A Marriage of Convenience: β-Subunits and Voltage-dependent K⁺ Channels*

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The movement of ions across cell membranes is essential for a wide variety of fundamental physiological processes, including secretion, muscle contraction, and neuronal excitation. This movement is possible because of the presence in the cell membrane of a class of integral membrane proteins known as ion channels. Ion channels, thanks to the presence of aqueous pores in their structure, catalyze the passage of ions across the otherwise ion-impermeable lipid bilayer. Ion conduction across ion channels is highly regulated, and in the case of voltage-dependent K⁺ channels, the molecular foundations of the voltage-dependent conformational changes leading to their open (conducting) configuration have provided most of the driving force for research in ion channel biophysics since the pioneering work of Hodgkin and Huxley (Hodgkin, A. L., and Huxley, A. F. (1952) J. Physiol. 117, 500–544). The voltage-dependent K⁺ channels are the prototypical voltage-gated channels and govern the resting membrane potential. They are responsible for returning the membrane potential to its resting state at the termination of each action potential in excitable membranes. The pore-forming subunits (α) of many voltage-dependent K⁺ channels and modulatory β-subunits exist in the membrane as one component of macromolecular complexes, able to integrate a myriad of cellular signals that regulate ion channel behavior. In this review, we have focused on the modulatory effects of β-subunits on the voltage-dependent K⁺ (Kv) channel and on the large conductance Ca²⁺- and voltage-dependent (BKCa) channel.

General Properties of Kv and BKCa Channels

Voltage-dependent K⁺ channels are tetrameric channels (reviewed in Ref. 2), with each α-subunit containing a voltage sensor and contributing to the central pore. This pore is formed by four α-subunits that encircle a central ion conduction pathway. K⁺ channels can be recognized by certain common features like the pore-lining P-loops, which have a consensus amino acid sequence, -TXGYGD-, called the K⁺-channel “signature sequence” (3). The Kv channel α-subunit contains six transmembrane regions (TM3; S1–S6), with both N and C termini on the intracellular side of the membrane (a tetrameric 6TM architecture). Although conserving the general structure of Kv channels (i.e. they have a voltage sensor (S1–S4) and pore modules (S5-P-S6)), BKCa channels are an exception inside the S4 superfamily of ion channels. BKCa channels contain seven transmembrane segments (S0–S6) with the N terminus facing the extracellular side (reviewed in Ref. 4). The S4 segment of Kv and BKCa channels contains positively charged amino acids (Arg or Lys) at every third position and is part of the voltage sensor responsible for voltage-dependent gating (1) (reviewed in Ref. 5).

Potassium channels may be considered the guardians of the cellular electrical homeostasis, and thus K⁺ channel diversity is of great importance in determining the variety of electrical responses of cells when subjected to stimuli. The possible mechanisms that originate the immense voltage-dependent K⁺ channel diversity are: (a) multiple genes, (b) alternative splicing, (c) formation of heteromultimeric channels, and (d) co-expression with accessory subunits.

β-Subunits of Kv Channels

Kv channel properties can be modified by accessory proteins that regulate their channel gating and/or subcellular distribution (reviewed in Ref. 6). The β-subunits of Kv channels (Kvβ) are cytoplasmic proteins that have a mass of ~40 kDa. The proteins β1, β2, and β3 are coded by different genes, and additional variability is produced by alternative splicing on the N-terminal region (7, 8). The Kv β-subunits form a tetrameric structure and are associated in 1:1 ratio with the α-subunit (9, 10) (Fig. 1A).

Kv β-Subunits Modify the Biophysical Properties of Kv Channels

Two main types of inactivating Kv channels have been described: 1) delayed rectifiers showing slow (second time scale) inactivation and 2) rapidly inactivating (A-type) Kv channels (reviewed in Ref. 7). The co-expression of some Kv β-subunits with Kvα changes the inactivation kinetics in slow inactivating channels, inducing a fast A-type inactivation (11). In addition, these subunits regulate the surface expression and voltage sensitivity of Kv1 channels (reviewed in Ref. 6; see below).

Kvβ1.1 binds to the N terminus of the Kv1 subfamily but not to Kv2, Kv3, or Kv4, indicating that Kvβ1 interaction with Kvα channels is restricted to Kv1 family members. The Kv β1.1-subunit modifies the rate of inactivation in delayed rectifier channels like Kv1.4, but the voltage dependence of this process remains unchanged (8, 11). Similar to Kvβ1.1, the Kv β1.2- and Kvβ1.3-subunits accelerate inactivation in Kv1.4 (12) and induce inactivation in non-inactivating Kv1.1 and Kv1.5 chan-

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3 The abbreviations used are: TM, transmembrane; Kv, voltage-dependent K⁺ channel; BKCa, large conductance Ca²⁺- and voltage-dependent K⁺ channel; Kvβ, β-subunits of Kv channels; α-DTX, α-dendrotoxin; BKCaβ, β-subunits of BKCa channels; BKCaβ2, N terminus of the BKCa β2-subunit; ChTX, charybdotoxin; IßTx, iberiotoxin; AA, arachidonic acid; ER, endoplasmic reticulum; PKA, cAMP-dependent protein kinase.
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Kv β-Subunit Pharmacology

The α-dendrotoxin (α-DTX) block a class of fast inactivating aminopyridine-sensitive K⁺ channels. By sedimentation analysis of α-DTX acceptors isolated from bovine cortex, two species are identified: a large subunit (α) and a “novel” subunit (β) (9). The Kv β-subunits have been related with changes in blockade induced by anesthetics. Bupivacaine induces internal and external blockade in the Kvα1.5 channels. The internal blockade induced by bupivacaine decreases (~4-fold) when Kvα1.5 is assembled with Kvβ1.3. Quinidine is also less potent (~8-fold) in blocking channels formed by Kvα1.5/Kvβ1.3 than channels consisting only of α-subunits (15). In dorsal root ganglion neurons, the Kv β1-subunit decreased the Kv1.1 sensitivity to the local anesthetic n-butylnitinobenzoate used in treatment of chronic pain (16).

Structure and Redox Properties of Kv β-Subunit

The structure of the isolated Kvβ1 N terminus (amino acids 1-62) was solved using NMR spectroscopy (17). The N terminus of Kvβ1.1 does not exhibit a well defined, unique, three-dimensional structure, indicating a fast conformational equilibrium between weakly structured substrates. The lack of a well defined structure can be an advantage in view of the long trajectory that is followed by the N terminus before reaching its blocking site (10, 18). The crystallization of the Kv β2-subunit showed that it forms a 4-fold symmetric tetramer composed of four triose-phosphate isomerase barrels, each having eight parallel β-strands that form a central core and intervening α-helices encircling the perimeter of the barrel (19). At the front face of each Kv β2-subunit, there is a tightly bound NADP⁺ molecule. The crystal structure of the Kv1.2 channel in complex with the Kv β2-subunit shows that the N terminus of the Kv1.2 α-subunit forming the T1 domain is like a docking platform for the Kv β2-subunit (10). As observed in the isolated structure of the Kv β2-subunit (19), the active site contains an NADP⁺ cofactor and catalytic residues for the hydride transfer. Therefore, Kv β-subunits can be important for catalytic function behaving as a redox sensor and allowing direct coupling of membrane electrical activity to the redox state of the cell (20-22). Some of the main properties of Kv β-subunits are summarized in Table 1.

β-Subunits of BKCa Channels

Regulatory β-subunits of BKCa channels (BKCaβ) contain 191-235 amino acids sharing a predicted membrane topology, with the N and C termini oriented toward the cytoplasm (Fig. 1B). They have two putative TM segments connected by a 112-123-residue extracellular “loop” that contains three or four putative glycosylation sites and disulfide linkages arising from conserved cysteine residues (23). At present, four BKCa β-subunits have been cloned in mammals (β1-β4, coded by genes KCNMB1-4) (Reviewed in Ref. 23). The BKCaβ3 family comprises four distinct subunits (β3α-d) that arise as a consequence of alternative splicing of a single gene.

Changes in Biophysical Properties of BKCa Channels Induced by BKCa β-Subunits

The BKCa β1-subunits induce an increase of the apparent Ca²⁺ sensitivity, a decrease of the voltage dependence of the channel, and slowing of the macroscopic kinetics (reviewed in Refs. 4 and 23). The BKCaβ1 effects seem to result from a stabilization of voltage sensor activation both when the channel is...
closed and when open. Ca$^{2+}$ sensitivity in these channels is increased because, at all voltages, less Ca$^{2+}$-binding energy is necessary to open the channel (24).

BK$_{Ca} \beta 2$ increases the Ca$^{2+}$ and voltage sensitivity of BK$_{Ca}$ channels and slows the kinetics of the channel (25, 26). Moreover, this subunit induces fast and complete inactivation (27). The N terminus of the BK$_{Ca} \beta 2$-subunit (residues 1–45, BK$_{Ca} \beta 2$N) blocks the BK$_{Ca}$ channel via interaction with a receptor site in the α-subunit, which becomes accessible once the channel is in the open state. BK$_{Ca} \beta 2$N structure was studied by NMR spectroscopy and consists of two domains connected by a flexible linker (Glu$^{17}$–Arg$^{19}$) (28) (Fig. 1B). Orio et al. (29) suggested that N- and C-terminal domains from BK$_{Ca} \beta 1$ and BK$_{Ca} \beta 2$-subunits modulate the steady-state and kinetic parameters of BK$_{Ca}$ channels.

BK$_{Ca} \beta 3\alpha$–c induce channel inactivation to BK$_{Ca}$ currents and also produce an outward rectification of the open channel currents. The inactivation process is faster than BK$_{Ca} \beta 2$-induced inactivation albeit incomplete (25). BK$_{Ca} \beta 3$-subunit induces a small and consistent decrease in activation time constants at all Ca$^{2+}$ concentrations, and it does not affect channel deactivation (25). β3-Subunit confers a non-linearity on instantaneous current-voltage properties that is regulated by extracellular segment of this β-subunit (30).

The human BK$_{Ca} \beta 4$-subunit has a complex Ca$^{2+}$ concentration-dependent effect on BK$_{Ca}$ channel current. This subunit decreases apparent Ca$^{2+}$ sensitivity at low Ca$^{2+}$ concentrations but induces an increase in the apparent sensitivity at high Ca$^{2+}$ concentrations (25, 31, 32). Human BK$_{Ca} \beta 4$ also slows down activation and deactivation kinetics (25, 31).

**BK$_{Ca} \beta$-Subunit Pharmacology**

Charybdothixin (ChTX), a toxin isolated from the scorpion Leiurus quinquestratus (33), made possible the isolation and purification of the first BK$_{Ca} \beta$-subunit reported (reviewed in Ref. 34). By the inhibition of high-Ca$^{2+}$-ChTX binding to BK$_{Ca}$ channels, a natural product identified as dehydrosoyasaponin was discovered. Dehydrosoyasaponin is a triterpene glycoside that increases the mean open time of BK$_{Ca}$ channels but only when it is added into the intracellular face of the channel and when the β-subunit is present (35). Iberiotoxin (IbTx), a scorpion toxin isolated from the scorpion Buthus tamulus, is another potent BK$_{Ca}$ channel blocker with the advantage of being highly selective for BK$_{Ca}$ (34). BK$_{Ca} \beta 1$-, β2-, and β4-subunits altered ChTx and IbTx binding in electrophysiological and biochemical studies (36–38). BK$_{Ca}$ channels are modulated by external binding of 17 β-estradiol. The presence of 17 β-estradiol elicits an increase in the currents recorded in patches expressing α- and β-subunits but not in those expressing only the α-subunit (39). The chemotherapeutic xenoestrogen tamoxifen also increased the BK$_{Ca}$ probability of opening only in the BK$_{Ca} \beta 1$-subunit presence (40). Cells expressing BK$_{Ca} \alpha \beta 4$ channels confer particular sensitivity to the adrenal glucocorticoids cortisol and corticosterone and are potentiated to a lesser degree by other sex and stress steroids (41). Fatty acids such as arachidonic acid (AA) alter BK$_{Ca} \beta$-subunit modulation of BK$_{Ca}$ channel inactivation. Currents induced by channels formed by α+β2 and α+β3 were affected by AA (42). BK$_{Ca}$ channel inactivation may be a specific mechanism by which AA and other unsaturated fatty acids influence neuronal death/survival in neuropathological conditions (42). Recently, the fluorescent dye voltage-sensitive DiBAC$_{4}$ (3) was reported as a BK$_{Ca}$ channel selective activator only when the regulatory rBK$_{Ca}$β1 or rBK$_{Ca}$β4, but not rBK$_{Ca}$β2, were co-expressed with rBK$_{Ca}$α in HEK293 cells (43). Some of the main properties of BK$_{Ca} \beta$-subunits are summarized in Table 1.

**β-Subunit Kv and BK$_{Ca}$ Channel Trafficking**

β-Subunits of Kv channels, in addition to modulating the channel activity at the cell surface, control the surface expression of the α-subunit (44). The interaction of Kv1 α-subunit and Kv β-subunit polypeptides is an early event in Kv1 biosynthesis, occurring in the endoplasmic reticulum (ER) (44, 45) (Fig. 2A). Despite dramatic differences in their effects on channel gating, each of the Kv β-subunits displays robust trafficking effects. Kv 1.1-, 1.2-, 1.4-, and 3β-subunits increase the membrane expression and the mature form of Kv1.2 when they are co-expressed (44, 46–48). The interaction with Kv2 β-subunits results in increased stability of Kv1.2 α-subunits. There is a dramatic difference in the degradation rates of the free Kv1.2 pool (non-bonded to Kv β2-subunit, t$_{1/2}$ ~ 3 h) and the Kv1.2 associated with Kvβ2 (t$_{1/2}$ ~ 15 h) (44). Therefore, although some cytoplasmic Kv1 channel β-subunits affect the inactivation kinetics of α-subunits, a more general and perhaps more

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**TABLE 1**

Summary of principal functions and tissue expression of Kvβ and BK$_{Ca} \beta$ subunits

| β-Subunit | Gene       | Binds to | Tissue expression | Functions                                      |
|-----------|------------|----------|-------------------|------------------------------------------------|
| Kvβ1      | KCNAB1     | All Kvα1 subfamily | Heart (Kvβ1.1, Kv β1.2, Kvβ1.5) | Confers fast inactivation                     |
| Kvβ2      | KCNAB2     | Kvα1, Kvα4 | Nervous system and T-lymphocytes | Increases surface expression of Kv             |
| Kvβ3      | KCNAB3     | Kvα1.2, Kvα1.3, Kvα1.4, Kvα1.5 | Human heart, rat brain | Confers fast inactivation; increases surface expression of Kv |
| BK$_{Ca}$β1 | KCNMB1 | BK$_{Ca}$α | Smooth muscle, trachea, aorta coronary | Increases Ca$^{2+}$ sensitivity in Ca$^{2+}$ > 300 nM; decreases voltage dependence; high affinity by ChTx, but IbTx sensitivity is reduced |
| BK$_{Ca}$β2 | KCNMB2 | BK$_{Ca}$α | Chromaffin cells, ovary, and brain | Confers inactivation; Increases Ca$^{2+}$ sensitivity; low ChTx affinity |
| BK$_{Ca}$β3 | KCNMB3 | BK$_{Ca}$α | Testis, pancreas, and spleen | Confers inactivation; induces current rectification; speeds activation kinetics |
| BK$_{Ca}$β4 | KCNMB4 | BK$_{Ca}$α | Brain | No effect in ChTx/IbTx sensitivities decreases ChTx and IbTx binding; in low [Ca$^{2+}$], decreases Ca$^{2+}$ sensitivity; in high Ca$^{2+}$, increases Ca$^{2+}$ sensitivity |
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A fundamental role is to mediate the biosynthetic maturation and surface expression of voltage-gated K⁺ channel complexes.

The Kv β-subunits (Kvβ1.1, Kvβ1.2, Kvβ2, and Kvβ3) can also induce the targeting of Kv1.2 to axons in cultured hippocampal neurons, recapitulating the subcellular localization of Kv1.2 observed in mammalian central and peripheral neurons (46). Axonal targeting of Kv1 channels (reviewed in Ref. 49) is affected by mutations that disrupt the NADP⁺ binding site but not by mutations in the putative catalytic active site (46). Regarding BK_{Ca} β-subunit trafficking, two reports have appeared indicating that BK_{Ca}β1 and BK_{Ca}β2 are able to reach the plasma membrane when they are expressed alone in HEK293 cells (50, 51). Co-expressing BK_{Ca}β1 with BK_{Ca} α-subunit reduces steady-state BK_{Ca} channels surface expression levels by means of an endocytic mechanism. This result shows that BK_{Ca}β1 can also regulate BK_{Ca} surface expression levels (50) (Fig. 2B). In addition, the co-expression of BK_{Ca}β1-subunit with BK_{Ca} α-subunit splice variant SV1 (that is retained in ER) exhibits dominant-negative properties on BK_{Ca}β1 surface expression. This study provides important insights into BK_{Ca} subunit assembly and suggests the early assembly of BK_{Ca} and β1-subunits in the ER (52).

Knock-out Models

Mouse genetic models have played an important role in the elucidation of molecular pathways underlying human disease; gene deletions have also underscored the physiological relevance of Kv and BK_{Ca} channel β-subunits. This approach has been used to determine the effects of Kvβ1 (53) and Kvβ2 (54) removal on Kv1 family Kv currents. Kvβ1.1-deficient mice show normal synaptic plasticity, but they show impaired learning, indicating that the Kv β1.1-subunit contributes to certain types of learning and memory (53). In aged mice, the deletion of the auxiliary potassium channel subunit Kvβ1.1 resulted in increased neuronal excitability, synaptic plasticity, and learning (55). The phenotype of Kvβ2-null mice includes reduced life spans, occasional seizures, and cold swim-induced tremors similar to that observed in Kv1.1-null mice (54).

Regarding BK_{Ca} channels, deletion of the smooth muscle BK_{Ca} β1-subunit causes slight hypertension and increased contractile response to vasoactive agonists (56, 57). BK_{Ca}β4 knock-out mice, on the other hand, display abnormal neuronal firing properties and temporal lobe seizures, indicating that the gating properties conferred by the β4-subunits are essential to normal neuronal function (58).

Coda

The properties of native Kv and BK_{Ca} channels are profoundly influenced by associated β-subunits controlling their subcellular distribution and channel gating. Here we have reviewed the most important examples of Kv β- and BK_{Ca} β-subunit modulation of channel gating, pharmacological properties, and channel trafficking. β-Subunits are expressed in many tissues, and in some cases, they are tissue-specific, allowing the involvement of K⁺ channels in a variety of different physiological processes. Three different genes that code for Kvβ (KCNAB1–3) and four genes that code for BK_{Ca}β (KCNMB1–4) have been reported. Additional diversity of β-subunits is produced by alternative splicing. β-Subunits are, however, only one piece of a protein network that is associated with ion channels. The pore-forming subunits of Kv and BK_{Ca} channels are components of large protein complexes in the plasma membrane. It is very important to know the changes in Kv and BK_{Ca} channel function induced by partners sharing the same protein complex. Identification of these partners and determination of their influence in channel properties will not only provide us with new insights about channel function but can also lead us to unravel new disguises of these molecular machines in cell physiology and pathophysiology.

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