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Function and regulation of large conductance Ca^{2+} -activated K^+ channel in vascular smooth muscle cells

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Abstract

Large conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels are abundantly expressed in vascular smooth muscle cells. Activation of BK_{Ca} channels leads to hyperpolarization of cell membrane, which in turn counteracts vasoconstriction. Therefore, BK_{Ca} channels have an important role in regulation of vascular tone and blood pressure. The activity of BK_{Ca} channels is subject to modulation by various factors. Furthermore, the function of BK_{Ca} channels are altered in both physiological and pathophysiological conditions, such as pregnancy, hypertension and diabetes, which has dramatic impacts on vascular tone and hemodynamics. Consequently, compounds and genetic manipulation that alter activity and expression of the channel might be of therapeutic interest.

Keywords

BK_{Ca} channel; vascular tone; regulation; cardiovascular dysfunctions; therapeutics

Ca^{2+} -activated K^+ (K_{Ca}) channels have an important role in cell excitability by sensing and reacting to changes in intracellular Ca^{2+} concentrations. Based on their conductance the K_{Ca} family is divided into small-conductance (SK_{Ca} , ~4–14 pS), intermediate-conductance (IK_{Ca} , ~32–39 pS) and large-conductance (BK_{Ca} , ~200–300 pS) channels. Whereas BK_{Ca} channels are activated by membrane depolarization and/or Ca^{2+} binding to the channel, SK_{Ca} and IK_{Ca} channels are voltage-insensitive and stimulated by Ca^{2+} binding to calmodulin constitutively bound to the channels [1]. SK_{Ca} , IK_{Ca} and BK_{Ca} channels are all present in vasculature with preferential expression of BK_{Ca} channels in vascular smooth muscle cells (VSMCs) and SK_{Ca} and IK_{Ca} channels in endothelial cells [2–4]. All these channels contribute significantly but differently to the regulation of vascular tone. Activation of BK_{Ca} channels in VSMCs enables K^+ efflux, resulting in membrane hyperpolarization. By contrast, activation of SK_{Ca} and IK_{Ca} channels indirectly triggers hyperpolarization of VSMCs through endothelium-dependent mechanisms involving generation of nitric oxide (NO) in the endothelium and endothelium-mediated hyperpolarization of VSMCs [2,5]. The hyperpolarization of VSMCs conferred by the

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Teaser Sentence: BK_{Ca} channels in VSMCs are key players in both cardiovascular adaption and dysfunctions during physiological and pathophysiological conditions and constitutes a potential therapeutic target for cardiovascular disorders associated with altered BK_{Ca} channel activity.

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endothelium possibly involves electrical coupling between the endothelium and VSMCs through myoendothelial gap junctions and/or activation of inwardly rectifying K^1 (Kir) channels as the result of accumulation of K^1 in the intercellular space owing to the activity of SK_{Ca} and IK_{Ca} channels in endothelial cells. As a result of VSMC hyperpolarization, the voltage-gated Ca^{21} channel (Cav) is closed, leading to a reduction of intracellular Ca^{21} concentrations and subsequent vasodilation. In this article we focus on the function and regulation of BK_{Ca} channels in VSMCs, and the readers are referred to excellent reviews on the roles of SK_{Ca} and IK_{Ca} channels in the regulation of vascular function [2,3,5].

Overview on BK_{Ca}

BK_{Ca} channels are implicated in a variety of physiological functions ranging from regulating tone of vascular beds to modulating release of hormones and neurotransmitters [6]. BK_{Ca} channels are a tetramer formed by pore-forming α -subunits along with accessory β -subunits, activated by membrane depolarization and/or an increase in intracellular Ca^{21} [7]. The α -subunit, ubiquitously expressed in mammalian tissues, is encoded by a single gene (KCNMA1) with various spliced isoforms. Four distinct genes have been identified for β -subunits (KCNMB1–4), and expression of β -subunits is tissue-specific [8]. The α -subunit contains a transmembrane domain sensing membrane potential and conducting ion and a cytoplasmic domain sensing Ca^{21} . The β -subunits consist of two transmembrane domains (TM1 and TM2) with a large extracellular loop. The α -subunit transmembrane domain is comprised of seven membrane-spanning segments (S0–S6), which contribute to voltage-sensing (S1–S4) and pore-forming (S5–S6) [9]. The cytoplasmic domain of α -subunit comprises two regulator of K^1 conductance (RCK) domains, and it is believed that Ca^{21} -sensing is achieved through two high affinity Ca^{21} binding sites located in both RCK1 and RCK2 [10].

Disulfide cross-linking studies revealed the locations of β -subunit in BK_{Ca} channels: TM1 next to S1 and to S2 and TM2 close to S0 [11]. Theoretically, α - and β -subunits of BK_{Ca} channels could co-assemble in a 1:1 stoichiometry. However, studies carried out in native tissues and heterologous expressing systems revealed that the population of BK_{Ca} channels is not homogenous [12,13]. Fractions of the BK_{Ca} channel can exist with less than four β -subunits [14]. Lines of evidence suggest that stoichiometry of α - to β -subunits of the BK_{Ca} channel is dynamic. Expression of $\beta 1$ -subunit is selectively up- or downregulated in VSMCs without affecting α -subunit expression under various physiological and pathophysiological conditions and hormone stimulation, leading to enhanced or depressed BK_{Ca} channel activity [15–17]. BK_{Ca} channels undergo extensive alternative splicing, and splice variants displays different biophysical properties, such as voltage sensitivity, Ca^{21} sensitivity and kinetics [18,19]. Channel protein trafficking [19,20], expression [21] and phosphorylation [19,22] also vary among splice variants. The association of β -subunits to α -subunit further expands the diversity of BK_{Ca} channel properties. Sensitivity of the channel to voltage and Ca^{21} was increased by the $\beta 1$, $\beta 2$ and $\beta 4$ subunits but not by $\beta 3$ subunit [23–25]. Pharmacological profiles of BK_{Ca} channels are also altered by β -subunits. BK_{Ca} channels formed by α and $\beta 4$ subunits are resistant to iberitoxin [25].

BK_{Ca} channels in VSMCs and their physiological roles

Expression and physiological roles of BK_{Ca} channels in VSMCs

BK_{Ca} channels are expressed in virtually all VSMCs, and both α - and β -subunits of the BK_{Ca} channel have been cloned [26–28]. The predominant β -subunit in VSMCs is $\beta 1$ -subunit [29,30]. Low levels of $\beta 2$ and $\beta 4$ transcripts are detected in VSMCs and their expression at protein level is undetectable or negligible [31,32]. In VSMCs, BK_{Ca} channels are primarily activated by an elevation of intracellular Ca^{21} concentration ($[Ca^{21}]_i$) due to

spontaneous [33] or agonist-stimulated [34] Ca^{2+} release from intracellular stores. In addition, Ca^{2+} influx through L-type voltage-gated Ca^{2+} channel ($\text{Ca}_v1.2$) also stimulates BK_{Ca} channel activity (Figure 1) [35]. Activities of BK_{Ca} channels contribute substantially to regulation of vascular function owing to its large single channel conductance (200–300 pS) and high protein expression [36,37]. The opening of the BK_{Ca} channel enables K^+ efflux, leading membrane hyperpolarization. This membrane potential change closes $\text{Ca}_v1.2$, which in turn reduces $[\text{Ca}^{2+}]_i$ and induces vasorelaxation. By contrast, closing of the BK_{Ca} channel causes membrane depolarization which opens $\text{Ca}_v1.2$, resulting in an increase in $[\text{Ca}^{2+}]_i$ and consequent vasoconstriction. In general, BK_{Ca} channels in VSMCs participates in setting resting membrane potential, regulating vascular tone and functioning as a compensatory mechanism to buffer vasoconstriction.

Vascular tone is the major determinant of resistance to blood flow through the circulation, and has a crucial role in both regulating blood pressure and distributing blood flow within the tissues and organs. In resistant arteries blockade of the BK_{Ca} channel with inhibitors of BK_{Ca} channel depolarized the membrane of VSMCs, leading to vasoconstriction [38]. In addition, the blockade of the BK_{Ca} channel enhanced vasoconstriction induced by vasoconstrictors [39]. By contrast, activation of BK_{Ca} channels with channel opener NS1619 caused membrane hyperpolarization, resulting in vasodilation and/or reduced vasoconstriction [40,41]. The importance of BK_{Ca} channels in VSMCs is further demonstrated by targeted deletion of genes of BK_{Ca} channel. VSMCs from the BK_{Ca} α -subunit null mice exhibited loss of spontaneous transient outward currents (STOCs) and more depolarized membrane potential, which accounted for the increased systemic blood pressure [42]. BK_{Ca} channels in VSMCs deprived of the $\beta 1$ subunit also displayed decreased channel activity which was associated with reduced Ca^{2+} sensitivity and impaired coupling between Ca^{2+} sparks and BK_{Ca} channel activity, leading to elevated systemic blood pressure in the transgenic mice [29,43]. An aorta from $\beta 1$ subunit knockout mice exhibited a heightened contractile response to norepinephrine [43]. These observations further support a negative feedback mechanism of BK_{Ca} channels in limiting excessive vasoconstriction. However, the significance of $\beta 1$ subunit in regulating blood pressure is challenged by a recent observation that $\beta 1$ -subunit knockout mice are not hypertensive [44].

BK_{Ca} channels as the target of vasoconstrictors and vasodilators

BK_{Ca} channels also have an important role in both vasodilation and vasoconstriction induced by vasoactive substances. In general, vasodilators activate BK_{Ca} channels, whereas vasoconstrictors inhibit the channels. BK_{Ca} channels in VSMCs are the major mediator of vasodilators. Activity of the channels was enhanced by isoproterenol [45,46], bradykinin [47], calcitonin gene related peptide [48], and prostaglandin E2 [49]. Accordingly, suppressing BK_{Ca} channel activity with tetraethylammonium (TEA) impaired vasorelaxation in response to isoproterenol [45]. By contrast, vasoconstriction induced by 5-hydroxytryptamine [50], phenylephrine [50], angiotensin II [50–52], endothelin-1 [51], thromboxane A2 [53], and 20-hydroxyeicosatetraenoic acid (20-HETE) [54] was accompanied by an inhibition of BK_{Ca} channel activity.

Regulation of BK_{Ca} channel activity in VSMCs

Phosphorylation

A variety of phosphorylation sites have been identified in cytoplasmic domain of BK_{Ca} channel α -subunit [55]. BK_{Ca} channels in VSMCs is regulated by various protein kinases, including cyclic AMP-dependent protein kinase A (PKA), cyclic GMP dependent protein kinase G (PKG), diacylglycerol and/or Ca^{2+} -dependent protein kinase C (PKC), and tyrosine kinase c-Src. Depending on the particular protein kinase involved, phosphorylation of BK_{Ca}

channels could lead to altered Ca^{2+} - and voltage-sensitivity [55], which in turn increases or decreases channel activity. In general, BK_{Ca} channel activity in VSMCs was stimulated by PKA [48,56] and PKG [57,58] but inhibited by PKC [59,60] and c-Src [50]. However, excitatory effects of PKC and c-Src on BK_{Ca} channel were also observed. Activation of PKC increased BK_{Ca} channel activity in rat pulmonary arterial myocytes [61]. In addition, c-Src was found to enhance BK_{Ca} channel activity in a heterologous expression system [62]. Interestingly, complex cross-talks exist among protein kinases in modulating BK_{Ca} channel activity. In coronary and pulmonary arterial myocytes, cAMP was found to cross-activate BK_{Ca} channel through PKG [46,63]. In addition, PKG mediated PKC-stimulated BK_{Ca} channel in rat pulmonary arterial myocytes [61]. Furthermore, cAMP-induced activation of BK_{Ca} channel in coronary and pulmonary arterial myocytes was inhibited by PKC [59,64]. Whether these cross-talks occur *in vivo* has yet to be determined. A variety of vasoactive substances exerted their actions involving phosphorylation of BK_{Ca} channel (Figure 2). For example, vasoconstriction induced by 20-HETE, 5-hydroxytryptamine, angiotensin II, and phenylephrine involved inhibition of the BK_{Ca} channel by PKC and c-Src [50,52,54]. By contrast, vasodilation induced by isoproterenol and NO required activation of the BK_{Ca} channel by PKA and PKG, respectively [46,57].

Steroid hormones

Accumulating evidence has also implicated steroid hormones as important physiological and pathophysiological regulators of cardiovascular functions [65]. These hormones have been shown to alter vascular function in part by regulating BK_{Ca} channel activity in VSMCs through both genomic and non-genomic mechanisms.

Acute administration of 17- β -estradiol resulted in vasorelaxation and/or vasodilation which could be inhibited by blockade of BK_{Ca} channel [66,67]. Electrophysiological techniques_[RE1] revealed stimulation of BK_{Ca} channel activity by estrogen and xenoestrogens in various vascular beds, such as coronary artery [68–70], cerebral artery [71], uterine artery [72]. Enhanced BK_{Ca} channel activity by acute estrogen exposure did not involve changes in channel expression [73]. A variety of non-genomic mechanisms have been implicated in acute vascular responses exerted by estrogen. At pharmacological (micromolar) concentrations, estrogen exerted its stimulatory action through interacting with $\beta 1$ subunit [74]. Estrogen at physiological concentrations could stimulate generation of NO in VSMCs; and NO in turn enhanced BK_{Ca} channel activity through the cGMP-PKG signaling pathway [68,72]. Estrogen could also enhance BK_{Ca} channel activity in VSMCs in part through increasing NO release from endothelial cells [66]. Furthermore, a recent study revealed that activation of the G-protein coupled estrogen receptor stimulated BK_{Ca} channel activity in coronary arterial myocytes independent of NO-cGMP-PKG pathway, leading to vasorelaxation [70]. Acute administration of estrogen increased coronary blood flow [75]. Major portion of vasodilation of coronary artery and subsequent increased blood flow induced by estrogen were conferred by activation of BK_{Ca} channel. Thus, estrogen-induced vasodilation likely has a role in reducing both myocardial infarct size and ventricular arrhythmias.

Chronic exposure to estrogen also alters BK_{Ca} channel function in VSMCs. Estrogen responsive sequences have been identified in the promoters of mouse α -subunit gene, and estrogen strongly stimulated mSlo promoter activity through activation of estrogen receptor α ($\text{ER}\alpha$) [76]. However, in contrast to genomic upregulation of mSlo transcription, stimulation of hSlo gene expression by estrogen involved a nongenomic PI3K signaling pathway and other mechanisms [77]. In ovariectomized nonpregnant sheep, prolonged exposure to 17 β -estradiol increased expression of $\beta 1$, but not α -subunit in uterine artery [73]. Similarly, chronic treatment of nonpregnant ovine uterine arteries with physiologically relevant concentrations of 17 β -estradiol in an *ex vivo* tissue culture system augmented BK_{Ca}

channel activity, which was accompanied by increased protein expression of $\beta 1$ subunit [17]. By contrast, similar treatment of ovariectomized guinea pig increased expression of α but not $\beta 1$, subunit in aorta [78], suggesting species- and/or tissue-dependent upregulation of BK_{Ca} channels by estrogen. However, a 48-hour exposure of cultured human coronary arterial myocytes to a pharmacological concentration of 17β -estradiol led to downregulation of BK_{Ca} channels [79].

Acute progesterone exposure also induced vasorelaxation and/or vasodilation of coronary artery through endothelium-independent, nongenomic mechanism [80]. Activity of BK_{Ca} channel in coronary artery was significantly enhanced by progesterone [81]. Testosterone-induced vasorelaxation was sensitive to blockade by BK_{Ca} channel blockers [82,83]. BK_{Ca} channel activity was stimulated by testosterone in coronary artery [82]. A recent study revealed that hydrogen sulfide (H_2S) was involved in the vasodilatory effect of testosterone [84]. It is likely that activation of BK_{Ca} channels by this gasotransmitter leads to vasodilation induced by testosterone.

Chronic treatment of rat aorta with glucocorticoids, such as corticosterone and 11-dehydrocorticosterone resulted in enhanced contractile response to phenylephrine and angiotensin II [85]. A later study demonstrated that expression of α -subunit in cultured rat aortic myocytes was decreased after chronic exposure to glucocorticoids [86], which in part accounted for the enhanced contractile response induced by glucocorticoids. The enhanced vasoactivity by glucocorticoids could attribute to occurrence of hypertension during clinical application of glucocorticoids [87]. In addition, a 24-hour treatment of cultured VSMCs with aldosterone decreased expression of BK_{Ca} channels [88].

Redox and ROS

Reactive oxygen species (ROS), generated in vascular cells including endothelial and smooth muscle cells, exert both physiological and pathophysiological impacts on vascular function by altering intracellular reduction and/or oxidation (redox) status [89]. One of the ROS targets in blood vessels is BK_{Ca} channels in VSMCs. The activity of BK_{Ca} channels in VSMCs was stimulated by hydrogen peroxide (H_2O_2) [90,91], which in turn could hyperpolarize cell membrane leading to vasodilation. Indeed, H_2O_2 caused vasorelaxation and/or vasodilation which was attenuated by BK_{Ca} channel blockers [90,92[RE2]]. H_2O_2 could directly bind to the channel to modify BK_{Ca} channel activity [90]. In addition, activation of BK_{Ca} channels by H_2O_2 also involved PLA2-arachidonic acid signaling pathway and metabolites of lipoxygenase [91]. BK_{Ca} channel activity in pulmonary arterial myocytes was stimulated by oxidizing agents, such as 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) and oxidized glutathione (GSSG), where it was inhibited by reducing agents, such as dithiothreitol (DTT), reduced glutathione (GSH;) and NADH [93]. By contrast, the opposite was observed for heterologously expressed BK_{Ca} channels [94,95]. Peroxynitrite ($OONO^-$), an oxidant formed by the reaction of NO and superoxide, contracted cerebral artery [96]. Furthermore, $OONO^-$ inhibited BK_{Ca} channels in cerebral and coronary arterial myocytes [97,98]. The impaired BK_{Ca} channel activity by $OONO^-$ could depolarize the cell membrane, resulting in vasoconstriction.

Excessive generation of ROS has been observed in a variety of physiological and pathophysiological conditions, such as aging, diabetes, hypoxia, and hypertension [89]. Therefore, alternation of BK_{Ca} channel function by ROS might contribute to the development of cardiovascular dysfunctions associated with those conditions. Indeed, oxidation of cysteine residues was found to be a contributing factor in altered BK_{Ca} function of VSMCs in diabetes [95].

Gases (NO and CO)

In circulatory system, NO is primarily synthesized by nitric oxide synthase (NOS) in the endothelium, which diffuses to VSMCs once released [99]. The interaction of NO with VSMCs causes hyperpolarization and vasorelaxation [100]. BK_{Ca} channels have been implicated in the action of NO in VSMCs. Vasorelaxation and/or vasodilation induced by NO was attenuated by blockade of BK_{Ca} channel [66,101]. NO stimulated the BK_{Ca} channel activity in various vascular beds [101–103]. Two types of modulation of BK_{Ca} channel by NO have been reported: (i) PKG-dependent [58,66,101] and (ii) direct interaction of NO with the channel protein [102,103]. The direct modulation possibly involved nitrosylation of sulfhydryl groups in BK_{Ca} channels [102,104]. Furthermore, knockdown of β -subunit with antisense abolished the stimulatory effect of NO [104], implicating β -subunit as a mediator of NO.

Carbon monoxide (CO) is generated in both endothelial cells and VSMCs through metabolism of heme by heme oxygenase (HO) [105]. As similar to NO, CO also functions as a vasodilator, and inhibition of BK_{Ca} channels ablated CO's vasorelaxant effect [106,107]. Furthermore, BK_{Ca} channel activity in VSMCs was stimulated by CO [108,109]. Ca²⁺ sensitivity was increased [109], due to an enhanced coupling Ca²⁺ sparks to BK_{Ca} channels [110]. The modulation of BK_{Ca} channels by CO is independent of β -subunit [104] and does not involve PKG [108]. Several potential sites in α -subunit for CO interaction have been identified: (i) extracellular histidine residues [111], (ii) H365, D367 and H394 in RCK1 [112]; and (iii) H911 in RCK2 [113]. Considering the role of RCK1 and RCK2 in regulating Ca²⁺ affinity, it is not surprising that the contact of CO with residues within these two domains increase Ca²⁺ sensitivity. BK_{Ca} channel activity appears to be under a constant control of endogenously generated CO. Inhibition of HO depressed BK_{Ca} channel activity in renal artery, which could be reversed by exogenous CO [114].

Arachidonic acid and its metabolites

Arachidonic acid and its metabolites, such as 20-HETE_[RE3] and epoxyeicosatrienoic acids (EETs) are potent vasoactive substances and have important roles in regulation of vascular function. Whereas 20-HETE is a vasoconstrictor, arachidonic acids and EETs are vasodilators [115]. In isolated VSMCs, arachidonic acid activated BK_{Ca} channels [116,117]. Moreover, arachidonic acid-induced vasorelaxation was attenuated by blockade of BK_{Ca} channels [118], suggesting involvement of BK_{Ca} channels in vasoactive action of arachidonic acid. BK_{Ca} channel activity was enhanced by EETs [119]. EETs increased sensitivity of BK_{Ca} channels to Ca²⁺ and voltage. The enhanced BK_{Ca} channel activity could account for the vasodilatory response induced by EETs since the vasodilation was reduced by BK_{Ca} channel blockers [120].

By contrast, BK_{Ca} channel activity in VSMCs was inhibited by 20-HETE [54,121]. The inhibition of BK_{Ca} channels by 20-HETE was mediated by PKC [54]. As a result, vasoconstriction induced by 20-HETE was absent after blockade of BK_{Ca} channels [121]. In renal artery, both activation of BK_{Ca} channels and vasodilation induced by NO was largely due to depressed formation of 20-HETE by NO [122]. Decreased generation of 20-HETE during exposure to acute hypoxia and subsequently reduced inhibition of BK_{Ca} channel activity also accounted for hypoxia-induced vasodilation of pulmonary artery [123].

pH

The pH in VSMCs fluctuates, and a change in intracellular proton [H⁺]_i concentration could have an impact on vascular tone [124]. In general, acidification leads to vasorelaxation, whereas alkalization results in enhanced vasoconstriction [124]. Interestingly, BK_{Ca} channel activity in cultured coronary [125] and rabbit basilar [126] arterial myocytes was stimulated

by increasing H^+ concentration. Those observations suggest that activation of BK_{Ca} channels by H^+ could contribute to acidosis-induced vasorelaxation. A mutagenesis study revealed that H^+ exerted its action by interacting with His365 and His394 in addition to Asp367, a residue participating high-affinity Ca^{2+} sensing, in the RCK1 domain [127]. However, inhibitory effect of acidosis on BK_{Ca} channels was reported in human internal mammary [128] and rat tail [129] arterial myocytes. The physiological relevance of acidosis-induced BK_{Ca} channel inhibition is not clear.

Macromolecular signaling complex: a new form of BK_{Ca} channel regulation

Formation of macromolecular complexes by signaling molecules has a crucial role in cellular signaling, which enables rapid, specific and local regulation [130]. BK_{Ca} channels in VSMCs have been reported to form macromolecular complexes with various signaling components, including receptors, ion channels, enzymes, anchoring proteins and heme (Figure 3). The association of these components to BK_{Ca} channels influences ion channel activation, phosphorylation and location.

BK_{Ca} channels were found to associate with a variety of ion channels that mediate Ca^{2+} influx across plasma membrane and Ca^{2+} release from intracellular Ca^{2+} stores. BK_{Ca} channels and $Ca_v1.2$ formed a complex in rat aorta and in cultured human VSMCs; and β_2 -adrenergic receptor (β_2AR) functioned as a scaffold to enable the physical association of these two channels [131]. The association of BK_{Ca} channels with transient receptor potential canonical 1 (TRPC1), a Ca^{2+} -permeable cation channel mediating store-operated Ca^{2+} entry was observed in cultured rat aortic myocytes [132]. BK_{Ca} channels were also found to form a complex with ryanodine receptor (RyR) in myocytes of vas deferens and bladder [133] and trachea [134]. Activation of BK_{Ca} channels requires a $[Ca^{2+}]_i$ concentration of $10 \mu M$ [135]. Opening of Ca^{2+} -permeable channels in the complex leads to Ca^{2+} influx across the plasma membrane or release from the sarcoplasmic reticulum Ca^{2+} store, which could create high local concentrations of Ca^{2+} (Ca^{2+} microdomains) [136]. Physically associating BK_{Ca} channels with those Ca^{2+} -permeable channels ensures that BK_{Ca} channels are in close proximity to the Ca^{2+} microdomains, enabling rapid and specific activation. Consistently, both Ca^{2+} influx mediated by $Ca_v1.2$ [35,137] and TRPC1 [132], and Ca^{2+} release mediated by RyR [137] activated BK_{Ca} channels in smooth muscle cells. Although type 1 inositol 1,4,5-trisphosphated receptor (IP_3R) and BK_{Ca} channels were associated in cerebral arterial myocytes, activation of IP_3R -stimulated BK_{Ca} channel activity was independent of Ca^{2+} release from sarcoplasmic reticulum store [138].

Some G protein-coupled receptors (GPCRs) also associate with BK_{Ca} channels to form signaling complexes. BK_{Ca} channels and thromboxane A2 receptor (TP) were found to form a complex in myocytes of human coronary artery and rat aorta [139]. Activation of TP led to a G protein-independent trans-inhibition of BK_{Ca} channels, involving interaction between first intracellular loop and C terminus TP and voltage-sensing conduction cassette of BK_{Ca} channels. A macromolecular complex containing BK_{Ca} channels, $Ca_v1.2$, β_2AR , A-kinase-anchoring protein (AKAP79/150) was described in rat aorta and bladder in addition to in cultured human VSMCs [131]. The third intracellular loop of β_2AR was involved in BK_{Ca} channel- β_2AR interaction. To achieve full functional regulation of BK_{Ca} channels, possibly involving phosphorylation mediated by PKA, co-localization of β_2AR , BK_{Ca} channel, and AKAP79 is required. A recent study demonstrated existence of a macromolecular complex containing BK_{Ca} channels, angiotensin II type 1 receptor (AT1R), $G_{\alpha q/11}$, nonphagocytic NAD(P)H oxidases (NOX-1), and c-Src kinases (c-Src) in the caveolae of rat aortic myocytes [52]. Angiotensin II stimulated activity of NOX-1 leading to generation of ROS, which subsequently inhibited BK_{Ca} channel activity. Furthermore, physical association between BK_{Ca} channels and AT1R was enhanced in the vasculature of streptozotocin-

induced diabetic rats, resulting in heightened AT1R-mediated oxidative modification and impaired BK_{Ca} channel function.

Formation of kinase-ion channel complex permits fine control of phosphorylation state of the channel. Association of BK_{Ca} channels and c-Src has been demonstrated in myocytes of human coronary artery [50] and rat aorta [52]. Such a c-Src-BK_{Ca} channel complex arrangement may facilitate tyrosine phosphorylation of BK_{Ca} channels and subsequent channel inhibition stimulated by angiotensin II, 5-hydroxytryptamine, and phenylephrine. Direct evidence for association of other kinases to BK_{Ca} channels in VSMCs is lacking. However, the observations of participation of AKAP79 in formation of β 2AR-BK_{Ca} channel complex and requirement of AKAP79 in activation of β 2AR-stimulated BK_{Ca} channel activity [131] suggest that PKA is likely to be a component of the macromolecular complex. In mouse cardiac myocytes, PKA was a signaling component of a macromolecular complex which also included caveolin-3, Ca_v1.2, β 2-AR, G-protein α s, adenylyl cyclase, and protein phosphatase 2a [140].

BK_{Ca} channels contain two caveolin-binding motifs in its cytoplasmic domain. Association between caveolin-1 and BK_{Ca} channels was observed in rat aortic myocytes [52,141]. By forming such a complex, caveolin-1 has an important role in both targeting BK_{Ca} channels to caveolin-rich caveolae and reducing cell surface expression of the channel [141]. BK_{Ca} channel-mediated vasodilation was increased in gracilis muscle arterioles from caveolin-1 knockout mice [142]. By contrast, expression of caveolin-1 was dramatically increased in aorta from streptozotocin-induced diabetic rats, which was accompanied by impaired BK_{Ca} channel function [52]. It is not surprising that genetic ablation of caveolin-1 gene resulted in increased current density of BK_{Ca} channel in cerebral arterial myocytes [143] and protected vascular BK_{Ca} channel function in diabetes [52].

Heme could inhibit BK_{Ca} channel activity in VSMCs activity through binding to a heme binding site located between two RCKs; and binding of CO to heme relieved the inhibitory effect [144]. In addition, HO catalyzed metabolism of heme to produce CO in the presence of O₂ [145]. HO-2 and BK_{Ca} channels formed a complex in carotid body and HO-2 acted as an O₂ sensor to regulate BK_{Ca} channel activity [146]. The generation of CO by HO-2 bound to BK_{Ca} channel could provide a rapid and specific modulation of the channel. Although HO-2 and BK_{Ca} channel could form a complex in pulmonary artery, hypoxic pulmonary vasoconstriction was not altered in HO-2-deficient or BK_{Ca}-deficient mice [147]. Thus, this mechanism did not contribute to modulation of BK_{Ca} channels in VSMCs during exposure to hypoxia.

BK_{Ca} channel function in physiological conditions

Development and aging

Aging and development are accompanied by complex changes of vascular structure and function. Development alters the contribution of BK_{Ca} and voltage-gated K¹ (K_V) channels to setting resting membrane potential in pulmonary arterial myocytes. [RE4]Whereas BK_{Ca} currents predominate in ovine fetal myocytes, K_V currents became the major player in adult myocytes [148]. The expression of BK_{Ca} and K_V channels followed the same pattern [149,150]. By contrast, BK_{Ca} activity in systemic vasculature increased during development. The human adult aortic myocytes exhibited a higher BK_{Ca} current density and longer open duration than in the fetal cells [151]. Similarly, activity of BK_{Ca} channels is progressively upregulated in rat aortic myocytes after birth [152].

Vasorelaxation was attenuated with the advance of age [153]. Correspondingly, activity of BK_{Ca} channels in VSMCs significantly decreased during aging [154,155].

Electrophysiological studies revealed that compared with young F344 rats, current density of BK_{Ca} channel in coronary arterial myocytes significantly decreased in the old rats [156–158]. Accordingly, expression of α - and β -subunits was decreased. Dramatic reduction in BK_{Ca} channel expression was also observed in aged human coronary artery [156]. Vasoconstriction of coronary artery induced by iberiotoxin in the old rat was also blunted compared with the young rat [156,157], suggesting a significantly decrease in the contribution of BK_{Ca} channels in regulation of vascular tone. Therefore, downregulated BK_{Ca} channel activity and expression likely have important roles in an increased risk of coronary spasm and myocardial ischemia in the elderly. The protein levels of BK_{Ca} α -subunit were also downregulated with age in arterioles from rat skeletal muscles [159]. However, BK_{Ca} channel function and expression remained unchanged in the cerebral artery [160].

Pregnancy and ovarian cycle

Uterine blood flow increases substantially during pregnancy to meet metabolic requirements for the growth and survival of the fetus. Pregnancy was associated with decreased myogenic tone of ovine uterine artery, which was abolished by blockade of BK_{Ca} channels [17]. Moreover, a significant portion of the increased uterine blood flow was attenuated by intra-arterial infusion of TEA [161,162]. Therefore, enhanced BK_{Ca} channel function has an important role in the adaptive changes of uterine circulation. Electrophysiological studies confirmed this notion by demonstrating enhanced BK_{Ca} channel activity in uterine arterial myocytes during pregnancy [17]. RT-PCR and Western blot revealed a significant increased expression of β 1 subunit in this vascular bed [17,31]. The increased β 1 subunit expression enhanced Ca²⁺-sensitivity and BK_{Ca} channel activity, which in turn led to decreased vascular tone and increased blood flow. Considering increased eNOS_[RE5] expression and activity [163,164] and increased cGMP and PKG in uterine artery during pregnancy [31], it is likely that modulation of BK_{Ca} channel activity by augmented NO-cGMP-PKG signaling pathway could provide additional regulation on uterine vasoreactivity.

Uterine blood flow also increases during follicular phase of the ovarian cycle [165]. Coincidentally, expression of BK_{Ca} channels, β 1, but not α -subunit was also increased in addition to upregulated cGMP-PKG signal pathway. This increased uterine blood flow could be mimicked by administering 17 β -estradiol systemically or directly in the uterine circulation in ovariectomized nonpregnant ewes, and large portion of it was mediated by 17 β -estradiol-stimulated activation of BK_{Ca} channels in uterine arterial myocytes [72].

Pulmonary circulation adjustment at birth

Fetal pulmonary vasculature remains constricted *in utero*. At birth, resistance in the pulmonary vasculature decreases, leading to vasodilation and perfusion of the lung. Pulmonary vasodilation induced by increasing fetal oxygen tension in acutely instrumented fetal sheep was blocked by BK_{Ca} channel blockers [166], suggesting the involvement of activated BK_{Ca} channels. [RE6]Electrophysiological recording confirmed this notion. BK_{Ca} channel activity in fetal ovine and rabbit pulmonary arterial myocytes was increased by elevating oxygen partial pressure (pO₂) [166,167]. The enhanced BK_{Ca} channel function in turn caused membrane hyperpolarization.

Fetal pulmonary arterial myocytes cultured under hypoxic condition or under normoxic environment in the presence of hypoxia-inducible factor-1 (HIF-1) mimetic deferoxamine resulted in increased expression of both α and β 1 subunit transcripts [168]. HIF-1 is the main transcription factor sensing changes in pO₂, and putative HIF-1 α -responsive sequences are present on the 5' sequence of the gene of β 1 subunit [169]. This upregulation of BK_{Ca}

channel expression was proposed to prepare pulmonary vasculature to respond to perinatal vasodilator stimuli [170].

Exercise

In exercise-trained rats, acetylcholine-induced vasorelaxation of rat mesenteric artery and aorta was enhanced, which was partially suppressed by BK_{Ca} channel blockers [171]. Moreover, exercise training enhanced BK_{Ca} channel blocker-induced contraction of coronary artery from Yucatan miniature swine [172]. BK_{Ca} channel current density was increased in coronary and aortic myocytes of exercise-trained rats and swine [158,173]. The enhanced BK_{Ca} channel activity was largely due to increased Ca²⁺- and voltage-sensitivity [173]. Furthermore, exercise can have a beneficial role in improving cardiovascular function in aging and dislipidemia. In the rat model, aging caused a reduction of BK_{Ca} current density in coronary artery, which was accompanied by reduced expression of both α and β 1 subunits [158]. Both depressed functional and molecular expression of BK_{Ca} channel could be partially reversed by exercise training. Diabetic dyslipidemia decreased STOCs mediated by BK_{Ca} channel in swine coronary myocytes [174]. The reduced BK_{Ca} channel function subsequently increased coronary tone. Exercise training effectively prevented both increased vascular tone and impaired BK_{Ca} channel function.

BK_{Ca} channel function in pathological conditions

Hypertension

Hypertension is the most prevalent risk factor for cardiovascular diseases, such as stroke and myocardial infarction. Considering the role of BK_{Ca} channels in regulating membrane potential and vascular tone, it is reasonable to rationalize that dysfunctional BK_{Ca} channels_[RE7] in VSMCs could contribute to development of hypertension. Gene knockout studies demonstrated that targeted gene deletion of both α and β 1 subunits resulted in increased vascular tone and elevated blood pressure [29,42,43]. Conversely, a genetic polymorphism in β 1 subunit of human BK_{Ca} channel (E65K), a gain-of-function mutation, was associated with a low prevalence of diastolic hypertension [175]. Some hypertension models also implicated BK_{Ca} channels in pathogenesis of hypertension. BK_{Ca} channel function in rat mesenteric artery was depressed in N^ω-nitro-L-arginine-induced hypertension [176]. This impaired BK_{Ca} function was attributed to reduced expression of α subunit [177]. Rats chronically infused with angiotensin II demonstrate_[RE8]d raised systolic blood pressure, and iberiotoxin-induced constriction of cerebral artery was attenuated [178]. Consistent with this observation, expression of β 1, but not α , subunit in cerebral arterial myocytes was profoundly decreased, and the coupling of Ca²⁺ sparks to BK_{Ca} channels was impaired. Similarly, expression of β 1 subunit was depressed in cerebral artery of spontaneously hypertensive rats (SHR) [15]. However, selective loss of β 1 subunit might not be sufficient to cause hypertension. A recent study suggests that β 1 subunit null mice were not hypertensive based on continuous 24-hour blood pressure measurements [44]. In contrast to the findings in angiotensin II- and N^ω-nitro-L-arginine-induced hypertensive rats, other experimental data suggest that vascular BK_{Ca} channel function was upregulated in both spontaneously hypertensive and deoxycorticosterone acetate (DOCA)-salt hypertensive rats. Enhanced vasoconstriction induced by BK_{Ca} channel blockers [179–181] and heightened BK_{Ca} channel activity [179,182,183] was observed in various blood vessels. In line with these findings, expression of α or β 1 subunits was elevated [181–183]. The upregulated BK_{Ca} channel function was suggested to represent a compensatory mechanism to limit excessive vasoconstriction in hypertension [182].

Diabetes

Both type 1 and type 2 diabetes cause vascular dysfunction. Myogenic tone was increased in cerebral arteries from streptozotocin-induced type 1 diabetic rats [184] and mice [185] and type 2 diabetic BBZDR/Wor rats [186] and mesenteric artery from type 2 diabetic C57BL/KsJ-db/db mice [187]. In both type 1 and type 2 diabetes, vasoreactivity mediated by BK_{Ca} channels was impaired. Compared with non-diabetic animals, augmentation of agonist-induced vasoconstriction in mesenteric artery by iberiotoxin was lost, and membrane hyperpolarization by NS 1619 was decreased in Zucker Diabetic Fatty (ZDF) rats [188]. Vasoconstriction of cerebral artery induced by blockade of BK_{Ca} channels was attenuated in streptozotocin-induced diabetic rats [185,189]. Similarly, vasodilation by BK_{Ca} channel opener BMS-191011 was absent in retinal artery of streptozotocin-induced diabetic rats [190]. Therefore, capability of BK_{Ca} channels to oppose vasoconstriction is weakened in both types of diabetes. Electrophysiological experiments demonstrated that BK_{Ca} function was impaired in both type 1 [52,185,189] and type 2 [191,192] diabetic VSMCs. It appears that diabetes specifically targets the β 1 subunit of the BK_{Ca} channel. The expression of β 1, but not α subunit was reduced in type 1 [185,193,194] and insulin-resistant and/or type 2 diabetes [189,192]. Considering the role of β 1 subunit in regulating BK_{Ca} activity, it is not surprising that impaired BK_{Ca} channel function was associated with reduced Ca²⁺ and voltage sensitivity [185,189,192,193]. The coupling of Ca²⁺ sparks to BK_{Ca} channel was also impaired [185]. Diabetes was associated with enhanced production of ROS in VSMCs [195]. ROS acted as potent inhibitors of BK_{Ca} channels and produced a response similar to deletion of the β 1 subunit [196]. Rotenone, a mitochondrial electron transport chain inhibitors, partially reversed generation of H₂O₂ and restored BK_{Ca} function in cerebral artery from streptozotocin-induced diabetic mice [195]. Hyperglycemia has an important role in development of BK_{Ca} channel dysfunction in diabetic patients. In the recombinant system, culturing HEK 293 cells expressing hSlo_{1RE9} in high glucose enhanced superoxide dismutase and suppressed catalase (CAT) expression, leading to increased production of ROS, which in turn inhibited channel function through interacting C911 located in the cytoplasmic domain of BK_{Ca} channels [95]. The actions of high glucose was mimicked by H₂O₂ and restored by CAT gene transfer. Functional studies also demonstrated that impaired BK_{Ca} channel-mediated vasodilation could be restored by superoxide dismutase and CAT treatment [197]. Long-term exposure cultured human coronary arterial myocytes to high glucose also led to downregulation of β 1, but not α subunit through ubiquitination [194]. Attenuation of CO-induced vasorelaxation of tail artery from streptozotocin-induced diabetic rats was accompanied by a decreased sensitivity of BK_{Ca} channel to CO, which was mimicked by chronic exposure of the cultured VSMCs to high glucose [198]. A recent study revealed that BK_{Ca} channels, AT1R, caveolin-1 and c-Src are co-localized in caveolae of VSMCs, and upregulation of caveolin-1 expression in streptozotocin-induced rats with diabetes promote stronger association between BK_{Ca} channel and AT1R, leading to enhanced inhibition of BK_{Ca} channel by angiotensin pathway [52].

Hypoxia

BK_{Ca} channels could function as a chemosensor to detect changes in pO₂ [199]. Modulation of BK_{Ca} channel activity in VSMCs by oxygen is crucial in regulating tissue perfusion and regional blood flow. Hypoxia is caused by a variety of physiological and pathophysiological conditions, such as ischemia, residence at high altitude and chronic obstructive pulmonary disease. BK_{Ca} channels contribute differently to hypoxic responses depending on the nature of hypoxia (acute or chronic) and vascular beds (pulmonary or systemic).

In general, acute hypoxia causes pulmonary vessels to constrict and systemic vessels to dilate to maintain adequate O₂ delivery [199]. Altered BK_{Ca} channel activity in part accounts for the vascular responses induced by acute hypoxia. Acute hypoxia inhibited

BK_{Ca} channel activity in pulmonary arterial myocytes [200,201], whereas it enhanced BK_{Ca} function in myocytes of cerebral and pial arteries [123,202]. Several potential mechanisms can account for the inhibitory effect of acute hypoxia in pulmonary myocytes. Acute hypoxia could alter the cytosolic redox state, which in turn inhibited BK_{Ca} channel activity in pulmonary myocytes [93]. In addition, a decrease in pO₂ could directly exert its action on BK_{Ca} channels. In a recombinant system, acute hypoxia has been shown to reversibly inhibit BK_{Ca} channels activity through a decreased Ca²⁺-sensitivity that was independent of soluble cytosolic factors [203].

Hemeoxygenase-2 (HO-2) and BK_{Ca} channels could form a complex in heterologous expressing system and HO-2 was proposed to function as an O₂ sensor in carotid body [146]. Hypoxia exerts its inhibitory action on BK_{Ca} channel by reducing generation of CO, a BK_{Ca} channel activator, due to decreased supply of HO-2 substrate O₂. However, targeted deletion of HO-2 nor BK_{Ca} channel genes failed to alter acute and chronic vascular responses to alveolar hypoxia in the lung [147]. The enhanced BK_{Ca} channel activity in cerebral artery likely involves altered generation of ROS and 20-HETE [123]. Generation of ROS in cerebral arterial myocytes was increased during exposure to acute hypoxia. In addition, hypoxia-stimulated BK_{Ca} channel activity was abrogated by a superoxide dismutase mimic. Furthermore, hypoxia caused a dramatic decrease endogenous 20-HETE level [RE10] which led to removal of the inhibitory effect of 20-HETE on BK_{Ca} channels [123]. These findings suggest that both redox modulation of BK_{Ca} channels by ROS and alleviation of 20-HETE inhibitory effect contribute to hypoxia simulated-BK_{Ca} function in cerebral artery. By contrast, acute hypoxia was observed to inhibit BK_{Ca} channel activity in cerebral artery by uncoupling Ca²⁺ sparks to BK_{Ca} channel [204]. However, the physiological impact of hypoxia-mediated inhibition on BK_{Ca} channel in cerebral artery remains unclear since acute hypoxia causes cerebral vasodilation.

Chronic hypoxia depressed BK_{Ca} channel activity in rat and human pulmonary artery [205,206]. Although K_V is the predominant K⁺ channel in regulating resting membrane potential in adult pulmonary arterial myocytes [148], depressed BK_{Ca} channel activity during prolonged exposure to hypoxia could also contribute to pulmonary vasoconstriction, leading to pulmonary hypertension. BK_{Ca} channel function in systemic vasculature is also altered by chronic hypoxia. Relaxation mediated by BK_{Ca} channel in both adult and fetal sheep cerebral arteries was impaired by long-term high altitude hypoxia [207]. Cultured rat basilar arterial myocytes displayed reduced BK_{Ca} channel activity after chronic exposure to hypoxia [208]. The reduced BK_{Ca} channel activity was associated with decreased Ca²⁺- and voltage-sensitivity of the channel [205]. Chronic hypoxia also depressed BK_{Ca} channel activation stimulated by PKA and PKG [205,209]. In addition to post-translational modification of the channel, chronic hypoxia also alters BK_{Ca} channel expression. In response to chronic hypoxia expression of α subunit was reduced in rat pulmonary artery [210]. Expression of β 1 subunit was also decreased in cultured myocytes from rat aorta, rat basilar artery and human mammary artery after prolonged hypoxia treatment [208].

Ischemia presents a hypoxic insult to tissues and organs. BK_{Ca} channel activity and vasorelaxation mediated by BK_{Ca} channels were depressed following ischemia for 1 hour in porcine coronary myocytes [211]. However, vasorelaxation of human coronary artery induced by BK_{Ca} channel opener NS 1619 was not affected after cardiopulmonary bypass, and expression of both α and β 1 subunits remain unchanged [212]. In addition, BK_{Ca} channel function in cerebral artery was largely not affected by ischemia [213].

Shock

Vascular BK_{Ca} channels have been proposed to contribute to development of endotoxic and hemorrhagic shock [214]. VSMC membrane became hyperpolarized in both endotoxic and

hemorrhagic shock rats [16,215]. In addition, both types of shock were associated with vascular hyporeactivity to norepinephrine. BK_{Ca} channel blockers partially reversed both membrane hyperpolarization and attenuated vasoconstriction induced by norepinephrine. Similarly, impaired vascular reactivity to norepinephrine was restored by BK_{Ca} channel blockers in human brachial artery during endotoxemia [216]. Those findings implicate BK_{Ca} channels in the development of hypotension. Further evidence came from electrophysiological experiments, which demonstrated that endotoxin lipopolysaccharide increased BK_{Ca} channel activity in cerebral arterial myocytes [217]. BK_{Ca} channel activity was also increased in mesenteric artery from hemorrhagic shock animals [16]. The elevation of BK_{Ca} activity was the result of increased expression of β 1 subunit and enhanced coupling of Ca²⁺ sparks to BK_{Ca} channel.

Other cardiovascular dysfunctions

Both myogenic tone and membrane depolarization of cerebral artery increase after subarachnoid hemorrhage [218,219]. It was found that blockade of BK_{Ca} channel caused constriction of cerebral artery from control, but not subarachnoid hemorrhage animals. In addition, both Ca²⁺ sparks and STOCs were reduced [220]. Those findings imply impaired BK_{Ca} channel function in cerebral artery following subarachnoid hemorrhage. However, functional properties and expression of BK_{Ca} channels remained unaltered in cerebral artery from several models of subarachnoid hemorrhage [218,220]. Cirrhosis upregulated BK_{Ca} channel α -subunit expression in rat mesenteric artery and enhanced BK_{Ca} channel-mediated vasodilation [221]. BK_{Ca} channel activity was reduced in rabbit and porcine coronary arterial myocytes during left ventricular hypertrophy [222,223]. The impaired BK_{Ca} channel function was not due to alternation of BK_{Ca} channel expression, but conferred by reduced Ca²⁺-sensitivity of the channel.

BK_{Ca} channel as a therapeutic target of cardiovascular dysfunction

As mentioned above, BK_{Ca} channels play an important role in the regulation of membrane potential of VSMCs which in turn controls vascular tone; and various physiological and pathophysiological conditions, including hypertension, shock, diabetes, ischemic heart disease, which are associated with altered BK_{Ca} channel function in VSMCs. Therefore, BK_{Ca} channels constitute a major target for treatment of cardiovascular diseases. Several strategies are potentially useful in combating these cardiovascular dysfunctions.

The most common and convenient approach is pharmacological manipulation. A variety of compounds have been developed to either activate or inhibit BK_{Ca} channels [224]. Hypoactivity of BK_{Ca} channels could be boosted by BK_{Ca} channel openers, whereas hyperactivity of the channel might be suppressed by BK_{Ca} channel blockers. The prototype BK_{Ca} channel opener benzimidazolones, such as NS 1619 and NS 004 interact with α -subunit to enhance channel function [225]. However, their clinical use is hampered by low efficacy and lack of selectivity. In fact, clinical trials involving other BK_{Ca} channel openers, such as NS 8, BMS204352 and TA1702 have been discontinued [224]. The BK_{Ca} channel α -subunit is ubiquitously expressed whereas β 1 subunit is predominantly expressed in smooth muscle cells [29,30,226]. Therefore, development of molecules that selectively target the β 1 subunit would be favorable for treating cardiovascular diseases associated with altered BK_{Ca} channel function in VSMCs. The studies by Dopico and colleagues are encouraging; and the authors reported that cholane-derived steroid lithocholate stimulated BK_{Ca} channel activity by selectively interacting with β 1 but not β 2–4 subunits [227]. Thus, lithocholate could serve as a template for developing subunit-selective BK_{Ca} channel openers.

By contrast, a local delivery of a BK_{Ca} channel gene could provide an alternative treatment for impaired BK_{Ca} channel activity. Intracorporal microinjection of naked BK_{Ca} channel gene (pcDNA-hSlo) enhanced erectile function [228]. Preliminary results from a human clinical trial were promising and showed safety and efficacy [229]. Recently, work from the same group demonstrated the effectiveness of smooth muscle-specific gene transfer with the human BK_{Ca} channel in atherosclerotic cynomolgus monkeys using a vector containing smooth muscle α -actin promoter [230]. Therefore, tissue-specific delivery of BK_{Ca} channel gene together with application BK_{Ca} channel modulators could provide an efficient treatment of BK_{Ca} channel-related cardiovascular dysfunctions.

Altered BK_{Ca} channel function could also be corrected indirectly. Endothelium of blood vessels is the sources of a variety of vasodilators, such as NO, prostacyclin, and cytochrome P-450 metabolites EETs [231]. Drugs that increase production of those molecules would then heighten BK_{Ca} channel activity in VSMCs. Furthermore, delivery of cytochrome P-450 epoxygenase gene into VSMCs increased 14,15-EET release and subsequently enhanced BK_{Ca} channel activity [232]. This approach could provide another possibility for managing dysfunction of BK_{Ca} channel in VSMCs.

Concluding remarks

In summary, BK_{Ca} channels have been implicated in setting resting membrane potential and regulating vascular tone. Vascular responses exerted by various vasoactive substances involve activation or inhibition of BK_{Ca} channel in VSMCs. In addition, BK_{Ca} channels in VSMCs are subjected to a variety of modulations involving genomic and non-genomic mechanisms. Furthermore, alterations of BK_{Ca} channels in VSMCs have been documented in both physiological and pathophysiological conditions, such as aging and/or development, pregnancy, hypertension, diabetes, and hypoxia. However, precise mechanisms underlying the altered functional and molecular expression of BK_{Ca} channel in VSMCs remains to be elucidated in future studies. Information obtained from these studies will expand our understanding of physiological and pathophysiological roles of BK_{Ca} channels in cardiovascular adaptation and dysfunctions, and facilitate translating the knowledge into design of effective therapeutics. Effective manipulation of BK_{Ca} channel activity by development of subunit-specific BK_{Ca} channel modulators and tissue-specific gene delivering techniques will be key to the treatment of cardiovascular diseases associated with altered expression and activity of BK_{Ca} channels in VSMCs.

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Biographies

Dr Zhang is professor of Pharmacology and Physiology at Loma Linda University School of Medicine. He was the President of the Western Pharmacology Society in the USA in 2008. He has been members in the various study sections of grant review for US National Institutes of Health and American Heart Association for more than 15 years. Dr Zhang is the author/coauthor of over 480 scientific articles, book chapters and abstracts. His research interests focus on the molecular mechanisms in the uteroplacental circulation and developmental programming of adult cardiovascular disease.

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Highlights

- BK_{Ca} channels are abundantly expressed in vascular smooth muscle cells
- BK_{Ca} channels participate in regulation of vascular tone and blood pressure
- BK_{Ca} channels are a key player in both cardiovascular adaption and dysfunctions
- Developing therapeutics is crucial to treat diseases due to BK_{Ca} channel alterations

Figure 1. Sources of Ca^{21} in activation of BK_{Ca} channels in VSMCs

The major cause for activation of BK_{Ca} channels in VSMCs is an increase in intracellular Ca²¹ concentrations in the vicinity of BK_{Ca} channel (Ca²¹ microdomain). Such a localized increase in Ca²¹ could be achieved by spontaneous or agonist-stimulated Ca²¹ release from intracellular stores through RyR or IP₃R on SR and Ca²¹ influx through L-type Ca_v1.2. Activation of BK_{Ca} channels enables K¹ efflux due to the K¹ electrochemical gradient, resulting in hyperpolarization of the myocyte membrane and subsequent closure of Ca_v1.2.

Abbreviations: BK_{Ca}: Large-conductance Ca²¹-activated K¹; Ca_v1.2: voltage-gated Ca²¹ channel; IP₃R: inositol 1,4,5-trisphosphate receptor; RyR: ryanodine receptor; SR: sarcoplasmic reticulum; VSMCs: vascular smooth muscle cells.

Figure 2. Regulation of BK_{Ca} channels in VSMCs

BK_{Ca} channels in VSMCs are a regulatory target of vasodilators and vasoconstrictors through GPCRs. These vasoactive agents alter BK_{Ca} channel activity through phosphorylation of the channel by activation of protein kinases. Generally, BK_{Ca} channel activity is enhanced by activation of PKA or PKG, but depressed by activation of PKC and c-Src. In addition, BK_{Ca} activity is modulated by a variety of factors involving both non-genomic and genomic mechanisms. An elevation and/or generation of NO, CO, H⁺, EETs and estrogen stimulates BK_{Ca} channel activity, whereas an increase production of 20-HETE and ROS and a decrease in pO₂ inhibit BK_{Ca} channel function. However, exceptions do exist. As a result, activation of BK_{Ca} channels hyperpolarizes the myocyte membrane leading to vasodilation, whereas inhibition of BK_{Ca} channel causes depolarization of the membrane resulting in vasoconstriction.

Abbreviations: BK_{Ca}: Large-conductance Ca²⁺-activated K⁺; CO: carbon monoxide; EETs: epoxyeicosatrienoic acids; GPCR: G-protein coupled receptors; 20-HETE: 20-hydroxyeicosatetraenoic acid; NO: nitric oxide; pO₂: oxygen partial pressure; PKA: protein kinase A; PKC: protein kinase C; ROS: reactive oxygen species; VSMCs: vascular smooth muscle cells.

Figure 3. Regulation of BK_{Ca} channels by forming macromolecular complexes in VSMCs

BK_{Ca} channels in VSMCs could form macromolecular complexes with other signaling components including GPCRs, ion channels, enzymes, G proteins, and anchor proteins. The association of Ca²¹-permeable channels (Ca_v1.2 and RyR) and BK_{Ca} channel enables creation of a high concentration of Ca²¹ in the vicinity of BK_{Ca} channels (Ca²¹ microdomain). The formation of GPCR-BK_{Ca} channel complex permits direct modulation of the channel by thromboxane A2 receptor (TP) or indirect modulation by β2AR-associated PKA or AT1R-associated c-Src. The inclusion of nonphagocytic NAD(P)H oxidases (NOX-1) in the AT1R-BK_{Ca} complex provide additional modulation of BK_{Ca} channels by ROS, which might have a role in the pathophysiology of cardiovascular dysfunction in patients with diabetes.

Abbreviations: AT1R: angiotensin II type 1 receptor β2AR: β2-adrenergic receptor; GPCR: G-protein coupled receptors; PKA: protein kinase A; ROS: reactive oxygen species; VSMCs: vascular smooth muscle cells.