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Modulation of BK_{Ca} channel gating by endogenous signaling molecules

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Abstract

Large-conductance Ca^{2+}- and voltage-activated K^+ (BK_{Ca}, MaxiK or Slo1) channels are expressed in almost every tissue in our body and participate in many critical functions such as neuronal excitability, vascular tone regulation and neurotransmitter release. The functional versatility of BK_{Ca} channels owes in part to the availability of a spectacularly wide array of biological modulators of the channel function. In this review, we focus on modulation of BK_{Ca} channels by small endogenous molecules, emphasizing their molecular mechanisms. The mechanistic information available from studies on the small naturally occurring modulators is expected to contribute to our understanding of the physiological and pathophysiological roles of BK_{Ca} channels.

Introduction

Large-conductance Ca^{2+}- and voltage-activated K^+ (BK_{Ca}, MaxiK, $K_{Ca}1.1$) 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 synergic activation mechanism encompassing membrane depolarization and intracellular Ca^{2+}/Mg^{2+} (98). The inter-dependent activation mechanism enables BK_{Ca} channels to typically exert a negative feedback influence on cellular excitability (98,118). The importance of BK_{Ca} 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 BK_{Ca} channel activity (5,101). Conversely, enhancement or up regulation of the channel function in select cells may offer protection against some of the aforementioned disorders (20,25,41,132).

Structurally, BK_{Ca} channels are composed of four pore-forming Slo1 (α) subunits, each of which contains 7 transmembrane segments (S0–S6) and a large C-terminal cytoplasmic region (98). 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 K^+" (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, Ca^{2+}-dependent conformational changes in a recombinant Slo1 RCK2 protein have been detected (135). The detailed activation mechanism of the BK_{Ca} channel is yet to be elucidated but the atomic structural information available from voltage-gated Kv1.2/2.1 channels (73,74) and Ca^{2+}-gated MthK channels (56,57) forms a basis of the following

model of BK_{Ca} 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 Ca^{2+} probably involves two distinct high-affinity Ca^{2+} sensors per subunit: the RCK1 sensor (130) and the Ca^{2+} bowl sensor (102). Activation of the Ca^{2+} 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). Ca^{2+} binding facilitates opening of the gate, and, conversely, opening of the gate facilitates binding of Ca^{2+} 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 Ca^{2+}, the gate may open once every few minutes on the average (47). Furthermore, open probability (P_o) of the channel approaches unity at sufficiently depolarized voltages without Ca^{2+}, and the channel opens frequently at negative voltages without VSD activation with high concentrations of Ca^{2+}.

Only one gene (KCNMA1) (23) codes for the pore-forming Slo1 subunit but the functional properties of the BK_{Ca} channel are exceptionally diverse, encompassing many mechanisms, from transcriptional regulation (67), microRNA-mediated regulation (11), 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 BK_{Ca} channel complexes often contain auxiliary β subunits (KCNMB) in a tissue-specific manner (66). Four β subunits, β_1–β_4 (10,21,65,66,89,119), have been identified, each of which contains two transmembrane segments connected by a large extracellular loop, leaving both N and C termini in the intracellular side (89). Functionally, the presence of β subunits markedly alters the channel's gating and pharmacological characteristics (10,84,89,117). Recent evidence also shows that native BK_{Ca} 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. Once assembled, the BK_{Ca} channel activity is subject to modulation 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 BK_{Ca} channels has been studied extensively and summarized (103). This review primarily focuses on examples of regulation of BK_{Ca} 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_i may occur as a consequence of normal cell function (26). Depolarization by action potentials and synaptic potentials may noticeably increase intracellular H^+ concentration ($[H^+]_i$) (26). Additionally, a large fall in pH_i, 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).

By contrast, BK_{Ca} 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 BK_{Ca} channels, reported that intracellular H^+ decreased ionic currents through BK_{Ca} channels (27). Recent studies utilizing heterologously expressed Slo1 channels as well as

native BK_{Ca} 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 BK_{Ca} channels in the absence of Ca^{2+} without altering the single-channel current size (*i*) (8,44,51,92). The increase in open probability (P_o) caused by H^+ is accompanied by a 40~50 mV shift in the macroscopic conductance-voltage curve (GV) to the negative direction with an EC_{50} value of pH_i 6.5 and a Hill coefficient of >2 such that the nearly full effect is observed between pH_i = 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 Ca^{2+}-dependent activation (130), as the necessary residues, with His365 and His394 accounting for 2/3 and 1/3 of the shift in GV, respectively. Mutation of His365/His394 to neutral Ala approximated the voltage dependence at pH_i7.2 whereas that to positively-charged Arg produced a voltage dependence similar to that at pH_i6.2. Combined with the finding that the stimulatory effect of H^+ diminishes with increasing ionic strength, it has been suggested that the protonated imidazole side chains of His365 and His394 electrostatically interact with nearby electronegative elements. One of the electrostatic interaction partners appears to be Asp367, which is a critical component in high-affinity Ca^{2+} sensing (130). In summary, it is envisioned that intracellular H^+ protonates the side chains of His365 and His394 located within the high-affinity Ca^{2+}-sensor site in the RCK1 domain and then the positively charged side chains interact with Asp367. This interaction in part mimics the action of Ca^{2+}, expanding the gating ring and promoting opening of the gate.

The RCK1 sensor in Slo1 responds to both Ca^{2+} and H^+. This multi-ligand nature of the RCK1 sensor with regards to H^+ and Ca^{2+} is probably physiologically significant as the intracellular concentrations of these two ions are also reciprocally regulated (7,134). The H^+ sensitivity of the BK_{Ca} channel may also play an important role in pathophysiological conditions, such as in cerebral ischemia during which significant increases in both intracellular H^+ and Ca^{2+} 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 BK_{Ca} channels (50,114). Bioinformatic inspection 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 P_o in cell-free membrane patches with a high affinity (IC_{50} = ~70 nM) without altering *i* (114). The modulatory effect of heme is independent of the redox status of 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/electron paramagnetic resonance (EPR) spectroscopic measurements (114) and thin-layer chromatography/mass spectroscopy assays (55) performed on a model peptide whose sequence corresponds to the putative heme binding segment. The detailed mechanism of the heme action was addressed by Horrigan et al. (48), who isolated the contribution of each allosteric gating component to the overall gating. The measurements all together showed that heme is a modulator, not a simple inhibitor, of the Slo1 channel function such that it increases P_o at negative voltages and decreases P_o at more positive voltages. Interaction of heme with the RCK1-RCK2 linker segment may expand the gating ring and impede the gating ring-VSD interaction that normally accompanies activation of the channel (48).

While the molecular mechanism of the heme action of the BK_{Ca} channel is relatively clear, its physiological significance is less certain. If heme is in fact a signaling molecule, a mechanism to activate the signaling cascade and a mechanism to terminate the signal should exist. One way to activate the heme signaling pathway may be influx of heme across the plasma membrane using heme transporters (95,104). The heme signal can be terminated by the action of heme oxygenase (HMOX)(79), thus removing any direct influence of heme on BK_{Ca} channels. HMOX, however, produces a number of heme degradation products, including CO, a putative gaseous messenger, which may exert distinct effects on the channels.

Carbon monoxide, CO

One of the well-known effects of 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 PKG-consensus Ser residues in Slo1 located near the Ca^{2+} bowl sensor (87) and at the distal C-terminus (38) increases P_o by a few folds. 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 BK_{Ca} channels, implicating that the channels themselves are gas sensors. CO, applied as a gas or using CO-releasing molecules (CORMs) (58), increases P_o (54,122,129) even in cell-free membrane patches (120,123,124,126), suggestive of the possibility that CO modulates 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 (Fe^{2+}) interacts with CO (16). Thus, Jaggar et al. postulated that the heme bound to the BK_{Ca} 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, BK_{Ca} channels treated with the oxidant H_2O_2, 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 BK_{Ca} channel insensitive to heme fails 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 RCK1 Ca^{2+} sensor in Slo1 is required for the stimulatory action of CO (52); mutation of His365, His394 or Asp367, the residues in the RCK1 sensor involved in the H^+ and Ca^{2+} sensitivity, also eliminates the CO sensitivity (52). This finding suggests that both CO and H^+ increase P_o by mimicking the action of Ca^{2+} on the RCK1 sensor (52). The essential roles of His365 and His394 in the CO sensitivity are consistent with the earlier observations that low pH_i and the histidine modifier DEPC antagonize 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 BK_{Ca} channel to multiple ligands: Ca^{2+}, H^+ and CO. This finding has been interpreted to indicate that the RCK1 sensor is multi-ligand in nature and accommodates Ca^{2+}, 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 transmitted to the channel's gate. Several lines of results, including that the effects of mutations affecting the Ca^{2+}/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 requires 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 BK_{Ca} 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 RCK1 ligand 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_o even at saturating concentrations of Ca^{2+} (123), which cannot be easily explained by the effect of CO as a Ca^{2+} mimetic for the RCK1 sensor (52).

In many cell types, hypoxia inhibits BK_{Ca} channels and the direct stimulation of the BK_{Ca} channel by CO represents one of the 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 O_2-dependent manner (61). It has been postulated that under normoxia, CO generated by HMOX2 continuously stimulates the BK_{Ca} channel and that hypoxia inhibits HMOX2 and removes the stimulatory influence of CO on the BK_{Ca} 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 the dominant one *in vivo* because mice with the HMOX2 gene constitutively disrupted show relatively normal hypoxic responses (82,90).

Reactive oxygen/nitrogen species (ROS/RNS)

The aerobic existence inevitably creates reactive molecules capable of oxidizing cellular constituents including proteins (45). While 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 Fe^{2+}/Fe^{3+}, which may be present as contaminating species. It is not surprising then that treatment of cells/membrane patches containing BK_{Ca} channels with reactive species such as H_2O_2 has been reported to produce a myriad of effects (9,19,24,68,121,128,136). A consensus effect of H_2O_2, a physiological oxidant produced during normal oxygen metabolism, applied to heterologously-expressed BK_{Ca} channels is inhibitory, attributed largely to a decrease in P_o (19,33,77,106,112,113) and, to a lesser extent, a decrease in the number of channels available to open (*N*) (106,137). Typically, the diminished P_o by H_2O_2 persists after wash, indicative of amino-acid modification, but the gating change is reversed by reducing agents such as the physiological reducing agent glutathione (GSH) (19,33) as well as DTT (33,77,106,113), all of which are capable of regenerating a free sulfhydryl group (-SH) in cysteine from the oxidized side chain sulfenic acid (-SOH). A variety of thiol modifying agents, such as NEM, DTNB and MTSEA (33,113,121), also decrease P_o, thus implicating that the absence of free sulfhydryl groups in BK_{Ca} channel is critical. A systematic Cys-to-Ala mutagenesis of Slo1 showed that oxidation of Cys911 near the Ca^{2+} bowl sensor in the distal C-terminus decreased the energetic contribution of the Ca^{2+} bowl sensor to the channel activation (113). The proximity of Cys911 to the Ca^{2+} bowl sensor is in line with the Ca^{2+} dependence of the inhibitory effect; in the absence of Ca^{2+}, the redox status of Cys911 plays little role but the effect becomes greater with increasing

concentrations of Ca^{2+} while leaving that Ca^{2+} dependence mediated by the RCK1 sensor intact (113). In addition to Cys911 at the distal C-terminus, Cys430 in the RCK1 domain also contributes to the oxidation sensitivity of the Ca^{2+} dependence of the BK_{Ca} channel (137). Both Cys430 and Cys911 are susceptible to air oxidation and account for the rundown phenomenon following patch excision (137). Additional Cys residues, whose oxidation alters the channel gating, are also present in the channel. One interesting example is the biologically modulated inclusion of the STREX exon in the cytoplasmic region of Slo1 (131). The STREX inclusion introduces additional Cys residues and potentiates the inhibitory effect of oxidation (36).

Reactive molecules other than H_2O_2 have been reported to affect BK_{Ca} channels: $O_2^{\bullet -}$ (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 (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 BK_{Ca} channels failed to increase P_o (52).

Direct biochemical/proteomic evidence that any of the Cys residues in the BK_{Ca} channels are dynamically oxidized and reduced under physiological conditions is not yet available. Pathophysiologically, Cys oxidation in the BK_{Ca} 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 BK_{Ca} 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* P_o in the absence of Ca^{2+} 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 β1 (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, phospholipids 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 BK_{Ca} channels (17). The interest in modulation of BK_{Ca} channels by lipids is further stimulated by the recent finding that voltage-dependent gating of ion channels critically depends on membrane phospholipids (133).

1. 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), dihydroxyeicosatrienoic acids (DHETs), exert a variety of effects (93,97), including stimulation of BK_{Ca} channels in pituitary tumor cells (32,127), artery smooth muscle cells (3,6,29,46,63), and heterologous expression systems (39,42). The stimulatory action of various fatty acids on the BK_{Ca} channels has been typically attributed to an increase in P_o (3,29,32) and/or to an increase in N (3). 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, have not been fully explored.

Despite the large number of fatty acids capable of enhancing the BK_{Ca} 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 BK_{Ca} P_o by several folds (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 (59), 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 BK_{Ca} 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 BK_{Ca} 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.

2. Phospholipids

Phospholipids are an important constituent of cell membranes and have been known to alter the functions of BK_{Ca} channels in many ways (40,85,91). Phosphatidylinositol 4, 5-bisphosphate (PIP_2) 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-triphosophate (IP_3) 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), which showed that application of exogenous PIP_2 to the cytoplasmic side at a physiological concentration (~10 μM) (83) increases currents through both native vascular and heterologously-expressed BK_{Ca} channels. The current enhancing effect of PIP_2 depends on its negative phosphate 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 (RKK) in the Slo1 S6-RCK1 linker segment. Neutralization of the charged residues noticeably diminished the overall effect of PIP_2, perhaps suggesting that the sequence RKK is a PIP_2 interaction site. The involvement of the S6-RCK1 linker in the PIP_2 action is reminiscent of the results obtained in KCNQ, another voltage-gated K^+ channel (138). Biophysically, PIP_2 increases P_o in the BK_{Ca} channel by shifting GV to the negative direction by ~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 S6-

RCK1 linker is shortened (88); 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.

3. 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), and dehydroepiandrosterone (62), 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 (17β-estradiol) relaxes vascular smooth muscle in a low μM range (64) and activates native vascular BK_{Ca} channels and heterologously-expressed Slo1-β1 channels with an EC_{50} of a few μM 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 17α-estradiol is less effective than 17β-estradiol (117). The pore-forming subunit Slo1 is not sufficient for the 17β-estradiol sensitivity but the robust 17β-estradiol sensitivity requires coexpression of a β subunit, either β1, β2 or β4 (10,62,117). The observation that a membrane impermeant analog of 17β-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-β complex (117), distinct from the PIP_2 interaction site located near the cytoplasmic side of S6 (116).

At low concentrations of Ca^{2+}, 17β-estradiol shifts GV to the negative direction without altering *i* (31,62,117). In the presence of 17β-estradiol, marked changes in the single-channel kinetics, such as an increase in the mean burst duration have been noted (31). However, how the gating changes by 17β-estradiol can be accounted for by the prevailing allosteric gating scheme (47) is unclear. In addition, a β subunit-independent inhibitory effect of estrogen mediated by the Slo1 pore probably exists (34). Other steroids such as corticosterone (62,75), dehydroepiandrosterone (62) and lithocholate (22) also activate BK_{Ca} channels in a β subunit-dependent manner.

Concluding remarks

Acute modulation of BK_{Ca} channels greatly expands their functional repertories, 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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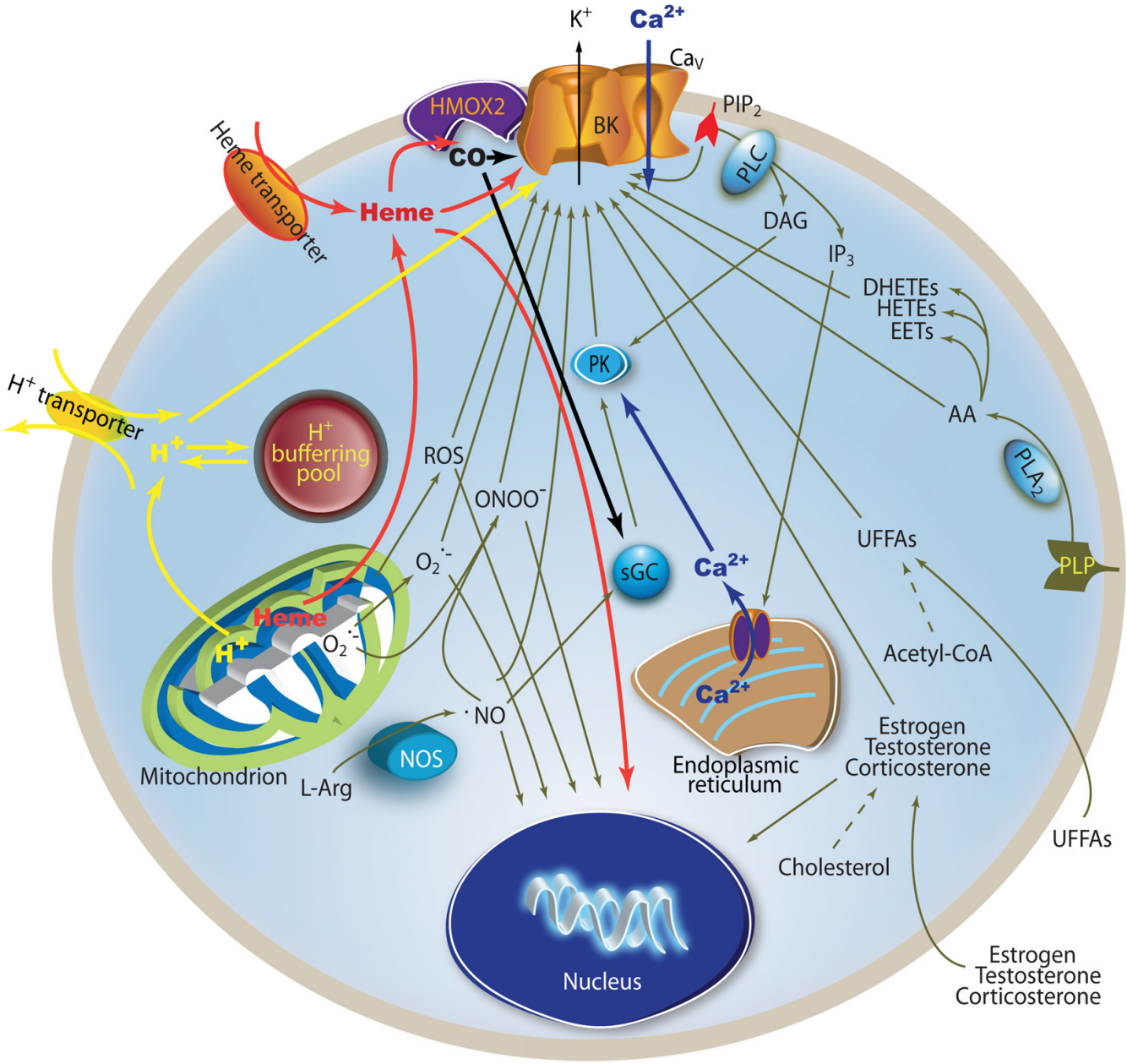

Figure 1.

Multiple intracellular signaling cascades modulate BK_{Ca} channels. PLP (phospholipids) UFFAs (unsaturated free fatty acids) NOS (nitric oxide synthase), PK (protein kinase), sGC (soluble guanylyl cyclase), PLA_2 (Phospholipase A_2), PLC (Phospholipase C), IP_3 (Inositol triphosphate), DAG (diglyceride), L-Arg (L-arginine)

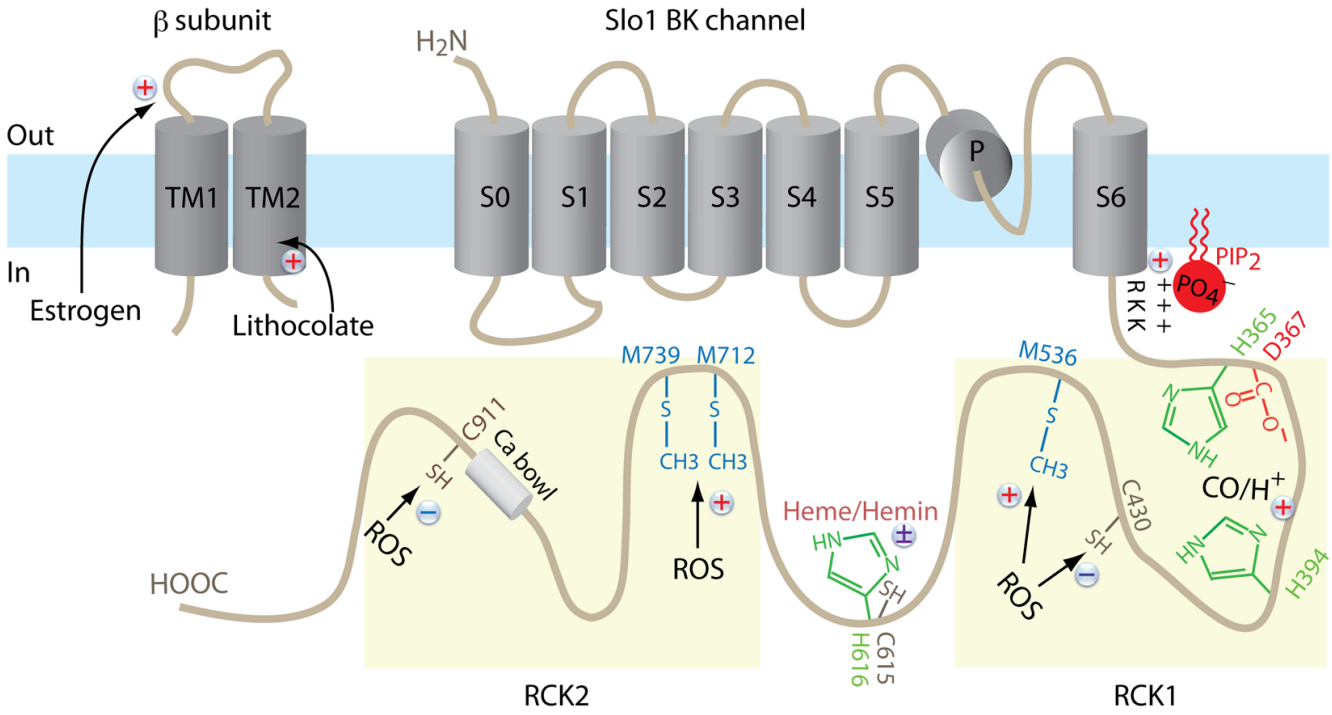

Figure 2.

The schematic diagram shows the key amino acid residues involved in modulation of BK_{Ca} channel by select intracellular messengers including H^+, heme/hemin, CO, ROS, and PIP_2. Stimulatory modulators are indicated by "+", inhibitory modulators are indicated "–"and mixed-effect modulators are indicated by "±". The residue numbers are according to NP 002238.