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Chapter

Ion Channels and Their Regulation in Vascular Smooth Muscle

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Abstract

Vascular smooth muscle excitability is exquisitely regulated by different ion channels that control membrane potential (E_m) and the magnitude of intracellular calcium inside the cell to induce muscle relaxation or contraction, which significantly influences the microcirculation. Among them, various members of the K^+ channel family, voltage-gated Ca^{2+} channels, and transient receptor potential (TRP) channels are fundamental for control of vascular smooth muscle excitability. These ion channels exist in complex with numerous signaling molecules and binding partners that modulate their function and, in doing so, impact vascular smooth muscle excitability. In this book chapter, we will review our current understanding of some of these ion channels and binding partners in vascular smooth muscle and discuss how their regulation is critical for proper control of (micro)vascular function.

Keywords: ion channels, signal transduction, vascular tone, vascular smooth muscle, microcirculation

1. Introduction

Vascular smooth muscle cells wrapping around small resistance arteries and arterioles are crucial for vascular reactivity [1]. These cells enable dynamic, moment-to-moment control of vessel diameter and pressure-induced contraction (e.g., vascular tone). This control is central to autoregulation of resistance vessels, maintenance of vessel caliber independently of changes in blood pressure, and proper perfusion to meet the metabolic demands of a given tissue.

To regulate arterial diameter, vascular smooth muscle receives and integrates many inputs, including changes in intraluminal pressure, vasoconstrictor and vasodilatory signals from endothelial cells lining the inner arterial wall, and nerve terminals innervating the vessels [2]. These inputs regulate vascular smooth muscle excitability, at least in part, by modulating the activity of a number of ion channels to control membrane potential (E_m) and the magnitude of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) [1]. Among the many ion channels, transient receptor potential (TRP) channels, voltage-gated (K_V), Ca^{2+}-activated (BK_{Ca}) and inward rectifier (K_{ir}) K^+ channels, and voltage-gated Ca^{2+} channels (VGCC) are fundamental in transducing mechanical force, establishing E_m, and regulating $[Ca^{2+}]_i$ [1]. Mechanisms for the regulation of vascular smooth muscle ion channels, including those mentioned above, involve agonist-independent and agonist-dependent activation of G_q and/or G_s protein-coupled receptors (G_xPCR) [3]. The optimal

activation and regulation of these mechanisms are often dependent on the formation of macromolecular complexes driven by scaffold proteins to target and compartmentalize proteins/signaling events to specific regions and substrates [4].

In this chapter, we provide a brief overview of our current understanding of the role of different subunits of TRP channels, several K^+ channel subtypes, as well as key VGCCs in control of vascular smooth muscle excitability and (micro)vascular function. We will also discuss their regulation by signaling pathways and macromolecular complexes. Note that many more studies than those cited here can be found in the scientific literature. Comprehensive studies exploring additional aspects of vascular ion channel regulation, vascular smooth muscle excitability, and (micro) vascular function in health and disease can be found in recent reviews [1, 5–9].

2. TRP channels

The TRP channel superfamily is composed of 28 members divided into 6 subfamilies based on their molecular and biophysical properties [10]. Functional TRP channels are composed of four subunits, each with six membrane-spanning helices that can exist in a homomeric or heteromeric form. Vascular smooth muscle cells express a number of these channels, including members of the TRPC, TRPV, TRPM, and TRPP subfamilies [7, 9]. The function of TRP channels in vascular smooth muscle ranges from regulating contractility to modulating the proliferative state of the cells. In this section, we focused our discussion on the functional role and regulation of specific TRP channels in vascular smooth muscle excitability.

2.1 Functional role of vascular smooth muscle TRP channels

The function of different TRP channels in vascular smooth muscle has been unmasked using conventional and innovative molecular, pharmacological, and genetic approaches. These approaches revealed distinctive roles of specific TRP channel subfamilies in modulating vascular smooth muscle excitability and vascular reactivity. For instance, studies have found that TRPC3 channels are not essential for pressure-induced constriction. Yet, TRPC3 channels play a key role in receptor-mediated vasoconstriction of resistance arteries upon activation of various G_qPCRs, including purinergic receptors, endothelin (ET_A) receptors, and angiotensin II (AT_1) receptors [7, 11, 12]. The mechanisms for TRPC3 channel activation involve direct coupling of the channel with IP_3 receptors located in the sarcoplasmic reticulum (SR) in a process that does not require SR Ca^{2+} release [13]. TRPC5 channels, in association with TRPC1 channels, have been shown to contribute to store-operated Ca^{2+} entry in vascular smooth muscle from arterioles [14], which may modulate cell excitability. Expression of TRPP channels in vascular smooth muscle contributes to stretch-activated cation currents, which causes vascular smooth muscle membrane depolarization. The activity of these channels has been associated with stretch-dependent regulation of vascular tone in cerebral arteries and control of systemic blood pressure [15, 16]. The nonselective cation channel TRPC6 is involved in Ca^{2+} mobilization leading to vascular smooth muscle contraction [17]. More recently, these channels have been shown to be part of a mechanosensation complex [18]. In this model (see **Figure 1**), stretch is "sensed" by AT_1 receptors. These receptors then induce the activation of TRPC6 channels to bolster Ca^{2+} release from the SR, which triggers TRPM4 channel activity and vascular smooth muscle contraction. This model reveals an exquisite and finely orchestrated macromolecular complex for control of stretch-induced contraction.

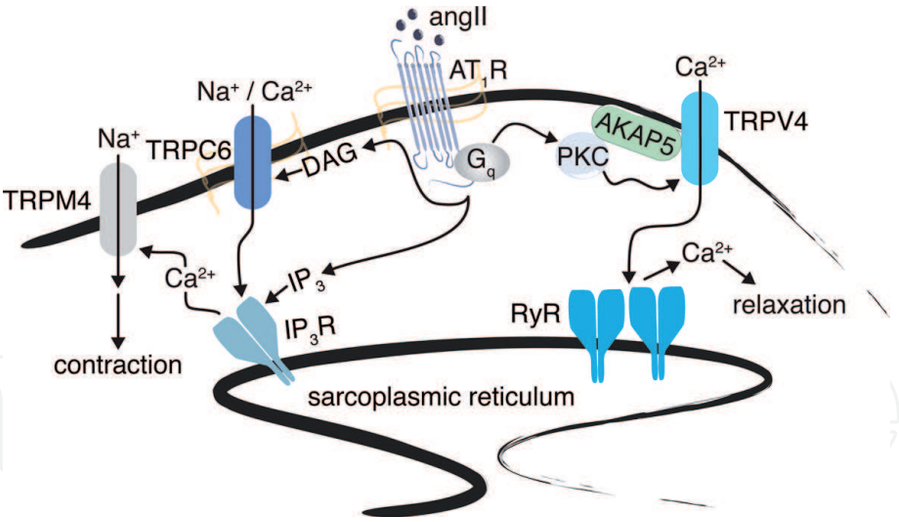

Figure 1.
Regulation of vascular smooth muscle excitability by TRP channels. Diverse TRP channels contribute to vascular smooth muscle relaxation or contraction. Ca^{2+} influx via TRPV4 channels is associated with an increase in the frequency of Ca^{2+} release through ryanodine receptors (RyR) located in the sarcoplasmic reticulum (e.g., Ca^{2+} sparks). The production of Ca^{2+} sparks triggers the activity of adjacent BK_{Ca} channels at the surface membrane to promote K^+ efflux, membrane potential hyperpolarization, and vascular smooth muscle relaxation [18]. TRPV4 channel activity can be stimulated by activation of AT_1 receptors (AT_1R) by angiotensin II (angII) via an AKAP5-mediated PKC signaling pathway [20, 25]. This AT_1R/AKAP5/PKC/TRPV4 axis may serve as a negative feedback mechanism to offset stimulation of L-type Ca^{2+} channel $Ca_V1.2$ (LTCCs) leading to contraction in response to angII. AT_1R, independent of angII, has also been implicated in a mechanosensitive pathway that activates TRPC6 channels to boost cytosolic Ca^{2+} via stimulation of IP_3 receptors (IP_3R). This increase in cytosolic Ca^{2+} promotes the activity of TRPM4 channels leading to vascular smooth muscle membrane depolarization and subsequent contraction in response to stretch [17]. The orange wavelike lines highlight stretch-sensing proteins. The model was generated by taking in consideration studies cited and described above. Na^+ = sodium; Ca^{2+} = calcium; AKAP5 = A kinase anchoring protein 5; PKC = protein kinase C; DAG = diacylglycerol; IP_3 = inositol trisphosphate.

Contrary to the contractile influences of the TRP channels described above, activation of TRPV4 channels is associated with vascular smooth muscle relaxation (**Figure 1**) [19]. The underlying mechanism involves the formation of a macromolecular complex between TRPV4 and BK_{Ca} channels in the plasma membrane and ryanodine receptors (RyR) in the SR. This complex does not depend on direct protein-protein interactions between TRPV4 and BK_{Ca} channels and RyR but requires their close physical proximity. The close association between these proteins facilitates Ca^{2+} influx via TRPV4 channels that stimulates RyR activity resulting in the generation of Ca^{2+} sparks. The ensuing Ca^{2+} sparks activate BK_{Ca} channels leading to vascular smooth muscle membrane potential hyperpolarization and relaxation [20]. Because of the high Ca^{2+} permeability of TRPV4 channels in vascular smooth muscle [21], they could potentially activate adjacent BK_{Ca} channels directly to regulate cell excitability and vascular reactivity. Examination of this exciting possibility is an excellent opportunity for further research. Moreover, additional work is needed to comprehensively define the role of TRP channels in vascular smooth muscle in the microcirculation during physiological and pathological conditions.

2.2 Regulation of TRP channels in vascular smooth muscle

It has been well documented that agonist-dependent activation of G_qPCRs/protein kinase C (PKC) and G_sPCRs/protein kinase A (PKA) signaling regulates TRP channels [10]. Protein kinase G (PKG) modulation of TRP channels has also been reported. However, how these signaling pathways control TRP channel activity in vascular smooth muscle, as well as the underlying consequences in vascular

reactivity are not well understood. A limited number of studies have shown that PKG inhibits TRPC3 channels and that this may contribute to nitric oxide (NO)-mediated relaxation, but the mechanisms require further examination [22]. PKA signaling stimulates the activity of several members of the TRPV channel subfamily while inhibiting TRPC5 and TRPC6 channels in vascular smooth muscle [7]. PKA-mediated inhibition of TRPC6 channels in response to agonist stimulation was shown to be dependent on phosphorylation of the TRPC6 subunit at threonine 69, which resulted in a reduction in angiotensin II (angII)-induced vasoconstriction [23], thus revealing a comprehensive mechanism for the regulation of TRP channels and the underlying effects in vascular reactivity.

PKC activity inhibits TRPC3 channels and activates TRPM4 and TRPV4 channels in smooth muscle [7, 9, 21, 24]. TRPM4 activation by PKC proceeds, at least in part, by stimulating channel trafficking and membrane translocation via a mechanism requiring PKCδ [25]. This PKCδ-dependent anterograde TRPM4 trafficking is functionally relevant as it promotes vascular smooth muscle contraction. PKC-dependent regulation of TRPV4 channels has been suggested to be critical for counteracting the vasoconstriction stimulated by angII [21]. Intriguingly, TRPV4 channels are found in complex with the scaffold A kinase anchoring protein 5 (AKAP5 = human AKAP79 and murine AKAP150) in vascular smooth muscle (**Figure 1**) [21, 26]. This scaffold protein provides a platform for targeting and compartmentalization of signaling molecules (e.g., PKA, PKC, protein phosphatase 2B = PP2B) to specific substrates (e.g., ion channels) [4]. With a suggested distance between them of ~200 nm [26], optimal AKAP5-anchored PKC modulation of TRPV4 activity is highly dependent on the distance between the targeted kinase and the ion channel. This tight regulation of TRPV4 activity by AKAP5 may be essential for vascular smooth muscle excitability given the high Ca^{2+} permeability of these channels, as mentioned previously. Mechanisms regulating TRPP channels by PKA and/or PKC in vascular smooth muscle are currently unclear. The studies discussed above reveal the complex functional role of TRP channels in vascular smooth muscle and how their regulation alters vascular function and highlights unique opportunities for further research. For instance, it will be important to define the role of the AKAP5/PKC/PKA complex and its association with other TRP channels in regulating vascular smooth muscle excitability and vascular reactivity. It also remains to be determined whether dynamic trafficking of other TRP channels and the involvement of the AKAP5/PKC/PKA complex in this process also play a role in fine-tuning vascular reactivity.

3. K^+ channels

The activity of K^+ channels determines vascular smooth muscle membrane potential and is therefore key regulators of vascular tone [1]. By setting and controlling the membrane potential, these channels influence the levels of $[Ca^{2+}]_i$ and therefore, vascular smooth muscle contraction. Intriguingly, a subset of K^+ channels has also been linked to regulation of vascular smooth muscle proliferation (see recent review on this topic in [27, 28]). Vascular smooth muscle cells express a wide variety of isoforms from several classes of K^+ channels, including K_V, BK_{Ca}, and K_{ir} channels (see **Figure 2**). In the following sections, we will describe the expression, function, and regulation of these K^+ channels and their control of vascular function.

3.1 K_V channels

A number of K_V channels isoforms are expressed in vascular smooth muscle, including members of the K_V1 ($K_V1.1$, $K_V1.2$, $K_V1.3$, $K_V1.5$, $K_V1.6$), K_V2 ($K_V2.1$), and

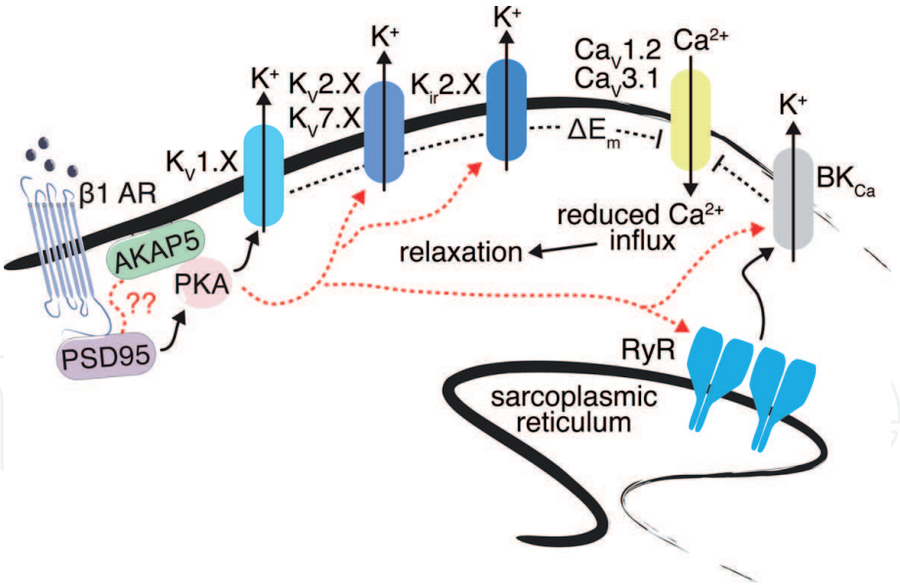

Figure 2.
Regulation of vascular smooth muscle excitability by K+ channels. Vascular smooth muscle cells express a number of K^+ channels that, upon activation, provide negative feedback regulation of membrane potential and vascular tone. K^+ channel activation results in efflux of K^+ ions that hyperpolarize the membrane potential (ΔE_m; denoted by dotted black lines) leading to a reduction in the open probability of LTCC $Ca_V1.2$ and TTCC $Ca_V3.1$ that results in a decrease in global $[Ca^{2+}]_i$ leading to vascular smooth muscle relaxation [32, 95]. As mentioned in ***Figure 1****, activation of ryanodine receptors (RyR) K^+ channels can be distinctively regulated by vasoactive agents acting through the G_s/AC/PKA, NO/sGC/PKG, and G_q/PLC/PKC axes to either increase or decrease their activity. An example is the regulation of $K_V1.X$ channels by β adrenergic signaling (e.g., through β1 adrenergic receptors) [44–46]. Here, $K_V1.X$ channel regulation by β1 adrenergic signaling requires PKA targeting to the channel by postsynaptic density protein 95 (PSD95). Whether PSD95 as well as further scaffolding of proteins by AKAP5 is required for PKA regulation of $K_V1.X$ and other K^+ channels (e.g., $K_V2.X$, $K_V7.X$, $K_{ir}2.X$) as well as RyR is unclear (denoted by dotted red lines in the cartoon). The model was generated by taking in consideration studies cited and described above. K^+ = potassium; Ca^{2+} = calcium; AKAP5 = A kinase anchoring protein 5; BK_{Ca} = large-conductance Ca^{2+} activated potassium channels; AC = adenylyl cyclase; PKA = protein kinase A; NO = nitric oxide; sGC = soluble guanylyl cyclase; PKG = protein kinase G; PLC = phospholipase C; PKC = protein kinase C.*

K_V 7 (K_V7.1–5), as well as several silent K_V subunits (e.g., K_V9.3) [1]. K_V channels are formed by a homo- or heterotetrameric assembly of pore-forming α subunits and regulatory β subunits. Key examples with functional relevance in vascular smooth muscle excitability from multiple vascular beds are K_V channels assembled by K_V1.2/K_V1.5 subunits, K_V2.1 subunits with K_V9.3, and K_V1/K_V7 subunits. These subunit compositions confer functional and pharmacological diversity essential for fine-tuning vascular smooth muscle excitability and vascular reactivity [29–31]. The mechanisms for this involve activation of K_V channels by a depolarizing stimulus (e.g., stretch-induced depolarization) and their regulation by vasoactive agents [1, 32, 33]. The resultant K^+ efflux hyperpolarizes the membrane potential of vascular smooth muscle leading to a decrease in the open probability of voltage-gated L-type Ca^{2+} channels $Ca_V1.2$ (LTCCs), which contributes to decrease $[Ca^{2+}]_i$ and relaxation (**Figure 2**). Conversely, their inhibition depolarizes the membrane potential, which will increase the open probability of LTCCs and lead to an increase in global $[Ca^{2+}]_i$ and vascular smooth muscle contraction. This negative feedback regulation, together with other K^+ channels, is essential for fine control of vascular smooth muscle excitability and vascular reactivity. However, in the context of the microcirculation, not much is known regarding the expression, functional composition, physiological role, and regulation of K_V channels in arteriolar vascular smooth muscle. Consideration of these issues is important as recent studies have implicated that impairment in K_V channel expression and/or function in the development of channelopathies are associated with small vessel

diseases [34]. Moreover, additional pathologies such as hypertension, metabolic disorders, and diabetic hyperglycemia impair (micro)vascular function, at least in part, by altering the expression/function of K_V channels, but mechanisms remain not fully understood (see review in [5]).

Many vasoactive agents modulate vascular function by acting on K_V channels expressed in vascular smooth muscle. For instance, agents that trigger activation of G_qPCRs, such as angII, phenylephrine, and endothelin 1, are known to stimulate vasoconstriction, at least in part, by decreasing the expression and/or function of K_V channels, particularly those of the K_V1, K_V2, and K_V7 subfamilies (**Figure 2**) [1, 35–37]. The effects of these vasoconstrictors are related to PKC-mediated phosphorylation and/or changes in surface expression of K_V subunits [36, 38–40], perhaps via engagement of different PKC isoforms [41]. This may contribute to selective control of K_V channel activity in response to different stimuli. In addition, increases in $[Ca^{2+}]_i$ have also been associated with inhibition of K_V channels [42, 43]. Considering that activation of G_qPCRs also increases $[Ca^{2+}]_i$, these data suggest that both PKC activity and elevated $[Ca^{2+}]_i$ could synergize to exacerbate K_V channel inhibition, which will result in membrane potential depolarization, activation of LTCCs, and vascular smooth muscle contraction.

In stark contrast to G_q signaling, activation of NO/PKG and G_s/PKA signaling is typically associated with stimulation of vascular K_V channels, including the members of the K_V1 and K_V7 subfamilies [1]. The functional consequence of this regulation is vascular smooth muscle relaxation. Phosphorylation of K_V channels by PKA is opposed by protein phosphatases such as PP2B, which will dephosphorylate the different subunits [44]. Whether G_sPCR/PKA/PP2B regulation of K_V channels requires scaffolding proteins that could target all components of the signaling complex within close proximity to the channels is not well understood. A recent series of studies have demonstrated that the scaffold protein postsynaptic density 95 (PSD95), which was thought to be a neuronal-specific protein, is expressed in vascular smooth muscle (**Figure 2**) [45, 46]. Intriguingly, PSD95 was found to be necessary for basal- and isoproterenol-induced PKA-mediated activation of $K_V1.X$ channels that resulted in vascular smooth muscle relaxation [45–47]. This was due to the formation of a distinctive PSD95-mediated signaling complex involving the β1-adrenergic receptor (β1 AR)-, PKA-, and $K_V1.2$-containing channels [45, 47]. Since PSD95 is associated with AKAP5 in neurons [48], the argument was made that the PSD95-AKAP5 complex may be essential for PKA targeting and regulation of $K_V1.2$ function and that this will have an impact on vascular smooth muscle excitability and vascular reactivity [47]. However, additional studies have found that K^+ currents produced by $K_V1.X$ and $K_V2.X$ subunits and BK_{Ca} channels are of similar magnitude in wild-type and AKAP5-depleted ($AKAP5^{-/-}$) vascular smooth muscle cells [5, 49, 50]. These results suggest that, at least basally, AKAP5 is not necessary, and PSD95 may be sufficient for PKA-dependent regulation of K^+ channels in these cells. β-Adrenergic stimulation has also been found to regulate K_V7 channels leading to vasorelaxation [51], but whether a scaffold protein is mediating these effects is unknown and thus requires further examination. In addition, it is also unclear how K_V2-containing channels, which contribute about 70% of the K_V current in mouse cerebral and mesenteric vascular smooth muscle [49], are regulated by PKA signaling, presenting another area of further research.

3.2 BK_{Ca} channels

BK_{Ca} channels are abundantly expressed in vascular smooth muscle cells [52]. The pore-forming α subunit (BK_{Ca} α1.1) assembles into tetramers to form a functional channel, but unlike TRP and K_V subunits, it contains seven transmembrane

domains and a heavily regulated long carboxyl terminal. Further regulation of BK_{Ca} channel function is conferred by the association of the α subunit with accessory β1 and LRRC26 γ subunits, which increases the Ca^{2+} sensitivity of the channel [52]. BK_{Ca} channels are sensitive to voltage and Ca^{2+}. The Ca^{2+} source to activate BK_{Ca} channels comes from the release of Ca^{2+} from the SR via RyRs (**Figure 2**) [20]. This complex does not depend on direct protein-protein interactions between BK_{Ca} channels and RyR but requires that both of these ion channels are in close physical proximity to each other. As mentioned above, data also have suggested that TRPV4 is responsible for triggering RyR activity [19], and thus, these channels have been proposed to form part of the same signaling complex with BK_{Ca} and RyRs in vascular smooth muscle. Intriguingly, LTCCs have been found to have an indirect or "loose" coupling with RyRs that could enable their activation [53], perhaps by modulating the SR Ca^{2+} concentration [54]. Additional components of the same or similar signaling complexes (see T-type Ca^{2+} channels section) with divergent roles have been identified [1, 7, 13, 55], but an integrated model remains to be defined. Regardless, the activation of these channels should result in K^+ efflux that hyperpolarizes the plasma membrane of vascular smooth muscle cells leading to relaxation. The physiological influence of BK_{Ca} channels in control of membrane potential and vascular tone, however, seems to be species-, stimulus-, and vessel-dependent, even when compared between different orders of the same vascular tree. Indeed, studies have reported a clear involvement of BK_{Ca} channels in tonic negative feedback regulation of membrane potential and pressure-induced constriction, even in human resistance arteries, whereas others have failed to establish a relationship (exemplary studies in [56–62]). The differences may be associated with disparities in the BK_{Ca} channel's Ca^{2+} sensitivity and/or subunit expression levels and composition in the different vascular beds. A recent and broader discussion on this topic can be found in [1]. Thus, given the distinctive role of BK_{Ca} channels in the vasculature, further research on how these channels are regulated to control vascular tone in different vascular beds during physiological and pathological conditions is warranted.

Generally, vasoactive agents that act via activation of the G_s/adenylyl cyclase (AC)/PKA and NO/soluble guanylyl cyclase (sGC)/PKG axes potentiate BK_{Ca} channel activity, whereas those acting through the G_q/phospholipase C (PLC)/PKC axis inhibit the channel [1, 52]. Vasoactive agents may also regulate BK_{Ca} channel activity indirectly by modulating the function of RyR in the SR or other ion channels (e.g., L-type $Ca_V1.2$ and T-type $Ca_V3.2$ channels) in the plasma membrane that are involved with direct activation of RyR or SR Ca^{2+} refilling [54, 63, 64]. Although there is some evidence that scaffold proteins such as AKAP5 may help target signaling molecules to BK_{Ca} channels [65], whether this indeed occurs in native vascular smooth muscle cells, as well as its functional relevance, is unclear. Considering the role of BK_{Ca} channels in negative feedback regulation of vascular tone, the physiological implications of vasoactive agents acting through distinct G_xPCRs on BK_{Ca} channels will be either vascular smooth muscle relaxation or contraction.

3.3 K_{ir} channels

As their name implies, K_{ir} channels produce an inward current. This current is observed at a potential negative to the K^+ equilibrium potential that helps stabilize the resting E_m [1, 66]. They also produce a small outward current at depolarizing potentials that serves as an electrical amplifier to magnify hyperpolarization. Inward rectification occurs due to voltage-dependent blockade of the channel by polyamines and Mg^{2+}. K_{ir} channels are regulated by lipids (e.g., phosphatidylinositol 4,5 bisphosphate (PIP_2) and cholesterol) [1, 66]. Intriguingly, a recent study found that cholesterol, but not PIP_2, regulates K_{ir} channel activity in the cerebral

vascular smooth muscle [67], suggesting that these channels may be distinctively modulated by lipids depending on their tissue distribution. A functional K_{ir} channel is formed when four pore-forming α subunits, each containing two membrane-spanning domains, come together. Two main α subunits (e.g., $K_{ir}2.1$ and $K_{ir}2.2$) have been identified in vascular smooth muscle from multiple species [68–71]. Intriguingly, the expression of these subunits in a specific vascular bed may be species-dependent. Accordingly, although K_{ir} subunit expression and channel activity have been extensively reported in murine cerebral vascular smooth muscle [67–69, 71], minimal, if any, K_{ir} subunit expression and channel activity were found in the human cerebral vascular smooth muscle [72]. The functional implication of the activation of these channels in vascular smooth muscle is relaxation. K_{ir} channel activity can be modulated by vasoactive agents with those acting through the G_q/PLC/PKC axis, inducing channel inhibition, and those acting on the G_s/AC/PKA pathway, promoting channel activity [1]. The physiological relevance of these regulatory mechanisms on K_{ir} channels and their control of vascular function are less well understood and therefore are in need of further evaluation.

4. Voltage-gated Ca^{2+} channels

Vascular smooth muscle cells express several subtypes of VGCCs [9]. These channels have been shown to be important for vascular smooth muscle contraction, and some subtypes have been implicated in relaxation mechanisms. In this section, we will focus on the role of two key subtypes of VGCCs, namely, LTCCs and T-type Ca^{2+} channels (TTCCs), in regulation of vascular smooth muscle excitability.

4.1 L-type Ca^{2+} channel $Ca_V1.2$

The L-type Ca^{2+} channel $Ca_V1.2$ (i.e., LTCCs) is essential for vascular smooth muscle contraction and vascular reactivity. Therefore, they play a key role in controlling blood flow and blood pressure [33, 73]. LTCCs are comprised of a pore-forming α_{1c} subunit and auxiliary β, $\alpha_2\delta$, and γ subunits that modulate channel function and trafficking [74]. The α_{1c} subunit contains four homologous domains (I, II, III, IV). Each domain comprises of six membrane-spanning segments (S1–S6) with intracellular amino- and carboxyl termini, which contain many regions relevant for channel regulation and control of cell excitability. In vascular smooth muscle, expression of the α_{1c} subunit is critical for pressure-induced constriction as evidenced by an absence of myogenic response after LTCC blockade and depletion of the $Ca_V1.2$-α_{1c} subunit in mice (e.g., SMAKO mouse) [33, 73, 75]. The auxiliary subunits α2 and δ are the product of the same gene that gets proteolytically cleaved after translation but remains connected by disulfide bonds, which give rise to the mature subunit. The $\alpha_2\delta$ subunit has been linked to regulation of α_{1c} subunit surface expression that controls Ca^{2+} influx in vascular smooth muscle and the level of myogenic constriction [76]. The β subunit, which remains cytoplasmic, also contributes to the α_{1c} subunit surface expression and channel regulation and therefore can modulate vascular smooth muscle excitability in health and disease [77, 78]. Unlike the other subunits, the expression, regulation, and function of the γ subunit in vascular smooth muscle are unclear and likely the subject of further research.

LTCCs in vascular smooth muscle are distinctively regulated by the G_s/AC/PKA, NO/sGC/PKG, and G_q/PLC/PKC axes [9, 79]. Accordingly, the NO/sGC/PKG signaling axis has been shown to inhibit vascular LTCCs [80]. This has been associated with a reduction in $[Ca^{2+}]_i$ that may be part of the vasodilatory mechanism underlying the activation of this pathway [79]. Receptor-mediated signaling via the

G_q/PLC/PKC axis typically results in potentiation of LTCC activity [9, 79, 81]. The functional effects of this G_q/PLC/PKC-mediated activation of LTCCs are vascular smooth muscle contraction and an increase in vascular tone. Intriguingly, activation of the G_s/AC/PKA axis has been shown to inhibit, activate, or produce no effect on vascular LTCC activity (**Figure 3**) [79]. Irrespectively of this however, PKA signaling has been generally linked with vasodilation, thus raising questions about the functional relevance, if any, of this kinase in the regulation of vascular LTCCs. Intriguingly, recent studies revealed that elevations in extracellular D-glucose (HG) potentiate LTCC activity via a G_s/AC/PKA pathway in vascular smooth muscle [82–85]. This HG-induced PKA-dependent activation of LTCCs resulted in increased global $[Ca^{2+}]_i$ and vasoconstriction, thus providing the first example of a PKA-dependent pathway underlying vascular smooth muscle contraction. Future studies should further examine the in vivo relevance of this pathway.

LTCC regulation by G_s/AC/PKA and G_q/PLC/PKC axes in vascular smooth muscle is mediated by AKAP5 (**Figure 3**) [85, 86]. The involvement of the scaffold in this regulation was initially speculated from total internal reflection fluorescence (TIRF) microscopy experiments that optically recorded the activity of single or clusters of LTCCs [87, 88]. From these experiments, it was clear that the activity and location of functional LTCCs were heterogeneous throughout the surface membrane of vascular smooth muscle cells [81, 87, 89]. Whereas some LTCCs showed stochastic activity with low Ca^{2+} flux and duration of events, others had persistent activity characterized by increased Ca^{2+} flux and events with prolonged open time that were produced by the opening of two or more channels [81, 87, 89–91]. The

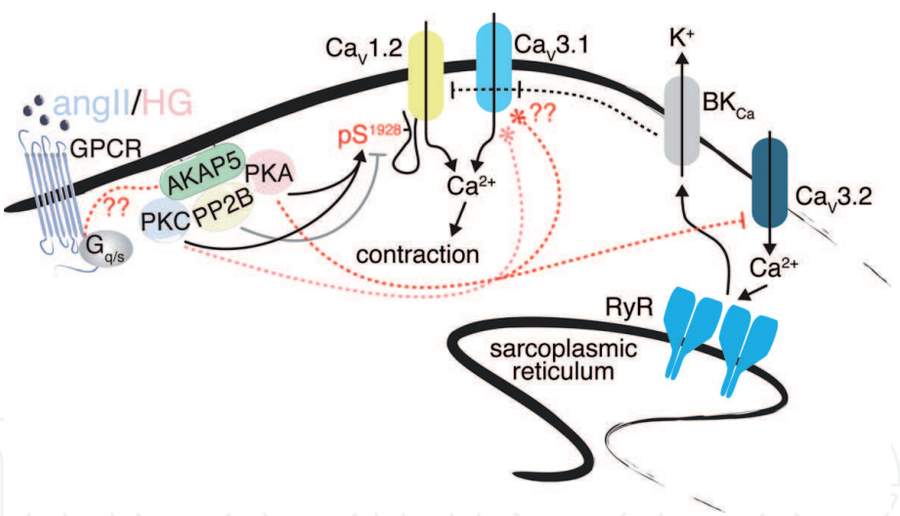

Figure 3.
Regulation of vascular smooth muscle excitability by voltage-gated Ca2+ channels. Ca^{2+} influx via L-type Ca^{2+} channel $Ca_V1.2$ is essential for vascular smooth muscle contraction [33]. Their activity is regulated by G_s/PKA and G_q/PKC signaling pathways upon activation of a specific GPCR by a given stimulus (e.g., angII or HG). The association of $Ca_V1.2$ with these signaling pathways is orchestrated by AKAP5 [86]. PKA (and perhaps PKC) augments L-type Ca^{2+} channel $Ca_V1.2$ activity by increasing $Ca_V1.2$ phosphorylation at serine 1928 (pS^{1928}) [82, 85]. The phosphatase PP2B suppresses enhancement of L-type Ca^{2+} channel $Ca_V1.2$ activity presumably by preventing/opposing channel phosphorylation in vascular smooth muscle cells (gray line). Two TTCC subtypes are expressed in vascular smooth muscle, with $Ca_V3.1$ contributing to contractile mechanisms and $Ca_V3.2$ forming a complex with RyR in the sarcoplasmic reticulum and BK_{Ca} channels in the surface membrane to foster relaxation [95, 100]. Although PKA has been shown to inhibit $Ca_V3.2$, whether this requires AKAP5 function is unclear (dotted red lines with perpendicular line at the end). It is also unclear whether AKAP5-anchored PKC and PKA regulate $Ca_V3.1$ channel activity (dotted light and dark red lines with star near $Ca_V3.1$). Finally, whether the GPCRs activated by angII and HG are targeted to specific complexes by AKAP5 is unclear (red dotted line with ?? symbols). The model was generated by taking in consideration studies cited and described above. GPCR = G-protein coupled receptors; angiotensin II = angII; high glucose = HG; AKAP5 = A kinase anchoring protein 5; protein kinase A= PKA; protein kinase C = PKC; protein phosphatase 2B; PP2B; phosphorylation at $Ca_V1.2$ serine 1928 = pS^{1928}; K^+ = potassium; Ca^{2+} = calcium; ryanodine receptors = RyR.

stochastic and persistent activity of LTCCs was modulated by membrane potential [92]. However, the occurrence of LTCCs with persistent activity is limited to specific regions of the surface membrane and has been demonstrated to be highly dependent on PKC activity and AKAP5 expression [81, 86]. The activity of phosphatases, such as PP2B, that are targeted to the channel by AKAP5, counteracts anchored kinase activity and restricts persistent LTCC activity (**Figure 3**) [89]. Accordingly, in vascular smooth muscle in which PKC is inhibited or cells from mice with genetically depleted PKC or AKAP5, the frequency of persistent LTCC activity is minimal [86, 87, 93]. In addition, PP2B inhibition stimulates persistent LTCC events in cells from wild type but not AKAP5$^{-/-}$ mice, suggesting that removing this "brake" facilitates kinase-mediated potentiation of channel activity [86, 89]. These results suggest an important role for AKAP5-anchored PKC and PP2B activity in modulating basal persistent LTCC activity. The physiological significance of these findings is underscored by data indicating that persistent LTCC events account for 50% of the total dihydropyridine-sensitive (e.g., LTCCs) Ca^{2+} influx at physiological membrane potentials [92], which is critical for vascular smooth muscle contractility in health and disease [82, 84, 86, 93].

4.2 T-type Ca^{2+} channels

T-type Ca^{2+} channels are formed by pore-forming α_1 subunits with similar topology as that of the LTCC α_{1c} subunit, but with no known auxiliary subunits that modulate channel function [74]. TTCCs are activated at more hyperpolarized potentials and show similar conductance with Ca^{2+} or Ba^{2+} as charge carriers. Vascular smooth muscle cells express several TTCC α_1 subunits, including $Ca_V3.1$ (α_{1G}) and $Ca_V3.2$ (α_{1H}) [9, 94–98]. Intriguingly, $Ca_V3.1$, which is found in murine vascular smooth muscle, seems to be replaced by $Ca_V3.3$ (α_{1I}) in human cells [96], suggesting that expression of TTCC α_1 subunits is species-dependent. TTCCs have been shown to contribute to vascular smooth muscle excitability in several vascular beds from different species [9, 98]. However, rigorous analysis revealed that different $Ca_V3.X$ subunits may have very divergent physiological responses. For instance, whereas $Ca_V3.1$ ($Ca_V3.3$) mediates low-pressure-induced constriction, $Ca_V3.2$ contributes to the negative feedback regulation of vascular tone by stimulating the RyR/BK_{Ca} axis (**Figure 3**) [64, 95, 96]. TTCCs can also be regulated by signaling molecules. Indeed, the NO/PKG and AC/PKA axes both inhibit vascular TTCCs [99, 100], which may have key implications in vascular smooth muscle excitability. Whether these signaling molecules are organized and targeted by scaffold proteins such as AKAPs to areas near TTCCs to fine-tune their function is unclear and therefore the subject of future studies.

5. Conclusions

Vascular smooth muscle excitability is exquisitely controlled by a repertoire of ion channels, which in themselves, are regulated by several vasoactive agents. The precise regulation of ion channels in vascular smooth muscle cells is essential for the dynamic adjustment of vascular tone necessary to maintain adequate tissue perfusion and blood pressure. Here, we have provided a brief overview of our current knowledge of key ion channels and their regulation by receptor-mediated signaling pathways that are activated by various vasoactive agents to modulate vascular smooth muscle excitability and therefore vascular tone. We focused on several TRP channels, multiple K^+ channel subtypes, and various classes of VGCCs. We emphasized ion channel regulation by signaling pathways associated with the G_s/AC/PKA,

NO/sGC/PKG, and G_q/PLC/PKC axes given their important role in modulating vascular smooth muscle excitability. When possible, we identified key gaps in knowledge, even in areas that have been extensively studied, which are fertile ground for further research. Because of the importance of all the ion channels and signaling pathways discussed above on vascular control, understanding how they are affected during pathological conditions is essential for the development of rational therapies to treat (micro)vascular diseases.

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Conflict of interest

None.

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