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Potassium Channels in the Peripheral Microcirculation

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Abstract

Vascular smooth muscle (VSM) cells, endothelial cells (EC), and pericytes that form the walls of vessels in the microcirculation express a diverse array of ion channels that play an important role in the function of these cells and the microcirculation in both health and disease. This brief review focuses on the K^+ channels expressed in smooth muscle and endothelial cells in arterioles. Microvascular VSM cells express at least four different classes of K^+ channels, including inward-rectifier K^+ channels (K_{IR}), ATP-sensitive K^+ channels (K_{ATP}), voltage-gated K^+ channels (K_V), and large conductance Ca^{2+} -activated K^+ channels (BK_{Ca}). VSM K_{IR} participate in dilation induced by elevated extracellular K^+ and may also be activated by C-type natriuretic peptide, a putative endothelium-derived hyperpolarizing factor (EDHF). Vasodilators acting through cAMP or cGMP signaling pathways in VSM may open K_{ATP} , K_V , and BK_{Ca} , causing membrane hyperpolarization and vasodilation. VSM BK_{Ca} may also be activated by epoxides of arachidonic acid (EETs) identified as EDHF in some systems. Conversely, vasoconstrictors may close K_{ATP} , K_V , and BK_{Ca} through protein kinase C, Rhokinase, or c-Src pathways and contribute to VSM depolarization and vasoconstriction. At the same time K_V and BK_{Ca} act in a negative feedback manner to limit depolarization and prevent vasospasm. Microvascular EC express at least 5 classes of K^+ channels, including small (sK_{Ca}) and intermediate (IK_{Ca}) conductance Ca^{2+} -activated K^+ channels, K_{IR} , K_{ATP} , and K_V . Both sK and IK are opened by endothelium-dependent vasodilators that increase EC intracellular Ca^{2+} to cause membrane hyperpolarization that may be conducted through myoendothelial gap junctions to hyperpolarize and relax arteriolar VSM. K_{IR} may serve to amplify sK_{Ca} - and IK_{Ca} -induced hyperpolarization and allow active transmission of hyperpolarization along EC through gap junctions. EC K_{IR} channels may also be opened by elevated extracellular K^+ and participate in K^+ -induced vasodilation. EC K_{ATP} channels may be activated by vasodilators as in VSM. K_V channels may provide a negative feedback mechanism to limit depolarization in some endothelial cells.

Keywords

arterioles; endothelium; ion channels; microcirculation; vascular smooth muscle; vasoconstriction; vasodilation

INTRODUCTION

Ion channels play an important role in the regulation of microvascular function. First, ion channels in the plasma membrane, as well as ion channels in the membrane of the smooth endoplasmic reticulum provide the major source of cytoplasmic Ca^{2+} in smooth muscle (14, 54,93), pericytes (78,79,127,130,157,163) and endothelial cells (117) that form the vessels in the microcirculation. In smooth muscle cells (14,54,150), and pericytes (79,157), intracellular Ca^{2+} determines the state of contraction, or tone, while in endothelial cells, intracellular

Ca^{2+} controls the production and release of endothelial cell autacoids, such as NO (44,59), PGI_2 (59), and epoxides of arachidonic acid (21) as well as impacting the barrier function of these cells (120,144). Furthermore, intracellular Ca^{2+} likely regulates gene expression in all types of microvascular cells (22,126,145,151). Second, ion channels play a central role in setting and modulating membrane potential (69). Membrane potential, in turn controls the activity of voltage-gated Ca^{2+} channels in vascular smooth muscle cells (69) and pericytes (78,79,127,163), modulating Ca^{2+} influx, intracellular Ca^{2+} , and microvascular tone.

Studies of macrovascular endothelial cells, primarily in culture, suggest that membrane potential also impacts Ca^{2+} influx in these cells by affecting the electrochemical gradient for Ca^{2+} diffusion into endothelial cells through nonselective cation channels (although this may be different in microvascular endothelial cells—see below) (117). Membrane potential also has been suggested to impact other aspects of Ca^{2+} homeostasis, including Ca^{2+} release from intracellular stores and Ca^{2+} sensitivity of smooth muscle contraction (47,89,119,159,160). Thus, ion channels participate in all aspects of microvascular function from control of blood flow to exchange of solutes and water to interactions of endothelial cells with inflammatory cells.

The cells that make up the walls of vessels in the microcirculation (smooth muscle cells, pericytes, and endothelial cells) express a diverse array of ion channels. For example (Figure 1), arteriolar smooth muscle cells have been shown to express at least four different classes of potassium channels (67,69,115), one or more types of voltage-gated Ca^{2+} channels (63), one or more types of Cl^- channels (82), and several nonselective cation channels from the transient receptor potential family (TRP) of channels (5,64). Although not as well studied, pericytes (78,79,121,127,130,157,163) and microvascular endothelial cells (117) also express a similar complexity of ion channels. Furthermore, the function or expression of ion channels in the microcirculation may differ from those in conduit vessels. There is substantial evidence from the pulmonary circulation for differences in expression and function of K^+ channels as one moves from conduit vessels toward the microcirculation (11,12,29,107,137). In the coronary circulation, there is a higher density of inward rectifier K^+ channel currents in smooth muscle cells from resistance arteries compared to large coronary arteries (123), and the density of currents through L-type Ca^{2+} channels increases progressively from conduit arteries to large arterioles (16). The Ca^{2+} threshold for activation (i.e., the Ca^{2+} setpoint) of large-conductance Ca^{2+} -activated K^+ channels is 10-fold higher in smooth muscle cells from skeletal muscle arterioles, compared to smooth muscle isolated from conduit arteries (71). These examples support the hypothesis that ion channels in the cells that make up the walls of vessels in the microcirculation display unique function or expression patterns that remain largely unexplored. For the purposes of this brief review, I focus on potassium channel expression and function in the plasma membrane of smooth muscle and endothelial cells in arterioles in the peripheral microcirculation. Readers are directed to other sources for more comprehensive, in depth reviews of K^+ channels and other ion channels in the cardiovascular system (15,63,69,70, 115–118,124,138).

POTASSIUM CHANNELS IN ARTERIOLAR SMOOTH MUSCLE CELLS

Smooth muscle cells in the walls of arterioles in the microcirculation play a central role in the regulation of total peripheral resistance (and hence blood pressure), the distribution of blood flow between and within tissues and organs; and microvascular exchange. Potassium channels importantly contribute to the regulation of smooth muscle tone and, hence, all of these functions (69,70). As in most cells in the body, K^+ channels are the dominant ion channels expressed in the plasma membrane of arteriolar smooth muscle cells, and currents through K^+ channels contribute substantially to the membrane potential in these cells (Figure 2). Because of the high input resistance of the plasma membrane in smooth muscle cells (on the order of 10 G Ω (67,

69,115,116)), it is important to remember that only small changes in steady-state membrane current are required to produce significant changes in membrane potential. Membrane potential, in turn, regulates the activity of voltage-gated Ca^{2+} channels, controlling Ca^{2+} influx and intracellular Ca^{2+} . In smooth muscle cells, because of the K^+ electrochemical gradient, opening of K^+ channels leads to K^+ efflux from cells and membrane hyperpolarization. This closes voltage-gated Ca^{2+} channels, reducing intracellular Ca^{2+} and leading to vasodilation. Conversely, closure of open K^+ channels causes membrane depolarization, opening Ca^{2+} channels and leading to Ca^{2+} -dependent vasoconstriction. Studies over the past 20 years have identified at least four different classes of K^+ channels expressed by arteriolar smooth muscle cells. These include inward rectifier K^+ (K_{IR}) channels, ATP-sensitive K^+ (K_{ATP}) channels, Ca^{2+} -activated K^+ (K_{Ca}) channels, and voltage-activated K^+ (K_{V}) channels, as summarized in Table 1.

SMOOTH MUSCLE K_{IR} CHANNELS

Inward-rectifier K^+ channels derive their name from the fact that at membrane potentials negative to the potassium equilibrium potential, these channels conduct K^+ ions into cells, whereas at more positive potentials, outward K^+ current flow is limited (124). Recent studies suggest that the K_{IR} channel isoform expressed in smooth muscle is KIR 2.1 (17,161). These channels are blocked by Ba^{2+} ions at micromolar concentrations and are activated by increases in extracellular K^+ (124). In coronary and cerebral microcirculations, smooth muscle K_{IR} channels act as sensors for increases in extracellular K^+ , leading to membrane hyperpolarization and vasodilation when extracellular K^+ is elevated from 5 mM to 8–15 mM (38,84,115,124,125). Current density through K_{IR} channels in coronary smooth muscle increases from conduit arteries into small, resistance arteries, as noted above (123). This difference in K^+ channel expression largely explains the observation that conduit arteries have little response to small elevations in extracellular K^+ , whereas resistance arteries display a robust dilation (123,124). In skeletal muscle microcirculation, K_{IR} channels appear to play a more modulatory role affecting primarily the duration and kinetics of K^+ -induced smooth muscle hyperpolarization and vasodilation (20). Inward-rectifier K^+ channels also may be activated by C-type natriuretic peptide, a putative endothelium-derived hyperpolarizing factor (EDHF) (24). Bradykinin may activate K_{IR} channels in coronary arterioles, and it has been proposed that these channels participate in propagation of hyperpolarizing signals along arterioles (128). In other systems, K_{IR} channels can be modulated by protein kinases (156) or G-proteins (77), suggesting that their vascular counterparts may also be regulated. This hypothesis is supported by recent observations showing that in some blood vessels, NO may activate K_{IR} channels (134). Inward rectifier K^+ channels may be downregulated during hypertension (106,138).

SMOOTH MUSCLE K_{ATP} CHANNELS

ATP-sensitive K^+ channels close with increases in intracellular ATP, hence their name (124). They also are modulated by a myriad of other intracellular signals, including ADP, H^+ , and Ca^{2+} (124). These channels in smooth muscle are likely composed of a tetramer of KIR 6.1 subunits that form the ion conductive pore (111,139), and complementary regulatory sulfonylurea receptor (SUR) subunits, SUR 2B (111). K_{ATP} channels are blocked by sulfonylureas like glibenclamide and opened by activators such as pinacidil and cromakalim (124). In skeletal muscle and the heart, K_{ATP} channels are open at rest and contribute to resting smooth muscle membrane potential and tone (43,66,73,88,129,146,147). These channels also appear to play a role in the mechanism of action of both vasodilators (66,70,73) and vasoconstrictors (70,115,142,155). Studies have shown that K_{ATP} channels may be activated by protein kinase A and c-GMP-dependent protein kinase and have been implicated in the mechanism of action of endogenous vasodilators such as adenosine (34,66), PGI_2 (66),

calcitonin-gene-related-peptide (CGRP) (115), and NO (115). Conversely, activation of protein kinase C (27,56,115,142) and elevation of intracellular Ca^{2+} (acting through calcineurin) (155) by vasoconstrictors such as norepinephrine (68), vasopressin (148), endothelin (113), and angiotensin II (56,112) close these channels. Smooth muscle ATP-sensitive K^+ channels also appear to be downregulated in diseases such as diabetes (33,104, 105,111,164) and hypertension (138), potentially contributing to end-organ problems in these diseases.

SMOOTH MUSCLE K_{Ca} CHANNELS

The dominant K_{Ca} channels expressed by microvascular smooth muscle cells appear to be large (Big) conductance, BK_{Ca} channels. These channels have a high single-channel conductance (240 pS, hence their name) and are activated by membrane depolarization and increases in intracellular Ca^{2+} (69,115). They are composed of Slo1 subunits that form the pore, and Slo β_1 subunits that modulate the Ca^{2+} sensitivity of the channel (19,141). BK_{Ca} channels are blocked by millimolar concentrations of tetraethyl ammonium ions (55); scorpion toxins such as charybdotoxin (also blocks other K^+ channels)(109) and iberiotoxin (highly selective) (45); and indoles such as penitrem A and paxilline (83). These channels are activated by compounds such as NS 1619 (115).

Like voltage-gated Ca^{2+} channels (35,36,80), BK_{Ca} channels likely exist in signaling complexes with voltage-gated Ca^{2+} channels, protein kinases (protein kinase A, cGMP-dependent protein kinase, protein kinase C, cSrc, etc.), phosphatases, and other signaling proteins (6,80,98). These macromolecular signaling complexes also appear to be located adjacent to smooth endoplasmic reticulum Ca^{2+} -activated, Ca^{2+} -release channels (ryanodine receptors) such that focal release of Ca^{2+} from ryanodine receptors triggered by Ca^{2+} influx through voltage-gated Ca^{2+} channels (i.e., Ca^{2+} sparks) can activate BK_{Ca} channels in smooth muscle (75).

Studies of microcirculatory beds *in vivo* have shown that microvascular BK_{Ca} channels do not appear to contribute to resting membrane potential under normal conditions (71,72,100,122). This appears to result, at least in part, from the channels having a high threshold for activation by Ca^{2+} , compared to what has been observed in smooth muscle from conduit arteries (71). However, BK_{Ca} channels in the microcirculation are opened and play an important negative feedback role during active agonist- and stretch-induced vasoconstriction (70,71,85,154,158). The impact of vasoconstrictor-induced activation of BK_{Ca} channels due to membrane depolarization and elevated intracellular Ca^{2+} may be moderated by the activity of protein kinase C, which appears to inhibit these channels in some systems (91,110). In addition, recent *in vitro* studies suggest that these channels may be closed by cSrc tyrosine kinases that are activated by vasoconstrictors such as serotonin (6). These observations are complicated by the findings that tyrosine kinases like cSrc (96), Pyk2, and Hck (95) enhance the activity of BK_{Ca} channels expressed in HEK 293 cells.

A number of vasodilators, including NO (10), CO (149) and epoxides of arachidonic acid (EETs) (162), appear to activate BK_{Ca} channels in some systems either directly or by activation of protein kinases (see (70)). In hypertension, the functional expression of these channels is upregulated (31,138) and they contribute substantially to resting membrane potential and tone in this disease state (31). Recent data suggest that hypertension also may alter the expression of the modulatory β_1 subunits, affecting the Ca^{2+} sensitivity of these channels in this disease (7,9). Expression and function of BK_{Ca} channels also may be altered in hypercholesterolemia and atherosclerosis (138).

Some microvascular smooth muscle also may express small conductance K_{Ca} (sK_{Ca}) channels (50). These channels require calmodulin for Ca^{2+} sensitivity (unlike BK_{Ca} channels that have

an intrinsic Ca^{2+} sensor), have a single-channel conductance of 10 pS, and are blocked by the bee venom peptide, apamin (15). Their physiological function in smooth muscle has not been well studied but recent evidence suggests that in some smooth muscle cells, sK_{Ca} channels may be activated by arachidonic acid (49).

SMOOTH MUSCLE K_V CHANNELS

Smooth muscle cells in the microcirculation also express a variety of K_V channels, including members of the K_V 1.5 and 1.6 families (25,26), and likely others (8,81,143). Like BK_{Ca} channels, K_V channels are composed of a tetramer of $\text{KV}\alpha$ subunits that form the ion conductive pore of the channels, along with accessory $\text{KV}\beta$ subunits (31,143). These channels are activated by membrane depolarization and likely participate in the negative feedback regulation of membrane potential along with BK_{Ca} channels. Blockers of microvascular smooth muscle K_V channels include 4-aminopyridine (25,26,31,72,115), correolide, and agitotoxin-2 (25,26). *In vitro* studies have demonstrated that the activity of K_V channels contributes to resting membrane potential and smooth muscle tone (25,26,67,72). These channels appear to be activated by the cAMP-protein kinase A signaling pathway (1–3) such that they may be involved in the mechanism of action of vasodilators such as adenosine, PGI_2 and CGRP. Decreased intracellular pH activates K_V channels in coronary myocytes (13). As with K_{ATP} channels, vasoconstrictors tend to close K_V channels through signaling pathways involving protein kinase C (4) and Ca^{2+} (30). Recent studies indicate that Rho kinase also may close these channels (101). All of these effects likely contribute to vasoconstrictor-induced depolarization of arteriolar smooth muscle. There appears to be a functional downregulation of smooth muscle K_V channels in hypertension, despite evidence that expression of the channel protein is increased (31). This functional downregulation may be related to increased intracellular Ca^{2+} observed in myocytes from hypertensive animals (31). Hyperglycemia has been shown to impair K_V channel function and expression in small coronary arteries (99). The function of K_V channels in the microcirculation *in vivo* has not been established due to the ubiquitous expression of multiple classes of these channels and due to the lack of selective inhibitors/activators.

ARTERIOLAR ENDOTHELIAL CELL K^+ CHANNELS

Endothelial cells also express a diverse array of K^+ channels (Figure 3). However, relatively little is known about the channels expressed and their function in arteriolar endothelial cells. Data from studies of endothelial cells from different tissues suggest that arteriolar endothelial cells express K_{ATP} , K_V , K_{IR} , and two types of K_{Ca} channels (see below) (117). Potassium channels in endothelial cells appear to play an important role in endothelial cell signaling through regulation of endothelial cell membrane potential (117). Endothelial cells are electrically coupled to one another by gap junctions (117), thus changes in endothelial cell membrane potential have the ability to be summed and transmitted along the endothelium providing a mechanism for long-distance transmission of signals in the microcirculation (41, 97). In addition, endothelial cells may be electrically coupled to overlying smooth muscle cells by myoendothelial gap junctions such that changes in endothelial cell membrane potential may be transmitted to smooth muscle cells to modulate arteriolar tone accordingly (40,131,132).

Through their impact on membrane potential, endothelial cell K^+ channels also have been proposed to regulate Ca^{2+} influx into endothelial cells (117). Although there are a few reports that cultured endothelial cells express voltage-gated Ca^{2+} channels (see (117) for references), most data suggest that calcium influx into endothelial cells occurs through non-selective cation channels, such as those formed by the transient receptor potential (TRP) family of ion channel proteins (117,118). Ion flow through these channels is determined by the electrochemical gradient across the cell membrane, which is determined by the concentration gradient for the

ion (Ca^{2+} in this case), the membrane potential, and the conductance of the ion channel (117). Thus, changes in membrane potential, by altering the electrochemical gradient, can influence the movement of ions through the channel. In cultured endothelial cells (59,117), and perhaps in microvascular endothelial cells *in situ* (57,58), it has been shown that endothelial cell membrane potential strongly influences Ca^{2+} influx through nonselective cation channels: depolarization inhibits Ca^{2+} influx and hyperpolarization increases Ca^{2+} influx. However, recent data suggest that this may not be the case in endothelial cells in small arteries and arterioles where Ca^{2+} signaling appears to be independent from changes in membrane potential (see below for more on this topic) (28,102). Table 1 summarizes the K^+ channels expressed in endothelial cells and factors that modulate these channels.

ENDOTHELIAL K_{ATP} CHANNELS

In addition to their presence in smooth muscle cells, microvascular endothelial cells also may express K_{ATP} channels (76,92,133). For the most part, their presence and function has been inferred by effects of the sulphonylurea K_{ATP} channel antagonist, glibenclamide, and by effects of K_{ATP} channel agonists such as pinacidil and cromakalim. Endothelial K_{ATP} channels have been implicated in arteriolar dilation induced by hyperosmolarity (65), adenosine (90, 103), and isoflurane (46). In cultured cells, shear stress has been shown to increase expression of these channels (23). Effects of disease states on endothelial K_{ATP} channel expression or function have not been studied.

ENDOTHELIAL K_{V} CHANNELS

Several recent studies suggest that microvascular endothelial cells may express several different classes of K_{V} channels (25,26,37,42,62). However, their physiological function has not been established. It has been suggested that these channels may participate in membrane potential oscillations and negative feedback regulation of membrane potential (37).

ENDOTHELIAL K_{IR} CHANNELS

Endothelial cells in arterioles appear to express strongly rectifying K_{IR} channels, probably $\text{K}_{\text{IR}} 2.1$ (117). At first glance, the function of these channels in arterioles is not apparent because the resting membrane potential of arteriolar endothelial cells is on the order of -30 mV (40, 152), which is outside of the membrane potential range where these channels significantly contribute to membrane currents with normal extracellular K^+ concentrations (124) (Figure 4). However, as in smooth muscle, endothelial cell K_{IR} channels may function as sensors for elevated extracellular K^+ and provide a hyperpolarizing signal to alter microvessel function (Figure 4). In fact, in small mesenteric arteries, endothelial K_{IR} channels may contribute to K^+ -induced dilation as smooth muscle cells in these vessels lack these channels (32). Interestingly, in smooth muscle cells isolated from cremasteric arterioles (20), while K_{IR} channel currents can be recorded, they lack the outward “hump” that is observed in endothelial cells isolated from the same vessels (see Figure 4) and that is required for these currents to contribute to membrane hyperpolarization. Thus, it is possible that endothelial cell K_{IR} channels contribute to K^+ -induced dilation in cremasteric arterioles as well.

In addition to serving as sensors for extracellular K^+ , K_{IR} channels have the potential to act as amplifiers of hyperpolarization initiated by the opening of other K^+ channels, in particular sK_{Ca} and IK_{Ca} channels (Figures 3 and 4). Hyperpolarization-induced activation of outward currents through K_{IR} channels also may contribute to conduction of hyperpolarizing signals along endothelial cells as proposed for smooth muscle K_{IR} channels (128) (Figure 3).

Studies of cultured macrovascular endothelial cells also suggest that K_{IR} channels may be involved in shear-stress-induced hyperpolarization of endothelial cells (74), and their

expression may be modulated by fluid shear (61), although this has not been established in native microvascular endothelial cells. Vasoconstrictors such as angiotensin II, vasopressin, endothelin, and histamine have been reported to inhibit K_{IR} channels in endothelial cells by a G-protein-dependent mechanism (117). However, the functional significance of this phenomenon has yet to be established.

ENDOTHELIAL K_{Ca} CHANNELS

Endothelial cells appear to express at least two classes of calcium-activated K^+ channels: small conductance (sK_{Ca} , probably $sK3$ (87)) and intermediate conductance (IK_{Ca} , probably $IK1$ (87)) K^+ channels. Distinct from BK_{Ca} channels, these channels have a smaller single channel conductance (10 pS for sK_{Ca} and 30–80 pS for IK_{Ca} (117)), are not voltage-activated, and require the Ca^{2+} binding protein, calmodulin, to display Ca^{2+} sensitivity (117). They also have a distinct pharmacology. Both channels are insensitive to iberiotoxin and blockade by millimolar TEA. The bee venom peptide apamin blocks sK_{Ca} channels (117), as do tetrabutylammonium ions (53). Charybdotoxin, which blocks, among other things, BK_{Ca} channels, also potently blocks IK_{Ca} channels (117). IK_{Ca} channels also are antagonized by clotrimazole and its derivatives such as TRAM 39 and TRAM 34 (39,60). Depending on the vessel and the species studied, sK_{Ca} (Cor de Wit, personal communication), IK_{Ca} (102) or both channels mediate agonist-induced hyperpolarization of endothelial cells and play a major role in arteriolar dilation induced by endothelium-dependent vasodilators, such as acetylcholine, substance P, bradykinin, and histamine (21,135,136) (Figure 3). The hyperpolarization induced by Ca^{2+} -triggered opening of these channels may be conducted along the endothelium via endothelial gap junctions (40) and transmitted to smooth muscle cells through myoendothelial gap junctions to cause vasodilation (40,102,131,132) (Figure 3). As noted above, in studies of macrovascular endothelial cells, this hyperpolarization also has been shown to increase the driving force for endothelial cell Ca^{2+} entry and autacoid production (117). However, recent studies suggest that arteriolar endothelial cell Ca^{2+} signaling may be independent from changes in endothelial cell membrane potential (28,102,140). This suggests that arteriolar endothelial cells may have novel Ca^{2+} influx pathways, or that Ca^{2+} handling in these cells may be different from what has been described previously in cultured cells.

There is a paucity of information on regulation of endothelial IK_{Ca} channels by factors other than Ca^{2+} . In cultured cells, shear stress has been demonstrated to increase the expression of IK_{Ca} channels (18). The impact of disease states on endothelial K_{Ca} channel expression and function in the microcirculation has not been established. However, IK_{Ca} channel expression is increased in mesenteric endothelial cells from patients with colonic cancer (86). Conversely, sK_{Ca} and IK_{Ca} channel expression and function are diminished in regenerated endothelium after balloon injury (87).

QUESTIONS FOR THE FUTURE

Thus, ion channels participate in all aspects of microvascular function. However, given the diversity in expression patterns and multiple mechanisms of regulation of the array of ion channels found in cells that make up microvessels, it is clear that much remains unknown. The use of genomic (52,94) and proteomic approaches, applied specifically to the microcirculation, appear to be efficient approaches to examine the large number and the diversity of expression patterns of ion channels in microvascular smooth muscle cells, pericytes, and endothelial cells. In addition, the use of gene silencing methods, such as antisense oligonucleotides (51) and siRNA (108), in conjunction with conventional pharmacology provides a powerful set of tools to examine the function of specific ion channels in the microcirculation. Indeed, the antisense approach has already proven successful in identification of a component of stretch-activated cation channels in resistance artery smooth muscle cells *in vitro* (153). These approaches, along

with the simultaneous measurement of membrane potential, intracellular Ca^{2+} , and diameter (or other functional assay of vessel function) must also be taken *in vivo*, so that the true role played by ion channels in the microcirculation in health and disease states can be established.

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Figure 1.

Ion channels expressed in arteriolar smooth muscle cells. Schematic diagram indicating the families of ion channels expressed in smooth muscle cells in the wall of arterioles. Current evidence (see text for details and references) indicate that arteriolar smooth muscle cells express inward rectifier K^+ channels, ATP-sensitive K^+ channels (K_{ATP}), several different types of voltage-gated K^+ channels (K_V), one or more types of calcium-activated K^+ channels (K_{Ca}), one or more types of voltage-gated Ca^{2+} channels (VGCC), two or more types of Cl^- channels (CLC), and several different types of nonselective cation channels (NSCC) likely from the transient receptor potential (Trp) family of channels. Intracellular ion channels include Ca^{2+} -activated Ca^{2+} release channels (ryanodine receptors, RYR) and inositol 3,4,5-trisphosphate receptors (IP_3R). It should also be noted that other channels also are likely expressed in intracellular membranes, including those of mitochondria (not shown).

Figure 2. Potassium channels regulate arteriolar smooth muscle tone by affecting membrane potential. Schematic diagram of an arteriolar muscle cell and cross sections of arterioles with open or closed K⁺ channels. VGCC, voltage-gated Ca²⁺ channel.

Figure 3.

Ion channels expressed in arteriolar endothelial cells. Schematic of a longitudinal section of an arteriole showing cross sections of endothelial cells and smooth muscle cells. Ion channels expressed by microvascular endothelial cells include ATP-sensitive K^+ channels (K_{ATP}), voltage-gated K^+ channels (K_V), inward rectifier K^+ channels (K_{IR}), small conductance (sK_{Ca}) and intermediate conductance (IK_{Ca}) Ca^{2+} -activated K^+ channels, and several types of non-selective cation channels (NSCC), likely from the transient receptor potential (Trp) family of channels. Intracellular ion channels include IP_3 receptors (IP_3R). Also shown are pathways for activation of endothelial K^+ channels leading to endothelial membrane hyperpolarization (see text for references). Agonists such as acetylcholine, substance P, or bradykinin bind to G-protein coupled receptors ($G_{q/11}$) and activate phospholipase $C\beta$ ($PLC\beta$) that acts on membrane phospholipids (PIP_2) to form IP_3 . This second messenger binds to IP_3 receptors (IP_3R) in the membrane of the smooth endoplasmic reticulum, releasing stored Ca^{2+} . Release of Ca^{2+} from intracellular stores, as well as G-protein-mediated events and activation of other signaling cascades open nonselective cation channels (NSCC), allowing extracellular Ca^{2+} to diffuse into the cells. Release of Ca^{2+} and increased Ca^{2+} influx increase intracellular Ca^{2+} , leading to formation of endothelium derived autacoids such as NO, PGI_2 and epoxides of arachidonic acid (EETS) (not shown in figure). Elevated intracellular Ca^{2+} also activates IK_{Ca} and/or sK_{Ca} channels, leading to K^+ efflux and membrane hyperpolarization. The K^+ released through these channels and the membrane hyperpolarization may activate outward currents through K_{IR} channels, supporting the hyperpolarization. Because cells in the walls of arterioles are coupled by gap junctions, the endothelial cell hyperpolarization may be transmitted to other endothelial cells and to smooth muscle cells, leading to smooth muscle hyperpolarization, closure of voltage-gated Ca^{2+} channels (VGCC), a reduction in intracellular Ca^{2+} , and vasodilation. Transmission of hyperpolarization through gap junctions to adjacent endothelial cells also may activate K_{IR} channels in these cells and allow conduction of hyperpolarizing signals for long distances along the endothelium.

Figure 4.

Inward rectifier K^+ (K_{IR}) channel currents in arteriolar endothelial cells. Data are means $\pm$ SE ($n = 5$). Ba^{2+} -sensitive ($100 \mu M$) difference currents measured in the presence of 5 or 15 mM K^+ in the extracellular fluid as indicated. Endothelial cells were isolated enzymatically from hamster cremaster arterioles and membrane currents studied using the amphotericin B perforated patch technique as described previously for arteriolar smooth muscle cells (72). Note that at normal extracellular K^+ (5 mM), at the resting membrane potential of these cells (-30 mV), there is little or no outward current. However, with an increase in extracellular K^+ , the current–voltage relationship shifts to the right such that outward current through the K_{IR} channels is present. This would tend to hyperpolarize the cells toward, in this case, -45 mV. Note also that in physiological K^+ (5 mM), membrane hyperpolarization induced by opening of other K^+ channels could recruit outward current through K_{IR} channels, amplifying the original hyperpolarization.

Table 1

Potassium channels in arteriolar smooth muscle and endothelial cells

Ion channel ^a	VSM ^b	ENDO ^c	Additional subunits	Outward currents activated by:	Outward currents inhibited by:	Antagonists
BK _{Ca}	Slo1 (19,141)	- or ?	Slo-β (19,141)	Depolarization, ↑[Ca ²⁺] _{in} (69,115), NO(10), CO (149), EETs(162), PKA(115), PKG(115), cSrc (96)?	Hyperpolarization, ↓[Ca ²⁺] _{in} (115), PKC (91,110), cSrc (6)?	Iberitoxin(45), charybdotoxin (109), penitrem A(83), paxillin (83), 1 mM TEA (115)
SK _{Ca}	- or ?	SK3 (87)	Calmodulin (117)	↑[Ca ²⁺] _{in} (117)	↓[Ca ²⁺] _{in} (117)	Apamin(117), d-tubocurarine (48), TBA(53)
IK _{Ca}	—	IK1(87)	Calmodulin (117)	↑[Ca ²⁺] _{in} (117)	↓[Ca ²⁺] _{in} (117)	TRAM-39(39,60), TRAM-34 (39,60), charybdotoxin(117), clotrimazole(39,60), TBA(114)
K _V	KV 1.5, 1.6 (25,26) + ?	KV 1.5, 1.6? (25,26,37,42,62)	K _v β (31,143)	Depolarization (115), PKA (1-3), ↓pH (13)	Hyperpolarization (115), PKC (4), ↑[Ca ²⁺] _{in} (30), Rho kinase (101)	4-aminopyridine (25,26,115), correolide, Agitoxin-2 (25,26)
K _{ATP}	KIR 6.1 (111, 139)	KIR 6.1 or 6.2? 1997 (92,133)	SUR2b (111)	↓ATP, ↑ADP, PKA, PKG (115, 124), pinacidil, cromakalim, etc. (115)	↑ATP, ↓ADP (124), ↑[Ca ²⁺] _{in} (via calcineurin) (155), PKC (27,56, 115,142),	Glibenclamide, tolbutamide, TPA, Ba ²⁺ (115,124)
K _{IR}	KIR 2.1 (161)	KIR 2.1 (117)	—	Hyperpolarization, ↑[K ⁺] _{out} (124), CNP (24), NO (134)	Depolarization, ↓[K ⁺] _{out} (124), ?	Ba ²⁺ , quinidine, phenylcyclidine (115,124)

Note. PKA, protein kinase A; PKG, cGMP-activated protein kinase; PKC, protein kinase C (see text for other abbreviations). Inhibitor abbreviations: TEA, tetraethyl ammonium; TBA, tetrabutyl ammonium; TPA, tetrapentyl ammonium. —, not present; ?, present, but specific isoform unclear, or mechanism unclear (see text for references or more information).

^aSee text for definitions of channel abbreviations.

^bV_{vascular} smooth muscle.

^cEndothelium.