New Disguises for an Old Channel: MaxiK Channel β-Subunits

Patricio Orio,[1,2] Patricio Rojas,[1,2] Gonzalo Ferreira,[4] and Ramón Latorre[1,2,3]

[1]Centro de Estudios Científicos (CECS), Valdivia; [2]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile; [3]Department of Physiology and Anesthesiology, UCLA School of Medicine, Los Angeles, California 90095; and [4]Departamento de Biofísica, Facultad de Medicina, Universidad de la Republica, Montevideo, Uruguay

Ca^{2+}-activated K^+ channels of large conductance (MaxiK or BK channels) control a large variety of physiological processes, including smooth muscle tone, neurosecretion, and hearing. Despite being coded by a single gene (Slowpoke), the diversity of MaxiK channels is great. Regulatory β-subunits, splicing, and metabolic regulation create this diversity fundamental to the adequate function of many tissues.

Ca^{2+} entry into cells mediated by voltage-dependent Ca^{2+} channels (VDCCs) is essential for life because it permits, for example, neurosecretion to occur, smooth muscle contraction to take place, and the process of hearing to develop. However, some mechanisms must be put into action to control Ca^{2+} influx, either to stop or to dampen the physiological effect of the increase in cytoplasmic Ca^{2+}. In many cases, this is accomplished by one of the most broadly expressed K^+ channels in mammals: the large-conductance, voltage- and Ca^{2+}-activated K^+ channel, also known as the MaxiK (or BK) channel because of its large single-channel conductance (>200 pS in 100 mM symmetrical K^+). This channel increases its activity on membrane depolarization or an increase in cytosolic Ca^{2+}, and its activation dampens the excitatory events that elevate the cytosolic Ca^{2+} concentration and/or depolarize the cell membrane. Except in heart myocytes, MaxiK channels are almost ubiquitously expressed among mammalian tissues, and they play a variety of roles, of which only a few have been extensively studied.

Depending on the cell type, different MaxiK currents can be observed, differing mainly in Ca^{2+} sensitivity and macroscopic kinetics. Moreover, inactivating MaxiK currents have been observed. This variety of MaxiK channel types became intriguing when it was realized that there is only one mammalian gene, called *Slowpoke* (*Slo*) (8), coding for the MaxiK channel protein. This suggested that MaxiK channel diversity was a consequence of alternative splicing and/or interaction with regulatory subunits. In fact, both mechanisms account for MaxiK variability, but the interaction with a special kind of regulatory subunit is what causes the most dramatic changes in MaxiK properties. These are membrane-integral proteins called MaxiK β-subunits (7). Also, other regulatory proteins, posttranslational metabolic regulation, and modifications add more variety to this multifaceted channel (8).

Until 1999, only one β-subunit was known at the molecular level. This subunit (now known as the β1-subunit) accounted mainly for MaxiK properties in vascular smooth muscle cells (VSMCs), where it is highly expressed. In the past few years, three more β-subunits have been cloned and characterized. Here we will review and discuss this and other recent landmarks regarding the regulation of MaxiK channels by β-subunits and their physiological importance.

Molecular properties of MaxiK channels

The minimal molecular component necessary and sufficient for MaxiK activity is its pore-forming α-subunit, and functional channels are formed as tetramers of this protein. First cloned from *Drosophila*, the gene coding for MaxiK was called *Slowpoke* or *Slo*, and after the description of the *Slo2* and *Slo3* channels (see below) it was renamed *Slo1*. The presence of a positively charged fourth transmembrane segment makes the MaxiK channel a member of the S4 superfamily. Most members of this superfamily have six transmembrane segments, called S1 to S6, but MaxiK channels have a seventh transmembrane segment at the NH_2 terminus, called S0. Thus the NH_2 terminus of the protein is placed in the extracellular side of the membrane (Fig. 1). The intracellular COOH-terminal domain comprises two-thirds of the protein and contains four hydrophobic segments and several alternative splicing sites (Fig. 1).

The pore domain of the channel is assigned to the region contained between S5 and S6 segments, which includes the signature sequence of K^+ channels (TVGYG). As in all other voltage-dependent K^+ channels, the S4 segment is (or is part of) an intrinsic voltage sensor, which is the actual trigger for MaxiK activation (8). Gating and ionic currents in MaxiK channels can be elicited by membrane depolarization in the absence of Ca^{2+}, suggesting that this is a voltage-dependent channel. The divalent cation acts as a modulator able to decrease the necessary energy to open the channel, as can be inferred from the leftward shift in the open probability vs. voltage (P_o-V) relationships (Fig. 2).

Recently, a tetramerization domain has been identified for the MaxiK channel in a hydrophilic region located between the S6 and S7 hydrophobic segments (10). This is the only hydrophilic region capable of self-association, forming stable tetramers in solution. In functional assays with truncated channels, this domain proved to be necessary for a dominant-negative feature, suggesting its involvement in tetramerization.

0886-1714/02 5.00 © 2002 Int. Union Physiol. Sci./Am. Physiol. Soc. www.nips.org

This region was named BK-T1, resembling the T1 domain of voltage-dependent K^+ channels, which is also involved in tetramerization. However, these domains are different both in amino acid sequence and localization.

The region from the S8-S9 linker to the end of the protein is called the "tail" and can be expressed as a separable domain. Its coexpression with the rest of the channel (the "core") produces functional channels, whereas neither of the parts alone forms functional channels. The Ca^{2+}-binding site has been located to this large intracellular domain, between the hydrophobic segments S9 and S10 (8). This region, frequently called the "Ca^{2+} bowl" (Fig. 1), is highly conserved in all cloned MaxiK channels and contains an Asp-rich (-QDDDDDP-) sequence motif, emerging as a very good candidate for a Ca^{2+}-binding site. A study using chimerical channels between Slo1 and Slo3 confirmed the localization of the Ca^{2+} sensitivity to a region comprising the end of the S9-S10 linker and part of the S10 hydrophobic segment (13). On the other hand, direct $^{45}Ca^{2+}$-binding studies were performed by using a fusion protein consisting of the last 280 amino acid residues of the *Drosophila* Slo1 (dSlo1) channel in an overlay assay (1). By replacing all Asp residues with the uncharged residue Asn, they showed that the Ca^{2+} bowl motif accounts for only 56% of $^{45}Ca^{2+}$ binding. This implies the existence of more than one Ca^{2+}-binding site per tail domain: a low-affinity and a high-affinity site (the latter being the Ca^{2+} bowl).

The Slo family

The MaxiK channel is not the only member of its family. Another two members have been described: Slo2 and Slo3 (12, 20). The *Slo2* gene was cloned from the worm *Caenorhabditis elegans*, and *Slo3* was identified from a testis cDNA library.

Slo2 needs the presence of internal Cl^- to be activated by Ca^{2+}, and the sensitivity to these two ions is strictly coupled. Slo2 lacks a S0 domain and an apparent intrinsic voltage sensor since the S4 domain is uncharged (Fig. 1). Surprisingly, Slo2 is a voltage-dependent channel, albeit with a smaller voltage dependence than Slo1. The *Slo2* gene has a mammalian ortholog called *Slack*.

Slo3 shares the same membrane topology with Slo1 and is also voltage dependent. However, Slo3 is regulated by intracellular pH instead of internal Ca^{2+}; increasing pH increases P_o.

Sources of MaxiK channel diversity

It is not only changes in membrane potential and Ca^{2+} concentration that result in modulation of MaxiK channel activity. There is evidence of changes in MaxiK channel activity by phosphorylation and/or interaction by G proteins, by mechanical stretch, and by various endothelium-derived vasoactive substances (8, 17). All of these modulatory mechanisms allow fast integration of multiple stimuli in many tissues, including neurons, VSMCs, and cochlear hair cells. The *Drosophila* α-subunit (dSlo) can interact with two different accessory proteins, known as dSlip1 and Slob. Both are soluble proteins that can physically interact with MaxiK channel by its intracellular

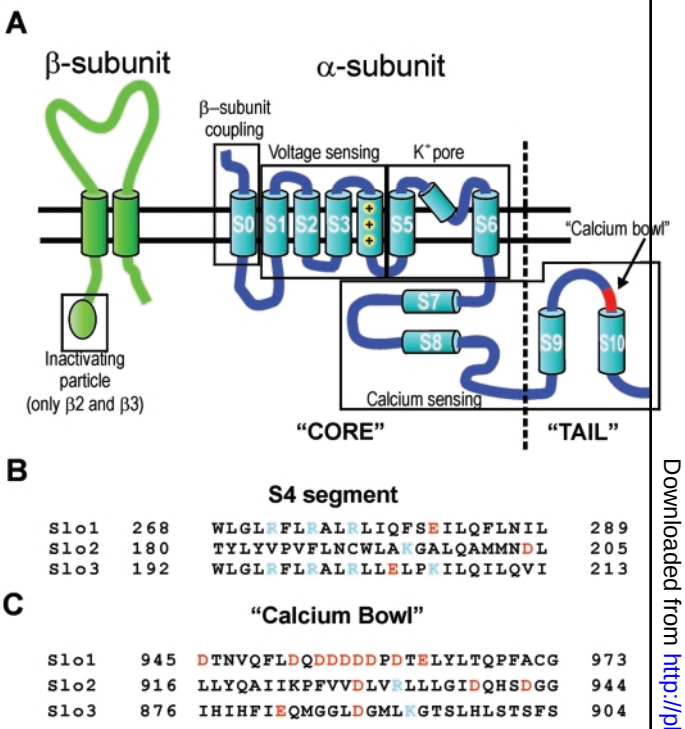

FIGURE 1. Molecular design of the MaxiK channel. *A*: proposed topology of α- and β-subunits. The channel is formed by 4 α-subunits and probably 4 β-subunits. *B*: alignment of S4 segments of the 3 members of Slo family. Note that Slo2 lacks positive charges in this segment. *C*: alignment of the region located at beginning of S10 segment. The Ca^{2+} bowl present in Slo1 is absent in Slo2 and Slo3.

face. Whereas Slob increases the channel P_o, dSlip1 seems to decrease the number of MaxiK channels in the plasma membrane.

Alternative splicing creates channels with different kinetics and Ca^{2+} sensitivity. Most splicing sites are located in the COOH terminus of the Slo1 protein, and several sites reside between hydrophobic domains S8 and S9 (8). However, the differences between splice variants are modest when compared with the effect of coexpression with β-subunits. It is the interaction with these accessory proteins that creates really different MaxiK channels, which, with the other regulatory mechanisms, can be finely tuned to perform their physiological roles.

The β-subunits

Regulatory β-subunits share a putative membrane topology, with two transmembrane segments connected by a 120-residue extracellular "loop" and with NH_2 and COOH terminals oriented toward the cytoplasm (Fig. 1). The loop has three or four putative glycosylation sites. At present, four β-subunits have been cloned in mammals (2, 7, 9, 15, 18).

Sequence similarities are major between β1-β2 and β2-β3, respectively. β4 is the most distantly related of all β-subunits. β-Subunit orthologs have not been described in *Drosophila* or in the worm *C. elegans*, suggesting that this protein is a "novel" acquisition in evolution.

The first β-subunit was identified as a protein associated with

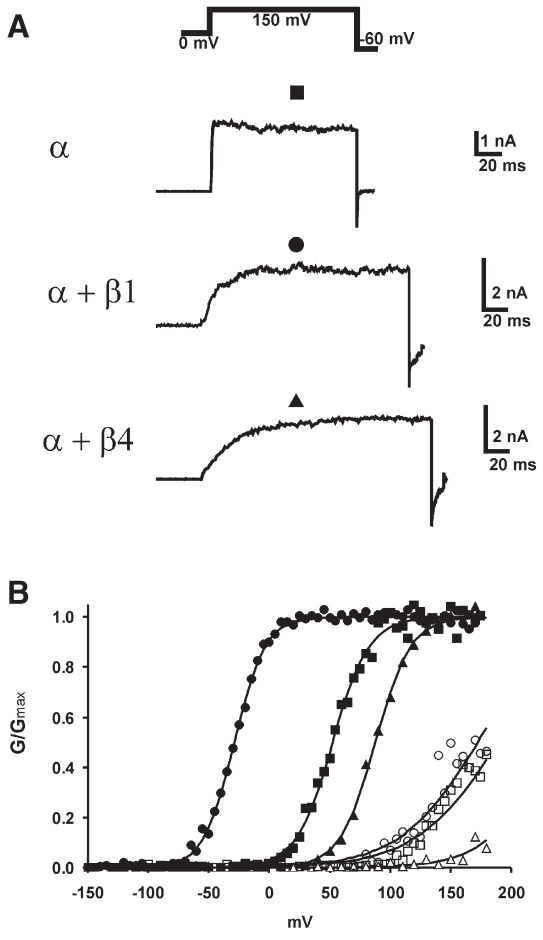

FIGURE 2. Modulation of voltage and Ca^{2+} dependences given by β-subunits. *A*: current traces elicited by a voltage pulse of 150 mV at 4 μM intracellular Ca^{2+} for channels formed by α-, α + β1-, and α + β4-subunits. *B*: open probability-voltage (P_o-V) relationships at 4 μM (filled symbols) and 7 nM (open symbols) intracellular Ca^{2+} concentration for channels formed by α- (squares), α + β1- (circles), and α + β4- (triangles) subunits.

MaxiK channels in smooth muscle membrane preparation with high affinity for charybdotoxin (CTX), a MaxiK peptide blocker. This protein was called β-subunit, but with the recent cloning of new family members, it was renamed β1. Coexpression of this subunit with the α-subunit produces a leftward shift of the P_o-V curves, an effect that it is particularly dramatic at Ca^{2+} concentrations > 1 μM. However, functional coupling between α- and β-subunits can take place in the absence of Ca^{2+} (4). In this condition, the β1-subunit increases P_o, but because of its low magnitude this change is almost imperceptible in macroscopic records. The primary effect of the β1-subunit is to increase the stability of the open states, although small changes in Ca^{2+} affinity of both closed and open states appear to be functionally important (4). Besides increasing the apparent Ca^{2+}/voltage sensitivity of the α-subunit, the β-subunit also modifies MaxiK channel kinetics and alters its pharmacological properties. The β-subunit slows down the activation and the deactivation kinetics of the channel (Fig. 2). The presence of the β-subunit is also a requirement for internal binding of the MaxiK channel opener dehydrosoyasaponin (a triterpene glycoside) (17) and for external binding of the agonist 17β-estradiol (16).

The next cloned β-subunit was identified by searching homologues to the β1-subunit in human expressed sequence tag (EST) databases. This new subunit is expressed preferentially in chromaffin cells and brain and was dubbed β2 (18). The most notorious difference from the β1-subunit is an NH_2-terminal domain that contains a hydrophobic region followed by positively charged residues. This type of sequence is characteristic of inactivation peptides that can occlude the conduction pathway of *Shaker* K^+ and of MaxiK channels. Consequently, coexpression of α- and β2-subunits produces inactivating currents, such as those seen in some chromaffin cells (see below). Removal of the NH_2-terminal domain, either by trypsin or molecular biology techniques, results in a β-subunit that does not inactivate the channel, so the currents are sustained and more suitable for kinetic and Ca^{2+} sensitivity comparisons. Ca^{2+} sensitivity and gating kinetics of channels formed by α- and β2-subunits are similar to those of channels formed by α- and β1-subunits (18). In contrast to the β1 subunit, this subunit confers low CTX affinity compared with channels formed only by α-subunits (18).

The β3-subunit was cloned from human EST databases. It was detected in testis, pancreas, and spleen (19), and it is phylogenetically more related to β2 than to β1 (15). There are four splice variants (β3a–d), whose differences are in the NH_2-terminal region. Each splice variant confers different inactivation properties to the MaxiK channel. Whereas the β3a and β3c subunits confer similar inactivation properties, β3b induces a faster and incomplete inactivation process that becomes evident only at large depolarizations (19). It is unclear whether or not the β3d-subunit interacts with the α-subunit since coexpression of α- and β3d-subunits does not produce changes in the Ca^{2+} activation curves or in the gating kinetics of the MaxiK channel.

The β4-subunit, also cloned from human EST databases, is expressed mainly in brain. Its coexpression with the α-subunit decreases the apparent Ca^{2+} sensitivity of the MaxiK channel (2, 9). Although this subunit slows down channel activation kinetics in a manner similar to the β1-subunit, the deactivation kinetics is very fast (similar to that observed in absence of the β-subunit; see Fig. 2) (2). These results indicate that the functional coupling of this subunit with the α-subunit is different from that induced by the other β-subunits. The β4-subunit decreases the CTX binding strength. If the external loops of the β1- and β4-subunits are exchanged (chimeras β1Lβ4 and β4Lβ1), the phenotypes obtained regarding toxin binding correspond to their respective loops (e.g., chimera β1Lβ4 has a toxin sensitivity corresponding to the β4 subunit). These results suggest that the loops of the β-subunits determine the characteristic of toxin binding. Also, because CTX is a pore-blocking toxin, it is suggested that the extracellular loop of β-subunits (at least β1 and β4) faces the pore and is very close to it (9).

β-Subunits therefore alter the Ca^{2+} sensitivity and gating kinetics of MaxiK channels, greatly contributing to MaxiK channel diversity. On the other hand, they modify the MaxiK channel pharmacological properties, changing toxin binding and acting as receptors for drugs. Most importantly, they allow the MaxiK channel to play important physiological roles, as we will see below.

Physiological role of β-subunits

In most native cells, MaxiK channels are functionally coupled to VDCC, an association that makes MaxiK channels function as negative feedback for VDCC activation and Ca^{2+} entry into the cell. VDCC activation leads to inward Ca^{2+} currents, which then promote membrane depolarization and accumulation of intracellular Ca^{2+}. Both events then promote MaxiK channel activation, leading to a hyperpolarization that closes VDCC.

To have a significant effect on MaxiK channel activity, intracellular Ca^{2+} concentrations must be >10 μM. Those elevated intracellular Ca^{2+} concentrations can indeed be reached in small "compartments" inside the cells, the Ca^{2+} microdomains, where VDCC and MaxiK channels are often tightly colocalized. Space and time characteristics of such compartments depend on the entry and exit of diffusing free Ca^{2+} and Ca^{2+} buffer properties (binding rate, mobility, etc.).

Not only VDCCs from the cell membrane are coupled with MaxiK channels. Ryanodine receptors from the sarcoplasmic reticulum are very close (<20 nm) to MaxiK channels in the plasma membrane. This association, confirmed by structural and functional evidence, is the basis for the proposed role of MaxiK channels in the control of vascular smooth muscle contraction. Below we discuss the negative feedback imposed by MaxiK channels in smooth muscle, chromaffin cells, and cochlea.

Smooth muscle

In VSMC, MaxiK channels provide the adequate regulation of the contractile tone. Local Ca^{2+} increases, called Ca^{2+} sparks, generate spontaneous transient outward currents (STOCs), which are generated by MaxiK channels. This hyperpolarizes the membrane and promotes relaxation (discussed in Ref. 6). As mentioned earlier, in these cells highly Ca^{2+}-sensitive MaxiK channels are formed mostly by α- and β1-subunits. The physiological relevance of β1-subunits in modulating the Ca^{2+} sensitivity of the MaxiK channel was shown with β1 knockout mice (3). The cerebral artery myocytes of these mice exhibited a decrease in the Ca^{2+} sensitivity of the MaxiK channels and a low Ca^{2+} spark-STOC coupling (Fig. 3). The absence of the β1-subunit also promoted an increment in arterial tone and blood pressure.

Chromaffin cells

Adrenal chromaffin cells are excitable cells that release neurotransmitters such as catecholamines in response to electrical stimulation. In these cells, MaxiK channels are important for the rapid termination of the action potential. Even though four different subtypes of VDCC (L, N, P, and Q types) are found in these cells, MaxiK channels are functionally coupled only to L- or Q-type VDCC. In rat chromaffin cells, there is a cell population that has inactivating MaxiK currents ($MaxiK_i$) and a pop

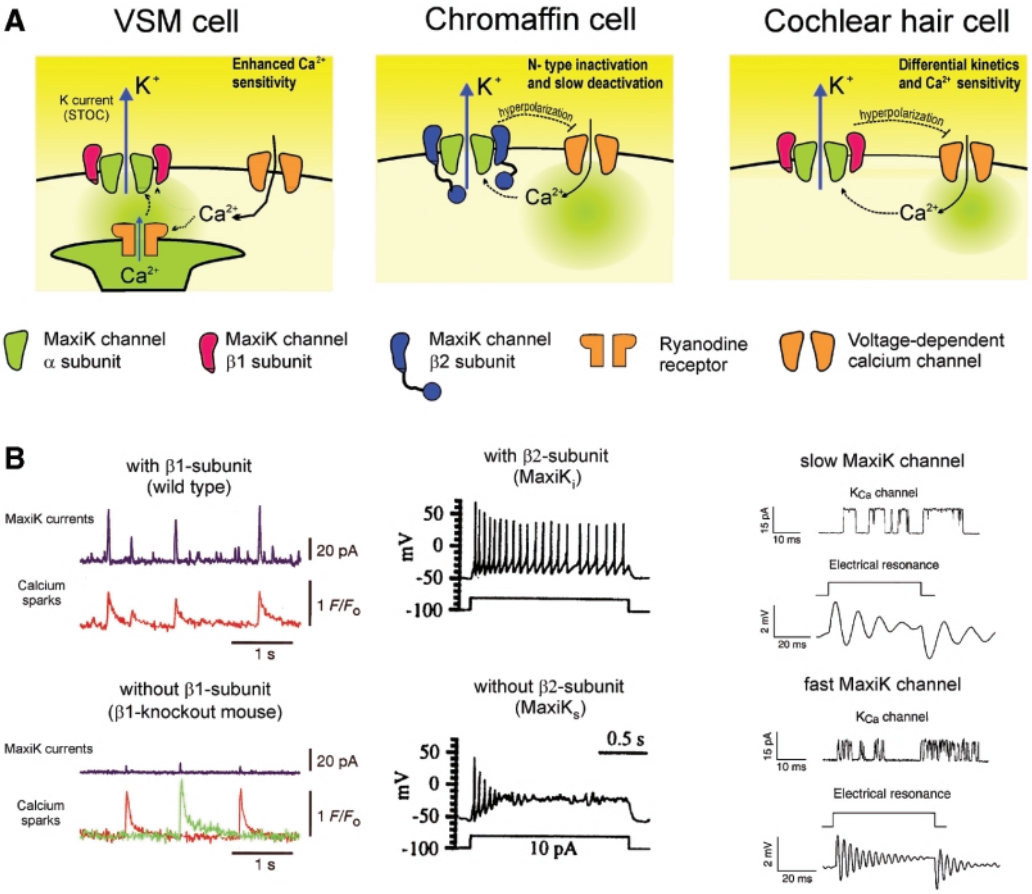

FIGURE 3. Proposed physiological roles of MaxiK channel β-subunits depicted as cartoons (*A*) and as examples on the impact of β-subunits on channel electrophysiology (*B*). In vascular smooth muscle (VSM) cells, β1-subunit confers the required Ca^{2+} sensitivity for effective coupling between Ca^{2+} sparks and spontaneous outward currents. In chromaffin cells, slower MaxiK deactivation kinetics allow β2-subunit-expressing cells to fire repeatedly. In auditory outer hair cells, combination of MaxiK splice variants and β1-subunit expression gives rise to a variety of MaxiK channel kinetics, allowing each cell to electrically tune to a characteristic frequency. See text for further details. *B*, *left*: reproduced with permission from Ref. 3. *B*, *middle*: reproduced with permission from Ref. 14. *B*, *right*, reproduced with permission from Ref. 5

ulation with noninactivating MaxiK currents ($MaxiK_s$) (17). In the $MaxiK_i$ cells, the β2-subunit is expressed. As a consequence, MaxiK channels in these cells show rapid inactivation and slow deactivation kinetics (18). Because of the much slower deactivation of the MaxiK channel, the afterhyperpolarization phase is prolonged. This relieves Na^+ channel inactivation and allows repetitive firing. Thus while $MaxiK_i$ are tonically firing cells, $MaxiK_s$ are phasically firing cells (Fig. 3) (14).

Cochlea

In the frog, chick, and turtle auditory systems, which have been used as models of study of this sense, frequency tuning is performed almost exclusively by the hair cells. Each different hair cell has a characteristic tuning frequency. In these animals, electrical resonance is achieved through the interplay of L-type VDCC and MaxiK channels operating, as for example in the turtle, at frequencies from 30 to 600 Hz (5). The opening of L-type VDCC induced by depolarization increases internal Ca^{2+} concentration, which in turn activates MaxiK channels. Activation of MaxiK channels hyperpolarizes the cell, closing Ca^{2+} channels and promoting the membrane potential oscillation. Subsequent oscillations are damped, because fewer MaxiK channels are recruited in each cycle (Fig. 3) (5). The number and type of MaxiK channels control the resonant frequency of a particular hair cell. Higher frequencies are present in cells containing a large number of MaxiK channels with faster activation kinetics. The origin of this wide range of MaxiK gating kinetics is not completely clear at present but most probably is accomplished by differential expression of distinct splice variants of the Slo1 α-subunit, together with an expression gradient of a β-subunit (5, 11). In fact, a number of MaxiK channel splice variants with different activation kinetics and Ca^{2+} sensitivities have been identified in different places in the cochlea (5). In chicken, an ortholog of the β1-subunit that presents an expression gradient across the cochlea has been identified (11). As mentioned earlier in this review, the β1-subunit increases Ca^{2+} sensitivity and slow deactivation kinetics. β-Subunits add to the system a level of variability that cannot be achieved only with a splice variant expression gradient. It is of interest to note here that, as the frequency range gets wider, more diversity in the MaxiK channel is needed. Thus Fettiplace and Fuch (5) modeled the electrical tuning in the turtle with five MaxiK splice variants, but in the chicken basilar papilla (150–4,000 Hz), they needed a minimum of nine MaxiK splice variants.

Coda

In this review, we have tried to convey to the physiologist the importance of a voltage-dependent K^+ channel with a probability of opening also controlled by cytoplasmic Ca^{2+}. This tetrameric channel, formed by a protein coded by a single gene (*Slowpoke*), attains its large diversity essentially through three mechanisms: alternative splicing, metabolic modulation, and forming a molecular complex with β-subunits (β1–β4). Of course, one or more of the above-mentioned mechanisms acting at the same time can also obtain more multiplicity of MaxiK channels. In particular, the biophysical properties of MaxiK channels are drastically modified by the most studied and well-characterized β1-subunit. This subunit, by decreasing the energy necessary to open the channel and by slowing down channel deactivation, sets the tone in smooth muscle (a fact firmly demonstrated with the help of β1-subunit knockout mice). Extraordinary in its beauty is the molecular mechanism used by turtles, frogs, and chicks to generate in the cochlea the fundamental resonance frequencies. In those animals, gradients of spliced MaxiK channels and β1-subunits across the cochlear epithelia talk to VDCC to produce the different oscillatory voltage responses in the hair cells.

As we discussed, in many tissues the physiological role for β2-, β3-, and β4-subunits can be inferred from the impact of these subunits in the electrophysiology of the channel. However, the real physiological impact will not be known until more detailed studies in native cells are undertaken. For example, the β3-subunit has been detected in testis, but native MaxiK currents in this tissue have not been described in detail. The implication of β2- and β3-subunits in spleen and pancreas, where β3 is located at secreting β-cells (15), is also not clear. In these cells, macroscopic MaxiK currents have been described, but their physiological role remains obscure. We are confident that all of the present unknowns regarding this field will be understood in the near future, unveiling new mechanisms of how the electrical activity of the cell is coupled to its metabolism.

We are supported by Fondecyt Grants 1000890 (R. Latorre), 2000063 (P. Orio), and 2010006 (P. Rojas); TWAS-99-301 RG/BIO/LA (G. Ferreira); Human Frontiers in Science Program (R. Latorre); and Cátedra Presidencial (R. Latorre). P. Orio is recipient of a Ph.D. fellowship from Fundación Andes. P. Rojas is recipient of a Ph.D. fellowship from Conicyt. CECS is a Millennium Institute. CECS also benefits from generous support by Empresas CMPC.

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