REVIEW

K+ channels in biological processes: vascular K+ channels in the regulation of blood pressure

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Abstract: Appropriate supply of blood to organs and tissues is highly dependent on arterial blood pressure and therein of the peripheral blood vessel resistance. Of the two main components of vessel resistance, the active resistance results from the modulation of the contractility level of vascular smooth muscle cells (VSMCs). The intracellular level of Ca^{2+} in VSMCs is an essential component of muscle contraction and is tightly regulated through modulation of the membrane potential. Since resting membrane potential of vascular cells is mainly dependent on K^+ ions, ion channels permeable to K^+ ions have a significant impact on contractility of smooth muscle cells and therefore on vascular diameter and blood pressure. Activation of K^+ channels on both endothelial cells and VSMCs is generally associated to hyperpolarization and relaxation of vascular smooth muscle. Several types of K^+ channels are expressed in VSMCs and endothelial cells, and they are classified based on their pharmacological and biophysical properties. Voltage-dependent K^+ channels are activated by depolarization and are mainly involved in negative-feedback mechanisms. Ca^{2+}-activated K^+ channels can be divided into three groups, with BK_{Ca} being activated by both intracellular Ca^{2+} and depolarization. On the other hand, $K_{Ca}2.x$ and $K_{Ca}3.1$ channels (small and intermediate Ca^{2+}-activated K^+ channels, respectively) are almost strictly dependent on rises in intracellular Ca^{2+} levels to increase their open probability. K_{ir} and adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels, members of the same family, have a significant impact on VSMC membrane potential. The more recently studied two-pore K^+ channels are thought to be metabolic sensors (like K_{ATP} channels) and would be involved in acute regulation of local blood flow. This review will summarize the main K^+ channels expressed in vascular cells and their relevance in the control of vascular tone and blood pressure.

Keywords: membrane potential, Ca^{2+}, vascular tone

Blood pressure
Regulation of blood pressure by vascular tone

Blood pressure can be defined as the pressure applied by circulating blood on blood vessels walls. It is dependent on the blood volume, the blood flow, and the resistance of the blood vessels. Blood pressure and blood flow are regulated by the constriction and dilation of resistance arteries, usually small arteries with internal diameters $<300 \, \mu m$.[1] Physiological elevations in intravascular pressure or augmented sympathetic activity promotes smooth muscle depolarization, increases myocyte intracellular Ca^{2+}, and thus vasoconstriction. The subsequent increase in total peripheral resistance within the vasculature increases blood pressure.[2] The vascular smooth muscle cells (VSMCs) within the blood vessel wall provide structural integrity to the vessel and regulate vascular tone and blood pressure by their contractile state. From a basal resting tone,

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the VSMCs are constantly in a semi-contracted state and can contract further, leading to a reduced blood vessel diameter and increased blood pressure (Figure 1). On the other hand, relaxation of VSMCs leads to a larger vessel diameter and hence to a reduction of blood pressure. Although the systemic outcome of this change in vessel resistance is a modification in blood pressure, generally, vascular tone – and hence VSMC contractile state – is finely tuned in order to maintain or adapt blood flow to the tissue perfused by the artery involved. Indeed, according to Poiseuille's Law, with all other parameters constant, a reduction of the vessel radius by a factor of two would result in a decrease in blood flow by a factor of 16.

Impact of intracellular calcium on vascular tone

Contraction and relaxation of the VSMCs are tightly regulated by the intracellular calcium concentration ($[Ca^{2+}]_i$) emphasizing the importance of the homeostasis regulatory mechanisms in the control of vascular tone. Vasoconstrictors like epinephrine, endothelin-1, angiotensin II, or thromboxane A2 act through increasing $[Ca^{2+}]_i$ by opening Ca^{2+} channels located in the cytoplasmic and sarcoplasmic membrane as well as on the apparent calcium sensitivity of the contractile apparatus in VSMCs, whereas relaxing factors have the opposite effect. Interestingly, an elevation in endothelial $[Ca^{2+}]_i$ induces relaxation of the adjacent VSMCs through endothelium-derived relaxing factors such as nitric oxide (NO), prostacyclin (PGI_2) and endothelium-derived

Figure 1 Relationship between VSMC contraction and blood vessel diameter.
Notes: From a basal resting tone – and semi-contracted state – the VSMCs can either contract further, leading to a reduced blood vessel diameter and increased blood pressure, or relax, leading to a larger vessel diameter and reduced blood pressure. B, Basal resting tone, C, contraction, and R, relaxation of VSMCs.
Abbreviation: VSMC, vascular smooth muscle cell.

hyperpolarizing factor (EDHF).[3–6] Therefore, fine tuning of $[Ca^{2+}]_i$ in both cell types is imperative for precise regulation of blood pressure and organ and tissue perfusion.

Role of the membrane potential on vascular cells and its impact on intracellular calcium and on blood vessel diameter

The membrane potential of vascular cells is dependent on the intracellular and extracellular concentration of ions as well as their respective permeability across the lipid bilayer. A resting membrane potential (E_m) is generated by well-balanced influx and efflux of ions through ion channels along their gradients as well as by active ion transport through ion pumps.

In VSMCs, E_m (−45 mV) is primarily determined by K^+ efflux through several plasma membrane K^+ channels, including the voltage-gated K^+ (K_V) channels, the large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, inwardly-rectifying K^+ (K_{ir}) channels, ATP-sensitive K^+ (K_{ATP}) channels, and two-pore domain K^+ (K_2P) channels.[7–10] With a reversal potential for K^+ around −89 mV, opening of K^+ permeable channels will hyperpolarize E_m. Excitation of VSMCs followed by the depolarization of the E_m by influx of cations (Ca^{2+} and Na^+) or efflux of Cl^- promotes the opening of voltage-dependent Ca^{2+} channels (VDCCs). The opening of VDCCs allows Ca^{2+} influx, causing a rise in $[Ca^{2+}]_i$ and activation of the cellular contractile machinery. Depolarization also leads to the voltage-dependent opening of K_V channels, and the corresponding increase in $[Ca^{2+}]_i$ through VDCCs in combination with the depolarization opens the voltage- and Ca^{2+}-sensitive BK_{Ca} channels. Opening of these K^+ channels causes a compensatory hyperpolarizing current that reduces open probability of VDCCs to oppose vasoconstriction.[7]

In vascular endothelial cells (ECs), hyperpolarization rather than depolarization is associated with raised $[Ca^{2+}]_i$. This is attributed to the increase in the inward electrochemical gradient for Ca^{2+}, as ECs generally lack VDCCs. The increase in EC $[Ca^{2+}]_i$ is of major importance for physiological function in blood vessels. It initiates and sustains vasodilation by stimulating the synthesis of NO and PGI_2. It also activates small-conductance K^+ ($K_{Ca}2.3$) and intermediate-conductance K^+ ($K_{Ca}3.1$) channels, generating a hyperpolarization known as the endothelium-derived hyperpolarization factor (EDHF), which spreads to and relaxes the adjacent VSMCs.[5,6] However, it is important to note that EDHF is not limited to $K_{Ca}2.3$ and $K_{Ca}3.1$ channels but is rather an amalgam of different
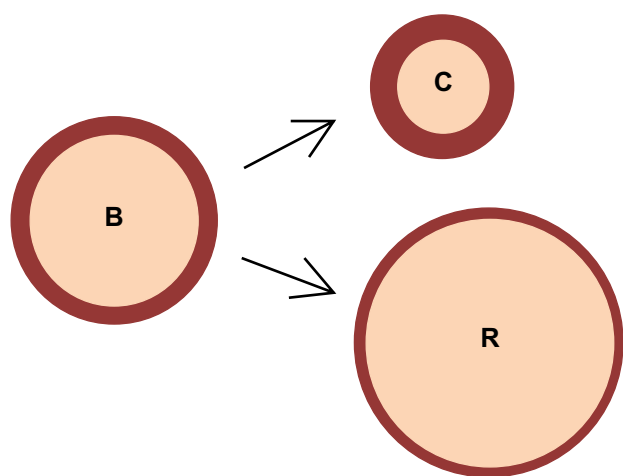
mechanisms which have in common the ability to induce a hyperpolarization of the underlying smooth muscle cells. The following sections of this review will focus on the different types of K+ channels expressed in VSMCs and ECs and their role in regulating blood pressure and vascular tone.

Potassium channels

Voltage-dependent K+ channels

Voltage-dependent K+ channels (K_V) consist of a large super-family of 12 subfamilies containing several members each in addition to splice variants.[11] However, vascular K_V channels mainly consist of K_V1, K_V2, and K_V3 isoforms. More recently, K_V7 (KCNQ) and K_V11 (HERG) family members have also been identified in VSMCs.[12,13] Archetypical K_V channels have cytoplasmic carboxy and amino termini and are six transmembrane domain proteins, with segment S4 being the voltage-sensor, and their quaternary structure consists of four α subunits.[14] The complexity of K_V currents is due to the heteromultimerization of α subunits, modifying biophysical and pharmacological properties. Additional potential convolution is provided through modulation of the K_V heteromultimers by β subunits. K_V channels are activated by depolarization and are therefore an optimal component of a negative-feedback mechanism in response to a contractile-prone VSMC depolarization.[7] Their activity is, however, subject to regulation by protein kinases like PKA, PKC, and PKG.

In VSMCs, the main isoforms of K_V channels expressed are K_V1.2, K_V1.5, K_V1.6, K_V2.1, and K_V3.1, but expression pattern is variable depending on the vascular bed.[15] However, studies have suggested that functional expression of K_V channels can be regulated through heteromultimerization with "silent" K_V5, 6, 8, and 9. Indeed, heteromultimerization with "silent" α subunits appears to modulate K_V current expression, drug sensitivity, as well as biophysical properties such as inactivation.[16]

Blocking of K_V channels with 4-aminopyridine significantly increases myogenic tone.[17] K_V7 are also suggested to play a prime role in hypoxia-induced vasodilation, as XE991, a K_V7-specific inhibitor, abolishes dilation.[18] On the other hand, in pulmonary arteries, K_V1.2, K_V2.1, K_V3.1, and K_V7 resting currents are essential to resting membrane potential and are involved in the hypoxia-induced vasoconstriction of pulmonary arteries.[19,20] Interestingly, despite numerous ex vivo evidence, there is currently little in vivo evidence of a physiological role for vascular K_V channels.[21,22] Although expression of K_V1.3 and 1.5 has been reported in ECs, a role for endothelial K_V channels remains unclear, but a depolarization feedback has been proposed.[23,24] Further investigation is

however necessary to better understand K_V channel function in endothelium.

Calcium-dependent K+ channels

The human genome holds eight genes that encode Ca^{2+}-dependent K+ channels. Based on their genetic relationship, mechanisms of calcium sensing, pharmacology, and single-channel conductances, these channels can be divided into two distinct but only remotely related groups.[25] The first group contains the large-conductance (single-channel conductance >150 pS) Ca^{2+}-dependent BK_{Ca} (K_{Ca}1.1) channel, and the related Slack (K_{Ca}4.1), Slick (K_{Ca}4.2), and Slo3 (K_{Ca}5.1) channels. The latter three are activated by increases in intracellular Na^+ and/or Cl^- or by alkalization rather than by intracellular Ca^{2+} and will not be discussed any further in this review. The second group of Ca^{2+}-dependent channels, the SK/IK (K_{Ca}2.x/3.1) group, contains the three small-conductance (single-channel conductance <50 pS) Ca^{2+}-dependent K+ channels, K_{Ca}2.1 (SK1), K_{Ca}2.2 (SK2), and K_{Ca}2.3 (SK3), as well as the intermediate-conductance (single-channel conductance 50–150 pS) K_{Ca}3.1 (SK4/IK) channel. All four channels from this second group have the same calmodulin-mediated calcium sensing mechanism, which is different to the intrinsic calcium sensing mechanism of the BK_{Ca} channel (see next section). While all K_{Ca}2.x and K_{Ca}3.1 channels are expressed in neuronal tissue, only K_{Ca}2.3 and K_{Ca}3.1 are expressed in vascular tissue, where they play important roles in regulating blood pressure.[26]

K_{Ca}2.3 and K_{Ca}3.1 channels

Functional homomeric K_{Ca}2.3/K_{Ca}3.1 channels are formed by the association of four subunits, each of them containing six transmembrane domains with intracellularly located amino and carboxy termini. It has been suggested that different K_{Ca}2.x channel subunits (K_{Ca}2.1–K_{Ca}2.3) could form heteromeric channels, but this has not been shown in vascular cells.[27] K_{Ca}2.x/K_{Ca}3.1 channels lack voltage sensitivity despite the presence of some charged amino acids in the fourth transmembrane domain, and absence of a Ca^{2+}-binding motif would suggest insensitivity to [Ca^{2+}]. However, K_{Ca}2.x channels are constitutively tethered with calmodulin, working as a Ca^{2+}-sensing subunit, and induce Ca^{2+}-dependent channel opening.[28] Indeed, half maximal effective concentration (EC_{50}) values for [Ca^{2+}] range from 300 to 500 nM,[29] which are similar to the EC_{50} values for calmodulin and are within the physiological range of [Ca^{2+}]$_i$ levels in vascular cells.[28,30] K_{Ca}3.1 channels are also constitutively connected with calmodulin, and their EC_{50} value for

[Ca^{2+}] is about 740 nM.[28,30,31] The lack of voltage dependence allows these channels to remain in an open state at more negative membrane potentials than K$_V$ and BK$_{Ca}$ channels. Consequently, these channels can hyperpolarize the membrane toward values near the K$^+$ equilibrium potential of −89 mV. K$_{Ca}$2.3 and K$_{Ca}$3.1 channels are expressed in vascular ECs that require hyperpolarization to sustain Ca^{2+} influx through transient receptor potential (TRP) channels or Ca^{2+} release from intracellular stores during cellular activation. The transmission of hyperpolarization through gap junctions is known as a component of EDHF.[22,23]

The coexpression of K$_{Ca}$2.3 and K$_{Ca}$3.1 channels in ECs was initially viewed as a redundancy, but a growing body of evidence suggests that the two endothelial channels are located in spatially different microdomains and contribute in different signaling pathways.[5] K$_{Ca}$2.3 is found at interendothelial connections and has been shown to co-localize with TRP channels (Figure 2).

In this position, K$_{Ca}$2.3 has been suggested to detect local Ca^{2+} increases in response to shear stress stimulation.[32] A very recent work by Sonkusare et al[33] shows that activation of as few as three vanilloid (TRPV) family member TRPV4 channels per cell can cause activation of EC K$_{Ca}$2.3 and K$_{Ca}$3.1 channels followed by maximal dilation of pressurized resistance arteries. However, in this study both channels seemed to be involved by TRPV4 channel opening.
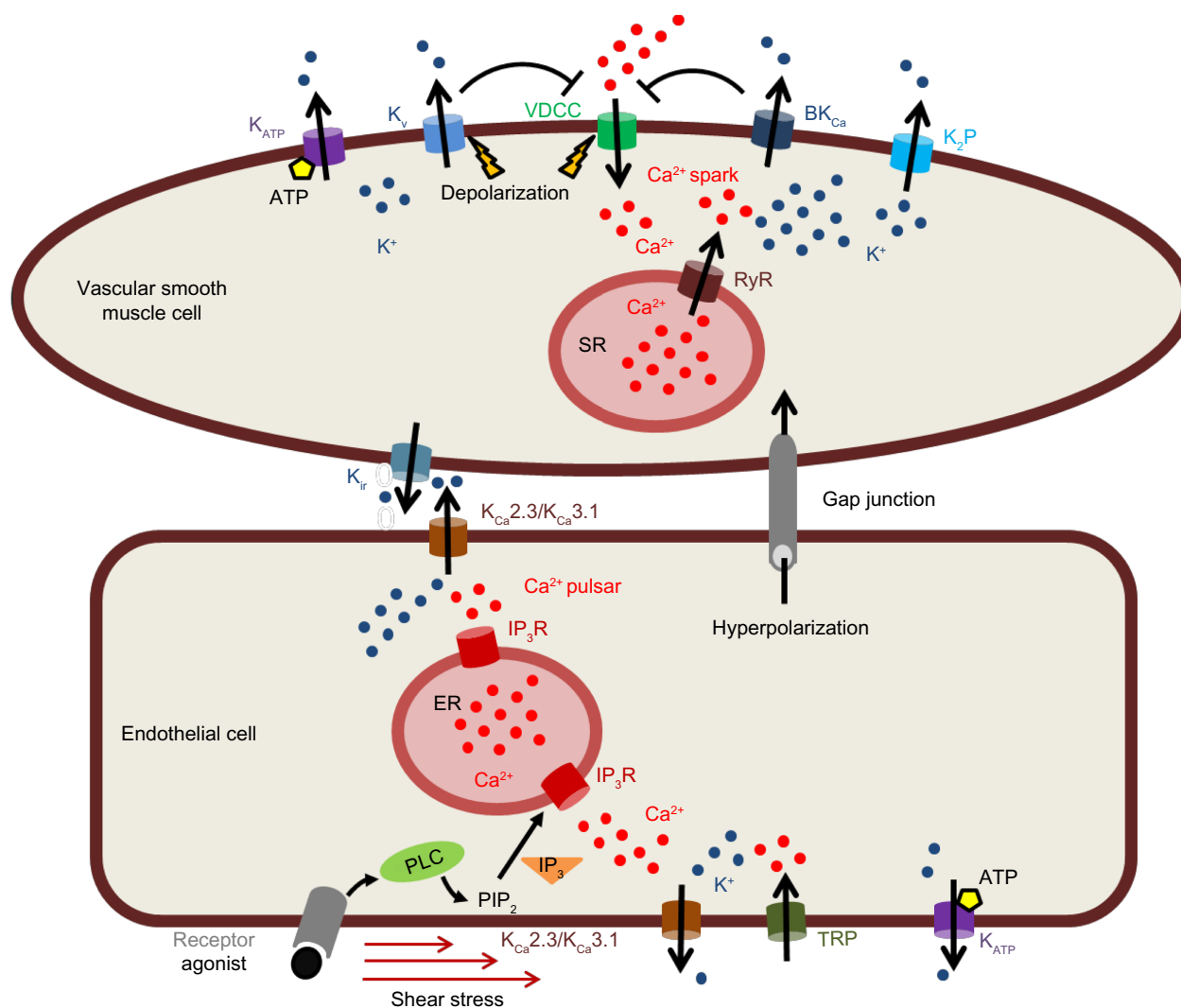

Figure 2 Modulation of membrane potential by K$^+$ channels in vascular cells.
Notes: Endothelial cell: activation of K$_{Ca}$2.3/K$_{Ca}$3.1 channels in response to IP$_3$R-mediated Ca^{2+} release from the ER after receptor stimulation or in response to Ca^{2+} influx through TRP channels, and communication to adjacent smooth muscle cell via gap junctions and K$_{Ca}$2.3/K$_{Ca}$3.1 signaling. Smooth muscle cell: activation of K$_{ATP}$, K$_V$, VDCCs, and K$_2$P channels. Activation of BK$_{Ca}$ channels by RyR-mediated Ca^{2+} sparks from the SR leads to hyperpolarization of the membrane potential and closure of VDCCs.
Abbreviations: ATP, adenosine triphosphate; BK$_{Ca}$, large-conductance Ca^{2+}-activated K$^+$ channel; ER, endoplasmic reticulum; IP$_3$, inositol 1,4,5-trisphosphate; IP$_3$R, IP$_3$ receptor; PLC, phospholipase C; PIP2, phosphatidyl-inositol-bisphosphate; K$_2$P, two-pore domain K$^+$ channel; K$_{ATP}$, ATP-sensitive K$^+$; K$_{Ca}$, Ca^{2+}-activated K$^+$ channel; K$_V$, voltage-gated K$^+$ channel; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; TRP, transient receptor potential; VDCC, voltage-dependent Ca^{2+} channel.

$K_{Ca}3.1$ channels are predominantly localized in EC–VSMC connections, where Ca^{2+} release through inositol 1,4,5-trisphosphate receptors (IP_3Rs) regulates the function of these channels (Figure 2). Ledoux et al[34] have identified local Ca^{2+} release events ("Ca^{2+} pulsars") in vascular ECs, which are mediated by IP_3Rs in the endothelial endoplasmic reticulum (ER) in cellular projection through the internal elastic lamina and making contact to adjacent VSMCs to pass on EDHF through gap junctions. The authors showed that the endothelium-dependent vasodilator acetylcholine elevated IP_3 followed by increased frequency of Ca^{2+} pulsars, whereas blunting IP_3 signaling or depleting ER Ca^{2+} inhibited these events. $K_{Ca}3.1$ channels also co-localized to the endothelial projections, and blocking these channels caused cellular depolarization. Inhibition of Ca^{2+} pulsars also depolarized ECs, but additional block of $K_{Ca}3.1$ channels had no further effect in the absence of pulsars.[34]

Despite different intracellular localization of $K_{Ca}2.3$ and $K_{Ca}3.1$ channels and their different signal transduction pathways, activation of either channel initiates hyperpolarization and subsequent vasodilatory responses through EC hyperpolarization spreading to the underlying VSMCs, promoting closure of VDCCs, and ensuring relaxation and vasodilation.[5,35] The significance of this $K_{Ca}2.3/K_{Ca}3.1$-mediated EDHF for the systemic regulation of blood pressure is demonstrated by the observation of an elevated blood pressure in $K_{Ca}3.1$- and/or $K_{Ca}2.3$-deficient mice.[32] Particularly, deficiency of either channel increases mean arterial blood pressure (MAP) by 7–9 mmHg. Lack of $K_{Ca}2.3$ increases MAP by increasing systolic pressure (SP) as well as diastolic pressure (DP) but without a change in pulse pressure (PP), whereas lack of $K_{Ca}3.1$ seems to increase MAP because of a higher SP as well as a higher PP, but at a normal DP.[36] Interestingly, lack of both channels increased SP, DP, and PP, but did not increase MAP further, suggesting other compensatory mechanisms. These findings suggest that the blood pressure changes caused by the deficiency of either channel are not additive and that the loss of one channel cannot be compensated by the other.

Normal (quiescent) contractile VSMCs do not express $K_{Ca}2.3$ or $K_{Ca}3.1$ channels.[36] Instead, the majority of Ca^{2+}-dependent K^+ currents in these cells is carried by BK_{Ca} channels. However, the expression pattern of Ca^{2+}-dependent K^+ channels changes drastically if VSMCs undergo phenotypic changes. For instance, proliferation of smooth muscle cells is accompanied by an upregulation of $K_{Ca}3.1$ as determined by mRNA and protein expression of this channel.[37–39] This phenotypic change can trigger restenosis after angioplasty and atherosclerosis as stimulation of $K_{Ca}3.1$ expression was also detected in proliferating neointimal smooth muscle cells in balloon-catheterized coronary arteries of swine and carotid arteries of rats.[36–39] Furthermore, induction of $K_{Ca}3.1$ expression was also observed in human neointima smooth muscle cells from coronary bypass vessels.[40] These findings suggest that induction of $K_{Ca}3.1$ expression in atypical smooth muscle growth is a common feature and an indicator of a phenotypic alteration in a number of animal species as well as in humans.[41]

BK_{Ca} channel

Functional BK_{Ca} channels are heteromultimers consisting of four α-subunits and four β-subunits.[42] The α-subunit contains eleven hydrophobic domains (S0–S10), with an extracellular amino terminus and an intracellular carboxy terminus. S0–S6 are located in the cytoplasmic membrane, with a pore domain between S5 and S6.[42] Like other voltage-gated channels (see section on K_v channels), the S4 most likely functions as the voltage sensor of the channel. Several studies have suggested that the α-subunit has also an intrinsic Ca^{2+} sensor, a "Ca^{2+} bowl" located in the tail region of the protein (for a detailed review of these studies see Knaus et al, 1994).[42] As mentioned previously, only one gene encodes for the α-subunit, but many splice variants with different properties were detected. The existence of splice variants could explain different BK_{Ca} channel properties like voltage sensitivity or phosphorylation by different kinases.[43,44] In blood vessels, BK_{Ca} channels are primarily expressed in smooth muscle cells. Only a few studies have shown expression of the channel in the endothelium of pig (summarized in Köhler and Ruth, 2010).[36] However, the BK_{Ca} channel conducts most of the Ca^{2+}-sensitive K^+ currents in healthy contractile VSMCs. The β-subunit of the BK_{Ca} channel contains two transmembrane domains with a long extracellular linker, while the amino and the carboxy termini are located in the cytoplasm. Four β-subunit genes have been identified, but the predominant subunit in VSMCs is the β1 isoform.[45] The β1-subunit increases the voltage and calcium sensitivity of the channel by interacting with the α-subunit.[46]

In VSMCs, BK_{Ca} channels regulate the membrane potential by producing spontaneous transient outward currents (STOCs) in response to highly localized releases of Ca^{2+} (Ca^{2+} sparks) through ryanodine receptors (RyR) from the sarcoplasmic reticulum (SR) (Figure 2).[47] This spark-to-STOC coupling is only possible when BK_{Ca} channels, located in the cell membrane, and the RyR, located in the SR membrane, are in very close proximity.[48] One single Ca^{2+} spark, which measures roughly 2 μm in diameter, generates

a spatially restricted (1% of the cell) large increase in $[Ca^{2+}]_i$ (>10 μM), while the global Ca^{2+} is raised by only 2 nM.[47,48] At negative membrane potentials, BK_{Ca} channels are normally less sensitive to $[Ca^{2+}]_i$ than $K_{Ca}2.3/K_{Ca}3.1$ channels and thus are less active around resting membrane potential of VSMCs.[49,50] But BK_{Ca} channels are also activated by membrane potential depolarization. The voltage-sensitivity is variable, as it is modulated by $[Ca^{2+}]_i$.[51] Increase of $[Ca^{2+}]_i$ shifts the voltage-dependent parameters to more negative voltages and allows the channel to open under a physiological range of membrane potentials.[52,53]

The hyperpolarization resulting from the BK_{Ca} channel activation provides an important negative feedback on Ca^{2+} influx by closing the VDCCs and thus counteracting vasoconstriction. Therefore, BK_{Ca} channels in VSMCs are deeply involved in blood pressure control and have been associated with other EDHF-dilator responses, as BK_{Ca} channels are the anticipated targets of diffusible EDHFs (eg, EETs, H_2O_2, and NO).[54,55] However, it is currently not clear whether lack of BK_{Ca} channels results in a loss of blood vessel dilation mediated by these diffusible EDHFs.

Nevertheless, the relevance of BK_{Ca} channels in regulating blood pressure is demonstrated by the following studies using BK_{Ca} channel knockout mice and a variety of hypertensive animal models. BK_{Ca} channel deficiency causes mild hypertension, and accordingly, hypertensive disease models show a reduced BK_{Ca} channel function or expression. Mice deficient of the pore-forming α-subunit have increased systemic blood pressure as a consequence of the loss of the VSMCs hyperpolarizing K^+ current, and by primary hyperaldosteronism.[56] Furthermore, BK_{Ca} channel–deficient mice show substantial erectile dysfunction and reduced sensitivity to the phosphodiesterase-5 inhibitor sildenafil.[57,58] VSMCs from mice lacking the auxiliary β1-subunit of the BK_{Ca} channel also show reduced numbers of STOCs and thus have a more depolarized resting membrane potential. Consequently, vascular smooth muscle contractility is augmented and the systemic arterial blood pressure is elevated in these mice.[46,59] In contrast, decreased BK_{Ca} channel function in VSMCs has been described in numerous models of hypertension, and hence, upregulation of BK_{Ca} α-subunits could represent a mechanism to lessen hypertension.[55] In angiotensin II–hypertensive rats and in spontaneous hypertensive rats, reduced BK_{Ca} channel activity in VSMCs has been observed and has been related to a downregulation of the expression of the β1-subunit.[60,61] In L-nitro-arginine-treated hypertensive rats and in rats with pulmonary hypertension, a downregulation of the expression

of the pore-forming α-subunit was demonstrated.[62,63] In aged rats, lower expression levels of both α- and β-subunits have been reported.[64]

Moreover, two single nucleotide-polymorphisms in the human BK_{Ca} channel α-subunit gene (IVS17 + 37T>C and C864T) and one in the β1-subunit gene (E65K) have been identified.[65,66] The genetic changes in the α-subunit gene are associated with increased severity of systolic and general hypertension as well as increased risk of myocardial infarction. Contrarily, the nucleotide-polymorphism in the β1-subunit gene causes enhanced channel activity (gain-of-function mutation) and is protective against diastolic hypertension in post-menopausal women. Consequently, these discoveries undoubtedly demonstrate a substantial role of BK_{Ca} channel α- and β-subunits in controlling blood pressure and prove that both subunits represent possible drug targets for a blood pressure–lowering therapy in hypertension.

Other K^+ channels
K_{ir} and K_{ATP} channels

The pore-forming proteins of both K_{ir} and K_{ATP} channels belong to the same gene family, the inwardly rectifying potassium channels (K_{ir}). Six subfamilies are currently recognized, designated $K_{ir}1.0$ to $K_{ir}6.0$. Their name originates from the steep inward rectification of their current-voltage relation, hence they conduct inward K^+ current much more readily than outward current.[67] This inward rectification is in fact the consequence of intracellular polyamines and Mg^{2+} ions blocking K_{ir} pore in response to depolarizing membrane potential. K_{ir} channel subunits comprise only two transmembrane domains, and functional channels are built by tetramers.[67] Expression studies suggest that one channel of the $K_{ir}2.0$ family ($K_{ir}2.1$, but not $K_{ir}2.2$ or $K_{ir}2.3$), is expressed in VSMCs, and the channel is activated by moderate elevations of extracellular K^+ (from 6 to 15 mM).[67] The elevation of external K^+ causes a graded shift of the voltage-dependency of $K_{ir}2.1$ conductance, which leads to hyperpolarization and vasodilation. This outcome might appear unanticipated, since an increase in extracellular K^+ levels would predict a decrease in the electrochemical gradient for K^+, therefore reducing the overall influence of K^+ channels on the VSMC membrane potential and consequently on the vascular tone. However, activation of the K_{ir} channels exceeds this effect and the overall result of a somehow modest increase in extracellular K^+. The expression of K_{ir} channels is inversely proportional to the artery diameter. Moreover, their K^+ sensitivity makes K_{ir} a powerful metabolic sensor as they can trigger an increase in blood flow in response to K^+ accumulation following neuronal activity

(ie, neurovascular coupling), for example.[68,69] Lack of K$_{ir}$2.1 in mice completely abrogated the K$_{ir}$ currents, and arteries from these animals did not dilate in response to elevated K$^+$.[70] However, whether blood pressure is changed in these animals is not known. Endothelial K$_{ir}$ channels are thought to be involved in the flow-mediated dilation as they are also activated by shear stress, and they can be found in several but not all ECs.[34] Wu et al proposed that cerebral VSMCs also express K$_{ir}$2.2, and K$_{ir}$2.4 expression has been detected in cultured human pulmonary smooth muscle cells.[71,72] However, unequivocal evidence that K$_{ir}$ channels play a relevant role in the regulation of systemic blood pressure is still lacking.

K$_{ATP}$ channels are hetero-octameric complexes comprising four pore-forming subunits (K$_{ir}$6.1 or K$_{ir}$6.2) and four regulatory sulphonylurea receptors (SUR2A and SUR2B), which are proteins from the ATP-binding cassette family.[67] K$_{ATP}$ channels in VSMCs most likely result from the association of SUR2B with K$_{ir}$6.1/K$_{ir}$6.2 subunits (1:1 ratio). Showing a weaker inward rectification than their K$_{ir}$2 family channel counterpart, substantial interest has been directed toward these channels as they exhibit activity under normal physiological conditions and significantly contribute to the control of membrane potential, arterial diameter, and blood pressure regulation.[67,73] Similarly to K$_{ir}$2 channels, K$_{ATP}$ channels are physiologically important through their capacity to couple cellular metabolism and electrical excitability, although different mechanisms are involved. Hence, change in intracellular ATP/ADP ratio is the main stimulus to K$_{ATP}$ channel opening, membrane potential hyperpolarization and vasodilation in conditions of high metabolic activity. Binding of ATP to the K$_{ir}$6 subunits blocks the channel, but this is compensated by the activating influence of the ADP/SUR interaction.[73] Therefore, a decrease in ATP intracellular levels or an increase in ADP cytoplasmic concentration would both result in an increase in K$_{ATP}$ open probability. Furthermore, recent findings also indicate that the ATP/ADP ratio may not be the only physiological regulator of K$_{ATP}$ channels, as the nucleotide sensitivity of the channels is modulated by changes in membrane levels of phosphatidyl-inositol-bisphosphate (PIP$_2$) and fatty acyl CoA esters, phosphorylation by protein kinases as well as by caveolin-1.[74,75] Alternatively, K$_{ATP}$ channels are also sensitive to shear stress, pH, and hyperosmolarity and induce a subsequent vasodilation. Altogether, these studies suggest that K$_{ATP}$ channels can be finely tuned to significantly modulate blood flow in health and disease.

Although K$_{ATP}$ channels are expressed in both VSMCs and ECs, the current understanding of the endothelial function of K$_{ATP}$ channels remains incomplete. Indeed, transgenic mice expressing an endothelial-specific dominant negative K$_{ir}$6.1 subunit show a significant increase in coronary resistance as evidenced by an elevated basal coronary perfusion pressure.[76] This observation suggests that endothelial K$_{ir}$6.1 channel is more important for coronary than systemic circulation. In addition, endothelial K$_{ir}$6.2 channels are suggested to be important in the mechanotransduction of shear stress in the pulmonary microcirculation, since lack of endothelial K$_{ir}$6.2 channel function may cause incorrect adjustment to interrupted blood flow in the lung.[77]

K$_2$P channels

Structurally distinct from other vascular K$^+$ channels, K$_2$P channels are characterized by their two pore regions (P1 and P2; hence their name) per functional unit. Each subunit consists of homo- or heterodimers of four transmembrane domain (M1–M4) subunits. Studies have identified 10 out of the 15 members of the K$_2$P channel family in vascular cells.[78] K$_2$P channels are classified into six separate subfamilies based on their structural and functional properties: TWIK, TASK, TREK, THIK, TALK, and TRESK subfamilies.[78] Numerous research studies have shown that K$^+$ channels of the K$_2$P channel family are expressed in several vascular beds, including cerebral, mesenteric, and pulmonary VSMCs.[79–83] K$_2$P channels are considered responsible for background leak currents and are therefore involved in controlling and stabilizing the membrane potential.[10] Although their gating is independent of membrane potential, K$_2$P channel activity is modulated by a wide variety of factors (eg, chemical stimuli like pH, mechanical stimuli, phospholipids, and polyunsaturated fatty acids).[10] Moreover, the sensitivity of the K$_2$P channels to the different stimuli varies according to the K$_2$P channel class. Interestingly, K$_2$P channels have been suggested as important players in hypoxic vasoconstriction of pulmonary vasculature.[82] Indeed, while hypoxia generally induces vasodilation in most vascular beds, low pO$_2$ (partial pressure of oxygen) will be associated with a vasoconstriction in pulmonary arteries. It has also been suggested that TASK-1 inhibition could be involved in the pathogenicity of ET-1, leading to pulmonary arterial hypertension.[83] TREK-1 appears to be highly expressed in ECs and involved in endothelium-dependent vasodilation. Hence, recent studies using TREK-1 deficient mice showed a reduced NO production and vasodilatory response to bradykinin in cutaneous and cerebral arteries, indicators generally associated with an endothelial dysfunction.[84] Surprisingly, despite this endothelial dysfunction, arterial blood pressure in TREK-1

deficient mice remains unaltered. The authors explained this discrepancy by a more substantial role of TREK-1 in the cerebral circulation, with only a minor role in the regulation of systemic blood pressure. However, opposing results have been reported where no changes have been observed in cerebral vasculature from TREK-1 knockout mice, supporting further studies to better understand the role of K_2P channels in VSMCs.[85]

Future research directions

The key factor in the active regulation of blood pressure is the contractile state of VSMCs, making membrane potential a brilliant tool to manipulate vascular tone in a fast, efficient, and precise manner. K^+ channels are major components in this regulatory process (Figure 2), in both endothelium and VSMCs. The multitude of distinct K^+ channels expressed in the vasculature (Figure 2) allows fine-tuning of the vascular response to the direct environment as well as to ensure a constant blood flow. Moreover, the impressive complexity of the numerous combinations of K^+ channels expressed represents a fascinating toolbox given by Mother Nature to compensate pathological conditions or to respond to various physiological conditions.

Opportunities for future investigations on the regulation of vascular tone, especially through K^+ channels, are numerous. Recent developments of experimental tools will allow us to further push the limit of our understanding of ion channel regulation. We should also better understand the interaction of the proteins with molecules inhibiting or activating the channels, leading to the development of new therapeutic avenues. However, better pharmacological tools may not necessarily be the consequence of more specific compounds, due to possible heteromultimer formation (ie, K_V channels). The microenvironment, as briefly alluded to in this review, is also a critical component of ion channel activity modulation. Indeed, multiprotein complexes including – but not limited to – anchoring proteins will most likely be a target of future studies, as they could result in a better understanding of the different regulatory proteins. Ion channel microenvironment also includes lipid environment and intracellular microdomains (eg, Ca^{2+} and cyclic nucleotides). Evidently, increasing interests focus on dynamic regulation of ion channel function through intracellular microdomains such as Ca^{2+} pulsars, wavelets, sparks, and sparklets.

Finally, in our current pharmacogenomic era, it appears essential to identify genetic regulators of K^+ channel expression to better understand the intrinsic variability in the human genome and its impact on pathophysiology. Again,

such knowledge will be fertile in future developments and will undeniably have a significant clinical impact on improving human conditions and treatments of vascular diseases.

Disclosure

The authors have nothing to disclose.

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