HHS Public Access

Author manuscript

Annu Rev Physiol. Author manuscript; available in PMC 2019 February 19.

Published in final edited form as:

Annu Rev Physiol. 2019 February 10; 81: 113–137. doi:10.1146/annurev-physiol-022516-034038.

Regulation of BK Channels by Beta and Gamma Subunits

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Abstract

Ca^{2+}- and voltage-gated K^+ channels of large conductance (BK channels) are expressed in a diverse variety of both excitable and inexcitable cells, with functional properties presumably uniquely calibrated for the cells in which they are found. Although some diversity in BK channel function, localization, and regulation apparently arises from cell-specific alternative splice variants of the single pore–forming α subunit (*KCa1.1*, *Kcnma1*, *Slo1*) gene, two families of regulatory subunits, β and γ, define BK channels that span a diverse range of functional properties. We are just beginning to unravel the cell-specific, physiological roles served by BK channels of different subunit composition.

Keywords

BK channels; Ca^{2+}- and voltage-dependent K^+ channels; auxiliary subunits; *KCa1.1*; beta subunits; gamma subunits; *KCNMB*; *LRRC26*

INTRODUCTION

BK channels (also called maxi-K or Slo1 channels) are K^+ channels of unusually large single-channel conductance that are distinctive in being regulated by two physiological stimuli: elevations in cytosolic Ca^{2+}, and membrane depolarization. Although either Ca^{2+} or voltage can independently increase channel activation, typically both signals act in concert to sculpt the contributions of BK current activation during normal cellular electrical activity. BK channels, encoded by a single *KCa1.1* gene (also called *Kcnma1* or *Slo1*), are also expressed in an unusually broad range of cell types, including both excitable and inexcitable cells (1–3). In fact, it is possible that, among cation channels, *KCa1.1*-encoded BK channels are found in a larger range of cell types than any other plasma membrane ion channel, including neurons with a variety of diverse firing behaviors (4–6), neuroendocrine cells (7, 8), glia cells (9), skeletal muscle cells (10), smooth muscle cells (SMCs) of all types (11–13), principal cells (PCs), and intercalated cells (ICs) in collecting ducts of the kidney (14–16), and a variety of epithelial cells (17–20). There is also a growing literature on BK channels in membranes of intracellular organelles, including nuclei (21), mitochondria (22),

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

and lysosomes (23). Among their various loci of expression, BK channels are thought to play distinct physiological roles that are tailored to the requirements of the given cell type and depend on cell-specific membrane potentials and cytosolic Ca^{2+} concentrations. With such broad diversity in likely physiological roles, it would seem remarkable that a single gene product could accomplish these tasks. Although a variety of mechanisms, including alternative splicing of the *KCa1.1* gene (22, 24–27) and posttranslational modifications (28–31), may contribute to this functional diversity, auxiliary subunits—the focus here—play a major role.

For most ion channels, functional identity is defined by properties conferred by the pore-forming subunits of the channel. In contrast, the enormous diversity in BK channel function arises from coassembly with non-pore-forming regulatory subunits. In fact, the same BK pore–forming subunit can participate in channels that could be considered to be entirely functionally distinct, as a simple consequence of the "wardrobe" of regulatory subunits that can decorate the pore-forming subunits. For BK channels, the pore-forming Slo1 α subunits (Figure 1*a*) can coassemble with at least two distinct regulatory subunit families, β and γ (Figure 1*b*), into a tetramer of pore-forming subunits (Figure 1*c*) surrounded by 0–4 β and 0–4 γ subunits per channel (Figure 1*d*). Although all BK channels share similar large single-channel conductance with similar voltage sensitivity, they differ in a number of other attributes that define potential physiological roles. Figure 1*e–g* compares conductance-voltage (GV) curves for α alone (32) and then for the two founding members of the β and γ subunit families, α+β1 (33) and α+γ1 (34). Based on experimentally measured activation parameters, the figure highlights the powerful effects of BK regulatory subunits. The large variation in the BK channel gating range among different subunit combinations contrasts with the narrower activation range that exists for a variety of Kv channels, which all arise from distinct genes (Figure 1*h*). In essence, BK channels of different subunit composition define completely different kinds of channels—different not only in range of activation, but also in rates of channel activation or deactivation, inactivation single-channel current rectification properties, and aspects of pharmacological sensitivity (Table 1).

This diversity poses challenges both to understanding the physiological roles of BK channels and using BK channels as targets for therapeutic interventions. First, the widespread expression of BK channels and their participation in a variety of fundamental physiological processes mean that any attempt to use a BK modulator to ameliorate symptoms arising from one particular cell type, tissue, or organ system will also impact other loci unrelated to the pathology, perhaps with highly undesirable consequences. Second, in many locations we really do not know the underlying regulatory subunit composition of the BK channels. An additional challenge is that, with their large single-channel conductance, robust BK currents can be generated by a relatively small population of channels. Consequently, biochemical and visualization methods that depend on protein abundance can be less advantageously employed.

Recent reviews have addressed the allosteric basis of BK channel regulation (35–40), provided an overview of how auxiliary subunits may regulate BK gating (35, 41–44), including the specific role of β1-containing BK channels in smooth muscle and kidney (39, 45), and considered the regulation and physiological roles of BK channels (29, 35, 42, 46).

Here, we focus on (*a*) a description of functional properties conferred on BK channels by regulatory subunits that may aid in identification of BK channels of particular composition and (*b*) efforts to understand the specific physiological roles played by BK channels of specific auxiliary subunit composition through the use of knockout (KO) mouse models.

A BIT OF HISTORY: THE BK CHANNEL α SUBUNIT

Preceding the full impact of patch-clamp methodologies, a combination of electrophysiology and pharmacology revealed a growing catalog of functionally distinct K^+ channels available to produce nuanced regulation of cellular excitability. This included outward currents dependent on cytosolic Ca^{2+} elevations (47, 48). Among these Ca^{2+}-dependent K^+ (K_{Ca}) channels were those of particularly large single-channel conductance, which led to the big K^+ (BK) or maxi-K designations, making them among the first to be studied with single-channel recording methods (10, 49, 50). A hallmark of these channels was their sensitivity to the scorpion toxins, charybdotoxin (ChTx) (51) and iberiotoxin (IbTx) (52). The ease with which such channels could be recorded made them a favored preparation of biophysics for several decades, advantages that still hold true for investigation of mechanisms of modulation and drug action.

A first step toward identification of the molecular substrate of such channels was made when a mutation in the gene responsible for the *Drosophila slowpoke* behavioral phenotype was associated with the loss of a ChTx-sensitive K_{Ca} current present in *Drosophila* flight muscle (1). The *slowpoke* gene was then shown to encode a protein with homology to voltage-dependent K^+ channels (53, 54), and heterologous expression of this gene resulted in a K_{Ca} current and single channels of large conductance (2) with similarities to mammalian BK channels (10, 49, 50). Subsequently, a highly homologous mouse *Slo1* gene (now termed *KCa1.1*) was identified (3). Distinguishing features of the KCa1.1 α subunit include an extra S0 transmembrane segment, resulting in an extracellular N terminus and a large cytosolic domain (Figure 1*a*) that form the so-called gating ring responsible for ligand dependence (55–57).

Discovery of the β Subunit Family

Almost cotemporaneous with identification of *Kcnma1*/*Slo1*, ChTx was exploited as a tool for biochemical purification of the molecular components of BK channels from tracheal and aortic smooth muscle (58, 59). This led to identification of peptide fragments corresponding to both the *Slo1* α subunit and an associated β subunit (58, 59), leading to a full-length, 191-amino acid β subunit protein (60). The deduced sequence predicted a protein with two transmembrane sequences, cytosolic N and C termini, and a large cysteine-rich extracellular loop (Figure 1*b*) with two N-linked glycosylation sites. Heterologous coexpression of the bovine β1 subunit with mouse *Slo1* α resulted in BK currents that activated at a given $[Ca^{2+}]$ at voltages approximately 70 to 90 mV more negative than for *Slo1* α subunit alone (61), demonstrating that a β subunit could be a functionally important determinant of BK channel properties. Furthermore, the β1 subunit conferred sensitivity to dehydrosoyasaponin I (DHS-I), a medicinal herb that potently activates some BK channels (62). That an auxiliary subunit

could define unique pharmacological sensitivities now motivates work seeking to exploit subunit composition to identify more specific activators or inhibitors of BK channels.

Identification of the β1 subunit was a major advance in accounting for functional properties of smooth muscle BK channels. However, additional features of BK currents in other native cells implied there was more to discover. For example, inactivating K_{Ca} currents were noted in guinea pig hippocampal pyramidal cells (63) and rat hippocampal neurons (64). Subsequently, single BK channels and macroscopic BK currents in both adrenal medullary chromaffin cells (CCs) (7, 65) and clonal pancreatic β cells (66) established that some BK channels exhibit inactivation, with some features similar to rapid inactivation of some Kv channels (65, 67). Furthermore, bilayer recordings of channels obtained from rat brain plasma membrane vesicles revealed BK channels that differ in gating kinetics at a given Ca^{2+} and also in sensitivity to ChTx (68, 69). These demonstrations that BK channels exhibit significant functional and pharmacological diversity suggested that additional determinants of BK channel function remained to be identified.

The availability of cDNA libraries and expressed sequence tag (EST) databases subsequently led to identification of three additional mammalian genes, *Kcnmb2*, *Kcnmb3*, and *Kcnmb4*, each encoding proteins with homology to the β1 subunit (70–76). *Kcnmb2* encodes the BK β2 subunit whose cytosolic N terminus mediates BK inactivation (70, 72, 77) and accounts for BK channel inactivation in adrenal CCs (72, 78) and clonal pancreatic endocrine cells (66). In humans and primates, the *Kcnmb3* gene encodes four distinct alternative splice variants, β3a–d (75, 79), each with different cytosolic N termini. β3a (75, 80), β3b (73, 75), and β3c (73, 75) mediate kinetically distinct forms of inactivation. In mice, alternative splice variants corresponding to the β3c and β3d isoforms appear to be absent (79), and even the existence of a rodent β3b isoform remains tenuous. Finally, the *Kcnmb4* gene encodes a β4 subunit, which is generally considered the predominant brain β subunit isoform (71, 74, 76).

Functional Signatures of β Subunits

Each of the four β subunits defines functional features presumably suited for specific physiological roles. However, in large measure, such specific physiological roles remain to be fully elucidated. A better understanding of the functional properties of BK channels of particular subunit composition is ultimately essential for recognizing the roles of such channels in native cells. Here, key features of each β subunit are summarized, with a particular focus on those properties that may be useful for the identification and study of such channels in native cells (summarized in Table 1).

β1-Containing channels.—At elevated cytosolic Ca^{2+} concentrations (1 μM and higher), β1-containing BK channels exhibit a shifted voltage-of-half activation (V_h) of approximately −70 to −90 mV relative to α-alone channels (Figure 1*e,f*; Figure 2*a,b*). However, at 0 Ca^{2+}, V_h is little changed (33, 61), reflecting the combination of slowing of both channel closure and opening (81). At all $[Ca^{2+}]_i$, β1-containing channels will exhibit longer single-channel bursts and slower deactivation kinetics compared with α alone (Figure 2*i,j*). β1-containing channels exhibit neither inactivation nor instantaneous current

rectification (Figure 2*g*), distinguishing them from β2- and β3-containing channels. β1-Containing channels are sensitive to ChTx and IbTx, albeit with somewhat different blocking kinetics than α alone (72).

β2-Containing channels.—β2-Containing BK channels share with β1 a shift in gating at elevated Ca^{2+} (Figure 2*c*), albeit of approximately −50 to −70 mV (70, 72, 82) and, like β1, also slow both activation and deactivation (82) (Figure 2*i*). The distinguishing feature of β2-containing BK channels is robust inactivation, reaching limiting time constants of ~ 20–30 ms at positive voltages and 10 μM Ca^{2+} (Figure 2*c*) (72, 77). With strong depolarizations at elevated Ca^{2+}, channels inactivate to a very low steady-state open probability (Figure 2*l*). Recordings that show persistent BK current arising from α+β2 channels probably reflect incomplete stoichiometric assembly of β2 subunits in the channel population, with some fraction of channels lacking β2 subunits. Because the steady-state inactivation properties of the α+β2 channels shift with cytosolic Ca^{2+} in accordance with the channel activation curves (83), more than 90% of the channel population will be inactivated at a holding potential of −50 mV with 10 μM cytosolic Ca^{2+}. Consequently, without an adequate prepulse to potentials of −100 or more negative (83), α+β2 channels can be overlooked when cytosolic Ca^{2+} is high. However, with buffered 0 Ca^{2+} cytosolic solutions, essentially no inactivation of α+β2 channels will occur until above +20 mV (83). This set of properties places some constraints on the types of roles that α+β2 channels may play. For example, with action potential (AP) durations of 5–10 ms, minimal fractional inactivation of BK channels would be expected and, at AP frequencies of about 10–20 Hz, most inactivated BK channels would recover from inactivation between sequential APs. Another hallmark of α+β2 channels that is shared with α+β3 channels is instantaneous outward current rectification (Figure 2*g,h*) following repolarization (84). This rectification arises from properties of β2 and β3 extracellular loops affecting inward current flux (85). For assessment of the contributions of α+β2 channels in a given cell, careful characterization of the intrinsic inactivation and activation properties remains essential to evaluate how such channels may impact cell excitability. As discussed below, the average β2:α subunit ratio in expressed BK channels must also be taken into consideration in terms of how β2-containing cells may impact excitability among different cells. This also holds true for other β subunits.

β3-Containing BK channels.—Despite having fascinating properties, β3-containing BK channels are the least understood in terms of potential loci and roles in native cells. The properties of inactivation of both human and mouse β3a (79, 80, 86) and also human β3b (73, 87) have been studied in some detail (Figure 2*d,e*). For both, inactivation is best described by a two-step mechanism involving both a preinactivated open state and then an inactivated state. Although the biophysical details may not seem physiologically important, the presence of these two open states has important consequences for the properties of BK currents during recovery from inactivation, most clearly exemplified by β3a (Figure 2*d,k*). Although open β3a-containing BK channels close at rates comparable to α alone (Figure 2*i*), repolarization from a steady-state inactivated condition results in an instantaneous unblock, reflecting rapid return to the preinactivated open state, followed by a tail current that persists for tens or even hundreds of milliseconds (compare Figure 2*i–j*). This behavior is most obvious at the single-channel level (Figure 2*k*). As a consequence, β3a-mediated tail

currents exhibit a large increase in total net tail current integral compared to what is seen for recovery from Kv channel inactivation, and such a slow tail would be expected to have major consequences on cell excitability. As yet, there are no recordings of β3a-containing BK currents from native cells, although the β3a N terminus is the most highly conserved of all β3 isoforms among mammalian species (79). For human β3b-containing BK channels (Figure 2*e*), when β3b expression is robust and probed with strong activation conditions (depolarization positive to +100 mV and 10 μM Ca^{2+}), β3b-containing BK channels exhibit a very rapid (0.5–2 ms at room temperature) inactivation that relaxes to a voltage-dependent steady-state current level, reflecting very rapid block and unblock (73). At weaker depolarizations with slower BK activation, time-dependent inactivation is convolved with the current activation time course, and no obvious inactivation is observed. However, in such cases, the steady-state current at positive potentials will still exhibit voltage-dependent reduction characteristic of fast block (Figure 2*l*), a signature feature of human β3b-containing BK channels. Given the profound steady-state block produced by β3 N-terminal variants, trypsin-mediated removal of inactivation may be a useful tool for diagnosis of such currents (Figure 2*m*). Less is known about either the inactivating β3c variant or the noninactivating β3d variant (73, 75). An additional functional signature shared by all β3 subunits is outward current rectification, i.e., a reduced ionic conductance in the inward current direction (Figure 2*g,h*) (84). This property may also be useful for identification of candidate β3-containing BK currents.

β4-Containing BK channels.—The pervasive presence of β4 message in the brain makes β4 of central importance to understanding the role of BK channels in terms of excitability in central nervous system (CNS) neurons. Although some reports differ regarding specific properties of heterologously expressed β4-containing BK channels, on balance, most work suggests that β4-containing channels exhibit a positive GV shift of approximately +30 to +40 mV at 0 Ca^{2+}, while exhibiting a modest −20 to −30 mV negative shift at Ca^{2+} above 1 μM, when studied with symmetric K^+ solutions (41, 74, 88). The rightward shift at low Ca^{2+} appears to arise from a more marked slowing of activation of β4-containing BK channels at 0 Ca^{2+} relative to other β subunits, in conjunction with a less marked prolongation of tail currents in comparison to β1 and β2. The slowing of activation at low Ca^{2+} may be the primary contributor to the physiological roles of β4-containing BK channels. At more elevated Ca^{2+}, the slowing of deactivation produced by β4 appears somewhat less than that for β1 and β2 (Figure 2*i*). Unlike β2 and β3, but like β1, β4-containing BK channels exhibit no instantaneous current rectification (Figure 2*g–h*), but these channels are distinguished by almost complete resistance to ChTx and IbTx inhibition (71, 76).

β:α Stoichiometry Adds to Functional Diversity

The number of β subunits in a BK channel complex (β:α) adds to the repertoire of functional diversity. β:α stoichiometry was defined through use of the β2 N-terminal inactivation domain as a kinetic reporter that varies as a function of the number of β2 subunits per channel (89). This established that 0–4 β2 subunits can be present in single BK channels (89). Variable stoichiometry also occurs in native cells, as shown for rat (90) and mouse adrenal CCs (78). Differences in β1:α stoichiometry may also underlie BK channel

differences among mesenteric, coronary, and cerebral arteries (91). To what extent variations in average β:α ratios occur for BK currents in other native cells remains largely unexplored.

β subunit stoichiometry impacts BK channel function in multiple ways. The dependence of channel open probability (Po) on voltage for single BK channels shifts in accordance with β subunit stoichiometry (89), with each individual β2 subunit contributing in an energetically independent fashion to shift BK gating leftward (Figure 1*d*). Thus, the average number of β2 (and presumably other β) subunits in a population should influence both magnitude and duration of any afterhyperpolarizations (AHPs). For CCs, cells with a larger average number of β2 subunits in the channel population (more rapid inactivation and more left-shifted gating) have, on average, stronger AHPs and higher rates of AP firing due to recovery of Na_V channels from inactivation (7, 78). As a corollary, the average β:α stoichiometry will impact differences in rates of BK activation, deactivation, inactivation, and instantaneous current rectification.

An unexplored issue is whether variable stoichiometry may also impact the pharmacological identification of BK channels. β4 subunits confer almost complete insensitivity to IbTx (71), but BK channels in the brain, where β4 is abundant, can be either sensitive or resistant to the scorpion toxins ChTx and IbTx (68, 92). One possibility is that only channels containing four β4 subunits may be fully resistant to these toxins. In Kv channels, ChTx binds in any of four indistinguishable orientations (93). If the presence of fewer than four β subunits allows accessibility to some orientations, this would result in channels with toxin sensitivity intermediate between α alone and α+β, in accordance with whether there are 1, 2, 3, or 4 available orientations (Figure 3*a*). Whereas channels with four β4 subunits would be IbTx resistant (Figure 3*a–c*), channels with 1–3 β4 subunits (Figure 3*b–c*) or a population of channels with mixed stoichiometries (Figure 3*d–e*) would exhibit toxin sensitivity rather similar to channels totally lacking β4 subunits. With IbTx concentrations typically used experimentally, many BK currents identified as IbTx sensitive might, in fact, reflect β4-containing BK channels. This issue highlights the challenges faced in terms of unambiguous identification of the composition and functional properties of BK channels in native cells.

New Kids on the Block: The γ (LRRC) Family of BK Family Channel Regulators

In some cell types, including breast and prostate tumor cells (94, 95) and lacrimal gland cells (96), BK current activation occurs at potentials negative to 0 mV even with 0 cytosolic Ca^{2+}, which is inconsistent with known BK α splice variants or α+β subunit combinations. Searching for additional BK regulatory partners, Yan & Aldrich (34) identified such a subunit, LRRC26 (leucine-rich repeat-containing protein 26), in the LNCaP prostate tumor cell line. Coexpression of LRRC26 with Slo1 produces currents that are shifted ~120 mV in the negative direction at both 0 and elevated Ca^{2+} (Figure 1*g*, Figure 2*f*), matching the properties of BK channels found in LNCaP cells. Moreover, three additional genes (*LRRC38*, *LRRC52*, and *LRRC55*) were identified that encode subunits related to LRRC26, now all termed γ subunits. Two of these, *LRRC52* (γ2) and *LRRC55* (γ3), produce gating shifts when heterologously coexpressed with Slo1 α subunits (97).

γ Subunits (97), completely distinct from β subunits, are a subset within the extensive superfamily of LRRC proteins (98). The four γ subunits [gene names *LRRC26* (γ1),

LRRC52 (γ2), *LRRC55* (γ3), and *LRRC38* (γ4)] share an N-terminal cleavable signal sequence, an extracellular LRRC domain with a set of six LRRs repeats flanked by an N-terminal cysteine-rich segment (LRRNT) and a C-terminal cysteine-rich segment (LRRCT), a single transmembrane segment, and a short cytosolic C-terminal tail (Figure 1*b*). Although no solved structures exist for any of the γ subunits, the γ1 subunit extracellular domain shares similarities with the hagfish variable lymphocyte receptor B (99). The function of the γ1 subunit LRRC domain is unknown, but it is tempting to consider that natural ligands that bind to γ subunits may exist.

γ1-Containing BK channels.—The most notable functional feature of the γ1 subunit is the ~120-mV leftward-gating shift of γ1-containing BK channels compared to α-alone BK channels (34). The V_h shift occurs at both 0 and elevated cytosolic Ca^{2+}, largely distinguishing γ1 subunit effects on V_h from those of β subunits at 0 Ca^{2+} (Figure 1*g*, Figure 2*f*). Although some β subunits markedly influence channel kinetic properties at 0 Ca^{2+} (81), the overall effect on V_h is rather minor. In contrast to the incremental effects of β subunits on BK gating, as the mole fraction of LRRC26 message is increased relative to BK α, the characteristic Boltzmann shape of BK current activation splits into two components (Figure 1*d*), one identical to currents arising from the α subunit alone and the other characteristic of channels fully shifted by LRRC26 (100). Recently, it has been shown that a single γ1 subunit is sufficient to produce the all-or-none gating shift, but channels can accommodate up to four γ1 subunits (101, 102). The presence of a single γ1 subunit apparently produces a concerted change in the BK channel that strongly favors channel activation. Both γ1 and β2 subunits can coassemble in the same BK channels, with γ1 producing normal gating shifts even in the presence of four β2 subunits (103).

Of the four proposed γ subunits, only γ1 is definitively an established BK channel regulatory subunit in native cells. Three specific functional properties define γ1-containing BK channels: (*a*) the large magnitude of the gating shift, (*b*) the fact that the shift also occurs at 0 Ca^{2+}, and (*c*) resistance to activation by the natural toxin, mallotoxin (104). BK currents in parotid acinar cells of the salivary gland exhibit both the leftward-gating shifts characteristic of γ1-containing BK channels and insensitivity to mallotoxin (105, 106). Similarly, native transepithelial K^+ currents in tracheal epithelial cells are resistant to mallotoxin, which is likely indicative of γ1-containing BK channels (107, 108). The large leftward-gating shift and resistance to mallotoxin are essential functional signatures diagnostic of the presence of γ1-containing BK channels in native cells.

γ2−γ3 Subunits also shift BK gating, but the roles of γ2 and γ3 remain unclear.—γ2 (LRRC52) is a regulatory partner of the SLO3 voltage- and pH-regulated K^+ channel that underlies sperm KSper current (109, 110). Knockout of γ2 (LRRC52) shifts KSper current in mouse sperm rightward and results in a fertility deficit (111), confirming its role as a native SLO3 partner. Although mRNA expression suggests that γ2 expression is limited to testis and sperm in both mice and humans, the ability of LRRC52 to shift BK gating approximately −100 mV at 0 Ca^{2+} leaves open the possibility that it may partner with Slo1 subunits in some unidentified loci. For γ3 (LRRC55), message distribution (97, 112) and in situ hybridization (113) suggest potential roles in excitable cells, although γ3-induced

gating shifts are modest compared to those in both γ1 and γ2 (97). At present, γ3 remains simply an interesting candidate for a BK regulatory subunit. Because the effect of γ4 (LRRC38) on BK gating is limited (97), its role as a BK regulatory subunit remains murky.

Functional Roles Played by BK Channels of Specific Regulatory Subunit Composition in Native Tissues

Given the functionally diverse properties conferred on BK channels by regulatory subunits, it is expected that channels of a given subunit composition may be tailored for specific physiological roles. We now focus on cell/tissue systems where KO animals have been used in conjunction with electrophysiological approaches to specifically assess the potential role of BK channels of particular subunit composition in those loci. One impediment to this goal is that standard whole-cell approaches in native cells may not allow BK channel properties to be adequately defined within the background of other currents, while excised patch recordings that might be helpful are less routinely obtained. For neurons, in particular, this can be a challenging problem.

BK β1 subunits, smooth muscle tone, and hypertension.—β1 subunits are abundantly expressed in SMCs, including those in the trachea, aorta, cerebral arteries, and urinary smooth muscle. The shifted range of activation of β1-containing BK channels compared to *Slo1* α subunit alone (61) makes them well suited to play a role in smooth muscle relaxation following elevation of SMC Ca^{2+}. In support of this idea, genetic deletion of the β1 subunit established that β1-containing BK channels are critical in regulating contractility in arterial (114, 115), tracheal (13, 116), and bladder (117) smooth muscle. Smooth muscle tone reflects a balance between factors favoring vasoconstriction, including elevation of cytosolic Ca^{2+} via voltage-dependent Ca^{2+} channels and release of sarcoplasmic reticulum (SR) Ca^{2+}, and factors favoring vasodilation, including activation of BK channels in response to Ca^{2+} elevation (39). A fascinating aspect of BK activation in vascular smooth muscle is that it is the localized ryanodine receptor (RYR)–mediated release of Ca^{2+} from SR Ca^{2+} stores and not influx through Ca_V channels that appears to be the essential source of Ca^{2+} for BK activation (118, 119). The opening of a single SR RYR channel releases a packet of Ca^{2+} into the cytosol (termed a Ca^{2+} spark, detected optically using fluorescent Ca^{2+}-binding dyes) (118), resulting in a spontaneous transient outward K^+ current (STOC) that reflects coupled activation of a cluster of perhaps 30 BK channels (45). The relationship between Ca^{2+} influx, SR Ca^{2+} filling, spark release, and STOCs in regulating SMC tone can be summarized as follows (120). Ca^{2+} influx elevates global cytosolic Ca^{2+}, which can promote vasoconstriction via activation of myofilaments. However, at normal SMC membrane potentials, β1-containing channels will not be activated. The elevation of cytosolic Ca^{2+} results in gradual filling of the ER, and Ca^{2+} spark frequency increases as Ca^{2+} in the SR increases (121). The localized elevation of Ca^{2+} into the range of 10–30 μM (122, 123) corresponding to the Ca^{2+} sparks is then sufficient to cause localized activation of β1-containing BK channels. In cerebral arteries from β1-KO mice, Ca^{2+} spark frequency and amplitude are unaffected (114), but there is a reduction in average STOC amplitude and an increase in the number of Ca^{2+} sparks that fail to activate any BK current (114, 115). Thus, the presence of the β1 subunit is critical for ensuring that smooth muscle BK channels respond appropriately to local changes in Ca^{2+}.

Despite the contributions of β1-containing BK channels to vasodilation and SMC tone, the consequences of general β1 KO on overall physiology may involve changes in multiple organ systems. For example, although elevations in mean arteriole pressure have been noted in β1-KO mice (114, 115) along with heart enlargement consistent with essential hypertension (114), in other work, longer-term monitoring revealed no increase in mean arteriole pressure in β1-KO mice and no cardiac enlargement (124). This raised the possibility that any hypertension arising from β1 KO may be more closely linked to deficient K^+ secretion in the kidney (125). Furthermore, blood pressure elevation in BK α-subunit KO mice has been attributed to hyperaldosteronism with decreased serum K^+, despite an associated depolarization and attenuated vasorelaxation in SMCs (126). Resolution of some of these issues might benefit from the use of conditional KO models.

Irrespective of the origins of experimental hypertension and the relative contributions of SMCs, kidney, or other players, gain-of-function β1 mutations have been reported to protect against hypertension (127, 128). In addition, an age-related decrease in β1 expression in humans was reported to increase hypertension (129), and direct activation of BK channels by omega-3 fatty acids lowers blood pressure (130). Overall, the results suggest that β1-containing BK channels play an important role in maintaining blood pressure.

β1 (and β4)-Containing BK channels and the kidney.—Although detailed electrophysiological studies of BK channel properties in kidney epithelial cells are not available, brief mention is warranted because of the potential importance of BK channels in kidney function. BK channels are thought to mediate flow-induced K^+ secretion (FIKS) through the apical membrane of epithelial cells into the lumen of collecting ducts of the kidney in response to increased fluid movement (131). Although it is clear that FIKS occurs and BK channels contribute to the increase in luminal K^+, many details of FIKS remain uncertain. Key questions include: (*a*) What are the relative roles of ICs and PCs in FIKS? (*b*) What is the BK channel subunit composition in ICs and PCs and how might that be important for differential functional roles between these cells? (*c*) What are the mechanisms by which flow increases BK activation?

The presence of BK channels in kidney epithelial cells was first established in the early days of patch-clamp recording (132–134). Cell-attached patch recordings from rat cortical collecting tubule epithelial cells indicated that, at normal resting cytosolic Ca^{2+} levels, there is little BK channel activation until well above 0 mV (14). Furthermore, BK channels were more frequently found in patches from likely ICs than PCs (14). Although whole-cell recording methods have rarely been applied to kidney epithelial cells, in one case, using a patch pipette with 10 μM Ca^{2+} revealed a clear tetraethylammonium-sensitive, high-variance current (consistent with BK) in ICs but not in PCs (14), and no current was detected in ICs with 100 nM Ca^{2+}. As yet, the functional properties observed for BK channels in kidney cells do not allow conclusions regarding likely auxiliary subunit composition or specific roles β1-versus β4-containing BK channels might play, although immunohistochemical results support the idea that β4 and β1 are present in ICs and PCs, respectively (135).

A key question regarding the potential physiological roles of BK channels in kidney cells concerns the conditions under which BK channels are activated. In ICs, fluid flow in the

collecting ducts is thought to stimulate K^+ efflux into the lumen, and there are several hypotheses to explain how this might happen. In both cells of the medullary thick ascending limb and ICs, BK channels in patches exhibit increases in Po in response to increased pressure on a patch membrane and osmotic changes (14, 136). One possibility is that a kidney BK splice variant (137) known as the STREX exon (30, 138) may confer mechanosensitivity on BK channels. Although this BK splice variant mediates stretch-induced BK activation in chick heart (139), its role in the mechanosensitivity in mammalian BK channels is unknown. Another hypothesis is that Ca^{2+} influx through TRPV4 (vanilloid-type transient receptor potential channels) in kidney epithelial cells may mediate BK activation (140). Whether one or both of these mechanisms, or some other mechanism, contributes to flow-induced BK activation remains incompletely understood.

Both β1- and β4-KO mice exhibit alterations in urinary function compared to wild-type (WT) controls (125, 141, 142). This prompted the suggestion that hypertension in β1-KO mice may arise from deficient K^+ secretion in the kidney and associated aldosteronism (125), rather than exclusively reflecting the contribution of vascular smooth muscle. Similarly, β4 KO produces a mild hypertension that may also be related to kidney function (143). If β1- and β4-containing BK channels are, in fact, segregated between PCs and ICs, respectively, this raises the question of what unique property of each form of BK channel is critical for the cells in which it is found.

β2-Containing BK Channels

The identification of the β2 (*Kcnmb2*) subunit (70, 72) established the molecular basis for inactivation of BK channels in rodent CCs (90) and pancreatic β cells (66), which was later confirmed with β2-KO animals (78). However, except for work on suprachiasmatic nucleus (SCN) neurons (144) and rat dorsal root ganglion neurons (145), we are unaware of any other reports describing native BK channels that have the unambiguous hallmarks of β2-containing BK channels, as were itemized above. Although KCa currents with inactivating features have been noted in a variety of other loci, including hippocampus, amacrine, and cerebellar Purkinje cells (64, 146–148), the properties of these currents do not allow firm conclusions regarding the underlying auxiliary subunit composition. Better descriptions of the underlying functional properties of inactivating BK currents in native cells in conjunction with the use of animals with KO of specific regulatory subunits would help advance our understanding.

β2-Containing BK channels in chromaffin cells: impact on evoked firing and bursting.—Rodent CCs express both inactivating and noninactivating BK channels, with some cells expressing almost exclusively inactivating (BKi), others exclusively noninactivating (BK_s), and others intermediate channels (7, 90). Cells with predominantly inactivating BK current are better able to fire repetitive APs when stimulated with constant current injection, suggesting that the shifted gating range of α+β2 channels compared to α alone may contribute to more robust AHPs in the BK_i cells, thereby supporting recovery from inactivation of voltage-dependent inward current. Comparisons of WT and β2-KO cells (78) also support this hypothesis, with the unexpected twist that CCs from β2-KO animals exhibit spontaneous slow-wave bursts of activity, also noted in the small fraction of WT CCs

with noninactivating BK current. The ionic basis for such slow-wave bursts and the requirement for BK channels lacking β2 subunits warrant further investigation.

Kinetic differences between α-alone and β2-containing BK channels may also shape AP properties. Because β2-containing channels activate a bit more slowly than α alone, the peak AP in cells with β2-containing BK channels is somewhat higher, perhaps facilitating more Ca_V channel activation. However, none of the functional effects of β2 subunits present in CCs appear linked to the intrinsic inactivation behavior of the channels. At normal resting potentials and resting Ca^{2+}, β2-containing channels are not inactivated, and during the 5-ms halfwidth of CC APs, little inactivation will occur, even at frequencies up to 10 Hz or so. Thus, on the basis of work performed in CCs, at present, we remain ignorant about the potential role of β2-mediated BK inactivation.

β2–Containing BK channels in the suprachiasmatic nucleus.—BK channels in neurons of the SCN have been implicated in circadian rhythmicity (149). SCN neurons in WT animals exhibit reduced spontaneous firing rates (and increased BK protein) during nighttime compared to daytime, and KO of the BK α subunit results in nighttime firing rates indistinguishable from daytime rates (149). Subsequently, it was shown that BK currents in SCN neurons exhibit some inactivation during daytime, but not during nighttime (144). β2 subunit KO decreases SCN neuron firing during daytime, making firing similar to the diminished WT nighttime firing. The reduced firing of WT SCN neurons during nighttime and β2-KO neurons during daytime was proposed to reflect increased BK activity, while the increased daytime firing in WT neurons was thought to show suppression of BK activity by β2-mediated inactivation (144). However, based on a consideration of the rates of β2-mediated inactivation and its voltage and Ca^{2+} dependence, it has been suggested that the increased firing in cells during daytime may reflect a more negative gating range of β2-containing BK channels, thereby increasing firing rates (150). The latter evaluation suggests that the known biophysical properties of β2-mediated inactivation are inconsistent with substantial inactivation occurring during normal SCN firing. Although it is unambiguous that SCN BK channels contain some complement of β2 subunits, how the known underlying properties of β2-containing BK channels may fit with changes in excitability is not fully answered.

Inactivating BK channels and spike broadening.—One early hypothesis was that rapid inactivation of BK channels may contribute to use-dependent spike broadening (151–153), a potential mechanism by which nerve terminal transmitter release might be enhanced. In both hippocampal pyramidal cells (152) and lateral amygdala (153), AP trains occurring at frequencies above 30 Hz exhibit AP broadening of approximately 30–50% over the first three APs. Supporting the involvement of BK currents, the percent increase in AP duration is reduced with BK inhibition by paxilline or IbTx or by manipulations that reduce Ca^{2+} influx or strongly buffer cytosolic Ca^{2+}. However, even when BK activation is reduced, some AP broadening still occurs. In hippocampal cells, 4-AP, a blocker of Kv but not BK channels, produces a more pronounced AP prolongation than BK inhibition, suggesting that BK is not the primary current involved in AP repolarization (152). The specific inactivation behavior of any current that may underlie spike broadening in these cells remains unknown. Although

simulations support the idea that a putative inactivating BK current could underlie AP prolongation (152), these simulations were not grounded in existing knowledge about the functional effects that known inactivating β subunits may confer on BK channels. If inactivating BK channels do, in fact, contribute to spike broadening in some cells, what properties must they have? First, inactivation must be appreciable during APs with a 1-ms halfwidth. Second, inactivation must reach steady state by the third AP in trains where sequential APs occur within less than ~25–30 ms. Neither the properties of β2-containing nor β3-containing BK channels appear consistent with these requirements. For β2-containing BK channels, the onset of β2 inactivation is too slow to allow appreciable inactivation during single APs or a rapid approach to the steady state. For β3-containing BK channels, rapid inactivation of the human β3b isoform would probably allow steady-state inactivation to be achieved during a single AP, but complete recovery would occur between APs. For more slowly inactivating β3 isoforms, the concerns raised in regard to β2 would apply. The availability of β2-KO mice, and perhaps β3-KO mice in the future, may allow such issues to be more unambiguously addressed. Overall, this topic underscores the fact that, to understand the physiological roles of BK channels in native tissues, not only must the specific subunit composition of BK channels in any tissue of interest be defined, but how the biophysical properties of such channels dictate the types of physiological roles a channel can play must be assessed.

β3-Containing BK Channels

β3-KO mice have not yet been described. Similarly, there are no recordings from native cells of BK currents that unambiguously contain β3 subunits. Given that rodents may have only one of the four human β3 N-terminal splice variants (75, 79), this will limit the utility of mice for investigation of β3 physiology, except for the highly conserved β3a subunit. Although early work suggested that all four β3 variants can be found in a broad range of human tissues, including spleen, kidney, pancreas, testis and, with lower abundance, liver, lung, placenta, and brain, we remain largely ignorant regarding the cells that may specifically express β3-containing BK channels. In humans, β3-containing BK channels have been proposed to have an impact on epilepsies (154), insulin resistance (155), and rheumatoid arthritis (156). In the last case, β3b-containing BK channels were proposed to be present in synoviocytes from patients, based largely on detection of mRNA and protein by real-time polymerase chain reaction (RT-PCR) and immunostaining, respectively. Yet recordings of BK currents from synoviocytes, either with or without silencing of β3 expression, have not revealed any functional features that would be considered diagnostic of the presence of β3b-containing BK channels (e.g., Figure 2*l*). Future advances will depend on clear identification of loci in the expression of β3-containing BK channels and demonstration of currents with properties reflecting the presence of β3-containing BK channels.

β4-Containing BK Channels

A series of studies have used β4-KO animals to make inferences regarding the role of IbTx-resistant, β4-containing BK channels in the CNS (92, 157–160). Whereas WT hippocampal dentate gyrus granule cells express IbTx-resistant BK channels, single BK channels from β4-KO animals are IbTx sensitive, consistent with expectations based on heterologous

expression (157). Furthermore, the absence of β4 is associated with a change in channel kinetics to a fast-gating mode characteristic of IbTx-sensitive channels (68). The absence of the β4 subunit also results in a shortening of dentate gyrus AP duration, along with a reduced AHP duration such that, whereas WT cells fire at AP frequencies of less than 20 Hz during constant current injection, β4-KO cells fire at frequencies in excess of 30 Hz (157). The idea is that the slower activation of β4-containing BK channels in WT cells means less BK current is activated during an AP, thereby slowing repolarization. This in turn leads to increased activation of conductances such as apamin-sensitive SK channels that contribute to longer duration AHPs in WT cells. An uncertainty about the shorter duration APs in β4-KO cells is whether this arises exclusively from the faster-gating kinetics of BK channels lacking β4 subunits, or whether an increased surface density of BK channels in the KO cells might also contribute (158). β4-KO mice exhibit temporal loop seizures, generally consistent with the increased excitability of dentate gyrus neurons (157), whether arising from more rapid BK gating kinetics or a net increase in BK current density. The idea that faster-gating kinetics of BK channels lacking β4 subunits might account for the increased firing in β4-KO animals is also supported by computational tests (160, 161).

A complexity in the interpretation of the functional properties of β4-containing BK channels expressed heterologously is that GV shifts and BK gating kinetics obtained with symmetrical K^+ gradients (88) appear to differ from those obtained when physiological K^+ gradients are used (161). This topic requires future attention and needs to be evaluated for other BK auxiliary subunits. In the case of γ1-containing BK channels, the GV properties of heterologously expressed γ1-containing channels (100) are essentially indistinguishable from those of γ1-containing channels in native cells (20).

An apparent challenge in assessing the role of β4-containing channels is that, to date, few studies have successfully isolated macroscopic, voltage-activated, β4-containing BK current in native cells. A clear demonstration of much slower activation of macroscopic BK current in the WT versus β4-KO cells would be an important confirmation of the muting effect of β4 on BK channel activation.

γ1-Containing BK Channels

An LRRC26-KO mouse model has helped define loci of LRRC26 expression (20). A combination of quantitative RT-PCR, β-galactosidase reporter gene activity, protein chemistry, and electrophysiology suggests that γ1-containing BK channels may be exclusively localized to secretory epithelial cells. BK currents recorded from WT lacrimal gland, parotid gland, and submandibular gland cells were activated over voltages consistent with the presence of γ1-containing BK channels, while currents recorded from cells from LRRC26-KO animals were shifted rightward approximately 120 mV. In both parotid and submandibular glands, LRRC26 KO recapitulated the reduction in salivary K^+ secretion associated with KO of BK α subunits (162, 163). Although negative results cannot fully exclude the presence of γ1-containing BK channels in nonepithelial cells, at present there is no functional evidence to support that possibility. In cells postulated to express γ1-containing BK channels, a necessary test is the simple functional demonstration of BK channel activation at negative potentials in cells with 0 cytosolic Ca^{2+}. The expression of

γ1-containing BK channels in specific epithelial cells promises to allow new tests of the physiological roles of such cells. For the other members of the γ subunit family, there is currently no information regarding specific roles in BK channel regulation.

CONCLUDING REMARKS

As the remarkable diversity of BK channel functional properties has been unveiled, the challenge has been to determine the physiological roles that BK channels of particular subunit compositions play in native cells. In some cells, e.g., SMCs, with β1 and secretory epithelial cells with γ1, the sets of conductance are simple enough to allow a clear assessment of BK channel contributions. In excitable cells, the challenges are more complex, given the palette of other conductances, including Kv currents, that may pose challenges to clear identification of the specific BK contributions. A theme of this review is that any such attempt requires not only careful determination of the identity of subunits in a given locus, but also careful attention to the biophysical/functional properties of the native channels. It is the biophysical/functional properties that define what physiological roles a given channel can play. Once the likely functional properties of BK channels in a given native cell are understood, illumination of the contributions of such channels might benefit from more routine application of AP voltage-clamp waveforms and dynamic-clamp approaches as tools to assess the temporal contributions of BK channels to complicated patterns of excitability. Another challenge is that, because of low abundance, knowledge about loci of expression of BK regulatory subunits remains rather rudimentary in many cases. In the future, it is hoped that small genetically encoded, functionally inert optical tags incorporated in regulatory subunits might provide more direct views of where specific regulatory subunits are located.

ACKNOWLEDGMENTS

This work was supported by the US National Institute of General Medical Sciences (NIGMS) under the US National Institutes of Health (NIH; grant GM-118114) to C.J.L.

Glossary

BK	big conductance voltage- and Ca^{2+}-activated K^+
SMC	smooth muscle cell
PC	principal cell
IC	intercalated cell
KO	knockout
K_{Ca}	Ca^{2+}-dependent K^+ channel
ChTx	charybdotoxin
IbTx	iberiotoxin
Kv	voltage-dependent K^+
CC	chromaffin cell

AP	action potential
AHP	afterhyperpolarization
LRRC	leucine-rich repeat-containing
SR	sarcoplasmic reticulum
FIKS	flow-induced K^+ secretion
SCN	suprachiasmatic nucleus

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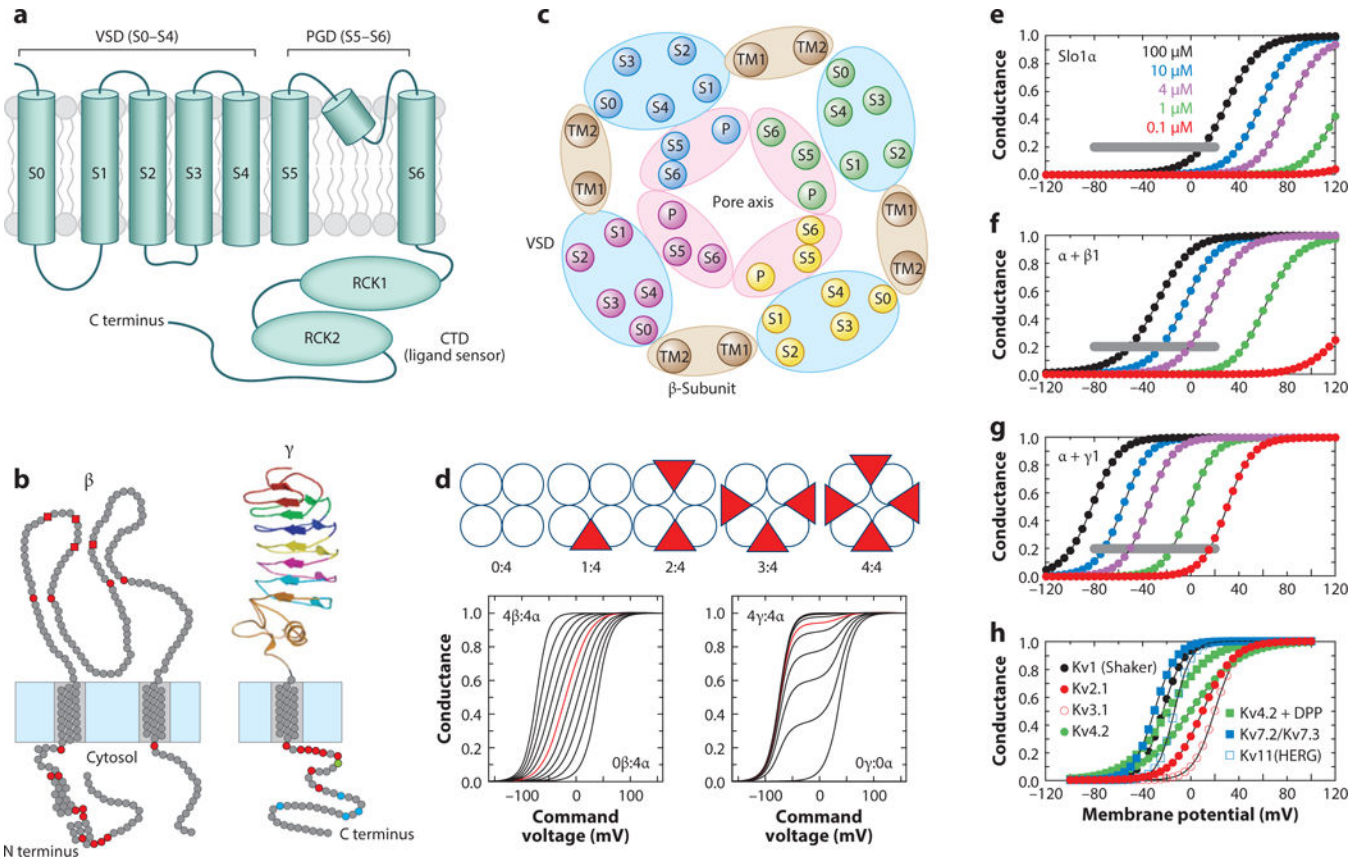

Figure 1.

Two families of regulatory subunits generate BK channel functional diversity. (*a*) General topology of a single BK α subunit, showing the voltage-sensor domain (VSD) formed by transmembrane segments S0–S4, a pore-gate domain (PGD) arising from S5–S6 bracketing the selectivity filter, and a cytosolic domain (CTD) involving regulator of conductance for potassium ligand–sensing modules. Panel adapted from Reference 103 under the terms of the Creative Commons Attribution 4.0 International License, http://creativecommons.org/licenses/by/4.0. (*b*) General transmembrane arrangement of β and γ subunits, with basic residues in red, acidic residues in blue, and extracellular β subunit cysteines in orange. Residues correspond to β3a and γ1. Model of γ1 extracellular structure follows an LRRC domain in a hagfish lymphocyte receptor (99). Panel adapted from Reference 80 (*left*) and Reference 102 (*right*). (*c*) BK channels are tetramers, with up to four β subunits per channel. Positions of β subunits were inferred from cross-linking experiments (164). Panel adapted from Reference 102. (*d*) Both β and γ subunits can assemble with 1–4 subunits per BK channel. Each β subunit in a BK channel incrementally shifts gating (*bottom left*), while a single γ1 subunit is sufficient to produce a gating shift similar to a set of four γ1 subunits. The red curve indicates equimolar α and either β or γ subunit. Panel adapted from Reference 100. (*e–g*) Idealized GV curves for (*e*) BK α-only subunits (32), (*f*) β1-containing BK channels (33), and (*g*) γ1-containing BK channels (34), with a gray bar highlighting the range from −80 to +20 mV. (*h*) GV curvevs for various Kv channels encoded by distinct genes.

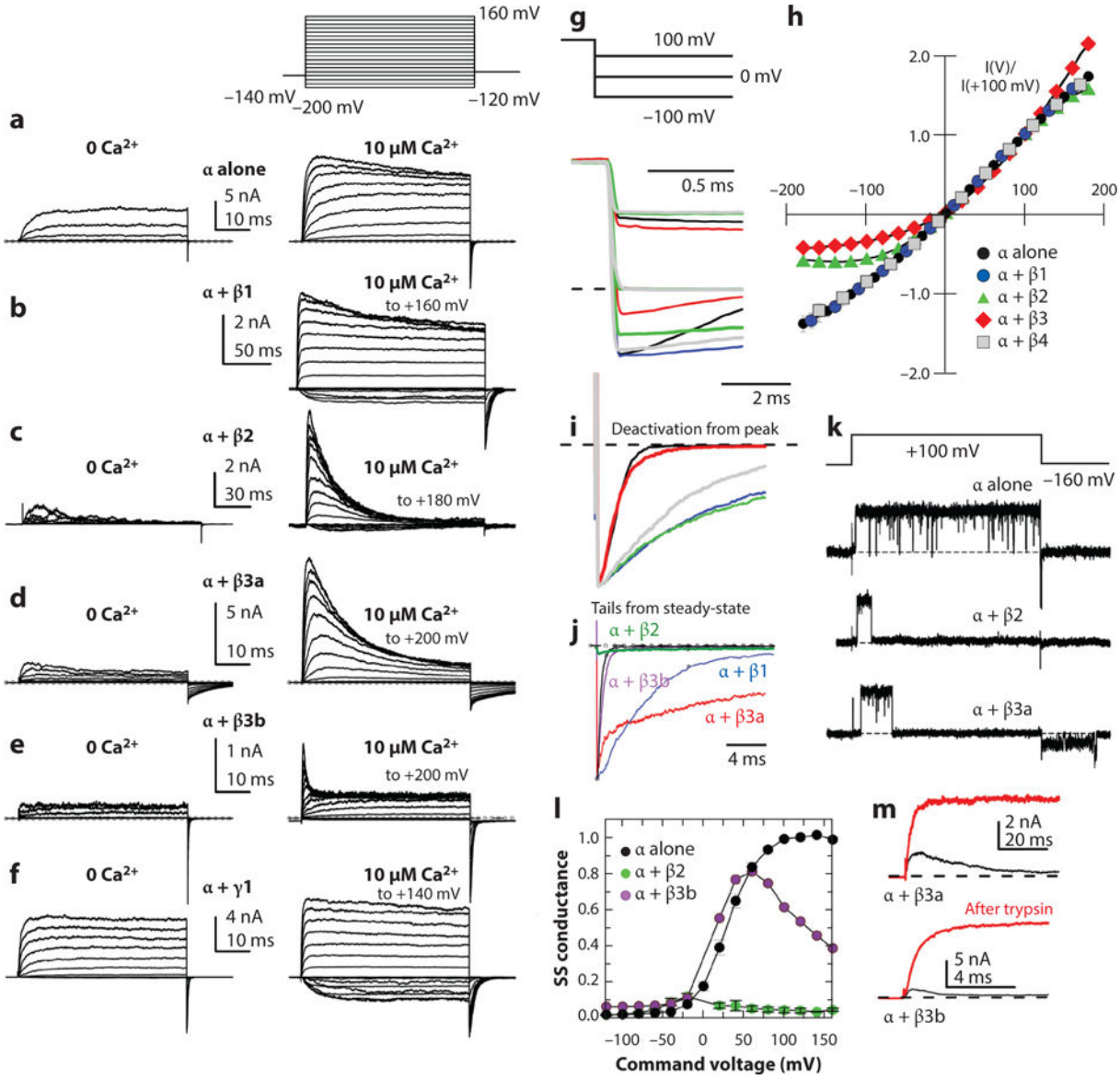

Figure 2.

Functional signatures of various BK regulatory subunits. (*a–f*) Activation at 0 (*left*) and 10 μM Ca^{2+} is shown for the indicated BK channel composition and voltage protocol (maximum voltage in each case indicated on the right-hand family of traces). Traces for β1 and β2 are on a slower time base. Note inward current for β1, β2, and γ1 indicative of leftward-gating shifts and the prominent and kinetically distinct inactivation for β2, β3a, and β3b. (*g*) Instantaneous tail currents are shown at +100, 0, and −100 mV after depolarization to +180 mV at 10 μM Ca^{2+}, highlighting outward rectification of β2 and β3 variants. Both β2 and β3 constructs had their N-terminal inactivation domain removed. (*h*) Full instantaneous IV curves for different β subunits. (*i*) Normalized deactivation for different α + β currents following repolarization from peak current activation. Note slowing of deactivation by β1, β2, and β4 (all at 10 μM Ca^{2+}) but not β3. (*j*) Normalized deactivation following repolarization from steady-state current at +180 mV. Note absence of appreciable

tail current for β2 and pronounced slowing with β3a, while β3b is similar to α alone. (*k*) Single-channel traces showing the absence of tail reopenings for β2-containing channels, but the unusual reduced current burst for β3a that accounts for its tail prolongation. (*l*) Steady-state conductance for β2 and β3b at 10 μM Ca^{2+}, indicating that steady-state activity reflects strong inactivation for β2 channels and rapid voltage-dependent block for β3b. (*m*) Magnitude of outward current for β3a and β3b is dominated by inactivation, which can be readily removed by brief cytosolic trypsin application. Panels *a*, *d*, *e*, and *j* are modified from Reference 80; panels *c* and *f* from Reference 103 under the terms of the Creative Commons Attribution 4.0 International License, http://creativecommons.org/licenses/by/4.0; and panels *g*, *i*, and *j* from Reference 84.

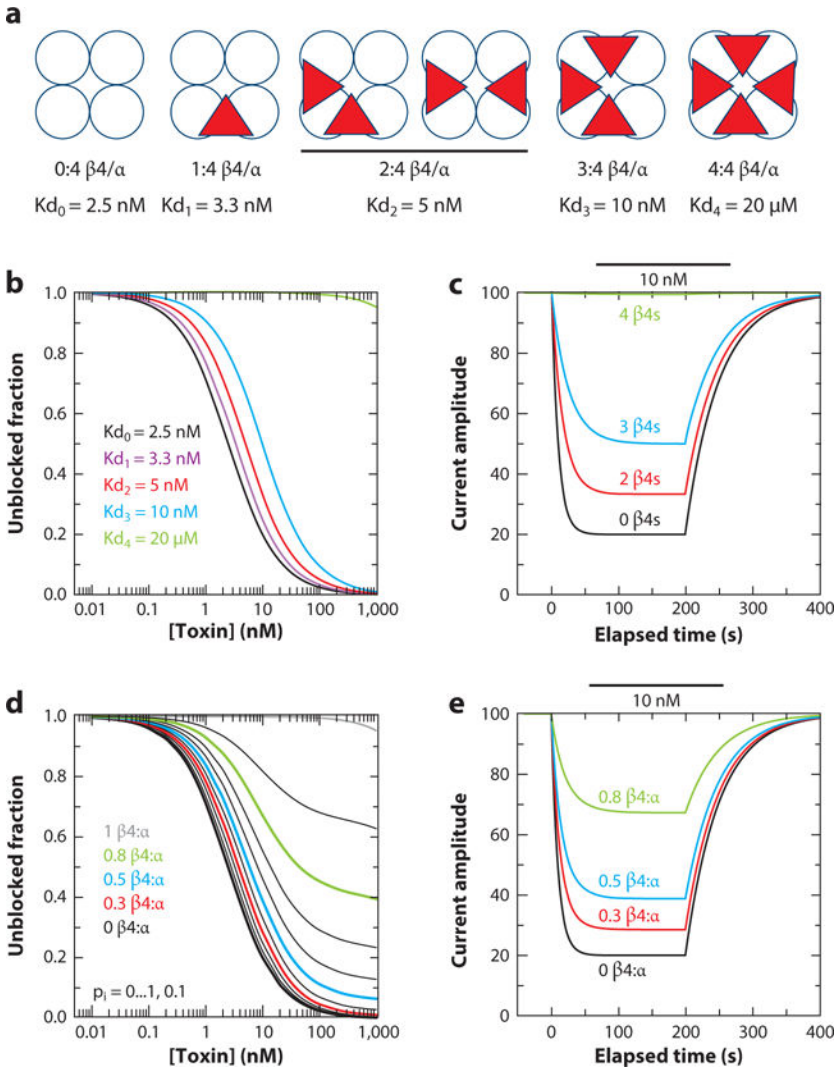

Figure 3.

Potential impact of subunit stoichiometry on BK pharmacology. (*a*) Model of toxin sensitivity in which channels lacking a β4 subunit have four potential toxin-binding orientations, while the addition of 1–4 β4 subunits reduces the available binding orientations, resulting in different forward rates of block and Kds. (b) Calculated toxin inhibition for channel populations, each of a given stoichiometry shown in panel *a*. (c) Calculated onset and recovery of inhibition by 10 nM of nominal toxin, showing that even channels (all of an identical stoichiometry) with up to three β4 subunits may exhibit appreciable inhibition. The single-site forward rate was assumed as 2×10^6 $M^{-1}s^{-1}$, with a 0.02 s^{-1} unblock rate. (*d*) Toxin inhibition for a population of channels with regulatory subunits distributed in a binomial fashion for β4 subunit mole fractions of 0 to 1 in steps of 0.1. (*e*) Calculated onset and recovery based on populations of channels with different mole fractions of β4 subunits.

Table 1

Functional hallmarks of BK channels with particular regulatory subunits

BK channel	Gating shifts relative to α alone	Kinetic features	Inactivation	Instantaneous current rectification	Pharmacology
α-Alone	NA	NA	None	Linear	ChTx (2–4 nM)
β1-Containing	−70-mV leftward shift at elevated Ca^{2+}	Prolonged single channel bursts and tail currents even at 0 Ca^{2+}	None	Linear	DHS activation ChTx (~8 nM)
β2-Containing	−50-mV leftward shift at elevated Ca^{2+}	Absence of tail current during recovery from inactivation	20–40 ms inactivation Complete inactivation with β2:α approaching 1	More than β1 and β4	Some ChTx/IbTx resistance ChTx (30–60 nM)
β3-Containing	None for human variants Mouse shifts gating −30-mV leftward at both 0 and elevated Ca^{2+}	β3a produces prolonged tail currents during recovery from inactivation	Human: β3a: 40–50 ms (incomplete) β3b: 2 ms (but incomplete) β3c: 60 ms Mouse: β3a: 40 ms (complete) β3b: no inactivation	Greater than β2, β1, or β4	ChTx (60–80 nM)
β4-Containing	+20-mV rightward shift at low Ca^{2+}, but a −25- to −50-mV leftward shift at higher Ca^{2+}	Slowed activation and deactivation	None	Linear	Almost complete resistance to IbTx and ChTx block (could be stoichiometry dependent)
γ1-Containing	−120-mV leftward shift at both 0 and elevated Ca^{2+}	Faster activation and slowed deactivation	None	Linear	Mallotoxin resistance
γ2-Containing	−100-mV leftward shift at all Ca^{2+}	Similar to γ1	None	Not described	None

Estimates largely based on recordings with symmetrical 140 mM K^+ in excised patches. Shading highlights strongly diagnostic features.

Abbreviations: ChTx, charybdotoxin; DHS, dehydrosoyasaponin; IbTx, iberiotoxin; NA, not applicable.