Large-conductance Ca²⁺- and voltage-activated K⁺ (BKCa) channels are well known for their roles in regulation of membrane excitability, partly owing to their large conductance, 250–300 pS in symmetrical 150 mM K⁺, and to the synergistic activation mechanism encompassing membrane depolarization and intracellular Ca²⁺/Mg²⁺ (98). The interdependent activation mechanism enables BKCa channels to typically exert a negative feedback influence on cellular excitability (98, 118). The importance of BKCa channels in regulation of vascular tone, determination of action potential duration and frequency, and neurotransmitter release has been well documented (98). Consistent with the functional importance, notable phenotypes, such as hypertension, erectile dysfunction, and urinary incontinence, are associated with inhibition or down-regulation of the BKCa channel activity (5, 101).

Conversely, enhancement or upregulation of the channel function in select cells may offer protection against some of the aforementioned disorders (20, 25, 41, 132).

Structurally, BKCa channels are composed of four pore-forming Slo1 (α) subunits, each of which contains 7 trans-membrane segments (S0–S6) and a large COOH-terminal cytoplasmic region (98) (see Figure 2). S1–S4 form the primary voltage-sensor domain (VSD), and S5, P, and S6 together form the main ion permeation domain. The cytoplasmic area is postulated to harbor two homologous structural units termed "regulators of conductance for Ca²⁺" (RCK1 and RCK2) based on partial sequence similarity to the bacterial K⁺ channel MthK whose high-resolution structures are known (56, 57). Four sets of RCK1/RCK2 dimers are envisioned to form a moving structure termed a "gating ring." Consistent with the gating ring hypothesis, Ca²⁺-dependent conformational changes in a recombinant Slo1 RCK2 protein have been detected (135). The detailed activation mechanism of the BKCa channel is yet to be elucidated, but the atomic structural information available from voltage-gated Kv1.2/1.1 channels (73, 74) and Ca²⁺-gated MthK channels (56, 57) forms a basis of the following model of BKCa channel activation. The VSDs transduce changes in membrane potential and, their movements are electromechanically coupled to the permeation gate within the pore domain. Extensive mutagenesis studies suggest that the activation by Ca²⁺ probably involves two distinct high-affinity Ca²⁺ sensors per subunit: the RCK1 sensor (130) and the Ca²⁺-bowl sensor (102). Activation of the Ca²⁺ sensors induces conformational changes in the gating ring and leads to opening of the permeation gate near/within S6 via the linkers connecting the gating ring and S6 (78, 88, 130). The interactions among the gate, VSDs, and divalent cation sensors are allosteric and reciprocal (78). Ca²⁺ binding facilitates opening of the gate, and, conversely, opening of the gate facilitates binding of Ca²⁺ to its sensors by increasing the binding affinity. However, the coupling between the gate and the voltage/intracellular ligand sensors is such that, even without VSD activation and Ca²⁺, the gate may open once every few minutes on the average (47). Furthermore, open probability (Pₒ) of the channel approaches unity at sufficiently depolarized voltages without Ca²⁺ and the channel opens frequently at negative voltages without VSD activation with high concentrations of Ca²⁺.

Only one gene (KCNMA1) (23) codes for the pore-forming Slo1 subunit, but the functional properties of the BKCa channel are exceptionally diverse, encompassing many mechanisms from transcriptional regulation (67), microRNA-mediated regulation (11), and alternative splicing (1) to acute modulation of gating by signaling molecules. Alternative splicing of the RNA produces numerous transcripts, translating to diverse Slo1 proteins with distinct functional properties in different tissues and in different hormonal states (105, 115, 131). To further increase the diversity, native BKα channel complexes (KCNMB) in a wide variety of tissues and species contain both NH1 and NH2-domains, each of which is flanked by several NH2-terminal segments containing both N1 and N2 domains (89). Further, the NH1-domain also shows that the molecular signal (4, 76, 124) propagates to the NH2-domain, and following factors are involved: the NH2-domain having local and spatial features, the NH1-domain having local and spatial features.
channel complexes often contain auxiliary β subunits (KCNMB) in a tissue-specific manner (66). Four β subunits, β1–4 (10, 21, 65, 69, 119), have been identified, each of which contains two transmembrane segments connected by a large extracellular loop, leaving both NH2 and COOH termini in the intracellular side (97). Functionally, the presence of β subunits markedly alters the channel’s gating and pharmacological characteristics (16, 84, 89, 117). Recent evidence also shows that native BKCa channels are part of macromolecular signaling complexes that include enzymes (4, 76, 124) and/or ion channels (13, 37) to mediate local and spatially directed signaling (FIGURE 1). Once assembled, the BKCa channel activity is subject to regulation by a wide spectrum of biologically relevant factors such as serine/threonine/tyrosine phosphorylation, cysteine/methionine oxidation, steroid hormones, and gases [oxygen, nitric oxide (NO), and carbon monoxide (CO)] (52, 69, 120, 123, 124). Of the modulatory factors, phosphorylation of BKCa channels has been studied extensively and summarized (103). This review primarily focuses on examples of regulation of BKCa channels by other molecules: H+, heme, CO, reactive oxygen and nitrogen species, and lipids.

Protons, H+

H+ is a vitally important ion, and its intracellular concentration is under a tight control. However, some fluctuations in pH may occur as a consequence of normal cell function (26). Depolarization by action of Kv1.2/2.1 channels (58, 65, 122) changes in voltage and intracellular 

\[
\text{Ca}^{2+} \rightarrow \text{Ca}^{2+} \text{S6 sensor} \rightarrow \text{Ca}^{2+} \text{bowl sensor} \rightarrow \text{Ca}^{2+} \text{sensor} \rightarrow \text{Ca}^{2+} \text{influx} \rightarrow \text{Ca}^{2+} \text{channel} \rightarrow \text{Ca}^{2+} \text{release} \rightarrow \text{Ca}^{2+} \text{activation} \rightarrow \text{Ca}^{2+} \text{effects}.
\]

In this model, \text{Ca}^{2+} influx is the rate-limiting step in the activation of the channel, and the open probability appears to be determined by the number of open states reached by the channel, which in turn depends on the number of open states reached by the channel. Once the channel is open, it remains open for a few milliseconds, during which it allows \text{Ca}^{2+} to flow down its concentration gradient. The closed state of the channel is reached at the end of this period, and the channel is ready to open again (11). The channel is activated by a change in voltage, and its activity is subject to regulation by a wide spectrum of biologically relevant factors such as serine/threonine/tyrosine phosphorylation, cysteine/methionine oxidation, steroid hormones, and gases [oxygen, nitric oxide (NO), and carbon monoxide (CO)] (52, 69, 120, 123, 124). Of the modulatory factors, phosphorylation of BKCa channels has been studied extensively and summarized (103). This review primarily focuses on examples of regulation of BKCa channels by other molecules: H+, heme, CO, reactive oxygen and nitrogen species, and lipids.

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H+ is a vitally important ion, and its intracellular concentration is under a tight control. However, some fluctuations in pH may occur as a consequence of normal cell function (26). Depolarization by action of Kv1.2/2.1 channels (58, 65, 122) changes in voltage and intracellular
potentials and synaptic potentials may noticeably increase intracellular H+ concentration ([H+]i) (26).

Additionally, a large fall in pH as much as one unit, is possible under pathological conditions, such as cerebral ischemia (111). Not surprisingly, numerous types of ion channels are sensitive to intracellular H+, an increase in [H+]i typically decreases ionic currents. The current inhibition is most frequently attributed to a rapid permeation-pore blocking action of H+ (108).

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By contrast, BKCa channels are robustly activated by intracellular H+, a characteristic shared only by a few others among the K+ channel family (80). Early studies, often using native BKCa channels, reported that intracellular H+ decreased ionic currents through BKCa channels (27). Recent studies utilizing heterologously expressed Slo1 channels as well as native BKCa channels now show that H+ stimulates opening of the channel (8, 44, 51, 92). It is uncertain what accounts for the seemingly contradictory results.

Intracellular H+ increases ionic currents through BKCa channels in the absence of Ca2+ without altering the single-channel current size (i) (8, 44, 51, 92). The increase in open probability (Po) caused by H+ is accompanied by a 40- to ~50-mV shift in the macroscopic conductance-voltage curve (GV) to the negative direction with an EC50 value of pH i ~6.5 and a Hill coefficient of ~2 such that the nearly full effect is observed between pH = 6.0 and 7.2 (8), a physiologically feasible range. A mutagenesis study identified His365 and His394 (using NP 002238 numbering) in the RCK1 sensor domain, which is important in the Ca2+-sensitive range (130). Asp367. This interaction in part mimics the action of Ca2+, expanding the gating ring and promoting opening of the gate. The RCK1 sensor in Slo1 responds to both Ca2+ and H+. This multi-ligand nature of the RCK1 sensor with regard to H+ and Ca2+ is probably physiologically significant since the intracellular concentrations of these two ions are also reciprocally regulated (7, 134). The H+ sensitivity of the BKCa channel may also play an important role in pathophysiological conditions, such as in cerebral ischemia during which significant increases in both intracellular H+ and Ca2+ concentrations are observed (71).

Heme

Like H+, heme is a fundamentally important molecule, typically as a stable protein prosthetic group. Emerging evidence suggests that free intracellular heme may function as a non-genomic signaling molecule, acutely modulating BKCa channels (50, 114). Bioinformatic studies of the Slo1 primary sequence suggested that the sequence CKACH (114, 125) in the linker region between the cytoplasmic RCK1 and RCK2 segments might be capable of coordinating heme. Indeed, heme applied to the cytoplasmic side decreased Po in cell-free membrane patches with a high affinity (IC50 ~70 nM) without altering i (114). The modulatory effect of heme is independent of the redox status of the iron center, but substitution of the iron with other metals generally interferes with its modulatory ability. Mutations in the sequence CKACH disrupted the sensitivity of the channel to heme (55, 114, 123), suggesting that the sequence may be part of the heme binding site. This idea was further corroborated by UV-vis/electrophysiological measurements (114) and thin-layer chromatography/immunoblotting assays (55) performed on a model peptide whose sequence corresponds to the putative heme binding domain. The detailed mechanism of the heme action was addressed by Horrigan et al. (48), who isolated the contribution of each heme group by CO-releasing molecule (CRMO; 120) and CH3CN. Additionally, a large fall in pH, as much as one unit, is possible under pathological conditions, such as cerebral ischemia (111). Not surprisingly, numerous types of ion channels are sensitive to intracellular H+, an increase in [H+]i typically decreases ionic currents. The current inhibition is most frequently attributed to a rapid permeation-pore blocking action of H+ (108).
interact with the action of the nitric oxide synthase (NOS) to modulate Ca2+ homeostasis in the cytosol. In the state of hypoxia, the BKCa channel displays a decreased sensitivity to Ca2+, which is believed to be due to the action of HMOX2 (124), which catalyzes heme to produce CO in an O2-dependent manner (61). It has been postulated that under normoxia, CO generated by HMOX2 (124), which cannot be easily explained by the effect of hypoxia inhibiting BKCa channels, implicating that the channels themselves are gas sensors. CO, applied as a gas or using CO-releasing molecules (CORMs) (38), increases Po by a few fold even at saturating concentrations of Ca2+ (51, 94), favor the earlier observations that low pH and the histidine modifier DEPC agonize the CO action (52, 120).

The results summarized above collectively show that the BKCa channel is sensitive to both Ca2+ and CO, a putative gaseous messenger, which may exert distinct effects on the channels. Carbon Monoxide

One of the well known effects of carbon monoxide (CO) is to relax blood vessels (70). CO, like another gaseous messenger NO, binds to the heme iron center in soluble guanylyl cyclase (sGC) and increases its activity, leading to an increased level of cGMP and of phosphorylation by cGMP-dependent protein kinase (PKG) (96). Experimental phosphorylation of the PKG-consensus Ser residues in Slo1 located near the Ca2+ binding site (87) and at the distal COOH terminus (38) increases P on by a few fold. The underlying mechanism of the PKG-mediated regulation is not yet known and neither is whether the aforementioned residues are dynamically phosphorylated in vivo in response to CO.

In addition to the PKG-dependent mechanism, electrophysiological results suggest CO directly stimulates BKCa channels, implicating that the channels themselves are gas sensors. CO, applied as a gas or using CO-releasing molecules (CORMs) (38), increases Po (54, 122, 129) even in cell-free membrane patches (120, 123, 124, 126), suggestive of the possibility that CO mediates the channel directly or indirectly through those entities intimately associated with the channel proteins, possibly in the same macromolecular complex. Typically, CO-sensitive proteins, such as sGC, are heme proteins in which the reduced iron center (Fe2+) interacts with CO (16). Thus Jaggar et al. (54) postulated that the heme bound to the BKCa channel acts as a sensor for CO, enhancement of the channel activity by CO reflects the ability of CO to remove the inhibitory influence of heme. However, this hypothesis is not consistent with more recent results. For example, BKCa channels treated with the oxidant H2O2, which is expected to oxidize the heme iron and disrupt the CO-protein interaction (16), remain sensitive to CO (52). Furthermore, mutations that render the BKCa channel insensitive to heme fail to disrupt the CO sensitivity (52, 123); the effects of heme and CO are mediated by different molecular loci. A recent study has shown that the BKCa sensor in Slo1 is required for the stimulatory action of CO (52); mutation of His386, His384, or Asp367, the residues in the BKCa sensor involved in the H+ and Ca2+ sensitivity, also eliminates the CO sensitivity (52). This finding suggests that both CO and H+ increase P by mimicking the action of Ca2+ on the BKCa sensor (52). The essential roles of His386 and His384 in the CO sensitivity are consistent with the earlier observations that low pH, and the histidine modifier DEPC agonize the CO action (52, 120).

The results summarized above collectively show that the RCK1 sensor encompassing His365, His394, and Asp367 is essential for the high sensitivity of the BKCa channel to multiple ligands: Ca2+, H+, and CO. This finding has been interpreted to indicate that the RCK1 sensor is multi-ligand in nature and accommodates Ca2+, H+, or CO (51, 52). Alternatively, it may be postulated that a separate sensor for each ligand exists and that the binding information converges on the aforementioned His and Asp residues and is transmitted to the channel’s gate. Several lines of results, including that the effects of mutations affecting the Ca2+/H+ sensitivity are generally additive (51, 94), favor the former multi-ligand postulate. The physicochemical mechanism by which CO interacts with the RCK1 sensor remains elusive. It is conventionally believed that CO-sensing proteins require a metal or heme cofactor (16). The available structures of the bacterial channel MthK, which shows a reasonable degree of primary sequence similarity to the BKCa channel, do not suggest that the Slo1 RCK1 sensor harbors any metal or heme cofactor (56, 57). As an alternative idea, an electrostatic interaction between the weak dipole moment of CO and the RCK sensor pocket comprised of His365, His394, and Asp367 has been suggested to contribute (52). In addition to the RCK sensor, an additional CO interaction site may exist in the Slo1 channel (123). CO may increase P even at saturating concentrations of Ca2+ (121), which cannot be easily explained by the effect of CO as a Ca2+ mimic for the RCK1 sensor (52).

In many cell types, hypoxia inhibits BKCa channels, and the direct stimulation of the BKCa channel by CO is a cellular mechanisms of oxygen sensing (124). In oxygen-sensing carotid body glomus cells, Slo1 proteins are found closely associated with HMOX2 (124), which catalyzes heme to produce CO in an O2-dependent manner (61). It has been postulated that under normoxia, CO generated by HMOX2 continuously stimulates the BKCa channel and that the binding inhibits HMOX2 and removes the stimulatory influence of CO on the BKCa channel (124). The overall scheme is supported by several lines of evidence, including the results of HMOX2 gene-knockdown experiments (124). However, the HMOX2-mediated oxygen-sensing mechanism may not be the dominant one in vivo because mice with the HMOX2 gene constitutively disrupted show relatively normal hypoxic responses (82, 90).

**Reacti ve Oxygen/Nitrogen Species**

The aerobic existence inevitably creates reactive molecules capable of oxidizing cellular constituents including proteins (45). Although excess concentrations of the reactive molecules are clearly deleterious,
causing oxidative stress, cells utilize some of the reactive molecules at low concentrations as vital signaling molecules (53).

Redox modulation of cell function has been difficult to study in part because the experimental tools available to manipulate the levels of reactive species often lack desired specificity and because reactive species are capable of readily modifying multiple amino acid residues, including cysteine, methionine, histidine, tryptophan, and tyrosine. Furthermore, some oxidation reactions are critically dependent on multivalent cations, such as Fe²⁺/Fe³⁺, which may be present as contaminating species. It is not surprising then that treatment of cells/membrane patches containing BKCa channels with reactive species such as the physiological reducing agent glutathione (GSH) (19, 33) as well as DTT (33, 77, 106, 113), all of which are sensitive to oxidation in Slo1 is drastically potentiated by the recent physiological processes including gating of BKCa channels, oxidation of any of one of the three Met residues M536, M712, and M739 located in the RCK1 domain by chloramines increases Po in the absence of Ca²⁺ in part by shifting GV to the negative side chain to a rigid and polar one, roughly equivalent to the side chain of lysine (15). Methionine oxidation has marked functional effects in many proteins, including calmodulin (14), calcium/calmodulin-dependent kinase (35), and ion channels (28, 49, 60) and is implicated in many phenomena including aging and neurodegenerative diseases (49, 107). In BKCa channels, oxidation of any of one of the three Met residues M536, M712, and M739 located in the RCK1 and RCK2 domains by chloramines increases Po in the absence of Ca²⁺ in part by shifting GV to the negative direction by ~50 mV (100). The overall shift in the voltage dependence is caused by stabilization of the activated state of VSD and of the open state of the gate (100). Interestingly, the stimulatory effect of methionine oxidation in Slo1 is drastically potentiated by coexpression of the auxiliary subunit ß1 (99) but a mechanistic interpretation of the finding remains to be developed.

Reactive molecules other than H₂O₂ have been reported to affect BKCa channels: O₂⁻ (72, 113), NO (2, 18, 19, 69), and peroxynitrite (ONOO⁻) (72, 77, 113). Many of the effects are inhibitory in nature, and some of the inhibitory effects are mediated by Cys911 (77, 113). In addition, nitrothiosylation (for review, see Ref 81) of yet an unidentified cysteine residue induced by NO-releasing compounds may increase the channel activity (2, 18, 69). It may be noted that application of a NO-releasing compound to heterologously expressed BKCa channels failed to increase P₀ (52).

Direct biochemical/protein evidence that any of the Cys residues in the BKCa channels are dynamically oxidized and reduced under physiological conditions is not yet available. Pathophysiologically, Cys oxidation in the BKCa channel is likely to be a contributing factor in those disease states where oxidative stress is implicated. One such condition is diabetes-induced vascular dysfunction where oxidative stress is an important contributing factor and suggested to induce oxidation of Cys911, leading to impaired vasorelaxation (77). Methionine is another amino acid readily susceptible to oxidation. Oxidation of methionine to methionine sulfoxide by the addition of an oxygen atom to its reactive sulfur atom changes its flexible and nonpolar side chain to a rigid and polar one, roughly equivalent to the side chain of lysine (15). Methionine oxidation has marked functional effects in many proteins, including calmodulin (14), calcium/calmodulin-dependent kinase (35), and ion channels (28, 49, 60) and is implicated in many phenomena including aging and neurodegenerative diseases (49, 107). In BKCa channels, oxidation of any of one of the three Met residues M536, M712, and M739 located in the RCK1 and RCK2 domains by chloramines increases P₀ in the absence of Ca²⁺ in part by shifting GV to the negative direction by ~50 mV (100). The overall shift in the voltage dependence is caused by stabilization of the activated state of VSD and of the open state of the gate (100). Interestingly, the stimulatory effect of methionine oxidation in Slo1 is drastically potentiated by coexpression of the auxiliary subunit ß1 (99) but a mechanistic interpretation of the finding remains to be developed.

**Fatty acids:**

Anchidonic acid, from dietary membranes and action of phospholipase A2, is oxidized by transition metals, such as Fe²⁺/Fe³⁺, which may be present as contaminants. It is not surprising then that treatment of cells/membrane patches containing BKCa channels with reactive species such as the physiological reducing agent glutathione (GSH) (19, 33) as well as DTT (33, 77, 106, 113), all of which are sensitive to oxidation in Slo1 is drastically potentiated by the recent physiological processes including gating of BKCa channels, oxidation of any of one of the three Met residues M536, M712, and M739 located in the RCK1 and RCK2 domains by chloramines increases P₀ in the absence of Ca²⁺ in part by shifting GV to the negative direction by ~50 mV (100). The overall shift in the voltage dependence is caused by stabilization of the activated state of VSD and of the open state of the gate (100). Interestingly, the stimulatory effect of methionine oxidation in Slo1 is drastically potentiated by coexpression of the auxiliary subunit ß1 (99) but a mechanistic interpretation of the finding remains to be developed.

**Lipids and Metabolites**

Lipids are a structurally diverse group of molecules that include fatty acids, glycerophospholipids, and steroids, and many lipids and lipid-related metabolites are recognized as cellular signaling molecules involved in regulation of a variety of physiological and pathophysiological processes including gating of BKCa channels (17). The interest in modulation of BKCa channels by lipids is further stimulated by the recent finding that voltage-dependent gating of ion channels critical for neurotransmission is modulated by fatty acids as well as phosphatidic acid (EET). In addition, DHA (15) increases P₀ in BKCa channels in a manner similar to that observed with the patch excision of Slo1 (99) but a mechanistic interpretation of the finding remains to be developed.
channels critically depends on membrane phospholipids (133).

**Fatty acids: arachidonic acid and its metabolites**

Arachidonic acid, a fatty acid, initially synthesized from dietary sources in select cells, is stored in cell membranes and released to the cytoplasm by the action of phospholipases. Once released, arachidonic acid as well as its metabolites such as hydroxyeicosatetraenoic acids (HETEs), epoxyeicosatrienoic acid (EET), and dihydroxyeicosatrienoic acids (DHETs) exert a variety of effects (93, 97), including stimulation of BKCa channels in pituitary tumor cells (32, 127), artery smooth muscle cells (3, 6, 29, 46, 63), and heterologous expression systems (39, 110). The stimulatory action of various fatty acids on the BKCa channels has been typically attributed to an increase in $P_o$ (3, 29, 32) and/or to an increase in $N$. How the changes in $P_o$ correlate with the changes in the functional domains of the channel, the gate, the VSDs, and the gating ring has not been fully explored.

Despite the large number of fatty acids capable of enhancing the BKCa channel activity, considerable structural specificity has been reported, suggesting that the fatty acid effector, presumably the channel itself, has specific interaction sites. To effectively increase $P_o$, fatty acids should have a cis conformation (32), a relatively long tail group (C > 8), and a negatively charged head group (29). For example, oleic acid (C18), arachidonic acid (C20), and eicosapentaenoic acid (C20) meet these structural requirements and increase BKCa $P_o$ by several fold (29, 32). The location of the double bonds in fatty acids may be also important (39, 127).

The biophysical mechanism and the molecular components necessary for the stimulatory action of the fatty acids are not yet clear. Because fatty acids may "flip" across cell membranes (39), it has been difficult to determine whether the fatty acid interaction sites face the intracellular side or extracellular side. Additionally, there is no clear consensus whether the auxiliary subunits of the BKCa channel, β1-4, are required for the fatty acid action. The predominantly vascular auxiliary subunit β1 may not be required for 17,18-epoxyeicosatetraenoic acid, one metabolite of arachidonic acid, to activate the BKCa channel, suggesting that the pore-forming Slo1 subunit is sufficient (46). In contrast, arachidonic acid increases $P_o$ when Slo1 is expressed with β2 or β3 but not when expressed alone or coexpressed with β4 (110). One likely but complicating possibility is that fatty acids differ in their β-subunit requirement.

**Phospholipids**

Phospholipids are an important constituent of cell membranes and have been known to alter the functions of BKCa channels in many ways (40, 85, 91). Phosphatidylinositol 4,5-bisphosphate (PIP2) in particular has been a subject of intense investigation because this negatively charged phospholipid influences numerous ion channels and because it serves as a precursor for inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG), both of which in turn modulate...
many ion channels. This multifunctional nature of PIP$_2$ is physiologically noteworthy but has hindered execution of well controlled experiments (109). Nevertheless, an emerging paradigm is that PIP$_2$, especially when phosphorylated by lipid kinases, directly affects many ion channels (109), and BK$_{Ca}$ channels appear to be no exception. This is illustrated in a recent study by Vaithianathan et al. (116), who showed that application of exogenous PIP$_2$, to the cytosolic side at a physiological concentration (~10 $\mu$M) (83) increases currents through both native vascular and heterologously expressed BK$_{Ca}$ channels. The current enhancing effect of PIP$_2$ depends on its negative phospho group and the inositol moiety, and the washout kinetics is influenced by the acyl chain length. The importance of the negative phosphate group in PIP$_2$ prompted Vaithianathan et al. to mutate a cluster of three positively charged residues (BDK) in the Slo1 BK$_{Ca}$ linker segment. Neutralization of the charged residues noticeably diminished the overall effect of PIP$_2$, perhaps suggesting that the sequence BDK is a PIP$_2$ interaction site. The involvement of the 56-RCK1 linker in the PIP$_2$ action is reminiscent of the results obtained in KCNQ, another voltage-gated K$^+$ channel (130). Biophysically, PIP$_2$ increases $P_o$ in the BK$_{Ca}$ channel by shifting $G_0$ to the negative direction by $\sim 15$ mV at an intermediate concentration of Ca$^{2+}$. It is interesting to note that a similar shift in the voltage dependence is observed when the 56-RCK1 linker is shorted (98), binding of PIP$_2$ to the linker region may affect the coupling process between the gate in the pore module and the cytoplasmic gating structure.

**Steroid hormones**

Steroid hormones are well known for their genomic effects, but their acute, non-genomic mode of action involving direct binding to membrane-bound effectors, including BK$_{Ca}$ channels, is starting to be appreciated (86). Multiple steroid hormones, including estrogen (117), testosterone (43), dehydroepiandrosterone (62), and glucocorticoids (75), have been reported to acutely affect BK$_{Ca}$ channels. Because estrogen may offer a cardioprotective effect (30), the non-genomic action of estrogen on BK$_{Ca}$ channels has been extensively investigated. Acute application of estradiol (17$\beta$-estradiol) relaxes vascular smooth muscle in a low micromolar range (64) and activates native vascular BK$_{Ca}$ channels and heterologously expressed Slo1-$\beta_1$ channels with an EC$_{50}$ of a few micromolar in a Ca$^{2+}$-dependent manner with the current enhancing effect diminishing at higher concentrations of Ca$^{2+}$ (31, 62, 117). Importantly, clear structural specificity exists because 17$\alpha$-estradiol is less effective than 17$\beta$-estradiol (117). The pore-forming subunit Slo1 is not sufficient for the 17$\beta$-estradiol sensitivity, but the robust 17$\beta$-estradiol sensitivity requires coexpression of a $\beta$ subunit, either $\beta_1$, $\beta_2$, or $\beta_4$ (16, 62, 117). The observation that a membrane impermeant analog of 17$\beta$-estradiol applied from the extracellular side activates the BK$_{Ca}$ channel suggests that the estradiol interaction site may lie near the extracellular side of the Slo1-$\beta_3$ complex (117), distinct from the PIP$_2$ interaction site located near the cytoplasmic side of S6 (116).

At low concentrations of Ca$^{2+}$, 17$\beta$-estradiol shifts $G_0$ to the negative direction without altering $I_{Ca}$ (31, 62, 117). In the presence of 17$\beta$-estradiol, marked changes in the single channel kinetics, such as an increase in the mean burst duration, have been noted (31). However, the gating changes by 17$\beta$-estra diol can be accounted for by the prevailing allosteric gating scheme (47) is unclear. In addition, a $\beta$ subunit-independent inhibitory effect of estrogen mediated by the Slo3 pore probably exists (34). Other steroids such as corticosterone (62, 75), dehydroepiandrosterone (62), and lithocholate (22) also activate BK$_{Ca}$ channels in a $\beta$ subunit-dependent manner.

**Concluding Remarks**

Acute modulation of BK$_{Ca}$ channels greatly expands their functional repertoire, allowing the channels to contribute to multitudes of physiological and pathophysiological phenomena. Important mechanistic insights into the channel regulation by small molecules such as Ca$^{2+}$, Mg$^{2+}$, and H$^+$ are now available, and the mechanisms of action of other modulatory agents on the BK$_{Ca}$ channel gating should become better elucidated in the near future. The BK$_{Ca}$ channel gating is allosterically mediated by three major domains of the channel, the pore, VSDs, and the gating ring, all of which undergo rapid and marked conformational changes, and any modifications of the energetics and kinetics of the functional domains could change the channel current size. Thus additional modulatory phenomena of BK$_{Ca}$ channels are certainly waiting to be discovered. Such future studies will incorporate the realization that BK$_{Ca}$ channels form macromolecular complexes with other signaling molecules, such as voltage-dependent Ca$^{2+}$ channels (12, 13), HMOX2 (124), and protein kinases/phosphatases (76). The macromolecular assembly formation itself may be dynamic and subject to modulation. The knowledge obtained from studies of the modulator action could contribute to rational design of therapeutically useful low-molecular weight compounds targeting BK$_{Ca}$ channels. Dysfunction of a modulatory pathway may underlie a disease state, and synthetic compounds could be designed to regulate the pathway in a predictable manner. Much work and excitement lie ahead.

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