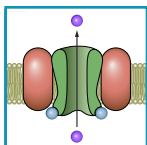


MOLECULAR DETERMINANTS OF BK CHANNEL FUNCTIONAL DIVERSITY AND FUNCTIONING

Ramon Latorre, Karen Castillo, Willy Carrasquel-Ursulaez, Romina V. Sepulveda, Fernando Gonzalez-Nilo, Carlos Gonzalez, and Osvaldo Alvarez

Centro Interdisciplinario de Neurociencia de Valparaíso and Doctorado en Ciencias Mención Neurociencia, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile; Universidad Andres Bello, Facultad de Ciencias Biologicas, Center for Bioinformatics and Integrative Biology, Avenida Republica 239, Santiago, Chile and Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile



Latorre R, Castillo K, Carrasquel-Ursulaez W, Sepulveda RV, Gonzalez-Nilo F, Gonzalez C, Alvarez O. Molecular Determinants of BK Channel Functional Diversity and Functioning. *Physiol Rev* 97: 39–87, 2017. Published November 2, 2016; doi:10.1152/physrev.00001.2016.—Large-conductance Ca^{2+} - and voltage-activated K^+ (BK) channels play many physiological roles ranging from the maintenance of smooth

muscle tone to hearing and neurosecretion. BK channels are tetramers in which the pore-forming α subunit is coded by a single gene (*Slowpoke*, *KCNMA1*). In this review, we first highlight the physiological importance of this ubiquitous channel, emphasizing the role that BK channels play in different channelopathies. We next discuss the modular nature of BK channel-forming protein, in which the different modules (the voltage sensor and the Ca^{2+} binding sites) communicate with the pore gates allosterically. In this regard, we review in detail the allosteric models proposed to explain channel activation and how the models are related to channel structure. Considering their extremely large conductance and unique selectivity to K^+ , we also offer an account of how these two apparently paradoxical characteristics can be understood consistently in unison, and what we have learned about the conduction system and the activation gates using ions, blockers, and toxins. Attention is paid here to the molecular nature of the voltage sensor and the Ca^{2+} binding sites that are located in a gating ring of known crystal structure and constituted by four COOH termini. Despite the fact that BK channels are coded by a single gene, diversity is obtained by means of alternative splicing and modulatory β and γ subunits. We finish this review by describing how the association of the α subunit with β or with γ subunits can change the BK channel phenotype and pharmacology.

I.	INTRODUCTION	39
II.	BK CHANNEL PHYSIOLOGY AND...	41
III.	ALLOSTERIC MODEL FOR BK...	46
IV.	INFERENCES ABOUT THE...	51
V.	MOLECULAR DETERMINANTS OF...	57
VI.	MOLECULAR DETERMINANTS OF...	61
VII.	BK CHANNELS ALTERNATIVE...	62
VIII.	MODULATORY β SUBUNITS	66
IX.	MODULATORY γ SUBUNITS	70
X.	BK CHANNEL OPENERS	73
XI.	CONCLUSIONS	74

I. INTRODUCTION

Evidence of Ca^{2+} -activated conductance in cells was initially reported in *Helix* and *Aplysia* in the 1970s (300, 301). Ca^{2+} injections into neurons and an increase in intracellular Ca^{2+} induced by metabolic poisoning promoted an increase in the K^+ conductance, and Meech (299) in a seminal review proposed that this Ca^{2+} -activated conductance was the link between cell metabolism and cell electrical activity. The improvements on the patch-clamp technique in the

early 1980s (166) allowed the characterization of the electrical activity of single channels activated by voltage and internal Ca^{2+} , observations that were reported the same year by Marty in chromaffin cells (288) and Pallota et al. (342) in cultured muscle cells (see also Ref. 19). Almost at the same time, these channels were incorporated into lipid bilayers from a muscle membrane preparation enriched in t tubules, and they were found to have almost identical properties as those described in cells (245). Dubbed BK (for big K^+) (35) or Maxi K^+ (242), these channels proved to have unsuspected characteristics compared with other K^+ channels, such as an extremely large single-channel conductance (~ 250 pS in symmetrical 100 mM K^+) and an exquisite K^+ selectivity (34, 111, 496). Similar to other K^+ channels, BK channels were blocked by internal and external tetraethylammonium (TEA) and Ba^{2+} (34, 329, 330, 451–454, 496). These ions, as well as scorpion toxins [charibdotoxin and iberiotoxin (58, 143, 144, 277, 278, 307, 310)] were fundamental in unveiling the BK channel's gross architecture, which proved to contain a large internal vestibule and a rather shallow external mouth connected by a narrow selectivity filter. The lack of state-dependent blockage by

large quaternary ammonium (QA) ions has been more recently deemed to be a strong indication that the activation gate is not internal to the selectivity filter as it is for voltage-dependent K^+ (Kv) channels, but rather it is the selectivity filter itself (252, 426, 430, 469).

Cloning the BK channel from *Drosophila* (1, 13) with the help of the *Slowpoke* mutant, which is a mutant deficient in a Ca^{2+} -activated conductance (113), showed that the BK channel-forming protein, α subunit, has a topology very similar to that of Kv channels (FIGURE 1A). Showing that the BK channel is conserved through the evolutionary ladder, a gene encoding a BK channel was also isolated from brain

and skeletal muscle of mouse (*mSlo1*), becoming clear that all the variants of *mSlo1* cDNAs were the product of a single gene [*Slowpoke*, *KCNMA1* (56)]. The hydrophobicity profile of the BK channel protein showed six transmembrane domains with a conserved positively charged S4 transmembrane segment, a well-defined pore domain containing the signature sequence TVGYGD typical of K^+ channels, and a very large COOH terminus. However, further analysis of the protein hydrophobicity pattern showed the presence of an extra transmembrane domain, S0 (455), leaving the NH_2 terminus of the protein pointing towards the external side. As in Kv channels, the α subunit forms tetrameric BK channels (362, 398, 461). It is at present clear

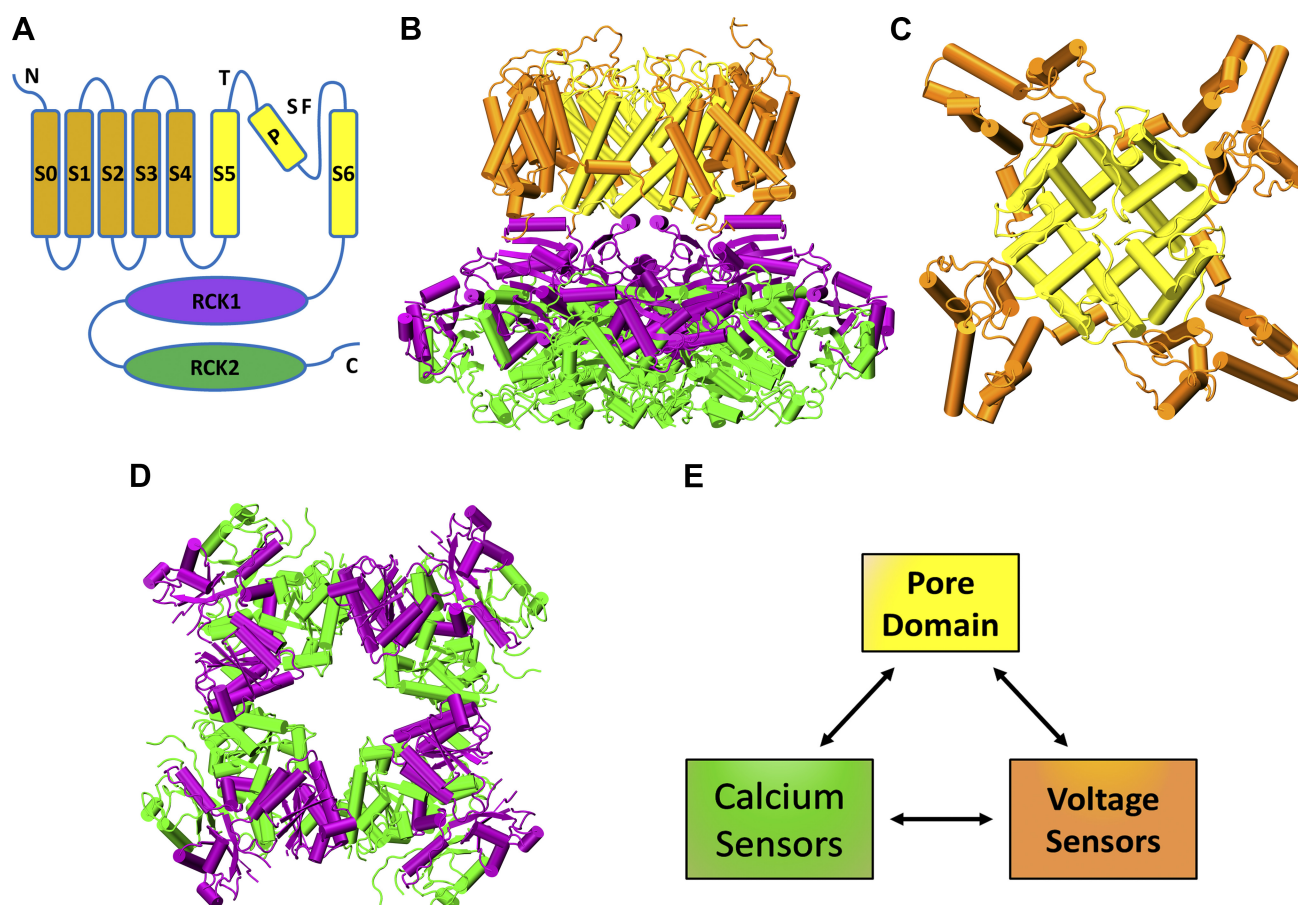


FIGURE 1. The modular nature of BK channel. **A:** topology of the BK channel predicted by hydrophilicity profiles. Alpha helices are represented as rectangles, S0 to S4 form the voltage sensor domain. The turret (T) is the loop joining S5 with the pore helix (P). SF is the selectivity filter. SF and S6 form the internal entryway. NH_2 terminal is extracellular, and COOH terminal is cytosolic. Intracellular domain consists of a pair of RCK domains. **B:** full homotetrameric BK channel tertiary structure is composed of a transmembrane and a cytosolic region. The transmembrane region comprised the pore domain (yellow) and the voltage sensors (orange). The large cytosolic region contains the calcium sensors, which is composed of two regulators of potassium conductance domains, RCK1 and RCK2 (violet and green, respectively). **C:** structure (top view) of the transmembrane region. The pore was modeled using the multiple sequence alignment done by Carvacho et al. (61) [with the MthK crystallographic structure (355) (PDB ID: 4HYO)]. The S1–S4 sequence was aligned against Shaker chimera Kv1.2/Kv2.1 crystallographic structure (266) (PDB ID: 2R9R) based on a multiple sequence alignment made by Lu et al. (271). The S0 segment was built as an alpha helix using secondary structure. **D:** structure (top view) of the Ca^{2+} -bound gating ring with the RCK1 in violet and the RCK2 in green. The coordinates were obtained from the crystal structure (PDB code: 3U6N) and rendered using the Visual Molecular Dynamics, version 1.9.2 (200). **E:** a modular model for BK channel proposed that each domain correspond to a specific structural domain and they interact with one another.

that the S0–S4 domain forms a well-defined voltage sensor and that the COOH terminus creates a tetrameric gating ring containing two high-affinity Ca^{2+} binding sites (32, 69, 241, 389, 508).

Very early on, it was realized that the BK channel gating kinetics was very complex (280, 281, 313). However, the pioneering studies by Magleby's group (279, 293, 297, 298, 371, 372) and those of the group of Aldrich (71, 185, 187) paved the way for the two-tiered allosteric model of Horrigan and Aldrich (186). In this type of mechanism, it is assumed that there are several modules that can undergo independent rearrangements between two or more different equilibrium conformations. These modules can communicate their conformational states to other modules affecting their equilibria, in similar ways as described for allosteric enzymes (315). Usually there are correspondences between structure and function in the sense that the parts of the channels that undergo different equilibria are contained in different structures (see **FIGURE 1**). In the case of BK channels, these modules have been identified through a diversity of approaches, and some of the interactions between them have been revealed (243). Thus, by taking advantage of the tetrameric structure of BK channels, the model considers the existence of four modular voltage sensors and four Ca^{2+} binding sites. Activation of these sensors can independently lead to channel opening, and communication between the sensors and the pore, as well as between sensors, would be allosteric (186). In other words, increasing voltage or Ca^{2+} binding can activate the pore gate by decreasing the free energy of the closed-open transition. This allosteric model has been extremely successful in accounting for the experimental data obtained under the most different experimental conditions. Allosteric models are reviewed in section III and used throughout the review as a theoretical framework to explain the variety of BK biophysical characteristics originating in posttranscriptional and posttranslational processing, as well as association with accessory subunits.

The physiological importance of this channel was on solid grounds when its ubiquitous distribution in several different types of cells and tissues was confirmed (244, 292). Most importantly, the pore forming α subunit in smooth muscle was observed to be accompanied by an auxiliary β subunit, namely, the β_1 subunit (135, 227, 339). The capability of β_1 to increase the apparent Ca^{2+} sensitivity of the channel proved to be crucial in the control of the smooth muscle tone. Later with the discovery of other three β subunits (β_2 – β_4), the diversity and physiological role of BK channels increased greatly (188, 339, 439, 474). BK channels are now known to be involved in repetitive firing (117), neurotransmitter release (195, 363), hormone secretion, K^+ secretion in the kidney (183), and hearing (311). BK channels also play important roles in ethanol tolerance (103),

nociception (419), and the regulation of circadian rhythm (66, 120, 305, 316, 468). The discovery of the γ subunit (γ_1 – γ_4) made BK channels important to cells in which the internal Ca^{2+} is not subject to large changes. BK channels formed by α and γ_1 subunits have a large open probability even at the cell resting potential and at physiological intracellular Ca^{2+} concentrations (485, 511). Thus, despite the fact that α subunit is the product of a single gene (*Slowpoke* in *Drosophila*), a large channel diversity has been attained not only by the presence of auxiliary subunits, but also by alternative splicing (128) and metabolic regulation (408). Since BK channels are activated by cytoplasmic Ca^{2+} and depolarizing voltages, they are the perfect damping machine of excitatory processes such as muscle contraction or neurosecretion.

In this review, we first summarize the physiological importance of BK channels in different cells and tissues emphasizing that the malfunctioning of BK channels is responsible for several diseases. Central to its physiological importance is its allosteric activation by both Ca^{2+} and voltage, best explained by allosteric gating models. Because of this, we thought appropriate to give next a detailed description of how these models can be used to analyze channel gating since they reflect the modular nature of the BK channel-forming protein. Moreover, allosteric models have been the basis to explain the effects on channel gating of mutations and auxiliary subunits. In what follows, we have mostly covered what is currently known regarding the structure of the BK channel and its relation to its function as an allosteric protein, focusing on the individual characteristics of each protein module and the communication mechanisms among themselves. In addition, we reviewed how alternative splicing and accessory subunits can increase channel diversity and modify BK channel phenotypes.

II. BK CHANNEL PHYSIOLOGY AND RELATED CHANNELOPATHIES

BK channel expression is ubiquitous. As can be inferred from this assertion, BK malfunctioning or abnormal expression gives rise to several pathophysiological conditions where auxiliary proteins can also have a role. Indeed, BK channel dysfunctions generate alterations in different tissues and organs as will be described in the following paragraphs.

A. Smooth Muscle

Expressed mainly in smooth muscle, BK α subunit associated with the β_1 subunit proved to be crucial in maintaining smooth muscle arterial tone. The degree of muscle tension is crucial for the regulation of blood pressure. In vascular smooth muscle cells, Ca^{2+} -dependent relaxation is due to the transient release of Ca^{2+} from the sarcoplasmic reticu-

lum (Ca^{2+} sparks) which activates BK channels (FIGURE 2) (49, 353). These Ca^{2+} sparks are produced in the boundaries of BK channels formed by a complex of α and $\beta 1$ subunits, $[(\alpha+\beta 1)\text{BK}]$; for the interested reader, β subunits are discussed in sect. VIII], where the concentration of the divalent cation can reach micromolar concentrations (348). The spontaneous transient outward K^+ currents (STOCs) (37) mediated by BK channels and induced by the Ca^{2+} sparks disappear in $\beta 1$ knockout mice, leading to hypertension. It is worth mentioning here that acidosis dilates brain parenchymal arterioles (PAs). This is due to a conversion of the Ca^{2+} waves observed at normal physiological pH (7.4) in PAs of smooth muscle cells, to mainly Ca^{2+} sparks at more acidic pHs. These Ca^{2+} sparks lead to the activation of BK channels with the consequent vasodilation of cerebral PAs (88).

Patients with primary hypertension present an increase in heart weight, showing a reduction of $\beta 1$ levels in vascular smooth muscle cells. This is linked to decreases in STOCs (493). On the other hand, a $\beta 1$ gain of function mutant (E65K) has been associated with low prevalence of moderate and severe diastolic hypertension (122, 219, 394).

BK channels provide a negative feedback to urinary smooth muscle contraction, limiting contraction amplitude and duration, and urinary bladder spontaneous and nerve-evoked contractions, which are enhanced in smooth muscle cells lacking BK channels (174, 304). Accordingly, BK channel dysfunction is implicated in some forms of urinary bladder pathologies leading to urinary incontinency, such as overactive bladder (OAB) and detrusor overactivity (350). OAB is among the top 10 most prevalent chronic human health conditions that increases with age (73, 350). This is consistent with findings that aging reduces BK channel density in the cell membrane of coronary and corporal smooth muscle cells (90, 350, 436).

BK channels are highly expressed in airway smooth muscle (ASM; Ref. 148). Interferon- γ -mediated reduction of BK channel activity in airway epithelial cells leads to mucociliary dysfunction (285). Goldklang et al. (148) demonstrated that systemic administration of the BK channel agonist rottlerin reduced methacholine-induced airway hyperreactivity (AHR) in ovalbumin- and house dust mite-sensitized mice. These findings suggest that rottlerin, with both anti-

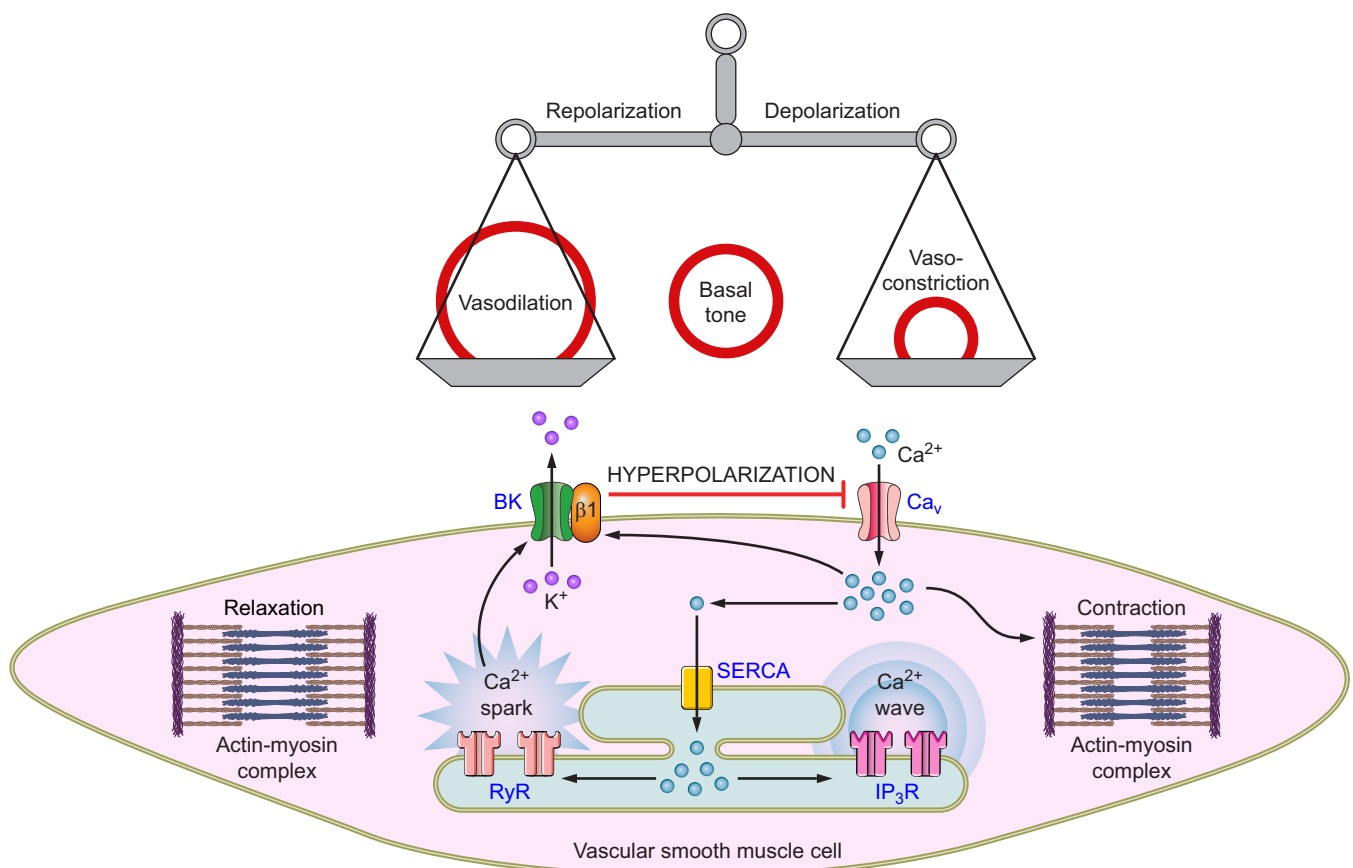


FIGURE 2. A model for the role of BK channel in vascular smooth muscle cells. Under conditions supporting vasoconstriction, global Ca^{2+} increase through voltage-activated calcium channels (Ca_v) and calcium-induced calcium release mechanism through inositol trisphosphate receptor (IP_3R), promoting cell contraction. When vasodilatation is favored, local calcium-induced calcium release mechanism through ryanodine receptor (RyR) leads the $(\alpha+\beta 1)\text{BK}$ channel activation, which hyperpolarizes the membrane, inhibiting Ca_v channels.

inflammatory and ASM relaxation properties, may benefit asthma treatment.

B. Kidney

BK channels are expressed in the intercalated cells (IC) of the cortical collecting duct (CCD) and in the connecting tubule (CNT) where they play an important role in K^+ secretion (reviewed in Refs. 183, 467). $(\alpha + \beta 1)$ BK channels are expressed in the apical membrane of mouse CNT principal cells (PCs) and in the initial collecting ducts. $(\alpha + \beta 4)$ BK channels can be found in intercalated cells (FIGURE 3). The hypertension observed in the $\beta 1$ knockout mice is exacerbated by a high- K^+ diet due to a defective K^+ handling by the kidney and to aldosteronism (157). $(\alpha + \beta 4)$ BK channels in intercalated cells are activated in an external Ca^{2+} -dependent fashion by a shear stress induced by high tubule fluid flow (262). This BK channel activation is absent in $\beta 4$ knockout mice (182). The loss of intracellular K^+ mediated by the activation of these channels produces a decrease in cell volume that in turn increases the luminal diameter with the consequent reduction in resistance to

flow (184). On the basis of the finding that the transient receptor potential vanilloid 4 (TRPV4) forms a complex with BK channels in smooth muscle (108) and is present in ICs, Holtzclaw et al. (184) advanced the hypothesis that shear stress activates Ca^{2+} -permeable TRPV4. It has been reported that Ca^{2+} permeating through TRPV4 channels is responsible for BK channel activation in ICs (see FIGURE 3). Alternatively, primary cilia in PCs have been proposed as the flow sensor, and the bending of these cilia is thought to activate the Ca^{2+} influx through TRPV4 channels, which produce a Ca^{2+} -induced Ca^{2+} release from intracellular stores (31, 263). In turn, the Ca^{2+} -dependent activation of MAPK promotes the release of PGE_2 from the PCs, which activates BK channels in ICs (60). It has also been proposed that shear stress produces a connexin channel-mediated ATP efflux from the ICs and a K^+ extrusion via $(\alpha + \beta 4)$ BK channels with the concomitant decrease in cellular volume and increase in tubular flow (181).

Hypertension of $\beta 1^{-/-}$ mice is linked to deficient K^+ secretion, aldosteronism, and an enhanced fluid retention (157, 158). End-stage renal disease (ESRD) is one of the multiple

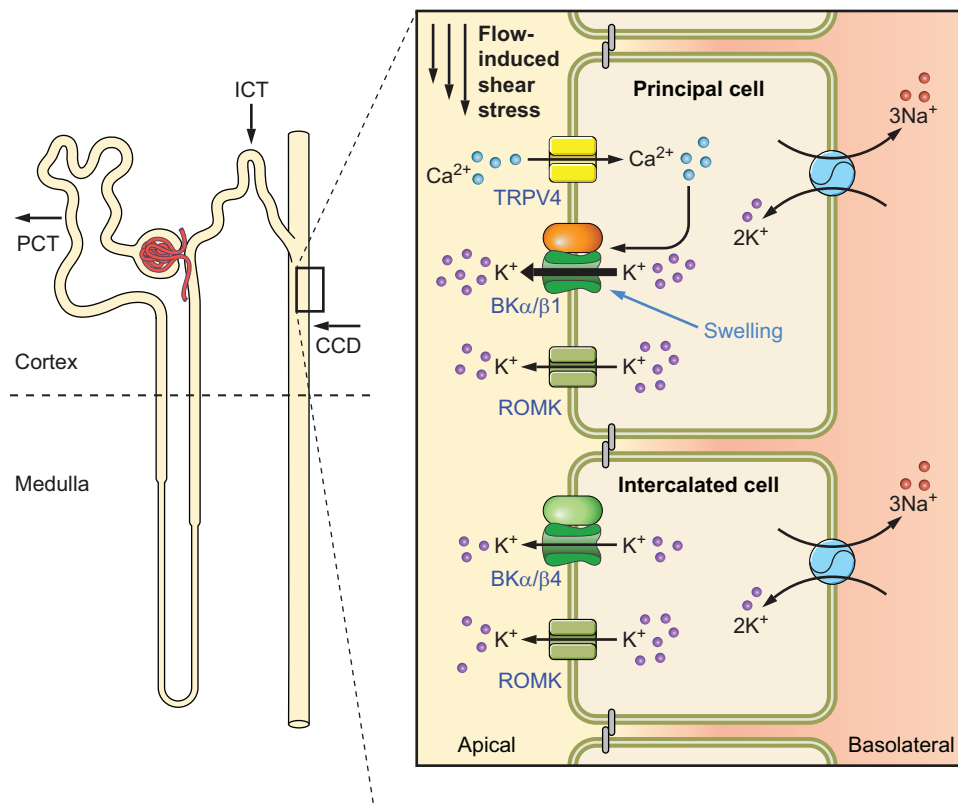


FIGURE 3. BK channel plays a key role in renal K^+ flux within the nephron. BK channels in the apical membrane of epithelial cells of the cortical collecting duct (CCD) are activated by tubule fluid flow-induced shear stress Ca^{2+} entry (possible through TRPV4 channel) and cell swelling. In basal conditions, ROMK is thought to be the primary responsible for K^+ secretion. Under increased K^+ secretion demanded by higher flow, high aldosterone, or others, it is proposed that both ROMK and BK play a role. The physiological role of BK in the proximal cells (PC) or intercalated cells (IC) in the distal nephron is defined by the β subunit coexpressed with the channel. Arrows illustrate places with higher K^+ flux in the nephron. PCT, proximal convoluted tubule; ICT, initial collecting tubule; CCD, cortical collecting duct.

consequences of sustained hypertension. The colon potassium secretion capacity increases in end-stage renal disease, to the extent that it makes a substantial contribution to K^+ homeostasis (289). Apart from the indirect effect of BK channels in ESRD, due to the previously explained influence in blood pressure, an increased expression of apical BK channels in the intestinal epithelium is in part responsible of the enhanced colonic K^+ secretion in ESRD (289).

C. The Nervous System

Localized mainly in axons and synaptic terminals (442), BK channels have been implicated in neurotransmitter release (156, 495). Additionally, and in central neurons, they contribute to repolarization of action potentials (AP) (222, 396, 420) and mediate the fast phase of AP afterhyperpolarization (161, 239, 470). In some neurons, such as Purkinje, vestibular, and cerebral Golgi cells, inhibiting BK channels speeds AP firing accompanied by a reduction in afterhyperpolarization (199, 328, 470). On the other hand, in hippocampal CA1 pyramidal, GABAergic and intracardiac autonomic neurons, inhibiting BK channels has the effect of deaccelerating firing rate (146, 161, 349). Recently, Kimm et al. (222) have shown that in dopaminergic neurons of the substantia nigra pars compacta (SNc) inhibition of BK channel leads to a pronounced AP broadening, a more negative afterhyperpolarization, and an increase in firing rate. The results clearly indicate that the increase in AP duration is due to an enhancement of the slowly deactivating K_{v2} current, which is short circuited when the fast-activating BK channels are present. Thus we should keep in mind when analyzing the functional role of the BK channel that this needs to be defined in the context of the other conductances in the cell.

In the central nervous system, BK channels colocalize with voltage-dependent Ca^{2+} channels (Ca_v) (30, 159). It has been argued that this colocalization is essential to raise the local concentration of Ca^{2+} to levels at which the channel open probability (P_o) reaches reasonable values at membrane voltages in the -50 to 0 mV range (29). Assuming an apparent dissociation constant for Ca^{2+} of $10\ \mu\text{M}$ and a Hill coefficient of 3, a $10\ \mu\text{M}$ internal Ca^{2+} concentration would be required to reach a $P_o = 0.5$ at 0 mV. In fact, BK channels from rat brain tissue were found to be associated with L-, P/Q-, and N-type Ca_v channels (30). Moreover, Berkefeld and Fakler (27), by coexpressing BK and N-type of Ca_v channels and using an experimental paradigm meant to mimic the physiological conditions, showed that BK channels operate as ligand-activated channels gated by intracellular Ca^{2+} (27). P/Q- and L-type of Ca_v channels have different activation characteristics, and these differences, when BK- Ca_v complexes are present, are translated into BK K^+ currents with different kinetics and voltage dependence (28). P/Q- and N-type of Ca_v channels are mainly found in pre- and postsynaptic compartments in

cerebral Purkinje cells as well as cholinergic interneurons, whereas L-type Ca_v channels are found in cell soma and dendrites of hippocampal pyramidal neurons. It is then expected that BK channels will show a functional variability, generated by the coassembly of BK with different Ca_v channel partners in different types of neurons (29). Quantification of the distribution of P/Q-type of Ca_v channels and BK channels in rat cerebellar Purkinje cells using electron microscopy showed that clusters of P/Q channels in the protoplasmic face of soma and primary dendrites colocalize with BK channels, with the nearest neighbor distance of ~ 40 nm (204).

Although neuronal BK channels can be formed by the α subunit alone giving rise to iberitoxin-sensitive, marten toxin-insensitive fast-activated channels, the contribution of BK channels to AP repolarization and to the firing rate is greatly determined by the BK channel's composition (458). For example, the $\beta 4$ subunit (see sect. VIII for details) is highly expressed in the brain, where it provides BK channels with their slow gating characteristics, low IbTx sensitivity, and reduced contribution to action potential repolarization (47, 80, 286, 303, 466) (FIGURE 4). In particular, ($\alpha + \beta 4$)BK channels are expressed in trigeminal ganglia that project primary terminal afferents into the trigeminal caudal nucleus (TCN). In TCN, ($\alpha + \beta 4$)BK channels modulate glutamate release. Excitatory neurotransmitters and calcitonin gene-related peptides (CGRP) released from TCN synaptosomes are inhibited by BK channel activation and stimulated by BK channel blockers such as iberitoxin (IbTX) (379, 478). Since CGRP has been involved in the transmission of pain, it has been proposed that BK channels may represent a potential target for drug therapy for migraines and orofacial pain (379, 478). In the hippocampus dentate gyrus, deletion of the $\beta 4$ subunit transforms slow-gated BK channels into fast-gated BK channels, indicating that this subunit limits the contribution of the BK channel to repolarization after action potential (47). Thus $\beta 4$ protects cells against hyperexcitability in the dentate gyrus which, when uncontrolled, can lead to temporal lobe seizures. The failure of IbTX to increase spontaneous electrical activity, to bind to rat striatal membranes, or to modulate the acetylcholine release in rat striatal slices (127, 381) made the role of BK channels in the regulation of neurotransmitter release uncertain. However, recalling that $\beta 4$ is highly expressed in the brain, ($\alpha + \beta 4$)BK channels proved to be resistant to IbTX action. The low sensitivity of $\alpha + \beta 4$ channels to IbTX is mainly due to a decrease in the toxin association rate constant by $\sim 1,000$ -fold, explaining the low sensitivity to the toxin of some BK channels present in the nervous system (303) (see sect. VIII).

BK channels in the nervous system can be seen as an “emergency break” which prevents transmitter-related hyperexcitability and concomitant cell toxicity (25). Genetic variations in BK channels have been recently implicated in men-

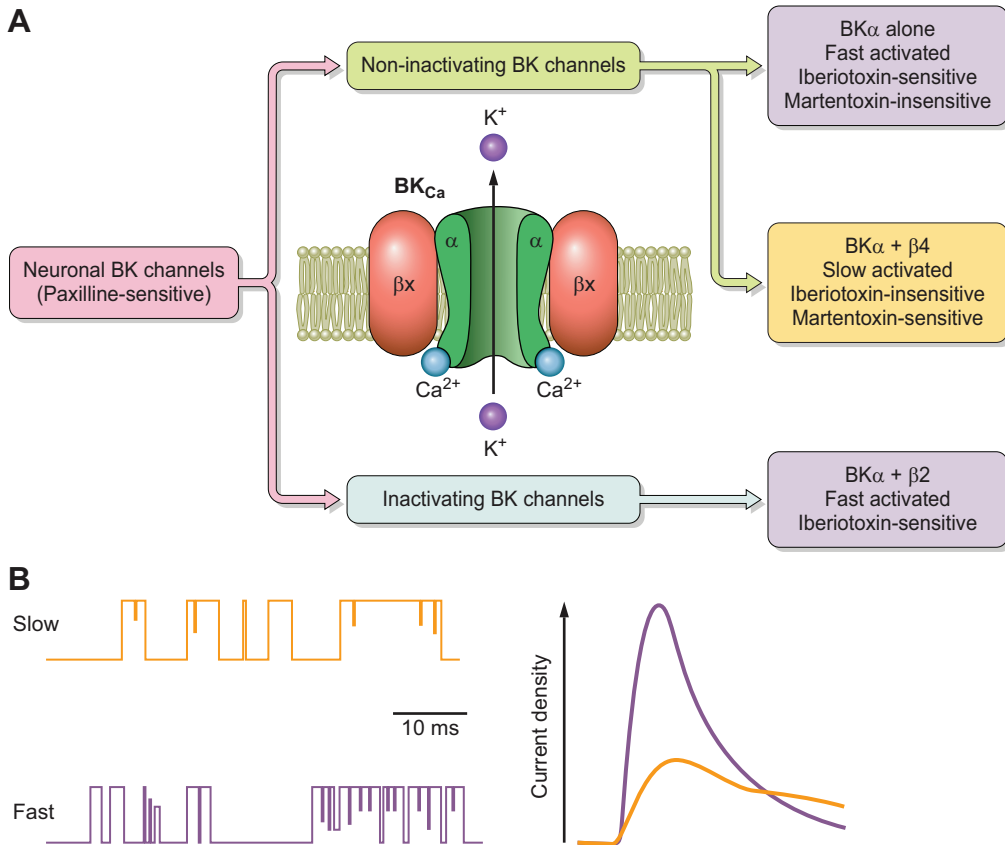


FIGURE 4. BK channels and its β auxiliary subunits are pivotal in neuronal excitability. **A:** diagram chart of BK neuronal channels, pharmacology of scorpion toxins, and general gating features. **B:** illustration of single-channel recordings and current densities for fast and slow activated neuronal BK.

tal retardation, autism, and schizophrenia, which all come along with severe cognitive impairments (445). Patients suffering from a variety of neurological diseases often develop neurogenic detrusor over activity (NDO) (194). NDO is associated with decreased detrusor smooth muscle (DSM, the smooth muscle found in the wall of the urinary bladder) BK channel expression and function leading to increased DSM excitability and contractility (193).

BK α subunit knockout mice have impaired motor coordination and exhibit ataxia (382), a result that has been confirmed in transgenic mice lacking BK channels exclusively in the Purkinje cells of the cerebellum (83). In fact, Dell'Orco et al. (92) have shown in a mouse model of ataxia type 1 that the neuronal atrophy is a consequence of a reduction in BK channel expression and subthreshold-activated K^+ channels that brings about neuron depolarization and the subsequent cell loss. BK channel deficiency does not impair working memory in mice, but showed significantly slower training, so it appears that BK channels play a crucial role in learning but not in memory storage or recollection (445).

The expression of BK α subunit mRNA in the suprachiasmatic nuclei (SCN), the master pacemaker that drives the circadian control of physiology and behavior in mammals, is regulated in a diurnal manner. BK transcripts increase during the night, and concomitantly the average spontaneous firing frequency (SFF) of SCN neurons drops during the night (154, 343). The molecular nature of the channel in-

involved in the regulation of the SFF was unveiled with the help of *KCNMA1*-deficient mice (305). Meredith et al. (305) confirm that BK channels were upregulated at night, and they also found that circadian amplitude of locomotor activity in the *KCNMA1*^{-/-} mice decreased and that the SFF increased during subjective night compared with wild-type animals. As we will describe in detail in the section on β subunits, the β 2 subunit imparts an N-type inactivation to BK channels. Whitt et al. (468) have recently showed that inactivating BK currents predominate during the day. This inactivation decreases during the night, leading to larger BK currents. This diurnal variation of BK currents is abolished in β 2 knockout mice, showing that the BK inactivation process is used as a switch to set the circadian variations in SCN excitability. These data suggest that BK modulators could influence the endogenous rhythm structure, and perhaps be useful in patients with circadian and sleep dysfunctions.

Both BK loss-of-function and gain-of-function have been linked to epilepsy. It can be assumed that loss of BK channel activity to be the underlying cause of hyperactivity and epilepsy (25). In temporal lobe epilepsy (TLE) patients, a downregulation of BK channels was observed (322), but on the other hand, human idiopathic generalized epilepsy is directly associated to a D434G gain-of-function mutation in the BK α subunit, which increases Ca^{2+} sensitivity and mean open time of the channel (106). It has been suggested that by increasing the open probability of the BK channel at

constant voltage, the D434G mutation allows for a more rapid repolarization of the action potential, enabling faster removal of inactivation of Na^+ channels and in turn increasing brain excitability. The D434G mutation deserves further analysis, since it shows how this channelopathy helped elucidate the coupling mechanism between Ca^{2+} binding and BK pore opening [see sect. VB (491)]. In humans, a truncation mutation in *KCNMB3*, the gene coding for the $\beta 3$ subunit, has been described to confer susceptibility to common idiopathic generalized epilepsy forms (247, 269, 427). More recently, it was found that the activity of BK channels is suppressed in Alzheimer's disease model mice, but cognitive recovery can be achieved by chronic activation of BK channels (460).

D. Cochlea

In the cochlea of birds, reptiles, and amphibians, the tonotopic map is defined by the interplay between BK and voltage-dependent Ca^{2+} channels contained in sensory hair cells (125). Although the proposal that the different tuning frequency is determined by BK splice variants with different Ca^{2+} sensitivities received support from different laboratories (e.g., Refs. 327, 370), this hypothesis has been shown to be untenable. Splicing of transcripts encoding BK channels in birds appears to be only a minor determinant of the electrical tuning of cochlear hair cells. Ramanathan et al. (364) advanced the hypothesis that since β subunits are preferentially expressed by low-frequency hair cells, the adequate distribution of αBK , $(\alpha + \beta 1)\text{BK}$ and $(\alpha + \beta 4)\text{BK}$ channels along the cochlea could determine the electrical tuning of hair cells. The evidence indicates that the difference in BK gating kinetics is given by the differential expression of the $\beta 1$ subunit, which is profusely expressed towards the low-frequency apex of the cochlea (311).

Although its role is less well understood, BK channels are also expressed in the inner hair cells (IHCs) and outer hair cells of mammals and transcripts for the α , $\beta 1$, and $\beta 4$ subunits have been identified in these cells (42, 240, 410). Using α and $\beta 1/\beta 4$ subunit knockout mice, Pyott et al. (358) were able to show that BK channels are not required for normal hearing in mammals but that α knockout mouse showed an increased resistance to noise-induced hearing loss. Nonetheless, more recently and using targeted deletion of BK channels in inner and outer hair cells of mice, Kurt et al. (235) have shown that BK channels in IHCs play a role in defining the temporal fine structure and the dynamic range of auditory information.

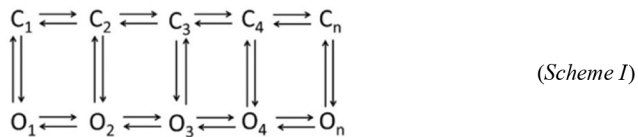
III. ALLOSTERIC MODEL FOR BK CHANNEL ACTIVATION BY VOLTAGE AND INTRACELLULAR Ca^{2+}

BK channels are constituted by a voltage sensor domain, a pore domain, and an intracellular COOH terminus domain

containing calcium binding sites (see **FIGURE 1**). Their activity (i.e., the opening that allows the passage of K^+ ions) is pivotal for the homeostasis and electrical balance of excitable and nonexcitable cells. Models based on allostery have been developed in efforts to understand how the different modules communicate among each other to control BK channel gating. Coupling between different BK channel modules provides the basis for the transduction of physicochemical stimuli into changes in membrane potential, and hence define the channel's functional properties. It is therefore necessary to have a conceptual framework to analyze channel gating and the kinetics models used to date. The best model that reflects the allosteric nature of BK channel activation is the Horrigan and Aldrich (HA model) (186). The HA model has also been crucial in understanding the effects of auxiliary subunits, mutations, and modulators. We provide a detailed description of this model below.

A. Gating Kinetics in BK Channels Is Complex

Despite considering several open and closed states, early kinetic models differed regarding the nature of BK channel voltage dependence. Thus Moczydlowski and Latorre (314), based on their analysis of single-channel data, proposed that voltage dependence resides in the movement of Ca^{2+} from the internal milieu to binding sites located inside the electric field of the membrane. On the other hand, Methfessel and Boheim (306) attributed the voltage dependence to a charged gate. Other laboratories proposed separate activation steps consisting of Ca^{2+} binding and voltage sensing (33, 68, 98, 302, 341, 405, 465). Moreover, there were previous suggestions of the existence of a calcium-independent component in the activation of the channel (19, 341). Therefore, an intrinsic voltage-dependent mechanism was postulated (126, 302, 341, 471). The existence of this mechanism is evident for BK at Ca^{2+} concentrations below 100 nM (71, 77, 302, 417). An important observation regarding the number of kinetic states and how they were connected was the inverse correlation between open and closed dwell times (293, 302). On average, long open dwell times were found adjacent to short closed dwell times, and long closed dwell times occurred adjacent to short open dwell times (293). These observations exclude kinetic schemes, where only one pathway from closed to open states is proposed to explain channel gating. This kind of linear model predicts no correlation between open and closed dwell times. Hence, the observed correlation is consistent with models where two or more closed states make direct contact with two or more open states (293). Based on these considerations, McManus and Magleby (293) proposed a general kinetic scheme to account for calcium activation and the open and closed dwell times as illustrated in Scheme I.



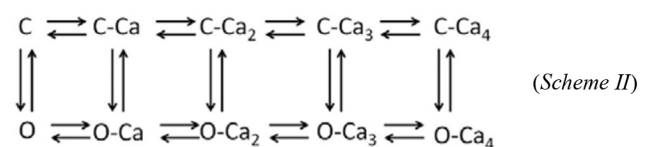
This model reproduces the observed dwell-time correlations if C_1 is the longest-lived shut time and O_1 the shortest living open state, where closed times get shorter from left to right and open times grow longer from left to right. Calcium drives the equilibrium toward the right to account for the calcium activation of the channel according to Scheme I.

Meera et al. (302) demonstrated that BK channel activation becomes calcium independent and purely voltage gated at calcium concentrations lower than 100 nM. This finding indicates that at least one of the leftmost close to open transitions in Scheme I can occur in the absence of calcium, and must be voltage dependent. Cui et al. (77) reported additional evidence of the existence of a voltage-dependent transition that is independent of calcium. They demonstrated that the position of the half-activation voltage fails to be a function of the logarithm of calcium concentration, as required for systems where all the voltage dependence resides on a voltage-dependent calcium binding constant (471). Furthermore, depolarization can activate maximally BK channels in the absence of calcium (77). Based on these observations, Cui et al. (77) proposed a model where the vertical transitions, observed in Scheme I, are voltage dependent, and the horizontal ones are calcium binding reactions. Activation and deactivation kinetics reveal that the channel has two main states: open and closed. Analysis of macroscopic currents show that both activation and deactivation kinetics display single exponential time courses, indicating that the vertical transitions are slow compared with the horizontal transitions, and that the latter appear to be in equilibrium with the opening reaction (77). This is correct to a first approximation since a closer inspection of the activation kinetics reveals that there is a delay in the onset of the activation current (77, 417).

B. Two-Tiered Allosteric Model

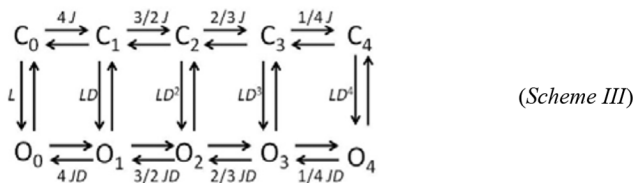
As we will discuss in detail in section V, when describing the gating ring and Ca^{2+} binding sites, the existence and characterization of Ca^{2+} binding sites contained in the COOH-terminal region clearly demonstrate that calcium and voltage sensors are located in separate domains of the protein (FIGURE 1, A-D). This is the basic concept of Monod-Wyman-Changeux (MWC) allosteric mechanisms (315). Cox et al. (71) proposed a successful voltage-dependent MWC model to explain the main steady-state and kinetic characteristics of the macroscopic currents of BK channels. This model considers that BK channels are tetramers of α subunits in which each subunit has a single calcium-binding site and a single voltage-sensing element (FIGURE 1A). A single Ca^{2+} binding site per subunit is consistent with the esti-

mated Hill number (larger than 2) which is necessary to fit the Ca^{2+} activation curve and the tetrameric nature of the BK channel structure (19, 280, 281, 296, 398). A single voltage-sensing element per subunit is justified since the structure of transmembrane domains S1-S4 of the α subunit is similar to that of the voltage sensor domain (VSD) of K_v channels (1, 13, 56, 290, 444). Each subunit with one calcium-binding site and one voltage-sensing element has four different possible states: 1) Ca^{2+} bound, 2) voltage activated, 3) Ca^{2+} bound and voltage activated, and 4) neither Ca^{2+} bound nor voltage activated. Considering all the possible states and their connections, the working kinetic model contains 256 states. A kinetic model with such a large number of states would be impractical, and the model had to be simplified to be tractable. The number of different states was reduced if a fourfold symmetry was assumed in analogy to K_v channels (172, 275, 276). A fourfold symmetric tetramer with all the voltage sensors inactive could reside in six bound states: Ca^{2+} bound to adjacent subunits or Ca^{2+} bound to diagonally opposed subunits. If the two forms with two Ca^{2+} were taken to be equivalent, then the number of states would decrease to five. Since the macroscopic activation and deactivation kinetics are mono-exponential, the channel seems to have only two main states: closed and open (77). This observation implies that the voltage-dependent closed-to-open transition proceeds in one step. In other words, the four voltage sensors activate simultaneously in a highly orchestrated fashion. With these simplifications, Cox et al. (71) reduced the number of states to 10: 5 with all voltage sensors at resting state and 5 with the four voltage sensors activated. The resulting kinetic scheme (Scheme II) is a voltage-dependent version of the MWC allosteric mechanism (315). Vertical close-to-open transitions in this scheme are voltage dependent and imply the concerted activations of the four voltage sensors.



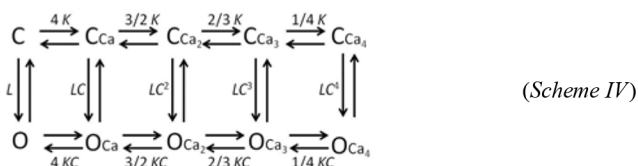
Additionally, Stefani et al. (417) demonstrated that voltage sensor activation and channel opening occur at different time courses. Replacing K^+ with Cs^+ led to the observation of gating currents and ionic currents on the same patch because cesium conductance is only 1/400 of the K^+ conductance. Gating currents precede, in time, the activation of ionic currents, an observation incompatible with Scheme II which requires that voltage sensor activation and channel opening occur simultaneously. Furthermore, a detailed study of BK single channel kinetics in the absence of Ca^{2+} showed the presence of multiple open and closed states (425), again a result incompatible with a MWC mechanism with a central voltage-dependent concerted step conceived by Scheme II. Accordingly, Horrigan et al. (187) studied the kinetics of activation of BK channels and found additional

characteristics incompatible with Scheme II. They found that activation of ionic currents according to Scheme II must follow an exponential time course. However, the experimental results revealed that it proceeds along an exponential time course but after a short delay. In addition, the voltage-dependent activation time constant evidenced that there is more than one limiting step in channel gating. The slope of the logarithm of the time constant versus voltage relationship is characterized by a charge displacement of $0.41 e_0$ for voltages ranging from -30 to $+140$ mV. But this decreases when reaching a limiting slope consistent with a charge movement of $0.14 e_0$ for voltages ranging from -20 mV down to -500 mV. A similar behavior was reported for the logarithm of the open channel probability versus voltage relationship where the associated charge displacement is $2 e_0$ for voltages from 0 to $+100$ mV, but falls to a limiting slope with a charge movement of only $0.4 e_0$ (187). These results were explained with a gating scheme where the voltage-dependent close-to-open transitions are allosterically modulated by the activation of four independent voltage sensors. The charge associated with the limiting slope corresponds to the intrinsic voltage dependency of the closed-to-open transition. Scheme III, shown below, is consistent with the steady-state observations for BK open channel probability in the absence of calcium (185, 187).



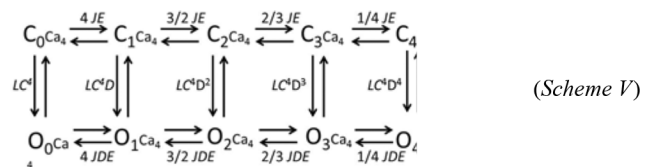
In this scheme, C_0 to C_4 represent the closed states with 0, 1, 2, 3, and 4 active voltage sensors, while O_0 to O_4 indicate 0, 1, 2, 3, and 4 open state transitions. J is the voltage-dependence equilibrium constant for the resting (R) to active (A) transition of each voltage sensor, defined as the A/R probability ratio for the closed unbound channel. L is the equilibrium constant for the closed to open transition with no active voltage sensor defined as the O_0/C_0 probability ratio. The allosteric factor D accounts for the allosteric interaction between the voltage sensors and the channel gate.

Calcium binding can open BK channels even with all voltage sensors inactive. Scheme IV describes the calcium-dependent gating system at extreme negative voltages when no voltage sensors are active, assuming one calcium-binding site at each subunit.



In this scheme, C to CCa_4 represent the closed states with 0, 1, 2, 3, and 4 calcium binding sites, and O to O_Ca_4 illustrate open states with 0, 1, 2, 3, and 4 Ca^{2+} -bound sites. K is the equilibrium constant for the unbound to calcium-bound transition for each calcium-binding site defined as the bound/unbound probability ratio for each calcium-binding site for the closed channel with all voltage sensors at rest. K depends on calcium concentration, $[Ca^{2+}]$, and the Ca^{2+} dissociation constant, K_D , $K = [Ca^{2+}]/K_D$. The allosteric factor C accounts for the allosteric interaction between the calcium-binding site and the channel gate.

At saturating $[Ca^{2+}]$, calcium occupies the four calcium-binding sites, as described in Scheme V, for the calcium-saturated condition. In Scheme V, the allosteric factor E is included to account for the interaction between the voltage sensors and the calcium binding sites.



Schemes III, IV, and V are extreme cases of a general dual allosteric model where the channel gate, four voltage sensors, and four Ca^{2+} binding sites interact through allosteric factors. Three equilibrium constants L , J , and K and three allosteric factors C , D , and E determine the open channel probability, as shown in the following equation

$$P_o = \frac{L(1 + KC + JD + JKCDE)^4}{L(1 + KC + JD + JKCDE)^4 + (1 + K + J + JKE)^4} \quad (1)$$

A family of P_o vs. membrane potential (V) at different $[Ca^{2+}]$ is shown in **FIGURE 5, A AND B**, calculated by using Equation 1 and the parameters reported by Horrigan and Aldrich (186). The parameters in Equation 1 could be determined with a global curve fit to a family of P_o vs. V relationships measured at different calcium concentrations, as those shown in **FIGURE 5A**. However, it is recommendable to choose experimental conditions that allow for the selection of only one or a few parameters. Since interactions between the different parameters are expected, this strategy would help to better constrain their values.

C. Experimental Approaches to Determine Gating Parameters of the Allosteric Model for BK Channel Gating

The following paragraphs describe procedures to determine these parameters in independent experiments.

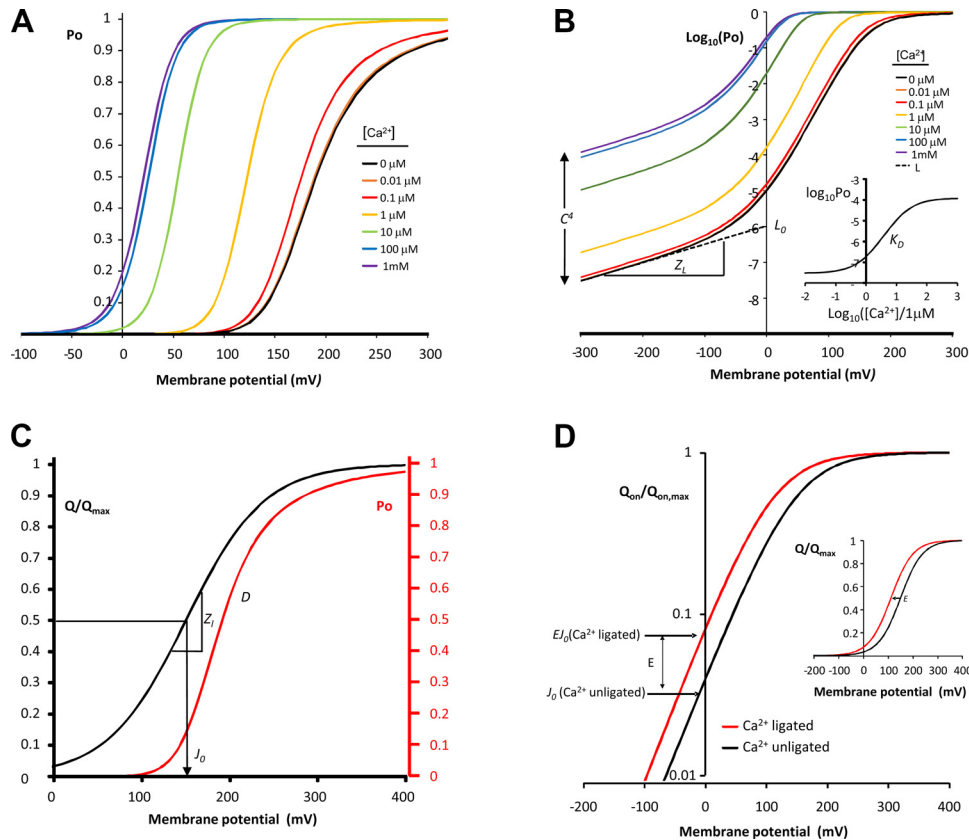


FIGURE 5. This figure illustrates a procedure to determine the BK equilibrium parameters of the Horrigan and Aldrich (1986) allosteric model. The plots were drawn using Equation 1 and the Horrigan and Aldrich parameters: $L_O = 9.8 \times 10^{-6}$, $z_L = 0.3$, $V_h(J) = 150$ mV, $z_J = 0.58$, $K_D = 11$ μM , $C = 8$, $D = 25$, $E = 2.4$. **A:** a family of open channel probability (P_o) as a function of voltage showing the displacement to the left for increasing Ca^{2+} concentrations. **B:** P_o vs. V data plotted on a semi-logarithmic scale to display the effect of voltage and Ca^{2+} at very negative voltages where P_o is very low. Data to construct these plots can be collected from membranes with a large number of channels, N , and measuring the time-averaged number of open channels, NP_o , at negative voltages. The slope of the dashed line drawn over the $P_o(V)$ curve at 0 Ca^{2+} is proportional to the charge z_L and the extrapolation to $V = 0$ is L_O (linear transformation of Equation 2). The total vertical displacement of the curves from 0 to 1 mM Ca^{2+} is a measure of the allosteric parameter C . *Inset* shows P_o measured at -300 mV as a function of Ca^{2+} concentration. From this curve the calcium dissociation constant (K_D) can be calculated (Eq. 3). **C:** normalized Q vs. V curve for closed channels ($Q/Q_{\max}$), in the absence of calcium. From this curve, the parameters J_O and z_J are obtained (Eq. 6). The P_o vs. V in the absence of calcium is determined by L , J_O , z_J , and the allosteric factor D , which can be found using curve fitting procedure (Eq. 4). **D:** semi-logarithmic plot of $Q/Q_{\max}$ at 0 Ca^{2+} (black line) and saturating Ca^{2+} concentration (red line). The parameter E determines the vertical displacement of the 0 V intercept of the curves (Eqs. 7 and 8) and the left shift on the linear plot shown in the *inset*.

BK channels do open, albeit with a very low P_o even when the voltage sensors are at rest, $J = 0$, and in the absence of calcium, $K = 0$. In this condition, Equation 1 simplifies to $P_o = L$. Data obtained under this extreme condition are collected by counting the number of open channels, NP_o , at large negative potentials in membranes with a known number of channels, N . As illustrated in **FIGURE 5B**, L is an exponential function of voltage with the following form

$$L = L_O e^{\frac{z_L F V}{RT}} \quad (2)$$

In Equation 2, L_O is the equilibrium constant for $V = 0$ and z_L is the charge displacement associated with the closed to open transition. The value of these two parameters can be determined from the slope and the vertical axis intercepts of

the semilogarithmic plot of P_o vs. voltage drawn for very negative voltages (**FIGURE 5B**). Values reported for these parameters are $L_O = 9.8 \times 10^{-6}$ and $z_L = 0.3$ (186), where $L = 4.8 \times 10^{-6}$ at a cell resting potential of -60 mV.

Allosteric factor C can be determined at very large negative potentials, where the voltage sensors are at rest, $J = 0$, by comparing the open probability in the absence and in the presence of an excess of calcium. In the former case $P_o = L$ and as well as the latter case, for very large values of K , Equation 1 simplifies to $P_o = LC^4$. The method to determine allosteric parameter C is shown in **FIGURE 5B**. Horrigan and Aldrich (186) reported a value for $C = 7.4$, which indicates that the BK channel open probability in the pres-

ence of saturating calcium concentrations is 3,000 times larger than in the absence of calcium.

For intermediate calcium concentrations and $J = 0$, Equation 1 reduces to

$$P_0 = \frac{L(1 + KC)^4}{L(1 + KC)^4 + (1 + K)^4} \quad (3)$$

We illustrate in the *inset* of **FIGURE 5B** how the equilibrium constant, K , and the binding constant K_D , $K = [\text{Ca}^{2+}]/K_D$, can be determined from the P_0 vs. V curve as indicated by Equation 3 using the already determined C value. Horrigan and Aldrich (186) reported a $K_D = 9.3 \mu\text{M}$ for Ca^{2+} . There is evidence that there are at least two calcium-binding sites per monomer with different dissociation constants (18, 279, 401, 482). Equation 3 describes the experimental data up to $73 \mu\text{M}$ Ca^{2+} , but fails to explain the results obtained at higher calcium concentrations (312, 423).

The voltage sensor equilibrium constant J and the allosteric factor D can be determined by measuring P_0 as a function of voltage in the absence of calcium, where $K = 0$. For this condition, Equation 1 simplifies to Equation 4

$$P_0 = \frac{L(1 + JD)^4}{L(1 + JD)^4 + (1 + J)^4} \quad (4)$$

The following expression defines J as a function of voltage

$$J = J_0 e^{\frac{z_J F V}{RT}} \quad (5)$$

Here J_0 is the equilibrium constant for $V = 0$, and z_J is the charge displacement associated with the resting to active voltage sensor transition. Values reported for these parameters are $J_0 = 3.1 \times 10^{-2}$ and $z_J = 0.55$. The estimated value that best adjusts this, so that allosteric factor D can fit the P_0 vs. V data, is 25. According to Scheme III, the equilibrium constant for the closed-to-open channel, when all four voltage sensors are activated, is 25^4 (4×10^5) times larger than when all voltage sensors are at rest. At 300 mV, when all the voltage sensors are active, $L = 3.6 \times 10^{-4}$, $LD^4 = 1.4 \times 10^2$, and $P_0 \sim 1.0$. Therefore, voltage can fully open the BK channels even in the absence of calcium.

Gating currents (I_g) were observed by replacing potassium with cesium ions (417). I_g and cesium currents can be separated because I_g develop with orders of magnitude faster than ionic currents, where the latter present a delay and are 1/3,000 of the potassium currents (417). I_g determination provides a direct measure of the voltage sensor equilibrium constant. In the absence of calcium, the ON component of I_g displays a single exponential decay. However, in the presence of calcium, a second exponential with a longer time constant appears, revealing the allosteric interaction of the pore gate with the voltage sensors (186).

J can be determined from the gating charge displaced on a membrane depolarization step. This is legitimate, since the ON I_g vanishes before the ionic current starts to develop, i.e., when channels are still closed (67, 186, 418). The fraction of the total charge displaced, $Q_{on}/Q_{on,max}$, is a measure of the probability to find the voltage sensor in its activate state. Therefore, $Q_{on}/Q_{on,max}$ can be written as a Boltzmann function

$$\frac{Q_{on}}{Q_{on,max}} = \frac{1}{1 + J^{-1}} \quad (6)$$

A plot for this function is shown in **FIGURE 5C** (left curve). The parameters J_0 and z_J defining J can be determined using a nonlinear curve fitting procedure. The red line (curve at right) in **FIGURE 5C** is a plot of P_0 vs. voltage in the absence of calcium, drawn by using Equations 4 and 5, where the only unknown parameter is D , which can be determined from this plot.

Comparing the I_g of the fast component of ON I_g , in the absence and presence of calcium, makes it possible to evaluate allosteric factor E , which describes the interaction between the voltage sensors and the calcium binding sites with the pore gate closed since the fast I_g ends before the channels begin to open. Boltzmann functions are adequate descriptions of the charge-voltage curves, where the effect of calcium shifts the half voltage, V_b , to the left, at which point the half of the maximum charge mobilizes (see the *inset* of **FIGURE 5D**). The left shift of V_b implies $E > 1$. This ΔV_b is only -20 mV. A more accurate estimation of the ΔV_b produces a left shift of -33 mV (186), obtained from admittance measurements (123) of I_g . This ΔV_b implies an allosteric factor $E = 2.1$, meaning a weak interaction between voltage sensors and calcium binding sites. However, stronger interaction between the voltage sensor and Ca^{2+} binding site have also been reported ($E = 10$ – 24 ; Refs. 59, 385).

The last parameter to be determined is E , the allosteric parameter connecting the voltage sensors and the calcium binding sites. Calcium binding is expected to shift the $Q_{on}/Q_{on,max}$ vs. V curve to the left, as shown in the *inset* of **FIGURE 5D**. The equilibrium of the resting-to-active transition with calcium bound is the product JE . For $V = 0$, the $Q_{on}/Q_{on,max}$ ratio is a small value ($1/J_0$), and the following approximations of Equation 6 are legitimate

$$\frac{Q_{on}}{Q_{on,max}(\text{Ca} \ll K_d)} = J_0 \quad (7)$$

$$\frac{Q_{on}}{Q_{on,max}(\text{Ca} \gg K_d)} = EJ_0 \quad (8)$$

Allosteric factor E can be determined from the $Q_{on}/Q_{on,max}$ ratio measured under saturating calcium and no calcium

conditions, as illustrated from the vertical displacement of the logarithmic plot in **FIGURE 5D**.

ON and OFF I_g mobilize the same amount of charge for short (≈ 1 ms) depolarizing pulses. For longer depolarizing pulses, the charge collected by the integration of OFF I_g grows following a double exponential time course. The fast time constant corresponds to the decay time of ON I_g . The charge of the slow component reveals a charge mobilization during the depolarizing pulse, which is too slow to be a measurable I_g (185). The slow time constant is 100 times longer than the fast one and is comparable with the time constant of the ionic current activation, suggesting that this slow charge movement is limited by channel opening (185). This slow charge corresponds to the charge displaced in the closed-to-open transition, plus the charge displaced between open states. This situation is illustrated in Scheme III. Starting at a holding potential such as C_0 is the only populated state, where a depolarization will shift the distribution of the closed states with C_2 being the most populated state, because the equilibrium constant J is a function of voltage. The charge associated with the transition from C_0 to C_2 is the fast component of the gating charge. Now, the C_2 to O_2 transition occurs, and the equilibrium of the open states is shifted to O_4 , as the equilibrium constant J is multiplied by allosteric factor D . The OFF charge movement is, therefore, limited by the open-to-closed transition giving origin to the slow component of the OFF charge as a function of the depolarizing pulse duration. Actually, the time constant of the slow I_g component is similar to the time constants of the potassium current activation. However, in the presence of auxiliary subunits such as $\beta 1$, $\beta 2$, and $\beta 4$, the slow component develops in advance of and faster than ionic current activation, suggesting that BK channel opening proceeds in two steps (67). This observation indicates that Schemes III to V should include an additional tier of closed states. This type of three-tiered schemes was also suggested (279) as an explanation for the short-lived flicker observed in open channels (372, 397). Contreras et al. (67) worked out the complete three-tiered model and used it to explain the effects of β subunits on BK channel kinetics and equilibrium.

IV. INFERENCES ABOUT THE NATURE OF THE CONDUCTION SYSTEM AND GATES

A. Selectivity and Conduction

The BK channel is the most selective K^+ channel found to date and the one with the largest conductance (~ 250 pS in symmetrical 100 mM KCl), hence the name of BK or Maxi K. Na^+ is essentially impermeable, and the single-channel conductance ratio $g_{\text{K}}/g_{\text{Na}}$ is ~ 300 (34, 112, 417, 496). The calculated relative permeability sequence using the Goldman-Hodgkin-Katz equation is $\text{Ti}^+:\text{K}^+:\text{Rb}^+:\text{NH}_4^+ = 1.2:1:0.7:0.11$ (34), and agrees well with that obtained by using bionic potentials (112). On the other hand, the conductance sequence is $\text{K}^+:\text{Ti}^+:\text{NH}_4^+:\text{Rb}^+ = 1:0.41:0.18:0.07$ (112).

Since BK channels were first characterized, it was surprising to be confronting an ion channel with such exquisite selectivity and, paradoxically, with such a huge conductance compared with other K^+ channels (242). We still do not have a full answer regarding how this high conductance is generated, but we have some hints, most of them are from studies focused on the internal vestibule. The neutralization residue D292N located directly outside the selectivity filter reduces around 40% of the single-channel conductance, which can be explained by a reduction of the surface charge density (170, 171). Comparing the primary sequence of the pore regions of BK and K_v channels, it is evident that BK channels, unlike K_v channels, hold two glutamate residues that give rise to two negatively charged rings with a total of eight negative charges at the entrance of the internal BK channel vestibule (**FIGURE 6**). Neutralization of these two glutamates causes a decrease in channel conductance to half the value obtained with the wild-type (WT) BK from ~ 300 to 150 pS in symmetrical 150 K^+ (46, 331). Maximal conductance of the WT and the double mutant channel are, however, identical. This indicates that the negative charges increase K^+ concentration in the vestibule through long range electrostatic interactions. The finding that these negative charges contribute to single-channel BK conductance

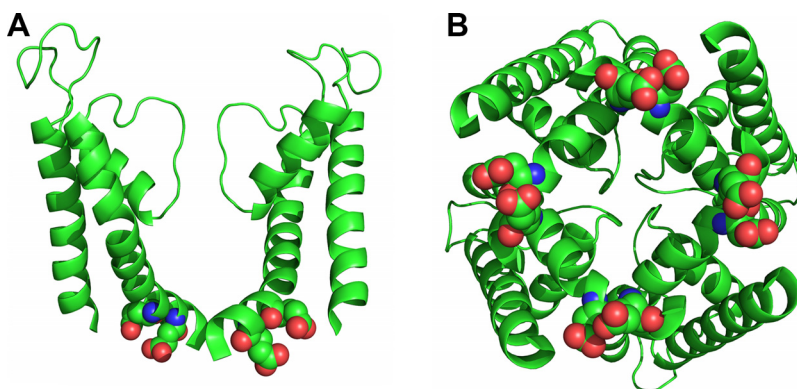


FIGURE 6. Eight negatively charged glutamate residues are located at the inner entrance of BK channel (E386 and E389). **A:** secondary structure of two subunits of the pore domain are shown, extracellular side. **B:** the pore domain viewed from the intracellular side. Atomic coordinates were taken from the BK channel homology model (61). Glutamate atoms are shown as spheres using this color code: N = blue, C = white, O = red. Illustrations were prepared using The PyMOL Molecular Graphics System, version 1.7.4, Schrödinger, LLC.

was further corroborated by replacing E321 and E324 by cysteines and exposing them to negative and positive thiol reagents. Internal addition of maleimide propionic acid, a negative thiol reagent, increases channel conductance, whereas ethyl methanethiosulfonate, a positive thiol reagent, decreases it (139).

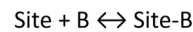
To calculate the electrostatic potential along the internal cavity, Carvacho et al. (61) built a molecular model of the BK channel pore and solved the Poisson-Boltzmann equation. The calculated electrostatic profile agrees with the electrostatic potentials obtained using TEA and Ba^{2+} blockade, and amounts to -22 mV at the internal channel entrance. This potential is enough to raise K^+ concentration at the channel entrance by about twofold.

On the other hand, the structure of the MthK channel revealed that the internal entrance in this channel is much wider than in K_v channels by ~ 10 Å (209, 267). The accessibility experiments of Zhou et al. (516) strongly suggest that the radius of the internal entrance of BK channels is even larger than that of MthK. Thus, in BK channels, as in MthK channels, most of the electric field drops in the selectivity filter (61, 94, 209). The size of the internal cavity can be an important factor in determining channel conductance, and this can, at least in part, explain the large difference in single-channel conductance between K_v and BK despite the high degree of conservation of the selectivity filter (FIGURE 7). In fact, reducing the diffusion coefficient of K^+ , decreasing the effective volume of the BK inner cavity by using sucrose or decreasing the size of the inner BK vestibule by increasing the side chain of the amino acids in positions 321/324 has been observed to reduce channel conductance (44, 139). The estimates indicate that in Shaker K^+ channels (~ 12 pS in symmetrical 100 mM K^+), most of the electrical resistance resides in the inner cavity, whilst only 7% of the electrical resistance lies in the internal cavity of BK channels (44, 94, 139).

B. Blockade

H^+ , Na^+ , Cs^+ , and Ba^{2+} block BK channels by interacting with the channel conduction pathway (45, 64, 309, 452, 497). These cations have been fundamental in determining the multi-ion pore nature of the selectivity filter of BK channels. The internal block of the BK channel by Na^+ can be relieved by external K^+ . External K^+ does not affect the entry rate of Na^+ , but only increases the exit rate of Na^+ from the channel, indicating that both monovalent ions can coexist simultaneously inside the selectivity filter of BK (497).

If the ion blockade is described by a simple first-order process of the type



(Scheme VI)

where B is the blocking ion interacting with a site located in the electric field then, the voltage-dependent equilibrium constant, $K(V)$, that describes the reaction depicted in SCHEME VI, is given by the equation (472)

$$K(V) = K(0)e^{\frac{z\delta FV}{RT}} \quad (9)$$

where $K(0)$ is the equilibrium constant at zero applied voltage, z is the ion valence, and δ is the distance traveled by the ion in the electric field (strictly “an electrical distance”). The product $z\delta V$ is the electric potential difference between the binding site and the bulk solution from where the blocker is added. For monovalent ions, $z\delta$ cannot attain a value larger than 1. Deviations from Equation 9 (where $\delta > 1$) would indicate that the conduction system of the channel can be simultaneously occupied by more than one ion. In other words, we would be in the presence of a channel that behaves as a multi-ion pore (179).

External Cs^+ promotes a voltage-dependent blockage of BK channels with a $z\delta$ that can reach values of 1.3 at 10 mM Cs



FIGURE 7. Alignment of potassium channels selectivity filter. The primary sequence of seven human and two prokaryote potassium channels are aligned from residues surrounding the pore: Slo1 or BK (hKCNMA1, B7ZMF5), Slo3 (hKCN1, A8MYU2), Shaker-related (hKCN7, B2RU13), Kv1.2 (hKCN2, P16389), Kv2.1 (hKCN1, Q14721), EAG (hKCNH1, Q95259), Kv1.4 (hKCN4, P22459), KcsA (POA334, from *Streptomyces lividans*), and MthK (O27564 from *Methanothermobacter thermautotrophicus*). Primary structures were aligned using CLUSTAL O (1.2.1) multiple sequence alignment. Selectivity filter is enclosed in the black box. The phenylalanine residue 380 in BK (Slo1) channel is highlighted in green.

(64, 497). It should be highlighted that the current-voltage relationship shows an upturn at negative voltages, which is large enough to indicate that Cs^+ blockage is relieved by the influx of K^+ ions. In other words, Cs^+ is forced to permeate through the BK channel pushed by the incoming K^+ ions. In a two-site vacancy diffusion model, the $z\delta > 1$ originates from the fraction of the electric field where K^+ and Cs^+ travel before reaching their respective binding sites. If the two sites are very near, it can reach a maximum value of ~ 2 ($z\delta$ for both ions is ~ 1). It is also possible that if enough kinetic energy is supplied to the K^+ ions entering the channel, K^+ can knock off the blocking ion from the BK channel (10, 64, 180). In this case $z\delta$ can reach values larger than 2, and a knock-off model offers the best explanation for the characteristics of Cs^+ ions that block BK channel (64).

Internal as well as external Ba^{2+} is able to block BK channels (517), although with very different affinities. While millimolar amounts of external Ba^{2+} are required to block BK channels, micromolar internal Ba^{2+} suffices to induce appreciable blockade (452). The experiments using Ba^{2+} as a blocking ion have been extremely useful in defining the degree of ion occupancy of BK channel selectivity filters. In particular, Neyton and Miller (330) made the observation that the exit of Ba^{2+} towards the external side is slowed by increasing external K^+ concentration. This observation is most economically explained by assuming the existence of a K^+ binding site located external to Ba^{2+} , which once occupied is able to hinder the exit of the blocking ion (a “lock-in site”). In other words, the selectivity filter of BK channels is able to contain Ba^{2+} and K^+ simultaneously. Since this lock-in site is in equilibrium with the external K^+ when the channel is blocked by Ba^{2+} , it is possible to determine the dissociation constant (K_D) for K^+ at the lock-in site. Vergara et al. (451) measured a K_D of $\sim 3 \mu\text{M}$ by buffering external K^+ using a crown ether that binds K^+ with high affinity. This implies that K^+ binds to BK channels very tightly. As C-type inactivation is viewed as a collapse of the selectivity filter in the absence of permeant ions, this tight K^+ binding to the sites contained in the selectivity filter has been suggested to be the cause of the lack of C-type inactivation (e.g., Ref. 268) in BK channels. In fact, when the external K^+ concentration is reduced below the contaminant external K^+ concentration ($\sim 4 \mu\text{M}$), BK channels enter a quiescent state reminiscent of C-type inactivation (451).

C. TEA and Analogs

Both external and internal TEAs block BK channels, although with different affinities (34, 453, 454, 496). The dissociation constant of the external TEA site is $\sim 0.2 \text{ mM}$ with a $z\delta = 0.1$, whereas internal TEA blocks BK channels with a K_D of $\sim 30 \text{ mM}$ and a $z\delta$ of 0.3. High affinity of external TEA is given by the presence of a tyrosine residue located at the external entrance of the BK pore and near the

selectivity filter Tyr-309 in mBK (398). The external TEA site is selective for TEA and appears to discriminate between the different TEA analogs by size. Modifications of the ethyl side chain, either by increasing or decreasing its size, promote reductions in binding strength. On the other hand, the potency of the different TEA analogs in blocking BK channels from the internal side is determined by their hydrophobicity, as in K_v channels. Blocking potency is increased as the compound is made more hydrophobic (454). These results gave the idea that the basic architecture of BK channels is similar to that of K_v channels, i.e., wide and large internal vestibules and a rather shallow external mouth with a well-defined TEA binding site (FIGURE 8).

D. Where Is the Activation Gate?

1. State-independent quaternary ammonium ions block in BK channels

Blocking ions can also provide strong hints regarding the location of the activation gate in BK channels. The pioneering work by Armstrong (9, 10) using quaternary ammonium (QA) ions was the first to shed light on the gross

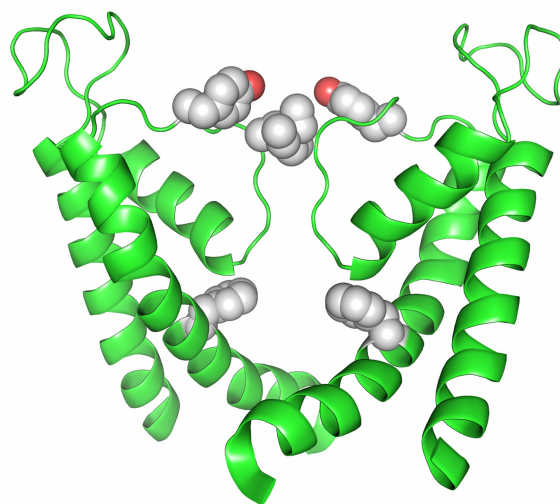


FIGURE 8. TEA binding site, modeled after Crouzy et al. (75). External TEA blocks K^+ channel binding to the entrance of the selectivity filter surrounded by a ring of four tyrosine residues. The figure also shows the tyrosine in position 380. This residue plays an important role in gating and conduction (59). Atomic coordinates of the protein atoms were taken from the BK channel homology model (61). TEA was coupled to BK channel by means docking simulations. The software used was Autodock Vina (443). Afterwards, a short molecular dynamics simulation was performed using AutoMD plugin from VMD 1.9.2 (200) and NAMD 2.9 software (NAMD was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign, see Ref. 352). TEA parameters were assigned according Paramchem results (449). TEA, tyrosine, and phenylalanine atoms are shown as spheres using this color code: N = blue, C = white, and O = red. Illustrations were prepared using The PyMOL Molecular Graphics System, version 1.7.4, Schrödinger, LLC.

architecture of K_v channels by proposing the existence of an internal gate and a large internal vestibule able to accommodate bulky and hydrophobic QA ions. This basic topology of K_v channels proposed by Armstrong was confirmed by the structural data, which showed that the activation gate in K^+ channels is in the bundle crossing constituted by the S6 transmembrane segments (105, 266, 267). Therefore, in K_v channels QA ions that are added internally can only reach their binding site when channels are open; hence, they are open-channel blockers.

The blockade of BK channels by QA ions was further studied by Aldrich's group (253, 469) that found the following: 1) binding or unbinding of large QA ions in BK channels is much faster than in K_v channels; 2) deactivation of BK channels is not modified by the presence of QA ions; and 3) by using a very large hydrophobic QA, *N*-(4-[benzoyl]benzyl)-*N,N,N*-tributylammonium bromide (bbTBA), they showed that BK channels can close unimpeded by the presence of bbTBA and that there is no evidence of bbTPA trapping after BK channel closing. Taken together, these results suggest that unlike K_v channels, QA blockage is state-independent, the internal vestibule of BK channels is larger than that of K_v channels, and the activation gate is located beyond the QA binding site, possibly at the selectivity filter. However, a further characterization of the bbTBA blockage suggested that to reach the binding site, bbTBA requires partial BK activation since fully closed channels cannot be blocked (426). Notably, although tetrabutylammonium (TBA) and the Shaker "ball" peptide (BP) exhibit open-channel blockage of BK channels in the presence of K^+ , replacing Rb^+ with K^+ induces a state-independent blockage by these two compounds (430). This effect is specific to BK because in Shaker K^+ channels the state dependency of the blockade by TBA and BP is independent from the permeant ion. As stated by Thompson and Begenisich (430), these results represent a positive test for selectivity filter gating. However, they do not discard the possibility of an enlargement of the intracellular entrance upon gating in the presence of K^+ ions.

It should be noted here that Ba^{2+} was also used to determine the location of the activation gate in K^+ channels (12). These experiments demonstrate that Ba^{2+} can reach its binding site when added externally, independent of the conformational channel state. However, Ba^{2+} added internally can only block open channels. These results confirmed the conclusions reached by using QA ions regarding the existence of an internally located activation gate. In BK channels, on the other hand, and in agreement with the hypothesis that the selectivity filter is or is part of the activation gate, Ba^{2+} is unable to block closed channels when added internally or externally. Ba^{2+} can be trapped inside BK channels by closing them during a blocking period with a large negative voltage pulse. The trapped divalent cation

cannot be dissociated from its binding site until after the channel opens (308, 309).

2. Shaker ball peptide

Single-channel studies showed that Shaker ball peptides (BP) and mutational homologs are able to interact with BK internal vestibules (437, 438). Similar to the blockage induced by these polypeptides of the Shaker K^+ channel (320), blocking potency increased either by making the BP NH_2 terminus more hydrophobic or by adding more positively charged residues to the BP $COOH$ terminus. Also, increasing the peptide association rate constant (k_{ON}) and hydrophobicity, augmented the mean blocking time with no effect on k_{ON} (437). These results revealed the presence of negative charges in or near the peptide binding site and of a peptide-interacting hydrophobic surface in the internal BK vestibule. Li and Aldrich (252) found that a peptide containing two positive charges (more than the ball peptide) can induce a slow blockage in addition to increasing binding potency. The blockade produced by this peptide, dubbed enhanced ball peptide (EBP), is state dependent and consistent with an open-channel blocking mechanism. Furthermore, EBP slowed the deactivation kinetics, a result suggestive of peptide trapping. Taking into account the results obtained by using QA ions, these observations are consistent with a conformational rearrangement that enlarges the intracellular entrance of the channel upon opening. Thus, although the passage of the small K^+ ion is hindered at the level of the selectivity filter, the pore-lining S6 helices undergo some conformational changes related to channel gating. There is no doubt, however, as indicated by state-dependent methanethiosulfonate ethyltrimethylammonium (MTSET), that the BK channel inner pore is much larger than that of other K^+ channels with known crystal structure (516).

E. Toxins

Scorpion toxins have played an important role in the development of our detailed knowledge of BK channels. In particular, charybdotoxin (ChTX) was fundamental in the isolation, purification, and identification of BK channels in different tissues (137). Isolated from the venom of the scorpion *Leiurus quinquestratus*, ChTX was the first peptide toxin found to block BK channels (310). ChTX is a 37-amino acid peptide containing 6 cysteine residues responsible for stabilizing the toxin tertiary structure and several positively charged residues, providing the peptide with a net charge of +5 (FIGURE 9). BK channel sensitivity to ChTX greatly decreases (~30-fold) when the α subunit is coexpressed with $\beta 2$ or $\beta 3$ subunits (456, 480; see sect. VIII). ChTX, however, turned out to be a rather unspecific toxin, being able to inhibit other K^+ channels including $K_{Ca3.1}$, voltage-dependent K^+ channels, $K_{v1.2}$, $K_{v1.3}$ and $K_{v1.6}$ (216) as well as some small-conductance SK_{Ca} channels (173).

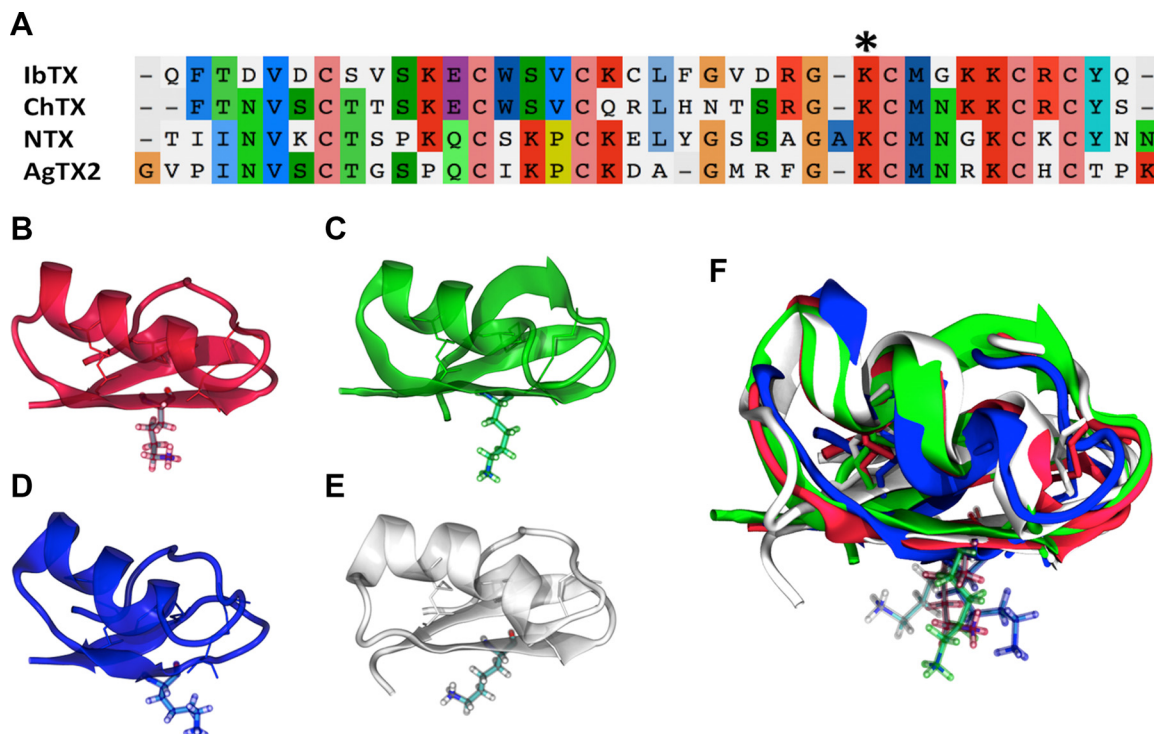


FIGURE 9. A: alignment of primary structure of scorpion toxins targeting the BK channel. The primary sequence of iberitoxin (IbTx; Uniprot code P24663), charybdotoxin (ChTx; P13487), noxiustoxin (NTx; P08815), and agitoxin (AgTx2; P46111) are shown. Residues with conserved identity are highlighted in color boxes. The asterisk marks the lysine residue which penetrates into the selectivity filter and blocks the channel. Multiple sequence alignment was done using CLUSTAL O (1.2.1). B–E: toxin structures. The lysine residue involved in binding to BK selectivity filter is shown as sticks. The highly conserved disulfide bonds are showed in thin sticks. B: charybdotoxin [red; PDB ID code 2CRD (38)]. C: agitoxin [green; PDB ID code 1AGT (231)]. D: iberitoxin [blue; homology model]. E: noxiustoxin [white; PDB ID code 1SXM (89)]. F: superimposition of the structure of the four toxins reveals a conserved secondary structure. The iberitoxin homology model was constructed under MODELLER 9v7 (378) using as reference the ChTx structure (2A9H), the alignment proposed by Johnson and Sugg 1992 (211). Illustrations were drawn using Visual Molecular Dynamics, version 1.9.2 (200).

Unlike ChTX, iberitoxin (IbTX) obtained from the venom of the African scorpion *Buthus tamulus* inhibits BK channel selectively with great potency: $K_D \sim 1.2$ nM (58, 131, 143, 217). IbTX has a 68% sequence identity with ChTX and the same number of residues, but possesses five acidic amino acids and one less basic residue compared with ChTX (**FIGURE 9A**). Another member of the ChTX toxin family is slotoxin, which has a K_D of ~ 1.5 nM (136) and is obtained from the Mexican scorpion *Centruroides noxious*, exhibiting a 54 and 75% identity with ChTX and IbTX, respectively. Slotoxin reversibly blocks α BK channels and irreversibly blocks $(\alpha + \beta_1)$ BK channels, whilst weakly blocking $(\alpha + \beta_4)$ BK channels (136). Additionally, martentoxin (MarTX), a 37-amino acid peptide purified from the venom of the Chinese scorpion *Buthus martensii*, shares a 39 and 45% identity with ChTX and IbTX, respectively. Remarkably, MarTX specifically blocks $(\alpha + \beta_4)$ BK channels with almost no effect on α BK channels, with a K_D of ~ 100 nM (400, 429). Unlike ChTX or IbTX whose effects are insensitive to $[\text{Ca}^{2+}]_i$, MarTX inhibits BK channels at low $[\text{Ca}^{2+}]_i$ but enhances BK channel activity at high $[\text{Ca}^{2+}]_i$ (208, 400). On the other hand, $(\alpha + \beta_1)$ BK channels are

activated by MarTX, with a dose-response curve with a K_D of ~ 500 nM (428). A toxin isolated from the venom of the Chinese scorpion *Buthus martensii*, BmP₀₉, is also able to specifically block BK channels albeit with less potency, exhibiting a K_D of ~ 27 nM (494). In contrast to the other peptide toxins, BmP₀₉ was defined as a long-chain toxin, since it is composed of 85 amino acid residues and in addition may be posttranslationally modified.

1. Toxin mode of action and structural considerations

Early on it was recognized that ChTX binds to the BK channel with 1) 1:1 channel:toxin stoichiometry, 2) long-range electrostatic interactions involved in toxin binding, 3) voltage-dependent binding reactions, and 4) toxin binding to the closed and open conformation of the channel (7). Regarding the voltage dependence of ChTX block, MacKinnon and Miller (277) showed that this is actually produced by a destabilization of the toxin:channel complex mediated by the flow of K^+ ions entering the conduction system from the intracellular side since the voltage depen-

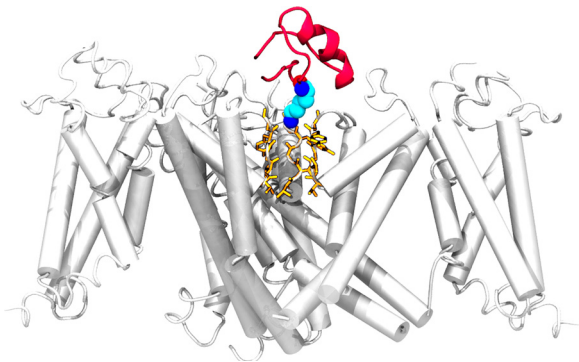


FIGURE 10. Schematic representation of charybdotoxin (PDB ID code 2CRD) secondary structure (red) docked to the selectivity filter (orange sticks) of BK with a stoichiometry of 1:1. Secondary structure of BK is represented as cylinders. Atoms of Lys27, the main residue implied in the BK channel blockade, are represented as spheres using the following color code: N = blue, O = red, C = cyan. See **FIGURE 8** legend for details on docking and drawing.

dence of the blockage disappears if internal K^+ is replaced with Na^+ . These observations are consistent with the hypothesis that ChTX acts by binding to and occluding the BK pore. Mutagenesis of ChTX showed that neutralization of ChTX Lys-27, which is an amino acid residue conserved in all members of the ChTX-toxin family, greatly decreases potency and makes the toxin block insensitive to voltage (347). From these experiments, it became clear that the positive charge of Lys-27 is interacting with a K^+ binding site located in the selectivity filter of the channel (**FIGURE 10**). Studies with other toxins (e.g., agitoxin2, AgTX2) showed that the mechanism by which this positively charged residue binds to the channel selectivity filter is common to all toxins of this type, applying to other K^+ channels as well (177, 365). Actually, the NMR structures of AgTX2, ChTX, IbTX, and noxiustoxins have been determined, showing that the peptide is held together by three or four disulfide bonds exhibiting nearly superimposable structures (**FIGURE 9A**) (39, 89, 211, 231). The extracellular vestibule of the Shaker K^+ channel was mapped by using double-mutant cycles between the channel and ChTX and AgTX2 (177, 323). These toxins were used as molecular calipers to determine distances of particular residues in the channel from the pore axis, as well as to define the channel-toxin surface of interaction. Four structural motifs involved in toxin binding were identified in Shaker K^+ channels: 1) the turret (F425, K427 residues), 2) the pore helix (D431 amino acid), 3) the selectivity filter (Y445, D447 residues), and 4) the loop connecting the selectivity filter with S6 (M448, T449, and V451 residues), as illustrated in **FIGURE 1A**. The question regarding the preference of certain toxins, such as IbTX preference for BK channels over K_v channels, was addressed by Giangiacomo et al. (143), who found novel IbTX binding sites in the BK turret. The BK turret contains five amino acids more than its counterpart in K_v channels (61, 143). More recently, Banerjee et al. (15) determined the crystal structure of the complex $K_{v1.2}/K_{v2.1}$ chimera-ChTX complex and found, as predicted from biophysical experiments, that Lys-27 projects its side chain

into the pore, leaving the amino group near the top of the first K^+ binding site (see **FIGURE 10**). Notably, this site appears empty in the channel-toxin complex. The absence of K^+ in S1 is hypothesized to be due to electrostatic repulsion between the amino group and the incoming K^+ and hydrogen bonding formation between the four carbonyl oxygens of the selectivity filter and the Lys-27 amino group (15). These four carbonyl groups would otherwise coordinate K^+ in S1.

2. Paxilline

Paxilline, panitrem, and lolitrem B are fungal indole alkaloids able to inhibit BK channels at low nanomolar concentrations (203, 228) in a highly specific manner (cf. Refs. 115, 396). Paxilline stimulates the binding of ChTX, suggesting that the inhibitor acts at a site different from that of ChTX (228). Applied internally, paxilline blocks BK channels with a K_D of 1.9 nM and a Hill coefficient of ~ 1 . Sanchez and McManus (380) reported that increasing internal Ca^{2+} concentration decreases paxilline potency, suggesting that the toxin binds preferentially to the closed state, and proposed an allosteric interaction between the divalent cation and paxilline. In a recent study, Zhou and Lingle (514) showed convincingly that paxilline inhibition can be fully reversed by increasing the open probability of the channel (P_o). Paxilline binds tightly to closed channels with a K_D of ~ 10 nM, and the K_D increases to ~ 10 μ M at P_o near its maximum value. Interpretations of these results in terms of the HA allosteric model suggest that paxilline allosterically modifies the BK channel closed-open equilibrium by stabilizing the closed configuration of the BK channel (decreasing L ; see Scheme III). Both the large QA ion bbTBA and high concentrations of sucrose that occupy the inner vestibule hinder the access of paxilline to its binding site, suggesting that this lipophilic molecule reaches its blocking position from the internal solution. Therefore, this alkaloid differs from the mode of action of, for example, lipophilic local anesthetics in that it can reach its site from the lipid moiety (178, 393). Paxilline, however, does not impede the entrance of MTS reagents like MTSET (514). A partial occlusion of the internal entrance of the channel is consistent with this result and with the fact that Sanchez and McManus (380) found a decrease in the single-channel conductance, in the presence of paxilline.

Considering the finding that the pH-activated Slo3 channel is insensitive to paxilline, Zhou et al. (515) by making chimeras of Slo1 and Slo3, found that paxilline binding requires the S6 transmembrane segment of the BK channel. Point mutations performed in S6 identified the mSlo1 G311 residue as essential to maintaining high-affinity inhibition by paxilline. These results strongly suggest that paxilline binds to a position located within the BK channel's internal vestibule.

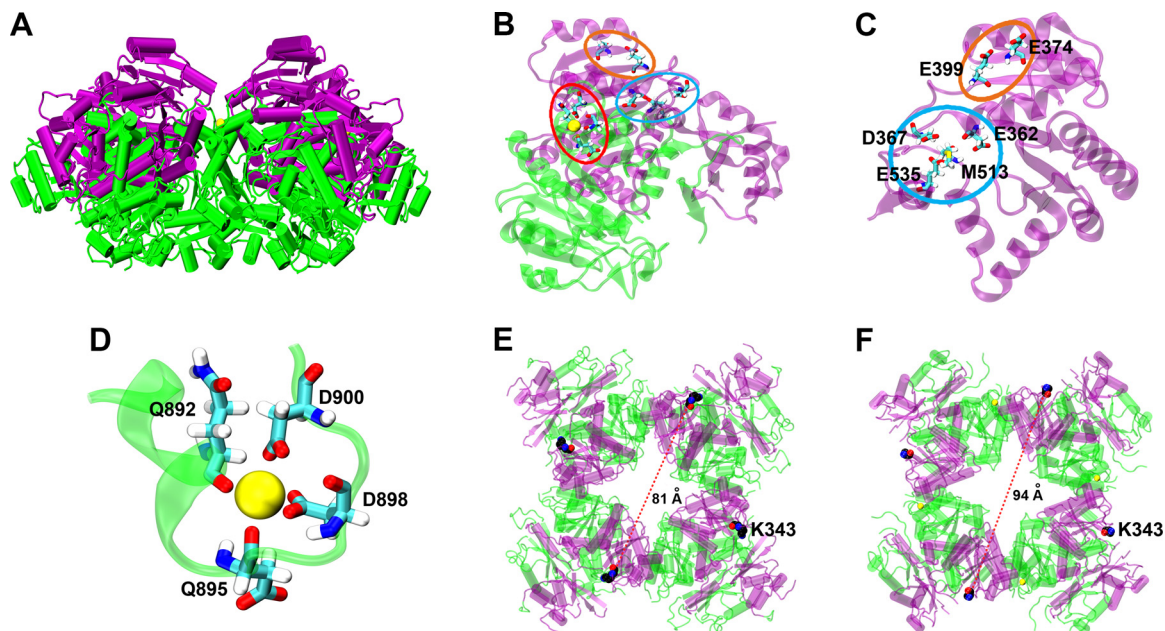


FIGURE 11. Ca^{2+} binding sites in the gating ring of BK channels. **A:** side view of two subunits forming the gating ring, RCK1 in violet and RCK2 in green. **B:** ribbon representation of RCK domains of one subunit, with the residues participating in the three calcium binding sites represented as sticks; the yellow sphere depicts the calcium ion. The high-affinity binding site in RCK1 (blue oval), the high-affinity binding site in the RCK2 or “calcium bowl” (red oval), and the low-affinity calcium and magnesium binding site (orange oval) are shown. **C:** closer view of RCK1 domain, rotated 90 degrees around y -axis from position in **B**. High-affinity calcium binding site residues (D362, D367, M513, and E535) and the low-affinity calcium and magnesium binding site residues (E399 and E374) are shown as sticks and enclosed in ovals as in **B**. **D:** calcium bowl on the RCK2 domain. The four residues that coordinate Ca^{2+} in the crystal structure of BK gating ring are shown as sticks, while Ca^{2+} is represented by the yellow sphere. **E:** crystal structure of the BK channel gating ring in the closed, Ca^{2+} -free, conformation (PDB code: 3NAF), showing the position of lysine 343 and the distance between alpha carbons of K343 residues of diagonally opposite subunits. **F:** crystal structure of the BK channel gating ring in the open, Ca^{2+} -bound, conformation (PDB code: 3U6N). As in **E**, the position of K343 residues and the distance between them in diagonally opposite subunits is shown. K343 is located at the NH_2 terminus of the RCK1 and connects with the COOH terminus of S6. The distance between K343 residues of two opposite subunits increases 13 Å when the gating ring binds calcium and is believed that it propagates inducing rearrangements in the S6 segments, resulting in channel opening. Atomic coordinates used in **A**, **B**, **C**, **D**, and **F** were read from PDB code 3U6N, and atomic coordinates used in **E** were read from PDB code 3NAF. Structures were drawn using the Visual Molecular Dynamics, version 1.9.2 (200).

V. MOLECULAR DETERMINANTS OF Ca^{2+} GATING

As stated before, BK channels can be activated by calcium, which binds to two putative sites in each subunit in the COOH -terminal domains. Two regulators of K^+ conductance elements in this segment harbor the calcium binding sites and form the gating ring as described below.

A. RCK Domains Form a Gating Ring: Two High-Affinity Ca^{2+} Binding Sites

In BK channels, the COOH terminus of each subunit is composed of two nonidentical regulators of K^+ conductance (RCK1 and RCK2), as shown in **FIGURE 11**, **A** and **B** (210, 477, 498, 499). The first identified “high-affinity” Ca^{2+} binding site is contained in RCK2 and consists of a 28-amino acid domain (from T883 to Q910 in mBK) that comprises a string

of five aspartic acid residues dubbed the calcium bowl (389). Mutations of these aspartate residues produced BK channels with a decreased Ca^{2+} sensitivity. However, the neutralization mutants [called $+$ -shifted mutants since they rightward shift the $G(V)$ curves] and the wild-type channel have the same sensitivity to Cd^{2+} (389). Bian et al. (32) found that the channel still conserved some Ca^{2+} sensitivity even when the five aspartic acid residues in the calcium bowl were neutralized by asparagine residues (mutant D5N5). These results are a clear indication of the presence of a second Ca^{2+} binding site with a different affinity to Ca^{2+} . Additionally, binding of radioactive Ca^{2+} showed a reduction of only 56% in the D5N5 mutant protein, when assayed on 280 amino acid fusion proteins of the COOH terminus of the *Drosophila* BK channel, containing the Ca^{2+} bowl (32; see also Ref. 43).

To these findings, we must add those obtained using Slo1-Slo3 chimeras where the tails, defined as the region com-

posed by segments S9–S10, between these two channels are exchanged (391). We note here that the Slo3 channel is activated by protons and lacks a Ca^{2+} bowl (390, 391). Although the Slo1-Slo3 tail channel is activated by Ca^{2+} concentrations $>300 \mu\text{M}$, the $G(V)$ curve of these chimeric channels is leftward shifted by $\sim 60 \text{ mV}$ in the nominal absence of Ca^{2+} compared with the wild-type BK. This observation not only suggests the existence of a second Ca^{2+} binding site, but also indicates that the Slo1 tail, in the absence of Ca^{2+} , increases the energy necessary to open the BK channel. From a physiological point of view, this would have the advantage of reducing the BK channel open probability when the cell is at rest (low internal Ca^{2+} and negative membrane potential). A modulation BK channel gating by the tail (COOH terminal) was confirmed by Moss and Magleby (319), who found that replacing the Slo1 tail with the Slo3 tail increased the open probability in the absence of Ca^{2+} and reduced BK channel Ca^{2+} sensitivity.

A more detailed study in which all the aspartates contained in the stretch comprised between mBK amino acids D895 and D903 were mutated to alanine, confirmed the existence of high-affinity sites that were not associated with the presence of the Ca^{2+} bowl (18). Moreover, Bao et al. (18) found that mutating the mBK residue methionine 513 to isoleucine, together with deleting most of the Ca^{2+} bowl ($\Delta 898-903$), resulted in a low Ca^{2+} sensitivity channel (18). M513 is located at the end of S7 segment, being part of the RCK1 domain (FIGURE 11C). Analyses of the data obtained with the deleted Ca^{2+} bowl and the M513I mutant using the HA allosteric model indicate that each “site” contributes equally to channel opening (sect. III, Scheme IV). In other words, each one is responsible for half of the channel's Ca^{2+} sensitivity (18). Another BK channel mutant that markedly reduced mBK channel Ca^{2+} sensitivity is the double mutant D362A/D367A. These two aspartate residues are located in the RCK1 domain (FIGURE 11C) while the mutant D362A/D367A-D5N5 is only activated by Ca^{2+} at concentrations $>10^{-3} \text{ M}$ (482). It should be noted that not all the aspartate residues in the Ca^{2+} bowl when neutralized decrease the affinity to the same extent. When the experiments are performed in an M513I background, point mutations D898A and D900A suffice to obliterate high-affinity BK channel Ca^{2+} sensitivity (FIGURE 11D). In addition, these two residues are essential for radioactive Ca^{2+} binding to a fusion protein that contains the Ca^{2+} bowl (17). These results led Bao et al. (17) to propose a structural model for the Ca^{2+} bowl in which these two aspartate residues are directly involved in complexing Ca^{2+} . Remarkably, as we will see below, this hypothetical model was confirmed when the structure of the gating ring in the presence of Ca^{2+} was solved (499).

Selectivity of the BK channel Ca^{2+} binding sites to divalent cations was characterized by Oberhauser et al. (334),

who found that Cd^{2+} , Sr^{2+} , Mn^{2+} , and Fe^{2+} are able to regulate BK channels in the absence of Ca^{2+} . These observations, together with the finding by Salkoff's group showing that Cd^{2+} can activate wild-type BK as well as Ca^{2+} bowl mutants (389), were assessed again by Zeng et al. (508) taking advantage of the features of the D5N5 mutant as well as the D362A/D367A mutant. Based on the two high-affinity Ca^{2+} -binding sites hypothesis, the D362A/D367A mutant (FIGURE 11C) should be insensitive to Cd^{2+} . In agreement with this proposition, Zeng et al. (508) found that the D362A/D367A mutant can only be activated by Ca^{2+} and Sr^{2+} and that the D5N5 mutant is activated by Ca^{2+} , Cd^{2+} , and Sr^{2+} . To this, we must add the more recent finding that Ba^{2+} is able to activate BK channels by selectively binding to the Ca^{2+} bowl, suggesting that in this binding site only divalent cations of the largest radii can satisfy the interactions required to stabilize the active conformation (517).

Thus the finding that these two sites have a different selectivity to divalent cations gave strong support to the view that BK channels contain two different high-affinity Ca^{2+} sites. However, we should note here that the molecular nature of the RCK1 high-affinity Ca^{2+} binding site is still far from clear, because further mutagenesis studies indicated that mutation E535A in mBK reduces Ca^{2+} sensitivity as much as D367A, which casts doubts on the importance of the M513 residue as a possible Ca^{2+} coordinator (510). The proposal is that mutation M513I reduces Ca^{2+} sensitivity by modifying the structure of the Ca^{2+} binding site in the RCK1 domain. Furthermore, the divalent cation selectivity of this site was also revisited with the findings that, although mutations D367A and E535A (FIGURE 11C) almost eliminate Ca^{2+} sensitivity, they do not abolish Cd^{2+} binding, and although D362A reduces Ca^{2+} only slightly, it greatly reduces Cd^{2+} sensitivity (510). These observations suggest that the Ca^{2+} and Cd^{2+} binding sites in RCK1 are different even though they share some residues.

A careful characterization of the BK channel gating kinetics showed that at $[\text{Ca}^{2+}] < 10 \mu\text{M}$, the Ca^{2+} bowl accelerates channel activation, whereas at higher $[\text{Ca}^{2+}]$, the high-affinity Ca^{2+} binding site contained in RCK1 modifies both activation and deactivation (508). We understand these results in terms of the allosteric model (Scheme IV) in which the open-closed transition is a first-order reaction and where allosteric Ca^{2+} binding controls the forward and backward rate constants. Noting that these assumptions imply that the affinity of the Ca^{2+} sites must increase as the channel opens, the modulation of the activation and deactivation kinetics by different Ca^{2+} sites should indicate that the Ca^{2+} bowl undergoes a change in Ca^{2+} affinity faster than the RCK1 site when the channel opens (69). However, if the opening reaction occurs in two steps as proposed by Contreras et al. (67),

it is possible that the two different Ca^{2+} binding sites are modulating different conformational changes during BK channel activation.

Sweet and Cox (423) determined the calcium bowl and RCK1 dissociation constants from measurements of the open channel probability at various Ca^{2+} concentrations ranging from 10^{-8} to 10^{-3} M at fixed negative potentials (-80 mV), where the voltage sensors are mostly at rest. They observed that the experimental data were not described well by the HA allosteric model (186), using a single calcium dissociation constant for the closed channel, K_D , and a dissociation constant CK_D for the open channel, where C is an allosteric factor (see Eq. 3). Instead, they demonstrate that experimental results are best described by using two different dissociation constants for the closed channel, which is consistent with the presence of two different high-affinity calcium binding constants (423). The dissociation constant used in this curve fitting procedure were 3.7 and 51 μM Ca^{2+} for the closed channel and 0.7 and 21 μM Ca^{2+} for the open channel. These dissociation constants are in the same order of magnitude as the values reported using patch-clamp fluorometry or tryptophan fluorescence intensity decay of the isolated gating ring (207, 312). Using mutations that disabled the high-affinity calcium binding sites one at a time, Sweet and Cox (423) measured the calcium bowl and the RCK1 dissociation constants in separate experiments. They constructed a double mutant (E399N/D367A) to eliminate the RCK1 binding site and multiple mutations D897N/D898N/D899N/D900N/D901N (D5N5) as well as D898A/D900A (D2A2) to disable the calcium bowl. The calcium bowl has a higher affinity: K_D values are 3.1 and 0.88 μM Ca^{2+} for closed and open states, respectively. For the mutation D5N5 of the RCK1, the reported dissociation constants are 26.8 and 5.6 μM Ca^{2+} for the closed and open channel, respectively. For the D2N2 mutation of the RCK1, these dissociation constants were 23.2 and 4.9 μM Ca^{2+} (423). In addition, these researchers reported a negative cooperative interaction between the RCK1 and calcium bowl binding sites (423). Negative cooperativity between Ca^{2+} sensors contained in RCK1 and RCK2 was also inferred from the fit to the $G(V)$ and fluorescence-voltage curves by using an allosteric model that includes two Ca^{2+} binding sites per subunit (385). These last results contrast with those of Qian et al. (361), who found that there is positive cooperativity between Ca^{2+} sensors by using BK channels that contained two RCK1 sites and two Ca^{2+} bowl sites either in the same subunit or in different subunits. The reasons for the differences between these two set of results are not clear, but Sweet and Cox (423) have argued that the Ca^{2+} binding constants reported by Qian et al. (361) are incompatible with their Ca^{2+} dose-response curves taken at -80 and 0 mV.

B. Low-Affinity Ca^{2+} Binding Sites

Although a tendency has been observed of the half voltage (V_b) of the $G(V)$ curve to move toward saturation over the 70 – 100 μM Ca^{2+} range, it has also been observed that as internal Ca^{2+} increases to millimolar concentrations the $G(V)$ curves are again leftward shifted, which is suggestive of a low-affinity Ca^{2+} binding site. This shift is also produced by Mg^{2+} . In fact, millimolar concentrations of Mg^{2+} can shift V_b towards more negative voltages in the absence of Ca^{2+} by decelerating the rate of deactivation (399, 512). Thus these results indicate the presence of a rather unselective low-affinity Ca^{2+} binding site. Slo1-Slo3 tail chimera studies provided evidence that this low-affinity Ca^{2+} binding site is located in the BK channel core (401). Recalling that the Slo3 channel lacks a Ca^{2+} bowl and is not activated by Mg^{2+} , coexpression of the Slo1 core with the Slo3 tail produced channels with the same sensitivity to Mg^{2+} as the wild-type Slo1, suggesting that the low-affinity site resides in the BK channel core. Additionally, site-directed mutagenesis performed in the RCK1 domain showed that mBK mutants, E374A and E399A (FIGURE 11C), completely abolished Mg^{2+} -dependent activation at 110 μM Ca^{2+} without changes in the BK channel Ca^{2+} -dependent activation (401, 482). These two amino acid residues reside at the NH_2 terminus of the RCK1 domain. Confirming the existence of an unselective divalent cation binding site, Zeng et al. (508) showed that the E399A mutant not only abolishes the Mg^{2+} sensitivity of the site, but also the sensitivity to Mn^{2+} , Co^{2+} , and Ni^{2+} . In fact, besides Ca^{2+} and Mg^{2+} , Sr^{2+} , Cd^{2+} , Mn^{2+} , Co^{2+} , and Ni^{2+} are able to activate BK channels by binding to the low-affinity divalent cation site (508). We note that under physiological conditions this low affinity site would normally be occupied by Mg^{2+} .

C. Mg^{2+} -Dependent Activation of BK Channel and the Voltage Sensor

Unexpectedly, the mutation R213Q, known to decrease the voltage sensitivity of BK (274; see sect. VI), also eliminates Mg^{2+} -dependent activation without affecting Ca^{2+} sensitivity (196). Two observations are consistent with electrostatic interaction: 1) the leftward shift of the $G(V)$ curves induced by Mg^{2+} in the absence of Ca^{2+} is decreased by increasing ionic strength; and 2) replacing Q397, a residue that lies close to the Mg^{2+} binding site, by a positively charged residue K or R mimics the Mg^{2+} effect. These results strongly suggested that, upon binding, Mg^{2+} interacts electrostatically with R213 and that the Mg^{2+} site is located in the neighborhood of S4 segment (488). On the other hand, I_g measurements show that the presence of 10 mM Mg^{2+} does not affect the charge movement between closed states ($I_{g\text{closed}}$). However, the amplitude of $I_{g\text{OFF}}$ decreases as the depolarizing voltage pulse duration increases. The most parsimonious explanation of these findings is that Mg^{2+} interacts preferentially with open channels, hindering

voltage sensor deactivation, and thus facilitating channel opening (488). This observation also provides an explanation as to how Mg^{2+} is able to modify BK channel macroscopic current deactivation without affecting channel activation. However, Chen et al. (82), by using single-channel recordings, arrived at the conclusion that Mg^{2+} can also bind to closed channels, as long as some of the voltage sensors are in their active configuration (82). These results are in apparent contradiction with the I_g measurements, but Chen et al. (82) have argued that the effect of Mg^{2+} would not be manifested in $I_{g,closed}$, since Mg^{2+} action requires voltage sensor activation.

Further mutagenesis experiments revealed that the Mg^{2+} binding site is located at the interface between the voltage sensor domain of one subunit, and the RCK1 of an adjacent subunit (489). In addition to the two negatively charged residues in mBK, E374, and E399, Yang et al. (489) found that D99 positioned in the cytosolic long loop connecting S0 with S1 segments, as well as N172 in the S2–S3 loop of a neighboring subunit, also participate in Mg^{2+} binding. Additional functional studies showed that there is an interaction between D99 in the VSD and E374 in RCK1 with the N172 residue spatially close to E399 (492).

D. Coupling Between Ca^{2+} Sensors and Channel Opening

The structural and fluorescence data clearly indicate that Ca^{2+} binding alters the conformation of the gating ring (142, 207, 312, 385, 477, 498–501). The results to date appear to indicate that allosteric coupling between the gating ring and the pore gate is mainly mediated by the linker connecting these two domains. Thus the COOH-terminal domain is connected to the S6 through a linker (C-linker). Niu et al. (333) found that at 0 Ca^{2+} , shortening of the C-linker increases channel activity. In fact, as expected from a C-linker acting as a passive spring, the energy difference between closed and open states increases linearly with linker length. Additionally, increasing linker length decreases Ca^{2+} sensitivity of the channel.

We recall here that the gain-of-function mutation D434G associated with the human syndrome of generalized epilepsy and paroxysmal dyskinesia increases BK channel activity. The D434 residue is part of a 76-amino acid residue NH2 terminus that includes the secondary structures βA - αC (AC region) of RCK1. Molecular modeling indicates that the mutation reduces the flexibility of this region (491). This is evidence that the NH₂ terminus of the RCK1 domain that includes the C-linker mediates allosteric coupling. A reduction in flexibility of the AC region will consequently increase in the spring constant, which should have the same result as a decrease in the C-linker length.

According to Yuan et al. (498), upon Ca^{2+} binding there is a 10 Å expansion of the structure formed by the four RCK1 domains. Larger rearrangements of the gating ring have been proposed using patch-clamp fluorometry. In this case, the modeling of the FRET results suggests that a 30° rotation of each RCK1-RCK2 module brings the fluorophores together by 40 Å and moves the C-linker outwards by 5 Å (312). To these data we must add the experiments done with the isolated gating ring by the group of Olcese (207), indicating that Ca^{2+} promotes conformational changes, which are greatly reduced if the negatively charged residues of the Ca^{2+} bowl are neutralized. Notably, upon Ca^{2+} binding there is a reversible reduction in the hydrodynamic radius of the gating ring, a change consistent with the findings by Miranda et al. (312) indicating that the fluorescent probes at position 667 of the gating ring come closer together by 40 Å. Independent of the actual conformational change of the gating ring induced upon Ca^{2+} binding, its expansion will apply a mechanical force that will pull the C-linker to open the pore gate.

Until recently, different groups have tried and failed to express constructs of BK without the gating ring. In particular, a previous analysis showed that a construct truncated three residues after the S6 was unable to reach the plasma membrane or tetramerize (387). Other constructs, such as one containing the tetramerization domain (362), were able to form tetramers but not functional channels (387). The usefulness of a functional construct lacking the gating ring is evident since it should allow to study the properties of the structures responsible for the voltage dependence and conduction without the allosteric complications introduced by the presence of the gating ring.

BK channels formed only by subunits composed of S0-S6-tetramerization domain can give origin to functional BK channels when the last 74 residues or the motif (11 residues) for efficient processing and surface expression of the Kv1.4 are added to the COOH terminus of this “minimal” subunit (52). Channels in which the gating ring is replaced with 74 or 11 amino acid residues of the Kv1.4 channel are blocked by TEA and IbTx, and the activation kinetics is slowed down when coexpressed with the $\beta 1$ subunit but are insensitive to Ca^{2+} and Mg^{2+} . These results indicate that divalent cation binding is an exclusive property of the gating ring, but block and $\beta 1$ subunit effects are defined by the pore domain and the voltage sensor. The lack of the gating ring induces a rightward shift of the $G(V)$, which has been interpreted as arising from a passive tension to S6 through the RCK1-S6 linker (see above and Ref. 333).

E. Coupling Between Ca^{2+} and Voltage Sensors

Using voltage-clamp fluorometry, Savalli et al. (385) were able to show that a sudden increase in internal Ca^{2+} con-

centration leftward shifted both $G(V)$ and fluorescence-voltage curves. This is a clear indication of coupling between Ca^{2+} and voltage sensors. Notably, this coupling was lost when the Ca^{2+} binding site in RCK2 was eliminated but not in the case of the D362A/D367A mutant that obliterated the RCK1 Ca^{2+} binding site. Besides demonstrating that the two high-affinity Ca^{2+} binding sites are not functionally equivalent, these experiments suggest a strong coupling between the RCK1 Ca^{2+} binding site and the voltage sensor. Binding of Ca^{2+} to this site strongly stabilized the active configuration of the VSD (the allosteric factor that accounts for the coupling between RCK1 Ca^{2+} sensors and voltage sensors, $E1$ is 10, see scheme V). The allosteric coupling between RCK2 Ca^{2+} sensors and voltage sensors is weaker ($E2 = 2.3$). These differences in coupling strength between the two Ca^{2+} sensors and the voltage sensor can be accounted for by the physical nearness between RCK1 and the VSD as they both contribute to Mg^{2+} coordination (see above). Strong coupling between the Ca^{2+} and voltage sensors has also been reported by measuring BK gating currents in the absence and at saturating Ca^{2+} concentrations ($E = 24$, see Eqs. 7 and 8; Ref. 59).

VI. MOLECULAR DETERMINANTS OF VOLTAGE GATING

A. The Voltage Sensor Defined

As discussed in section III, depolarizing voltages are able to activate the BK channel in the absence of Ca^{2+} . This was the first demonstration that the BK channel structure contained an intrinsic voltage sensor (302, 341). Essentially, BK channels, at sufficiently high voltages, can be maximally activated by voltage in the absence of internal Ca^{2+} or the gating ring (52, 72, 77).

On the other hand, the measured number of gating charges per channel obtained using the maximum gating charge (Q) displaced during depolarization over the number of channels (N) (Q/N method) was ~ 4 (417). This was much less than the number obtained for the voltage-dependent Shaker K^+ channel, which is $\sim 12 e_0$ (2, 388, 395). Although BK lacks some important voltage-sensing residues present in Shaker including one in S4, this difference appears to be insufficient to explain the contrast in gating charge movement between these channels. BK has an S4 segment containing three arginine residues that match positively charged residues in the S4 of the Shaker. Moreover, there is no difference between the pattern of arginines in the S4 of BK channels compared with the S4 of the $\text{K}_v2.1$ channel, which has the same number of gating charges as the Shaker channel (205) (FIGURE 12A). Charged residue neutralization or charged reversal strategies showed that potential voltage-sensing residues in mBK are R213 in S4, D153 and R167 in S2, and D186 in S3 (95, 274) (FIGURE 12, B AND C). Thus, in agreement with accessibility experiments indicat-

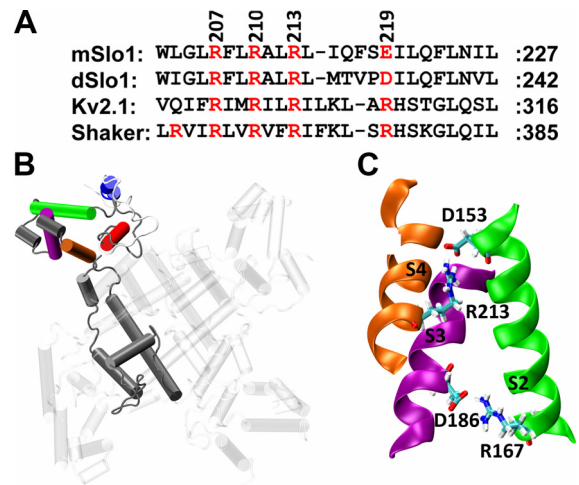


FIGURE 12. The voltage sensor of the BK channel. **A:** alignment of the S4 transmembrane segment of mouse BK channel, *Drosophila* BK channel, Kv2.1, and Shaker. The numbers indicate the charged residues in mouse BK channel. **B:** top view of the transmembrane region of BK channel S0, S1, S2, S3, and S4 are in blue, red, green, violet, and orange, respectively; the linkers and pore of the same subunit are in dark gray; and the rest of the channel is in light gray. **C:** the voltage sensing residues are located in the S2, S3, and S4 transmembrane segments. The positions were determined by Castillo et al. (62).

ing that R210 is modified only from the external solution and that R213 is modified only from the internal solution (196), in BK channels only one charge from the S4, R213, contributes to the gating charge. Considering that, in contrast to K_v channels, only $\sim 50\%$ of the total gating charge is contained in the S4 domain, Pantazis et al. (344) coined the term “decentralized” distribution of voltage-sensing residues. Therefore, the large difference in gating changes between BK and K_v channels may lie in structural differences in their voltage sensors. We recall here that BK channels have an additional transmembrane segment (S0), which has been proposed to modulate the equilibrium between resting and active conformations of the voltage sensors (the parameter J in the allosteric model; Ref. 230; see Eqs. 1 and 5). Thus, as we show below, the voltage sensor domain (VSD) in the BK channel can be structurally defined as the module comprised by transmembrane segments S0 to S4. In the lack of a crystal structure of the BK channel, the position of the S0 is uncertain; however, using the lanthanide resonance energy transfer technique (202), we (62) have been able to construct a spatial map of the extracellular face of the BK channel. In this map (FIGURE 12B), S0 lies between S1 and S2 (cf. Ref. 258).

B. Operation of the Voltage Sensor Viewed Using Fluorescence

Voltage-clamp fluorometry approaches (78, 134, 282) revealed that BK channels undergo voltage-dependent conformational changes in the S3–S4 region of the R207Q

mutant (384). As expected from fluorescence changes associated with changes in the voltage sensor equilibrium, fluorescence-voltage, $F(V)$, curves overlapped with gating charge-voltage relationships, $Q(V)$, and both were leftward shifted compared with the conductance-voltage curve. One interesting observation was made by Savalli et al. (384) who used the fluorescent probe PyMPO covalently linked to cysteine-202 to show that voltage-dependent fluorescent change is only observed in the presence of a tryptophan in position 203. This result indicates that the fluorescence of PyMPO is quenched by W203, most probably in the same subunit. This tryptophan-induced quenching, mediated by photoinduced electron transfer, has been used to detect conformational changes in proteins as well as a caliper to measure distance in macromolecules (101, 102, 283, 284).

By placing the fluorescent probe tetramethylrhodamine maleimide (TMRM) in the NH_2 -terminal of the S2 segment, and neutralizing the gating charge R213 in S4, or by placing TMRM in the NH_2 terminus of S4, and neutralizing the gating charge D153 in S2, it is possible to demonstrate that the conformational changes that occurred during gating by these two transmembrane segments are not independent (344). The large decrease in slope of the $F(V)$ curves (indicative of an impairment of the voltage dependence) of one segment when charge neutralization is performed in the other clearly establishes interactions between the S2 and S4 segments during VSD perturbation. There is no unique mechanistic interpretation of these results since either a mechanical coupling or a focusing of the electric field can equally well explain the results. In the former case, activation of one segment pushes the other, and in the latter, activation of one segment distorts the electric field causing the appearance of an aqueous crevice. The Olcese's group (345) also provided evidence that the residue W203, located at the extracellular end of S4, is able to quench the fluorescence of TMRM positioned at the NH_2 terminal of S0 only at hyperpolarized voltages. This result provides evidence that W203 in S4 moves away from S0 during BK channel activation. Using a similar strategy, Pantazis and Olcese (346) demonstrated that, during activation, S4 also moves away from S1 and S2, and S2 moves closer to S1.

C. Coupling of Voltage Sensor and Gate

How the voltage-driven conformational changes of the VSD couple to the opening the pore is still unclear, but it appears that the transmembrane segment S6 plays an important role in communicating the VSD with the activation gate. It is known that mutations in the S6 greatly modify BK channel gating. Mutations on the human BK F380 residue (256) (see **FIGURES 7 AND 8**) or on its corresponding residue in murine BK-F315 (457), have profound effects on gating and unitary conductance. Mutation F380I in human BK reduced the conductance to ~ 100 pS and made the mean

open times much shorter than wild-type values. Similarly, F380Y also reduced the single-channel conductance but required less Ca^{2+} to activate the channel, compared with the wild-type BK (256, 457). Replacing murine L312 or F315 in S6 corresponding to L377 and F380 in the human BK, respectively, with hydrophilic amino acids of a smaller side chain stabilizes the open state. On the basis of these results, Wu et al. (476) suggested that L312 interacts with F315 of a different subunit in the closed state. Accordingly, opening the pore implies breaking this interaction, and disruption of this interaction would stabilize the open state. In agreement with this hypothesis, substitution of L312 with polar, negatively or positively charged residues produced permanently open channels (84). This constitutive channel activity is not restricted to position 312 since the amino acid substitution of A313 or A316 with polar or charged amino acids also produced permanently open channels. However, Carrasquel-Ursulaez et al. (59) found that the mutation of F380 (see **FIGURES 7 AND 8**) by a small hydrophobic amino acid (A, I, or L) greatly hindered opening without appreciably affecting the workings of the voltage sensor. The use of the HA allosteric model indicated that these results are best explained by assuming that this mutation 1) considerably weakens coupling between the voltage sensor and the calcium binding site as well as between the voltage sensor and the channel gate (allosteric factors E and D), and 2) increases the free energy difference between closed and open states by 19 kJ/mol. In terms of the allosteric model, this energy increase implies a decrease in the equilibrium constant that defines the closed to open transition (L_o) by $\sim 1,700$ times. Note then that in contrast to what is proposed by Wu et al. (476), the disruption of the interaction between F380 and L377 by introducing small hydrophobic amino acids can stabilize the closed state. These results are in agreement with those of Zhou et al. (516), who found that mutating L312 and F315 to cysteine produced a large rightward shift of the $G(V)$ curves, implying a stabilization of the closed state. Moreover, a close inspection of the interaction between the corresponding residue pair in KscA (I100–F103) in the closed and open states in crystal structures revealed that the interaction between these residues is present in both channel structures (76, 105).

VII. BK CHANNELS ALTERNATIVE SPLICING

BK cDNA is the result of splicing 27 constitutive exons in vertebrates, most of which have conserved sequences across the species (128, 271). The S0 transmembrane segment is encoded by exon 1 and the voltage sensor and pore domain, that is, transmembrane segments S1–S6 are generated from exons 2 to 9. The COOH terminus of the BK channel is encoded by exons 9–27, including the RCK1 domain (exons 9–12), S7–S8 segments (exons 14–16), the RCK2 domain and calcium bowl (exons 21–24), as well as the S10 segment (exons 24–25) (**FIGURE 13A**).

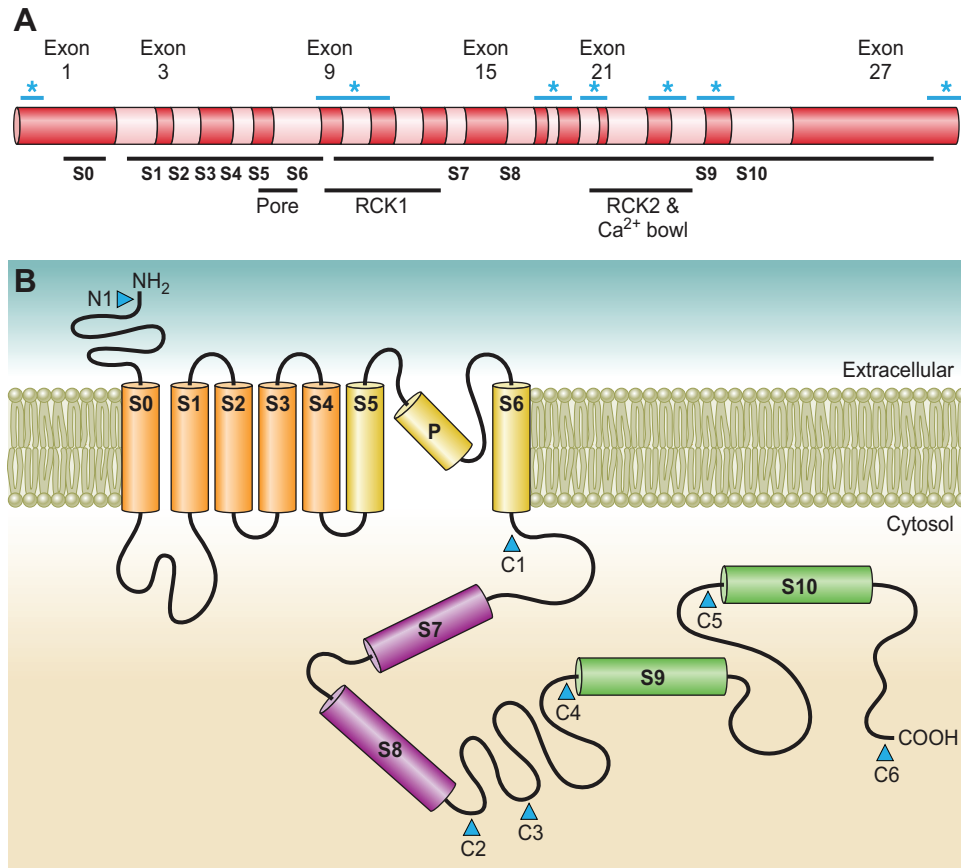


FIGURE 13. Schematic illustration of BK channel exons and sites of splicing variants. **A:** odd (red) and even (pink) exons are represented. The segments of the channel encoded by the different exons are indicated below. The length of each exon is proportional to its size. The diagram also shows the relative positions of the alpha helix segments, S0–S10, with respect to the 28 exons which codify for each of them. The approximate location on the primary structure of the pore region, RCK domains, and calcium bowl are indicated. Light blue asterisks show regions in which alternative splicing occurs. **B:** schematic representation of BK channel topology showing the sites of variable splicing (light-blue arrowheads). BK channels are alternately spliced at six main sites in the COOH terminus. C2 site is subjected to extensive alternation in splicing in different tissues generating among others ZERO and STREX variants. DEC variants are found at site C6. (See also Refs. 99, 128, 271, 311, 402.)

There are highly variable alternative splice regions in BK channels: the first lies at the NH_2 terminus before the starting site of exon 1, a second site is before RCK1 (between exons 9–11), another exists between exons 17 and 19, one is close to RCK2 (between exons 20–21), another is before the calcium bowl (between exons 23–24), and the last is at the COOH terminus immediately after exon 27 (128, 271). As can be envisioned, the existence of alternative splicing and splice variants in sites subjected to many regulatory inputs for channel activity, such as RCK domains in COOH terminus, can generate channels with different biophysical features.

A. Overview of the Splicing Variants

BK channel splicing variants are observed across species; although variants are not necessary of the same type between them, the sites where they occur are conserved (**FIGURE 13**). Unfortunately, there is no consensus about a

nomenclature used for the sites of splicing, which in addition to the number of splicing variants discovered makes it difficult to visualize as part of a common phenomenon. As an example, in *Drosophila* BK cDNA exons 17 and 23 are absent (128). In these insects, two alternative splicing sites located at the COOH terminus of BK channels were identified: the so-called site A locates between exons 18 and 19 and the site B between exons 19 and 20 (13, 128). Three additional alternative splicing sites between residues 327 and 797 in *Drosophila* BK channels have been identified, exhibiting significant differences in the mean open time when expressed in *Xenopus* oocytes (1). On the other hand, a large number of BK channel splice variants have been detected in mouse BK transcripts (56). By aligning the mouse and *Drosophila* BK sequences, the splice site equivalents for A and B of *Drosophila* and several alternative starting sequences before exon 1 at the NH_2 terminus were also identified in mice (56).

Four sites of alternative splicing in the COOH-terminal domain were also found in the human brain BK transcripts, where nine isoforms of BK channels named hbr1 to hbr9 were identified (444). The alternative splicing site 1 is located between exons 16 and 17, site 2 aligns with site A of *Drosophila* and mice, splicing site 3 aligns with site B of *Drosophila* and mice, and site 4 for alternative splicing resides between exons 23 and 24. A fifth alternative splicing site near the COOH terminus of BK channels in humans was identified in pancreatic islet cells and was named site 5 (124, 375).

In mouse *slo1* cDNA, splicing gave rise to nine different variants, showing high variability at the COOH-terminal end (56). Five alternative splicing sites in mouse BK mRNA were identified in adrenal medulla (483). An alternative splicing site near the NH₂ terminus of the protein between S0 and S1 was identified and called N1 (505). Interestingly, a 33-residue insertion at this site produces dominant-negative subunits and retains the channel in intracellular compartments. The splice sites located in the COOH terminus were named C1–C6 (402).

B. Physiological Relevance of BK mRNA Splicing

The biological and evolutionary significance of alternative splicing of BK channel transcripts was previously reviewed by Fodor and Aldrich (128). They examined the genes encoding for BK channel in *C. elegans*, *Drosophila*, chicken, mouse, rat, turtle, and human and concluded that constitutive exons underlying splicing are conserved. The alternatively spliced sequences, however, are not retained across the animal species, although the alternative splicing sites, related to functional domains of the protein, remain concentrated at identical locations (FIGURE 13; Ref. 128).

Alternative splicing of the gene *slo1*, together with metabolic modulation and auxiliary subunits, leads to the expression of BK channels with a wide variety of phenotypes (104, 147, 212, 232, 327, 370). Indeed, alternative splicing within the mammalian BK channel generates different BK channel isoforms (1, 56, 81, 165, 213–215, 238, 327, 370, 444, 463, 483, 505) because this process modifies the characteristics of BK channels, and hence their roles in a number of physiological processes, such as auditory tuning (370), neuronal excitability (444), secretion (483), and others. As it was stated before, most BK channel mRNA splicing occurs by sequence insertion at more than 10 sites within the intracellular COOH-terminal domain of the transcript (357, 402). This event can profoundly modify the functional properties of the protein, such as Ca²⁺ and voltage sensitivity (212, 402), phosphorylation susceptibility (433), membrane expression modulation (3, 5), intracellular trafficking, lipidation, arachidonic acid modulation (403, 435, 504), and signaling pathways

(392, 432, 433). Alternative splicing is also critical in localizing the channel in the plasma membrane or in intracellular organelles (407, 503), thus controlling the presence of the channel in the plasma membrane according to physiological demands (407, 503).

C. Relevant BK Channel Splicing Variants Found in Vertebrates

Although there are at least six splicing sites in the COOH terminus of BK channel, those found in site C2, located within the linker between RCK domains, are the most studied. They can generate at least five isoforms (81) which are briefly described below, and illustrated in FIGURE 13.

1. BK ZERO

The ZERO variant of the BK channel results from splicing exons 19–23 in site C2, leaving out exons 21 and 22 (81). This splice variant is expressed in the brain, kidneys, thymus, stomach, muscle, small intestine, testis, adrenal gland, uterus, prostate, pituitary, breasts, and embryonic tissues. The ZERO variant constitutes almost 90% of total BK mRNA levels in the adult brain (81) and is activated by PKA in excised inside-out patches and in the presence of intracellular cAMP. The activity of this channel is eliminated in the presence of a specific PKA inhibitor or in the absence of ATP (81). Additionally, in the presence of the $\beta 4$ subunit and at low [Ca²⁺], ZERO BK channel activity is reduced and activation slowed down. At high [Ca²⁺], however, $\beta 4$ shifts V_h towards negative potentials and deaccelerates the deactivation time constant (21, 48, 351, 459). Interestingly, the $\beta 4$ auxiliary subunit also makes this BK channel isoform sensitive to cAMP-dependent phosphorylation (351).

2. BK e21 (STREX)

The e21 exon (also known as STREX) is the most widely studied BK channel splice variant. This variant arises from an insert of 58 amino acids in splice site C2 (FIGURE 13) resulting from the inclusion of exon 21 between exons 19 and 23 (81). Its name derives from its splicing regulation by stress-axis hormones (483) that change the capability of the channel to respond to calcium, oxidation, and phosphorylation (114, 351). The e21 (STREX) insert is highly conserved across vertebrates, suggesting common functional roles (232, 270). STREX is predominantly expressed in endocrine tissues such as the adrenal gland, pituitary, prostate, breast, and vascular tissues (81, 351, 357), but it is also expressed in the brain, kidneys, stomach, muscle, intestines, lungs, and testis. During central nervous system development, BK splicing switches from the STREX phenotype to the ZERO phenotype. The inclusion of e21 (STREX) results in a large leftward shift in the conductance voltage curve with a V_h at 1 μ M Ca²⁺ of 11.2 ± 5.4 mV, compared with

ZERO which shows a V_h of 71.2 ± 6.8 mV when expressed in HEK 293T cells (81). STREX channels show considerable slower deactivation kinetics compared with ZERO. Supporting the latter, the presence of STREX increases apparent Ca^{2+} sensitivity of BK channels in rat adrenal chromaffin cells and PC12 cells compared with channels without STREX exon (167, 375). Unlike ZERO, the STREX variant shows inhibition by PKA, but like ZERO, the effect of cAMP is eliminated in the presence of a PKA inhibitor or in the absence of ATP (81). In addition, $\beta 4$ slows down the deactivation time in STREX at low calcium concentrations and makes it sensitive to dephosphorylation (351). The $\beta 4$ subunit makes STREX BK channels significantly more sensitive to dephosphorylation, in agreement with the inhibition of STREX BK channels by PKA activation (433).

3. BK e22

The e22 variant, also known as hbr5, is the result of a 29-amino acid insert at site C2, resulting from the inclusion of exon 22 between exons 19 and 23 (81, 99, 377). The e22 splice variant mRNA is expressed at high levels at embryonic stages in the mouse (81), but it is also found in different adult tissues, such as breasts, prostate, uterus, skin, small intestine, muscle, liver, and spleen (42, 56, 81, 291). This BK isoform displays increased Ca^{2+} and voltage sensitivity compared with ZERO (81). In spite of this, the response to PKA phosphorylation is similar to that of ZERO channels (432, 433). Compared with ZERO, both e22 and STREX have significantly slower deactivation kinetics (81).

4. BK $\Delta e23$

The splice variant $\Delta e23$ results from splicing exons 19 to 24, skipping exon 23, and introducing a stop codon on exon 24 (81). The truncated protein $\Delta e23$ is deficient in trafficking to the cell surface and acts as a dominant negative inhibitor of cell surface expression of other splice variants. The intracellular permanence of the $\Delta e23$ splice variant has been attributed to the lack of endoplasmic reticulum exit signals present in full-length BK channels (236), although it seems that $\Delta e23$ can assemble as tetramers (81). In humans, insertion of an alternatively spliced exon at site N1 also generates dominant negative BK channels with defective trafficking to the cell surface (503). In tissues with low total levels of BK channel mRNA expression, the predominant splice BK variant mRNA is $\Delta e23$ (81), suggesting that plasma membrane channel expression is suppressed in those tissues. This variant is expressed in the mouse myometrium, where total BK channel protein expression increases towards the end of pregnancy. The BK channel-induced K^+ current, however, decreases concomitantly with a decreased cell surface location, and an increased endoplasmic reticulum localization (22, 109).

5. BK hbr

The human brain BK variant, hbr, arises from alternative splicing at several sites that can be occupied by inserts of 0 or more amino acid residues (444). For site 1, located between exons 16 and 17, there are three variants with 0, 4, and 40 residue insertions. For site 2, located between exons 18 and 19, there are four different inserts that are 0, 3, 29, and 32 residues long. For site 3, located at the end of exon 19, two variants with 0 and 5 residues can be found. For site C4, located between exons 23 and 24, there are two variants with 0 and 27 amino acid residue insertions (128, 444). There are 48 possible isoforms generated by all the combinations of alternative splicing. However, only nine isoforms were found by Tseng-Crank et al. (444) and named hbr1-hbr9. The hbr5 variant is the most Ca^{2+} -sensitive splice variant and has four positively charged amino acids inserted in site C1, which is thought to affect the interaction with Ca^{2+} -binding sites conferring high Ca^{2+} sensitivity.

6. gBK

The gBK is a splice variant frequently expressed in glioma cells. It contains a unique 34-amino acid exon at splicing site C2 (FIGURE 13) and is 97% identical to hbr5 (264). gBK possesses all the pharmacological and biophysical properties of the native BK currents identified in glioma cells (50, 367), and when comparing gBK to hbr5, the former shows enhanced Ca^{2+} sensitivity (264), maintaining the unitary conductance of the channel (264). The V_h of glioma BK channel activation at $2.1 \mu\text{M}$ internal Ca^{2+} is -14 mV. This is far more negative than the V_h of BK channels in human smooth and skeletal muscles (201, 248), human macrophages (132), and hbr3 (444), which all require free Ca^{2+} concentrations of $10 \mu\text{M}$ to obtain negative V_h values in the G/V curve (366). Even compared with its nearest relative, the hbr5 variant, glioma BK channels have an unusually high Ca^{2+} sensitivity (366), which implies that gBK becomes active at resting potential and at low Ca^{2+} concentrations. This capability correlates with the Ca^{2+} -dependent process driving tumorigenesis in glioma and other types of cancer (51, 229, 415).

7. mitoBK

In adult cardiomyocytes, BK channels are found expressed in mitochondria but not in the plasma membrane (reviewed in Ref. 14). The mitochondrial BK channel is encoded by the *KCNMA1* gene, similar to the plasma membrane BK, and was identified as mitoBK (406). The mitoBK was first found to be related to cardioprotection from global ischemia and reperfusion injury (484). To generate mitoBK channels, *KCNMA1* is spliced to generate the DEC isoform (named because of its last three amino acids) (406). The DEC splice variant results in an insertion of 50 amino acids at the COOH-terminal end, which is required for mito-

chondrial targeting and the prevention of BK channel expression in the plasma membrane (406). The ability of the DEC exon to target mitoBK of mitochondria could vary depending on the cell type or associations with other subunits. In fact, the expression in CHO cells of a mitoBK variant cloned from hair cells showed prominent expression in mitochondria, but also at the cell periphery (218). In contrast, expression of a DEC variant in COS cells is confined to the endoplasmic reticulum (273). In the mouse cochlea, the DEC variant can be found at high levels after birth, decreasing according to hearing maturation (376). Thus the DEC sequence may not be the only factor defining the targeting of BK DEC to mitochondria, and cell-specific mechanisms could play a role. The mitoBK channel has been found to be expressed in the inner mitochondrial membrane of a large variety of different adult cells (20, 86, 87, 118, 119, 163, 335, 404, 409, 484). This splice variant exhibits different voltage and calcium sensitivity, compared with plasma membrane BK channels in glioma cells, reaching an NP_o of ~ 0.6 at -40 mV in the presence of $400 \mu\text{M}$ Ca^{2+} (162). It has been reported that the cardioprotective effect of ischemic preconditioning could be related to a mitoBK-mediated decrease of reactive oxygen species (ROS) production, because the production of ROS after anoxia is higher in the *KCNMA1* KO animal (414).

In addition, BK is expressed in the nuclear envelopment of hippocampal neurons, linking neuronal activities, nuclear Ca^{2+} signaling, dendritic arborization, and gene expression (249). Although it was not confirmed to be a novel splice

variant, the nuclear BK channel exhibits similar voltage and Ca^{2+} dependence, as well as single-channel conductance and pharmacological properties to plasma membrane BK channels. Animals deficient in BK expression confirmed the importance of nuclear BK channel activity in regulating gene expression (249).

VIII. MODULATORY β SUBUNITS

β Subunits generate BK channel diversity by modifying BK gating and pharmacology. In addition to alternative splicing, BK channel activity and modulation are profoundly affected by the presence of auxiliary proteins such as β subunits. Until now, four auxiliary β subunits, encoded by four different genes, have been cloned and characterized. These two-spanning membrane proteins exhibit the same predicted structure with two transmembrane segments, linked by an extracellular loop with COOH and NH_2 termini facing the intracellular side of the membrane (FIGURE 14). All of them contribute to generating BK channel diversity by modifying gating properties and pharmacological features.

A. $\beta 1$ Subunit

The $\beta 1$ subunit coded by the gene *Kcnmb1* (the first BK subunit cloned) was discovered by isolating and reconstituting BK channels from tracheal or aortic smooth muscle

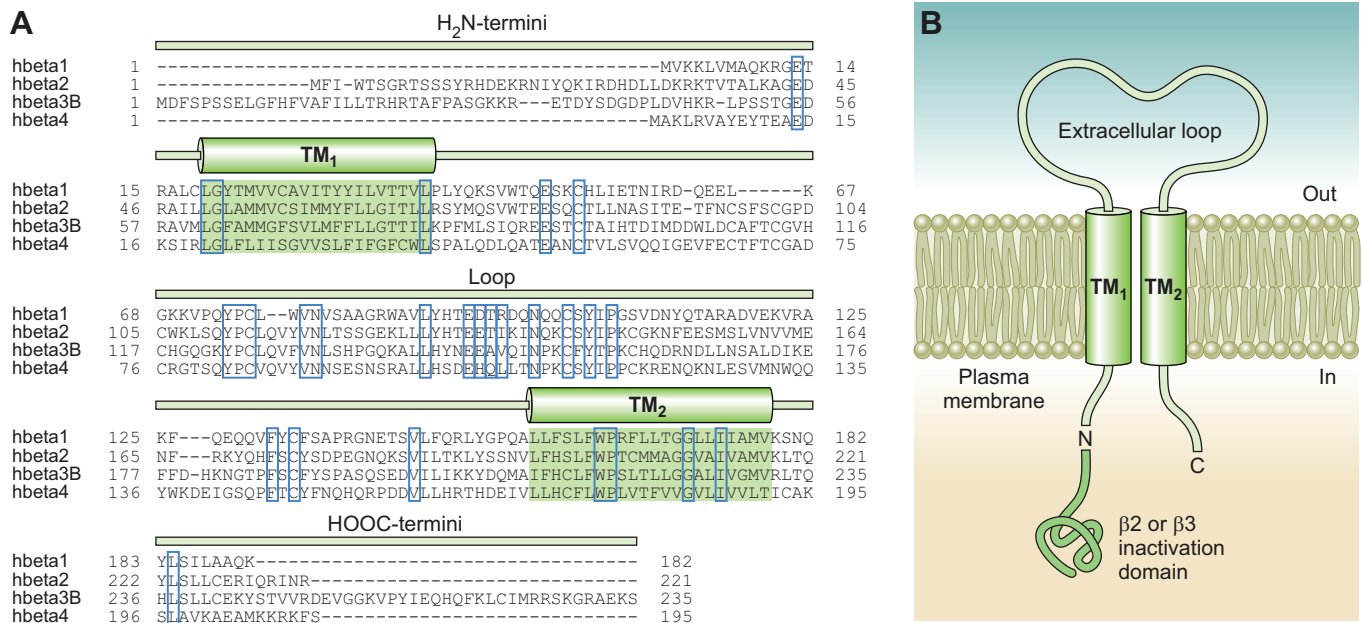


FIGURE 14. Topology of auxiliary β subunits. **A:** alignment of the primary structure of four known modulatory β subunits, highlighting the main domains of their predicted structure, intracellular COOH and NH_2 termini, transmembrane domains (TM1 and TM2), and the extracellular loop. Conserved amino acids are highlighted in light blue boxes. **B:** topological model shared by all β subunits: NH_2 and COOH terminus facing intracellular side, two transmembrane domains linked by an extensive extracellular loop. At the NH_2 terminus, $\beta 2$ and $\beta 3$ subunit contain additional amino acids that constitute the particle of inactivation.

(135, 226, 227). They identified a highly glycosylated polypeptide with a molecular weight of 31 kDa that coimmunoprecipitated with the BK α subunit. This proved to be a membrane protein containing two transmembrane segments connected by a long external loop, leaving NH₂ and COOH termini pointing to the cytoplasmic side [afterward named β 1 encoded by the *kcnmb1* gene (226)]. The secondary structure is shared by all BK β subunits (**FIGURE 14B**; reviewed in Refs. 188, 339, 439, 440). Heterologous expression of α and β 1 subunits in *Xenopus* oocytes showed that the β 1 subunit was able to increase the apparent Ca^{2+} sensitivity dramatically and conferred a new pharmacological profile to the BK channel (295). It was also noticed that differences in Ca^{2+} sensitivity between α BK and ($\alpha + \beta$ 1)BK channels started appearing at concentrations >300 nM of internal Ca^{2+} , and at lower Ca^{2+} concentrations the channel becomes Ca^{2+} insensitive without modifications of its voltage dependence (302). However, at [Ca^{2+}] <300 nM, the *G(V)* curves of ($\alpha + \beta$ 1)BK channels are consistently rightward shifted compared with α BK by ~ 50 mV (338). β 1 also slows down both activation and deactivation kinetics (48, 107). BK channels formed by α and β 1 subunits become sensitive to dehydrosoyasaponin, which is an active component of an herb known to be a potent agonist of BK channels (295). Coexpression of bovine β 1 with the α subunit increases ChTx potency by ~ 50 -fold, and in the case of h β 1 (human) the toxin dissociation rate constant is greatly slowed down without much change in the dissociation constant (107, 168, 303). Binding of other toxins is also altered by the presence of β 1. For example, in ($\alpha + \beta$ 1)BK channels the binding of slotoxin, which is a toxin isolated from the venom of *Centruroides noxious*, becomes irreversible (136). Altogether, these observations make it clear that this modulatory subunit generates channel diversity by dramatically modifying channel gating and channel pharmacology.

The binding of several other compounds of physiological or pharmacological importance also requires the presence of the β 1 subunit (439). Steroids such as 17 β -estradiol, tamoxifen, and lithocholic acid are compounds able to activate ($\alpha + \beta$ 1)BK but not α BK channels (54, 55, 91, 96, 97, 447). In particular, since 17 β -estradiol acutely restores impaired coronary flow in postmenopausal women (145), it is tempting to suggest that the targets of this hormone are the ($\alpha + \beta$ 1)BK channels that are profusely expressed in arterial smooth muscles.

In addition, β 1 modulates alcohol sensitivity by decreasing the capacity of ethanol to activate BK channels (53, 103, 121, 439). Phosphatidylinositol 4,5-bisphosphate (PIP₂) is a special case because it can behave as an inhibitor when BK channels are formed by the α subunit alone and as an agonist in the presence of the β 1 subunit (434). The mechanism(s) that mediate this and other cases of pharmacological modulation induced by the β 1 subunit will be discussed when describing the structural determinants responsible for β 1 modulation in the

BK channel. As discussed below, binding of some of these compounds is in some cases not specific to the β 1 subunit, and its effects are also seen in the presence of other β subunits.

B. β 2 Subunit

In chromaffin cells of the adrenal gland in pancreatic β -cell lines and in hippocampal neurons, it is possible to observe inactivating BK currents (175, 176, 254, 412). In particular, in culture rat chromaffin cells, inactivating (BK_i) and non-inactivating BK currents were detected, and repetitive firing was greatly enhanced in cells expressing BK_i (413). Inactivation on BK_i channels is conferred by the β 2 subunit encoded by the *kcnmb2* gene, and arises from its NH₂-terminal since deletion of the first 19 or 33 amino acid residues of this subunit completely eliminate inactivation (456, 480). More recently, the electrical properties of chromaffin cells have been characterized in wild-type and β 2 knockout adrenal medullary slices. As expected from previous findings in chromaffin cells, β 2 deficiency eliminates inactivation and decreases the rate of action potential firing (287). As in the case of Shaker K⁺ channels (192) and sodium channels (11), the inactivation process is removed by trypsin application to the internal side of the membrane, strongly suggesting the presence of an inactivating particle or gate (100, 412). Further demonstration of this inactivation mechanism comes from experiments in which β 1 was transformed into an inactivating subunit by fusing the first 19 residues of β 2 to its NH₂ terminal (456). It is remarkable to observe that inactivation is completely eliminated when the first three hydrophobic amino acids (FIW) from the NH₂ terminal are deleted, suggesting hydrophobic interactions with residues in the wall of the channel's internal vestibule (479). In addition, the observations that short, 16-amino acid residue NH₂-terminal peptides are able to induce inactivation and that inactivation cannot be removed by trypsin when channels are inactivated suggest that the inactivating peptides reach the inactivating site through side portals, just like in K_v channels (513). The structure of the isolated NH₂ terminal (first 34 amino acids) of the β 2 subunit was determined by using nuclear magnetic resonance. The peptide consists of a flexible NH₂ terminal (first 18 residues), the "ball" and an α -helical domain that connects the inactivating gate to TM1 (23).

Like β 1, β 2 also increases the apparent Ca^{2+} sensitivity of the BK channel and slows down gating kinetics (48, 456, 480). β 2 also reduces sensitivity to toxins, and in the case of ChTx β 2 increases the dissociation constant by about 10-fold (480). It has also been found that in ($\alpha + \beta$ 2)BK channels, arachidonic acid promotes a pronounced potentiation in the current and a slowdown in inactivation, apparently by directly interacting with the β 2 inactivation gate (422). Interestingly, the steroid hormone dehydroepiandrosterone (DHEA, a stress-related adrenal androgen), but not 17 β -estradiol, is able to activate the ($\alpha + \beta$ 2)BK channel (223).

C. $\beta 3$ Subunit

Alternative splicing of the gene coding for the $\beta 3$ subunit (encoded by the *kcnmb3* gene) generates four distinct $\beta 3$ ($\beta 3$ a-d) isoforms (21, 48, 446, 481). Although these subunits do not produce appreciable modifications in the BK channel Ca^{2+} sensitivity, three of them ($\beta 3a$, $\beta 3b$, and $\beta 3c$) are able to induce a rapid, albeit incomplete, inactivation process. Like $\beta 2$, $\beta 3b$ inactivation is mediated by the NH_2 terminal, and in channels formed by $\alpha + \beta 3b$ lacking the NH_2 terminal, the instantaneous current-voltage relationships show a marked outward rectification (481, 507). This instantaneous rectification is due to the particular structure of the $\beta 3$ external loop since reduction of its disulfide bridges with dithiothreitol irreversibly abolishes rectification (509). The data suggest that the inactivation process in ($\alpha + \beta 3b$)BK channels proceeds through a mechanism that involves a pre-inactivated open state (152, 255). Moreover, binding to the channel is stereospecific, since inactivating peptides composed of L-form amino acids, but not polypeptides composed entirely of D-amino acids, are able to bind to the pre-inactivated open state (152). It is important to note here that the inactivation process appears to be species dependent, because the mouse $\beta 3b$ does not promote BK current inactivation (506). At the single-channel level, ($\alpha + \beta 3b$)BK channels show very brief openings that cannot fully be resolved by conventional techniques (481).

D. $\beta 4$ Subunit

Highly expressed in the brain, $\beta 4$ encoded by the *kcnmb4* gene slows down the activation and deactivation kinetics of the BK channel (21, 48, 466). ($\alpha + \beta 4$)BK channels show a complex Ca^{2+} sensitivity. Indeed, at low internal Ca^{2+} concentrations ($<7 \mu\text{M}$), channels are less sensitive to Ca^{2+} than αBK channels, but at higher $[\text{Ca}^{2+}]_i$, ($\alpha + \beta 4$)BK channel activity is enhanced (48, 164, 459). It is important to note here that all the experiments reported above were conducted in the presence of symmetrical K^+ . When the external K^+ is replaced by Na^+ , $\beta 4$ induces a leftward shift of the $G(V)$ relationship at all Ca^{2+} concentrations (206).

The $\beta 4$ subunit makes the BK channel resistant to ChTx and IbTx by dramatically decreasing the toxin association rate constants (303). However, IbTx and ChTx blockade is essentially irreversible once the toxins bind. The $\beta 4$ domain responsible for toxin insensitivity is the external loop as swapping loops between $\beta 4$ and $\beta 1$ increases both the ChTx association and dissociation rate constants. Electrostatic interactions between positively charged residues contained in the $\beta 4$ loop and negative residues in the large BK turret have been proposed to help the formation of a “helmet” that hinders the toxin entrance to the channel’s external vestibule (133). Thus the toxin insensitivity showed by the slow-gated type of BK channels present in neurons (368) is conferred by the $\beta 4$ subunit.

Heteromeric ($\alpha + \beta 4$)BK channels, as is the case of ($\alpha + \beta 1$)BK channels, are activated by 17β -estradiol and by PIP_2 (21, 434). On the other hand, as the presence of $\beta 1$ determines an inhibitory effect of ethanol on BK channels (53), $\beta 4$ potentiates the response to ethanol (121, 441). The consequence of these observations is that the acute tolerance to ethanol of BK channels is induced in the absence of the $\beta 4$ subunits (286). Apparently, the high sensitivity to ethanol of ($\alpha + \beta 4$)BK channels is the result of a strong $\beta 4$ -dependent modulation of the effect of kinases, such as PKA and CaMKII, and phosphatases on ($\alpha + \beta 4$)BK channels (450). Martin et al. (286) pointed out that $\beta 4$ is crucial in the control of alcohol tolerance from the molecular to the behavioral levels, which is why it could be involved in individual differences in alcohol abuse and may represent a therapeutic target for alcoholism.

E. Structural Determinants of Functional Coupling Between α and β Subunits

Cox and Aldrich (70) and Nimigean and Magleby (332) were the first to provide strong evidence that the $\beta 1$ subunit-dependent increase in apparent Ca^{2+} sensitivity was not due to an increase in the true affinity of the BK channel for Ca^{2+} . Nimigean and Magleby (332) showed that the $\beta 1$ subunit was able to increase burst duration even in the absence of Ca^{2+} . Cox and Aldrich (70), on the other hand, found that although a small change in ($\alpha + \beta 1$)BK Ca^{2+} affinity was necessary to explain the results, the main effects of the $\beta 1$ subunit are on channel voltage sensitivity (see also Ref. 338). This was directly confirmed by comparing the gating charge versus voltage [$Q(V)$] curves obtained in αBK and in ($\alpha + \beta 1$)BK channels, which showed that $\beta 1$ induces a 70-mV shift to the left along the voltage axis of the curve (FIGURE 15). This stabilizes the voltage sensor both when the channel is open and when it is closed (16, 67). In addition, $\beta 2$ is also able to stabilize the voltage sensor in the active configuration, albeit to a lesser extent (67, 383, 384). In agreement with results demonstrating a lack of $\beta 3$ effects on the BK channel’s apparent Ca^{2+} sensitivity, I_g measurements showed that the characteristics of the $Q(V)$ curve of ($\alpha + \beta 3$)BK channels (FIGURE 15) are very similar to those of αBK channels (67). Although a fitting of the macroscopic current data to the HA allosteric model suggested the stabilization of the voltage sensor in its active configuration mediated by the $\beta 4$ subunit (459), I_g measurements showed that $\beta 4$ changes the slope of the $Q(V)$ curve without changes in the position of the curve in the voltage axis (FIGURE 15). In fact, the $Q(V)$ curve for ($\alpha + \beta 4$)BK is rightward shifted by 17 mV with respect to αBK . This slope modification amounts to a decrease in the number of apparent gating charges per voltage sensor from $0.63 e_0$ to $0.48 e_0$, and this decrease is enough to explain the complex BK channel Ca^{2+} sensitivity when α subunits are coexpressed with $\beta 4$ subunits (67).

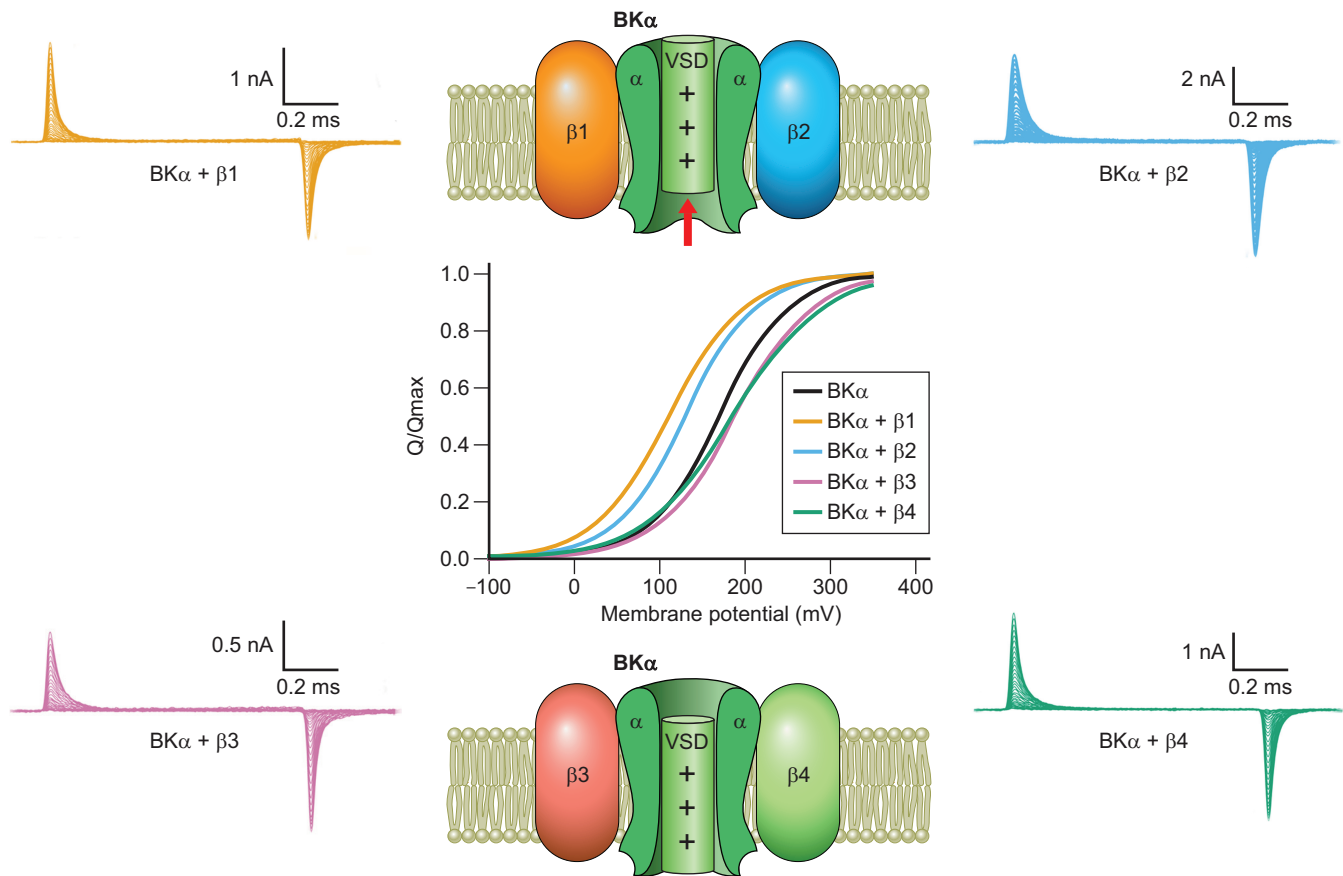


FIGURE 15. BK channel β subunits differentially modulate the voltage sensor domain of the channel. Gating currents (I_g) recordings elicited by 1-ms pulse duration to increasing voltages from -90 to 350 mV in increments of 10 mV are shown for BK α / $\beta 1$ (orange), BK α / $\beta 2$ (light-blue), BK α / $\beta 3$ (pink), and BK α / $\beta 4$ (green). The cartoon illustrates the activation of the voltage sensor domain (VSD) by $\beta 1$ and $\beta 2$ subunits (upper), and the null effect on VSD equilibrium of $\beta 3$ and $\beta 4$ (lower). The central plot shows the charge vs. voltage relationship for BK α alone and coexpressed by $\beta 1$ - $\beta 4$ subunits as indicated.

Furthermore, Bao and Cox (16) found that to fully account for the effects of $\beta 1$ on apparent Ca^{2+} sensitivity, it was also necessary to consider a decrease in the true Ca^{2+} affinity of the closed channel. The Ca^{2+} dissociation constant (K_C) of the closed ($\alpha + \beta 1$)BK channel was found to increase $\sim 30\%$ compared with that of the (α)BK channel. Although this change in K_C seems to be rather small, it actually produces a large leftward shift in the conductance-voltage [$G(V)$] curve. This is due to the fact that the equilibrium constant between open and closed states depends on the eighth power of the K_C/K_O ratio, where K_O is the Ca^{2+} dissociation constant of the open state (16). In the HA allosteric model (186), the K_C/K_O ratio is equal to the allosteric factor C that couples the binding of Ca^{2+} to the pore opening (see sect. III). A more detailed study considered mutagenesis to produce successive eliminations of the high Ca^{2+} selectivity sites contained in RCK1 (D367A; Ref. 482) and RCK2 [the calcium bowl (D898A/D900A); Ref. 17]. In the presence of the Ca^{2+} sites contained only in RCK1, the $\beta 1$ subunit promotes a decrease in the Ca^{2+} dissociation constant of the open channels. When the RCK1 Ca^{2+} sites are disrupted, $\beta 1$ induces an increase of the Ca^{2+} dissociation constant of the closed channels (424). These results

indicate that Ca^{2+} binding at either site has a larger impact on channel opening when the $\beta 1$ subunit is present.

Regarding the effects of $\beta 1$ on Ca^{2+} sensitivity, there are still some issues that remain to be solved. First, neutralization of the charges involved in BK voltage sensing eliminates the effect of the $\beta 1$ subunit even at saturating Ca^{2+} concentrations. This suggests that this subunit affects channel gating by mainly modifying voltage sensor conformation (490). To make these results consistent with those of Sweet and Cox (424), it is necessary to assume a high degree of coupling between voltage and Ca^{2+} sensors. In this regard, the situation is also confusing. Sweet and Cox (424) argue that there is no evidence for a large degree of coupling between both sensors since although the binding of Ca^{2+} to the RCK1 Ca^{2+} binding site is voltage dependent, binding of Ca^{2+} to the calcium bowl is not. On the other hand, the degree of coupling between voltage sensor activation and Ca^{2+} binding (allosteric factor E ; discussed in sect. III on kinetic models) is also under debate since the values vary between 2 (small coupling) to 20 (large coupling) (cf. Refs. 59, 186). Second, Qian and co-workers (359, 360) found that the presence of $\beta 1$ increased burst duration in BK chan-

nels containing a mutation in the Ca^{2+} bowl (D965N) that disrupted Ca^{2+} binding to this site. They also found that despite the fact that it was possible to recover the full effect of $\beta 1$ in BK channels formed by coexpressing the Slo1 core and tail, the modulatory effect of $\beta 1$ was lost in channels formed by coexpressing the Slo1 core and Slo3 tail. Third, the $\beta 1$ modulatory effect can still be observed in a triple mutant, in which Ca^{2+} sensitivity of the channel has been eliminated by introducing mutations in the Ca^{2+} bowl and in the RCK1 high-affinity Ca^{2+} binding site (359). These results indicate that the Ca^{2+} binding sites are not necessary for $\beta 1$ to promote an increase in apparent Ca^{2+} sensitivity through a lengthening of the burst duration and an increase in P_o . However, due to structural reasons, it is possible that the Slo1 tail, even with a nonfunctional Ca^{2+} bowl, is a requirement for proper $\beta 1$ BK modulation. These results are in clear disagreement with those of Sweet and Cox (424) who were unable to find a restoration effect of Ca^{2+} sensing by $\beta 1$ in a mutant that had both high-affinity Ca^{2+} binding sites obliterated. The reasons for this disagreement are at present unknown.

The stoichiometry of α and β subunits is in general assumed to be 1:1, but the expression of these two subunits in oocytes suggests that functional BK channels can arise with less than four β subunits (462). The leftward shift produced by increasing the $\beta 2$: α ratio is incremental, and in this case the number of $\beta 2$ subunits per channel also modifies steady-state inactivation and recovery from inactivation. This last result indicates that the process of inactivation mediated by the $\beta 2$ subunit is different from the inactivation process that takes place in K_v channels where it has been shown that the rate of recovery from inactivation is independent of the number of inactivation gates (276). Moreover, disulfide cross-linking indicates that all β subunits are located in between VSDs (S0–S4) and that their transmembrane (TM) segments interact with two adjacent VSDs. TM1 is located in the vicinity of S1 and S2, whereas TM2 is near S0 of the contiguous VSDs (258, 259, 473). Although these studies are a good indication of the proximity between transmembrane segments, they do not provide a clue about how strong the interactions between TMs are. Morera et al. (317) showed that there is a direct physical interaction between TM1 of $\beta 2$ and S1 of the α subunit by using the TOXCAT assay, a method used to detect the degree of transmembrane helix association (374). On the other hand, no binding of TM2, the external loop, or the COOH terminal of $\beta 2$ to any of the transmembrane segments of the α subunit was found. In this particular case, it is clear that there is noncovalent binding between TM1 and S1, but despite the nearness between TM2 and S0, there is no strong interaction between these two protein structures. Although at present there is no evidence whether the other β subunits show the same interactions with the α subunit as the $\beta 2$ subunit does, it is tempting to suggest that given that all β subunits are located in the same position with respect

to the α subunit, $\beta 1$, $\beta 3$, and $\beta 4$ are enabled to interact with the α subunit in the same manner.

Functional coupling between the α and $\beta 1$ subunits requires the S0 transmembrane segment and the external NH_2 terminal of the BK channel, since exchanges of these two segments between the *Drosophila* (dBK) and the human BK renders the latter insensitive to the presence of $\beta 1$ (455). The reverse chimera, the dBK channel containing the S0 transmembrane segment and the external NH_2 terminal of the hBK is fully modulated by $\beta 1$. Functional coupling between $\beta 1$ and the α subunit is also dramatically modified when the $\beta 1$ cytoplasmic regions are deleted. In this case, although the differences in the V_b at low Ca^{2+} concentrations ($<1 \mu\text{M}$) between the two subunits are maintained, the increase in apparent Ca^{2+} sensitivity promoted by the $\beta 1$ subunit at high Ca^{2+} concentrations is utterly lost (457). By exchanging different domains between the $\beta 1$ and $\beta 2$ subunits, Orio et al. (340) found that all that is required to recover the phenotype imparted to the BK channel by the parent subunit is the β subunit intracellular NH_2 and COOH terminals. In other words, a $\beta 2$ subunit containing the intracellular regions of the $\beta 1$ subunit gives rise to channels with a ($\alpha + \beta 1$)BK phenotype. More recently, by measuring I_g induced by channels formed by α , and chimeras between the $\beta 1$ and $\beta 3$ or $\beta 1$ and $\beta 2$ subunits, it has been possible to conclude that the structural determinants in the modulation of the BK voltage sensor induced by the $\beta 1$ subunits are in the NH_2 terminal. The residues responsible for this effect are two lysines (K3 and K4) contained in this $\beta 1$ subunit intracellular region (63). However, amino acid residues in the $\beta 1$ subunit, which reside in regions of the $\beta 1$ subunit external loop when mutated to alanine, promote appreciable changes in BK channel gating, suggesting that this $\beta 1$ structure may also interact with the external aspect of the voltage sensor (160).

IX. MODULATORY γ SUBUNITS

More recently described γ subunits also have deep effects on BK channel diversity and activity by modulating the voltage and calcium sensitivity of the α subunit. Four auxiliary γ subunits have been found to be encoded by four different genes (FIGURE 16A). These have one transmembrane segment with a huge extracellular domain formed by leucine-rich repeats (FIGURE 16B) and a cytoplasmic-facing shorter domain.

The presence and function of BK channels in nonexcitable cells was not well understood since these channels need a raise in internal $[\text{Ca}^{2+}]$ to be activated. The discovery of γ subunits, which displace to the left the BK activation curve, provided a plausible answer to the questions regarding the molecular basis of function of BK channels in nonexcitable cells (487).

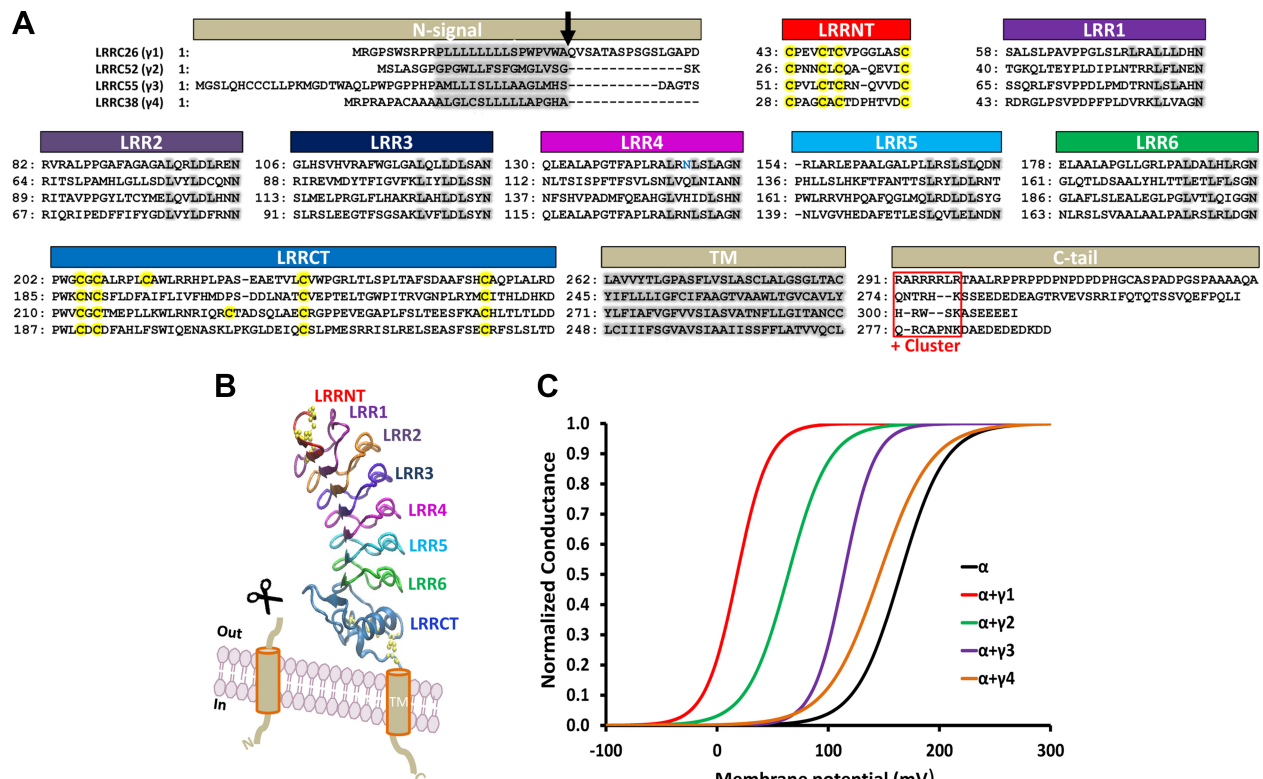


FIGURE 16. Structure of the accessory γ subunits. **A:** alignment of the primary structure of four known modulatory γ subunits. The main structures are highlighted using color rectangles on top of the primary structures. The predicted cleavage site is indicated with an arrow. **B:** general topological structure shared by all γ subunits: after cleavage, the protein contains only one transmembrane segment and the NH_2 terminal and the LRRCT domains are facing the extracellular side. LRRCT domain homology model was built using the crystal structure of the LRR domain of hagfish variable lymphocyte receptor B59 (PDB ID: 206S) (221) using the Visual Molecular Dynamics, version 1.9.2 (200). **C:** simulated normalized conductance-voltage curves from different γ subunits. Half-voltage activation and apparent activation charge were obtained from Yan and Aldrich (485).

These subunits are part of a group of leucine-rich repeat (LRR)-containing membrane proteins of around 35 kDa. They are dubbed γ_1 (LRRC26), γ_2 (LRRC52), γ_3 (LRRC55), and γ_4 (LRRC38) and have been enumerated according to their capability to promote a leftward shift of the BK channel conductance-voltage curve (FIGURE 16C; Ref. 485). γ Subunits are structurally unrelated to the previously known β subunits both in protein sequence and secondary structure, representing a new type of BK auxiliary subunit. The amino acid similarity between γ subunits is 30–40%. The predicted structure for the γ subunits consists of a single-span membrane protein with extracellular localization of its large NH_2 -terminal LRR domain, a single transmembrane domain, and a short intracellular COOH terminus (485) (FIGURE 16B). The NH_2 -terminal region of the immature protein contains a signal peptide that is absent in the mature protein and that is required for the translocation of the LRR domain to the extracellular side of the plasma membrane (79). The LRR domain of the γ subunit consists of six LRR motifs, of 24–25 residues long, containing a consensus sequence LxxLxLxxN (where x represents any amino acid) flanked by two cysteine-rich regions, called LRRNT and LRRCT, capping the NH_2 and COOH termi-

nals of the LRR domain, respectively (FIGURE 16, A AND B) (485). The LRR domain is the region with higher similarity between the four γ subunits, while the non-LRR regions are more divergent.

A. γ_1 Subunit (LRRC26)

The γ_1 subunit was the first gamma subunit to be discovered (486), and it is the most extensively characterized. Initially, LRRC26 was proposed as a cancer marker protein, because it is highly expressed in breast, prostate, colon, and pancreatic cancer tissue and cell lines. In normal human tissues, it is highly expressed in the prostate and salivary glands and very weakly in the colon, pancreas, and intestine (110). Recently, LRRC26 was found to be expressed in arterial smooth muscle (116). Because LRRC26 colocalizes with intermediate filaments and cytokeratins, it received the alternative name cytokeratin-associated protein in cancer (CAPC) (110).

Previous studies indicated the presence of an unusual BK phenotype in human lymph node carcinoma of the prostate

(LNCaP) cell lines, in the human breast cancer line T47D (141), and in rat and mouse auditory inner hair cells (431). Unlike conventional BK channels, these channels are activated at physiological membrane potentials in the absence of free cytosolic Ca^{2+} (141). Yan and Aldrich (486) found that the molecular determinant of this unusual BK phenotype in LNCaP cells was the presence of LRRC26. Since it is an auxiliary subunit of the BK channel, LRRC26 was named $\gamma 1$ to differentiate it from the previously known β subunits (486).

The most remarkable functional effect of $\gamma 1$ is a large negative shift in the voltage dependence of BK channel activation (~ 140 mV), consistent with the observed acceleration of the activation kinetics and the slow kinetic of deactivation. In the presence of $\gamma 1$, the Ca^{2+} sensitivity of BK channels does not change (486). This effect can be explained in the context of the HA model for BK channel (see sect. III; Ref. 186) by a 20-fold increase in the allosteric factor D that couples voltage sensor activation with the pore opening (486). This mode of action of $\gamma 1$ is different from the effects of β subunits, which modulate both voltage and Ca^{2+} sensitivities (16, 63, 67, 459).

The presence of $\gamma 1$ can selectively alter the pharmacology of the BK channel. BK channels in mouse parotid acinar cells (which expressed $\gamma 1$) are almost insensitive to mallotoxin (6), which is a potent BK activator in human smooth muscle cells (502). However, $\gamma 1$ produces smaller changes in BK sensitivity to NS-1619, an activator of the class of the benzimidazolones (421) in parotid acinar cells (6) and in LNCaP cells (141). Charibdotoxin, iberiotoxin, paxilline, and penitrem A are slightly less potent inhibitors of BK in LNCaP cells (141) compared with BK without $\gamma 1$ (107, 228, 257, 303, 421). Knocking down $\gamma 1$ expression in rat arterial myocytes reduces the vasoconstriction induced by iberiotoxin and the vasodilation induced by NS1619 (116). Unlike the effects of $\beta 1$ (190) and $\beta 4$ (189), which make BK channel sensitive to long-chain omega-3 polyunsaturated fatty acids, LRRC26 diminishes the activating effect of docosahexaenoic acid (DHA) (189).

B. $\gamma 2$ Subunit (LRRC52)

LRRC52 is mainly expressed in testis and skeletal muscle (485). This protein is a BK accessory subunit, albeit with less efficacy than $\gamma 1$ (485). Coexpression of BK with $\gamma 2$ causes a leftward shift of the half-activation voltage. This shift, although significant, is smaller than the shift caused by $\gamma 1$ (~ 101 vs. ~ 140 mV) (FIGURE 16C) (485). The effect of $\gamma 2$ is maximal in the absence of cytosolic Ca^{2+} , suggesting that this accessory subunit can modify the apparent calcium sensitivity. However, further research is necessary to determine the precise effect of $\gamma 2$ on BK channel gating in terms of the HA allosteric model (186).

C. $\gamma 3$ Subunit (LRRC55)

LRRC55 is mainly expressed in brain (485). This protein was described as a BK Slo1 accessory subunit, although with weaker effect than $\gamma 1$ and $\gamma 2$ on channel activity (485). In the absence of cytosolic Ca^{2+} , this accessory subunit causes a leftward shift of the half-activation voltage of BK channels of 51 mV, versus ~ 140 and ~ 101 mV shifts produced by $\gamma 1$ and $\gamma 2$, respectively (485). Like $\gamma 2$, the effect of $\gamma 3$ is maximal in the absence of cytosolic Ca^{2+} (485). Little is known about the participation of $\gamma 3$ in physiological process or diseases. A synonymous mutation of LRRC55 was found to be involved in stress fracture predisposition in humans (129).

D. $\gamma 4$ Subunit (LRRC38)

LRRC38 is mainly expressed in adrenal gland, thymus, and skeletal muscle (485). In the absence of cytosolic Ca^{2+} , $\gamma 4$ subunit causes a 19-mV leftward shift of the half-activation voltage of the BK channel opening (485). $\gamma 4$, as well as $\gamma 2$ and $\gamma 3$, changes the shape of the apparent charge of the G - V Boltzmann curve, suggesting some effects on the calcium sensitivity (485). The physiological role of $\gamma 4$ is unknown at present.

E. Structural Determinants of Functional Coupling Between α and γ Subunits

As mentioned above, although the four known γ subunits promote a leftward shift of the BK activation curve, they have very different efficacies. By swapping structural elements between γ subunits, Li et al. (250) found that the critical structural elements determining the γ subunits effects on BK α subunit activity are located in the transmembrane segment of γ and are adjacent to an intracellular cluster of positive charged amino acids. Furthermore, the replacement of the second TM segment of the unrelated $\beta 1$ by those structural elements produced chimera that fully retained $\gamma 1$ modulatory function on the BK channel. Chimeric constructs demonstrated moderated effects of the extracellular leucine-rich repeat domain as well as the rest of the intracellular COOH terminal on BK channel modulation (251).

The stoichiometry of α and γ subunits is unknown. Interestingly, an all-or-none effect of the γ subunit on BK channels has been observed when it is expressed in different α : $\gamma 1$ ratios (150). This result suggests that a single γ subunit is sufficient to produce the full regulatory effect on BK. This finding is different from the additive effects of association of the BK α subunit with one to four β auxiliary subunits (462). More recently, Gonzalez-Perez et al. (150, 151) found that $\beta 2$ and $\gamma 1$ subunit can coassemble simultaneously in the same BK channel, and since they are able to

modulate channel function independently, they provide BK channels with unique gating properties. Given the features that these two subunits provide to BK channels, the presence of channels formed by α , $\beta 2$, and $\gamma 1$ subunits in any given tissue would generate active channels at resting potentials, influencing cell excitability balance. Whether or not these two subunits are functionally expressed in the same cell type is still unknown, but the RNAs encoding for $\beta 2$ and $\gamma 1$ subunits have been shown to be present in testis and chromaffin cells (287, 487).

The role of K^+ channels in cell growth of many cancer cells has been demonstrated (234), and an upregulated expression of BK channels has been positively correlated with prostate, bone, and glioma tumors (36, 57, 464). Taking into account that cancer cells are not usually electrically excitable, and that γ subunits are strongly expressed in several cancer cell types, it is possible that γ subunit expression could be determining the activation of BK channels in these particular contexts.

X. BK CHANNEL OPENERS

Several BK channel openers, either from endogenous, natural, or synthetic origin, have been described in different studies, ranging from biophysics to physiology. Moreover, they have therapeutic potential in a large number of human pathophysiological conditions.

A. Endogenous Openers

Unsaturated *cis* free fatty acids (such as arachidonic, palmitoleic, oleic, linoleic, linolenic, eicosapenanoic, and eicosatetraenoic acids) have been found to increase BK channel activity, whereas saturated or *trans* isomers had no effects (93). Arachidonic acid was found to regulate vascular tone. Similarly, other fatty acids such as omega-3 docosahexaenoic acid (DHA) at nanomolar concentrations increase BK channel activity (190) in the presence of $\beta 1$ or $\beta 4$ (189). DHA decreases blood pressure in anesthetized wild-type, but not in BK-deficient mice (190). Interestingly, Hoshi et al. (191) found that the only point mutation able to dramatically reduce the DHA effect was one in the S6 transmembrane segment, namely, Y318S. The *Drosophila* BK has a serine in the equivalent position of S6 and is insensitive to DHA.

BK channels are modulated by 17β -estradiol when coexpressed with $\beta 1$ subunits (447). The presence of $\beta 2$ makes BK sensitive to corticosterone (142), and BK channels coexpressed with $\beta 4$ are potentiated by dehydroepiandrosterone, an adrenal androgen (223).

BK channels can also be activated by the endogenous antimicrobial peptide β -defensin 2, which was found to in-

crease the open probability of the BK channel coexpressed with the accessory subunit $\beta 1$, being dependent on two amino acids in the $\beta 1$ loop. This peptide, relevant in the innate immune system, has been found to be downregulated in serum from patients with hypertension. Notably, the administration of β -defensin 2 in monkeys significantly reduces blood pressure (261).

B. Synthetic Openers

Available commercial drugs, such as the therapeutic agent tamoxifen, used as a complement for cancer therapy, show BK channel activation only in the presence of $\beta 1$ (65, 96). Additionally, tamoxifen stimulates proliferation of MCF-7 cells (a breast cancer cell line) which shows high levels of BK expression (65). Similarly, other drugs such as zonisamide (an antiepileptic drug) (198), chlorzoxazone (a muscle relaxant) (265), and cilostazol (a phosphodiesterase III inhibitor) display BK channel activating characteristics (475), although their mechanisms are unknown.

Various companies have developed synthetic BK channel activators, such as the benzimidazolone derivatives NS004 (337) and NS1619 (336). The latter has been amply used to study functional effects on BK channels, and it is a potential therapeutic agent in pulmonary hypertension (369, 448), erectile dysfunction (149, 224, 416), bladder instability (237, 411), shock-induced vascular hyperactivity (197), migraines (272), inflammatory pain (4), and cytoprotection (138, 484). A recent report on another BK channel activator (Cym04) found that its activating effects, as well as those of NS1619, are dependent on the S6-RCK linker. However, it is not known whether the compound binds to the linker or if this part of the protein is required for conformational changes following drug binding to the channel (140).

The benzimidazolone derivative NS11021 is an improvement on NS1619. Being more specific and potent, it has received attention in efforts to improve the performance of BK channels in models of diseases associated with decreased activity of these channels (24, 142). NS11021 is able to reduce infarct sizes and improve cardiac performance after ischemia (8, 26, 40). In smooth muscle dysfunction, NS11021 enhances erectile responses in rats mainly through its BK activating effects (233), and induces an accentuated relaxation in human penile arteries (224), just like the phosphodiesterase type 5 inhibitor sildenafil, sold as Viagra. NS11021 also decreases the excitability and contractility of urinary bladder smooth muscle, which is an effect eliminated by IbTx (246). On the other hand, in the nervous system, NS11021 has been useful to establish a role for BK channels in migraines (260, 478).

A series of anthraquinone analogs, called the GoSlo-SR family of activators, are able to left shift the voltage dependence of BK activation by more than 100 mV in bladder smooth muscle cells (373).

The dye bis-(1,3-dibutylbarbituric acid)trimethine oxonol [DiBAC(4)(3)], which is a voltage sensitive opener, has activating effects only when $\beta 1$ and $\beta 4$ subunits are present (318). It is important to note that at saturating concentrations this compound induces a 300-mV left shift in the voltage-dependent activation curve, without depending on the presence of β subunits or calcium (386).

Other compounds lose their capability to increase activity on BK channels when coexpressed with β subunits as is the case of *N*-arylbenzamide derivatives (225). NS19504, identified in a drug screening (326), activates endogenous BK channels in native smooth muscle cells and reduces spontaneous contractions in bladder strips in an IbTX-sensitive manner.

C. Natural Openers

In addition to synthetic compounds, natural BK channel activators are found in herbs, roots, fungi, and leaves and have been used in popular medicine to treat asthma and smooth muscle disorders (324), as is the case of dihydrosoyasaponin (155). Other natural compounds such as DHS-I strongly increase the open probability of BK channels in smooth muscles when applied to the intracellular side in the presence of the $\beta 1$ subunit (294, 295).

D. BK Openers Used in Clinical Trials

Pharmaceutical companies have produced BK channel activators (e.g., BMS204352), which have shown neuroprotective effects in animal models (85) and have been introduced to phase III clinical trials for the treatment of acute ischemic stroke, but without success. A related compound, BMS223131, has been found to relax smooth muscle (41) and has been studied clinically for overactive bladder, but has not been developed.

Small molecules such as thioureas, tetrahydroquinolines, compound 36 (153), compound Z (354), terpenes, and benzofuroindoles are also able to activate BK channels. They have been intended mostly for the treatment of urinary incontinence, overactive bladder, erectile dysfunction, and stroke. Some of them entered into phase II clinical trials (325).

Unoprostone isopropyl (unoprostone), an approved prostanoid and synthetic docosanoid BK channel activator, has been used for the treatment of open-angle glaucoma and ocular hypertension (169). It also decelerates decreases in central retinal sensitivity in patients with retinitis pigmentosa in a phase II clinical study (220). Andolast, another BK channel opener, is being used for the treatment of asthma and is currently in phase III clinical trials (321).

Sildenafil has proven to be effective in patients with class II and III pulmonary arterial hypertension (130, 474). In ad-

dition, the anthelmintic compound emodepside has been suggested to inhibit neuronal and muscle activity by increasing the opening of BK channels, which was corroborated by rescue experiments (74).

BK channel openers have been shown to have possible therapeutic potential in conditions characterized by hyperexcitability and smooth muscle dysfunction. Translating preclinical findings to the clinic may be hampered by many factors as broad as the effects of targeting the channel, mainly because BK is ubiquitously expressed and it suffers many pre- and posttranslational modifications. To this must be added that BK channel performance changes upon coexpression with auxiliary subunits. These changes lead to an increase in BK channel molecular diversity and modulation by endogenous or exogenous compounds, which hampers the development of specific drugs.

XI. CONCLUSIONS

In recent years, remarkable advances in our understanding of the molecular aspects of gating, conduction, and the physiological roles played by BK channels in a variety of different cellular processes have been seen. The dual allosteric mechanism proposed for BK channel gating clearly explains the most salient features of channel behavior, in particular, the fact that the channel can open in the absence of sensor activation. Voltage-sensor activation and/or Ca^{2+} binding coupled to pore opening in a nonobligatory fashion decreasing the free energy necessary to activate the pore gate. Although we lack a crystal structure of the transmembrane domain of the BK channel, its similarity to K_v channels and the known structure of the gating ring allow us to say with certainty where the voltage sensor and the Ca^{2+} binding sites are located. These structures are in charge of transducing electrical and chemical free energy into mechanical energy, leading to the pore opening. However, there are many open questions about the molecular nature of the structures that couple these energies to the channel activation gate. Once this is known, we will be able to give a physical meaning to the allosteric factors present in the allosteric model.

At present there is little doubt that the gate that hinders the passage of K^+ ions through the channel is located near or in the selectivity filter. This is further supported by the findings that indicate that in the Ca^{2+} -activated K^+ channel from bacteria, MthK, Ca^{2+} -dependent gating occurs at the selectivity filter (355, 356). What is worth noticing is that in MthK channel gating is also accompanied by a reduction in the size of the internal vestibule upon closing, apparently due to a movement of the intracellular helices. Some of the experiments described in this review suggest that BK channel closing also involves a reduction in size of the internal channel entryway formed by the S6 transmembrane segments.

By having gating charges distributed in the S2, S3, and S4 transmembrane segments, the voltage sensor of BK channels has been considered as a “descentralized” voltage sensor compared with the VSD of K_v channels, where most of the gating charges are concentrated in S4. Voltage-clamp fluorometry experiments indicate that during activation S2 interacts with S4 since, neutralization in one segment modifies the effective valence on the other. Electrical field focusing or mechanical coupling can account for these observations. The S0 segment appears also to be part of the VSD in BK channels as this structure also undergoes voltage-dependent changes similar to S2 and S4. The evidence indicates that the S6 segment is an important player in the coupling between the VSD and the pore gate. However, the molecular details of this process are still lacking.

The crystal structure of the BK gating ring gave a clear picture of one of the high-affinity Ca^{2+} binding sites, the Ca^{2+} bowl, and identified the important residues involved in divalent cation binding. However, the structure of the second high-affinity Ca^{2+} binding site is still a mystery. The evidence we have so far indicates that the coupling between the Ca^{2+} sensors and the activation gate is mediated by the S6-RCK1 linker. Expansion of the gating ring is thought to pull the linker, initiating the opening of the pore. The Mg^{2+} binding site has attracted special attention because of its particular location at the interface between the VSD and the COOH-terminal domain (CTD). Mg^{2+} , through long-range electrostatic interactions, modifies the workings of the voltage sensor, enhancing voltage sensor-activation gate coupling. It remains to be determined to what extent the VSD/CTD interface is involved in pore opening.

The physiological versatility of the BK channel is greatly increased by alternative splicing and its association with β and γ subunits. Alternative splicing gives rise to many different BK phenotypes, and it also appears that certain BK splice variants have as a target not the plasma membrane but organelles, as it occurs in the case of the mitochondrial BK channel. β Subunits, on the other hand, allow BK channels to be important players in the normal as well as the pathological function of smooth muscle and the nervous system and in determining their pharmacological profile. The more recently discovered γ subunits have revealed new roles for this ubiquitous channel, allowing it to open without much Ca^{2+} at negative voltages. Splicing, modulatory subunit interactions, and metabolic regulation allow BK channels to be involved in a wide array of physiological functions.

ACKNOWLEDGMENTS

Address for reprint requests and other correspondence: R. Latorre, Centro Interdisciplinario de Neurociencias de Valparaíso, Pasaje Harrington 287, Valparaíso 2366103, Chile (e-mail: ramon.latorre@uv.cl).

GRANTS

This work was supported by the Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), a Millennium Institute supported by the Millennium Scientific Initiative of the Ministerio de Economía, Fomento y Turismo. This work was also supported by Projects FONDECYT 1150273 (to R. Latorre), 1160261 (to C. Gonzalez), and 1131003 (to F. Gonzalez-Nilo); Grant RI-130006 (to R. Latorre and C. Gonzalez); and CONICYT PCHA/Doctorado Nacional 2013-21130631 fellowship (to R. Sepulveda).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

- Adelman JP, Shen KZ, Kavanaugh MP, Warren RA, Wu YN, Lagrutta A, Bond CT, North RA. Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron* 9: 209–216, 1992.
- Aggarwal SK, MacKinnon R. Contribution of the S4 segment to gating charge in the Shaker K^+ channel. *Neuron* 16: 1169–1177, 1996.
- Ahrendt E, Kyle B, Braun AP, Braun JE. Cysteine string protein limits expression of the large conductance, calcium-activated K^+ (BK) channel. *PLoS One* 9: e86586, 2014.
- Akerman S, Holland PR, Lasalandra MP, Goadsby PJ. Inhibition of trigeminovascular dural nociceptive afferents by Ca^{2+} -activated K^+ [MaxiK/BK(Ca)] channel opening. *Pain* 151: 128–136, 2010.
- Alioua A, Lu R, Kumar Y, Eghbali M, Kundu P, Toro L, Stefani E. Slo1 caveolin-binding motif, a mechanism of caveolin-1-Slo1 interaction regulating Slo1 surface expression. *J Biol Chem* 283: 4808–4817, 2008.
- Almasy J, Begenisich T. The LRR26 protein selectively alters the efficacy of BK channel activators. *Mol Pharmacol* 81: 21–30, 2012.
- Anderson CS, MacKinnon R, Smith C, Miller C. Charybdotoxin block of single Ca^{2+} -activated K^+ channels. Effects of channel gating, voltage, and ionic strength. *J Gen Physiol* 91: 317–333, 1988.
- Aon MA, Cortassa S, Wei AC, Grunnet M, O'Rourke B. Energetic performance is improved by specific activation of K^+ fluxes through K(Ca) channels in heart mitochondria. *Biochim Biophys Acta* 1797: 71–80, 2010.
- Armstrong CM. Interaction of tetraethylammonium derivatives with the potassium channels of squid giant axon. *J Gen Physiol* 58: 413–437, 1971.
- Armstrong CM. Potassium pores of nerve and muscle membranes. *Membranes* 3: 325–358, 1975.
- Armstrong CM, Bezanilla F, Rojas E. Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J Gen Physiol* 62: 375–391, 1973.
- Armstrong CM, Swenson RP Jr, Taylor SR. Block of squid axon K channels by internally and externally applied barium ions. *J Gen Physiol* 80: 663–682, 1982.
- Atkinson NS, Robertson GA, Ganetzky B. A component of calcium-activated potassium channels encoded by the *Drosophila* slo locus. *Science* 253: 551–555, 1991.
- Balderas E, Zhang J, Stefani E, Toro L. Mitochondrial BKCa channel. *Front Physiol* 6: 104, 2015.
- Banerjee A, Lee A, Campbell E, MacKinnon R. Structure of a pore-blocking toxin in complex with a eukaryotic voltage-dependent K^+ channel. *Elife* 2: e00594, 2013.
- Bao L, Cox DH. Gating and ionic currents reveal how the BKCa channel's Ca^{2+} sensitivity is enhanced by its beta1 subunit. *J Gen Physiol* 126: 393–412, 2005.

17. Bao L, Kaldany C, Holmstrand EC, Cox DH. Mapping the BKCa channel's "Ca²⁺ bowl": side-chains essential for Ca²⁺ sensing. *J Gen Physiol* 123: 475–489, 2004.
18. Bao L, Rapin AM, Holmstrand EC, Cox DH. Elimination of the BK(Ca) channel's high-affinity Ca²⁺ sensitivity. *J Gen Physiol* 120: 173–189, 2002.
19. Barrett JN, Magleby KL, Pallotta BS. Properties of single calcium-activated potassium channels in cultured rat muscle. *J Physiol* 331: 211–230, 1982.
20. Bednarczyk P, Koziel A, Jarmuszkiewicz W, Szewczyk A. Large-conductance Ca²⁺-activated potassium channel in mitochondria of endothelial EA.hy926 cells. *Am J Physiol Heart Circ Physiol* 304: H1415–H1427, 2013.
21. Behrens R, Nolting A, Reimann F, Schwarz M, Waldschutz R, Pongs O. hKCNMB3 and hKCNMB4, cloning and characterization of two members of the large-conductance calcium-activated potassium channel beta subunit family. *FEBS Lett* 474: 99–106, 2000.
22. Benkuský NA, Fergus DJ, Zuccherro TM, England SK. Regulation of the Ca²⁺-sensitive domains of the maxi-K channel in the mouse myometrium during gestation. *J Biol Chem* 275: 27712–27719, 2000.
23. Bentrop D, Beyermann M, Wissmann R, Fakler B. NMR structure of the "ball-and-chain" domain of KCNMB2, the beta 2-subunit of large conductance Ca²⁺- and voltage-activated potassium channels. *J Biol Chem* 276: 42116–42121, 2001.
24. Bentzen BH, Nardi A, Calloe K, Madsen LS, Olesen SP, Grunnet M. The small molecule NS11021 is a potent and specific activator of Ca²⁺-activated big-conductance K⁺ channels. *Mol Pharmacol* 72: 1033–1044, 2007.
25. Bentzen BH, Olesen SP, Ronn LC, Grunnet M. BK channel activators and their therapeutic perspectives. *Front Physiol* 5: 389, 2014.
26. Bentzen BH, Osadchii O, Jespersen T, Hansen RS, Olesen SP, Grunnet M. Activation of big conductance Ca²⁺-activated K⁺ channels (BK) protects the heart against ischemia-reperfusion injury. *Pflügers Arch* 457: 979–988, 2009.
27. Berkefeld H, Fakler B. Ligand-gating by Ca²⁺ is rate limiting for physiological operation of BK(Ca) channels. *J Neurosci* 33: 7358–7367, 2013.
28. Berkefeld H, Fakler B. Repolarizing responses of BKCa-Cav complexes are distinctly shaped by their Cav subunits. *J Neurosci* 28: 8238–8245, 2008.
29. Berkefeld H, Fakler B, Schulte U. Ca²⁺-activated K⁺ channels: from protein complexes to function. *Physiol Rev* 90: 1437–1459, 2010.
30. Berkefeld H, Sailer CA, Bildl W, Rohde V, Thumfart JO, Eble S, Klugbauer N, Reisinger E, Bischofberger J, Oliver D, Knaus HG, Schulte U, Fakler B. BKCa-Cav channel complexes mediate rapid and localized Ca²⁺-activated K⁺ signaling. *Science* 314: 615–620, 2006.
31. Berrout J, Jin M, Mamenko M, Zaika O, Pochynuk O, O'Neil RG. Function of transient receptor potential cation channel subfamily V member 4 (TRPV4) as a mechanical transducer in flow-sensitive segments of renal collecting duct system. *J Biol Chem* 287: 8782–8791, 2012.
32. Bian S, Favre I, Moczydlowski E. Ca²⁺-binding activity of a COOH-terminal fragment of the Drosophila BK channel involved in Ca²⁺-dependent activation. *Proc Natl Acad Sci USA* 98: 4776–4781, 2001.
33. Blair LA, Dionne VE. Developmental acquisition of Ca_v2.1-sensitivity by K1 channels in spinal neurones. *Nature* 315: 329–331, 1985.
34. Blatz AL, Magleby KL. Ion conductance and selectivity of single calcium-activated potassium channels in cultured rat muscle. *J Gen Physiol* 84: 1–23, 1984.
35. Blatz ALMK. Calcium-activated potassium channels. *Trends Neurosci* 10: 463–467, 1987.
36. Bloch M, Ousingsawat J, Simon R, Schraml P, Gasser TC, Mihatsch MJ, Kunzelmann K, Bubendorf L. KCNMA1 gene amplification promotes tumor cell proliferation in human prostate cancer. *Oncogene* 26: 2525–2534, 2007.
37. Bolton TB, Imaizumi Y. Spontaneous transient outward currents in smooth muscle cells. *Cell Calcium* 20: 1411–1452, 1996.
38. Bontems F, Gilquin B, Roumestand C, Ménez A, Toma F. Analysis of side-chain organization on a refined model of charybdotoxin: structural and functional implications. *Biochemistry* 31: 7756–7764, 1992.
39. Bontems F, Roumestand C, Gilquin B, Ménez A, Toma F. Refined structure of charybdotoxin: common motifs in scorpion toxins and insect defensins. *Science* 254: 1521–1523, 1991.
40. Borchert GH, Hlavackova M, Kolar F. Pharmacological activation of mitochondrial BK(Ca) channels protects isolated cardiomyocytes against simulated reperfusion-induced injury. *Exp Biol Med* 238: 233–241, 2013.
41. Boy KM, Guernon JM, Sit SY, Xie K, Hewawasam P, Boissard CG, Dworetzky SI, Natale J, Gribkoff VK, Lodge N, Starrett JE Jr. 3-Thio-quinolinone maxi-K openers for the treatment of erectile dysfunction. *Bioorg Med Chem Lett* 14: 5089–5093, 2004.
42. Brandle U, Frohnmayer S, Krieger T, Zenner HP, Ruppersberg JP, Maassen MM. Expression of Ca²⁺-activated K⁺ channel subunits and splice variants in the rat cochlea. *Hear Res* 161: 23–28, 2001.
43. Braun AP, Sy L. Contribution of potential EF hand motifs to the calcium-dependent gating of a mouse brain large conductance, calcium-sensitive K⁺ channel. *J Physiol* 533: 681–695, 2001.
44. Brelidze TI, Magleby KL. Probing the geometry of the inner vestibule of BK channels with sugars. *J Gen Physiol* 126: 105–121, 2005.
45. Brelidze TI, Magleby KL. Protons block BK channels by competitive inhibition with K⁺ and contribute to the limits of unitary currents at high voltages. *J Gen Physiol* 123: 305–319, 2004.
46. Brelidze TI, Niu X, Magleby KL. A ring of eight conserved negatively charged amino acids doubles the conductance of BK channels and prevents inward rectification. *Proc Natl Acad Sci USA* 100: 9017–9022, 2003.
47. Brenner R, Chen QH, Vilaythong A, Toney GM, Noebels JL, Aldrich RW. BK channel beta4 subunit reduces dentate gyrus excitability and protects against temporal lobe seizures. *Nat Neurosci* 8: 1752–1759, 2005.
48. Brenner R, Jegla TJ, Wickenden A, Liu Y, Aldrich RW. Cloning and functional characterization of novel large conductance calcium-activated potassium channel beta subunits, hKCNMB3 and hKCNMB4. *J Biol Chem* 275: 6453–6461, 2000.
49. Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT, Aldrich RW. Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. *Nature* 407: 870–876, 2000.
50. Brismar T, Collins VP. Potassium and sodium channels in human malignant glioma cells. *Brain Res* 480: 259–267, 1989.
51. Brundage RA, Fogarty KE, Tuft RA, Fay FS. Calcium gradients underlying polarization and chemotaxis of eosinophils. *Science* 254: 703–706, 1991.
52. Budelli G, Geng Y, Butler A, Magleby KL, Salkoff L. Properties of Slo1 K⁺ channels with and without the gating ring. *Proc Natl Acad Sci USA* 110: 16657–16662, 2013.
53. Bukiya AN, Liu J, Dopico AM. The BK channel accessory beta1 subunit determines alcohol-induced cerebrovascular constriction. *FEBS Lett* 583: 2779–2784, 2009.
54. Bukiya AN, Singh AK, Parrill AL, Dopico AM. The steroid interaction site in transmembrane domain 2 of the large conductance, voltage- and calcium-gated potassium (BK) channel accessory beta1 subunit. *Proc Natl Acad Sci USA* 108: 20207–20212, 2011.
55. Bukiya AN, Vaithianathan T, Toro L, Dopico AM. Channel beta2-4 subunits fail to substitute for beta1 in sensitizing BK channels to lithocholate. *Biochem Biophys Res Commun* 390: 995–1000, 2009.
56. Butler A, Tsunoda S, McCobb DP, Wei A, Salkoff L. mSlo, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science* 261: 221–224, 1993.
57. Cambien B, Rezzonico R, Vitale S, Rouzaire-Dubois B, Dubois JM, Barthel R, Karimjee BS, Mograbi B, Schmid-Alliana A, Schmid-Antomarchi H. Silencing of hSlo potassium channels in human osteosarcoma cells promotes tumorigenesis. *Int J Cancer* 123: 365–371, 2008.
58. Candia S, Garcia ML, Latorre R. Mode of action of iberiotoxin, a potent blocker of the large conductance Ca²⁺-activated K⁺ channel. *Biophys J* 63: 583–590, 1992.
59. Carrasquel-Ursulaez W, Contreras GF, Sepulveda RV, Aguayo D, Gonzalez-Nilo F, Gonzalez C, Latorre R. Hydrophobic interaction between contiguous residues in the S6 transmembrane segment acts as a stimuli integration node in the BK channel. *J Gen Physiol* 145: 61–74, 2015.

60. Carrisoza-Gaytan R, Liu Y, Flores D, Else C, Lee HG, Rhodes G, Sandoval RM, Kleyman TR, Lee FY, Molitoris B, Satlin LM, Rohatgi R. Effects of biomechanical forces on signaling in the cortical collecting duct (CCD). *Am J Physiol Renal Physiol* 307: F195–F204, 2014.
61. Carvacho I, Gonzalez W, Torres YP, Brauchi S, Alvarez O, Gonzalez-Nilo FD, Latorre R. Intrinsic electrostatic potential in the BK channel pore: role in determining single channel conductance and block. *J Gen Physiol* 131: 147–161, 2008.
62. Castillo JP, Sanchez-Rodriguez JE, Hyde HC, Zaelzer CA, Aguayo D, Sepulveda RV, Luk LY, Kent SB, Gonzalez-Nilo FD, Bezanilla F, Latorre R. Beta I-subunit-induced structural rearrangements of the Ca^{2+} - and voltage-activated K^+ (BK) channel. *Proc Natl Acad Sci USA* 113: E3231–E3239, 2016.
63. Castillo K, Contreras GF, Pupo A, Torres YP, Neely A, Gonzalez C, Latorre R. Molecular mechanism underlying beta I regulation in voltage- and calcium-activated potassium (BK) channels. *Proc Natl Acad Sci USA* 112: 4809–4814, 2015.
64. Cecchi X, Wolff D, Alvarez O, Latorre R. Mechanisms of Cs^+ blockade in a Ca^{2+} -activated K^+ channel from smooth muscle. *Biophys J* 52: 707–716, 1987.
65. Coiret G, Borowiec AS, Mariot P, Ouadid-Ahidouch H, Matifat F. The antiestrogen tamoxifen activates BK channels and stimulates proliferation of MCF-7 breast cancer cells. *Mol Pharmacol* 71: 843–851, 2007.
66. Colwell CS. BK channels and circadian output. *Nat Neurosci* 9: 985–986, 2006.
67. Contreras GF, Neely A, Alvarez O, Gonzalez C, Latorre R. Modulation of BK channel voltage gating by different auxiliary beta subunits. *Proc Natl Acad Sci USA* 109: 18991–18996, 2012.
68. Cornejo M, Guggino SE, Guggino WB. Modification of Ca^{2+} -activated K^+ channels in cultured medullary thick ascending limb cells by *N*-bromoacetamide. *J Membr Biol* 99: 147–155, 1987.
69. Cox DH. The BKCa channel's Ca^{2+} -binding sites, multiple sites, multiple ions. *J Gen Physiol* 125: 253–255, 2005.
70. Cox DH, Aldrich RW. Role of the beta I subunit in large-conductance Ca^{2+} -activated K^+ channel gating energetics. Mechanisms of enhanced Ca^{2+} sensitivity. *J Gen Physiol* 116: 411–432, 2000.
71. Cox DH, Cui J, Aldrich RW. Allosteric gating of a large conductance Ca -activated K^+ channel. *J Gen Physiol* 110: 257–281, 1997.
72. Cox DH, Cui J, Aldrich RW. Separation of gating properties from permeation and block in mslo large conductance Ca -activated K^+ channels. *J Gen Physiol* 109: 633–646, 1997.
73. Coyne KS, Sexton CC, Bell JA, Thompson CL, Dmochowski R, Bavendam T, Chen CI, Quentin Clemens J. The prevalence of lower urinary tract symptoms (LUTS) and overactive bladder (OAB) by racial/ethnic group and age: results from OAB-POLL. *NeuroUrol Urodyn* 32: 230–237, 2013.
74. Crisford A, Murray C, O'Connor V, Edwards RJ, Kruger N, Welz C, von Samson-Himmelstjerna G, Harder A, Walker RJ, Holden-Dye L. Selective toxicity of the anthelmintic emodepside revealed by heterologous expression of human KCNNM1 in *Caenorhabditis elegans*. *Mol Pharmacol* 79: 1031–1043, 2011.
75. Crouzy S, Bernèche S, Roux B. Extracellular blockade of K channels by TEA: results from molecular dynamics simulations of the KcsA channel. *J Gen Physiol* 118: 207–217, 2001.
76. Cuello LG, Jogini V, Cortes DM, Perozo E. Structural mechanism of C-type inactivation in K^+ channels. *Nature* 466: 203–208, 2010.
77. Cui J, Cox DH, Aldrich RW. Intrinsic voltage dependence and Ca^{2+} regulation of mslo large conductance Ca -activated K^+ channels. *J Gen Physiol* 109: 647–673, 1997.
78. Cha A, Bezanilla F. Characterizing voltage-dependent conformational changes in the Shaker K^+ channel with fluorescence. *Neuron* 19: 1127–1140, 1997.
79. Chan DV, Somani AK, Young AB, Massari JV, Ohtola J, Sugiyama H, Garaczi E, Babinneau D, Cooper KD, McCormick TS. Signal peptide cleavage is essential for surface expression of a regulatory T cell surface protein, leucine rich repeat containing 32 (LRRC32). *BMC Biochem* 12: 27, 2011.
80. Chang CP, Dworetzky SI, Wang J, Goldstein ME. Differential expression of the alpha and beta subunits of the large-conductance calcium-activated potassium channel: implication for channel diversity. *Brain Res* 45: 33–40, 1997.
81. Chen L, Tian L, MacDonald SH, McClafferty H, Hammond MS, Huibant JM, Ruth P, Knaus HG, Shipton MJ. Functionally diverse complement of large conductance calcium- and voltage-activated potassium channel (BK) alpha-subunits generated from a single site of splicing. *J Biol Chem* 280: 33599–33609, 2005.
82. Chen RS, Geng Y, Magleby KL. $\text{Mg}(2+)$ binding to open and closed states can activate BK channels provided that the voltage sensors are elevated. *J Gen Physiol* 138: 593–607, 2011.
83. Chen X, Kovalchuk Y, Adelsberger H, Henning HA, Sausbier M, Wietzorrek G, Ruth P, Yarom Y, Konnerth A. Disruption of the olivo-cerebellar circuit by Purkinje neuron-specific ablation of BK channels. *Proc Natl Acad Sci USA* 107: 12323–12328, 2010.
84. Chen X, Yan J, Aldrich RW. BK channel opening involves side-chain reorientation of multiple deep-pore residues. *Proc Natl Acad Sci USA* 111: E79–88, 2014.
85. Cheney JA, Weisser JD, Bareyre FM, Laurer HL, Saatman KE, Raghupathi R, Gribkoff V, Starrett JE Jr, McIntosh TK. The maxi-K channel opener BMS-204352 attenuates regional cerebral edema and neurologic motor impairment after experimental brain injury. *J Cereb Blood Flow Metab* 21: 396–403, 2001.
86. Cheng Y, Gu XQ, Bednarczyk P, Wiedemann FR, Haddad GG, Siemen D. Hypoxia increases activity of the BK-channel in the inner mitochondrial membrane and reduces activity of the permeability transition pore. *Cell Physiol Biochem* 22: 127–136, 2008.
87. Cheng Y, Gulbins E, Siemen D. Activation of the permeability transition pore by Bax via inhibition of the mitochondrial BK channel. *Cell Physiol Biochem* 27: 191–200, 2011.
88. Dabrand F, Nelson MT, Brayden JE. Acidosis dilates brain parenchymal arterioles by conversion of calcium waves to sparks to activate BK channels. *Circ Res* 110: 285–294, 2012.
89. Dauplais M, Gilquin B, Possani LD, Gurrola-Briones G, Roumestand C, Menez A. Determination of the three-dimensional solution structure of noxiustoxin: analysis of structural differences with related short-chain scorpion toxins. *Biochemistry* 34: 16563–16573, 1995.
90. Davies KP, Stanevsky Y, Tar MT, Chang JS, Chance MR, Melman A. Ageing causes cytoplasmic retention of MaxiK channels in rat corporal smooth muscle cells. *Int J Impot Res* 19: 371–377, 2007.
91. De Wet H, Allen M, Holmes C, Stobbart M, Lippiat JD, Callaghan R. Modulation of the BK channel by estrogens: examination at single channel level. *Mol Membr Biol* 23: 420–429, 2006.
92. Dell'Orco JM, Wasserman AH, Chopra R, Ingram MA, Hu YS, Singh V, Wulff H, Opal P, Orr HT, Shakkottai VG. Neuronal atrophy early in degenerative ataxia is a compensatory mechanism to regulate membrane excitability. *J Neurosci* 35: 11292–11307, 2015.
93. Denson DD, Wang X, Worrell RT, Eaton DC. Effects of fatty acids on BK channels in GH₃ cells. *Am J Physiol Cell Physiol* 279: C1211–C1219, 2000.
94. Diaz-Franulic I, Sepulveda RV, Navarro-Quezada N, Gonzalez-Nilo F, Naranjo D. Pore dimensions and the role of occupancy in unitary conductance of Shaker K channels. *J Gen Physiol* 146: 133–146, 2015.
95. Diaz L, Meera P, Amigo J, Stefani E, Alvarez O, Toro L, Latorre R. Role of the S4 segment in a voltage-dependent calcium-sensitive potassium (hSlo) channel. *J Biol Chem* 273: 32430–32436, 1998.
96. Dick GM, Rossow CF, Smirnov S, Horowitz B, Sanders KM. Tamoxifen activates smooth muscle BK channels through the regulatory beta I subunit. *J Biol Chem* 276: 34594–34599, 2001.
97. Dick GM, Sanders KM. (Xeno)estrogen sensitivity of smooth muscle BK channels conferred by the regulatory beta I subunit: a study of beta I knockout mice. *J Biol Chem* 276: 44835–44840, 2001.
98. DiChiara TJ, Reinhart PH. Distinct effects of Ca^{2+} and voltage on the activation and deactivation of cloned Ca^{2+} -activated K^+ channels. *J Physiol (Lond)* 489: 403–418, 1995.
99. Dinardo MM, Camerino G, Mele A, Latorre R, Conte Camerino D, Tricarico D. Splicing of the rSlo gene affects the molecular composition and drug response of Ca^{2+} -activated K^+ channels in skeletal muscle. *PLoS One* 7: e40235, 2012.
100. Ding JP, Li ZW, Lingle CJ. Inactivating BK channels in rat chromaffin cells may arise from heteromultimeric assembly of distinct inactivation-competent and noninactivating subunits. *Biophys J* 74: 268–289, 1998.

101. Dose S, Neuweiler H, Sauer M. A close look at fluorescence quenching of organic dyes by tryptophan. *Chemphyschem* 6: 2277–2285, 2005.
102. Dose S, Neuweiler H, Sauer M. Fluorescence quenching by photoinduced electron transfer: a reporter for conformational dynamics of macromolecules. *Chemphyschem* 10: 1389–1398, 2009.
103. Dopico AM, Bukiya AN, Martin GE. Ethanol modulation of mammalian BK channels in excitable tissues: molecular targets and their possible contribution to alcohol-induced altered behavior. *Front Physiol* 5: 466, 2014.
104. Dopico AM, Widmer H, Wang G, Lemos JR, Treistman SN. Rat supraoptic magnocellular neurones show distinct large conductance, Ca^{2+} -activated K^{+} channel subtypes in cell bodies versus nerve endings. *J Physiol* 519: 101–114, 1999.
105. Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. The structure of the potassium channel: molecular basis of K^{+} conduction and selectivity. *Science* 280: 69–77, 1998.
106. Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Kotagal P, Luders HO, Shi J, Cui J, Richerson GB, Wang QK. Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat Genet* 37: 733–738, 2005.
107. Dworetzky SI, Boissard CG, Lum-Ragan JT, McKay MC, Post-Munson DJ, Trojnecki JT, Chang CP, Gribkoff VK. Phenotypic alteration of a human BK (hSlo) channel by hSlobeta subunit coexpression: changes in blocker sensitivity, activation/relaxation and inactivation kinetics, and protein kinase A modulation. *J Neurosci* 16: 4543–4550, 1996.
108. Earley S, Heppner TJ, Nelson MT, Brayden JE. TRPV4 forms a novel Ca^{2+} signaling complex with ryanodine receptors and BKCa channels. *Circ Res* 97: 1270–1279, 2005.
109. Eghbali M, Toro L, Stefani E. Diminished surface clustering and increased perinuclear accumulation of large conductance Ca^{2+} -activated K^{+} channel in mouse myotetrium with pregnancy. *J Biol Chem* 278: 45311–45317, 2003.
110. Egland KA, Liu XF, Squires S, Nagata S, Man YG, Bera TK, Onda M, Vincent JJ, Strausberg RL, Lee B, Pastan I. High expression of a cytokeratin-associated protein in many cancers. *Proc Natl Acad Sci USA* 103: 5929–5934, 2006.
111. Eisenman G, Dani JA. An introduction to molecular architecture and permeability of ion channels. *Annu Rev Biophys Chem* 16: 205–226, 1987.
112. Eisenman G, Latorre R, Miller C. Multi-ion conduction and selectivity in the high-conductance Ca^{2+} -activated K^{+} channel from skeletal muscle. *Biophys J* 50: 1025–1034, 1986.
113. Elkins T, Ganetzky B, Wu CF. A *Drosophila* mutation that eliminates a calcium-dependent potassium current. *Proc Natl Acad Sci USA* 83: 8415–8419, 1986.
114. Erxleben C, Everhart AL, Romeo C, Florance H, Bauer MB, Alcorta DA, Rossie S, Shipston MJ, Armstrong DL. Interacting effects of N-terminal variation and stress exon splicing on slo potassium channel regulation by calcium, phosphorylation, and oxidation. *J Biol Chem* 277: 27045–27052, 2002.
115. Essin K, Gollasch M, Rolle S, Weissgerber P, Sausbier M, Bohn E, Autenrieth IB, Ruth P, Luft FC, Nauseef WM, Kettritz R. BK channels in innate immune functions of neutrophils and macrophages. *Blood* 113: 1326–1331, 2009.
116. Evanson KW, Bannister JP, Leo MD, Jaggar JH. LRRC26 is a functional BK channel auxiliary gamma subunit in arterial smooth muscle cells. *Circ Res* 115: 423–431, 2014.
117. Faber ES, Sah P. Physiological role of calcium-activated potassium currents in the rat lateral amygdala. *J Neurosci* 22: 1618–1628, 2002.
118. Fahanik-Babaei J, Eliassi A, Jafari A, Sauve R, Salari S, Saghir R. Electro-pharmacological profile of a mitochondrial inner membrane big-potassium channel from rat brain. *Biochim Biophys Acta* 1808: 454–460, 2011.
119. Fahanik-Babaei J, Eliassi A, Saghir R. How many types of large conductance Ca^{2+} -activated potassium channels exist in brain mitochondrial inner membrane: evidence for a new mitochondrial large conductance Ca^{2+} -activated potassium channel in brain mitochondria. *Neuroscience* 199: 125–132, 2011.
120. Farajnia S, Meijer JH, Michel S. Age-related changes in large-conductance calcium-activated potassium channels in mammalian circadian clock neurons. *Neurobiol Aging* 36: 2176–2183, 2015.
121. Feinberg-Zadek PL, Martin G, Treistman SN. BK channel subunit composition modulates molecular tolerance to ethanol. *Alcohol Clin Exp Res* 32: 1207–1216, 2008.
122. Fernandez-Fernandez JM, Tomas M, Vazquez E, Orio P, Latorre R, Senti M, Marrugat J, Valverde MA. Gain-of-function mutation in the KCNMB1 potassium channel subunit is associated with low prevalence of diastolic hypertension. *J Clin Invest* 113: 1032–1039, 2004.
123. Fernandez JM, Bezanilla F, Taylor RE. Distribution and kinetics of membrane dielectric polarization. II. Frequency domain studies of gating currents. *J Gen Physiol* 79: 41–67, 1982.
124. Ferrer J, Wasson J, Salkoff L, Permutt MA. Cloning of human pancreatic islet large conductance Ca^{2+} -activated K^{+} channel (hSlo) cDNAs: evidence for high levels of expression in pancreatic islets and identification of a flanking genetic marker. *Diabetologia* 39: 891–898, 1996.
125. Fettiplace R, Fuchs PA. Mechanisms of hair cell tuning. *Annu Rev Physiol* 61: 809–834, 1999.
126. Findlay I, Dunne MJ, Petersen OH. High-conductance K^{+} channel in pancreatic islet cells can be activated and inactivated by internal calcium. *J Membr Biol* 83: 169–175, 1985.
127. Fischer HS, Saria A. Voltage-gated, margatoxin-sensitive potassium channels, but not calcium-gated, iberiotoxin-sensitive potassium channels modulate acetylcholine release in rat striatal slices. *Neurosci Lett* 263: 208–210, 1999.
128. Fodor AA, Aldrich RW. Convergent evolution of alternative splices at domain boundaries of the BK channel. *Annu Rev Physiol* 71: 19–36, 2009.
129. Friedman E, Moran DS, Ben-Avraham D, Yanovich R, Atzmon G. Novel candidate genes putatively involved in stress fracture predisposition detected by whole-exome sequencing. *Genet Res* 96: e004, 2014.
130. Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, Fleming T, Parpia T, Burgess G, Branzi A, Grimminger F, Kurzyna M, Simonneau G, Sildenafil Use in Pulmonary Arterial Hypertension Study Group. Sildenafil citrate therapy for pulmonary arterial hypertension. *N Engl J Med* 353: 2148–2157, 2005.
131. Galvez A, Gimenez-Gallego G, Reuben JP, Roy-Contancin L, Feigenbaum P, Kaczorowski GJ, Garcia ML. Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. *J Biol Chem* 265: 11083–11090, 1990.
132. Gallin EK. Calcium- and voltage-activated potassium channels in human macrophages. *Biophys J* 46: 821–825, 1984.
133. Gan G, Yi H, Chen M, Sun L, Li W, Wu Y, Ding J. Structural basis for toxin resistance of beta4-associated calcium-activated potassium (BK) channels. *J Biol Chem* 283: 24177–24184, 2008.
134. Gandhi CS, Olcese R. The voltage-clamp fluorometry technique. *Methods Mol Biol* 491: 213–231, 2008.
135. Garcia-Calvo M, Knaus HG, McManus OB, Giangiacomo KM, Kaczorowski GJ, Garcia ML. Purification and reconstitution of the high-conductance, calcium-activated potassium channel from tracheal smooth muscle. *J Biol Chem* 269: 676–682, 1994.
136. Garcia-Valdes J, Zamudio FZ, Toro L, Possani LD. SloTx1.1, a new scorpion peptide blocker of MaxiK channels that differentiates between alpha and alpha+beta (beta1 or beta4) complexes. *FEBS Lett* 505: 369–373, 2001.
137. Garcia ML, Giangiacomo KM, Hanner M, Knaus HG, McManus OB, Schmalhofer WA, Kaczorowski GJ. Purification and functional reconstitution of high-conductance calcium-activated potassium channel from smooth muscle. *Methods Enzymol* 294: 274–287, 1999.
138. Gaspar T, Domoki F, Lenti L, Katakam PV, Snipes JA, Bari F, Busija DW. Immediate neuronal preconditioning by NS1619. *Brain Res* 1285: 196–207, 2009.
139. Geng Y, Niu X, Magleby KL. Low resistance, large dimension entrance to the inner cavity of BK channels determined by changing side-chain volume. *J Gen Physiol* 137: 533–548, 2011.
140. Gessner G, Cui YM, Otani Y, Ohwada T, Soom M, Hoshi T, Heinemann SH. Molecular mechanism of pharmacological activation of BK channels. *Proc Natl Acad Sci USA* 109: 3552–3557, 2012.
141. Gessner G, Schonherr K, Soom M, Hansel A, Asim M, Baniahmad A, Derst C, Hoshi T, Heinemann SH. BKCa channels activating at resting potential without calcium in LNCaP prostate cancer cells. *J Membr Biol* 208: 229–240, 2005.

142. Ghatta S, Nimmagadda D, Xu X, O'Rourke ST. Large-conductance, calcium-activated potassium channels: structural and functional implications. *Pharmacol Ther* 110: 103–116, 2006.
143. Giangiacomo KM, Becker J, Garsky C, Schmalhofer W, Garcia ML, Mullmann TJ. Novel alpha-KTx sites in the BK channel and comparative sequence analysis reveal distinguishing features of the BK and KV channel outer pore. *Cell Biochem Biophys* 52: 47–58, 2008.
144. Giangiacomo KM, Garcia ML, McManus OB. Mechanism of iberiotoxin block of the large-conductance calcium-activated potassium channel from bovine aortic smooth muscle. *Biochemistry* 31: 6719–6727, 1992.
145. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO 3rd. Acute vascular effects of estrogen in postmenopausal women. *Circulation* 90: 786–791, 1994.
146. Gittis AH, Moghadam SH, du Lac S. Mechanisms of sustained high firing rates in two classes of vestibular nucleus neurons: differential contributions of resurgent Na, Kv3, and BK currents. *J Neurophysiol* 104: 1625–1634, 2010.
147. Glauser DA, Johnson BE, Aldrich RW, Goodman MB. Intragenic alternative splicing coordination is essential for *Caenorhabditis elegans* slo-1 gene function. *Proc Natl Acad Sci USA* 108: 20790–20795, 2011.
148. Goldklang MP, Perez-Zoghbi JF, Trischler J, Nkyimbangi T, Zakharov SI, Shiomi T, Zelonina T, Marks AR, D'Armiento JM, Marx SO. Treatment of experimental asthma using a single small molecule with anti-inflammatory and BK channel-activating properties. *FASEB J* 27: 4975–4986, 2013.
149. Gonzalez-Corrochano R, La Fuente J, Cuevas P, Fernandez A, Chen M, Saenz de Tejada I, Angulo J. Ca^{2+} -activated K^{+} channel (KCa) stimulation improves relaxant capacity of PDES inhibitors in human penile arteries and recovers the reduced efficacy of PDES inhibition in diabetic erectile dysfunction. *Br J Pharmacol* 169: 449–461, 2013.
150. Gonzalez-Perez V, Xia XM, Lingle CJ. Functional regulation of BK potassium channels by gamma1 auxiliary subunits. *Proc Natl Acad Sci USA* 111: 4868–4873, 2014.
151. Gonzalez-Perez V, Xia XM, Lingle CJ. Two classes of regulatory subunits coassemble in the same BK channel and independently regulate gating. *Nat Commun* 6: 8341, 2015.
152. Gonzalez-Perez V, Zeng XH, Henzler-Wildman K, Lingle CJ. Stereospecific binding of a disordered peptide segment mediates BK channel inactivation. *Nature* 485: 133–136, 2012.
153. Gore VK, Ma VV, Yin R, Ligutti J, Immke D, Doherty EM, Norman MH. Structure-activity relationship (SAR) investigations of tetrahydroquinolines as BKCa agonists. *Bioorg Med Chem Lett* 20: 3573–3578, 2010.
154. Green DJ, Gillette R. Circadian rhythm of firing rate recorded from single cells in the rat suprachiasmatic brain slice. *Brain Res* 245: 198–200, 1982.
155. Gribkoff VK, Lum-Ragan JT, Boissard CG, Post-Munson DJ, Meanwell NA, Starrett JE Jr, Kozlowski ES, Romine JL, Trojnecki JT, McKay MC, Zhong J, Dworetzky SI. Effects of channel modulators on cloned large-conductance calcium-activated potassium channels. *Mol Pharmacol* 50: 206–217, 1996.
156. Griguoli M, Sgritta M, Cherubini E. Presynaptic BK channels control transmitter release: Physiological relevance and potential therapeutic implications. *J Physiol* 594: 3489–3500, 2016.
157. Grimm PR, Irsik DL, Settles DC, Holtzclaw JD, Sansom SC. Hypertension of *Knmb1*^{−/−} is linked to deficient K secretion and aldosteronism. *Proc Natl Acad Sci USA* 106: 11800–11805, 2009.
158. Grimm PR, Sansom SC. BK channels and a new form of hypertension. *Kidney Int* 78: 956–962, 2010.
159. Grunnet M, Kaufmann WA. Coassembly of big conductance Ca^{2+} -activated K^{+} channels and L-type voltage-gated Ca^{2+} channels in rat brain. *J Biol Chem* 279: 36445–36453, 2004.
160. Gruslova A, Semenov I, Wang B. An extracellular domain of the accessory beta1 subunit is required for modulating BK channel voltage sensor and gate. *J Gen Physiol* 139: 57–67, 2012.
161. Gu N, Vervaeke K, Storm JF. BK potassium channels facilitate high-frequency firing and cause early spike frequency adaptation in rat CA1 hippocampal pyramidal cells. *J Physiol* 580: 859–882, 2007.
162. Gu XQ, Pamerter ME, Siemen D, Sun X, Haddad GG. Mitochondrial but not plasmalemmal BK channels are hypoxia-sensitive in human glioma. *Glia* 62: 504–513, 2014.
163. Gu XQ, Siemen D, Parvez S, Cheng Y, Xue J, Zhou D, Sun X, Jonas EA, Haddad GG. Hypoxia increases BK channel activity in the inner mitochondrial membrane. *Biochem Biophys Res Commun* 358: 311–316, 2007.
164. Ha TS, Heo MS, Park CS. Functional effects of auxiliary beta4-subunit on rat large-conductance Ca^{2+} -activated K^{+} channel. *Biophys J* 86: 2871–2882, 2004.
165. Ha TS, Jeong SY, Cho SW, Jeon H, Roh GS, Choi WS, Park CS. Functional characteristics of two BKCa channel variants differentially expressed in rat brain tissues. *Eur J Biochem* 267: 910–918, 2000.
166. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 391: 85–100, 1981.
167. Hanaoka K, Wright JM, Cheglakov IB, Morita T, Guggino WB. A 59 amino acid insertion increases Ca^{2+} sensitivity of rbslo1, a Ca^{2+} -activated K^{+} channel in renal epithelia. *J Membr Biol* 172: 193–201, 1999.
168. Hanner M, Vianna-Jorge R, Kamassah A, Schmalhofer WA, Knaus HG, Kaczorowski GJ, Garcia ML. The beta subunit of the high conductance calcium-activated potassium channel. Identification of residues involved in charybdotoxin binding. *J Biol Chem* 273: 16289–16296, 1998.
169. Harms NV, Toris CB. Current status of unoprostone for the management of glaucoma and the future of its use in the treatment of retinal disease. *Expert Opin Pharmacother* 14: 105–113, 2013.
170. Haug T, Olcese R, Toro L, Stefani E. Regulation of K^{+} flow by a ring of negative charges in the outer pore of BKCa channels. Part II: Neutralization of aspartate 292 reduces long channel openings and gating current slow component. *J Gen Physiol* 124: 185–197, 2004.
171. Haug T, Sigg D, Ciani S, Toro L, Stefani E, Olcese R. Regulation of K^{+} flow by a ring of negative charges in the outer pore of BKCa channels. Part I: Aspartate 292 modulates K^{+} conduction by external surface charge effect. *J Gen Physiol* 124: 173–184, 2004.
172. Heginbotham L, MacKinnon R. The aromatic binding site for tetraethylammonium ion on potassium channels. *Neuron* 8: 483–491, 1992.
173. Hermann A, Erxleben C. Charybdotoxin selectively blocks small Ca-activated K channels in *Aplysia* neurons. *J Gen Physiol* 90: 27–47, 1987.
174. Herrera GM, Heppner TJ, Nelson MT. Regulation of urinary bladder smooth muscle contractions by ryanodine receptors and BK and SK channels. *Am J Physiol Regul Integr Comp Physiol* 279: R60–R68, 2000.
175. Herrington J, Solaro CR, Neely A, Lingle CJ. The suppression of Ca^{2+} - and voltage-dependent outward K^{+} current during mAChR activation in rat adrenal chromaffin cells. *J Physiol* 485: 297–318, 1995.
176. Hicks GA, Marrion NV. Ca^{2+} -dependent inactivation of large conductance Ca^{2+} -activated K^{+} (BK) channels in rat hippocampal neurones produced by pore block from an associated particle. *J Physiol* 508: 721–734, 1998.
177. Hidalgo P, MacKinnon R. Revealing the architecture of a K^{+} channel pore through mutant cycles with a peptide inhibitor. *Science* 268: 307–310, 1995.
178. Hille B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J Gen Physiol* 69: 497–515, 1977.
179. Hille B, Schwarz W. Potassium channels as multi-ion single-file pores. *J Gen Physiol* 72: 409–442, 1978.
180. Hodgkin AL, Keynes RD. The potassium permeability of a giant nerve fibre. *J Physiol* 128: 61–88, 1955.
181. Holtzclaw JD, Cornelius RJ, Hatcher LI, Sansom SC. Coupled ATP and potassium efflux from intercalated cells. *Am J Physiol Renal Physiol* 300: F1319–F1326, 2011.
182. Holtzclaw JD, Grimm PR, Sansom SC. Intercalated cell BK-alpha/beta4 channels modulate sodium and potassium handling during potassium adaptation. *J Am Soc Nephrol* 21: 634–645, 2010.
183. Holtzclaw JD, Grimm PR, Sansom SC. Role of BK channels in hypertension and potassium secretion. *Curr Opin Nephrol Hypertens* 20: 512–517, 2011.

184. Holtzclaw JD, Liu L, Grimm PR, Sansom SC. Shear stress-induced volume decrease in C11-MDCK cells by BK- α /beta4. *Am J Physiol Renal Physiol* 299: F507–F516, 2010.
185. Horrigan FT, Aldrich RW. Allosteric voltage gating of potassium channels. II. Mslo channel gating charge movement in the absence of Ca^{2+} . *J Gen Physiol* 114: 305–336, 1999.
186. Horrigan FT, Aldrich RW. Coupling between voltage sensor activation, Ca^{2+} binding and channel opening in large conductance (BK) potassium channels. *J Gen Physiol* 120: 267–305, 2002.
187. Horrigan FT, Cui J, Aldrich RW. Allosteric voltage gating of potassium channels. I. Mslo ionic currents in the absence of Ca^{2+} . *J Gen Physiol* 114: 277–304, 1999.
188. Hoshi T, Pantazis A, Olcese R. Transduction of voltage and Ca^{2+} signals by Slo1 BK channels. *Physiology* 28: 172–189, 2013.
189. Hoshi T, Tian Y, Xu R, Heinemann SH, Hou S. Mechanism of the modulation of BK potassium channel complexes with different auxiliary subunit compositions by the omega-3 fatty acid DHA. *Proc Natl Acad Sci USA* 110: 4822–4827, 2013.
190. Hoshi T, Wissuwa B, Tian Y, Tajima N, Xu R, Bauer M, Heinemann SH, Hou S. Omega-3 fatty acids lower blood pressure by directly activating large-conductance Ca^{2+} -dependent K^{+} channels. *Proc Natl Acad Sci USA* 110: 4816–4821, 2013.
191. Hoshi T, Xu R, Hou S, Heinemann SH, Tian Y. A point mutation in the human Slo1 channel that impairs its sensitivity to omega-3 docosahexaenoic acid. *J Gen Physiol* 142: 507–522, 2013.
192. Hoshi T, Zagotta WN, Aldrich RW. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science* 250: 533–538, 1990.
193. Hristov KL, Afeli SA, Parajuli SP, Cheng Q, Rovner ES, Petkov GV. Neurogenic detrusor overactivity is associated with decreased expression and function of the large conductance voltage- and Ca^{2+} -activated K^{+} channels. *PLoS One* 8: e68052, 2013.
194. Hristov KL, Smith AC, Parajuli SP, Malysz J, Petkov GV. Large-conductance voltage- and Ca^{2+} -activated K^{+} channel regulation by protein kinase C in guinea pig urinary bladder smooth muscle. *Am J Physiol Cell Physiol* 306: C460–C470, 2014.
195. Hu H, Shao LR, Chavoshy S, Gu N, Trieb M, Behrens R, Laake P, Pongs O, Knaus HG, Ottersen OP, Storm JF. Presynaptic Ca^{2+} -activated K^{+} channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *J Neurosci* 21: 9585–9597, 2001.
196. Hu L, Shi J, Ma Z, Krishnamoorthy G, Sieling F, Zhang G, Horrigan FT, Cui J. Participation of the S4 voltage sensor in the Mg^{2+} -dependent activation of large conductance (BK) K^{+} channels. *Proc Natl Acad Sci USA* 100: 10488–10493, 2003.
197. Hu Y, Yang G, Xiao X, Liu L, Li T. Bkca opener, NS1619 pretreatment protects against shock-induced vascular hyporeactivity through PDZ-Rho GEF-RhoA-Rho kinase pathway in rats. *J Trauma Acute Care Surg* 76: 394–401, 2014.
198. Huang CW, Huang CC, Wu SN. Activation by zonisamide, a newer antiepileptic drug, of large-conductance calcium-activated potassium channel in differentiated hippocampal neuron-derived H19-7 cells. *J Pharmacol Exp Ther* 321: 98–106, 2007.
199. Hull CA, Chu Y, Thanawala M, Regehr WG. Hyperpolarization induces a long-term increase in the spontaneous firing rate of cerebellar Golgi cells. *J Neurosci* 33: 5895–5902, 2013.
200. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. *J Mol Graphics* 14: 33–38, 385895–27, 1996.
201. Hurley BR, Preiksaitis HG, Sims SM. Characterization and regulation of Ca^{2+} -dependent K^{+} channels in human esophageal smooth muscle. *Am J Physiol Gastrointest Liver Physiol* 276: G843–G852, 1999.
202. Hyde HC, Sandtner W, Vargas E, Dagcan AT, Robertson JL, Roux B, Correa AM, Bezanilla F. Nano-positioning system for structural analysis of functional homomeric proteins in multiple conformations. *Structure* 20: 1629–1640, 2012.
203. Imlach WL, Finch SC, Dunlop J, Dalziel JE. Structural determinants of lolitrem for inhibition of BK large conductance Ca^{2+} -activated K^{+} channels. *Eur J Pharmacol* 605: 36–45, 2009.
204. Indriati DW, Kamasawa N, Matsui K, Meredith AL, Watanabe M, Shigemoto R. Quantitative localization of Cav2.1 (P/Q-type) voltage-dependent calcium channels in Purkinje cells: somatodendritic gradient and distinct somatic co-clustering with calcium-activated potassium channels. *J Neurosci* 33: 3668–3678, 2013.
205. Islas LD, Sigworth FJ. Voltage sensitivity and gating charge in Shaker and Shab family potassium channels. *J Gen Physiol* 114: 723–742, 1999.
206. Jaffe DB, Wang B, Brenner R. Shaping of action potentials by type I and type II large-conductance Ca^{2+} -activated K^{+} channels. *Neuroscience* 192: 205–218, 2011.
207. Javaherian AD, Yusifov T, Pantazis A, Franklin S, Gandhi CS, Olcese R. Metal-driven operation of the human large-conductance voltage- and Ca^{2+} -dependent potassium channel (BK) gating ring apparatus. *J Biol Chem* 286: 20701–20709, 2011.
208. Ji YH, Wang WX, Ye JG, He LL, Li YJ, Yan YP, Zhou Z. Martenotoxin, a novel K^{+} -channel-blocking peptide: purification, cDNA and genomic cloning, and electrophysiological and pharmacological characterization. *J Neurochem* 84: 325–335, 2003.
209. Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. The open pore conformation of potassium channels. *Nature* 417: 523–526, 2002.
210. Jiang Y, Pico A, Cadene M, Chait BT, MacKinnon R. Structure of the RCK domain from the *E. coli* K^{+} channel and demonstration of its presence in the human BK channel. *Neuron* 29: 593–601, 2001.
211. Johnson BA, Sugg EE. Determination of the three-dimensional structure of iberitoxin in solution by ¹H nuclear magnetic resonance spectroscopy. *Biochemistry* 31: 8151–8159, 1992.
212. Johnson BE, Glauser DA, Dan-Glauser ES, Halling DB, Aldrich RW, Goodman MB. Alternatively spliced domains interact to regulate BK potassium channel gating. *Proc Natl Acad Sci USA* 108: 20784–20789, 2011.
213. Jones EM, Gray-Keller M, Art JJ, Fettiplace R. The functional role of alternative splicing of Ca^{2+} -activated K^{+} channels in auditory hair cells. *Ann NY Acad Sci* 868: 379–385, 1999.
214. Jones EM, Gray-Keller M, Fettiplace R. The role of Ca^{2+} -activated K^{+} channel spliced variants in the tonotopic organization of the turtle cochlea. *J Physiol* 518: 653–665, 1999.
215. Jones EM, Laus C, Fettiplace R. Identification of Ca^{2+} -activated K^{+} channel splice variants and their distribution in the turtle cochlea. *Proc Biol Sci* 265: 685–692, 1998.
216. Judge SI, Bever CT Jr. Potassium channel blockers in multiple sclerosis: neuronal Kv channels and effects of symptomatic treatment. *Pharmacol Ther* 111: 224–259, 2006.
217. Kaczorowski GJ, Garcia ML. Pharmacology of voltage-gated and calcium-activated potassium channels. *Curr Opin Chem Biol* 3: 448–458, 1999.
218. Kathiresan T, Harvey M, Orchard S, Sakai Y, Sokolowski B. A protein interaction network for the large conductance Ca^{2+} -activated K^{+} channel in the mouse cochlea. *Mol Cell Proteomics* 8: 1972–1987, 2009.
219. Kelley-Hedgpeeth A, Peter I, Kip K, Montefusco M, Kogan S, Cox D, Ordovas J, Levy D, Reis S, Mendelsohn M, Housman D, Huggins G. The protective effect of KCNMB1 E65K against hypertension is restricted to blood pressure treatment with beta-blockade. *J Hum Hypertens* 22: 512–515, 2008.
220. Khaitan D, Sankpal UT, Weksler B, Meister EA, Romero IA, Couraud PO, Ningaraj NS. Role of KCNMA1 gene in breast cancer invasion and metastasis to brain. *BMC Cancer* 9: 258, 2009.
221. Kim HM, Oh SC, Lim KJ, Kasamatsu J, Heo JY, Park BS, Lee H, Yoo OJ, Kasahara M, Lee JO. Structural diversity of the hagfish variable lymphocyte receptors. *J Biol Chem* 282: 6726–6732, 2007.
222. Kimm T, Khaliq ZM, Bean BP. Differential regulation of action potential shape and burst-frequency firing by BK and Kv2 channels in substantia nigra dopaminergic neurons. *J Neurosci* 35: 16404–16417, 2015.
223. King JT, Lovell PV, Rishniw M, Kotlikoff MJ, Zeeman ML, McCobb DP. Beta2 and beta4 subunits of BK channels confer differential sensitivity to acute modulation by steroid hormones. *J Neurophysiol* 95: 2878–2888, 2006.
224. Kiraly I, Pataricza J, Bajory Z, Simonsen U, Varro A, Papp JG, Pajor L, Kun A. Involvement of large-conductance Ca^{2+} -activated K^{+} channels in both nitric oxide and endothelium-derived hyperpolarization-type relaxation in human penile small arteries. *Basic Clin Pharmacol Toxicol* 113: 19–24, 2013.
225. Kirby RW, Martelli A, Calderone V, McKay NG, Lawson K. Large conductance Ca^{2+} -activated K^{+} channel (BKCa) activating properties of a series of novel N-arylbenzamides: channel subunit dependent effects. *Bioorg Med Chem* 21: 4186–4191, 2013.

226. Knaus HG, Folander K, Garcia-Calvo M, Garcia ML, Kaczorowski GJ, Smith M, Swanson R. Primary sequence and immunological characterization of beta-subunit of high conductance Ca^{2+} -activated K^{+} channel from smooth muscle. *J Biol Chem* 269: 17274–17278, 1994.
227. Knaus HG, Garcia-Calvo M, Kaczorowski GJ, Garcia ML. Subunit composition of the high conductance calcium-activated potassium channel from smooth muscle, a representative of the mSlo and slowpoke family of potassium channels. *J Biol Chem* 269: 3921–3924, 1994.
228. Knaus HG, McManus OB, Lee SH, Schmalhofer WA, Garcia-Calvo M, Helms LM, Sanchez M, Giangiacomo K, Reuben JP, Smith AB 3rd. Tremorgenic indole alkaloids potentially inhibit smooth muscle high-conductance calcium-activated potassium channels. *Biochemistry* 33: 5819–5828, 1994.
229. Komuro H, Rakic P. Intracellular Ca^{2+} fluctuations modulate the rate of neuronal migration. *Neuron* 17: 275–285, 1996.
230. Koval OM, Fan Y, Rothberg BS. A role for the S0 transmembrane segment in voltage-dependent gating of BK channels. *J Gen Physiol* 129: 209–220, 2007.
231. Krezel AM, Kasibhatla C, Hidalgo P, MacKinnon R, Wagner G. Solution structure of the potassium channel inhibitor agitoxin 2: caliper for probing channel geometry. *Protein Sci* 4: 1478–1489, 1995.
232. Kukuljan M, Taylor A, Chouinard H, Olguin P, Rojas CV, Ribera AB. Selective regulation of α Slo splice variants during *Xenopus* embryogenesis. *J Neurophysiol* 90: 3352–3360, 2003.
233. Kun A, Matchkov VV, Stankevicius E, Nardi A, Hughes AD, Kirkeby HJ, Demnitz J, Simonsen U. NS11021, a novel opener of large-conductance Ca^{2+} -activated K^{+} channels, enhances erectile responses in rats. *Br J Pharmacol* 158: 1465–1476, 2009.
234. Kunzelmann K. Ion channels and cancer. *J Membr Biol* 205: 159–173, 2005.
235. Kurt S, Sausbier M, Rüttiger L, Brandt N, Moeller CK, Kindler J, Sausbier U, Zimmermann U, van Straaten H, Neuhuber W, Engel J, Knipper M, Ruth P, Schulze H. Critical role for cochlear hair cell BK channels for coding the temporal structure and dynamic range of auditory information for central auditory processing. *FASEB J* 26: 3834–3843, 2012.
236. Kwon SH, Guggino WB. Multiple sequences in the C terminus of MaxiK channels are involved in expression, movement to the cell surface, and apical localization. *Proc Natl Acad Sci USA* 101: 15237–15242, 2004.
237. La Fuente JM, Fernandez A, Cuevas P, Gonzalez-Corrochano R, Chen MX, Angulo J. Stimulation of large-conductance calcium-activated potassium channels inhibits neurogenic contraction of human bladder from patients with urinary symptoms and reverses acetic acid-induced bladder hyperactivity in rats. *Eur J Pharmacol* 735: 68–76, 2014.
238. Lagrutta A, Shen KZ, North RA, Adelman JP. Functional differences among alternatively spliced variants of Slowpoke, a *Drosophila* calcium-activated potassium channel. *J Biol Chem* 269: 20347–20351, 1994.
239. Lancaster B, Nicoll RA. Properties of two calcium-activated hyperpolarizations in rat hippocampal neurones. *J Physiol* 389: 187–203, 1987.
240. Langer P, Grunder S, Rusch A. Expression of Ca^{2+} -activated BK channel mRNA and its splice variants in the rat cochlea. *J Comp Neurol* 455: 198–209, 2003.
241. Latorre R, Brauchi S. Large conductance Ca^{2+} -activated K^{+} (BK) channel: activation by Ca^{2+} voltage. *Biol Res* 39: 385–401, 2006.
242. Latorre R, Miller C. Conduction and selectivity in potassium channels. *J Membr Biol* 71: 11–30, 1983.
243. Latorre R, Morera FJ, Zaelzer C. Allosteric interactions and the modular nature of the voltage- and Ca^{2+} -activated (BK) channel. *J Physiol* 588: 3141–3148, 2010.
244. Latorre R, Oberhauser A, Labarca P, Alvarez O. Varieties of calcium-activated potassium channels. *Annu Rev Physiol* 51: 385–399, 1989.
245. Latorre R, Vergara C, Hidalgo C. Reconstitution in planar lipid bilayers of a Ca^{2+} -dependent K^{+} channel from transverse tubule membranes isolated from rabbit skeletal muscle. *Proc Natl Acad Sci USA* 79: 805–809, 1982.
246. Layne JJ, Nausch B, Olesen SP, Nelson MT. BK channel activation by NS11021 decreases excitability and contractility of urinary bladder smooth muscle. *Am J Physiol Regul Integr Comp Physiol* 298: R378–R384, 2010.
247. Lee US, Cui J. β -Subunit-specific modulations of BK channel function by a mutation associated with epilepsy and dyskinesia. *J Physiol* 587: 1481–1498, 2009.
248. Lerche H, Fahlke C, Iuzzo PA, Lehmann-Horn F. Characterization of the high-conductance Ca^{2+} -activated K^{+} channel in adult human skeletal muscle. *Pflügers Arch* 429: 738–747, 1995.
249. Li B, Jie W, Huang L, Wei P, Li S, Luo Z, Friedman AK, Meredith AL, Han MH, Zhu XH, Gao TM. Nuclear BK channels regulate gene expression via the control of nuclear calcium signaling. *Nat Neurosci* 17: 1055–1063, 2014.
250. Li Q, Fan F, Kwak HR, Yan J. Molecular basis for differential modulation of BK channel voltage-dependent gating by auxiliary gamma subunits. *J Gen Physiol* 145: 543–554, 2015.
251. Li Q, Guan X, Yen K, Zhang J, Yan J. The single transmembrane segment determines the modulatory function of the BK channel auxiliary gamma subunit. *J Gen Physiol* 147: 337–351, 2016.
252. Li W, Aldrich RW. State-dependent block of BK channels by synthesized shaker ball peptides. *J Gen Physiol* 128: 423–441, 2006.
253. Li W, Aldrich RW. Unique inner pore properties of BK channels revealed by quaternary ammonium block. *J Gen Physiol* 124: 43–57, 2004.
254. Li ZW, Ding JP, Kalyanaraman V, Lingle CJ. RINm5f cells express inactivating BK channels whereas HIT cells express noninactivating BK channels. *J Neurophysiol* 81: 611–624, 1999.
255. Lingle CJ, Zeng XH, Ding JP, Xia XM. Inactivation of BK channels mediated by the NH_2 terminus of the beta3b auxiliary subunit involves a two-step mechanism: possible separation of binding and blockade. *J Gen Physiol* 117: 583–606, 2001.
256. Lippat JD, Standen NB, Davies NW. A residue in the intracellular vestibule of the pore is critical for gating and permeation in Ca^{2+} -activated K^{+} (BKCa) channels. *J Physiol* 529: 131–138, 2000.
257. Lippat JD, Standen NB, Harrow ID, Phillips SC, Davies NW. Properties of BK(Ca) channels formed by bicistronic expression of hSloalpha and beta1-4 subunits in HEK293 cells. *J Membr Biol* 192: 141–148, 2003.
258. Liu G, Niu X, Wu RS, Chudasama N, Yao Y, Jin X, Weinberg R, Zakharov SI, Motoike H, Marx SO, Karlin A. Location of modulatory beta subunits in BK potassium channels. *J Gen Physiol* 135: 449–459, 2010.
259. Liu G, Zakharov SI, Yang L, Wu RS, Deng SX, Landry DW, Karlin A, Marx SO. Locations of the beta1 transmembrane helices in the BK potassium channel. *Proc Natl Acad Sci USA* 105: 10727–10732, 2008.
260. Liu H, Xin T, He W, Li F, Su ZQ. Myelinated Ah-type trigeminal ganglion neurons in female rats: neuroexcitability, chemosensitivity to histamine, and potential clinical impact. *Neurosci Lett* 567: 74–79, 2014.
261. Liu R, Zhang Z, Liu H, Hou P, Lang J, Wang S, Yan H, Li P, Huang Z, Wu H, Rong M, Huang J, Wang H, Lv L, Qiu M, Ding J, Lai R. Human beta-defensin 2 is a novel opener of Ca^{2+} -activated potassium channels and induces vasodilation and hypotension in monkeys. *Hypertension* 62: 415–425, 2013.
262. Liu W, Morimoto T, Woda C, Kleymann TR, Satlin LM. Ca^{2+} dependence of flow-stimulated K secretion in the mammalian cortical collecting duct. *Am J Physiol Renal Physiol* 293: F227–F235, 2007.
263. Liu W, Murcia NS, Duan Y, Weinbaum S, Yoder BK, Schwiebert E, Satlin LM. Mechanoregulation of intracellular Ca^{2+} concentration is attenuated in collecting duct of monocular-impaired orpk mice. *Am J Physiol Renal Physiol* 289: F978–F988, 2005.
264. Liu X, Chang Y, Reinhart PH, Sontheimer H, Chang Y. Cloning and characterization of glioma BK, a novel BK channel isoform highly expressed in human glioma cells. *J Neurosci* 22: 1840–1849, 2002.
265. Liu YC, Lo YK, Wu SN. Stimulatory effects of chlorzoxazone, a centrally acting muscle relaxant, on large conductance calcium-activated potassium channels in pituitary GH3 cells. *Brain Res* 959: 86–97, 2003.
266. Long SB, Campbell EB, MacKinnon R. Crystal structure of a mammalian voltage-dependent Shaker family K^{+} channel. *Science* 309: 897–903, 2005.
267. Long SB, Tao X, Campbell EB, MacKinnon R. Atomic structure of a voltage-dependent K^{+} channel in a lipid membrane-like environment. *Nature* 450: 376–382, 2007.

268. Lopez-Barneo J, Hoshi T, Heinemann SH, Aldrich RW. Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels. *Receptors Channels* 1: 61–71, 1993.
269. Lorenz S, Heils A, Kasper JM, Sander T. Allelic association of a truncation mutation of the KCNMB3 gene with idiopathic generalized epilepsy. *Am J Med Genet B Neuropsychiatr Genet* 144B: 10–13, 2007.
270. Lovell PV, McCobb DP. Pituitary control of BK potassium channel function and intrinsic firing properties of adrenal chromaffin cells. *J Neurosci* 21: 3429–3442, 2001.
271. Lu R, Alioua A, Kumar Y, Eghbali M, Stefani E, Toro L. MaxiK channel partners: physiological impact. *J Physiol* 570: 65–72, 2006.
272. Lu R, Lukowski R, Sausbier M, Zhang DD, Sisignano M, Schuh CD, Kuner R, Ruth P, Geisslinger G, Schmidtke A. BKCa channels expressed in sensory neurons modulate inflammatory pain in mice. *Pain* 155: 556–565, 2014.
273. Ma D, Nakata T, Zhang G, Hoshi T, Li M, Shikano S. Differential trafficking of carboxyl isoforms of Ca^{2+} -gated (Slo1) potassium channels. *FEBS Lett* 581: 1000–1008, 2007.
274. Ma Z, Lou XJ, Horrigan FT. Role of charged residues in the S1–S4 voltage sensor of BK channels. *J Gen Physiol* 127: 309–328, 2006.
275. MacKinnon R. Determination of the subunit stoichiometry of a voltage-activated potassium channel. *Nature* 350: 232–235, 1991.
276. MacKinnon R, Aldrich RW, Lee AW. Functional stoichiometry of Shaker potassium channel inactivation. *Science* 262: 757–759, 1993.
277. MacKinnon R, Miller C. Mechanism of charybdotoxin block of the high-conductance, Ca^{2+} -activated K^+ channel. *J Gen Physiol* 91: 335–349, 1988.
278. MacKinnon R, Miller C. Mutant potassium channels with altered binding of charybdotoxin, a pore-blocking peptide inhibitor. *Science* 245: 1382–1385, 1989.
279. Magleby KL. Gating mechanism of BK (Slo1) channels: so near, yet so far. *J Gen Physiol* 121: 81–96, 2003.
280. Magleby KL, Pallotta BS. Burst kinetics of single calcium-activated potassium channels in cultured rat muscle. *J Physiol* 344: 605–623, 1983.
281. Magleby KL, Pallotta BS. Calcium dependence of open and shut interval distributions from calcium-activated potassium channels in cultured rat muscle. *J Physiol* 344: 585–604, 1983.
282. Mannuzzu LM, Moronne MM, Isacoff EY. Direct physical measure of conformational rearrangement underlying potassium channel gating. *Science* 271: 213–216, 1996.
283. Mansoor SE, Dewitt MA, Farrens DL. Distance mapping in proteins using fluorescence spectroscopy: the tryptophan-induced quenching (TrIQ) method. *Biochemistry* 49: 9722–9731, 2010.
284. Mansoor SE, McHaourab HS, Farrens DL. Mapping proximity within proteins using fluorescence spectroscopy. A study of T4 lysozyme showing that tryptophan residues quench bimane fluorescence. *Biochemistry* 41: 2475–2484, 2002.
285. Manzanares D, Srinivasan M, Salathe ST, Ivonnet P, Baumlin N, Dennis JS, Conner GE, Salathe M. IFN-gamma-mediated reduction of large-conductance, Ca^{2+} -activated, voltage-dependent K^+ (BK) channel activity in airway epithelial cells leads to mucociliary dysfunction. *Am J Physiol Lung Cell Mol Physiol* 306: L453–L462, 2014.
286. Martin GE, Hendrickson LM, Penta KL, Friesen RM, Pietrzykowski AZ, Tapper AR, Treisman SN. Identification of a BK channel auxiliary protein controlling molecular and behavioral tolerance to alcohol. *Proc Natl Acad Sci USA* 105: 17543–17548, 2008.
287. Martinez-Espinoza PL, Yang C, Gonzalez-Perez V, Xia XM, Lingle CJ. Knockout of the BK beta2 subunit abolishes inactivation of BK currents in mouse adrenal chromaffin cells and results in slow-wave burst activity. *J Gen Physiol* 144: 275–295, 2014.
288. Marty A. Ca^{2+} -dependent K channels with large unitary conductance in chromaffin cell membranes. *Nature* 291: 497–500, 1981.
289. Mathialahan T, MacLennan KA, Sandle LN, Verbeke C, Sandle GI. Enhanced large intestinal potassium permeability in end-stage renal disease. *J Pathol* 206: 46–51, 2005.
290. McCobb DP, Featherston T, Lingle CL, Saito M, Krause JE, Salkoff L. A human calcium-activated potassium channel gene expressed in vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 269: H767–H777, 1995.
291. McCobb DP, Fowler NL, Featherstone T, Lingle CJ, Saito M, Krause JE, Salkoff L. A human calcium-activated potassium channel gene expressed in vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 269: H767–H777, 1995.
292. McManus OB. Calcium-activated potassium channels: regulation by calcium. *J Bioenerg Biomembr* 23: 537–560, 1991.
293. McManus OB, Blatz AL, Magleby KL. Inverse relationship of the durations of adjacent open and shut intervals for Ca^{2+} and K channels. *Nature* 317: 625–627, 1985.
294. McManus OB, Harris GH, Giangiacomo KM, Feigenbaum P, Reuben JP, Addy ME, Burka JF, Kaczorowski GJ, Garcia ML. An activator of calcium-dependent potassium channels isolated from a medicinal herb. *Biochemistry* 32: 6128–6133, 1993.
295. McManus OB, Helms LM, Pallanck L, Ganetzky B, Swanson R, Leonard RJ. Functional role of the beta subunit of high conductance calcium-activated potassium channels. *Neuron* 14: 645–650, 1995.
296. McManus OB, Magleby KL. Accounting for the Ca^{2+} -dependent kinetics of single large-conductance Ca^{2+} -activated K^+ channels in rat skeletal muscle. *J Physiol* 443: 739–777, 1991.
297. McManus OB, Magleby KL. Accounting for the Ca^{2+} -dependent kinetics of single large-conductance Ca^{2+} -activated K^+ channels in rat skeletal muscle. *J Physiol* 443: 739–777, 1991.
298. McManus OB, Magleby KL. Kinetic states and modes of single large-conductance calcium-activated potassium channels in cultured rat skeletal muscle. *J Physiol* 402: 79–120, 1988.
299. Meech RW. Calcium-dependent potassium activation in nervous tissues. *Annu Rev Biophys Bioeng* 7: 1–18, 1978.
300. Meech RW. Intracellular calcium injection causes increased potassium conductance in *Aplysia* nerve cells. *Comp Biochem Physiol A Comp Physiol* 42: 493–499, 1972.
301. Meech RW. The sensitivity of *Helix aspersa* neurones to injected calcium ions. *J Physiol* 237: 259–277, 1974.
302. Meera P, Wallner M, Jiang Z, Toro L. A calcium switch for the functional coupling between alpha (hsl) and beta subunits (Kv, cabeta) of maxi K channels. *FEBS Lett* 385: 127–128, 1996.
303. Meera P, Wallner M, Toro L. A neuronal beta subunit (KCNMB4) makes the large conductance, voltage- and Ca^{2+} -activated K^+ channel resistant to charybdotoxin and iberiotoxin. *Proc Natl Acad Sci USA* 97: 5562–5567, 2000.
304. Meredith AL, Thornehoe KS, Werner ME, Nelson MT, Aldrich RW. Overactive bladder and incontinence in the absence of the BK large conductance Ca^{2+} -activated K^+ channel. *J Biol Chem* 279: 36746–36752, 2004.
305. Meredith AL, Wiler SW, Miller BH, Takahashi JS, Fodor AA, Ruby NF, Aldrich RW. BK calcium-activated potassium channels regulate circadian behavioral rhythms and pacemaker output. *Nat Neurosci* 9: 1041–1049, 2006.
306. Methfessel C, Boheim G. The gating of single calcium dependent potassium channels is described by an activation/blockade mechanism. *Biophys Struct Mech* 9: 35–60, 1982.
307. Miller C. The charybdotoxin family of K^+ channel-blocking peptides. *Neuron* 15: 5–10, 1995.
308. Miller C. Trapping single ions inside single ion channels. *Biophys J* 52: 123–126, 1987.
309. Miller C, Latorre R, Reisin I. Coupling of voltage-dependent gating and Ba^{2+} block in the high-conductance, Ca^{2+} -activated K^+ channel. *J Gen Physiol* 90: 427–449, 1987.
310. Miller C, Moczydlowski E, Latorre R, Phillips M. Charybdotoxin, a protein inhibitor of single Ca^{2+} -activated K^+ channels from mammalian skeletal muscle. *Nature* 313: 316–318, 1985.
311. Miranda-Rottmann S, Kozlov AS, Hudspeth AJ. Highly specific alternative splicing of transcripts encoding BK channels in the chicken's cochlea is a minor determinant of the tonotopic gradient. *Mol Cell Biol* 30: 3646–3660, 2010.
312. Miranda P, Contreras JE, Plested AJ, Sigworth FJ, Holmgren M, Giraldez T. State-dependent FRET reports calcium- and voltage-dependent gating-ring motions in BK channels. *Proc Natl Acad Sci USA* 110: 5217–5222, 2013.

313. Moczyldowski E, Latorre R. Gating kinetics of Ca^{2+} -activated K^{+} channels from rat muscle incorporated into planar lipid bilayers. Evidence for two voltage-dependent Ca^{2+} binding reactions. *J Gen Physiol* 82: 511–542, 1983.
314. Moczyldowski E, Latorre R. Gating kinetics of Ca^{2+} -activated K^{+} channels from rat muscle incorporated into planar lipid bilayers. Evidence for two voltage-dependent Ca^{2+} binding reactions. *J Gen Physiol* 82: 511–542, 1983.
315. Monod J, Wyman J, Changeux JP. On the nature of allosteric transitions: a plausible model. *J Mol Biol* 12: 88–118, 1965.
316. Montgomery JR, Whitt JP, Wright BN, Lai MH, Meredith AL. Mis-expression of the BK K^{+} channel disrupts suprachiasmatic nucleus circuit rhythmicity and alters clock-controlled behavior. *Am J Physiol Cell Physiol* 304: C299–C311, 2013.
317. Morera FJ, Alioua A, Kundu P, Salazar M, Gonzalez C, Martinez AD, Stefani E, Toro L, Latorre R. The first transmembrane domain (TM1) of beta2-subunit binds to the transmembrane domain S1 of alpha-subunit in BK potassium channels. *FEBS Lett* 586: 2287–2293, 2012.
318. Morimoto T, Sakamoto K, Sade H, Ohya S, Muraki K, Imaizumi Y. Voltage-sensitive oxonol dyes are novel large-conductance Ca^{2+} -activated K^{+} channel activators selective for beta1 and beta4 but not for beta2 subunits. *Mol Pharmacol* 71: 1075–1088, 2007.
319. Moss BL, Magleby KL. Gating and conductance properties of BK channels are modulated by the S9–S10 tail domain of the alpha subunit. A study of mSlo1 and mSlo3 wild-type and chimeric channels. *J Gen Physiol* 118: 711–734, 2001.
320. Murrell-Lagnado RD, Aldrich RW. Interactions of amino terminal domains of Shaker K channels with a pore blocking site studied with synthetic peptides. *J Gen Physiol* 102: 949–975, 1993.
321. Mushtaq Y. The COPD pipeline. *Nature Rev Drug Discovery* 13: 253, 2014.
322. N'Gouemo P. Targeting BK (big potassium) channels in epilepsy. *Expert Opin Ther Targets* 15: 1283–1295, 2011.
323. Naranjo D, Miller C. A strongly interacting pair of residues on the contact surface of charybdotoxin and a Shaker K^{+} channel. *Neuron* 16: 123–130, 1996.
324. Nardi A, Calderone V, Chericoni S, Morelli I. Natural modulators of large-conductance calcium-activated potassium channels. *Planta Medica* 69: 885–892, 2003.
325. Nardi A, Olesen SP. BK channel modulators: a comprehensive overview. *Curr Med Chem* 15: 1126–1146, 2008.
326. Nausch B, Rode F, Jorgensen S, Nardi A, Korsgaard MP, Hougaard C, Bonev AD, Brown WD, Dyhring T, Strobaek D, Olesen SP, Christophersen P, Grunnet M, Nelson MT, Ronn LC. NS19504: a novel BK channel activator with relaxing effect on bladder smooth muscle spontaneous phasic contractions. *J Pharmacol Exp Ther* 350: 520–530, 2014.
327. Navaratnam DS, Bell TJ, Tu TD, Cohen EL, Oberholtzer JC. Differential distribution of Ca^{2+} -activated K^{+} channel splice variants among hair cells along the tonotopic axis of the chick cochlea. *Neuron* 19: 1077–1085, 1997.
328. Nelson AB, Krispel CM, Sekirnjak C, du Lac S. Long-lasting increases in intrinsic excitability triggered by inhibition. *Neuron* 40: 609–620, 2003.
329. Neyton J, Miller C. Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high-conductance Ca^{2+} -activated K^{+} channel. *J Gen Physiol* 92: 569–586, 1988.
330. Neyton J, Miller C. Potassium blocks barium permeation through a calcium-activated potassium channel. *J Gen Physiol* 92: 549–567, 1988.
331. Nimigean CM, Chappie JS, Miller C. Electrostatic tuning of ion conductance in potassium channels. *Biochemistry* 42: 9263–9268, 2003.
332. Nimigean CM, Magleby KL. Functional coupling of the beta(1) subunit to the large conductance Ca^{2+} -activated K^{+} channel in the absence of Ca^{2+} . Increased Ca^{2+} sensitivity from a Ca^{2+} -independent mechanism. *J Gen Physiol* 115: 719–736, 2000.
333. Niu X, Qian X, Magleby KL. Linker-gating ring complex as passive spring and Ca^{2+} -dependent machine for a voltage- and Ca^{2+} -activated potassium channel. *Neuron* 42: 745–756, 2004.
334. Oberhauser A, Alvarez O, Latorre R. Activation by divalent cations of a Ca^{2+} -activated K^{+} channel from skeletal muscle membrane. *J Gen Physiol* 92: 67–86, 1988.
335. Ohya S, Kuwata Y, Sakamoto K, Muraki K, Imaizumi Y. Cardioprotective effects of estradiol include the activation of large-conductance Ca^{2+} -activated K^{+} channels in cardiac mitochondria. *Am J Physiol Heart Circ Physiol* 289: H1635–H1642, 2005.
336. Olesen SP, Munch E, Moldt P, Drejer J. Selective activation of Ca^{2+} -dependent K^{+} channels by novel benzimidazolone. *Eur J Pharmacol* 251: 53–59, 1994.
337. Olesen SP, Munch E, Watjen F, Drejer J. NS 004—an activator of Ca^{2+} -dependent K^{+} channels in cerebellar granule cells. *Neuroreport* 5: 1001–1004, 1994.
338. Orio P, Latorre R. Differential effects of beta 1 and beta 2 subunits on BK channel activity. *J Gen Physiol* 125: 395–411, 2005.
339. Orio P, Rojas P, Ferreira G, Latorre R. New disguises for an old channel: MaxiK channel beta-subunits. *News Physiol Sci* 17: 156–161, 2002.
340. Orio P, Torres Y, Rojas P, Carvacho I, Garcia ML, Toro L, Valverde MA, Latorre R. Structural determinants for functional coupling between the beta and alpha subunits in the Ca^{2+} -activated K^{+} (BK) channel. *J Gen Physiol* 127: 191–204, 2006.
341. Pallotta BS. N-bromoacetamide removes a calcium-dependent component of channel opening from calcium-activated potassium channels in rat skeletal muscle. *J Gen Physiol* 86: 601–611, 1985.
342. Pallotta BS, Magleby KL, Barrett JN. Single channel recordings of Ca^{2+} -activated K^{+} currents in rat muscle cell culture. *Nature* 293: 471–474, 1981.
343. Panda S, Antoch MP, Miller BH, Su AI, School AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109: 307–320, 2002.
344. Pantazis A, Gudzenko V, Savalli N, Sigg D, Olcese R. Operation of the voltage sensor of a human voltage- and Ca^{2+} -activated K^{+} channel. *Proc Natl Acad Sci USA* 107: 4459–4464, 2010.
345. Pantazis A, Kohanteb AP, Olcese R. Relative motion of transmembrane segments S0 and S4 during voltage sensor activation in the human BK(Ca) channel. *J Gen Physiol* 136: 645–657, 2010.
346. Pantazis A, Olcese R. Relative transmembrane segment rearrangements during BK channel activation resolved by structurally assigned fluorophore-quencher pairing. *J Gen Physiol* 140: 207–218, 2012.
347. Park CS, Miller C. Interaction of charybdotoxin with permeant ions inside the pore of a K^{+} channel. *Neuron* 9: 307–313, 1992.
348. Perez GJ, Bonev AD, Nelson MT. Micromolar Ca^{2+} from sparks activates Ca^{2+} -sensitive K^{+} channels in rat cerebral artery smooth muscle. *Am J Physiol Cell Physiol* 281: C1769–C1775, 2001.
349. Perez GJ, Desai M, Anderson S, Scornik FS. Large-conductance calcium-activated potassium current modulates excitability in isolated canine intracardiac neurons. *Am J Physiol Cell Physiol* 304: C280–C286, 2013.
350. Petkov GV. Central role of the BK channel in urinary bladder smooth muscle physiology and pathophysiology. *Am J Physiol Regul Integr Comp Physiol* 307: R571–R584, 2014.
351. Petrik D, Brenner R. Regulation of STREX exon large conductance, calcium-activated potassium channels by the beta4 accessory subunit. *Neuroscience* 149: 789–803, 2007.
352. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K. Scalable molecular dynamics with NAMD. *J Computat Chem* 26: 1781–1802, 2005.
353. Pluger S, Faulhaber J, Furstenau M, Lohn M, Waldschutz R, Gollasch M, Haller H, Luft FC, Ehmke H, Pongs O. Mice with disrupted BK channel beta1 subunit gene feature abnormal Ca^{2+} spark/STOC coupling and elevated blood pressure. *Circ Res* 87: E53–60, 2000.
354. Ponte CG, McManus OB, Schmalhofer WA, Shen DM, Dai G, Stevenson A, Sur S, Shah T, Kiss L, Shu M, Doherty JB, Nargund R, Kaczorowski GJ, Suarez-Kurtz G, Garcia ML. Selective, direct activation of high-conductance, calcium-activated potassium channels causes smooth muscle relaxation. *Mol Pharmacol* 81: 567–577, 2012.
355. Posson DJ, McCoy JG, Nimigean CM. The voltage-dependent gate in MthK potassium channels is located at the selectivity filter. *Nature Struct Mol Biol* 20: 159–166, 2013.

356. Posson DJ, Rusinova R, Andersen OS, Nimigean CM. Calcium ions open a selectivity filter gate during activation of the MthK potassium channel. *Nat Commun* 6: 8342, 2015.
357. Poulsen AN, Wulf H, Hay-Schmidt A, Jansen-Olesen I, Olesen J, Klaerke DA. Differential expression of BK channel isoforms and beta-subunits in rat neuro-vascular tissues. *Biochim Biophys Acta* 1788: 380–389, 2009.
358. Pyott SJ, Meredith AL, Fodor AA, Vazquez AE, Yamoah EN, Aldrich RW. Cochlear function in mice lacking the BK channel alpha, beta1, or beta4 subunits. *J Biol Chem* 282: 3312–3324, 2007.
359. Qian X, Magleby KL. Beta1 subunits facilitate gating of BK channels by acting through the Ca^{2+} , but not the Mg^{2+} , activating mechanisms. *Proc Natl Acad Sci USA* 100: 10061–10066, 2003.
360. Qian X, Nimigean CM, Niu X, Moss BL, Magleby KL. Slo1 tail domains, but not the Ca^{2+} bowl, are required for the beta1 subunit to increase the apparent Ca^{2+} sensitivity of BK channels. *J Gen Physiol* 120: 829–843, 2002.
361. Qian X, Niu X, Magleby KL. Intra- and intersubunit cooperativity in activation of BK channels by Ca^{2+} . *J Gen Physiol* 128: 389–404, 2006.
362. Quirk JC, Reinhart PH. Identification of a novel tetramerization domain in large conductance K(Ca) channels. *Neuron* 32: 13–23, 2001.
363. Raffaelli G, Saviane C, Mohajerani MH, Pedarzani P, Cherubini E. BK potassium channels control transmitter release at CA3–CA3 synapses in the rat hippocampus. *J Physiol* 557: 147–157, 2004.
364. Ramanathan K, Michael TH, Jiang GJ, Hiel H, Fuchs PA. A molecular mechanism for electrical tuning of cochlear hair cells. *Science* 283: 215–217, 1999.
365. Ranganathan R, Lewis JH, MacKinnon R. Spatial localization of the K^{+} channel selectivity filter by mutant cycle-based structure analysis. *Neuron* 16: 131–139, 1996.
366. Ransom CB, Liu X, Sontheimer H. BK channels in human glioma cells have enhanced calcium sensitivity. *Glia* 38: 281–291, 2002.
367. Ransom CB, Sontheimer H. BK channels in human glioma cells. *J Neurophysiol* 85: 790–803, 2001.
368. Reinhart PH, Chung S, Levitan IB. A family of calcium-dependent potassium channels from rat brain. *Neuron* 2: 1031–1041, 1989.
369. Revermann M, Neofitidou S, Kirschning T, Schloss M, Brandes RP, Hofstetter C. Inhalation of the BK(Ca)-opener NS1619 attenuates right ventricular pressure and improves oxygenation in the rat monocrotaline model of pulmonary hypertension. *PLoS One* 9: e86636, 2014.
370. Rosenblatt KP, Sun ZP, Heller S, Hudspeth AJ. Distribution of Ca^{2+} -activated K^{+} channel isoforms along the tonotopic gradient of the chicken's cochlea. *Neuron* 19: 1061–1075, 1997.
371. Rothberg BS, Magleby KL. Gating kinetics of single large-conductance Ca^{2+} -activated K^{+} channels in high Ca^{2+} suggest a two-tiered allosteric gating mechanism. *J Gen Physiol* 114: 93–124, 1999.
372. Rothberg BS, Magleby KL. Kinetic structure of large-conductance Ca^{2+} -activated K^{+} channels suggests that the gating includes transitions through intermediate or secondary states. A mechanism for flickers. *J Gen Physiol* 111: 751–780, 1998.
373. Roy S, Morayo Akande A, Large RJ, Webb TJ, Camarasa C, Sergeant GP, McHale NG, Thornbury KD, Hollywood MA. Structure-activity relationships of a novel group of large-conductance Ca^{2+} -activated K^{+} (BK) channel modulators: the GoSlo-SR family. *Chem Med Chem* 7: 1763–1769, 2012.
374. Russ WP, Engelman DM. TOXCAT: a measure of transmembrane helix association in a biological membrane. *Proc Natl Acad Sci USA* 96: 863–868, 1999.
375. Saito M, Nelson C, Salkoff L, Lingle CJ. A cysteine-rich domain defined by a novel exon in a slo variant in rat adrenal chromaffin cells and PC12 cells. *J Biol Chem* 272: 11710–11717, 1997.
376. Sakai Y, Harvey M, Sokolowski B. Identification and quantification of full-length BK channel variants in the developing mouse cochlea. *J Neurosci Res* 89: 1747–1760, 2011.
377. Saleem F, Rowe IC, Shipston MJ. Characterization of BK channel splice variants using membrane potential dyes. *Br J Pharmacol* 156: 143–152, 2009.
378. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol* 234: 779–815, 1993.
379. Samengo I, Curro D, Barrese V, Tagliatela M, Martire M. Large conductance calcium-activated potassium channels: their expression and modulation of glutamate release from nerve terminals isolated from rat trigeminal caudal nucleus and cerebral cortex. *Neurochem Res* 39: 901–910, 2014.
380. Sanchez M, McManus OB. Paxilline inhibition of the alpha-subunit of the high-conductance calcium-activated potassium channel. *Neuropharmacology* 35: 963–968, 1996.
381. Saria A, Seidl CV, Fischer HS, Koch RO, Telser S, Wanner SG, Humpel C, Garcia ML, Knaus HG. Margatoxin increases dopamine release in rat striatum via voltage-gated K^{+} channels. *Eur J Pharmacol* 343: 193–200, 1998.
382. Sausbier M, Hu H, Arntz C, Feil S, Kamm S, Adelsberger H, Sausbier U, Sailer CA, Feil R, Hofmann F, Korth M, Shipston MJ, Knaus HG, Wolfer DP, Pedroarena CM, Storm JF, Ruth P. Cerebellar ataxia and Purkinje cell dysfunction caused by Ca^{2+} -activated K^{+} channel deficiency. *Proc Natl Acad Sci USA* 101: 9474–9478, 2004.
383. Savalli N, Kondratiev A, de Quintana SB, Toro L, Olcese R. Modes of operation of the BKCa channel beta2 subunit. *J Gen Physiol* 130: 117–131, 2007.
384. Savalli N, Kondratiev A, Toro L, Olcese R. Voltage-dependent conformational changes in human Ca^{2+} - and voltage-activated K^{+} channel, revealed by voltage-clamp fluorometry. *Proc Natl Acad Sci USA* 103: 12619–12624, 2006.
385. Savalli N, Pantazis A, Yusifov T, Sigg D, Olcese R. The contribution of RCK domains to human BK channel allosteric activation. *J Biol Chem* 287: 21741–21750, 2012.
386. Scornik FS, Bucciero RS, Wu Y, Selga E, Bosch Calero C, Brugada R, Perez GJ. DiBAC(4)(3) hits a “sweet spot” for the activation of arterial large-conductance Ca^{2+} -activated potassium channels independently of the beta(1)-subunit. *Am J Physiol Heart Circ Physiol* 304: H1471–H1482, 2013.
387. Schmalhofer WA, Sanchez M, Dai G, Dewan A, Secades L, Hanner M, Knaus HG, McManus OB, Kohler M, Kaczorowski GJ, Garcia ML. Role of the C-terminus of the high-conductance calcium-activated potassium channel in channel structure and function. *Biochemistry* 44: 10135–10144, 2005.
388. Schoppa NE, McCormack K, Tanouye MA, Sigworth FJ. The size of gating charge in wild-type and mutant Shaker potassium channels. *Science* 255: 1712–1715, 1992.
389. Schreiber M, Salkoff L. A novel calcium-sensing domain in the BK channel. *Biophys J* 73: 1355–1363, 1997.
390. Schreiber M, Wei A, Yuan A, Gaut J, Saito M, Salkoff L. Slo3, a novel pH-sensitive K^{+} channel from mammalian spermatocytes. *J Biol Chem* 273: 3509–3516, 1998.
391. Schreiber M, Yuan A, Salkoff L. Transplantable sites confer calcium sensitivity to BK channels. *Nat Neurosci* 2: 416–421, 1999.
392. Schubert R, Nelson MT. Protein kinases: tuners of the BKCa channel in smooth muscle. *Trends Pharmacol Sci* 22: 505–512, 2001.
393. Schwarz W, Palade PT, Hille B. Local anesthetics. Effect of pH on use-dependent block of sodium channels in frog muscle. *Biophys J* 20: 343–368, 1977.
394. Senti M, Fernandez-Fernandez JM, Tomas M, Vazquez E, Elosua R, Marrugat J, Valverde MA. Protective effect of the KCNNB1 E65K genetic polymorphism against diastolic hypertension in aging women and its relevance to cardiovascular risk. *Circ Res* 97: 1360–1365, 2005.
395. Seoh SA, Sigg D, Papazian DM, Bezanilla F. Voltage-sensing residues in the S2 and S4 segments of the Shaker K^{+} channel. *Neuron* 16: 1159–1167, 1996.
396. Shao LR, Halvorsrud R, Borg-Graham L, Storm JF. The role of BK-type Ca^{2+} -dependent K^{+} channels in spike broadening during repetitive firing in rat hippocampal pyramidal cells. *J Physiol* 521: 135–146, 1999.
397. Shelley C, Niu X, Geng Y, Magleby KL. Coupling and cooperativity in voltage activation of a limited-state BK channel gating in saturating Ca^{2+} . *J Gen Physiol* 135: 461–480, 2010.
398. Shen KZ, Lagrutta A, Davies NW, Standen NB, Adelman JP, North RA. Tetraethylammonium block of Slowpoke calcium-activated potassium channels expressed in *Xenopus* oocytes: evidence for tetrameric channel formation. *Pflügers Arch* 426: 440–445, 1994.

399. Shi J, Cui J. Intracellular Mg^{2+} enhances the function of BK-type Ca^{2+} -activated K^{+} channels. *J Gen Physiol* 118: 589–606, 2001.
400. Shi J, He HQ, Zhao R, Duan YH, Chen J, Chen Y, Yang J, Zhang JW, Shu XQ, Zheng P, Ji YH. Inhibition of martenotoxin on neuronal BK channel subtype ($\alpha + \beta 4$): implications for a novel interaction model. *Biophys J* 94: 3706–3713, 2008.
401. Shi J, Krishnamoorthy G, Yang Y, Hu L, Chaturvedi N, Harilal D, Qin J, Cui J. Mechanism of magnesium activation of calcium-activated potassium channels. *Nature* 418: 876–880, 2002.
402. Shipston MJ. Alternative splicing of potassium channels: a dynamic switch of cellular excitability. *Trends Cell Biol* 11: 353–358, 2001.
403. Shipston MJ. Ion channel regulation by protein S-acylation. *J Gen Physiol* 143: 659–678, 2014.
404. Siemen D, Loupatatzis C, Borecky J, Gulbins E, Lang F. Ca^{2+} -activated K channel of the BK-type in the inner mitochondrial membrane of a human glioma cell line. *Biochem Biophys Res Commun* 257: 549–554, 1999.
405. Singer JJ, Walsh JJ. Characterization of calcium-activated potassium channels in single smooth muscle cells using the patch-clamp technique. *Pflügers Arch* 408: 98–111, 1987.
406. Singh H, Lu R, Bopassa JC, Meredith AL, Stefani E, Toro L. MitoBK(Ca) is encoded by the *Kcnma1* gene, and a splicing sequence defines its mitochondrial location. *Proc Natl Acad Sci USA* 110: 10836–10841, 2013.
407. Singh H, Stefani E, Toro L. Intracellular BK(Ca) [iBK(Ca)] channels. *J Physiol* 590: 5937–5947, 2012.
408. Sitdikova GF, Fuchs R, Kainz V, Weiger TM, Hermann A. Phosphorylation of BK channels modulates the sensitivity to hydrogen sulfide (H_2S). *Front Physiol* 5: 431, 2014.
409. Skalska J, Bednarczyk P, Piwonska M, Kulawiak B, Wilczynski G, Dolowy K, Kudin AP, Kunz WS, Szewczyk A. Calcium ions regulate K^{+} uptake into brain mitochondria: the evidence for a novel potassium channel. *Int J Mol Sci* 10: 1104–1120, 2009.
410. Skinner LJ, Enee V, Beurq M, Jung HH, Ryan AF, Hafidi A, Aran JM, Dulon D. Contribution of BK Ca^{2+} -activated K^{+} channels to auditory neurotransmission in the Guinea pig cochlea. *J Neurophysiol* 90: 320–332, 2003.
411. Soder RP, Petkov GV. Large conductance Ca^{2+} -activated K^{+} channel activation with NS1619 decreases myogenic and neurogenic contractions of rat detrusor smooth muscle. *Eur J Pharmacol* 670: 252–259, 2011.
412. Solaro CR, Lingle CJ. Trypsin-sensitive, rapid inactivation of a calcium-activated potassium channel. *Science* 257: 1694–1698, 1992.
413. Solaro CR, Prakriya M, Ding JP, Lingle CJ. Inactivating and noninactivating Ca^{2+} - and voltage-dependent K^{+} current in rat adrenal chromaffin cells. *J Neurosci* 15: 6110–6123, 1995.
414. Soltysinska E, Bentzen BH, Barthmes M, Hattel H, Thrush AB, Harper ME, Qvortrup K, Larsen FJ, Schiffer TA, Losa-Reyna J, Straubinger J, Kniess A, Thomsen MB, Bruggemann A, Fenske S, Biel M, Ruth P, Wahl-Schott C, Boushel RC, Olesen SP, Lukowski R. KCNMA1 encoded cardiac BK channels afford protection against ischemia-reperfusion injury. *PLoS One* 9: e103402, 2014.
415. Soroceanu L, Manning TJ Jr, Sontheimer H. Modulation of glioma cell migration and invasion using Cl^{-} and K^{+} ion channel blockers. *J Neurosci* 19: 5942–5954, 1999.
416. Spector M, Rodriguez R, Rosenbaum RS, Wang HZ, Melman A, Christ GJ. Potassium channels and human corporeal smooth muscle cell tone: further evidence of the physiological relevance of the Maxi-K channel subtype to the regulation of human corporeal smooth muscle tone in vitro. *J Urol* 167: 2628–2635, 2002.
417. Stefani E, Ottolia M, Noceti F, Olcese R, Wallner M, Latorre R, Toro L. Voltage-controlled gating in a large conductance Ca^{2+} -sensitive K^{+} channel (hSlo). *Proc Natl Acad Sci USA* 94: 5427–5431, 1997.
418. Stefani E, Toro L, Perozo E, Bezanilla F. Gating of Shaker K^{+} channels. I. Ionic and gating currents. *Biophys J* 66: 996–1010, 1994.
419. Storer RJ, Immke DC, Goadsby PJ. Large conductance calcium-activated potassium channels (BKCa) modulate trigeminovascular nociceptive transmission. *Cephalalgia* 29: 1242–1258, 2009.
420. Storm JF. Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. *J Physiol* 385: 733–759, 1987.
421. Strobaek D, Christophersen P, Holm NR, Moldt P, Ahning PK, Johansen TE, Olesen SP. Modulation of the Ca^{2+} -dependent K^{+} channel, hSlo, by the substituted diphenylurea NS 1608, paxilline and internal Ca^{2+} . *Neuropharmacology* 35: 903–914, 1996.
422. Sun X, Zhou D, Zhang P, Moczydlowski EG, Haddad GG. Beta-subunit-dependent modulation of hSlo BK current by arachidonic acid. *J Neurophysiol* 97: 62–69, 2007.
423. Sweet TB, Cox DH. Measurements of the BKCa channel's high-affinity Ca^{2+} binding constants: effects of membrane voltage. *J Gen Physiol* 132: 491–505, 2008.
424. Sweet TB, Cox DH. Measuring the influence of the BKCa $\beta 1$ subunit on Ca^{2+} binding to the BKCa channel. *J Gen Physiol* 133: 139–150, 2009.
425. Talukder G, Aldrich RW. Complex voltage-dependent behavior of single unliganded calcium-sensitive potassium channels. *Biophys J* 78: 761–772, 2000.
426. Tang QY, Zeng XH, Lingle CJ. Closed-channel block of BK potassium channels by bbTBA requires partial activation. *J Gen Physiol* 134: 409–436, 2009.
427. Tano JY, Gollasch M. Calcium-activated potassium channels in ischemia reperfusion: a brief update. *Front Physiol* 5: 381, 2014.
428. Tao J, Shi J, Yan L, Chen Y, Duan YH, Ye P, Feng Q, Zhang JW, Shu XQ, Ji YH. Enhancement effects of martenotoxin on glioma BK channel and BK channel ($\alpha + \beta 1$) subtypes. *PLoS One* 6: e15896, 2011.
429. Tao J, Zhou ZL, Wu B, Shi J, Chen XM, Ji YH. Recombinant expression and functional characterization of martenotoxin: a selective inhibitor for BK channel ($\alpha + \beta 4$). *Toxins* 6: 1419–1433, 2014.
430. Thompson J, Begenisich T. Selectivity filter gating in large-conductance Ca^{2+} -activated K^{+} channels. *J Gen Physiol* 139: 235–244, 2012.
431. Thurm H, Fakler B, Oliver D. Ca^{2+} -independent activation of BKCa channels at negative potentials in mammalian inner hair cells. *J Physiol* 569: 137–151, 2005.
432. Tian L, Coghill LS, McClafferty H, MacDonald SH, Antoni FA, Ruth P, Knaus HG, Shipston MJ. Distinct stoichiometry of BKCa channel tetramer phosphorylation specifies channel activation and inhibition by cAMP-dependent protein kinase. *Proc Natl Acad Sci USA* 101: 11897–11902, 2004.
433. Tian L, Duncan RR, Hammond MS, Coghill LS, Wen H, Rusinova R, Clark AG, Levitan IB, Shipston MJ. Alternative splicing switches potassium channel sensitivity to protein phosphorylation. *J Biol Chem* 276: 7717–7720, 2001.
434. Tian Y, Ullrich F, Xu R, Heinemann SH, Hou S, Hoshi T. Two distinct effects of PIP2 underlie auxiliary subunit-dependent modulation of Slo1 BK channels. *J Gen Physiol* 145: 331–343, 2015.
435. Toro B, Cox N, Wilson RJ, Garrido-Sanabria E, Stefani E, Toro L, Zarei MM. KCNMB1 regulates surface expression of a voltage and Ca^{2+} -activated K^{+} channel via endocytic trafficking signals. *Neuroscience* 142: 661–669, 2006.
436. Toro L, Marijic J, Nishimaru K, Tanaka Y, Song M, Stefani E. Aging, ion channel expression, and vascular function. *Vasc Pharmacol* 38: 73–80, 2002.
437. Toro L, Ottolia M, Stefani E, Latorre R. Structural determinants in the interaction of Shaker inactivating peptide and a Ca^{2+} -activated K^{+} channel. *Biochemistry* 33: 7220–7228, 1994.
438. Toro L, Stefani E, Latorre R. Internal blockade of a Ca^{2+} -activated K^{+} channel by Shaker B inactivating “ball” peptide. *Neuron* 9: 237–245, 1992.
439. Torres YP, Granados ST, Latorre R. Pharmacological consequences of the coexpression of BK channel α and auxiliary β subunits. *Front Physiol* 5: 383, 2014.
440. Torres YP, Morera FJ, Carvacho I, Latorre R. A marriage of convenience: β -subunits and voltage-dependent K^{+} channels. *J Biol Chem* 282: 24485–24489, 2007.
441. Treistman SN, Martin GE. BK Channels: mediators and models for alcohol tolerance. *Trends Neurosci* 32: 629–637, 2009.
442. Trimmer JS. Subcellular localization of K^{+} channels in mammalian brain neurons: remarkable precision in the midst of extraordinary complexity. *Neuron* 85: 238–256, 2015.

443. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Computat Chem* 31: 455–461, 2010.
444. Tseng-Crank J, Foster CD, Krause JD, Mertz R, Godinot N, DiChiara TJ, Reinhart PH. Cloning, expression, and distribution of functionally distinct Ca^{2+} -activated K^{+} channel isoforms from human brain. *Neuron* 13: 1315–1330, 1994.
445. Typlt M, Mirkowski M, Azzopardi E, Ruettiger L, Ruth P, Schmid S. Mice with deficient BK channel function show impaired prepulse inhibition and spatial learning, but normal working and spatial reference memory. *PLoS One* 8: e81270, 2013.
446. Uebele VN, Lagrutta A, Wade T, Figueroa DJ, Liu Y, McKenna E, Austin CP, Bennett PB, Swanson R. Cloning and functional expression of two families of beta-subunits of the large conductance calcium-activated K^{+} channel. *J Biol Chem* 275: 23211–23218, 2000.
447. Valverde MA, Rojas P, Amigo J, Cosmelli D, Orio P, Bahamonde MI, Mann GE, Vergara C, Latorre R. Acute activation of Maxi-K channels (hSlo) by estradiol binding to the beta subunit. *Science* 285: 1929–1931, 1999.
448. Vang A, Mazer J, Casserly B, Choudhary G. Activation of endothelial BKCa channels causes pulmonary vasodilation. *Vasc Pharmacol* 53: 122–129, 2010.
449. Vanommeslaeghe K, Raman EP, MacKerell ADJ. Automation of the CHARMM General Force Field (CGenFF) II: assignment of bonded parameters and partial atomic charges. *J Chem Inf Model* 52: 3155–31268, 2012.
450. Velazquez-Marrero C, Seale GE, Treisman SN, Martin GE. Large conductance voltage- and Ca^{2+} -gated potassium (BK) channel beta4 subunit influences sensitivity and tolerance to alcohol by altering its response to kinases. *J Biol Chem* 289: 29261–29272, 2014.
451. Vergara C, Alvarez O, Latorre R. Localization of the K^{+} lock-In and the Ba^{2+} binding sites in a voltage-gated calcium-modulated channel. Implications for survival of K^{+} permeability. *J Gen Physiol* 114: 365–376, 1999.
452. Vergara C, Latorre R. Kinetics of Ca^{2+} -activated K^{+} channels from rabbit muscle incorporated into planar bilayers. Evidence for a Ca^{2+} and Ba^{2+} blockade. *J Gen Physiol* 82: 543–568, 1983.
453. Vergara C, Moczydlowski E, Latorre R. Conduction, Blockade and Gating in a Ca^{2+} -activated K Channel Incorporated into Planar Lipid Bilayers. *Biophys J* 45: 73–76, 1984.
454. Villarroel A, Alvarez O, Oberhauser A, Latorre R. Probing a Ca^{2+} -activated K^{+} channel with quaternary ammonium ions. *Pflügers Arch* 413: 118–126, 1988.
455. Wallner M, Meera P, Toro L. Determinant for beta-subunit regulation in high-conductance voltage-activated and Ca^{2+} -sensitive K^{+} channels: an additional transmembrane region at the N terminus. *Proc Natl Acad Sci USA* 93: 14922–14927, 1996.
456. Wallner M, Meera P, Toro L. Molecular basis of fast inactivation in voltage and Ca^{2+} -activated K^{+} channels: a transmembrane beta-subunit homolog. *Proc Natl Acad Sci USA* 96: 4137–4142, 1999.
457. Wang B, Brenner R. An S6 mutation in BK channels reveals beta1 subunit effects on intrinsic and voltage-dependent gating. *J Gen Physiol* 128: 731–744, 2006.
458. Wang B, Jaffe DB, Brenner R. Current understanding of iberiotoxin-resistant BK channels in the nervous system. *Front Physiol* 5: 382, 2014.
459. Wang B, Rothberg BS, Brenner R. Mechanism of beta4 subunit modulation of BK channels. *J Gen Physiol* 127: 449–465, 2006.
460. Wang L, Kang H, Li Y, Shui Y, Yamamoto R, Sugai T, Kato N. Cognitive recovery by chronic activation of the large-conductance calcium-activated potassium channel in a mouse model of Alzheimer's disease. *Neuropharmacology* 92: 8–15, 2015.
461. Wang L, Sigworth FJ. Structure of the BK potassium channel in a lipid membrane from electron cryomicroscopy. *Nature* 461: 292–295, 2009.
462. Wang YW, Ding JP, Xia XM, Lingle CJ. Consequences of the stoichiometry of Slo1 alpha and auxiliary beta subunits on functional properties of large-conductance Ca^{2+} -activated K^{+} channels. *J Neurosci* 22: 1550–1561, 2002.
463. Wang ZW, Saifee O, Nonet ML, Salkoff L. SLO-1 potassium channels control quantal content of neurotransmitter release at the *C. elegans* neuromuscular junction. *Neuron* 32: 867–881, 2001.
464. Weaver AK, Liu X, Sontheimer H. Role for calcium-activated potassium channels (BK) in growth control of human malignant glioma cells. *J Neurosci Res* 78: 224–234, 2004.
465. Wei A, Solaro C, Lingle C, Salkoff L. Calcium sensitivity of BK-type KCa channels determined by a separable domain. *Neuron* 13: 671–681, 1994.
466. Weiger TM, Holmqvist MH, Levitan IB, Clark FT, Sprague S, Huang WJ, Ge P, Wang C, Lawson D, Jurman ME, Glucksmann MA, Silos-Santiago I, DiStefano PS, Curtis R. A novel nervous system beta subunit that downregulates human large conductance calcium-dependent potassium channels. *J Neurosci* 20: 3563–3570, 2000.
467. Welling PA. Roles and regulation of renal K channels. *Annu Rev Physiol* 78: 415–435, 2016.
468. Whitt JP, Montgomery JR, Meredith AL. BK channel inactivation gates daytime excitability in the circadian clock. *Nat Commun* 7: 10837, 2016.
469. Wilkens CM, Aldrich RW. State-independent block of BK channels by an intracellular quaternary ammonium. *J Gen Physiol* 128: 347–364, 2006.
470. Womack MD, Hoang C, Khodakhah K. Large conductance calcium-activated potassium channels affect both spontaneous firing and intracellular calcium concentration in cerebellar Purkinje neurons. *Neuroscience* 162: 989–1000, 2009.
471. Wong BS, Lecar H, Adler M. Single calcium-dependent potassium channels in clonal anterior pituitary cells. *Biophys J* 39: 313–317, 1982.
472. Woodhull AM. Ionic blockage of sodium channels in nerve. *J Gen Physiol* 61: 687–708, 1973.
473. Wu RS, Liu G, Zakharov SI, Chudasama N, Motoike H, Karlin A, Marx SO. Positions of beta2 and beta3 subunits in the large-conductance calcium- and voltage-activated BK potassium channel. *J Gen Physiol* 141: 105–117, 2013.
474. Wu RS, Marx SO. The BK potassium channel in the vascular smooth muscle and kidney: alpha- and beta-subunits. *Kidney Int* 78: 963–974, 2010.
475. Wu SN, Liu SI, Huang MH. Cilostazol, an inhibitor of type 3 phosphodiesterase, stimulates large-conductance, calcium-activated potassium channels in pituitary GH3 cells and pheochromocytoma PC12 cells. *Endocrinology* 145: 1175–1184, 2004.
476. Wu Y, Xiong Y, Wang S, Yi H, Li H, Pan N, Horrigan FT, Ding J. Intersubunit coupling in the pore of BK channels. *J Biol Chem* 284: 23353–23363, 2009.
477. Wu Y, Yang Y, Ye S, Jiang Y. Structure of the gating ring from the human large-conductance Ca^{2+} -gated K^{+} channel. *Nature* 466: 393–397, 2010.
478. Wulf-Johansson H, Amrutkar DV, Hay-Schmidt A, Poulsen AN, Klaerke DA, Olesen J, Jansen-Olesen I. Localization of large conductance calcium-activated potassium channels and their effect on calcitonin gene-related peptide release in the rat trigemino-neuronal pathway. *Neuroscience* 167: 1091–1102, 2010.
479. Xia XM, Ding JP, Lingle CJ. Inactivation of BK channels by the NH2 terminus of the beta2 auxiliary subunit: an essential role of a terminal peptide segment of three hydrophobic residues. *J Gen Physiol* 121: 125–148, 2003.
480. Xia XM, Ding JP, Lingle CJ. Molecular basis for the inactivation of Ca^{2+} - and voltage-dependent BK channels in adrenal chromaffin cells and rat insulinoma tumor cells. *J Neurosci* 19: 5255–5264, 1999.
481. Xia XM, Ding JP, Zeng XH, Duan KL, Lingle CJ. Rectification and rapid activation at low Ca^{2+} of Ca^{2+} -activated, voltage-dependent BK currents: consequences of rapid inactivation by a novel beta subunit. *J Neurosci* 20: 4890–4903, 2000.
482. Xia XM, Zeng X, Lingle CJ. Multiple regulatory sites in large-conductance calcium-activated potassium channels. *Nature* 418: 880–884, 2002.
483. Xie J, McCobb DP. Control of alternative splicing of potassium channels by stress hormones. *Science* 280: 443–446, 1998.
484. Xu W, Liu Y, Wang S, McDonald T, Van Eyk JE, Sidor A, O'Rourke B. Cytoprotective role of Ca^{2+} -activated K^{+} channels in the cardiac inner mitochondrial membrane. *Science* 298: 1029–1033, 2002.
485. Yan J, Aldrich RW. BK potassium channel modulation by leucine-rich repeat-containing proteins. *Proc Natl Acad Sci USA* 109: 7917–7922, 2012.
486. Yan J, Aldrich RW. LRRC26 auxiliary protein allows BK channel activation at resting voltage without calcium. *Nature* 466: 513–516, 2010.

487. Yang C, Zeng XH, Zhou Y, Xia XM, Lingle CJ. LRRC52 (leucine-rich-repeat-containing protein 52), a testis-specific auxiliary subunit of the alkalization-activated Slo3 channel. *Proc Natl Acad Sci USA* 108: 19419–19424, 2011.
488. Yang H, Hu L, Shi J, Delaloye K, Horrigan FT, Cui J. Mg^{2+} mediates interaction between the voltage sensor and cytosolic domain to activate BK channels. *Proc Natl Acad Sci USA* 104: 18270–18275, 2007.
489. Yang H, Shi J, Zhang G, Yang J, Delaloye K, Cui J. Activation of Slo1 BK channels by Mg^{2+} coordinated between the voltage sensor and RCK1 domains. *Nature Struct Mol Biol* 15: 1152–1159, 2008.
490. Yang H, Zhang G, Shi J, Lee US, Delaloye K, Cui J. Subunit-specific effect of the voltage sensor domain on Ca^{2+} sensitivity of BK channels. *Biophys J* 94: 4678–4687, 2008.
491. Yang J, Krishnamoorthy G, Saxena A, Zhang G, Shi J, Yang H, Delaloye K, Sept D, Cui J. An epilepsy/dyskinesia-associated mutation enhances BK channel activation by potentiating Ca^{2+} sensing. *Neuron* 66: 871–883, 2010.
492. Yang J, Yang H, Sun X, Delaloye K, Yang X, Moller A, Shi J, Cui J. Interaction between residues in the Mg^{2+} -binding site regulates BK channel activation. *J Gen Physiol* 141: 217–228, 2013.
493. Yang Y, Li PY, Cheng J, Mao L, Wen J, Tan XQ, Liu ZF, Zeng XR. Function of BKCa channels is reduced in human vascular smooth muscle cells from Han Chinese patients with hypertension. *Hypertension* 61: 519–525, 2013.
494. Yao J, Chen X, Li H, Zhou Y, Yao L, Wu G, Chen X, Zhang N, Zhou Z, Xu T, Wu H, Ding J. BmP09, a “long chain” scorpion peptide blocker of BK channels. *J Biol Chem* 280: 14819–14828, 2005.
495. Yazejian B, Sun XP, Grinnell AD. Tracking presynaptic Ca^{2+} dynamics during neurotransmitter release with Ca^{2+} -activated K^{+} channels. *Nat Neurosci* 3: 566–571, 2000.
496. Yellen G. Ionic permeation and blockade in Ca^{2+} -activated K^{+} channels of bovine chromaffin cells. *J Gen Physiol* 84: 157–186, 1984.
497. Yellen G. Relief of Na^{+} block of Ca^{2+} -activated K^{+} channels by external cations. *J Gen Physiol* 84: 187–199, 1984.
498. Yuan P, Leonetti MD, Hsiung Y, MacKinnon R. Open structure of the Ca^{2+} gating ring in the high-conductance Ca^{2+} -activated K^{+} channel. *Nature* 481: 94–97, 2012.
499. Yuan P, Leonetti MD, Pico AR, Hsiung Y, MacKinnon R. Structure of the Hhuman BK channel Ca^{2+} -activation apparatus at 3.0 Å resolution. *Science* 329: 182–186, 2010.
500. Yusifov T, Javaherian AD, Pantazis A, Gandhi CS, Olcese R. The RCK1 domain of the human BKCa channel transduces Ca^{2+} binding into structural rearrangements. *J Gen Physiol* 136: 189–202, 2010.
501. Yusifov T, Savalli N, Gandhi CS, Ottolia M, Olcese R. The RCK2 domain of the human BKCa channel is a calcium sensor. *Proc Natl Acad Sci USA* 105: 376–381, 2008.
502. Zakharov SI, Morrow JP, Liu G, Yang L, Marx SO. Activation of the BK (SLO1) potassium channel by mallotoxin. *J Biol Chem* 280: 30882–30887, 2005.
503. Zarei MM, Eghbali M, Alioua A, Song M, Knaus HG, Stefani E, Toro L. An endoplasmic reticulum trafficking signal prevents surface expression of a voltage- and Ca^{2+} -activated K^{+} channel splice variant. *Proc Natl Acad Sci USA* 101: 10072–10077, 2004.
504. Zarei MM, Song M, Wilson RJ, Cox N, Colom LV, Knaus HG, Stefani E, Toro L. Endocytic trafficking signals in KCNMB2 regulate surface expression of a large conductance voltage and Ca^{2+} -activated K^{+} channel. *Neuroscience* 147: 80–89, 2007.
505. Zarei MM, Zhu N, Alioua A, Eghbali M, Stefani E, Toro L. A novel MaxiK splice variant exhibits dominant-negative properties for surface expression. *J Biol Chem* 276: 16232–16239, 2001.
506. Zeng X, Xia XM, Lingle CJ. Species-specific differences among KCNMB3 BK beta3 auxiliary subunits: some beta3 N-terminal variants may be primate-specific subunits. *J Gen Physiol* 132: 115–129, 2008.
507. Zeng XH, Ding JP, Xia XM, Lingle CJ. Gating properties conferred on BK channels by the beta3b auxiliary subunit in the absence of its NH_2 - and COOH termini. *J Gen Physiol* 117: 607–628, 2001.
508. Zeng XH, Xia XM, Lingle CJ. Divalent cation sensitivity of BK channel activation supports the existence of three distinct binding sites. *J Gen Physiol* 125: 273–286, 2005.
509. Zeng XH, Xia XM, Lingle CJ. Redox-sensitive extracellular gates formed by auxiliary beta subunits of calcium-activated potassium channels. *Nat Struct Biol* 10: 448–454, 2003.
510. Zhang G, Huang SY, Yang J, Shi J, Yang X, Moller A, Zou X, Cui J. Ion sensing in the RCK1 domain of BK channels. *Proc Natl Acad Sci USA* 107: 18700–18705, 2010.
511. Zhang J, Yan J. Regulation of BK channels by auxiliary gamma subunits. *Front Physiol* 5: 401, 2014.
512. Zhang X, Solaro CR, Lingle CJ. Allosteric regulation of BK channel gating by Ca^{2+} and Mg^{2+} through a nonselective, low affinity divalent cation site. *J Gen Physiol* 118: 607–636, 2001.
513. Zhang Z, Zhou Y, Ding JP, Xia XM, Lingle CJ. A limited access compartment between the pore domain and cytosolic domain of the BK channel. *J Neurosci* 26: 11833–11843, 2006.
514. Zhou Y, Lingle CJ. Paxilline inhibits BK channels by an almost exclusively closed-channel block mechanism. *J Gen Physiol* 144: 415–440, 2014.
515. Zhou Y, Tang QY, Xia XM, Lingle CJ. Glycine311, a determinant of paxilline block in BK channels: a novel bend in the BK S6 helix. *J Gen Physiol* 135: 481–494, 2010.
516. Zhou Y, Xia XM, Lingle CJ. Cysteine scanning and modification reveal major differences between BK channels and Kv channels in the inner pore region. *Proc Natl Acad Sci USA* 108: 12161–12166, 2011.
517. Zhou Y, Zeng XH, Lingle CJ. Barium ions selectively activate BK channels via the Ca^{2+} -bowl site. *Proc Natl Acad Sci USA* 109: 11413–11418, 2012.