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Ion channel remodeling in vascular smooth muscle during hypertension: Implications for novel therapeutic approaches

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Abstract

Ion channels are multimeric, transmembrane proteins that selectively mediate ion flux across the plasma membrane in a variety of cells including vascular smooth muscle cells (VSMCs). The dynamic interplay of Ca^{2+} and K^+ channels on the plasma membrane of VSMCs plays a pivotal role in modulating the vascular tone of small arteries and arterioles. The abnormally-elevated arterial tone observed in hypertension thus points to an aberrant expression and function of Ca^{2+} and K^+ channels in the VSMCs. In this short review, we focus on the three well-studied ion channels in VSMCs, namely the L-type Ca^{2+} ($Ca_V1.2$) channels, the voltage-gated K^+ (K_V) channels, and the large-conductance Ca^{2+}-activated K^+ (BK) channels. First, we provide a brief overview on the physiological role of vascular $Ca_V1.2$, K_V and BK channels in regulating arterial tone. Second, we discuss the current understanding of the expression changes and regulation of $Ca_V1.2$, K_V and BK channels in the vasculature during hypertension. Third, based on available proof-of-concept studies, we describe the potential therapeutic approaches targeting these vascular ion channels in order to restore blood pressure to normotensive levels.

Keywords

Hypertension; vascular smooth muscle cell; ion channel; calcium channel; potassium channel; gene therapy

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1. Introduction

Hypertension or high blood pressure is a multi-factorial disease that plagues more than 30% of adult Americans and about one quarter of people worldwide [1,2]. Hypertension arises because of complex interactions of various genes with the environment, and is considered a leading risk factor for cardiovascular and kidney diseases [3,4]. Hypertension with unknown etiology, also known as essential, primary or idiopathic hypertension, accounts for 95% of all human hypertension [2]. Blood pressure is determined by two important physical parameters, cardiac output (CO) and total peripheral resistance (TPR). Multiple pathways including the autonomic nervous system, renin-angiotensin system, aldosterone and other vasoactive substances affect CO and TPR to tightly regulate blood pressure and thus ensure appropriate flow of blood to various organs in the body [5–7]. In most clinical cases, CO is normal whereas TPR is elevated due to an abnormal constriction of the small arteries and arterioles [8,9]. The diameter of small arteries and arterioles is maintained mainly by the dynamic interplay of Ca^{2+} and K^+ channels expressed on the plasma membrane of vascular smooth muscle cells (VSMCs) [10]. The opening of K^+ channels in response to endogenous stimuli or pharmacological agents results in an efflux of K^+ from VSMCs, hyperpolarization of the plasma membrane, closure of Ca^{2+} channels, reduced intracellular Ca^{2+} levels and eventually vasodilation. Conversely, closure of K^+ channels depolarizes the plasma membrane resulting in the opening of more Ca^{2+} channels, increased intracellular Ca^{2+} levels, and vasoconstriction. The elevated vascular tone observed in human hypertension and in several experimental models of hypertension thus points to abnormalities in the expression and function of Ca^{2+} and/or K^+ channels in VSMCs [11]. Indeed, the VSMCs are more depolarized as a consequence of the 'ion channel remodeling' that occurs during chronic hypertension [12]. Several families of Ca^{2+} and K^+ channels are expressed in VSMCs (reviewed in [13,14]). In this review, we will only focus on three of the channel types in VSMCs that are reported to be altered in animal models of hypertension: the L-type Ca^{2+} ($Ca_V1.2$) channels, the voltage-gated K^+ (K_V) channels, and the large-conductance, Ca^{2+}-activated K^+ (BK) channels. We will limit our discussion to three important aspects of these channels: 1) their physiological role in VSMCs, 2) the alteration of these channels in VSMCs during hypertension, and 3) their potential as therapeutic targets for the treatment of hypertension.

2. Physiological role of vascular ion channels

VSMCs express different types of ion channels at the sacroplasmic reticulum and the plasma membrane to closely regulate intracellular Ca^{2+} levels, resting membrane potential (E_m) and cell contractility. In VSMCs, E_m is primarily determined by K^+ efflux through several plasma membrane K^+ channels, including the voltage-gated K^+ (K_V) channels and the large-conductance Ca^{2+}-activated K^+ channels, often referred to as "Maxi-K" or "Big K" (BK) channels[10,15]. While there are also inwardly-rectifying K^+ (KIR) channels [16,17], ATP-sensitive K^+ (K_{ATP}) channels [17,18], and two pore domain K^+ (K_{2P}) [19,20] channels present in VSMCs that likely also contribute to the final E_m, these channels are reviewed elsewhere [21]. Voltage-gated L-type Ca^{2+} ($Ca_V1.2$) channels open in response to membrane depolarization. Excitation of VSMCs results in depolarization, which leads to the voltage-dependent opening of $Ca_V1.2$ and K_V channels. The opening of $Ca_V1.2$ channels allows Ca^{2+} influx, causing a rise in global Ca^{2+} and activation of the cellular contractile machinery. The depolarization and the corresponding increase in intracellular Ca^{2+} through $Ca_V1.2$ channels both lead to BK channel opening, which causes a compensatory hyperpolarizing current that closes $Ca_V1.2$ channels to buffer vasoconstriction. The opening of K_V channels also allows K^+ to flow out of the cell and hyperpolarizes the VSMCs [10,15]. In this section, we review the structure and physiological function of these ion

channels in the VSMCs and when appropriate provide a brief comparison to the channels found in cardiomyocytes.

2.1. Voltage-gated L-type Ca^{2+} ($Ca_V1.2$) channels

Voltage-gated, long-lasting "L"-type Ca^{2+} ($Ca_V1.2$) channels are opened by depolarization, show a unitary conductance of 20–30 pS, and are slowly inactivating. Structurally, $Ca_V1.2$ channels are multimeric protein complexes, comprised of pore-forming α_1 subunits and auxiliary β, $\alpha_2\delta$ and γ subunits (Figure 1A). The α_1 subunits have four repeat domains each of which has six transmembrane sections (S1-S6). The α_1 subunits are responsible for voltage sensing, Ca^{2+} permeability, Ca^{2+}-dependent inactivation, and inhibition by Ca^{2+} channel blockers. Cytosolic accessory β subunits, of which there are four isoforms (β1-4), interact with the α_1 subunits to modulate channel properties such as plasma membrane targeting, channel inactivation and voltage-dependent gating [13,22–24]. The β subunits are classified as membrane-associated guanylate kinases, a class of scaffolding protein that has both guanylate kinase and Src homology 3 domains [25,26]. In VSMCs, both β2 and β3 subunits have been identified in bovine [27], mouse [28] and rabbit aortae [29]. More recently, β3 subunits have been identified in mouse mesenteric arteries as an important regulator of $Ca_V1.2$ subunits in hypertension [30]. Also present in the $Ca_V1.2$ complex are auxiliary $\alpha_2\delta$ ($\alpha_2\delta$1-4) and γ subunits, though their functions are less clear. Bannister *et al* observed that inhibition of the $\alpha_2\delta$-1 subunit caused vasodilation of pressurized rat cerebral arteries and reduced $Ca_V1.2$ currents in cerebral artery VSMCs [31]. Moreover, knockdown of $\alpha_2\delta$-1 reduced plasma membrane expression of $Ca_V1.2$, suggesting that $\alpha_2\delta$-1 is important for plasma membrane expression of functional $Ca_V1.2$ channels [31].

$Ca_V1.2$ channels open in response to depolarizing stimuli, which allows Ca^{2+} to flow inside the VSMC to initiate cell contraction and other Ca^{2+}-dependent processes. Calcium-dependent inactivation occurs when increasing intracellular Ca^{2+} causes closure of $Ca_V1.2$ channels, even when depolarization is maintained [32–34]; $Ca_V1.2$ channels can also inactivate *via* voltage-dependent inactivation [35,36]. Both Ca^{2+}-dependent inactivation and voltage-dependent inactivation limit the amount of Ca^{2+} entry into the VSMC, thus buffering constriction. The Ca^{2+}-binding protein, calmodulin, is also important in modulating $Ca_V1.2$ activity. The C-terminal lobe of calmodulin binds to the cytoplasmic carboxyl terminus of the $Ca_V1.2$ channel to cause Ca^{2+}-dependent inactivation. In addition, the amino-terminal lobe of calmodulin can bind to the carboxyl terminus of $Ca_V1.2$ to regulate Ca^{2+}-dependent facilitation of $Ca_V1.2$ [37–39].

Besides being regulated by Ca^{2+}, $Ca_V1.2$ channel activity can also be modulated by other proteins such as scaffolding proteins and signaling molecules [40]. Initially observed in neurons, a scaffolding protein, A-kinase anchoring protein (AKAP), was observed to bind several sites on the α_1 subunit of $Ca_V1.2$, including the amino terminus, the transmembrane I-II linker and the carboxy terminus [41]. This observation was further confirmed in VSMCs where AKAP150 anchors protein kinase C (PKC) to the sarcolemma, and is required for PKC activation of Ca^{2+} sparklets in a subset of $Ca_V1.2$ channels that importantly contribute to Ca^{2+} influx and regulation of $[Ca^{2+}]_i$ [42]. In addition to facilitating opening of $Ca_V1.2$ channels, AKAPs also regulate a Ca^{2+}-dependent negative feedback mechanism of $Ca_V1.2$ channels *via* calcineurin (also known as protein phosphatase 2B) [42,43]. In summary, $Ca_V1.2$ channel activity is tightly regulated in VSMCs by several mechanisms to control $[Ca^{2+}]_i$ and to carefully regulate vascular tone.

In cardiomyocytes, L-type Ca^{2+} channels play a major role in excitation-contraction coupling. $Ca_V1.2$ channels open in response to the depolarization of cardiomyocytes during an action potential. The increase in intracellular Ca^{2+} concentration *via* opening of $Ca_V1.2$ channels triggers Ca^{2+} release from sarcoplasmic ryanodine receptors (RyRs), which

increases intracellular Ca^{2+} sufficiently to allow Ca^{2+} binding to troponin C and initiation of cardiomyocyte contraction. Structurally, cardiac voltage-gated Ca^{2+} channels have similar subunit composition to vascular voltage-gated Ca^{2+} channels, with the α_{1C} ($Ca_V1.2$) subunit being the most abundant cardiac α subunit. Low voltage-activated Ca_V3 channels (T-type voltage-gated Ca^{2+} channels, α_{1G} and α_{1H}) are also expressed in the heart, but their expression is mainly limited to the sinoatrial pacemaker cells and Purkinje fibers, where they contribute to setting the frequency of action potential firing [44,45]. In cardiomyocytes, auxiliary β1, β2 and β3 subunits are expressed [46], and in the adult human myocardium, five β2 splice variants, $\beta2_a$ – $\beta2_e$ have been identified, with varying effects on channel activation and inactivation kinetics [47]. Other important auxiliary subunits that affect channel function are the $\alpha_2\delta$ and γ subunits. In cardiomyocytes, $\alpha_2\delta$ affects voltage dependence, current kinetics, and is required for normal cardiac excitation-contraction coupling. Differently from $\alpha_2\delta$ in VSMCs, cardiac $\alpha_2\delta$ is not involved in membrane targeting of $Ca_V1.2$ [48,49]. Three γ subunit isoforms are expressed in the rat heart (γ4, γ6, and γ7), while four isoforms (γ4, γ6, γ7, and γ8) have been detected in human cardiac tissue [50]. In heterologous expression systems, all of the γ isoforms in rat and human heart co-immunoprecipate with $Ca_V1.2$, but the effects of the γ subunit on channel activation and inactivation vary by isoform [50].

Similar to VSMCs, scaffolding proteins and other signaling molecules can modulate $Ca_V1.2$ channel function in cardiomyocytes. For example, calmodulin modulates $Ca_V1.2$ current in ventricular myocytes *via* binding to the C-terminus of $Ca_V1.2$ [51,52]. Similarly, AKAP15 directly anchors protein kinase A (PKA) to the $Ca_V1.2$ channels in cardiomyocytes and facilitate the increase in $Ca_V1.2$ current by β-adrenergic receptor activation [53,54]. Interestingly, K^+ channel interacting proteins (KChIPs), small Ca^{2+}-binding proteins that were originally identified as K_V4 channel auxiliary subunits, can directly regulate cardiac $Ca_V1.2$ channel current [55]. There are no reports to date regarding KChIP modulation of vascular $Ca_V1.2$ channel activity.

2.2. Voltage-gated K^+ (K_V) channels

The voltage-gated K^+ (K_V) channels are multimeric complexes that are activated by depolarization and allow K^+ conductance across the plasma membrane (Figure 1B). The pore-forming α subunits of K_V channels are transmembrane proteins that contain six transmembrane domains (S1–S6). The transmembrane domains S1 through S4 form the voltage sensor of the K_V channel, while the S5 and S6 domains form the channel pore. A functional K_V channel is comprised of four α subunits, which may be the same or different isoforms from the same gene family, of which there are several different gene families. Since the K_V channel α subunits can form homo- and hetero-tetramers, the biophysical properties, physiological regulation and pharmacological properties of these channels can vary. For example, while mRNA for $K_V1.1$–1.6 was detected in rat cerebral arteries, only protein for $K_V1.2$ and1.5 was detected suggesting that the functional K_V channel is a $K_V1.2/1.5$ heterotetramer in cerebral vasculature [56].

Physiologically, in rat mesenteric arteries, K_V channels contribute to resting membrane potential and vessel tone [57]. Plane *et al* observed that after blockade of K_V1 channels with either 4-aminopyridine (a selective K_V channel blocker) or correolide (a selective K_V1 channel blocker), the myogenic response, the constriction that occurs in response to increased intraluminal pressure, was greater compared to control conditions [58]. Similarly, the K_V2 channel blocker stromatoxin constricted pressurized, perfused rat cerebral arteries, suggesting an influence of K_V2 channels on cerebral myogenic tone [59]. In rat aortic VSMCs, $K_V2.1$ channels are expressed and blockade of K_V2 but not K_V1 channels induces oscillatory contractions [60].

K_V channels are also targets for phosphorylation by protein kinase A (PKA) and PKC [57,61–65]. PKC signaling leads to closure of K_V channels and smooth muscle constriction [57,63–65], while PKA signaling *via* G-protein coupled-receptor activation and adenylyl cyclase or guanylyl cyclase, which increase cAMP and cGMP, respectively, can open K_V channels to hyperpolarize the membrane and facilitate smooth muscle cell relaxation [57,61,62,65].

Recently, another class of K_V channels, the KCNQ (K_V 7) channels, were identified in VSMCs. The KCNQ family of K^+ channels are outwardly-rectifying, voltage-gated K^+ channels that regulate membrane excitability in cardiac, neuronal and inner ear cells. Initially identified in murine portal vein myocytes, KCNQ channels have since been identified in aorta [66], pulmonary [67], tibial [66], basilar [68,69], mid-cerebral [69] and mesenteric arteries [70,71] from rats, mice and humans. In rat cerebral arteries, the KCNQ channels contribute to the development of myogenic tone [69] and Mani *et al* suggest that KCNQ channel openers may be more effective for the treatment of cerebral vasospasm than the currently used calcium channel blocker nimodipine. Schleifenbaum *et al* suggest that H_2S, which may be the adipocyte-derived relaxing factor released from perivascular fat, inhibits vascular contraction *via* opening of KCNQ channels [66]. Jackson-Weaver *et al* on the other hand found that in small mesenteric arteries endothelial production of H_2S plays a major role in maintaining low vascular tone [72]. It is unknown if heritable mutations in KCNQ channels affect vascular function, possibly contributing to vascular dysfunction in disease states.

In non-vascular tissues and heterologous expression systems, the pore-forming α subunits of K_V channels are known to interact with several ancillary proteins including the smaller β subunits, K^+ channel interacting protein (KChIP) and accessory proteins (KChAP), the minimal K^+ channel protein (minK), the minK-related peptide (MiRP) and scaffolding proteins such as post synaptic density 95 (PSD-95) (reviewed in [73]). However, only a few of these ancillary proteins have been identified to complex with K_V channels in vascular tissues. Several vascular beds including the pulmonary, mesenteric and renal arteries express many β subunits either at the mRNA or protein level [74–76]. However, the physiological function(s) of β subunits in vascular tissues is yet to be identified. Our laboratory recently reported that PSD 95, a member of the membrane-associated guanylate kinases, complexes with the pore-forming α1.2 subunit of K_V1 channels at the plasma membrane of VSMCs in cerebral arteries [77]. Using *ex vivo* gene knockdown in cerebral arteries, we demonstrated that PSD-95 regulates K_V1 channel expression in cerebral VSMCs and modulate the membrane potential and diameter of cerebral arteries [77]. Clearly, future endeavors should be directed towards identifying the expression, and importantly the physiological function of ancillary proteins of K_V channels in the vascular tissues.

Owing to the diversity of the K_V channel gene family and many different combinations of subunits for heteromultimerization, the biophysical properties of K_V channels and their responses to pharmacological intervention may vastly differ between different tissue types. For example, in cardiomyocytes, at least seven different K_V channels have been cloned expressing two different outward currents, delayed rectifiers (K_V1.1, K_V1.2, K_V1.5, K_V2.1, K_VLQT1 and hERG) and transient rectifiers (K_V1.4, K_V4.2 and K_V4.3). In cardiomyocytes, potassium channels determine the magnitude and duration of action potentials. The diversity of types and levels of expression produce the heterogeneity of action potential configurations throughout the regions of the heart [78]. A consequence of this diversity on response to pharmacological intervention is demonstrated by hERG. Although hERG (K_V11.1) is expressed in smooth muscle cells, brain and endocrine cells its function in the heart makes it central to drug safety since a wide variety of prescription medications can block K_V11.1 causing drug-induced QT prolongation and an increased risk of sudden

cardiac arrest [79]. A more thorough treatment of K_V and other ion channels in cardiomyocytes can be found in other reviews [80,81].

2.3. High-conductance Ca^{2+}-sensitive K^+ (BK) channels

Another important ion channel for maintaining VSMC E_m is the large-conductance, Ca^{2+}-sensitive K^+ channel, also known as the "Maxi-K" or "Big K" (BK) channel (Figure 1C). Compared to K_V channels, BK channels have large single-channel conductance (100–300 pS). The pore-forming α subunits of BK channels have seven (S0–S6) transmembrane domains, which show partial homology with the six transmembrane domains, S1–S6, of K_V channels. While voltage-sensitivity and pore-formation of the BK channel are determined by the transmembrane domains of the α subunit, Ca^{2+}-sensitivity is conferred by four intracellular domains (S7–S10) and by the interaction of the first transmembrane domain (S0) of the α-subunit with an auxiliary β1 subunit. BK channels are encoded by the *hSlo* gene, and phenotypic diversity is generated by a high level of alternative splicing [82–84]. Similarly to K_V channels, opening of BK channels leads to efflux of K^+ out of VSMCs, hyperpolarizing the plasma membrane.

The BK channels are expressed in many vascular beds, including the small arteries and arterioles of the cerebral, coronary and renal circulations. The BK channels in some vascular beds, such as rat cerebral arteries, appear to be in close proximity to ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP_3Rs) in the sarcoplasmic reticulum (SR). The Ca^{2+} released by the RyRs, also known as Ca^{2+} sparks, stimulates opening of BK channels since blockade of RyRs with either ryanodine or depletion of intracellular Ca^{2+} with thapsigargin reduced Ca^{2+} sparks and BK currents [85–89]. IP_3Rs can also stimulate opening of BK channels, however this stimulation can be dependent on Ca^{2+} released from IP_3Rs as in basilar VSMCs [90] or independent of Ca^{2+} released from IP_3Rs as was observed in mouse and rat cerebral arteries [91]. In addition to interacting with SR Ca^{2+} release channels, BK channels couple to some plasma membrane ion channels, such as transient receptor potential (TRP) channels. For example, TRPC1 channels coupled to BK channels in cultured aortic VSMCs [92] and TRPV4 channels complex with RyRs and BK channels in rat cerebral artery VSMCs [93,94].

In addition to their voltage and Ca^{2+} sensitivity, BK channels are also regulated by signaling molecules such as PKA and PKC. PKA can both activate and inhibit BK channel opening, depending on the splice variant of the channel [95–98]. In the majority of arteries studied, activation of PKA and cGMP-dependent protein kinase leads to activation of BK channels [95]. However, the literature regarding the effect of PKC on BK channels is less clear and less studied. For example, in rat pulmonary arterial smooth muscle cells, forskolin, an activator of cAMP, induces PKC-mediated opening of BK channels [99] and PKC activators also open BK channels [100]. However, in rat tail artery smooth muscle cells, patch clamps studies demonstrate that the catalytic subunit of PKC significantly reduces single BK channel openings [101]. In addition, new signaling molecules continue to emerge such as endothelium-derived H_2S that targets BK channels to maintain low myogenic tone [72]. Thus, BK channels are regulated by several cellular processes and these can vary between vascular beds of interest.

Compared to VSMCs, there are very few studies of BK channels in cardiomyocytes. It is generally believed that the involvement of BK channels is limited in cardiomyocyte, possibly due to the low levels of BK channel expression in the whole heart [102–104]. However, some BK channels are found in the inner mitochondrial membrane of cardiomyocytes and may protect the heart against ischemic injury [105,106]. Another recent study utilizing whole animal and perfused heart preparations reported that the heart rate is significantly reduced by inhibiting BK channels in wild-type mice but not in BK knockout

mice [107]. Thus, BK channels may have important physiological functions in cardiomyocytes more than previously thought, and any therapies targeting vascular BK channels may have to consider cardiac effects as well.

3. Vascular ion channel remodeling during hypertension

Small arteries and arterioles undergo extensive biological and structural adaptation in response to elevated intraluminal perfusion pressure that occurs during chronic hypertension. The underlying pathophysiological processes appear to be complex, and likely involve vascular remodeling, endothelial dysfunction, smooth muscle cell hypertrophy, and changes in extracellular matrix composition and function [108,109]. The net effect of these adaptive changes is augmented vasoconstrictor responses and attenuated vasodilator responses to various physiological stimuli, resulting in elevated vascular tone in the arteries and arterioles that are exposed to persistent high blood pressure [110,111]. The elevated vascular tone observed in the small arteries of cerebral, coronary and renal circulations during hypertension is thought to buffer the transmission of the high systemic pressure to the fragile capillaries of the brain, heart and kidneys, respectively, to prevent pressure-induced damages [112,113]. However, in the case of small arteries and arterioles of the mesenteric and skeletal muscle beds that contribute substantially to total peripheral resistance, elevated vascular tone during hypertension would further accentuate the increase in systemic blood pressure. Similar to other components of the vasculature, during persistent high blood pressure, ion channels in the plasma membrane of VSMCs also undergo ‘electrical remodeling’ such that the arteries maintain a heightened vascular tone. In fact, evidence for ‘electrical remodeling in VSMC membranes exposed to high blood pressure was first observed in the early 1970’s when increased transmembrane Ca^{2+} and K^+ flux was observed in arteries from different experimental models of hypertension [114,115]. Subsequently, in the early 1980’s, it was first reported that VSMCs from arteries of spontaneously hypertensive rats (SHR), a genetic rodent model for hypertension, were depolarized, and generated Ca^{2+}-dependent tone [12,116]. Building on these pioneering studies, several important discoveries were reported regarding changes in ion channel expression in VSMCs during hypertension. In the following sections, we present a synopsis of our current understanding of the topic (summarized in Figure 2).

3.1 Upregulation of vascular Ca^{2+} channels during hypertension

Several years of research have conclusively demonstrated that an upregulation of L-type Ca^{2+} ($Ca_V1.2$) channel function in VSMCs is a hallmark feature of hypertension. A strong positive correlation exists between blood pressure and the number of functional $Ca_V1.2$ channels in the VSMCs *in vivo*. For example, systolic blood pressure was found to be linearly correlated to membrane densities of $Ca_V1.2$ channel currents in VSMCs from small mesenteric arteries of SHR and WKY rats [117]. Interestingly, a reduction in systolic blood pressure by treatment with ramipril, an angiotensin converting enzyme inhibitor, resulted in a concomitant decrease in $Ca_V1.2$ channel current densities in VSMCs of SHR [118]. A plethora of other studies have also shown that the profound increase in $Ca_V1.2$ channel function observed during hypertension is an abnormality shared among VSMCs of several vascular beds [13,117,119–123]. Electrophysiological studies have demonstrated elevated Ca^{2+} currents in freshly isolated VSMCs of cerebral, mesenteric, renal, skeletal and pulmonary arteries from various hypertensive animal models [13,117,119,120,123]. Single channel experiments and other studies have further shown that the elevated Ca^{2+} currents observed in VSMCs during hypertension are not the result of altered single channel conductance, open-time distribution or voltage sensitivity, but rather due to an increased number of $Ca_V1.2$ channel openings [124]. Complementing the electrophysiological studies, contractile studies in isolated arteries further proved that compared to normotensive animals, arteries from hypertensive animals develop more Ca^{2+}-dependent spontaneous tone that was

sensitive to $Ca_V1.2$ channel blockers [120]. For example, renal arteries from SHRs, a genetic model of hypertension, and aortic-banded rats, that develop hypertension due to elevated angiotensin II levels, develop more Ca^{2+}-dependent spontaneous tone that was reversed by nifedipine (1μmol/L), a $Ca_V1.2$ channel specific blockers, compared to their normotensive counterparts [13].

The profound upregulation in vascular $Ca_V1.2$ channel function observed in hypertensive animals is largely attributed to increased expression of the channel. Immunoblot analyses show that the expression of the pore-forming α_{1C} subunit of the $Ca_V1.2$ channel is elevated in arteries of hypertensive animals compared to age-matched normotensive animals [123,125]. For example, the protein expression of α_{1C} was higher in mesenteric, renal and skeletal muscle arteries of SHR compared to age-matched WKY rats [13]. In a separate study, increased expression of vascular tissue–specific exon 1-encoded α_{1C} protein was observed in aorta and mesenteric arteries of SHR compared to WKY rats [126]. Further, the increases in exon 1-encoded α_{1C} protein was specific to vasculature, and was not observed in brain or visceral smooth muscles. Increased expression of pore-forming α_{1C} subunit was also seen in renal arteries of aortic-banded rats compared to Sham rats, suggesting that the increased expression of vascular $Ca_V1.2$ channel is an anomaly shared between hypertensive animal models with different etiologies [120]. In the same study, the authors found that renal arteries that were exposed to high blood pressure for as little as 2 days exhibited increased expression of α_{1C} subunit, implying that the abnormal increase in vascular $Ca_V1.2$ channels may be an early event occurring during the development of hypertension.

Although the paradigm for functional upregulation of vascular $Ca_V1.2$ channels during hypertension is now well established, there is a paucity of information regarding the mechanisms that lead to increased protein expression of the pore-forming α_{1C} subunit. In this regard, even less information is available on cellular processes and proteins that regulate the expression of $Ca_V1.2$ channels in VSMCs during physiological conditions. Nevertheless, data available so far points to the involvement of multiple cellular mechanisms in the upregulated protein expression of vascular $Ca_V1.2$ channels during hypertension. Slight increases in α_{1C} transcript level were observed in mesenteric arteries of SHR compared WKY rats [125]. However, in this study, a 1.53-fold increase in α_{1C} transcript level was accompanied by an unmatched 3.4-fold increase in α_{1C} protein level in mesenteric arteries of SHR. Similarly, in neonatal piglets with hypoxia-induced pulmonary hypertension, a profound increase in the expression of α_{1C} protein in pulmonary arteries was accompanied by no change in transcript levels [123]. Thus, in addition to transcriptional activity, post-transcriptional mechanisms such as increased translation efficiency, increased trafficking of channel proteins to plasma membrane and increased stability of channel protein complex may also contribute to the upregulation of α_{1C} protein in the vasculature during hypertension. A recent study indicated a potential role of microRNAs (miRNAs) in determining α_{1C} protein expression at the post-transciptional level in the vasculature [127]. In this study, miR-328 was found to regulate hypoxic pulmonary hypertension by post-transcriptional repression of α_{1C} subunits in pulmonary arterial smooth muscle cells. Consequently, mice overexpressing miR-328 had remarkably decreased right ventricular systolic pressure and pulmonary artery wall thickness [127]. Enhanced anterograde trafficking of the channel protein complex to the plasma membrane *via* the ancillary β subunits may also contribute to increased $Ca_V1.2$ channel activity in the vasculature during hypertension. In heterologous expression systems, the β subunit has been shown to promote plasma membrane expression of $Ca_V1.2$ channels by binding to the pore-forming α_{1C} subunit and potentially masking an ER retention signal [128]. In a recent study, through the use of Ca_V β3 knockout ($\beta3^{-/-}$) mice, Kharade *et al* demonstrated that in mesenteric arteries from angiotension II-induced hypertensive mice, the β3 subunit is required for the upregulation of $Ca_V1.2$ channels, increased calcium-dependent tone, and the development of

high blood pressure [30]. Evidence from a recent study also points to clustering and coordinated gating of $Ca_V1.2$ channels in the plasma membrane of VSMCs during hypertension [129]. The authors in this study observed a higher frequency of coupled $Ca_V1.2$ channel gating events, measured as persistent Ca^{2+} sparklets, in VSMCs of angiotensin II-induced hypertensive animals, and raised the possibility of transient interaction of 2–6 adjacent $Ca_V1.2$ channels via their C-terminus during hypertension. Such a transient interaction of adjacent $Ca_V1.2$ channels and their simultaneous activation would result in enhanced Ca^{2+} influx per gating event and elevated myogenic tone during hypertension. Interestingly, persistent Ca^{2+} sparklet activity by $Ca_V1.2$ channels in VSMCs requires recruitment of PKCα by AKAP150 to the plasma membrane, suggesting that scaffolding proteins that 'organize' signaling molecules and ion channels may be important in mediating the vascular ion channel phenotype observed during hypertension [42]. Consistent with this hypothesis, AKAP150 knockout mice showed a lack of persistent Ca^{2+} sparklets, decreased myogenic tone, and did not develop angiotensin II-induced hypertension [42]. Although more mechanistic studies are needed in the future, information available thus far suggests the involvement of multiple regulatory pathways in the enhanced expression of vascular $Ca_V1.2$ channels during hypertension.

3.2 Alteration in vascular K_V channels during hypertension

The K_V channels are major contributors to resting membrane potential and the diameter of small arteries of several vascular beds including the cerebral, coronary, mesenteric and pulmonary circulations [14]. Pioneering electrophysiological studies by Harder *et al.* demonstrating that VSMCs from cerebral arteries of SHR are more depolarized than those from WKY rats prompted researchers to investigate the functional expression of vascular K^+ channels during hypertension [116]. Our laboratory, in addition to others, is particularly interested in determining if vascular K_V1 channels inactivate or down-regulate in response to elevated blood pressure, resulting in depolarized arteries and increased myogenic tone. In this regard, Post *et al.* initially reported that hypoxia-induced rises in cytosolic calcium inhibit K_V channel currents in patch-clamped VSMCs isolated from canine pulmonary arteries [130]. Cox *and* Petrou also observed that Ca^{2+} influx through L-type Ca^{2+} channels decreases K_V channel current in patch-clamped VSMCs isolated from rat mesenteric arteries [131]. These authors proposed that an elevated level of cytosolic calcium in the VSMCs of hypertensive rats may inactivate K_V1 channels resulting in membrane depolarization and arterial constriction, independent of changes in channel expression. Thus, one mechanism suggested to reduce vascular K_V1 channel function is through the inactivation of K_V1 channels by intracellular calcium. Alternatively, a loss of K_V1 channel function in VSMCs of arteries exposed to high blood pressure could be due to a reduced expression of channel proteins. Wang *et al.* reported that K_V channel current was attenuated in a rat model of pulmonary arterial hypertension (PAH) induced by hypoxia [132]. In this study, reduced expression of $\alpha_{1.2}$ and $\alpha_{1.5}$ mRNA and protein was reported in small pulmonary arteries of rats with hypoxia-induced PAH. A down-regulation of K_V1 channel function, mRNA and protein was also documented in small pulmonary arteries of humans with primary PAH [133]. In agreement with these findings, reduced densities of K_V current also have been reported in VSMCs from small mesenteric arteries and renal interlobar arteries of the SHR [134,135]. Furthermore, the mesenteric arteries of *N*ω-nitro-L-arginine-induced hypertensive rats exhibit a reduced expression of $\alpha_{1.5}$ that is associated with depolarization and enhanced contractile sensitivity [136,137]. The authors in these studies further reported that in whole cell patch-clamp experiments using a Ca^{2+}-free pipette solution, K^+ current attributed largely to voltage-dependent K^+ (K_V) channels was reduced by 60% in VSMCs of the hypertensive rats [137]. In this context, studies done in our laboratory also demonstrated that depolarization and elevated vascular tone seen in small cerebral arteries of SHR and renal hypertensive rats were associated with a loss of functional K_V1 channels [138]. We

further demonstrated that the loss of K_V1 channel function in cerebral arteries was due to depressed gene activity of the pore-forming α subunits. Many other studies revealed that in addition to K_V1 channels, *Shab*-type voltage-gated K^+ (K_V2) channels also contribute to membrane hyperpolarization and vessel diameter. For example, Amberg and Santana reported that K_V2 channels contribute to the resting diameter of rat middle cerebral arteries [59]. In their study, stromatoxin (ScTx) -induced block of the K_V2 channels reduced resting diameter by 13% in arteries of control rats. In contrast, cerebral arteries of angiotensin II-induced hypertensive rats showed only a 6% constriction in response to ScTx treatment and had a lower density of ScTx-sensitive K^+ current, suggesting attenuated K_V2 channel function. A separate study further suggested that activation of the transcription factor NFATc3 by Ca^{2+} entry via $Ca_V1.2$ channels would result in decreased K_V2 channel expression in cerebral arteries during hypertension pointing to an interdependency of functional ion channel expression [139]. Reduced gene expression of $\alpha_{2.1}$ and $\alpha_{2.2}$ leading to K_V2 channel dysfunction was also observed in myocytes of vasospastic cerebral arteries in a canine model of subarachnoid hemorrhage, a disorder often associated with chronic hypertension [140]. Recently, studies reported that many members of the K_V7 family are expressed in rodent and human blood vessels, and play an important role in controlling vascular tone [69–71]. Importantly, similar to K_V1 channels, a depression of K_V7 channel function was observed in aorta and mesenteric arteries of SHR and angiotensin II-induced mice, and the decreased K_V7 channel function was accompanied by a reduced expression of $K_V7.4$ protein levels [141]. Furthermore, a loss of K_V7 channel function as well as $K_V7.4$ protein expression levels were implicated in impaired β-adrenoceptor mediated vasodilation observed in renal arteries of SHR [142].

However, since the pore-forming α subunits of K_V channels are encoded by several genes and the α subunits that complex to form the heterotetrameric channel vary with vascular beds, predictably, the functional expression of vascular K_V channels in response to high blood pressure differs between hypertensive animal models [136,143]. For example, mesenteric arteries of *Nω*-nitro-L-arginine-induced hypertensive rats showed decreased expression of K_V $\alpha_{1.5}$ [136]; however increased K_V channel current and a negative shift in voltage dependence of activation accompanied by an increase in mRNA and/or protein levels of $\alpha_{1.2}$, $\alpha_{1.3}$, $\alpha_{1.5}$ and $\alpha_{2.1}$ was observed in mesenteric arteries of SHR [143,144]. Subtle differences in the biogenic process of K_V channels between different hypertensive animal models also has been reported in the cerebral arteries [138]. Experiments in our laboratory found that the decrease in K_V1 channel function in cerebral arteries of SHR is associated with reduced gene expression of $\alpha_{1.2}$ and not $\alpha_{1.5}$; however, depressed gene expression of $\alpha_{1.5}$ was observed as an additional abnormality in cerebral arteries of aortic-banded rats that had high blood pressure [138]. The expression of K_V channels also differs between vascular beds exposed to high blood pressure. For example, in SHR, protein expression of $\alpha_{2.1}$ was increased in mesenteric arteries, decreased in tail arteries, and unchanged in thoracic aorta compared to control WKY rats [143]. A recent study showed that mesenteric arteries in a genetic mouse model of hypertension were associated with *de novo* protein synthesis of the $K_V6.3$ subunit, and based on differences in current kinetics, the authors suggested that a switch from a homotetrameric $K_V2.1$ channel to a heterotetrameric $K_V2.1$/ $K_V6.3$ channel occurs during hypertension [145]. Another ‘silent’ subunit, namely K_V $\alpha_{9.3}$, was recently reported to complex with $K_V2.1$ channels in rat middle cerebral arteries; however the fate of K_V $\alpha_{9.3}$ during hypertension remains to be seen [146].

Even though several ancillary subunits are present for K_V channels in brain, only a few among them have been reported in vascular tissues. Importantly, the expression of ancillary β subunit in the vasculature during hypertension has been a subject of interest. In rats exposed to chronic hypoxia and in patients with primary pulmonary hypertension, a loss of K_V1 channel function was associated with a reduced gene activity of K_V1 α subunits, but no

change in $K_V\beta$ transcript levels [147,148]. Similarly, exposing cultured pulmonary VSMCs to hypoxia for 60 hours resulted in reduced K_V1 channel expression and function, but no changes in ancillary $K_V\beta1$, $K_V\beta2$ and $K_V\beta3$ mRNA levels [132]. However, the mRNA level of $K_V\beta1.1$ was increased in mesenteric and tail arteries, and thoracic aorta in SHR compared to normotensive WKY rats [144]. The mRNA levels of other K_V1 channel-accessory proteins, namely KChIP3 and KChAP were found to be reduced in aorta in a genetic mouse model of hypertension; however only KChAP mRNA levels were reduced in mesenteric arteries [145]. Collectively, the functional expression of K_V channels and associated proteins differs between vascular beds during hypertension, and highlights the complexities involved in the regulation of K_V channel function and expression.

3.3 Changes in vascular BK channels during hypertension

The BK channels are an important family of potassium channels that are densely expressed in VSMCs, and are activated by membrane depolarization and increased cytosolic Ca^{2+} concentration. The physiological function of vascular BK channels acting as a homeostatic mechanism to counteract myogenic constriction due to elevated intraluminal pressure, and thereby ensuring perfusion to critical organs is well recognized [149]. Several laboratories have studied BK channels in resistance and conduit arteries in different experimental models of hypertension [135,150–158]. A majority of the studies report that the functional expression of BK channels in VSMCs increases during hypertension [135,150–155]. In fact, as early as the 1970's, it was known that the smooth muscle cells from aorta, renal and caudal arteries of hypertensive rats had increased transmembrane K^+ ion flux that was sensitive to depletion of extracellular Ca^{2+} and Ca^{2+} channel blockers [114,159,160]. Subsequently, using isometric tension recording and vascular reactivity techniques along with BK channel specific pharmacological blockers such as tetraethylammonium (TEA), charybdotoxin and iberiotoxin, several studies demonstrated enhanced function of BK channels in arteries of experimental hypertensive animals [152,161–164]. For example, compared to control tissues, treatment with TEA robustly contracted proximal aortic segments from aortic-banded hypertensive rats, implying an accentuated dilator function of BK channels in aorta of hypertensive rats. Similarly, aortic rings from SHR contracted strongly to TEA, whereas aortic rings from normotensive WKY rats showed little to no constriction [161]. Concurrently, studies done in other vascular beds, such as the cerebral, femoral, carotid, renal and mesenteric vasculature, also showed elevated constrictor responses to BK channel specific blockers [152,162–164]. For example, treatment of endothelium-denuded carotid, femoral and mesenteric arterial segments from SHR with charybdotoxin elicited a concentration-dependent contraction, whereas the arterial segments from normotensive WKY rats did not contract [163]. Subsequent studies using the patch clamp technique provided additional evidence regarding increased BK channel function in VSMCs during hypertension. In agreement with vessel reactivity studies, results published from several laboratories indicate that compared to control, whole cell K^+ current through iberiotoxin-sensitive BK channels is elevated in VSMCs from aorta of SHR, stroke-prone SHR, and rats with renal hypertension [150,151,153,154,161]. Additionally, myocytes from mesenteric arteries of DOCA salt-induced hypertensive rats also exhibited elevated iberiotoxin-sensitive K^+ current compared to myocytes from Sham animals [165]. In fact, the levels of whole cell K^+ current through BK channels in VSMCs is positively correlated to blood pressure in hypertensive animals [152]. Lowering of blood pressure in SHR by the angiotensin-converting enzyme inhibitor ramipril normalized elevated BK current densities, in addition to abolishing TEA-induced contraction of aortic segments from SHR [152]. Single channel studies done by England *et al* clearly demonstrate that the accentuated iberiotoxin-sensitive K^+ current seen in VSMCs of SHR is not a result of altered BK channel conductance, but rather is due to an increase in the number of BK channel openings [150]. Ultimately, *in situ* studies of cerebral arterioles from hypertensive rats provided additional

evidence for upregulation of vascular BK channel function [155]. In this study, when iberiotoxin (10 nM) was topically applied to pial arterioles *in situ* by a cranial window preparation, arterioles from SHR constricted more compared to arterioles from normotensive WKY rats. Thus, the upregulation of vascular BK channel function seems to act as an adaptive mechanism to diminish elevated vascular excitability and vasospastic episodes in microcirculatory beds during hypertension, and thus ward off ischemic events of critical organs.

Biochemical and molecular biological studies further explored whether an increase in BK channel function in arteries during hypertension is due to elevated expression of the pore-forming subunit of the channel. Immunoblot analyses revealed that the densities of the 125-kDa immunoreactive band that represent the pore-forming α subunit is at least 2–3 times elevated in aorta of SHR compared to the normotensive WKY rats [154]. The expression of the pore-forming α subunit of BK channel is also increased in microcirculatory beds of SHR such the cerebral, mesenteric and coronary beds [11]. However, there is a scarcity of information regarding gene expression of the pore-forming α subunit of the BK channel in arteries during hypertension. Initial studies done in this regard suggest that the mRNA level of the α subunit of the BK channel is unchanged in aorta of SHR compared to WKY rats [154]. Thus, post transcriptional modifications and protein stability of the pore-forming α subunit of the BK channel, and/or its association with auxiliary regulatory proteins may play an important role in the increased functional expression of vascular BK channels during hypertension.

Even though the majority of the studies point to increased functional expression of BK channels in vasculature during hypertension, it is important mention that there are at least 3 studies to our knowledge that report decreased BK channel function in resistance arteries during hypertension [156–158]. Decreased BK channel current was reported in VSMCs of cerebral and mesenteric arteries from SHR and angiotensin II-infused hypertensive mice compared to normotensive Sprague Dawley rats and saline infused mice, respectively [156,157]. Similarly, compared to control rats, cerebral arteries from angiotensin II-infused hypertensive Sprague Dawley rats constricted less to iberiotoxin in isolated vessel reactivity studies [158]. The decreased BK channel function in these studies was explained as an effect of reduced mRNA expression of the ancillary BK β_1 subunit due to the activation of calcineurin/NFATc3 signaling pathway by elevated circulating angiotensin II [156] . As mentioned before, BK β_1 subunit confers Ca^{2+} sensitivity to BK channels, and mediates the coupling of Ca^{2+} sparks to BK channel activation [166]. Hence, reduced expression of the BK β_1 subunit would uncouple BK channels from L-type Ca^{2+} channels that produce Ca^{2+} sparks in VSMCs, resulting in disruption of the BK channel-mediated homeostatic mechanism to counteract myogenic constriction during higher intraluminal pressure. Accordingly, using acute blood pressure measurement techniques such as carotid arterial catheterization and tail cuff plethysmography, the BK β_1 knock out mice were initially reported to have a 10–20 mmHg increase in mean arterial pressure compared to wild-type age-matched controls [166–169]. However, a recent report using radiotelemetry to continuously measure blood pressure demonstrated that the 24-hour mean arterial pressure was similar between wild-type and BK β_1 knock out mice [170]. Thus, more studies are necessary to further elucidate the role of the ancillary BK β_1 subunit in the vasculature during hypertension. To add further complexity to the subject, intriguingly, the expression pattern of the ancillary BK β_1 subunit differs from that of the pore-forming α subunit in the vasculature during hypertension. For example, reduced mRNA expression of the β_1 subunit in cerebral and mesenteric arteries from SHR and angiotensin II-infused hypertensive mice was accompanied by no change in the expression of BK channel pore-forming α subunit mRNA in these animals [156–158]. Collectively, the information available from studies thus

far points to the intricacies associated with the expression and regulation of vascular BK channels during hypertension.

4. Vascular ion channels as antihypertensive targets

The prevalence of hypertension among adults in the United States is approximately 30% and increases with age to nearly 70% among adults 65 years and older [171]. Pharmacologic treatment and control of blood pressure has increased in the last 10 years [172]. There are currently more than one hundred molecules approved for the treatment of hypertension, however only one third of treated patients have their blood pressure normalized [172,173]. This is mainly due to low patient compliance to drug regimens, which require daily dosing and often multi-drug therapy [174,175]. Using gene therapy to control vascular tone by altering the expression and function of ion channels is an approach that could reduce the impact of patient adherence by avoiding the need for daily drug administration and reducing side effects associated with the non-specific effects of small molecule drugs [173,176]. This also avoids blood pressure fluctuations caused by short drug half-lives and inconsistent patient dosing intervals [176]. This approach has been demonstrated in proof of concept studies targeting $Ca_V1.2$, $K_V1.5$ and BK channels using knockdowns and virally mediated gene delivery of altered channel subunits [169,173,176–179]. Genetic epidemiological studies of BK subunit mutations also lend support to gene-targeted therapies for hypertension [180,181].

Calcium channels have been the main vascular ion channels to be directly targeted by drugs in the treatment of hypertension. Calcium channel blockers (CCB) have been extensively used for the control of hypertension for over two decades and were the most frequently prescribed antihypertensive as late as the 1990's [182]. However, the usefulness of CCB is complicated by their contraindications and some side effects [183]. CCBs, especially non-dihydropyridines, are contraindicated in patients with heart failure because of potential adverse effects such as atrioventricular nodal block and depressed contractility [183]. Tachycardia and edema are common side effects of some CCB including dihydropyridines. The dihydropyridines that selectively block vascular $Ca_V1.2$ channels have less direct action on the heart and cause reflex tachycardia through increase sympathetic activity due to the rapid onset of channel blockade [176]. Edema of the lower extremities, particularly the ankles, is thought to result from venous resistance to CCB-induced dilation [184]. CCBs are often given in combination with other classes of antihypertensive drugs to achieve an adequate level of blood pressure control through synergistic effects and to counteract side-effects of each component. CCB-induced reflex tachycardia is reduced by the addition of a β-adrenergic receptor blocker. CCBs are often paired with an angiotensin receptor blockers (ARB) or angiotensin converting enzyme inhibitors (ACEI) and are common multi-drug fixed dose pill formulations. The sympathetic activation of the renin system induced by CCBs can be buffered by these drugs while the reduced sodium balance produced by the CCBs augment the antihypertensive effects of ARB and ACEI [185]. Several studies suggest that these antihypertensive drugs may affect vascular ion channel expression independent of their effect on blood pressure through mitigating Ang II levels. Cox *et al.* used ramipril, an ACEI, and showed inihibition of Ang II leads to downregulation of Ca^{2+} and K^+ channels in mesenteric VSMCs in WKY and SHR [118]. A study involving aldosterone overexpressing transgenic mice showed aldosterone induced a concentration-dependent decrease in both BK-α and BK-β1 subunit mRNA levels suggesting the benefits of aldosterone antagonist treatment may be partially mediated through regulation of BK channel expression in VSMCs [186].

Gene therapy-based treatments aimed at reducing abnormal expression of L-type calcium channels in VSMCs may be a new way to treat hypertension with higher vascular selectivity

and fewer side effects than attempting to block Ca^{2+} influx with CCB [176]. Several studies targeting $Ca_V1.2$ serve as proof of concept for gene therapy approach to hypertension. Marsh and Telemaque used a gene therapy approach designed to reduce the availability of β_2-subunits required for $Ca_V1.2$ expression at the membrane. They conducted experiments using adenovirus delivery to establish dominant-negative mutated β_2-subunits of the L-type calcium channel [173,177]. Recombinant adenovirus expressing the full or C- / N- terminus truncated β_2-subunits were over expressed in HL-1 cells and used as α_{1C} chaperon decoys [177]. The dominant-negative function demonstrated in HL-1 cells illustrated that the β_2-subunit is a feasible molecular target for reducing the number of functional $Ca_V1.2$ channels in cardiomyocytes [177]. Studies focusing on reducing $Ca_V1.2$ expression selectively in noncardiac VSMCs have been conducted. Using a vascular smooth muscle specific enhancer/promoter, a viral vector mediated shRNA gene treatment selectively reduced the expression of $Ca_V1.2$ in A7r5 VSMCs with little effect in non-VSMCs including cultured cardiomyocytes [176]. Although siRNA is not a feasible long-term therapy due to its transient nature and systemic delivery, these studies demonstrate the proof of concept that vascular $Ca_V1.2$ expression and function can be selectively reduced in an aim to treat hypertension long-term without affecting cardiac $Ca_V1.2$ [176]. Bannister *et al.* targeted $Ca_V1.2$ e1b and $Ca_V1.2$ e1c subunit splice variants with shRNAs. They demonstrated in rat cerebral artery smooth muscle cells both $Ca_V1.2$ splice variants are located in the plasma membrane and shRNAs targeting each splice variant selectively reduces their expression. These knockdowns resulted in a decrease in $Ca_V1.2$ protein leading to vasodilation and reduced $Ca_V1.2$ currents suggesting exon 1 of $Ca_V1.2$ could be a novel antihypertensive target. Additionally, knockdowns of the $\alpha 2\delta$ subunit, which inserts both variants into the VSMC plasma membrane, reduce the surface expression of both $Ca_V1.2$ variants and currents making it an additional subunit target for gene therapy [178]. In particular, as Ca_V $\beta 3$ knockout mice showed a blunted increase in blood pressure in response to angiotensin II infusion, Ca_V $\beta 3$ subunits may also be targeted for potential antihypertensive therapy [30].

Potassium channels are another target for gene therapy for hypertension, especially for pulmonary hypertension. In chronic hypoxic pulmonary hypertension, the loss of $K_V1.5$ expression appears to be important to the pathogenesis. Studies in rodent models of hypoxia relevant to pulmonary hypertension in humans demonstrated reduced pulmonary hypertension despite continued hypoxia upon administration of cloned Kv1.5 from human pulmonary arteries. The augmentation of $K_V1.5$ was achieved by gene transfer to the pulmonary circulation via an adenovirus vector aerosol [179]. Large conductance potassium channels (BK) present another possible target for hypertensive gene therapy due to their proposed role as negative feedback regulators of vascular tone. In smooth muscle, an increase in BK channel activity is induced by local release of Ca^{2+} from the SR. This leads to K^+ efflux and hyperpolarization of the membrane closing L-type calcium channels and preventing further influx of Ca^{2+} [180]. BKβ1 subunits confer increased sensitivity to membrane potential and Ca^{2+} level changes to BK channels. The potential of BK channels as a target for gene therapy can be seen in knockout animal studies and human epidemiological studies.

The *in vivo* function of the BK β subunit has been assessed in knockout mice with a disrupted BKβ1 gene. BK$\beta1^{-/-}$ mice have abnormal Ca^{2+} spark/spontaneous transient outward K^+ current coupling which is shifted to more depolarized potentials. The elevated blood pressure seen between BKβ1−/− and +/+ mice were maintained over a wide range of blood pressure levels and different genetic backgrounds suggesting that the formation of heteromultimeric BKα and BKβ1 subunits is fundamental to the BK channel's negative feedback role in vascular tone [169]. Several genetic epidemiological studies have implicated variations in BK subunits in the prevalence of certain forms of hypertension. A single nucleotide substitution in the β1 gene (KCNMB1), corresponding to an E65K

mutation in the protein conferred a gain of function correlated with low prevalence of diastolic hypertension. The mutation showed increased Ca^{2+} sensitivity compared to wild-type without changes in channel kinetics [180]. The effect of this mutation is sexually dimorphic, protecting women and is influenced by estradiol and age. The genetic evidence for the different impact of the BK channel in the control blood pressure points to the E65K polymorphism as one of the strongest genetic factors correlated with reduced incidence of myocardial infarction and stroke [181]. The gain of function of the E65K polymorphism and reduced prevalence of diastolic hypertension suggests gene therapy modification of the BK β1 subunit as a potential target for essential hypertension.

As described above, evidence for the role of ion channel subunits in modulating blood pressure and proof-of-concept experiments demonstrating targeted modification of ion channel expression and function have laid a basic foundation for gene therapy treatment of hypertension. Future endeavors must resolve problems of efficacy and safety inherent in gene therapy. An ideal gene therapy vector would have attributes which include high titer, immunologically inert, sustained or regulatable expression, size capacity to accommodate the largest genes plus regulatory regions, cell type-specific targeting and the ability to transduce non-dividing post-mitotic cells. A final high value property is site specific chromosome integration. This allows faithful replication and segregation of the gene therapy vector during cell division, eliminates the uncertainty of random integration and allows endogenous regulatory regions to control expression under physiological conditions [187]. Limitations and safety concerns arise from deviation from this ideal. Low titer and low expression of transgenes can result in therapeutic failure while replication in non-target cells, immune activation, insertion mutagenesis and latent reactivation of the vector represent serious safety concerns [187,188]. Adeno-associated virus (AAV) in particular seems to avoid most of these safety concerns and is used in several preclinical or clinical trials [189]. Low titer of AAV and low gene transduction in vascular tissue are major roadblocks in using AAV for a systemic gene transduction in VSMCs. Modification of AAV tropism to target VSMC may help in achieving adequate expression for therapeutic efficacy [190].

Some studies discussed in this review utilized targeted gene knockout mice [30,169]. In general, care must be taken in interpreting results from knockout mice. For example, genetic background of the mice, interference by a selection marker gene, flanking genes or compensatory genetic loci may all influence the final phenotype in addition to the deleted gene [191,192]. Also, since hypertension is a disease with complex etiologies involving multiple genes and environmental factors, it is difficult to distinguish a direct causation between a gene and the disease from a mere correlation [193]. Furthermore, in global knockout mice where the target gene is removed from all tissues, developmental changes and the effects from tissues other than VSMCs, such as cardiomyocytes or neurons, may well contribute to the observed phenotypes. In this regard, smooth muscle-selective knockouts [194,195] or inducible, smooth muscle-selective knockouts [196] are available and may provide a cleaner picture of the complex mechanisms involved in hypertension.

5. Conclusion

The vasculature is an important part of blood pressure regulation. As outlined in this review, evidence suggests that ion channel subunits play an important role in modulating blood pressure and that the expression of those subunits change during hypertension, perhaps as a cause/effect and also as a compensatory mechanism. We have limited ourselves to considering three channel types, $Ca_V1.2$, K_V, and BK channels. There is a therapeutic potential for antihypertensive therapy targeting subunits or associated proteins of these channels pharmacologically or at the gene level. A better understanding of their regulation

in hypertension and the interaction with associated proteins will guide our endeavor in utilizing these ion channels for therapeutic purposes.

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Abbreviations

AAV	adeno-associated virus
ACEI	angiotensin converting enzyme inhibitors
AKAP	A-kinase anchoring protein
ARB	angiotensin receptor blockers
BK	large-conductance, Ca^{2+}-activated K^+ channel
$Ca_V1.2$	L-type Ca^{2+} channel
CCB	calcium channel blocker
CO	cardiac output
DOCA	deoxycorticosterone acetate
E_m	membrane potential
IP_3R	inositol 1,4,5-trisphosphate receptor
K_{ATP}	ATP-sensitive K^+ channel
KChAP	K^+ channel accessory protein
K_{IR}	inward-rectifying K^+ channel
K_V	voltage-gated K^+ channel
PAH	pulmonary arterial hypertension
PKA	protein kinase A
PKC	protein kinase C
RyR	ryonodine receptor
ScTx	stromatoxin
SHR	spontaneously hypertensive rat
TEA	tetraethylammonium
TPR	total peripheral resistance
TRP	transient receptor potential
VSMC	vascular smooth muscle cell
WKY	Wistar-Kyoto rat

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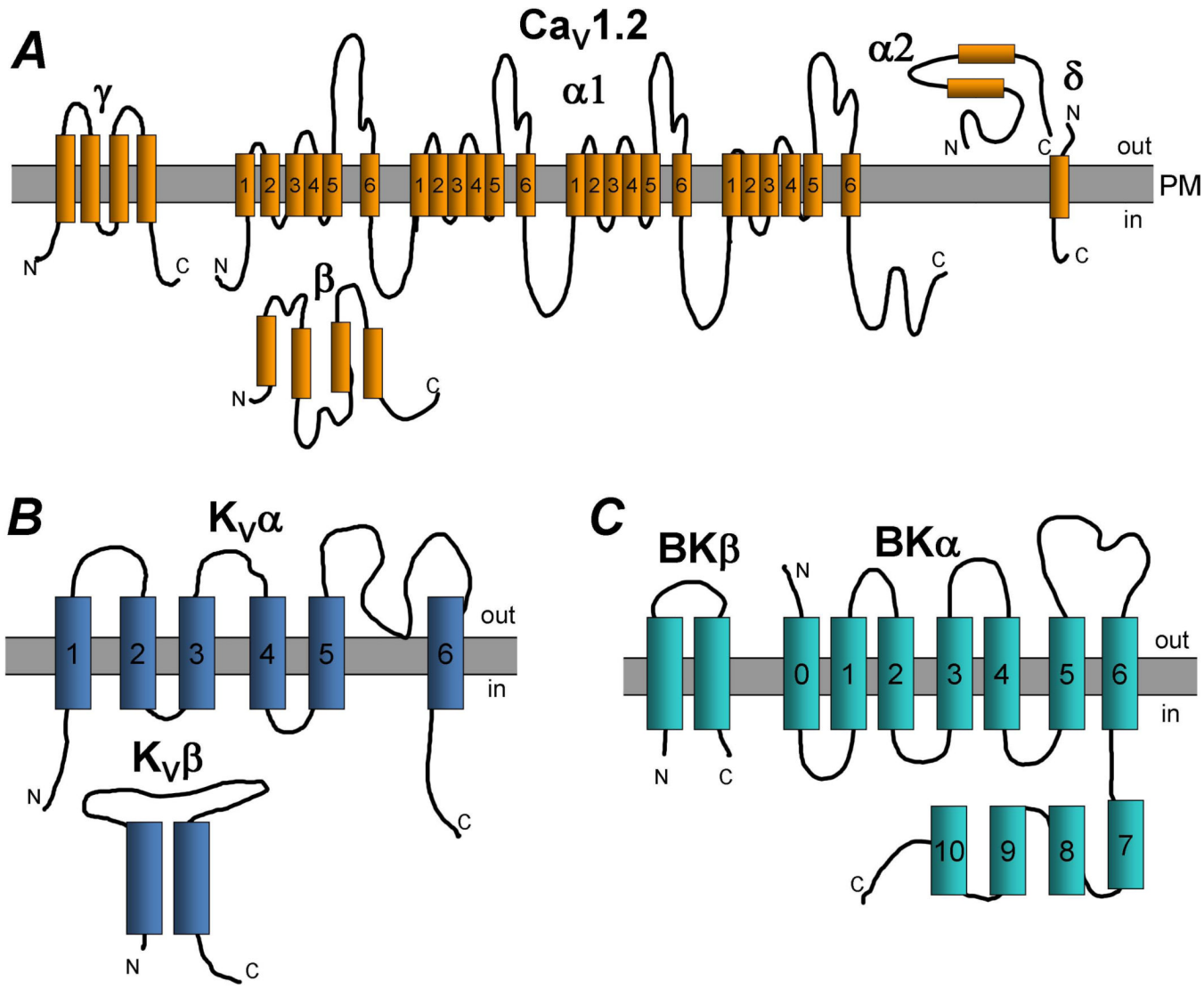

Figure 1.

Proposed topology of vascular ion channels. **A.** Voltage-gated L-type Ca^{2+} channel ($Ca_V1.2$) subunits α1, α2, β, γ, δ are depicted. PM: plasma membrane. **B.** Voltage-gated K^+ channel (K_V) α and β subunits. **C.** Large-conductance, Ca^{2+}-activated K^+ channel (BK) α and β subunits.

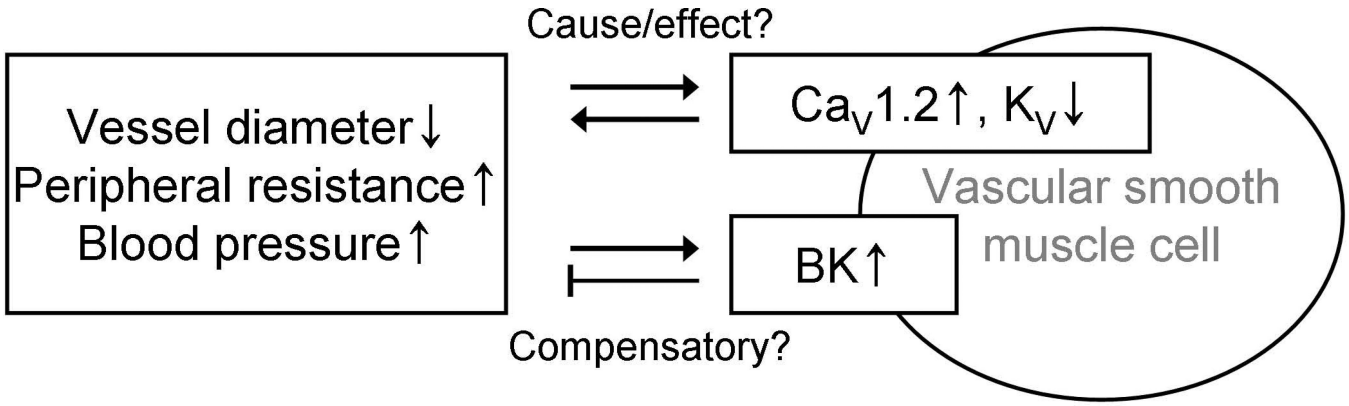

Figure 2.

Changes in vascular ion channel expression during hypertension. Voltage-gated L-type Ca^{2+} channels ($Ca_V1.2$) are upregulated and voltage-gated K^+ channels (K_V) are downregulated in hypertension. The changes in expression may be the cause of vasoconstriction and increased blood pressure or a result of sustained elevation in blood pressure. Large-conductance Ca^{2+} -activated K^+ channels (BK) are upregulated during hypertension, possibly as a compensatory mechanism inhibiting further increases in blood pressure.