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Regulation of voltage-activated K^+ channel gating by transmembrane β subunits

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Voltage-activated K^+ (K_V) channels are important for shaping action potentials and maintaining resting membrane potential in excitable cells. K_V channels contain a central pore-gate domain (PGD) surrounded by four voltage-sensing domains (VSDs). The VSDs will change conformation in response to alterations of the membrane potential thereby inducing the opening of the PGD. Many K_V channels are heteromeric protein complexes containing auxiliary β subunits. These β subunits modulate channel expression and activity to increase functional diversity and render tissue specific phenotypes. This review focuses on the K_V β subunits that contain transmembrane (TM) segments including the KCNE family and the β subunits of large conductance, Ca^{2+}- and voltage-activated K^+ (BK) channels. These TM β subunits affect the voltage-dependent activation of K_V α subunits. Experimental and computational studies have described the structural location of these β subunits in the channel complexes and the biophysical effects on VSD activation, PGD opening, and VSD–PGD coupling. These results reveal some common characteristics and mechanistic insights into K_V channel modulation by TM β subunits.

Keywords: channel, β subunit, K_v, KCNQ1, BK, KCNE, KCNMB, LRRC

INTRODUCTION

Cellular electrical signals organize and control activity in the nervous, muscular, and hormonal tissues. The voltage-activated K^+ (K_V) channels are a large group of transmembrane (TM) proteins that open in response to membrane depolarization to permit the selective efflux of potassium ions across the membrane. K_V channels play an important role in shaping the electric signals of excitable tissues and also contribute to the maintenance of ion homeostasis. Although K_V channels vary greatly in their activity, conductance and pharmacology, the basic structure of the channel and the mechanism of voltage-dependent activation are well conserved across the K_V family. K_V channels share a common topology of six TM α helices (S1–S6) that are organized into two structural domains, the voltage-sensing domain (VSD, S1–S4) and the pore-gate domain (PGD, S5 and S6). As seen in the available K_V channel crystal structures, four α-subunits coassemble to form a tetrameric complex with a central pore built from the PGDs of all four subunits and with the four VSDs located peripheral to the central pore (**Figure 1**).

Voltage-dependent activation of K_V channels involves three general molecular events (Zagotta et al., 1994). First, depolarization of the membrane potential drives the outward movement of the S4 segment of the VSD that contains conserved basic residues positioned within the electric field (Tombola et al., 2005; Bezanilla, 2008). Second, the conformational change during VSD activation is propagated to the PGD through interactions between the S4/S5 linker and the cytosolic side of S6 (Lu et al., 2001; Tristani-Firouzi et al., 2002; Long et al., 2005), the S4 and S5 helices (Ledwell and Aldrich, 1999; Soler-Llavina et al., 2006; Grabe et al., 2007), and the extracellular side of the S1 and the pore helix (Lee et al., 2009); this event is known as coupling. The third event of K_V channel activation is the opening of the PGD to allow ion permeation (Yellen, 1998).

Voltage-activated K^+ channel function is modulated by auxiliary β subunits (Pongs and Schwarz, 2010). Of particular interest to this review are the TM β subunits due to their ability to regulate the voltage-dependent activation of K_V channels. Here we review the major TM β subunits including the KCNE family and the β subunits of voltage- and Ca^{2+}-activated K^+ (BK) channels to summarize our current understanding of the location of β subunits in the channel complex and their impact on the three molecular events of K_V channel voltage-dependent activation.

KCNE β SUBUNITS

The KCNE family of β subunits consists of five members (KCNE1–5, also known as minK and minK related peptides 1–4) that all contain a single TM domain with an intracellular COOH terminus and an extracellular NH_2 terminus (Takumi et al., 1988; Abbott et al., 1999; Piccini et al., 1999; **Figure 1A**). KCNE subunits coassemble with and modulate K_V α subunits resulting in a diverse set of K_V channel phenotypes. Not only can one member of the KCNE family regulate multiple different K_V channels,

Abbreviations: AF, atrial fibrillation; CTD, cytosolic domain; *F–V*, fluorescence–voltage relationship; *G–V*, conductance–voltage relationship; K_V channels, voltage-activated K^+ channels; LQTS, long QT syndrome; PGD, pore-gate domain; P_o–*V*, open probability–voltage relationship; SQTS, short QT syndrome; TM, transmembrane; VCF, voltage-clamp fluorometry; VSD, voltage-sensing domain.

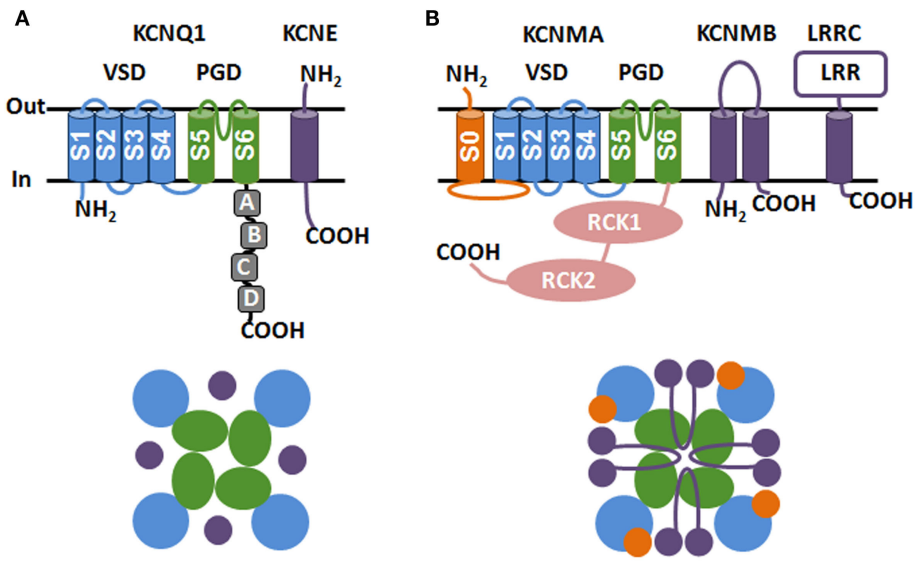

FIGURE 1 | Topology (top) and coassembly (bottom) of KCNQ1 (A) and BK channel (B) α subunits and β subunits. The voltage-sensing domain (VSD, S1–S4), pore-gate domain (PGD, S5 and S6), cytosolic domain (RCK1 and RCK2), S0 segment, and β subunits are colored as blue, green, pink, orange, and purple, respectively. The COOH terminal α-helices (A–D) of KCNQ1 and the leucine-rich-repeat domain (LRR) for LRRC are indicated. The coassembly of the heteromeric channel complex are illustrated as viewed from the extracellular face of the membrane (bottom).

but one K_V family member can be regulated by different KCNEs. For example, KCNQ1 (K_V7.1) can coassemble with all five of the KCNE family peptides; however, the effects of the different KCNEs on KCNQ1 function are very different (Barhanin et al., 1996; Sanguinetti et al., 1996; Schroeder et al., 2000; Tinel et al., 2000; Angelo et al., 2002; Grunnet et al., 2002). These effects range from constitutive, voltage-independent activation with KCNE3 (Schroeder et al., 2000), a shift of voltage-dependent activation to more positive voltages with KCNE1 (Barhanin et al., 1996; Sanguinetti et al., 1996) to inhibition of the ionic current by KCNE4 (Grunnet et al., 2002). Promiscuity in coassembly and diversity of functional regulation allows different tissues to carry unique electrophysiological phenotypes by expressing different KCNE–K_V combinations.

KCNE1 MODULATION OF KCNQ1

Of the many KCNE–K_V pairs, the channel formed by coassembly of KCNE1 with the KCNQ1 is the best studied due to the physiological importance of this channel, the dramatic effects of KCNE1 on the function of KCNQ1, and the fact that it was the first identified KCNE–K_V partnership. In the heart the heteromeric assembly of KCNQ1 + KCNE1 subunits generates the slow delayed-rectifier current (Barhanin et al., 1996; Sanguinetti et al., 1996), I_{Ks}, that is important for termination of the cardiac action potential (Sanguinetti and Jurkiewicz, 1990; Jost et al., 2007). In the inner ear KCNQ1 + KCNE1 channels play a role in the maintenance of endolymph potassium homeostasis (Vetter et al., 1996). Mutations in KCNQ1 (Wang et al., 1996b) or KCNE1 (Splawski et al., 1997) that compromise I_{Ks} function can cause long QT syndrome (LQTS) that manifests as prolongation of the QT interval on the surface electrocardiogram and a high risk of ventricular arrhythmias and sudden death. In some cases, the cardiac phenotype can be accompanied by congenital deafness (for review see Hedley et al., 2009). In contrast, I_{Ks} gain of function mutations cause premature repolarization and are associated with short QT syndrome (SQTS; Bellocq et al., 2004) and atrial fibrillation (AF; Chen et al., 2003; Hong et al., 2005; Lundby et al., 2007; Das et al., 2009).

The functional importance of KCNE1 effects on KCNQ1 channels is demonstrated by the existence of LQTS mutations in KCNE1 and by the remarkable modulation of KCNQ1 activity by KCNE1 in heterologous expression systems. Exogenous expression of KCNQ1 alone is sufficient to generate voltage-dependent potassium channels; however, coexpression of KCNE1 with KCNQ1 shifts the voltage-dependence of activation toward more depolarized potentials, slows activation and deactivation kinetics (Barhanin et al., 1996; Sanguinetti et al., 1996), removes inactivation (Pusch et al., 1998; Tristani-Firouzi and Sanguinetti, 1998), increases single channel conductance (Sesti and Goldstein, 1998; Yang and Sigworth, 1998), increases PIP_2 sensitivity (Li et al., 2011), renders the channel functionally sensitive to PKA phosphorylation (Kurokawa et al., 2003, 2009), and alters the channel pharmacology (Busch et al., 1997; Lerche et al., 2000). These functional changes result in a current that closely recapitulates the properties of the cardiac I_{Ks} current that are key to myocyte function *in vivo* (Silva and Rudy, 2005). In this brief review we will discuss the current evidence regarding the mechanism by which KCNE1 regulates the voltage-dependent activation of KCNQ1 as the best understood example of KCNE regulation of K_V channel gating. The study of KCNE3 and KCNE4 regulation of KCNQ1 has also provided similar mechanistic insight; for more details we refer the interested reader to another review to appear in this issue (Wrobel et al., submitted).

THE LOCATION OF KCNE1 IN THE KCNQ1 + KCNE1 CHANNEL

Biochemical and cysteine cross-linking assays have established proximity between various locations of the KCNE1 TM segment and KCNQ1 (Xu et al., 2008; Chung et al., 2009; Lvov et al., 2010; Chan et al., 2012; **Figure 1A**). On the extracellular side of the membrane, proximity between the KCNE1 NH_2 terminus (positions 36–43) and the tops of S1, S4, and S6 has been shown through disulfide cross-linking (Wang et al., 2011; Chan et al., 2012). An interaction between the distal KCNE1 COOH terminus and helix C of the KCNQ1 COOH terminus (**Figure 1A**) has been detected by co-immunoprecipitation (Haitin et al., 2009). However, truncation experiments indicate that removing either the extracellular NH_2 terminal domain (Takumi et al., 1991) or the cytosolic distal COOH terminus (Tapper and George, 2000; Chen et al., 2009) of KCNE1 does not prevent the right shift in voltage-dependence and slowing of activation kinetics upon coexpression with KCNQ1, indicating that these structures are not absolutely required for modulation of voltage-dependent activation in exogenous expression systems. Nevertheless, the distal COOH terminus of KCNE1 is required for imparting sensitivity to PKA phosphorylation (Kurokawa et al., 2009) downstream of β adrenergic stimulation, which is vital for the ability of I_{Ks} to regulate heart rhythm *in vivo* (Marx et al., 2002; Kurokawa et al., 2003; Volders et al., 2003). In addition, PKA-dependent phosphorylation can modify voltage-dependent activation by shifting the *G–V* curve toward less depolarized potentials and slowing deactivation kinetics (Kurokawa et al., 2003, 2009; Li et al., 2011).

In contrast, truncation and chimera studies show that the KCNE1 TM segment and proximal COOH terminus are both absolutely required for right shifting the KCNQ1 *G–V* and slowing of activation kinetics in heterologous expression systems (Tapper and George, 2000). Metal bridging of G55C in KCNE1 to C331 in KCNQ1 (Tapper and George, 2001) and mutant cycle analyses (Strutz-Seebohm et al., 2011) have provided evidence for an interaction between the KCNE1 TM segment and the outer aspect of the PGD. Cysteine cross-linking indicates that the proximal KCNE1 COOH terminus is in interaction with the KCNQ1 S4/S5 linker and S6 gate, key structures of the gating machinery (Lvov et al., 2010). An early study showed that mutation of the KCNE1 proximal COOH terminus strongly affects the activity of channels consisting of exogenously expressed KCNE1 and the KCNQ1 homolog that is endogenously expressed in the *Xenopus* oocyte (Takumi et al., 1991). Of note, the proximal COOH terminus is among the most highly conserved regions across the KCNE family, although this region may be of lower importance for the regulation of KCNQ1 activation by other KCNE family members (Melman et al., 2001, 2002; Gage and Kobertz, 2004).

These experimental results have been used to build various models of the KCNQ1 + KCNE1 channel complex using the atomic coordinates of $K_V1.2$ as a template for KCNQ1 (Kang et al., 2008; Xu et al., 2008; Chung et al., 2009; Strutz-Seebohm et al., 2011; Van Horn et al., 2011; Chan et al., 2012). These models consistently locate the KCNE1 TM segment in a cleft that is formed between two VSDs and adjacent to the outer face of the PGD (**Figure 1A**). From this position, KCNE1 participates in a broad set of interactions with KCNQ1 that span the entire length of the KCNE1 peptide. This interface includes interactions with both the VSD and the PGD, and contacts are made with as many as three KCNQ1 subunits of the tetramer. These structural models do not identify the likely mechanism for how KCNE1 modulates the voltage-dependent activation of KCNQ1 as the interactions with the VSD, PGD, and S4/S5 linker suggest that regulation of VSD movement, PGD opening, and electromechanical coupling are all plausible. These results are consistent with the biophysical experiments as reviewed below.

KCNE1 REGULATION OF VSD ACTIVATION

The biophysical effects of KCNE1 on the KCNQ1 VSD have been explored in several studies. First the state-dependent accessibility of a cysteine engineered into the NH_2 terminal part of S4 (positions 226–230) has been probed with MTS reagents to track S4 movement in response to membrane depolarization (Nakajo and Kubo, 2007; Rocheleau and Kobertz, 2008). In these studies, coexpression of KCNE1 with KCNQ1 slowed the rate of S4 cysteine modification. However, this result alone does not conclusively indicate that KCNE1 slows the movement of the KCNQ1 VSD since the kinetics of chemical modification are dependent on the solvent accessibility and chemical environment of the engineered cysteine (Rocheleau and Kobertz, 2008). In our hands, coexpression of KCNE1 with KCNQ1 exposes a cysteine engineered into the KCNQ1 VSD (E160C in S2) to extracellular MTS reagents (Wu et al., 2010a) indicating that KCNE1 can change either the solvent penetration into the VSD or the electrostatic environment within the VSD. Tryptophan and alanine scanning of the S4 segment with and without KCNE1 shows that KCNE1 changes the tolerance to mutation at positions within the VSD (Shamgar et al., 2008; Wu et al., 2010b) suggesting an alteration in the protein packing between S4 and the rest of the VSD or the PGD. These effects on protein packing were most dramatic for mutations of the intracellular half of S4 (Wu et al., 2010b). Changes in the solvent penetration or protein packing within the voltage sensor will change the profile of the electric field surrounding the S4 gating charges and thereby directly alter voltage sensation within the VSD. Recently, the voltage-clamp fluorometry (VCF) technique has been used to directly track VSD movement revealing that the voltage-dependence of VSD activation extends to far more hyperpolarized potentials in the presence of KCNE1 relative to KCNQ1 alone (Osteen et al., 2010). Together these results indicate that KCNE1 changes the structure and movements of the VSD. However, these changes do not offer a clear mechanism for the slow current kinetics and right shifted *G–V* relationship observed upon coexpression of KCNE1 with KCNQ1. As shown by MTS modification and VCF experiments, the kinetics of VSD activation are much faster than the current activation in the presence of KCNE1 (Rocheleau and Kobertz, 2008; Osteen et al., 2010), and the voltage-dependence of VSD movements is shifted by KCNE1 in the opposite direction (i.e., left) relative to the shift in the *G–V* relationship (Osteen et al., 2010).

KCNE1 REGULATION OF PGD OPENING

Study of either wild-type or mutant KCNE1 showed that KCNE1 alters single channel conductance (Sesti and Goldstein, 1998; Yang and Sigworth, 1998), ion selectivity (Goldstein and Miller, 1991), and channel block (Goldstein and Miller, 1991; Wang et al.,

1996a; Tai and Goldstein, 1998) suggesting that KCNE1 affects the structure of the KCNQ1 channel pore. Transplantation of the KCNQ1 PGD and cytosolic COOH terminus into K_V1.4 yielded a chimeric channel that coassembles with and is regulated by KCNE1 (Melman et al., 2004). Similarly, a recent study shows that KCNE1 shifts the *G–V* and activation kinetics of human and invertebrate KCNQ1 homologs differently, and chimeric channels between the two show that parts of the human KCNQ1 PGD (especially the S5–S6 pore loop) are required to endow the chimeric channel with the human phenotype of KCNE1 regulation (Nakajo et al., 2011). An interaction between KCNE1 and the PGD could modulate steady-state voltage-dependence by changing the energetic gap between the closed and open state of the PGD; also, this interaction could change activation kinetics by changing the energy of the transition between the open and closed state.

KCNE1 MODULATION OF COUPLING

As mentioned previously, disulfide cross-linking establishes an interaction between the KCNE1 proximal COOH terminus (residues 70–81) and the KCNQ1 S4/S5 linker (residues 251–257) as well as the S6 gate (residues 342–370; Lvov et al., 2010). The protein contacts between the S4/S5 linker and S6 gate are probably the major structural pathway for coupling VSD activation to PGD opening in KCNQ1, similar to electromechanical coupling in other K_V channels (see Introduction). Central to the coupling interface in the K_V1.2 crystal structure is a phenylalanine residue in the S6 gate that is highly conserved among K_V family channels (Haddad and Blunck, 2011; F481 in shaker, F351 in KCNQ1). Interestingly, an artificial mutation at this position in the KCNQ1 S6 (i.e., F351A; Boulet et al., 2007) as well as two naturally occurring LQTS mutations (R243C and W248R; Franqueza et al., 1999) and several artificial mutations (Labro et al., 2011) in the KCNQ1 S4/S5 linker all result in a right shifted *G–V* relationship and slow gating kinetics when the mutant α subunits were expressed alone. Thus, perturbing the internal coupling interface in KCNQ1 can mimic the phenotype of KCNE1 modulation of KCNQ1. Decoupling of PGD opening from VSD movements by KCNE1 is shown by the recent KCNQ1 VCF data as KCNE1 coexpression shifts the *F–V* (VSD activation) toward more hyperpolarized potentials while the *G–V* (PGD opening) is shifted to more depolarized potentials (Osteen et al., 2010).

A second interaction interface for coupling between the K_V VSD and the PGD has been identified between S1 and the pore loop (see Introduction). Interestingly, a cluster of gain of function KCNQ1 mutation that are associated with AF (S140G, V141M, Q147R) are located in this part of S1. Furthermore, these mutations have little impact on the function of homomeric KCNQ1 channels, but cause defective deactivation in the presence of KCNE1 (Chen et al., 2003; Hong et al., 2005; Lundby et al., 2007; Chan et al., 2012). These results indicate that KCNE1 may affect the coupling of the VSD and the PGD at both the intracellular and extracellular face of the membrane.

BK CHANNEL β SUBUNITS

BK channels have a unitary conductance of ∼100–300 pS (Marty, 1981). The opening of BK channels repolarizes the membrane and shuts down the voltage-dependent Ca^{2+} channels, thereby reducing Ca^{2+} influx. Through this negative feedback mechanism, BK channels regulate membrane excitability and intracellular Ca^{2+} signaling (Lancaster and Nicoll, 1987; Storm, 1987; Brayden and Nelson, 1992). BK channels are composed of pore-forming α subunits (Slo1) and accessory β subunits. The Slo1 subunit contains three structural domains, which are the VSD, the cytosolic domain (CTD) and PGD. In BK channels the opening of the PGD is controlled by voltage-sensing in the VSD, as in other K_V channels, and by Ca^{2+} binding in the CTD. In addition to the canonical VSD (S1–S4) of K_V channels, Slo1 contains a unique TM segment (S0; Wallner et al., 1996) which affects VSD activation (Koval et al., 2007; **Figure 1B**). A prominent feature of BK channel activation is the allosteric coupling between the VSD and the activation gate, i.e., the PGD can open when the VSDs are either in the resting or activated state but the activation of the VSDs promotes channel opening (Horrigan et al., 1999).

Four types of 2TM β subunit (β1–β4, encoded by the genes *KCNMB1–4*; Knaus et al., 1994; Wallner et al., 1999; Xia et al., 1999, 2000; Behrens et al., 2000; Brenner et al., 2000; Meera, 2000) and one 1TM β subunit (leucine-rich-repeat-containing protein 26, LRRC26; Yan and Aldrich, 2010) have been identified to date. Since the BK channel α subunit is encoded by a single gene (*slo1*), the tissue specific expression and differential modulation among these β subunits provide a major mechanism for generating a diversity of BK channel phenotypes in different tissues. The effects of β subunits on BK channel function are multifaceted, including altering voltage-dependent activation (Cox and Aldrich, 2000; Wang et al., 2006), changing Ca^{2+} sensitivity (McManus et al., 1995; Nimigean and Magleby, 1999; Wallner et al., 1999; Xia et al., 1999; Brenner et al., 2000), conferring inactivation (Wallner et al., 1999; Xia et al., 1999, 2000; Brenner et al., 2000), and endowing sensitivity to extracellular ligands (Valverde et al., 1999). Here we focus on the structural and functional interactions between different β subunits and Slo1 to elucidate how BK channel β subunits regulate voltage-dependent activation. Readers may refer to excellent reviews elsewhere for other perspectives of BK β subunits (Orio et al., 2002; Torres et al., 2007; Pongs and Schwarz, 2010; Wu and Marx, 2010).

LOCALIZATION OF 2TM β SUBUNITS IN BK CHANNELS

Among the four 2TM β subunits, β1, β2, and β3 share higher sequence homology (53% similarity between β2 and β1; 37% similarity between β3 and β1), whereas β4 is less conserved (less than 20% similarity with β1; Wallner et al., 1999; Brenner et al., 2000; Xia et al., 2000). However, all four β subunits adopt a similar membrane topology that includes two TM segments (TM1 and TM2), a large extracellular loop (116–128 amino acid residues) and short cytosolic NH_2 and COOH termini (Knaus et al., 1994; Xia et al., 1999; Brenner et al., 2000; Orio et al., 2002; **Figure 1B**).

Different Slo1 orthologs respond to β subunits modulation differently. For instance, the β1 and β2 subunits alter the voltage- and Ca^{2+}-dependent activation of mammalian Slo1 (human hSlo1 and mouse mSlo1) channels more prominently than drosophila Slo1 (dSlo1; Wallner et al., 1996; Lee et al., 2010). Studies of chimerical channels of mammalian Slo1 and dSlo1 revealed that the S0 TM segment is important for the functional effects of β subunits (Wallner et al., 1996; Morrow et al., 2006; Lee et al., 2010).

By engineering disulfide linkages, Marx and colleagues model S0 outside of the VSD adjacent to the S3 and S4 helices, while the two TM segments of the β subunits (TM1 and TM2) are packed close to each other at the mouth of the cleft between VSDs of two adjacent Slo1 subunits (Liu et al., 2008a, 2010; **Figure 1B**). Within this cleft, TM1 is close to S1 of one VSD and TM2 close to S0 of the adjacent VSD (Liu et al., 2008b, 2010; Wu et al., 2009; Zakharov et al., 2009). This model gives rise to the possibility of direct interactions between β subunits and the Slo1 VSD within the membrane. Interestingly, although experimental data suggest that the TM segments of β1, β2, and β4 subunits similarly localize with the Slo1 VSD, the functional effects of these β subunits on the voltage-dependent activation of BK channels differ (see below).

The extracellular loop of β subunits has been shown to alter BK channel block by charybdotoxin (CTX) and iberiotoxin (IbTX; Hanner et al., 1998; Meera, 2000; Chen et al., 2008). These peptide toxins block the channel pore by binding to the extracellular vestibule. The extracellular loop of β subunits interacts with these toxins to either enhance or reduce channel block, suggesting that the extracellular loop may extend from the TM domain at the channel periphery into the external vestibule and alter the entrance of the channel pore (**Figure 1B**). This picture was supported by another study regarding the rectification mechanism of BK channels in the presence of β3, in which the authors suggested that the extracellular loop of the β3 subunit associates with the Slo1 subunit near the axis of the permeation pathway to form gates blocking ion permeation (Zeng et al., 2003). The extracellular loop of the β1 subunit affects voltage-dependent activation. A recently published work (Gruslova et al., 2012) identified a functional domain in the extracellular loop of β1, consisting of residues Y74, S104, Y105, and I106, that alters voltage-dependent activation, possibly by promoting VSD activation and reducing the intrinsic open probability of the PGD. The extracellular loop connects the channel periphery to the pore so that it may modulate voltage-dependent activation by providing an additional linkage between the VSD and PGD or through direct interactions with these Slo1 structural domains.

The location of the cytosolic termini of β subunits relative to the Slo1 subunit is not clear except that the NH_2 terminus of β2 subunits is known to contain an inactivating domain that blocks the entrance to the intracellular pore with a "ball-and-chain" mechanism indicating that the NH_2 can reach the internal vestibule (Wallner et al., 1999; Xia et al., 1999, 2003; Bentrop et al., 2001; Zhang et al., 2006, 2009; Li et al., 2007). Nevertheless, deletion mutations of the cytosolic NH_2 and COOH termini eliminate the major effects of the β1 subunit on voltage-dependent activation (Wang and Brenner, 2006), indicating that the termini play an important role in Slo1-β subunits interactions. Consistently, a study of the chimeras between the β1 and β2 subunits showed that switching the cytosolic termini between the two β subunits switched the phenotypes of modulation of voltage-dependence and kinetics of activation (Orio et al., 2006).

2TM β SUBUNITS REGULATION OF VSD ACTIVATION, PGD OPENING, AND COUPLING

The β1 subunit

Among four types of 2TM β subunits, the β1 subunit has the largest effect on voltage-dependent activation. Recordings of macroscopic and single channel currents in the absence of Ca^{2+} show that the β1 subunit shifts the G–V or P_o–V relationship to more positive voltages (∼+20 mV) and decreases the steepness of these curves, indicating that the β1 subunit reduces voltage-sensitivity of the channel (McManus et al., 1995; Cox and Aldrich, 2000; Nimigean, 2000; Orio and Latorre, 2005). The β1 subunit may alter all three general steps of voltage-dependent activation based on the following evidence. First, gating current recordings show that the β1 subunit shifts the voltage-dependence of gating charge movements (Q–V curve) to more negative voltages in the absence of Ca^{2+} (Bao and Cox, 2005), indicating an impact of the β1 subunit on VSD activation. Second, at negative voltages, where the VSDs are at the resting state, the open probability of the channel is reduced in the presence of the β1 subunit, indicating that the intrinsic opening of the PGD is affected (Orio and Latorre, 2005; Wang and Brenner, 2006). Finally, evidence for the β1 subunit modulating the coupling between the VSD and the activation gate comes from fitting the above data to the HCA model (Horrigan et al., 1999) that describes the allosteric mechanism of voltage-dependent activation of BK channels. In the HCA model the VSDs activate when the channel is either open or closed, but at the open state VSD activation is more likely at any given voltage (the resting-activation equilibrium increases D fold, D is called the allosteric factor describing coupling between VSD activation and PGD opening). Studies from various laboratories show that the β1 subunit alters the allosteric factor D (Cox and Aldrich, 2000; Bao and Cox, 2005; Orio and Latorre, 2005; Wang and Brenner, 2006), indicating changes in the coupling between VSD activation and PGD opening.

The modulation of voltage-dependant activation may be a major mechanism underlying another predominant effect of the β1 subunit: increasing Ca^{2+} sensitivity, i.e., a Ca^{2+} concentration change from 0 to 100 μM elicits a larger shift of the G–V relation toward negative voltages in the presence of the β1 subunit (McManus et al., 1995). In studies by a number of laboratories, the effects of the β1 subunit were fitted to the HA model that describes allosteric voltage- and Ca^{2+}-dependent activation of BK channels (Horrigan and Aldrich, 2002). These model fittings show that changes of VSD activation and the intrinsic probability of PGD opening account for a large portion of increased Ca^{2+} sensitivity observed in experiments, while the changes in Ca^{2+} binding and its coupling to the activation gate are relatively minor (Orio and Latorre, 2005; Wang and Brenner, 2006). Such a correlation between altered VSD activation and increased Ca^{2+} sensitivity may not be a coincidence. Mutations in the Slo1 VSD that alter VSD activation (Ma et al., 2006) have been shown to reduce the effect of the β1 subunit on Ca^{2+} sensitivity; interestingly, the degree to which a mutation alters voltage-sensor activation is inversely correlated with the magnitude of the β1 subunit's effects on Ca^{2+} sensitivity of the mutant channel (Yang et al., 2008). These results suggest that the β1 subunit increases Ca^{2+} sensitivity of BK channels by altering VSD activation. However, the molecular mechanism of how changes in VSD activation alter Ca^{2+} sensitivity is not clear.

Other β subunits

Unlike the β1 subunit, the β2 subunit effects on voltage-dependent activation of BK channels are minimal (Orio and Latorre, 2005).

While both β subunits enhance Ca^{2+} sensitivity, β2 does not shift the *G–V* curve of BK channels in the absence of Ca^{2+} (Orio and Latorre, 2005; Lee et al., 2010). Mutations in the Slo1 VSD that alter VSD activation and reduce the β1 subunit modulation of Ca^{2+} sensitivity have little impact on the β2 subunit modulation of Ca^{2+} sensitivity (Yang et al., 2008). On the other hand, the NH_2 terminus of the CTD (the AC region) and the peptide linker between S6 and the CTD (the C-linker) are important for the modulation of Ca^{2+} sensitivity by β2 but not β1 (Lee et al., 2010). These results suggest that the β2 subunit targets the CTD, not the VSD, to modulate BK channel Ca^{2+} sensitivity. Nevertheless, using VCF, Savalli et al. (2007) showed that the β2 subunit shifts the *F–V* relationship toward hyperpolarized potentials when the S3–S4 linker of the Slo1 was labeled with thiol-reactive fluorescent dyes to track VSD activation. This result suggests that association with β2 subunits alters voltage-sensor activation. A further study comparing the gating current generated in the absence and in the presence of the β2 subunit may shed additional light on whether the β2 subunit alters voltage-dependent activation.

The β4 subunit reduces the intrinsic open probability of the PGD in the absence of Ca^{2+} binding as well as voltage-sensor activation (Wang et al., 2006), which reduces channel opening and shifts the *G–V* relation to more positive voltages at $[Ca^{2+}]$ of $< \sim 6\,\mu M$ (Brenner et al., 2000; Wang et al., 2006; Lee and Cui, 2009). However, at $[Ca^{2+}] > \sim 10\,\mu M$, the β4 subunit shifts the *G–V* relation to less positive voltages due to a shift of voltage-sensor activation that compensates for the reduced intrinsic opening of the PGD (Wang et al., 2006). Thus, the β4 subunit seems to modify voltage-dependent activation similarly as the β1 subunit.

1TM LRRCs MODULATION OF VOLTAGE-DEPENDENT ACTIVATION

Leucine-rich-repeat-containing protein 26 (Yan and Aldrich, 2010) is a newly identified BK channel β subunit; the protein sequence and structure of which are unrelated to the 2TM BK β subunits. LRRC proteins constitute a large protein family. LRRC26 belongs to an extracellular leucine-rich-repeat-only (Elron) cluster (Dolan et al., 2007) that consists of members a single TM segment, an extracellular LRR motif, and a short cytosolic COOH tail containing a stretch of acidic residues (**Figure 1B**). LRRC proteins have recently begun to gain appreciation as β subunits of K_V channels. LRRC52, another member of Elron, is enriched in testis and a β subunit of Slo3 (Yang et al., 2011). Slo3 is activated by voltage and H^+ and is the α subunit of Ksper, the voltage and acid activated K^+ channel in sperm (Schreiber et al., 1998; Navarro et al., 2007). LRRC52 association shifts the Slo3 *G–V* to negative voltages that match with the *G–V* relation of native Ksper *in vivo* (Yang et al., 2011). Amphoterin-induced gene and ORF (AMIGO) is a LRRC protein belonging to a large LRRC-IG/FN3 protein cluster (Kuja-Panula, 2003). AMIGO has a single TM domain, an extracellular LRR motif and an extracellular immunoglobulin motif. AMIGO associates with and also left shifts the *G–V* relation of the $K_V2.1$ channel (Peltola et al., 2011).

The leftward shift of the *G–V* relationship caused by LRRC26 is dramatic, around −140 mV, which makes BK channels activate at negative voltages without rises in Ca^{2+} concentration. The effect of LRRC26 on channel activation is independent of Ca^{2+} since mutations of both Ca^{2+} binding sites of BK channels (Ca^{2+} bowl deleted and D362A/D367A) have no effect on LRRC26 modulation. Fitting the HCA model showed that LRRC26 modulates BK channel activation mainly through a large enhancement (∼20-fold) of the allosteric factor *D*, suggesting that LRRC26 affects coupling between the VSD and PGD. The location of LRRC26 relative to Slo1 is not known. However, coexpression of Slo1 with both LRRC26 and the 2TM β1 subunit results in channels with the phenotype of Slo1 + β1, suggesting that the β1 subunit may compete with LRRC26 for a similar association site and prevent LRRC26 from binding. Functional analyses of LRRC26 deletion mutants indicate that except for the fifth leucine-rich-repeat (LRR5) motif and the cytosolic polyPD motif that contains multiple proline and aspartate repeats (residues 304–316), deletion of any region abolishes the function of LRRC26, while only the putative TM segment is necessary for association with Slo1 (Yan and Aldrich, 2010). These results further suggest that the TM segment of LRRC26 may be localized in the cleft between VSDs of the adjacent Slo1 subunits, similar to the 2TM BK channel β subunits and KCNE peptides.

CONCLUDING REMARKS

The studies of TM β subunits' regulation of K_V channels reveal common properties. First, the inter VSD cleft provides a docking site for the TM segment(s) offering the starting point for regulation of voltage-dependent activation. This conserved structural feature may be the basis for the promiscuity of some of the TM β subunits. Second, all three domains of the β subunits (extracellular, TM, intracellular) participate in the interactions with the K_V α subunit that modulate voltage-dependent activation; although, the specific interactions governing phenotypical changes are not entirely clear. Third, consistent with the structural understanding, β subunit interaction may affect all three general events of voltage-dependent activation; i.e., VSD activation, coupling, and PGD opening. All these results suggest that there is no single deciding interaction, or even a simple additive sum of individual interactions, that is responsible for the phenotype changes that are observed experimentally. Rather, the cooperation among multiple individual interactions may synergistically bring about the unique structure and function of the K_V α+β complex.

AUTHOR CONTRIBUTION

Mark A. Zaydman wrote the part on KCNEs; Xiaohui Sun wrote the part on BK channel β subunits; Jianmin Cui, Xiaohui Sun, and Mark A. Zaydman integrated the whole paper.

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