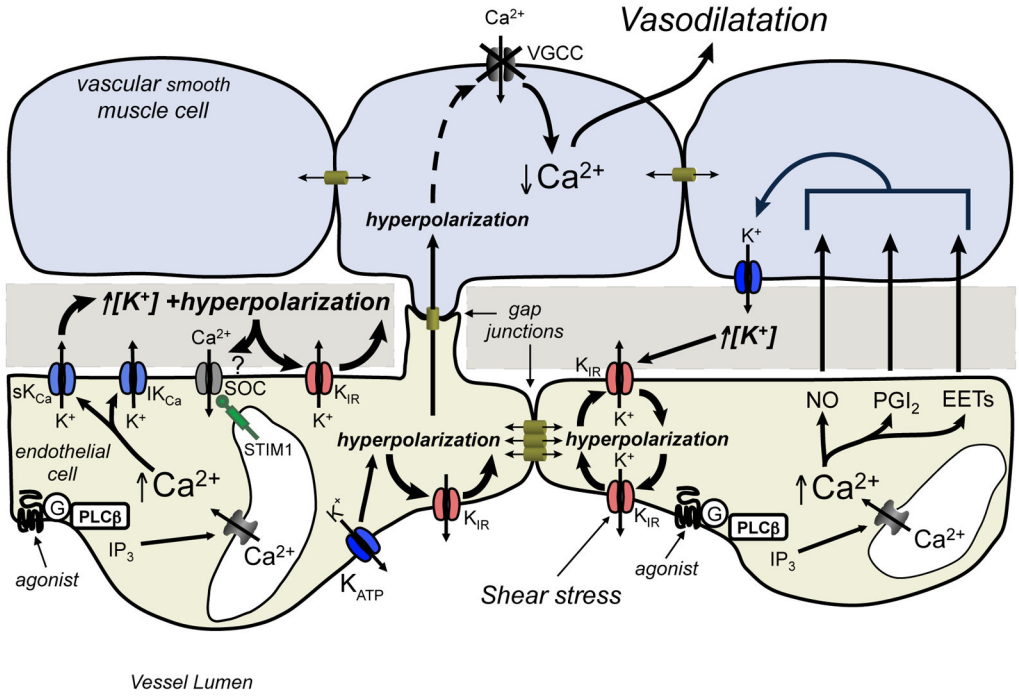

Figure 4.

Endothelial K_{IR} channels: Amplifiers and sensors of extracellular K^+. Hyperpolarization of endothelial cells, due to Ca^{2+}-dependent activation of endothelial cell IK_{Ca} and SK_{Ca}, or by activation of other endothelial cell K^+ channels such as ATP-dependent K^+ channels (K_{ATP}) can activate endothelial cell K_{IR} channels, amplifying the initial hyperpolarization in a positive feed-back manner. Conduction of this hyperpolarization to adjacent endothelial cells that are electrically coupled by gap junctions, can also recruit K_{IR} channels amplifying the hyperpolarization and promoting conduction of this electrical signal. Membrane hyperpolarization may promote Ca^{2+} entry into the endothelial cells through store-operated channels, although this is controversial. The resulting endothelial cell hyperpolarization can then be conducted, through myoendothelial gap junctions, to overlying smooth muscle cells, deactivating smooth muscle voltage-gated Ca^{2+} channels, reducing intracellular Ca^{2+} and promoting vasodilatation. Endothelial cell K^+ channels also can be recruited by elevation of extracellular K^+ concentration in their microenvironment by adjacent endothelial cell or smooth muscle cell K^+ channels. This mechanism may allow endothelium-derived vasodilators, such as NO, prostacyclin (PGI_2) or epoxides of arachidonic acid (EETs), which act, in part, by activating smooth muscle K^+ channels, to utilize endothelial cell K_{IR} channels in their mechanism of action. See text for more information. Figure redrawn and adapted from [56], with permission.