Maxi-K$_{Ca}$, a Unique Member of the Voltage-Gated K Channel Superfamily

L. Toro, M. Wallner, P. Meera, and Y. Tanaka

Large-conductance, voltage-, and Ca$^{2+}$-sensitive K$^{+}$ (maxi-K$_{Ca}$) channels regulate neuronal and smooth muscle excitability. Their pore-forming $\alpha$-subunit shows similarities with voltage-gated channels and indeed can open in the practical absence of Ca$^{2+}$. The NH$_2$ terminus is unique, with a seventh transmembrane segment involved in $\beta$-subunit modulation. The long COOH terminus is implied in Ca$^{2+}$ modulation.

Large-conductance, voltage-, and Ca$^{2+}$-sensitive K$^{+}$ (maxi-K$_{Ca}$) channels are ubiquitously distributed among tissues, except in heart myocytes. Their activity is triggered by depolarization and enhanced by an increase in cytosolic Ca$^{2+}$, providing a link between the metabolic and electrical state of cells. The physiological role of maxi-K$_{Ca}$ channels has been examined in smooth muscle, where they are particularly abundant and play a key role in setting contractile tone (7), and in neurons, where they are involved in spike shaping and neurotransmitter release (4). Their role in cellular processes has been determined in many cases using scorpion toxins like charybdotoxin and the selective blocker iberiotoxin (Ref. 4, see Fig. 4). Maxi-K$_{Ca}$ channels were first cloned from Drosophila; their primary sequence showed that they belong to the voltage-gated K$^{+}$ channel (Kv) superfamily (3, 4). Biochemical purification from smooth muscle tissues showed that maxi-K$_{Ca}$ channels are a protein complex formed by two integral membrane subunits, the pore-forming $\alpha$-subunit and a regulatory $\beta$-subunit (4). Current molecular studies have established several novel structural and functional features of maxi-K$_{Ca}$ channels. We have recently shown that, in contrast to other members of the Kv superfamily, the maxi-K$_{Ca}$ channel $\alpha$-subunit has a seventh transmembrane segment....

General molecular properties

After the Drosophila maxi-K$_{Ca}$ channel $\alpha$-subunit was cloned, many groups engaged in cloning maxi-K$_{Ca}$ (slo) channels from tissues of their interest, in the hope of finding the molecular basis of this channel function and diversity as related to specific cell types. The $\beta$-subunit was cloned after biochemical purification and increases the $\alpha$-subunit apparent Ca$^{2+}$ sensitivity (4).

Figure 1A shows a scheme of the $\alpha$-subunit primary characteristics. The primary sequence of S1–S6 is homologous to the corresponding regions in Kv channels; however, S0 is unique to maxi-K$_{Ca}$ channels and adds a seventh transmembrane segment that, together with the NH$_2$ terminus, allows $\beta$-subunit modulation (13). This region is a critical determinant of $\beta$-subunit modulation (13). It is now established that, in accordance with their sequence homology to other voltage-gated ion channels, the activation machinery of maxi-K$_{Ca}$ channels is triggered by voltage but not by Ca$^{2+}$ (9). Instead, Ca$^{2+}$ acts as a modulator: micromolar Ca$^{2+}$ switches the channel from a Ca$^{2+}$-independent state to a Ca$^{2+}$-modulated state. Only when the channel is in its Ca$^{2+}$-modulated state (Ca$^{2+}$ above 1µM) can the $\beta$-subunit upregulate its activity (5). The requirement for micromolar Ca$^{2+}$ suggests that colocalization or close approximation of maxi-K$_{Ca}$ channel $\alpha$-subunits with or without $\beta$-subunits with Ca$^{2+}$-conducting proteins in the plasma membrane or internal stores may be necessary for the channel to regulate cell excitability.

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conserved region among slo clones and seems to be involved in Ca\(^{2+}\) modulation (14,15).

Because native \(K_{\text{Ca}}\) channels have different properties within and among tissues, the failure to clone more than one maxi-\(K_{\text{Ca}}\) channel gene has been surprising. In contrast to other types of voltage-gated ion channels (\(Ca^{2+}, Na^{+},\) and \(K^{+}\) channels), which show a variety of isoforms, the maxi-\(K_{\text{Ca}}\) channel \(\alpha\)-subunit seems to be encoded by a single gene. Mammalian maxi-\(K_{\text{Ca}}\) channel \(\alpha\)-subunits have almost identical amino acid sequences among different species (>97%, Fig. 1B), whereas mammalian \(\beta\)-subunits have only 82–85% sequence identity. The striking sequence conservation of the \(\alpha\)-subunit may reflect a high evolutionary pressure to maintain an optimized function in mammals.

The fly slo channel (Dslo) differs from mammalian slo channels in its primary sequence, which is reflected in functional aspects such as single-channel kinetics, lack of \(\beta\)-subunit modulation (see Fig. 3), and insensitivity to nanomolar charybdotoxin and iberiotoxin (Fig. 1C).

Given the fact that the native maxi-\(K_{\text{Ca}}\) channels' functional differences in kinetics, \(Ca^{2+}\) sensitivities, and responses to phosphorylation cannot be explained by the existence of various genes, they may be explained by the expression of splice variants of the same gene and/or differential association with a modulatory subunit(s). Functional properties related to alternative splicing or the distribution of splice variants in tissues is starting to emerge. Changes in kinetics and/or apparent \(Ca^{2+}\) sensitivities have been reported for splice variants in brain (related to a 4-amino acid insert in splice site 1, Fig. 1A) (11) and in chromaffin cells (related to a 59-amino acid exon at splice site 2, Fig. 1A) (8). Also, their relative distribution in tissues has been examined using splice-site-specific polymerase chain reaction (8, 11). For example, the splice variant from chromaffin cells that has increased apparent \(Ca^{2+}\) sensitivity is present in brain and other tissues but not in spinal cord or smooth or skeletal muscles (8). However, the functional significance and the physiological relevance of most of the splice variants are still an open question.

In contrast to the maxi-\(K_{\text{Ca}}\) channel \(\alpha\)-subunit that has several splicing sites, analysis of the human \(\beta_{K_{\text{Ca}}}\) gene shows no evidence for alternative RNA splicing. Up to now, cloning of different genes for \(\beta\)-subunits has been elusive. However, related sequences for both subunits can be found in public data bases (14).

FIGURE 1. Basic properties of large-conductance, voltage-, and \(Ca^{2+}\)-sensitive (maxi-\(K_{\text{Ca}}\)) channels. A: diagram of slo channels. S0–S6 mark NH\(_2\)-terminal transmembrane segments. S0 is unique for maxi-\(K_{\text{Ca}}\) channels, and S1–S6 are homologous to voltage-dependent ion channels. S7–S10 are hydrophobic regions at the COOH terminus (two-thirds of the protein). Two separable domains, the “core” and “tail,” are marked. Triangles 1–6 mark alternative splice sites that lead to the insertion of 3–59 amino acids into the primary sequence. Putative sites for phosphorylation (~P) are in stars (protein kinase G) and in circles (protein kinase C). B: bar graph comparing the amino acid (aa) identity of human \(\alpha\)- (slo) and \(\beta\)-subunits with other species. GenBank accession numbers for \(\alpha\)- and \(\beta\)-subunits, respectively, are as follows: h (human), U11058 and U25138; b (bovine), U60105 and L26101; r (rat), U55995 and AF020712; m (mouse), L16912 and AF020711; c (canine), U41001 and U41002; ch (chicken), U73189; n (nematode, \textit{C. elegans}), see Ref. 14; d (\textit{D. melanogaster}), JH0697. C: blockade of Hslo, but not of Dslo, by 100 nM iberiotoxin (IbTX). Dissociation constant was ~1 nM (6).
Comparison with Kv channels. K+ channels are formed by the association of four \(\alpha\)-subunits; maxi-KCa channels seem to share this property. Kv as well as maxi-KCa channels are regulated by \(\beta\)-subunits (3). In contrast to Kv channels whose \(\beta\)-subunits are intracellular proteins and modulate inactivation (3), the \(\beta\)-subunit of maxi-KCa channels is a transmembrane protein that modulates Ca\(^{2+}\) sensitivity (see Fig. 3B), kinetics, and toxin binding (2, 4, 5) (Fig. 2A). The maxi-KCa channel \(\alpha\)-subunit shares sequence homology with Kv channels and other members of the S4 superfamily of voltage-gated ion channels, from transmembrane regions S1 to S6 (Fig. 2). The most striking similarities are 1) an S4 region with three critical charges that largely account for the voltage-sensor gating current, 2) conserved charged residues in S1, S2, and S3, which may form ionic interactions with charges in S4, and 3) a typical pore loop between S5 and S6 that forms the channel conduction pathway (3, 13).

These similarities point to the view that maxi-KCa channels possess an intrinsic voltage sensor and thus are voltage-gated channels. In fact, as is discussed below, depolarization of maxi-KCa channels leads to the outward movement of charged residues in the voltage sensor (Fig. 2A, arrow in S4), as in K channels, generating gating currents before pore opening (Fig. 2B) (9).

Novel topology

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Novel features of maxi-KCa channel \(\alpha\)-subunit topology. We have demonstrated that maxi-KCa channels possess an additional transmembrane region (S0) that leads to an exoplasmic NH\(_2\) terminus (6, 13). We have introduced a c-myc epitope (epitope tag) at the NH\(_2\) terminus and detected antibody binding of nonpermeabilized transfected cells using beads coated with secondary antibody. Because antibodies cannot cross cell membranes, labeling of living (nonpermeabilized) cells by antibodies is an indication that the epitope is extracellular. Figure 3A outlines the strategy and shows an example of bead-labeled cells.

Because S0 is highly conserved in all maxi-KCa (slo) channels, including the Drosophila and Caenorhabditis elegans homologues, the presence of a seventh transmembrane domain S0 leading to an exoplasmic NH\(_2\) terminus seems to be a key feature of this class of channels. As is discussed below, this additional domain S0 and the exoplasmic NH\(_2\) terminus are crucial for \(\beta\)-subunit modulation of the mammalian slo (Fig. 3B).

At the COOH terminus, maxi-KCa channels possess four hydrophobic regions (S7–S10). Our recent experiments show that at least S9 and S10 are cytoplasmic, since the in vitro translated protein containing these two regions (tail) (Fig. 1A) is soluble. Furthermore, in vivo reconstitution of channel activity can be obtained if a membrane...
patch of an oocyte expressing the “core” (Fig. 1A) is introduced into an oocyte expressing the tail (6).

Function-structure relationships

Expression of a large number of maxi-K<sub>Ca</sub> channels in heterologous systems has allowed the dissection of functional mechanisms that have been elusive for years. For example, it has been possible to answer the long sought after question: Are maxi-K<sub>Ca</sub> channels opened (gated) by voltage or by Ca<sup>2+</sup>?

Voltage-dependent gating. The measurement of gating currents, which reflect the movement of the voltage sensor by depolarization, is the experimental proof of a channel being voltage dependent. As shown in Fig. 2B, we have recently demonstrated that maxi-K<sub>Ca</sub> channels possess an intrinsic voltage sensor, since gating currents can be measured on cell depolarization (9). Gating currents and ionic currents (Fig. 2B, inset) are elicited independently of intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) when this divalent cation is practically absent ([Ca<sup>2+</sup>]<sub>i</sub> ≤ 100 nM) (5, 9); a large depolarization is required to maximally open the channel. If Ca<sup>2+</sup> increases to the micromolar level, the channel switches to a Ca<sup>2+</sup>-regulated state or “mode” in which less electrical energy is required to open the channel (5).

[Ca<sup>2+</sup>]<sub>i</sub> modulation. As stated before, Ca<sup>2+</sup> modulation of maxi-K<sub>Ca</sub> channels occurs only at concentrations above 100 nM free [Ca<sup>2+</sup>]<sub>i</sub> (5). It appears that the channel changes conformation between two states, from a Ca<sup>2+</sup>-independent state to a Ca<sup>2+</sup>-modulated state. The amino acid residues or structural determinants responsible for Ca<sup>2+</sup> binding are still an open question. A stretch of aspartic acids (D) between the S9 and S10 regions could be involved in Ca<sup>2+</sup> binding and sensitivity (14). However, natural splice variants, outside this aspartic acid reach region, have different apparent Ca<sup>2+</sup> sensitivities (8, 11), suggesting that the Ca<sup>2+</sup> binding site(s) is formed by a complex three-dimensional interaction of various protein sites.

β-Subunit modulation. The Ca<sup>2+</sup> sensitivity of maxi-K<sub>Ca</sub> channels is modulated by their β-subunit. This regulation takes place when the channel is in its Ca<sup>2+</sup>-modulated mode and occurs at concentrations higher than 1 µM (5). In addition to the effects on the channel’s apparent Ca<sup>2+</sup> sensitivity, the β-subunit also alters kinetics (5) and toxin binding (2) and makes the channels susceptible to activation by nanomolar dehydrosoyasaponin I (DHS-I; Ref. 4). Of these properties, the change in kinetics induced by the β-subunit is observed even at Ca<sup>2+</sup> concentrations lower than 1 µM, whereas upregulation by DHS-I occurs only if [Ca<sup>2+</sup>]<sub>i</sub> > 1 µM (5, 10). Using chimeric constructs from the nonregulated Dslo channel and the responsive Hslo channel (Fig. 3B), we have recently demonstrated that S0 and part of the exoplasmic NH<sub>2</sub> terminus of the α-subunit determine β-subunit modulation (13).

Pore blockers used to identify the pore domain. The region assigned as the pore domain of maxi-K<sub>Ca</sub> channels has the signature sequence (VGYGD) for the pore of K<sup>+</sup> channels (14). This assignment can be tested experimentally by modifying the response to known pore blockers. Ionic currents flowing through the pore of maxi-K<sub>Ca</sub> channels are blocked by tetraethylammonium, iberiotoxin (Fig. 1C), and/or charybdotoxin in all slo channels tested. One exception is Dslo, which is insensitive to nanomolar concen-
tractions of both iberiotoxin (Fig. 1C) and charybdotoxin. Experimental evidence showing that the assigned pore region in slo channels indeed forms the pore includes a chimeric construct where Hslo loses its sensitivity to iberiotoxin by exchanging its pore with the pore of Dslo (6).

Other mechanisms of regulation. Many studies in native cells have shown that maxi-KCa channels are modulated by various protein kinases. However, in cloned channels, this property is not yet clear. The majority of possible phosphorylation sites in the α-subunit of maxi-KCa channels are present in the COOH terminus (α, Fig. 1A). With the use of a low-stringency sequence for protein kinase A (PKA; R-X_{1-2}-S/T), Hslo was found to have 11 PKA possible phosphorylation sites but none using a strong consensus sequence (R-R/K-X-S*/T*). There is one strong protein kinase G (PKG) phosphorylation site (R/R-K-R-

Tissue distribution and physiological role

Functional maxi-KCa channels are ubiquitously distributed among tissues, except in the heart myocytes. This has been common knowledge for electrophysiologists and has been recently confirmed at the mRNA level. Electrophysiological experiments have shown that maxi-KCa channels are particularly abundant in smooth muscles, where they are thought to set the pace of contractile activity. Although they are expressed to a lesser extent in neurons, it is thought that they play important roles in the regulation of transmitter release and spike shaping.

At the mRNA level, both α- and β-subunits coexist in most tissues, although in brain the level of β-subunit mRNA is much lower than the level of α-subunit. In smooth muscles, both α- and β-subunit signals are strong (12). However, studies at the protein level are few and need to be performed for each tissue of interest. Important physiological questions to be addressed are, In which tissues are α- and β-subunits colocalized? Are both proteins evenly distributed in cells or spotted to certain regions? Are most of the maxi-KCa channels formed by α- and β-subunits?

In this regard, we have recently evaluated the molecular constituents of maxi-KCa channels in human coronary arteries (10). To investigate this...
point, we took advantage of two results derived from molecular studies in the cloned α- and β-subunits: 1) that DHS-I (nM range) only activates maxi-K$_{Ca}$ channels composed of α- and β-subunits (4), and 2) that the presence of the β-subunit makes the channel more sensitive to Ca$^{2+}$, requiring less voltage to open (5). Because ionic currents were activated by DHS-I and their Ca$^{2+}$ sensitivities increased dramatically when [Ca$^{2+}$]$_i$ was in the micromolar range, mimicking the behavior of expressed α- and β-subunits, we came to the conclusion that in human coronary smooth muscle most of the channels are composed of α- and β-subunits.

Maxi-K$_{Ca}$ channel blockers (e.g., iberiotoxin) effectively induce contractile activity of coronary and uterine smooth muscle (Fig. 4A). However, because channel activity in isolated cells is extremely low at resting Ca$^{2+}$ (nM range), these experiments were difficult to explain in terms of maxi-K$_{Ca}$ channels being "open at rest" and thus their blockade causing depolarization and contraction. The recent discovery of ryanodine-sensitive Ca$^{2+}$ spikes that relax smooth muscle through maxi-K$_{Ca}$ channels (7) and the fact that coexpression with the β-subunit dramatically increases the maxi-K$_{Ca}$ channel α-subunit Ca$^{2+}$ sensitivity, when tested in high external K+ (5), seem to give an answer to this puzzle. A local increase in [Ca$^{2+}$]$_i$, to micromolar levels in the vicinity of maxi-K$_{Ca}$ channels formed by α- and β-subunits may allow a significant K$^+$ flux through these channels, providing a very effective regulatory mechanism for the maintenance of smooth muscle tone. Studies using physiological ionic gradients and molecular anatomy studies should provide further evidence for this concept. It would be expected that maxi-K$_{Ca}$ channel α- and β-subunits are colocalized with Ca$^{2+}$ channels in the plasma membrane or in close approximation to Ca$^{2+}$ channels of internal membranes (ryanodine or inositol trisphosphate receptors) (Fig. 4B).

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References


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