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Functional Role of the β Subunit of High Conductance Calcium-Activated Potassium Channels

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Summary

Mammalian high conductance, calcium-activated potassium (maxi-K) channels are composed of two dissimilar subunits, α and β. We have examined the functional contribution of the β subunit to the properties of maxi-K channels expressed heterologously in Xenopus oocytes. Channels from oocytes injected with cRNAs encoding both α and β subunits were much more sensitive to activation by voltage and calcium than channels composed of the α subunit alone, while expression levels, single-channel conductance, and ionic selectivity appeared unaffected. Channels from oocytes expressing both subunits were sensitive to DHS-I, a potent agonist of native maxi-K channels, whereas channels composed of the α subunit alone were insensitive. Thus, α and β subunits together contribute to the functional properties of expressed maxi-K channels. Regulation of coassembly might contribute to the functional diversity noted among members of this family of potassium channels.

Introduction

The activity of calcium-activated potassium channels increases in response to an elevation of intracellular calcium. The hyperpolarization that results from increased activity of these channels thus constitutes an important feedback mechanism for the regulation of voltage-dependent calcium entry. Some types of calcium-activated potassium channels, such as the high conductance calcium-activated potassium (maxi-K) channel, are sensitive to both calcium and voltage, so that their apparent sensitivity to calcium is increased when the membrane is depolarized. Despite the high resolution of kinetic data obtained from electrophysiological recordings of maxi-K channel currents, the fundamental mechanisms underlying this dual regulation are not well understood. Recent advances in the biochemical and molecular biological characterization of maxi-K channels are yielding fresh insights. Biochemical purification of maxi-K channels from mammalian smooth muscle showed that they are composed of two structurally distinct subunits, α and β (Garcia-Calvo et al., 1994). The larger α subunit is homologous to the pore-forming subunits of other potassium channels (Knaus et al., 1994b). In fact, it is the mammalian homolog of the structural gene underlying the *slowpoke* (*slo*) phenotype in Drosophila (Atkinson et al., 1991). The smaller β subunit shows no homology with other ion channel subunits (Knaus et al., 1994a).

Although expression of the α subunit (*slo*) alone is sufficient to generate potassium channels that are gated by voltage and intracellular calcium (Adelman et al., 1992; Butler et al., 1993; Perez et al., 1994), the properties of these channels are only qualitatively similar to those recorded from native membranes. For example, the mouse *slo* currents are far less sensitive (~10-fold) to activation by calcium or voltage than are native channels. We report here that coexpression of a bovine smooth muscle maxi-K channel β subunit has dramatic effects on the properties of expressed mouse brain α subunits. Currents from cells expressing α/β heteromultimers more closely resemble those of native channels in both their gating and pharmacology. These data, therefore, provide functional evidence for association of the α and β subunits and also suggest a possible mechanism for generation of the observed diversity in the calcium sensitivity of maxi-K channels.

Results

Heterologous Expression of α and β Subunits in Xenopus Oocytes

Xenopus laevis oocytes injected with cRNA encoding an α subunit alone (mslo) expressed outward currents in response to ramp increases in membrane potential (Figure 1a). These currents were blocked by tetraethylammonium (TEA), charybdotoxin (ChTX), and iberiotoxin (IbTX), a specific blocker of maxi-K channels (Galvez et al., 1990; Giangiacomo et al., 1992; Candia et al., 1993). Cells injected with cRNA encoding only the β subunit (266 ± 24 nA at +80 mV; n = 3) did not differ from uninjected oocytes (322 ± 60 nA at +80 mV; n = 3) in their response to similar voltage ramps (Figure 1b). IbTX-sensitive currents recorded from oocytes coinjected with a mixture of cRNAs encoding both α and β subunits were similar in amplitude (7.3 ± 1.9 μA at +80 mV; n = 6) to currents recorded from α-injected oocytes (5.9 ± 1.3 μA at +80 mV; n = 6) and exhibited similar sensitivities to block by TEA (IC_{50} < 1 mM; data not shown) and ChTX (Table 1). The channels from coinjected oocytes activated at more hyperpolarized membrane potentials than those from oocytes injected with the α subunit alone (note the current at 0 mV).

β Subunit Modifies Voltage- and Calcium-Dependent Gating of Expressed mslo Channels

Two-electrode voltage-clamp recording from Xenopus oocytes is inadequate for the characterization of maxi-K channels, since the intracellular calcium concentration cannot be controlled. To compare the functional properties of expressed channels systematically, we recorded currents from isolated inside-out membrane patches. Patches

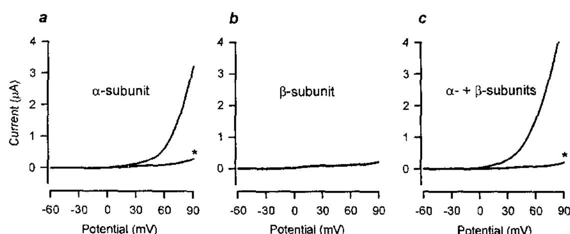

Figure 1. Expression of the α and β Subunits of Maxi-K Channels in *Xenopus Oocytes*

Currents were recorded from voltage-clamped oocytes that had been previously injected with cRNA encoding the α subunit alone (a), the β subunit alone (b), or both subunits together (c). Membrane current is plotted as a function of membrane potential, both of which were measured simultaneously using a two-electrode voltage clamp. Each trace shows the current elicited in response to a voltage ramp from −60 mV to 90 mV over 1 s. For (a) and (c), 50 nM IbTX was added to the bathing solution for at least 15 min prior to recording of the traces marked with an asterisk. Cells injected with cRNA encoding only the β subunit did not exhibit currents other than those endogenous to the oocyte (b). Large outward IbTX-sensitive currents were recorded from cells injected with either the α subunit alone (a) or both α and β subunits (c).

excised from cells injected with cRNAs encoding either the α subunit alone or a mixture of both α and β subunits exhibited maxi-K channels, with individual patches containing anywhere from 1 to several hundred channels. A typical patch contained about 50 channels, enough to produce macroscopic currents such as those shown in Figure 2. Such macropatches were used to study the sensitivity of the expressed channels to voltage and calcium, and to examine their ionic selectivity and pharmacological properties. Patches excised from β-injected oocytes lacked the

Table 1. Summary of Properties of Maxi-K Channels Formed by α Subunits Alone and by Coexpression of α and β Subunits

	α	α + β
Channel conductance	274 ± 7 pS (9)	262 ± 3 pS (12)
$Ca_{1/2}$	30 μM	3 μM
Activated by DHS-1	No (0/14)	Yes (11/11)
IC_{50} ChTX	1.9 ± 0.4 nM (4)	1.1 ± 0.1 nM (4)

$Ca_{1/2}$ is the calcium concentration required to cause a channel open probability of 0.5 at 0 mV, obtained by interpolation of the data in Figure 3a. The IC_{50} values for toxin block of the current were determined at +70 mV. The numbers of experiments are given in parentheses.

large currents observed in α-injected and coinjected oocytes. The currents recorded from α-injected versus coinjected oocytes were qualitatively similar in that they were both activated by depolarizing voltage steps and increasing concentrations of intracellular calcium, as expected for maxi-K channels (Latorre et al., 1989).

It is clear, however, from the comparisons shown in Figure 2 that the gating of channels from coinjected oocytes differed substantially from that of oocytes expressing only the α subunit. Inspection of the currents elicited by a family of depolarizing voltage steps (Figures 2a and 2c) reveals a large difference in channel open probability at the holding potential of 0 mV. With the β subunit present, many of the channels were open at the holding potential and turned off during steps to negative potentials. This produced the rapidly decaying inward currents seen at the beginning of steps to negative potentials in Figure 2c. Note that the currents in Figure 2a at large positive potentials are noisier than the corresponding currents in Figure 2c. This oc-

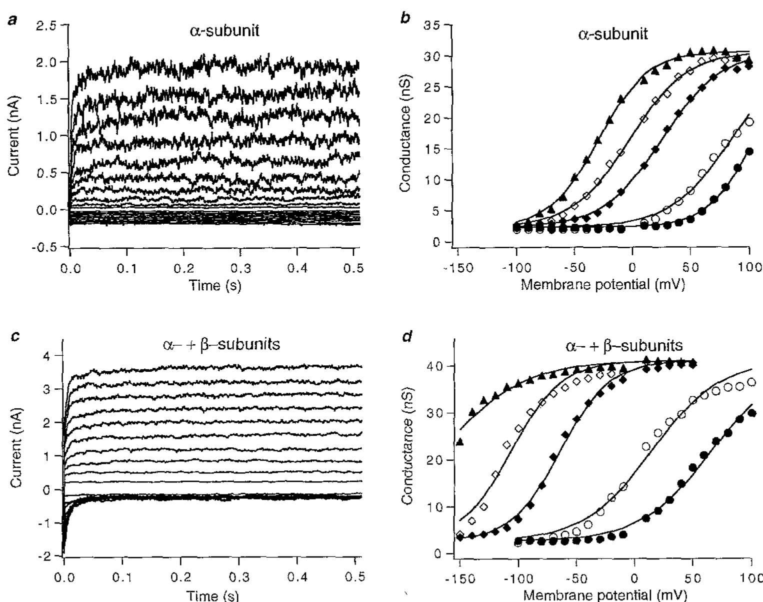

Figure 2. Macropatch Recording from Homomeric α Channels and Heteromeric α + β Channels

Currents were recorded from inside-out membrane patches excised from Xenopus oocytes injected with cRNA encoding either the α subunit (a and b) or a mixture of the α and β subunits (c and d). In (a) and (c), the membrane potential was held at 0 mV and stepped to command potentials that varied from −100 to +100 mV in 10 mV increments, with 3 μM internal calcium and 150 mM KCl on both sides of the membrane. (b) and (d) show the transformation of data from traces such as those shown in (a) and (c), in the presence of various calcium concentrations. Conductance was calculated as I/V_m, where I is the average current amplitude measured over the interval from 300–500 ms during the voltage step and V_m is the test potential. Continuous curves show the results of fitting each data set to a Boltzman function of the form: $G = G_{min} + G_{max}/\{1 + \exp[(V_{1/2} - V_m)/k]\}$, where G_{min} is the conductance measured at 1 μM calcium and −100 mV (where channel activation is minimal), G_{max} is the conductance measured at 100 μM calcium and +50 mV (where channels are maximally activated), V_m is the membrane potential, and k is a slope factor. Data are shown for homomeric (α) channels (b) and heteromeric (α + β) channels (d) with 1 μM (closed circles), 3 μM (open circles), 10 μM (closed diamonds), 30 μM (open diamonds), and 100 μM (closed triangles) calcium at the cytoplasmic side of the membrane. Open circles in (b) and (d) are data from (a) and (c), respectively.

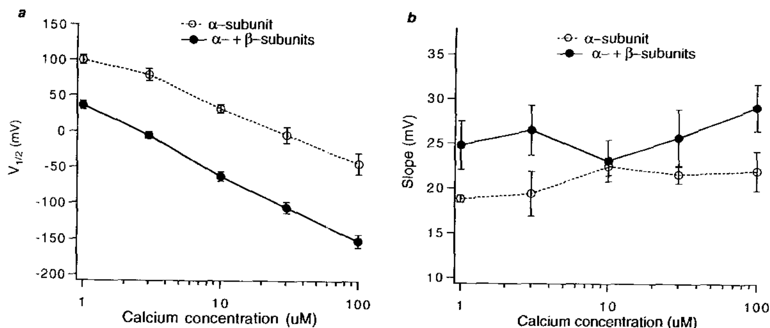

Figure 3. The β Subunit Shifts the Voltage Dependence of Channel Gating

The midpoints ($V_{1/2}$; a) and the slopes (k; b) of the fitted conductance-voltage curves for a number of experiments similar to the ones shown in Figure 2 are plotted against internal calcium concentration for channels composed of α subunits alone (open circles) and for heteromeric (α + β) channels (closed circles). Means ± SEM for 3–8 experiments at each condition are plotted.

curred because the open probability of α channels shown in Figure 2a is expected to be about 0.5 at +100 mV (see Figure 2b), where the variance is large, whereas the open probability of α + β channels is expected to be near 1 under these conditions (see Figure 2d), where the variance is small. Currents such as those shown in Figures 2a and 2c were obtained at various concentrations of internal calcium and were normalized to conductance–voltage plots, as shown in Figures 2b and 2d. The ranges of voltage and calcium used were adequate to determine the maximum conductance for each patch, as evidenced by the saturation of the conductance–voltage relation. After comparing the data from an α-injected (Figure 2b) versus a coinjected (Figure 2d) oocyte, it is apparent that coexpression of the β subunit resulted in a large shift in the voltage dependence of channel opening.

Figure 3a shows the combined results from several experiments such as the two shown in Figure 2. From this plot, it can be seen that the midpoint of the conductance–voltage curve ($V_{1/2}$) is shifted in the hyperpolarizing direction by coexpression of the β subunit, and that the magnitude of this shift depends on the calcium concentration. At 1 μM calcium, the difference in $V_{1/2}$ was 64 mV, whereas at 100 μM calcium, the difference in $V_{1/2}$ was 108 mV. Overall, the mean shift in $V_{1/2}$ associated with coexpression of the β subunit was −90 mV. This change in the voltage-dependence of channel activation is equivalent to that produced by a 10-fold increase in cytoplasmic calcium, as shown in Figure 3a. The slopes of the voltage dependencies were not statistically different when comparing α subunit versus α + β subunit channels at any calcium concentration. When data at all calcium concentrations were combined, the slopes from α + β patches (25.4 ± 6.8 mV; n = 30) were slightly higher ($p < .05$, unpaired t test) than those from α patches (22.3 ± 3.8 mV; n = 26).

A convenient measure of the calcium sensitivity of channel gating is the concentration of calcium required to open the channels half of the time at 0 mV ($Ca_{1/2}$; Table 1). The $Ca_{1/2}$ for channels encoded by the α subunit alone was about 30 μM, which is similar to the sensitivity of other cloned and heterologously expressed maxi-K channel α subunits (Adelman et al., 1992; Butler et al., 1993; Perez et al., 1994). However, the $Ca_{1/2}$ observed for channels in oocytes expressing both α and β subunits was about 10-fold lower (3 μM), which is within the range of values (1–5 μM) reported for native maxi-K channels from brain and smooth muscle (McManus, 1991).

Differences in channel gating were also observed at the single-channel level. For example, the open probability observed for individual α channels at 100 μM calcium and −20 mV was 0.39 ± 0.11 (mean ± SEM; 5 experiments), whereas under the same conditions, the open probability for channels formed by the α and β subunits was 0.88 ± 0.05 (mean ± SEM; 3 experiments). Individual maxi-K channels from native tissues tend to exhibit heterogeneity in gating, even when comparing channels within a single patch (McManus, 1991; Moczydlowski and Latorre, 1983; McManus and Magleby, 1991). We noted the same tendency in these experiments using cloned channels, but the presence of the β subunit caused a small reduction in the extent of this variability. For example, the calcium sensitivity of individual α channels varied over a 10-fold range, whereas the calcium sensitivity of the heteromultimeric channels varied over about a 4-fold range (data not shown).

Although a detailed kinetic analysis of the gating of the

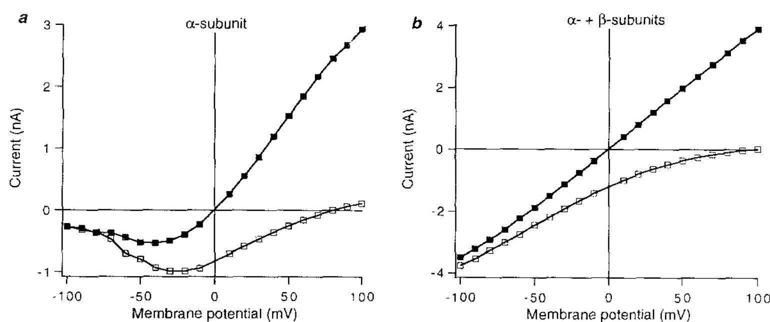

Figure 4. The Expressed Channels Are Selectively Permeable to Potassium over Sodium and Chloride

Current–voltage plots are shown for α channels (a) and heteromeric channels formed by α and β subunits (b) with 150 mM KCl on the external side and either 150 mM KCl (closed squares) or 150 mM NaCl (open squares) on the cytoplasmic side; 100 μM calcium was present on the cytoplasmic side.

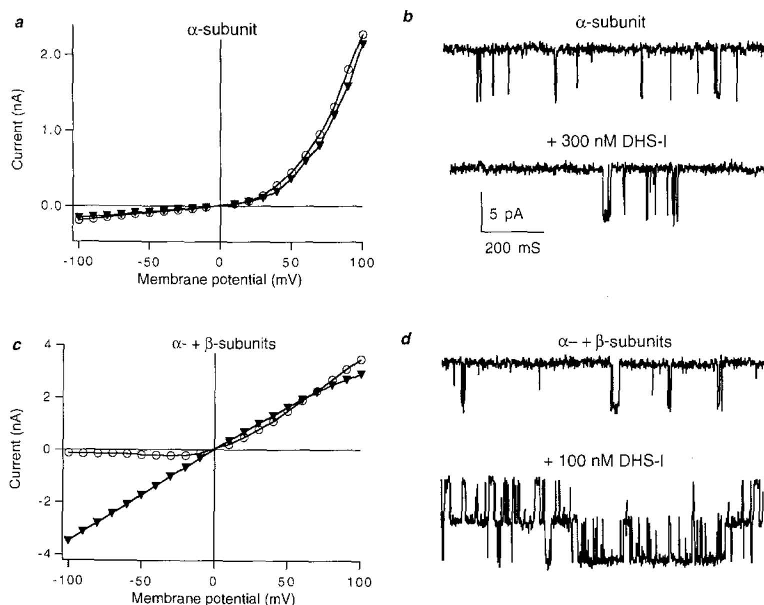

Figure 5. The β Subunit Confers Sensitivity to the Maxi-K Channel Agonist DHS-I

(a) Current–voltage relation in 3 μM internal calcium for a patch containing >100 α channels before (open circles) and after (closed triangles) addition of 300 nM DHS-I.

(b) Currents from a single α channel at −20 mV with 10 μM internal calcium, before (top) and after (bottom) addition of 300 nM DHS-I.

(c) Heteromeric channels; conditions and symbols as in (a).

(d) Patch with 2 α/β channels at −20 mV with 3 μM internal calcium, before (top) and after (bottom) addition of DHS-I. Analysis of the full record from which the data in (d) were excerpted revealed that 100 nM DHS-I caused a 45-fold increase in channel open probability.

All data are from excised, inside-out patches with 150 mM KCl on both sides of the membrane. In (b) and (d), channel opening is downward. DHS-I was always added to the internal face of the patch.

expressed channels has not been undertaken at the single-channel level, it is interesting to note that coexpression of the β subunit was associated with the disappearance of numerous brief closed events that are characteristic of expressed α channels. The significance of this apparent stabilization of the open state is unclear.

Selectivity

Coexpression of the β subunit did not grossly alter the ionic selectivity of the maxi-K channels. When NaCl was substituted for KCl on the cytoplasmic face of the patch (Figure 4), the apparent reversal potential of the currents shifted from 0 mV to greater than +80 mV, as expected for a potassium-selective channel. Such experiments were routinely performed and serve to demonstrate that the contribution of endogenous calcium-activated chloride current was too small to have influenced our conductance–voltage measurements. Patches with few channels were used to measure single-channel conductances. The values of the slope conductances of the α and heteromultimeric (α + β) channels were similar (Table 1).

β Subunit Confers Sensitivity to an Activator of Maxi-K Channels

In addition to its effects on channel gating, the β subunit conferred sensitivity to dehydrosoyasaponin I (DHS-I), a natural product isolated from a Ghanese medicinal herb that has been shown to be a potent agonist of maxi-K channels. When applied to the cytoplasmic face of native smooth muscle maxi-K channels, 100 nM DHS-I increases channel open probability by more than 50-fold (McManus et al., 1993). Interestingly, channels composed of only α subunits were insensitive to this compound. In 14 of 14 experiments, application of DHS-I at concentrations up to 500 nM produced either no change or a noticeable decrease in channel open probability (Figures 5a and 5b).

In contrast, channels from coinjected oocytes were quite sensitive to activation by DHS-I. In a typical experiment (3 μM calcium; Figure 5c), addition of 300 nM DHS-I increased the ensemble channel open probability at −100 mV from nearly 0 to maximal activity. At the single-channel level, DHS-I caused characteristic bursts of high channel open probability (Figure 5d), as previously described for native maxi-K channels. In 11 out of 11 similar experiments, 100–500 nM DHS-I caused large increases in channel open probability. Thus, the β subunit confers sensitivity of maxi-K channels to activation by DHS-I. It is not known whether DHS-I actually binds to the β subunit, or whether the β subunit instead exerts its effect on DHS-I sensitivity by allosterically modifying a site on the α subunit.

Discussion

Biochemical purification of smooth muscle maxi-K channels showed them to be composed of two subunits (Garcia-Calvo et al., 1994). Immunoprecipitation experiments indicate that the two subunits are tightly associated; antibodies directed against one subunit can precipitate both subunits (Knaus et al., 1994b). In this report we demonstrate the functional consequences of this structural association. Expression of the β subunit alone in Xenopus oocytes did not result in the appearance of functional potassium channels. However, coexpression of the β subunit with the α subunit led to formation of potassium channels with biophysical and pharmacological properties that were clearly distinct from channels formed by the α subunit alone. The properties of these α/β coexpressed channels more closely resembled those of native maxi-K channels. These data provide direct evidence that both α and β subunits contribute to the functional properties of maxi-K channels. Recent evidence indicates that sodium channels, calcium channels, and voltage-gated potassium

channels each can exist as heteromultimeric complexes (Isom et al., 1994). In each case, the accessory subunit appears to modify the gating or pharmacology of the pore-forming subunit. Thus, the heteromeric motif seems to be the rule rather than the exception. This effect of the maxi-K channel β subunit on channel activation and pharmacology is very unlike the effect of voltage-gated potassium channel β subunits on channel inactivation (Rettig et al., 1994). The large effect on sensitivity of the maxi-K channel to voltage and calcium conferred by its β subunit implies that the smaller subunit actually forms part of the transduction machinery of the channel.

Given the dramatic effects of the β subunit, it seems remarkable that expression of the α subunit alone gives rise to recognizable maxi-K channels. This raises the question whether inclusion of the β subunit in vivo is optional. Coassembly of maxi-K α and β subunits has been shown explicitly only in adult smooth muscle. It is not known for other tissues, or at other developmental stages, whether such coassembly is obligatory. This raises the possibility that regulated expression of β subunits constitutes a mechanism for generating functional diversity among mammalian maxi-K channels.

Experimental Procedures

Heterologous Expression

cRNAs were transcribed in vitro from linearized plasmids encoding either the α (Pallanck and Ganetzky, 1994) or β subunits (Knaus et al., 1994a) using the mCAP kit from Stratagene, then injected into Xenopus oocytes (15–100 ng per oocyte) using standard techniques. The expression plasmid for the α subunit cDNA contained mslo19(Δ5′), a fragment of the mslo19 (GenBank locus #MMU09383) cDNA from which all 5′ noncoding sequence (up to the second potential translation initiation site; Δ1–940) was removed. Oocytes injected with mslo19 cRNA containing the large 5′ untranslated region failed to produce currents. The translation initiation site used in this study corresponds to that of the mbr5 cDNA used by Butler et al. (1994). In coexpression experiments, a 5-fold molar excess of cRNA encoding the β subunit was used to drive coassembly of the subunits in the expressed channels. Injected cells were maintained at room temperature (19°C–22°C) in 50% L-15 (Specialty Media), 1 mM glutamine, 15 mM HEPES, 0.05 mg/ml gentamycin, and 5 mg/ml bovine serum albumin (pH 7.4). Currents were assayed 2–30 days following injection with standard two-microelectrode voltage-clamp techniques.

Two-Electrode Voltage Clamp

During recording, oocytes were bathed in a solution consisting of 96 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, and 0.1% bovine serum albumin (pH 7.4). In all cases, leakage currents were eliminated by subtracting the linear component of current recorded between −90 mV and −60 mV from each trace. Values are expressed as mean ± SEM. Experiments were performed at room temperature (19°C–22°C).

Patch Clamp

Following a brief exposure to hypertonic saline, the vittelin membrane was removed, and membrane patches were excised from the oocytes. Membrane currents were recorded with standard patch-clamp methods (Hamill et al., 1981) using pipettes with resistances of 1–3 MΩ. The current activated at a given voltage was calculated from the mean current measured from 300–500 ms in patches containing >50 channels, or from the average of 4–8 similar pulses in patches containing fewer channels. Data from experiments in which the maximum and minimum conductances were not clearly defined were excluded from analysis. The solutions on both sides of the membrane contained 150 mM KCl and 10 mM HEPES (pH 7.20), except where noted otherwise. External calcium concentration was 10 μM, and internal calcium concentration was varied. These solutions contained 0.3 μM calcium as a contaminant, which was determined by atomic absorption spectrophotometry and confirmed using maxi-K channels as biosensors. The contribution of this contaminant calcium was neglected. Experiments were done at 23°C–25°C.

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