INTRODUCTION

Auxiliary subunits often tune ion channel behavior to the needs of a particular cell type (Isom et al., 1994; Gurnett and Campbell, 1996; Xu et al., 1998). This method of generating functional diversity is particularly well exploited by the large-conductance Ca\(^{2+}\)-activated potassium channel, or BK\(_{\text{ca}}\) channel, which is composed of pore-forming \(\alpha\) and auxiliary \(\beta\) subunits (Knaus et al., 1994b; McManus et al., 1995). Four \(\alpha\) subunits are sufficient to form a fully functional channel complete with Ca\(^{2+}\) sensitivity, voltage sensitivity, and a large single-channel conductance (Shen et al., 1994; DiChiara and Reinhart, 1995; Cui et al., 1997). There is only a single gene for this subunit (Atkinson et al., 1991; Adelman et al., 1992; Butler et al., 1993). In different tissues, however, BK\(_{\text{ca}}\) channels display different phenotypes, and this is thought to be due primarily to the tissue-specific expression of some subset of four homologous BK\(_{\text{ca}}\) \(\beta\) subunits (\(\beta1-\beta4\)) (Knaus et al., 1994a; McManus et al., 1995; Wallner et al., 1995, 1999; Meera et al., 1996, 2000; Tanaka et al., 1997; Xia et al., 1999, 2000; Behrens et al., 2000; Brenner et al., 2000a; Uebele et al., 2000; Weiger et al., 2000).

The \(\beta2\) subunit, for example, confers rapid inactivation upon the BK\(_{\text{ca}}\) channels of adrenal chromaffin cells (Wallner et al., 1999; Xia et al., 1999), and \(\beta4\) renders many of the BK\(_{\text{ca}}\) channels of the brain insensitive to the scorpion toxin charybdoxin (Meera et al., 2000). Perhaps most profound, however, the BK\(_{\text{ca}}\) \(\beta1\) subunit, which is predominately expressed in smooth muscle, slows the BK\(_{\text{ca}}\) channel’s kinetic behavior and dramatically increases its Ca\(^{2+}\) sensitivity (McManus et al., 1995; Wallner et al., 1995; Meera et al., 1996; Cox and Aldrich, 2000; Nimigean and Magleby, 2000). In fact, mice that lack \(\beta1\) have hypertension, because the BK\(_{\text{ca}}\) channels of their vascular smooth muscle lack the Ca\(^{2+}\) sensitivity required for BK\(_{\text{ca}}\)-mediated feedback regulation of smooth muscle contraction (Brenner et al., 2000b). Thus, \(\beta1\) is important in the vascular system and indeed in many other smooth muscle-dependent systems as well (Nelson and Quayle, 1995; Snetkov and Ward, 1999; Bayguinov et al., 2001; Niu and Magleby, 2002; Meredith et al., 2004; Morales et al., 2004). Four \(\beta1\) subunits associate with a single BK\(_{\text{ca}}\) channel (Wang et al., 2002).

How \(\beta1\) enhances the BK\(_{\text{ca}}\) channel’s Ca\(^{2+}\) sensitivity is not well understood. Perhaps the simplest mechanism would be for it to increase the affinities of the channel’s Ca\(^{2+}\)-binding sites, but this does not appear to be the case. Nimigean and Magleby (1999, 2000) found that...
β1 increases the length of time that the BKCa channel spends in bursting states and that this effect persists in the absence of Ca²⁺. They suggested that it is this Ca²⁺-independent effect that underlies most of the channel's increased Ca²⁺ sensitivity. Furthermore, we found previously that as the Ca²⁺ concentration is raised, the concentration at which the BKCa channel’s conductance–voltage relation begins to shift leftward is essentially unaffected by β1 (Cox and Aldrich, 2000), a result that suggests that, at least when it is open, the channel’s affinity for Ca²⁺ is not greatly altered by β1. In fact, this study leads us to suggest that, rather than greatly altering the channel’s Ca²⁺-binding properties, β1 may be enhancing its voltage-sensing properties by shifting the equilibrium for voltage sensor activation, and therefore the channel’s gating charge vs. voltage relation (Q–V relation) and therefore the channel’s gating charge vs. voltage shifting the equilibrium for voltage sensor activation, thereby stabilizing the channel’s active conformation. In addition, however, in order to fully account for the increase in apparent Ca²⁺ affinity brought about by β1, we have also found it necessary to suppose that β1 decreases the true affinity of the closed channel for Ca²⁺.

MATERIALS AND METHODS

Channel Expression

Experiments were done with a BKCa channel α subunit clone from mouse mSlo-mbr5 (Butler et al., 1993) and a β1 subunit clone from bovine (ββ1) (Knaus et al., 1994a). In vitro transcription was performed with the nMessage mMachine kit with T3 or T7 RNA polymerase (Ambion). 0.05–250 ng of total cRNA was injected into Xenopus laevis oocytes 2–6 d before recording. Different amounts of cRNA were injected for different purposes, 0.05–0.2 ng for single channel recording, 10–50 ng for macroscopic current recording, and 150–250 ng for gating currents and limiting V$_{opt}$, recordings. ββ1 and mSlo cRNA were mixed in a molar ratio of 2:1 before injection.

Electrophysiology

All recording were done in the inside-out patch clamp configuration. Patch pipettes were made of borosilicate glass (VWR micropipettes) with 0.5–4 MΩ resistances that were varied for different recording purposes. The tips of the patch pipettes were coated with sticky wax (Sticky Wax) and fire polished. Data were acquired using an Axopatch 200B patch-clamp amplifier and a Macintosh-based computer system equipped with an ITG16 hardware interface (Instrutech) and Pulse acquisition software (HEKA Electoronik). For macroscopic current recording, data were sampled at 50 kHz and filtered at 10 kHz. In most macroscopic current experiments, capacity and leak currents were subtracted using a P/5 subtraction protocol with a holding potential of −120 mV and leak pulses opposite in polarity to the test pulse, but with BK$_{Ca+}$ currents recorded at 10 and 100 µM Ca²⁺ no leak subtraction was performed.

Unitary currents were sampled at 100 kHz and filtered at 10 kHz. For gating current recordings, voltage commands were filtered at 7.5 kHz to limit the size of fast capacity transients, and the data were sampled at 100 kHz and filtered at 5–10 kHz. Capacity and leak currents were subtracted using a P/5 protocol with a holding potential of −120 mV and leak pulses opposite in polarity to the test pulse. All experiments were performed at room temperature, 22–24°C.

Solutions

Gating current solutions were made according to Horrigan and Aldrich (1999). Pipette solutions contained (in mM) 127 TEAOH, 125 HMeSO$_4$, 2 HCl, 2 MgCl$_2$, 20 HEPES, pH 7.2 (adjusted with HMeSO$_3$ or TEAOH). The 0.5 nM Ca$_{1+}$ internal solution contained (in mM) 141 NMDG, 135 HMeSO$_4$, 6 HCl, 20 HEPES, 40 µM (+)-18-crown-6-tetracarboxylic acid (18C6TA), 5 EGTA, pH 7.2 (adjusted with NMDG and HMeSO$_3$).

K* current recording solutions were composed of the following (in mM): pipette solution, 80 KOH, 60 NMDG, 140 HMeSO$_4$, 20 HEPES, 2 KCl, 2 MgCl$_2$ (pH 7.20); internal solution, 80 KOH, 60 NMDG, 140 HMeSO$_4$, 20 HEPES, 2 KCl, 1 HEDTA or 1 EGTA, and CaCl$_2$ sufficient to give the appropriate free Ca$_{2+}$ concentration (pH 7.20). EGTA (Sigma-Aldrich) was used as the Ca$_{2+}$ buffer for solutions containing <1 µM free [Ca$^{2+}$]. HEDTA (Sigma-Aldrich) was used as the Ca$_{2+}$ buffer for solutions containing between 1 and 10 µM free Ca$^{2+}$, and no Ca$_{2+}$ chelator was used in solutions containing 100 µM free Ca$^{2+}$. 50 µM (+)-18-crown-6-tetracarboxylic acid (18C6TA) was added to all internal solutions to prevent Ba$^{2+}$ block at high voltages (Cox et al., 1997b).

The appropriate amount of total Ca$^{2+}$ (100 mM CaCl$_2$ standard solution; Orion Research Inc.) to add to the base internal solution containing 1 mM HEDTA to yield the desired free Ca$^{2+}$ concentration was calculated using the program Max Chelator (http://www.stanford.edu/~cpatton/maxc.html), and the solutions were prepared as previously described (Bao et al., 2004). To change Ca$^{2+}$ concentration, the solution bathing the cytoplasmic face of the patch was exchanged using a sewer pipe flow system (DAD 12) purchased from Adams and List Assoc. Ltd.

Data Analysis

All data analysis was performed with Igor Pro graphing and curve fitting software (WaveMetrics Inc.), and the Levenberg-Marquardt algorithm was used to perform nonlinear least-squares curve fitting. Values in the text are given ± SEM.

G–V Curves. Conductance–voltage (G–V) relations were determined from the amplitude of tail currents measured 200 µs after repolarizations to −80 mV following voltage steps to the test voltage. Each G–V relation was fitted with a Boltzmann function

$$G = \frac{G_{\text{max}}}{1 + e^{-\frac{V-V_{\text{half}}}{kT}}}$$
and normalized to the maximum of the fit. The half-activation voltage \((V_{1/2})\) and the effective gating charge \((z)\) were determined from the fitting.

**Q–V Curves.** The amount of activated gating charge \((Q)\) at a given voltage was determined from the area under the gating current trace between 0 and 300 \(\mu\)s after the initiation of the voltage step. Repolarizations were to \(-80\) mV. Each Q–V relation was fitted with a Boltzmann function and normalized to the maximum of the fit. The voltage sensor’s half-activation voltage \(V_c\) and the gating charge \(z_g\) were determined from the fitting.

**Limiting \(P_{open}^\alpha\) Analysis.** \(P_{open}^\alpha\) measurements <10\(^{-5}\) were made in 3 nM Ca\(^{2+}\) with patches that contained hundreds of channels. The number of channels in a given patch \((N)\) was determined by switching the patch into a solution that contained either 100 \(\mu\)M Ca\(^{2+}\) \((\alpha)\) or 10 \(\mu\)M Ca\(^{2+}\) \((\alpha+\beta1)\) and recording macroscopic currents at moderate to high voltages. \(N\) was then calculated as \(N = I/(IP_0)\), where \(i\) represents the single channel current at a given voltage and \(P_0\) represents the open probability at that same voltage. Both \(i\) and \(P_0\) were determined previously in separate experiments. In some experiments \(P_0\) was estimated from the G–V relation determined from the same patch. After determining the number of channels in a given patch, the membrane voltage was moved to lower voltages, the patch was superfused with a 3 nM Ca\(^{2+}\) solution, and unitary currents were recorded for 5–30 s at progressively more negative voltages. All points were then used to determine the probability of observing 0, 1, 2, 3, ... open channels at a given time, and true channel \(P_{open}\) was then determined as

\[
P_{open} = \frac{1(f_{open,1} + 2(f_{open,2} + 3(f_{open,3} + ... + n(f_{open,n}) + ... + N(f_{open,N}))}{N}
\]

where \(f_{open,n}\) represents the fraction of time \(n\) channels are open during the recording.

**Fitting \(\tau–V\) Curves Based on the Model of Horrigan et al. (1999).** Relaxation time constants as a function of voltage were calculated for Scheme I (see Fig. 5) assuming its horizontal steps equilibrate much more rapidly than its vertical steps, such that they are always at equilibrium (Cox et al., 1997a; Cui et al., 1997; Horrigan et al., 1999; Horrigan and Aldrich, 2002). Under this assumption, \(\tau(V)\) can be calculated as a weighted average of all the vertical rate constant in Scheme I as follows:

\[
delta = (\delta_0 \cdot f_{c0} + \delta_1 \cdot f_{c1} + \delta_2 \cdot f_{c2} + \delta_3 \cdot f_{c3} + \delta_4 \cdot f_{c4})
\]

\[
\gamma = (\gamma_0 \cdot f_{o0} + \gamma_1 \cdot f_{o1} + \gamma_2 \cdot f_{o2} + \gamma_3 \cdot f_{o3} + \gamma_4 \cdot f_{o4})
\]

\[
\tau = \frac{1}{\delta(V) + \gamma(V)}.
\]

where \(\delta_0\) and \(\gamma_0\) represent closed-to-open and open-to-closed rate constants, respectively, and \(f_{c0}\) and \(f_{o0}\) represent the fraction of closed or open channels occupying the state that precedes each transition. These fractions for the closed channel were calculated as follows:

\[
f_{c0} = \frac{4f \bar{e}^n}{(1 + 4f + 6f^2 + 4f^3 + f^4)}
\]

\[
f_{c1} = \frac{4f \bar{e}^n}{(1 + 4f + 6f^2 + 4f^3 + f^4)}
\]

\[
f_{c2} = \frac{6f \bar{e}^n}{(1 + 4f + 6f^2 + 4f^3 + f^4)}
\]

For the open channel, \(f_{o0} \cdot f_{o4}\) were calculated in the same way but using \(f_0\) rather than \(f\). Each vertical rate constant was also assigned a voltage dependence as follows

\[
\delta_i = \delta_i(0) e^{rac{xz_i V}{RT}}
\]

\[
\gamma_i = \gamma_i(0) e^{rac{xz_i V}{RT}}
\]

where \(x = (0, 1, 2, 3, 4, 5)\) and \(z_0 + z_x = z_x\). For a given vertical step, once the forward rate was specified, the backward rate was determined by the equilibrium parameters of the model.

\[
\delta_i = L_i \gamma_i
\]

\[
L_i = D_i L_o e^{rac{xz_i V}{RT}}
\]

Thus, to fit the \(\tau–V\) curves in Fig. 7, 11 independent parameters were required: \(L, V_{1/2}, D, z_2, z_3, z_4, \gamma_1(0), \gamma_2(0), \gamma_3(0), \gamma_4(0),\) and \(\gamma_4(0)\). For definitions of \(L, V_{1/2}, D, z_2, z_3,\) see RESULTS.

**RESULTS**

**Steady-state Effects of \(\beta1\)**

The BK\(_{Ca}\) channel is both Ca\(^{2+}\) and voltage sensitive, and the effects of these stimuli together are often displayed as a series of conductance–voltage \((G–V)\) relations determined over a series of Ca\(^{2+}\) concentrations (Barrett et al., 1982). Such a series, determined from currents recorded from channels expressed in Xenopus oocyte macropatches is shown in Fig. 1 C. These G–V curves were derived from channels composed of \(\alpha\) subunits alone. When the BK\(_{Ca}\) \(\beta1\) subunit is coexpressed with the \(\alpha\) subunit, the Ca\(^{2+}\)-induced leftward shifting evident in Fig. 1 C becomes more pronounced (Fig. 1, D and E; see also Table I) (McManus et al., 1995; Wallner et al., 1995; Meera et al., 1996; Cox and Aldrich, 2000), and thus it may be said that \(\beta1\) increases the Ca\(^{2+}\) sensitivity of the BK\(_{Ca}\) channel in that it increases its G–V shift in response to changes in Ca\(^{2+}\) concentration (McManus et al., 1995).

This increase in Ca\(^{2+}\) sensitivity can be viewed in a more conventional manner if a single voltage is considered. At \(-40\) mV, for example, \(\beta1\) dramatically in-

**TABLE I**

<table>
<thead>
<tr>
<th>([Ca])</th>
<th>(V_{1/2})</th>
<th>(Q(\epsilon))</th>
<th>(n)</th>
<th>(V_{1/2})</th>
<th>(Q(\epsilon))</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\muM)</td>
<td>mV</td>
<td></td>
<td></td>
<td>mV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>203 ± 5.3</td>
<td>0.95 ± 0.03</td>
<td>4</td>
<td>215 ± 3.4</td>
<td>0.66 ± 0.03</td>
<td>16</td>
</tr>
<tr>
<td>1</td>
<td>125 ± 4.7</td>
<td>1.35 ± 0.12</td>
<td>7</td>
<td>82 ± 3.4</td>
<td>0.99 ± 0.02</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>37 ± 5.6</td>
<td>1.26 ± 0.07</td>
<td>8</td>
<td>(-60 ± 1.6)</td>
<td>1.05 ± 0.05</td>
<td>7</td>
</tr>
<tr>
<td>100</td>
<td>1 ± 2.4</td>
<td>1.17 ± 0.03</td>
<td>22</td>
<td>(-100 ± 3.5)</td>
<td>1 ± 0.06</td>
<td>10</td>
</tr>
</tbody>
</table>

*The values shown are Boltzmann-fit parameters. They indicate mean ± SEM.*
Increases both the efficacy and apparent affinity of Ca\textsuperscript{2+} in activating the BK\textsubscript{Ca} channel (Fig. 2 A). Interestingly, however, and perhaps mechanistically telling, β1’s effects on Ca\textsuperscript{2+} sensing are not static with respect to voltage, but rather, they diminish as the membrane potential is made more positive (Fig. 2, B and C).

Conversely, if we examine β1’s effects at a single Ca\textsuperscript{2+} concentration, 100 μM (Fig. 2 D), we see that β1 powerfully shifts the channel’s G–V relation leftward along the voltage axis (~100 mV), and it reduces its maximum slope (20%). Here too, however, β1’s effects are not static with respect to the fixed stimulus. The β1-induced G–V shift decreases as the internal Ca\textsuperscript{2+} concentration is lowered (Fig. 2, E and F). In fact, at nominally 0 Ca\textsuperscript{2+} (3 nM in our experiments), β1 no longer produces a leftward G–V shift, but, instead, it creates a slight rightward G–V shift and a pronounced decline in G–V steepness (Fig. 2 G; Table I) (Cox and Aldrich, 2000; Nimigean and Magleby, 2000).

**β1’s Effects on BK\textsubscript{Ca} Gating Currents**

What does the β1 subunit do to the normal process of BK\textsubscript{Ca} channel gating that creates these large and physiologically important effects? Previously, we addressed...
Bao and Cox 397

this question by comparing the effects of $\beta_1$ to what happens to simulated currents when various parameters in models of $\text{BK}_{\text{Ca}}$ channel gating were altered (Cox and Aldrich, 2000). From this work we concluded that multiple aspects of gating are likely altered by $\beta_1$, including small changes in $\text{Ca}^{2+}$ binding, gating charge, and the intrinsic energetics of channel opening. One large change we predicted, however, was a large $\beta_1$-induced leftward shift in the channel’s charge–voltage ($Q$–$V$) relation. This, we supposed, would lower the free energy difference between open and closed states at most voltages, and thus lower as well the work $\text{Ca}^{2+}$ binding must do to open the channel (Cox and Aldrich, 2000).

Here, to directly test this prediction we have examined $\text{BK}_{\text{Ca}}$ gating currents in the absence and presence of $\beta_1$. A family of gating currents for the $\text{BK}_{\text{Ca}}$ channel is shown in Fig. 3 A. These currents were recorded in the essential absence of $\text{Ca}^{2+}$ (0.5 nM) with 1-ms voltage steps. Most notable, they are small and fast, 500–1,000 times smaller than the ionic currents we typically observe under the same conditions of channel expression (Fig. 1 A), and at $+160$ mV (Fig. 3 A, fourth trace down) the ON gating current decays with a time constant of $57.2 \pm 4.0$ s ($n = 16$), and the OFF gating current at $-80$ mV is similarly fast ($\tau(\text{off}) = 31.2 \pm 3.3$ $\mu$s, $n = 20$). Thus, care had to be taken to ensure that what we were observing was in fact gating current and not the result of capacity current subtraction errors. We are confident, however, that these currents are in-

![Figure 2.](image-url)

Figure 2. The effects of $\beta_1$ are both $\text{Ca}^{2+}$ and voltage dependent. (A–C) $\text{Ca}^{2+}$ dose–response curves determined for the $\text{BK}_{\text{Ca}}$ and $\text{BK}_{\text{Ca}+\beta_1}$ channel at (A) $-40$, (B) 0, and (C) $+40$ mV. Curves are fitted with the hill equation:

$$G = \frac{G_{\text{max}}}{1 + (K_d/\text{Ca})^n}.$$  

Fit parameters are as follows: $-40$ mV, $\text{BK}_{\text{Ca}}$ (Kd = 36.5 $\mu$M, $n = 1.24$); $\text{BK}_{\text{Ca}+\beta_1}$ (Kd = 6.89 $\mu$M, $n = 2.7$); 0 mV, $\text{BK}_{\text{Ca}}$ (Kd = 22.1 $\mu$M, $n = 1.5$); $\text{BK}_{\text{Ca}+\beta_1}$ (Kd = 3.4 $\mu$M, $n = 1.9$); $+40$ mV, $\text{BK}_{\text{Ca}}$ (Kd = 7.25 $\mu$M, $n = 1.6$); $\text{BK}_{\text{Ca}+\beta_1}$ (Kd = 3.68 $\mu$M, $n = 1.5$). (D–G) $G$–$V$ relations are shown for the $\text{BK}_{\text{Ca}}$ and the $\text{BK}_{\text{Ca}+\beta_1}$ channel at (D) 100 $\mu$M $\text{Ca}^{2+}$, (E) 10 $\mu$M $\text{Ca}^{2+}$, (F) 1 $\mu$M $\text{Ca}^{2+}$, and (G) 5 nM $\text{Ca}^{2+}$. The curves are fitted with Boltzmann functions as described in the legend to Fig. 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$L_2$</th>
<th>$L_3$</th>
<th>$\tau_j$</th>
<th>$\tau_i$</th>
<th>$V_{\text{th}}$</th>
<th>$V_{\text{th}}$</th>
<th>$D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{BK}_{\text{Ca}}$</td>
<td>$2 \times 10^{-6}$</td>
<td>$2.2 \times 10^{-6}$</td>
<td>$2.5 \times 10^{-6}$</td>
<td>$0.3 \times 10^{-6}$</td>
<td>$0.5 e$</td>
<td>$0.58 e$</td>
<td>$0.57 e$</td>
</tr>
<tr>
<td>$\text{BK}_{\text{Ca}+\beta_1}$</td>
<td>$0.4 e$</td>
<td>$0.41 e$</td>
<td>$0.41 e$</td>
<td>0</td>
<td>$155$ $\text{mV}$</td>
<td>$151$ $\text{mV}$</td>
<td>$80$ $\text{mV}$</td>
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<tr>
<td>Difference</td>
<td>$17$</td>
<td>$16.8$</td>
<td>$12.8$</td>
<td>$-4$</td>
<td></td>
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</tbody>
</table>

**TABLE II**

Comparing Voltage Gating Parameters
Alters Gating Currents, as they are not seen in uninjected oocytes (second trace down). They are not seen in response to voltage pulses of equal magnitude but opposite polarity (third trace down), and they have characteristics very similar to those reported previously for the BK channel (Horrigan and Aldrich, 1999, 2002) (Table II).

As shown in Fig. 3 B, we also recorded gating currents from channels composed of both α and β1 subunits, and remarkably they look very much like the BK currents. They are small and fast (see fourth traces down in Fig. 3, A and B; τ(on) = 54.7 ± 2.6 μs, n = 13, at +160 mV; τ(off) = 34.6 ± 3.0 μs, n = 14, at −80 mV), which is interesting because β1 slows ionic current relaxations (see Fig. 1, A and B and Fig. 7). Clearly, however, this slowing does not arise from a slowing of voltage sensor movement.

The gating currents we have recorded from both BKα and BKα+β1 channels have relaxation time constants close to the theoretical time resolution of our recording system τ ~ 40 μs, and so we have not analyzed the kinetics of these currents further, as they may be distorted by our hardware. What is clear, however, is that both with and without β1, BKCa gating currents are very fast.

An important consequence of the speed of the BKα and BKα+β1 channels’ gating charge movement is illustrated in Fig. 3 (C and D). In response to a strong depolarization, both channels’ voltage sensors move almost completely (Ig) before the channels begin to open (Ik). The time constants of channel opening are 18 (BKα) and 177 (BKα+β1) times larger than those of gating charge movement. Thus, for both channels, rapid ON gating currents reflect voltage sensor movement in the channel’s closed conformation (Stefani et al., 1997; Horrigan and Aldrich, 1999).

To determine Q–V relations we integrated both ON and OFF gating currents and plotted these integrals separately as a function of test potential. These integrals report the amount of gating charge that moves rapidly during each voltage pulse. Examples of Q–V curves from individual BKα and BKα+β1 patches are shown in Fig. 4 (A and B), and as is evident, both with and without β1, there is very little difference between the BKCa channels’ rapid ON and OFF Q–V curves. Thus, charge is not immobilized by depolarization in either case.

ON Q–V relations for each channel type were fitted with Boltzmann functions (Table III), and these curves were normalized to their maxima and averaged to yield the curves shown in Fig. 4 C. Of central importance, the two curves have the same shape, but the BKα+β1 curve lies far to the left of the BKα curve. Indeed, both curves are well fitted by Boltzmann functions, which
suggests that each channel's voltage sensors move independently, and a single voltage sensor behaves like a two-state system (Horrigan and Aldrich, 1999). For the BK$_a$ channel, the fit yielded values of $V_{1/2} = 151 \pm 1.9$ mV; BK$_{a+\beta_1}$: $z = 0.571 \pm 0.025$ e, $V_{1/2} = 80 \pm 2.4$ mV. The error bars in C represent SEM.

**Fig. 4.** The $\beta_1$ subunit shifts the closed channel's charge-voltage (Q-V) relation leftward without changing its shape. (A) On and Off Q-V relations determined from a single BK$_a$ channel patch. (B) On and Off Q-V relations determined from a single BK$_{a+\beta_1}$ channel patch. (C) Normalized averaged Q$_{on}$-V relations for the BK$_a$ (11 curves averaged) and BK$_{a+\beta_1}$ channels (14 curves averaged). Each curve in C is fitted with a Boltzmann function. The fit parameters are as follows: BK$_a$: $z = 0.577 \pm 0.025$e, $V_{1/2} = 151 \pm 1.9$ mV; BK$_{a+\beta_1}$: $z = 0.571 \pm 0.025$e, $V_{1/2} = 80 \pm 2.4$ mV. The error bars in C represent SEM.

**Table III**

Comparing Q-V Parameters

<table>
<thead>
<tr>
<th></th>
<th>$V_{1/2}$</th>
<th>$z$</th>
<th>$Q_{max}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK$_a$</td>
<td>156 $\pm$ 3.8</td>
<td>0.57 $\pm$ 0.02</td>
<td>49305 $\pm$ 6623</td>
<td>22</td>
</tr>
<tr>
<td>BK$_{a+\beta_1}$</td>
<td>80 $\pm$ 3.1$^a$</td>
<td>0.57 $\pm$ 0.01</td>
<td>27548 $\pm$ 4327$^a$</td>
<td>14</td>
</tr>
</tbody>
</table>

The values shown are Boltzmann-fit parameters. They indicate mean $\pm$ SEM. $Q_{max}$ represents the maximum gating charge measured per patch. $^a$Statistical significance between BK$_a$ and BK$_{a+\beta_1}$ values $P < 0.05$.

The Current View of BK$_a$ Voltage-dependent Gating

It is useful to view these results in the context of the current view of BK$_a$ voltage-dependent gating. A model represented by Scheme I (Fig. 5) has been shown by Horrigan, Cui, and Aldrich to very well approximate BK$_a$ gating and ionic currents in the absence of Ca$^{2+}$, over a very wide range of voltages (Horrigan and Aldrich, 1999, 2002; Horrigan et al., 1999). Horizontal transitions represent movement of the channel's four voltage sensors, each of which can be either active or inactive. Vertical transitions represent the conformational change by which the channel opens. The activation of a voltage sensor is not required for channel opening, but rather it promotes opening by lowering the free energy difference between open and closed.

**Fig. 5.** The allosteric model of BK$_{Ca}$ voltage-dependent gating of Horrigan et al. (1999). Horizontal transitions represent voltage sensor movement. Vertical transitions represent channel opening. For details of the model see RESULTS and MATERIALS AND METHODS.
Indeed, the factor by which the movement of a voltage sensor increases the closed-to-open equilibrium constant, referred to by Horrigan, Cui, and Aldrich as \( D_i \), is given simply by \( J_O/J_C \), where \( J_O \) is the equilibrium constant for voltage sensor activation at 0 mV when the channel is open, and \( J_C \) is this equilibrium constant when the channel is closed. \( J_C \) and \( J_O \) can be related to the midpoints of the open and closed channel’s Q–V relation as follows:

\[
J_C = \frac{z_f F (V - V_{ho})}{RT},
\]

(7)

\[
J_O = \frac{z_f F (V - V_{ho})}{RT},
\]

(8)

where \( R, T \), and \( F \) have their usual meanings, \( z_f \) is the gating charge associated with a single voltage sensor, and \( V_{ho} \) and \( V_{ho} \) are the half-maximal activation voltages of the channel’s Q–V relation, when the channel is either closed (\( V_{ho} \)) or open (\( V_{ho} \)). In order for voltage sensor movement to promote opening, \( V_{ho} \) must be more negative than \( V_{ho} \).

Completing then the mathematical description, the open probability (\( P_{open} \)) of the Horrigan, Cui, and Aldrich model as a function of voltage (\( V \)) is given by Eq. 9:

\[
P_{open} = \frac{1}{1 + \left( 1 + e^{\frac{z_f F (V - V_{ho})}{RT}} \right)^{1 - z_f F V / RT}},
\]

(9)

where \( L \) is the equilibrium constant between open and closed at 0 mV when no voltage sensors are active, and \( z_f \) is a small amount of gating charge associated with the closed-to-open conformational change.

As is the case for other voltage-gated \( K^+ \) channels, it is fairly clear that the S4 region, and probably part of S3, forms the \( BK_{Ca} \) channel’s voltage sensor, whose gating charge is designated here \( z_f \) (Stefani et al., 1997; Diaz et al., 1998; Horrigan and Aldrich, 1999; Hu et al., 2003). The physical basis for \( z_f \), however, has yet to be determined, but without assigning some voltage dependence to the closed-to-open conformational change, it is not possible to fit the \( BK_\alpha \) ionic current data at all well (Horrigan and Aldrich, 1999, 2002; Horrigan et al., 1999; Cox and Aldrich, 2000; Cui and Aldrich, 2000; Rothberg and Magleby, 2000). Thus, the equilibrium voltage dependence of \( BK_\alpha \) gating in the absence of \( Ca^{2+} \) is well described by five parameters, \( V_{ho}, V_{ho}, z_f, L, \) and \( z_f \), and to this point our data allows us to specify two of them \( V_{ho} \) and \( z_f \). To fully determine the effects of \( \beta_1 \) on the voltage-dependent aspects of \( BK_{Ca} \) channel gating at equilibrium, however, requires that we specify \( V_{ho} \), \( L \), and \( z_f \) for both channels as well. To do this we have performed the experiments described below.

Estimating \( \beta_1 \)’s Effects on the Closed-to-Open Conformational Change

At far negative voltages, where no voltage sensors are active, Eq. 9 reduces to Eq. 10 (Horrigan et al., 1999)

\[
P_{open} = Le^{\frac{z_f F V}{RT}},
\]

(10)

which can be rewritten as

\[
\log(P_{open}) = \frac{0.4342 z_f F V}{RT} + \log(L).
\]

(11)

Eq. 11 states that as we make the membrane voltage more and more hyperpolarized, a plot of \( \log(P_{open}) \) vs. voltage will begin to turn away from the voltage axis, and it will reach a limiting slope that is less than the maximum slope and reflects the voltage dependence of just the closed-to-open conformational change (Horrigan and Aldrich, 1999, 2002; Horrigan et al., 1999). That is, in this voltage range, the slope of the \( \log(P_{open}) \)–V relation will be determined only by \( z_f \), and the position of the curve on the vertical axis will be determined only by \( L \). Thus, as has been discussed previously (Horrigan and Aldrich, 1999, 2002), by determining the \( BK_{Ca} \) channel’s \( P_{open} \) vs. voltage relation at far negative potentials we can estimate \( z_f \) and \( L \) directly.

To do this, we recorded \( BK_\alpha \) and \( BK_{\alpha+\beta_1} \) macroscopic currents at depolarized voltages (+10 to +80 mV) and 10 or 100 \( \mu M \) \( Ca^{2+} \). From these currents we could determine the number of channels in a given patch (\( N \)) (see MATERIALS AND METHODS). Then, we lowered the \( Ca^{2+} \) concentration to 3 nM and the membrane voltage to negative values where the channels are rarely open, and recorded unitary currents. Such currents are shown in Fig. 6 (A and B). Notice, the \( BK_\alpha \) channels appear to open more frequently than the \( BK_{\alpha+\beta_1} \) channels, but the burst times of the \( BK_{\alpha+\beta_1} \) channels appear on average longer (expanded traces). From data like that in A and B, the probabilities of observing 1, 2, 3 ..., \( N \) open channels at a given time were determined with all-points histograms, and these probabilities and \( N \) were then used to determine the true mean open probability of the channels in each patch (see MATERIALS AND METHODS). In this way, we could measure open probabilities as low as \( 10^{-6} \).

\( \log(P_{open}) \) vs. voltage plots are shown in Fig. 6 (C and D) for the \( BK_\alpha \) and \( BK_{\alpha+\beta_1} \) channels. As the voltage becomes more negative, the \( BK_\alpha \) plot curves upward starting at \( -60 \) mV, and it reaches a limiting slope by \( -100 \) mV. Fitting just the most negative part of this curve with Eq. 11 yields values for \( L \) and \( z_f \) of \( 2.2 \times 10^{-6} \) and 0.410 \( e \) respectively, again similar to published values (Horrigan and Aldrich, 1999, 2002) (Table II) (for standard errors of fit parameters see figure legends). Surprisingly, however, in the \( BK_{\alpha+\beta_1} \) plot, no
Table 4. 

<table>
<thead>
<tr>
<th>Channel</th>
<th>Voltage (mV)</th>
<th>O 0 to C 0 Transition</th>
<th>C 4 to O 4 Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK Ca</td>
<td>-120</td>
<td>2.2e-06</td>
<td>0.41</td>
</tr>
<tr>
<td>BK Ca, B1</td>
<td>-100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
<td>-80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
<td>-60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
<td>-40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
<td>-20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
<td>0</td>
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</tr>
<tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
<td>40</td>
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<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BK Ca, B1</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

such submaximal limiting slope is attained. Instead, down to -120 mV, the BK Ca, B1 channel’s log(P open)–V relation remains roughly linear, with no clear inflection point that would suggest that the limiting slope is being approached. This is surprising, because Scheme I suggests that such an inflection point should exist, and the fact that we do not see it argues either that the B1 subunit fundamentally alters the gating behavior of the BKCa channel such that models of the form of Scheme I no longer apply, or we have yet to examine voltages negative enough to see the inflection point. Indeed, this latter possibility seems very real, as we have already discovered that it takes significantly lower voltages to hold the channel’s voltage sensors in their inactive state, at least for the closed channel, when the B1 subunit is present (Fig. 4 C). Unfortunately, however, we found it technically unfeasible to make accurate P open measurements for the BK Ca, B1 channel at voltages more negative than -120 mV, where P open approaches 10^-7. As described below, however, we have used ionic current kinetics to estimate where, if it exists, the inflection point should be.
constant of relaxation; but rather, as voltage sensors become active, a weighted average of all the vertical rate constants in Scheme I prevails. Thus, just as with the log($P_{\text{open}}$)–V relation, the model predicts that there will be a transition or inflection point in the channel's log($\tau$)–V relation at the voltage where voltage sensors start to become activated, and at voltages more negative than this the plot will reach a limiting slope that reflects now the portion of $z_l$ associated with the closing transition ($z_{c}$) (Horrigan and Aldrich, 2002).

Viewing Fig. 7C in this light, then, the $\text{BK}_{\alpha}$ curve has a limiting positive slope of $\sim0.11e$ (dashed line), which suggests that $z_{c} = 0.11e$ and it starts to veer upward at $\sim-60$ mV, which, as expected, is the same position as the inflection point in the $\text{BK}_{\alpha}$ channel’s log($P_{\text{open}}$)–V curve (Fig. 6C). Interestingly, however, the $\text{BK}_{\alpha+\beta1}$ curve appears to reach the same limiting slope at negative voltages as does the $\text{BK}_{\alpha}$ curve (dashed lines), so $z_{c}$ appears unchanged by $\beta1$. And, at the most positive voltages tested (although here it is not clear that limiting voltages have been reached) the two curves also have the same slope, which suggests that $z_{c}$ is also little altered. Thus, $\beta1$ does not appear to affect $z_{c}$, and we can estimate that it is $\sim0.41e$ for both channels. In

\begin{align*}
\text{Figure 7.} & \quad \text{The $\beta1$ subunit does not alter the voltage dependence of the closed-to-open conformational change. Macroscopic ionic currents were recorded from $\text{BK}_{\alpha}$ (A) and $\text{BK}_{\alpha+\beta1}$ (B) channels in excised oocyte macropatches. Voltage steps were varied as indicated, and the time courses of relaxation were fitted with single-exponential functions. Time constants ($\tau$) from the fits are plotted in C. For voltages of $+100$ mV and greater, activation time constants are plotted. Otherwise deactivation time courses are plotted. The $\tau$–V curves in C were fitted (solid lines) to a function that approximates the kinetics of Scheme I in the limit that voltage sensor movement is fast relative to channel opening and closing (see MATERIALS AND METHODS). The fit parameters were as follows: $\text{BK}_{\alpha}$; held $V_{\text{m}}$=151 mV, L = $2.2 \times 10^{-6}$, $z_{c} = 0.58e$, $z_{l} = 0.41e$, fitting yielded D = $12.6 \pm 0.43$, $z_{c} = 0.10 \pm 0.092$, $\gamma(0) = 7452.3$ s$^{-1}$, $\gamma(1) = 4121.4$ s$^{-1}$, $\gamma(2) = 5645.8$ s$^{-1}$, $\gamma(3) = 851$ s$^{-1}$, $\gamma(4) = 1025$ s$^{-1}$, $\delta(0) = 0.016$ s$^{-1}$, $\delta(1) = 0.114$ s$^{-1}$, $\delta(2) = 1.98$ s$^{-1}$, $\delta(3) = 3.76$ s$^{-1}$, $\delta(4) = 57.12$ s$^{-1}$; $\text{BK}_{\alpha+\beta1}$; held $V_{\text{m}} = 80$ mV, $z_{c} = 0.57e$, fitting yielded L = $3.3 \times 10^{-6}$ $\pm 6.4 \times 10^{-5}$, $z_{c} = 0.46$, D = $10.4 \pm 6.4$, $z_{c} = 0.17e$, $\gamma(0) = 931.7$ s$^{-1}$, $\gamma(1) = 213.2$ s$^{-1}$, $\gamma(2) = 547.8$ s$^{-1}$, $\gamma(3) = 335.5$ s$^{-1}$, $\gamma(4) = 126.7$ s$^{-1}$, $\delta(0) = 0.003$ s$^{-1}$, $\delta(1) = 0.007$ s$^{-1}$, $\delta(2) = 0.198$ s$^{-1}$, $\delta(3) = 1.251$ s$^{-1}$, $\delta(4) = 4.934$ s$^{-1}$. Both curves are also fitted (dashed lines) at far negative voltages with the following function:

$$
\tau = \frac{1}{\gamma(0)e^{-\Delta PV/R}}
$$

Fit parameters are as follows: $\text{BK}_{\alpha}$ (voltages $<-180$ mV) $\gamma(0) = 6940.8$ s$^{-1}$, $z = 0.11e$ $\text{BK}_{\alpha+\beta1}$ (voltages $<-280$ mV) $\gamma(0) = 1808.7$ s$^{-1}$, $z_{c} = 0.11e$.

\end{align*}
age sensor movement is much faster than channel opening and closing (Cox et al., 1997a; Horrigan and Aldrich, 2002; for details of the approximation see MATERIALS AND METHODS). As this condition applies for both channels over much of the voltage range, such an approximation is likely to be reasonably good. Indeed, Eq. 12 fits both curves quite well (Fig. 7 C, solid lines).

$$
\tau(I_C) = \left( \sum_{i=0}^{4} \gamma_i f_{ci} + \sum_{j=0}^{4} \delta_j f_{cj} \right)^{-1}
$$

(12)

Here $\delta_i$ and $\gamma_i$ are the forward and backward rate constants enumerated in Fig. 7 (bottom) and $f_{ci}$ and $f_{cj}$ represent the fraction of open channels or closed that occupy state $O_i$ or $C_i$ respectively. In fitting the BK curve we held $V_{ho}$, $z_f$, $L$, and $z_J$ to the values we estimated from gating current and limiting $P_{open}$ measurements, leaving only $V_{ho}$ to vary along with $z_e$ and five rate constants, one for each vertical step in Scheme I. The resulting fit yielded $z_f = 0.11$ and $V_{ho} = +39$ mV. For the BK fit we adjusted $V_{ho}$ to the value we obtained from our BK gating current measurements, $+80$ mV, $z_f$ was again fixed, and $L$ was now allowed to vary freely along with $z_J$, $V_{ho}$, $z_e$, and again five rate constants. The resulting fit yielded $V_{ho} = -25$ mV, $z_e = 0.15$, and $L = 3.3 \times 10^{-6}$, $z_J = 0.46$ (for standard deviations of fit parameters see figure legend). Thus, this analysis suggests, as we surmised above, that $z_J$ and $z_e$ change little, if any, with BK coexpression, but that $V_{ho}$ moves $\sim 64$ mV leftward, while $L$ perhaps increases slightly ($2.2 \times 10^{-6}$ to $3.3 \times 10^{-6}$) but remains on the order of $10^{-6}$.

A more direct way to measure $V_{ho}$ for either channel was to measure gating charge movement exclusively when the channel is open. This, however, requires large prolonged depolarizations that we have found technically unfeasible. Another way we can estimate $V_{ho}$, however, and indeed also $L$ for the BK channel, is to simply fit each channel’s $P_{open}$-V relation with Eq. 9, while holding the parameters we have already determined constant. Since for the BK channel we have specified four of five parameters, and for the BK channel three of five, we expect such fits to be very well constrained.

To do this we combined our limiting $P_{open}$ data (Fig. 6) with macroscopic current data (Fig. 2 G) to obtain $P_{open}$-V curves that are well determined over large ranges of both voltage and $P_{open}$. Fig. 8 shows these curves fitted with Eq. 9. For the BK fit in A, $V_{ho}$, $z_f$, $L$, and $z_J$ were set to the following values: $z_J = 0.58, V_{ho} = 151$ mV, $z_f = 0.41, L = 2.2 \times 10^{-6}$, and $D$ was allowed to vary freely. This yielded $D = 16.8$, which indicates a $V_{ho}$ value of $27$ mV similar to a previous report (Horrigan and Aldrich, 1999) (Table II). Similarly, for the BK fit (Fig. 8 B, solid line), $V_{ho}$, $z_f$, and $z_J$ were set as follows: $z_f = 0.57, V_{ho} = 80$ mV, $z_J = 0.41, e$, and the fit yielded a $D$ value of 12.8 that equates to $V_{ho} = -34$ mV, and $L = 2.5 \times 10^{-6}$. Thus $B \bar{1}$ is estimated here to shift $V_{ho}$ leftward 61 mV (see simulations in Fig. 8 C) but it has almost no effect on $L$.

To this point then our conclusions are strikingly simple. The $B \bar{1}$ subunit has little or no effect on the equilibrium constant of the conformational change by which the BK channel opens, and it does not affect

**Figure 8.** Estimating $L$ and $V_{ho}$ from $P_{open}$-V curve fits. (A) The $P_{open}$-V relation of the BK channel at 3 nM Ca$^{2+}$ is shown over a wide range of voltages. At $+60$ mV and below, the data points are from unitary current measurements; above $+60$ mV, the data are from macroscopic current measurements. The curve displayed represents the average of 35 experiments, and error bars represent SEM. The data are fitted with Eq. 9 as described in RESULTS. Free parameters: $D = 16.8 \pm 0.28$. (B) The $P_{open}$-V relation of the BK channel. The curve displayed represents the average of 43 experiments, and error bars represent SEM. As in A, the data are fitted with Eq. 9. Free parameters: $D = 12.8 \pm 0.55, L = 2.53 \times 10^{-6} \pm 0.55 \times 10^{-6}$. The dashed red curve is the fit from A placed here for ease of comparison. (C) Simulated Q-V relations for the open BK and BK channels demonstrating the $-61$ mV shift induced by $B \bar{1}$ that the fits in A and B predict. The functions displayed are

$$
\frac{Q}{Q_{max}} = \frac{1}{1 + e^{V_{ho} - V_{hc}/RT}}
$$

with $V_{ho}$ equal to 27 mV (BK) and $-34$ mV (BK) and $z_f = 0.58$ (BK), $z_f = 0.57$ (BK).
the gating charge on the channel’s voltage sensors, but it does stabilize voltage sensor activation both when the channel is open and when it is closed such that voltage sensors activate at more negative voltages with β1 present. Furthermore, β1 more effectively stabilizes the active voltage sensor when the channel is closed, than when it is open. That is, \(|\Delta V_{bc}| > |\Delta V_{bol}|\), and this reduces D the factor by which voltage sensor activation promotes channel opening by 24%, from 16.8 to 12.8.

β1 Affects Ca\(^{2+}\) Binding

The question still remains, however, as to whether these changes are enough to explain the changes in Ca\(^{2+}\) sensitivity described in Figs. 1 and 2. To address this issue we have incorporated our findings into a model of BK\(_{Ca}\) channel gating that takes into account both Ca\(^{2+}\) and voltage sensing.

Our recent work suggests that the BK\(_{Ca}\) channel has two sets of four high-affinity Ca\(^{2+}\) binding sites, which are structurally distinct but have similar binding properties (Bao et al., 2002, 2004; Xia et al., 2002). Assuming one site does not affect another, and the channel’s voltage sensors and Ca\(^{2+}\) binding sites also act independently, this information can be combined with Scheme I to produce a model of BK\(_{Ca}\) channel gating that considers both Ca\(^{2+}\) binding and voltage sensing (Cox and Aldrich, 2000; Cui and Aldrich, 2000; Rothberg and Magleby, 2000; Zhang et al., 2001; Morrigan and Aldrich, 2002). The open probability of such a model is given by Eq. 13:

\[
P_{open} = \frac{1}{1 + e^{-\frac{\Delta F(V-V_{th})}{RT}}}
\]

where now, as compared with Eq. 9, two terms has been added to the denominator that each contain a set of Ca\(^{2+}\) dissociation constants, one set for each binding site (\(K_{C1}, K_{O1}; K_{C2}, K_{O2}\)). Here, Ca\(^{2+}\) binding promotes opening by binding more tightly to the open channel than the closed, and thus two constants are specified for each type of site. The c subscript denotes the closed channel and the o subscript, the open channel. Because, both types of binding sites appear to have similar binding properties, we may simplify Eq. 13 to Eq. 14:

\[
P_{open} = \frac{1}{1 + e^{-\frac{\Delta F(V-V_{th})}{RT}}}
\]

where now eight equivalent binding sites are considered with binding constants \(K_{C}\) and \(K_{O}\).

To see, then, if the changes in \(V_{th}\) and \(V_{ho}\) that we have observed upon β1 coexpression are sufficient to account for the BK\(_{Ca}\) channel’s enhanced Ca\(^{2+}\) response, for each channel type, we fitted a series of G–V relations with Eq. 14 (Fig. 9). For the BK\(_{Ca}\) fit (Fig. 9 A), \(V_{th}, V_{ho}, z_e, L\), and \(z_l\) were held at the values we determined from our experiments in the absence of Ca\(^{2+}\) (Table II), and only \(K_{C}\) and \(K_{O}\) were allowed to vary. Still a reasonably good fit was obtained that captured well the shifting nature of the BK\(_{Ca}\) channel’s G–V relation as a function of Ca\(^{2+}\) concentration. This fit yielded \(K_{C} = 3.71 \mu M\) and \(K_{O} = 0.88 \mu M\), similar to our previous estimates (Bao et al., 2002).

When the BK\(_{Ca}\) fit is placed on the BK\(_{Ca+β1}\) data, of course it does not fit at all well (Fig. 9 B). Next, however, we adjusted \(V_{th}\) and \(V_{ho}\) and \(L\) to mimic the effects of β1. \(V_{th}\) was moved from +151 to +80 mV, \(V_{ho}\) was moved from +27 to −34 mV, and \(L\) was increased slightly, from 2.2 × 10\(^{-6}\) to 2.5 × 10\(^{-6}\). The important result is shown in Fig. 9 C. The effects of β1 on voltage sensing do greatly improve the G–V fits at low Ca\(^{2+}\) concentrations, 1 and 0.003 \(\mu M\). But at higher concentrations, the fit is not good, and it gets progressively worse as the Ca\(^{2+}\) concentration is increased. High Ca\(^{2+}\) concentrations do not shift the model’s G–V relation far enough leftward. Thus, β1’s effects on voltage sensing appear important, but they are not sufficient to account for all of the BK\(_{Ca+β1}\) channel’s enhanced Ca\(^{2+}\) sensitivity.

To determine then what is required to fully account for the BK\(_{Ca+β1}\) G–V relation as a function of Ca\(^{2+}\), we fit the BK\(_{Ca+β1}\) G–V relations with Eq. 14, now fixing the voltage-sensing parameters to their BK\(_{Ca}\) values (\(V_{th} = 80 \text{ mV}\) and \(V_{ho} = −34 \text{ mV}\), L = 2.5 × 10\(^{-6}\), \(z_l\) = 0.41) and allowing \(K_{C}\) and \(K_{O}\) to vary. The resulting fit is shown in Fig. 9 D. To account for the additional leftward shifting \(K_{C}\) increases from 3.71 to 4.72 \(\mu M\), while \(K_{O}\) declines slightly from 0.88 to 0.82 \(\mu M\). This causes the ratio \(K_{C}/K_{O}\) to increase from 4.2 to 5.7.

Thus, our analysis suggests that β1 has a very small effect (<10%) or perhaps no affect on Ca\(^{2+}\) binding when the channel is open, consistent with our earlier study (Cox and Aldrich, 2000), and it reduces the affinity of each binding site for Ca\(^{2+}\) when the channel is closed, increasing \(K_{C}\) ~27%. It is perhaps surprising that such small changes in affinity can have such a dramatic effect on the position of the channel’s G–V relation at high Ca\(^{2+}\) concentrations, but in fact this should be expected, as at saturating Ca\(^{2+}\) the equilibrium constant between open and closed depends on the eighth power of \((K_{C}/K_{O})\).

Perhaps only Half of the Channel’s Binding Sites Are Affected by β1

The estimates of β1’s effects on \(K_{C}\) and \(K_{O}\) just offered suppose that β1 affects all binding sites equally. However, the BK\(_{Ca}\) channel is thought to have two types of
high-affinity binding sites (Schreiber and Salkoff, 1997; Bao et al., 2002; Xia et al., 2002), and it could reasonably be that β1 affects only one type of site. In this case, to account for the effects of β1, the ratio $K_C/K_D$ for the site that is affected would have to increase more. To determine how much more we fitted the BK channel’s G–V relation as a function of Ca$^{2+}$ concentration without supposing any -independent gating. Fortunately, the physical sense, the BKCa $\Delta_z$-binding sites to be modified by $\beta_1$ alters various parameters of the model were altered to reflect the changes that occur as $\beta_1$ binds, $V_{V_h}=(27\rightarrow-34 \text{ mV}), V_{V_c}=(151\rightarrow80 \text{ mV}), L= (2.2 \times 10^{-6}, V_{V_c} = 27 \text{ mV}, z_f = 0.58, z_r = 0.41).$ (B) The fit from $\lambda$ is superimposed on a series of BK$_{c+1}$ G–V curves. (C) The voltage-sensing parameters of the model were altered to influence only half of the channels’ eight Ca$^{2+}$-binding sites. The data are fit with Eq. 13. For the fit we again used the BK$_{c+1}$ voltage-sensing parameters, but now we set one set of Ca$^{2+}$-binding constants to the values we determined for the BK channel ($K_{C1} = 3.71 \mu M$ and $K_{D1} = 0.88 \mu M$), and we let the second set vary. This yielded a fit similar to the one that was obtained when all sites were assumed to be affected equally (see Fig. 11 D), but now, with only half the sites affected, $K_C$ increased to 5.78 $\mu M$ and $K_D$ decreased to 0.73 $\mu M$. Thus, $K_C$ increased an additional 22%, and now a larger change in $K_D$ was also produced (0.88 $\mu M \rightarrow 0.73 \mu M, 17\%$ decline). To determine, however, whether this change in $K_D$ was in fact needed, we reran the fit, now also holding $K_D$ to 0.88 $\mu M$. The result is shown in Fig. 9 F. A similar fit is obtained, now with $K_C$ increased to 7.14 $\mu M$.

Thus, whether we allow all eight or only four of the model channel’s Ca$^{2+}$-binding sites to be modified by $\beta_1$, we can account for the general effects of $\beta_1$ on the position of the BK$\Delta_z$ channel’s G–V relation as a function of Ca$^{2+}$ concentration without supposing any change in $K_D$ and whether $K_D$ does in fact decrease as $\beta_1$ binds, we are as yet uncertain. What is clear, however, is that in order to account for the G–V shift produced by $\beta_1$ at 100 $\mu M$ Ca$^{2+}$, the closed affinity of at least one type of high-affinity Ca$^{2+}$-binding site must be lowered such that $K_C$ increases between 27% and 92%.

**DISCUSSION**

Here we have addressed the question of how, in a biophysical sense, the BK$\Delta_z$ $\beta_1$ subunit enhances the BK$\Delta_z$ channel’s Ca$^{2+}$ sensitivity. Because previous work suggested that much of this phenomenon may be due to effects on aspects of gating that are separate from Ca$^{2+}$ binding (Cox and Aldrich, 2000; Nimigean and Magleby, 2000; Qian et al., 2002), we focused first on determining to what extent $\beta_1$ alters various parameters that govern Ca$^{2+}$-independent gating. Fortunately, the
gating of the BK$_a$ channel in the absence of Ca$^{2+}$ had been carefully examined, and Horrigan, Cui, and Aldrich developed a model that provides both a good mathematical description of the BK$_{Ca}$ channel’s response to changes in voltage and an intuitive understanding of the various parameters that govern this process (Horrigan and Aldrich, 1999, 2002; Horrigan et al., 1999).

The Horrigan, Cui, and Aldrich model may be summarized as follows. The BK$_a$ channel opens and closes via a single conformational change that is weakly voltage dependent. This central conformational change is indirectly, or allosterically, affected by four voltage sensors, each of which can be either active or inactive. When a voltage sensor becomes active, it promotes opening by lowering the energy of the open conformation of the channel relative to the closed. For this to occur each voltage sensor’s half-activation voltage must become more negative as the channel opens.

With this model as a guide, our task then became twofold, to determine if the Horrigan and Aldrich model still describes the gating of the BK$_{Ca}$ channel when the β1 subunit is part of the channel, and if so, to then determine how the five parameters, $V_{h0}$, $V_{ho}$, $z_Y$, $L$, and $z_L$ that govern the model’s behavior at equilibrium change upon β1 coexpression. Calculations indicate that changes in any or all of these parameters could lead to changes in the apparent affinity of the channel for Ca$^{2+}$ at many voltages (Cox and Aldrich, 2000). To determine, however, which were actually changing, following the lead of Horrigan and Aldrich (1999), we used both gating and ionic currents to constrain the parameters of the model so that each could be well determined. Our results may be summarized as follows.

With β1 coexpression, the Horrigan, Cui, and Aldrich model (Fig. 5, Scheme I) is still applicable. β1 does not slow voltage sensor movement, which remains fast relative to opening and closing. It has very little or no effect on the parameters that govern the central conformational change at equilibrium ($L$ and $z_L$), and it has no effect on the gating charge carried by each voltage sensor ($z_Y$). It does, however, have an important effect on the energy required for voltage sensor activation, both when the channel is open and when it is closed. That is, β1 affects $V_{ho}$ and $V_{h0}$ both of which are shifted tens of millivolts leftward on the voltage axis. Interestingly, however, β1 shifts $V_{ho}$ ~10 mV further than it shifts $V_{h0}$, and this reduces by 24% the factor $D$ by which voltage sensor movement influences opening. As will be discussed below, this reduction in $D$ has consequences for the shape and position of the BK$_{Ca}$ channel’s G–V relation in the absence of Ca$^{2+}$.

We have also found, however, that, although changes in $V_{ho}$ and $V_{h0}$ can account for much of the effects of β1 on Ca$^{2+}$ sensing at low Ca$^{2+}$ concentrations (1 μM and below), at higher Ca$^{2+}$ concentrations, some effect of β1 on Ca$^{2+}$ binding must also be supposed. In particular, our data suggest that β1 either has no effect or a very small effect on the affinity of the channel’s Ca$^{2+}$ binding sites when the channel is open (a decrease in $K_o$ from ~0.9 μM to no less than 0.7 μM), but it increases the Ca$^{2+}$ dissociation constant of the closed channel by ~27% (3.7 to 4.7 μM), if all eight binding sites are affected, or by ~57–92% (3.7 to 5.8 μM or 7.1 μM), if only four sites are affected. These numbers correspond to a change in the factor $C$ by which Ca$^{2+}$ binding influences opening by 57–93%. Thus, paradoxically, BK$_{a+β1}$ channels are more sensitive to Ca$^{2+}$ throughout the physiological voltage range, because they bind Ca$^{2+}$ worse when they are closed and because their voltage sensors activate at lower voltages.

β1’s Effects in Energetic Terms

To gain an intuitive understanding of these results as they relate to the BK$_{Ca}$ channel’s G–V and Ca$^{2+}$ dose-response curves (Fig. 2, A–G), we have found it useful to consider the influence of Ca$^{2+}$ and voltage on channel gating in energetic terms. For this purpose, we may write for the eight–Ca$^{2+}$ binding site four–voltage sensor model the following equation, which relates the free energy difference between open and closed ($ΔG_{O–C}$) to Ca$^{2+}$ concentration ($[Ca^{2+}]$) and membrane voltage.

$$ΔG_{O–C} = -8R\ln\left(\frac{1 + [Ca]/K_o}{1 + [Ca]/K_C}\right) - 4R\ln\left(\frac{1 + e^{-\frac{(V_{ho} - V_{h0})}{RT}}}{1 + e^{-\frac{(V_{ho} - V_{h0})}{RT}}}\right) - z_LFV - RT\ln[L].$$

Eq. 15 has four terms. Term 1 represents the change in $ΔG_{O–C}$ that occurs as Ca$^{2+}$ is varied ($ΔΔG_{O–C}$ Ca$^{2+}$), term 2 the change in $ΔG_{O–C}$ that occurs as voltage sensors become active ($ΔΔG_{O–C}$ VS), term 3 the influence of voltage on the central conformational change, and term 4 the intrinsic energy difference between open and closed when no Ca$^{2+}$ ions are bound and no voltage sensors are active (Cui and Aldrich, 2000).

In Fig. 10 A, the sum of terms 3 and 4 is plotted as a function of voltage, and, as is evident, the direct influence of the membrane’s electric field on the central conformational change contributes linearly to $ΔG_{O–C}$. Because β1 does not change $L$ appreciably, or $z_L$, the same curve describes this influence for both channels, and only one curve has been drawn.

In Fig. 10 B term 2 is plotted using the gating parameters ($V_{h0}$, $V_{ho}$, $z_Y$) we have determined for each channel type (BK$_a$ and BK$_{a+β1}$). Marked on the figure (numbered ticks) are the voltages at which there are an aver-
age of 1, 2, 3, or 4 active voltage sensors (although individual channels may have more or less). Here we see that voltage sensor movement influences opening at lower voltages, when \( /H_{9252} \) is present, but the maximum influence that voltage sensor movement can have on \( /H_{11004} \), and therefore opening, is 7.2% less. This is due to the smaller difference between \( V_{hc} \) and \( V_{ho} \) in the \( /H_{9251}/H_{11001}/H_{9252} \) channel, which leads to a reduction in \( D \). Indeed, in the high voltage limit, term 2 becomes simply

\[
\Delta \Delta G_{OC}(VS) = -4RT \ln \left( \frac{e^{FV_{hc}/RT}}{e^{FV_{ho}/RT}} \right) = -4RT \ln [D].
\]

Also worth noting, at the bottoms of these curves, where nearly all four voltage sensors are active, the relationship between a change in membrane voltage and a change in \( \Delta G_{OC} \) becomes quite shallow, while in the middle of these curves, it is much steeper.

Combining now terms 2, 3, and 4 yields the plot in Fig. 10 C, which describes the energetics of gating in the absence of \( Ca^{2+} \). Superimposed on the plot is a box that indicates the range of \( \Delta G_{OC} \) over which the channel’s open probability is between 5% (upper edge) and 95% (lower edge, for the complete relation; Fig. 10 D). Outside the box, changes in \( \Delta G_{OC} \) have little effect on \( P_{open} \), while the progress of each curve through the box describes channel activation with voltage. At the dashed centerline \( \Delta G_{OC} = 0 \) and thus \( P_{open} = 0.5 \), so the voltage at which each curve intersects this line indicates each channel’s \( V_{1/2} \).

\( \beta 1 \) and the G–V at 0 \( Ca^{2+} \)?

With these plots in mind, then, we may consider such questions as why is the \( /H_{9251}/H_{11001}/H_{9252} \) G–V relation at 0 \( Ca^{2+} \) less steep than that of the \( /H_{9251} \) channel? And what determines the size of the \( \beta 1 \)-induced G–V shift? And why does it increase as \( Ca^{2+} \) is raised?

Beginning with the first question, we see in Fig. 10 C that in the absence of \( Ca^{2+} \), the \( /H_{9251}/H_{11001}/H_{9252} \) curve (blue) traverses the gating box at a shallower angle than does the \( /H_{9251} \) curve (red). This makes the \( /H_{9251}/H_{11001}/H_{9252} \) Channel’s \( P_{open} \)–V relation shallower than that of the \( /H_{9251} \) channel, and it creates a crossover between the two channels’ \( \Delta G_{OC} \)–V and \( P_{open} \)–V curves (see inset). Thus, the models recapitulate the data. This type of plot, however, makes it readily apparent why. The \( /H_{9251}/H_{11001}/H_{9252} \) chan-
nel’s G–V relation is shallower primarily because β1 reduces $D$, and this brings the shallow part of the BK$_{\alpha+\beta1}$ channel’s $\Delta G_{O-C-V}$ relation (Fig. 10 B), now combined with $L(V)$ to yield its $\Delta G_{O-C-V}$ relation (Fig. 10 C), into the gating box, while in the BK$_\alpha$ channel the larger influence of voltage sensor movement on channel opening places less of this shallow region in the box. Or to put it another way, when β1 is present, the effect of voltage sensor movement on $\Delta G_{O-C}$ is reduced, and this causes the channel’s $P_{\text{open}}-V$ relation in the absence of Ca$^{2+}$ to reflect in larger part the shallow voltage dependence of the central conformational change. Thus, in the absence of Ca$^{2+}$, β1 reduces the BK$_{\alpha}$ channel’s $P_{\text{open}}-V$ slope (or equivalently its G–V slope) because it shifts $V_h$ further leftward than it shifts $V_v$. Indeed as shown in Fig. 10 E, if this were not the case, if β1 shifted both $V_h$ and $V_v$ equally such that $D$ was unchanged, no crossover would occur, and the two $P_{\text{open}}-V$ relations would have similar slopes with the BK$_{\alpha+\beta1}$ curve now standing to the left of the BK$_\alpha$ curve (see inset).

**Differences between Mouse and Bovine β1 at 0 Ca$^{2+}$**

The converse case it also of interest. Suppose β1 shifted $V_h$ less than we have found it does, say −47 mV rather than −61 mV, while still shifting $V_v$ −71 mV. In this instance (Fig. 10 F) $D$ would be lowered further by β1, and the crossover point would now occur outside the gating box, such that the progress of the hypothetical BK$_{\alpha+\beta1}$ channel’s $\Delta G_{O-C-V}$ curve through the box would be determined almost solely by $L(V)$. That is, in such an instance voltage sensor movement would no longer lower $\Delta G_{O-C}$ enough to be observed as a change in $P_{\text{open}}$, and the channel’s $\Delta G_{O-C-V}$ relation and its $P_{\text{open}}-V$ relation would reflect predominately $z_p$. This would cause a large rightward G–V shift upon β1 coexpression (Fig. 10 F, inset), which is interesting, because this is what we think occurs with mouse β1.

Here we have studied the effects of the bovine β1 subunit, which under most conditions are very similar to those of the mouse β1. In the absence of Ca$^{2+}$, however, the G–V curve of the BK$_{\alpha+\beta1\text{mouse}}$ channel is much further right shifted than that of the BK$_{\alpha+\beta1\text{bovine}}$ channel, and it has a much shallower slope (unpublished data). We suggest therefore that perhaps mouse β1 reduces $D$ more than does bovine β1, and this gives rise to their different effects at 0 Ca$^{2+}$. Indeed, the BK$_{\alpha}$ β4 subunit also produces a large rightward G–V shift in 0 Ca$^{2+}$, perhaps for the same reason (Ha et al., 2004).

More generally, however, our data suggest that in the absence of Ca$^{2+}$, the energy imparted to BK$_\alpha$ channel opening by its voltage sensors (when combined with the effect of voltage on the central conformational change) is just enough to influence opening, but small changes in either the amount of energy required to open the channel (changes in $L$, or the addition of Ca$^{2+}$), or how much energy each voltage sensor’s movement contributes to channel opening (changes in $D$), are expected to have a profound effect on the shape and position of the BK$_\alpha$ channel’s $P_{\text{open}}-V$ relation.

β1’s Effect on the BK$_\alpha$ G–V curve as a Function of Ca$^{2+}$

Looking again at Fig. 10 C, what is also evident is that if the two $\Delta G_{O-C-V}$ curves were shifted downward, steeper parts of each curve would traverse the gating box, and the BK$_{\alpha+\beta1}$ curve would lie significantly to the left of the BK$_\alpha$ curve. Indeed, this is what occurs with Ca$^{2+}$ binding. Term 1 of Eq. 15 is plotted in Fig. 11 A as a function of Ca$^{2+}$. Here we see that as the Ca$^{2+}$ concentration increases, Ca$^{2+}$ binding lowers $\Delta G_{O-C}$ for the BK$_\alpha$ channel ≈6.7 kcal/mol, similar to what voltage sensor movement does (Fig. 10 B), while, owing to its larger $K_c$ value, it lowers $\Delta G_{O-C}$ for the BK$_{\alpha+\beta1}$ ≈8.2 kcal/mol, 22% more. On a plot of $\Delta G_{O-C}$ vs. voltage, Ca$^{2+}$ binding is seen as a downward shift along the energy axis, with a larger shift for higher Ca$^{2+}$. 1 μM Ca$^{2+}$, for example, shifts both channel’s $\Delta G_{O-C-V}$ curves downward 2.4 to 2.8 kcal/mol, such that they each now pass through the gating box at a steeper angle (Fig. 11 C). Thus, for both channels, the increase in G–V steepness observed between 0 and 1 μM Ca$^{2+}$ (Fig. 2, C and D) is seen here to be due to Ca$^{2+}$ lowering the energy that the voltage sensors must supply to open the channel that brings a steeper region of the $\Delta G_{O-C-V}$ relation (Fig. 10 B) into the gating box. The BK$_{\alpha+\beta1}$ $\Delta G_{O-C-V}$ curve, however, now lies to the left of the BK$_\alpha$ curve, because the BK$_{\alpha+\beta1}$ channel’s voltage sensors are activated at lower voltages.

Notice also in Fig. 11 C that the BK$_{\alpha+\beta1}$ curve is shifted downward by Ca$^{2+}$ slightly more than is the BK$_\alpha$ curve, but at 1 μM Ca$^{2+}$ this effect is not of importance, and if this were not the case (dashed line) the observed G–V shift due to β1 coexpression would be very close to the same. At higher Ca$^{2+}$ concentrations, however, (Fig. 11, D and E), two things become evident. The downward shifting effect of Ca$^{2+}$ on both curves means that there will be a leftward G–V shift in response to increases in Ca$^{2+}$ for both channels, but now, as Ca$^{2+}$ is increased, the shift will become progressively larger for the BK$_{\alpha+\beta1}$ channel. This is due to the β1-induced increase in $K_c$. In fact, indicated with a dashed line in Fig. 11 (D and E) is the $\Delta G_{O-C-V}$ curve that would be observed for the BK$_{\alpha+\beta1}$ channel if β1 affected only voltage sensing. Thus, at 10 μM Ca$^{2+}$, there would still be a leftward G–V shift in response to β1 coexpression, but it would not be nearly as far. To highlight this point further, in Fig. 11 F we have plotted the β1-induced $V_{1/2}$ shift predicted by the BK$_\alpha$ and BK$_{\alpha+\beta1}$ model channels at 0, 1, 10, and 100 μM Ca$^{2+}$, and indicated are the percentages of each shift that is due to β1’s effects on voltage...
sensing (green) and Ca$^{2+}$ binding (yellow). Thus, according to our analysis, the leftward G–V shifts observed in Fig. 2 (D–G) are due to β1’s effects on both voltage sensing and Ca$^{2+}$ binding. At low Ca$^{2+}$ concentrations, effects on voltage sensing are of primary importance, but at higher Ca$^{2+}$ concentrations, the percentage of each G–V shift that is due to β1’s effects on Ca$^{2+}$ binding increases until, at 100 μM Ca$^{2+}$, it is just >50%.

β1’s Effects on BKCa Ca$^{2+}$ Dose–Response Curves
By picking a single voltage on these plots, we can also see why at negative voltages Ca$^{2+}$ more effectively opens the channel when β1 is present (as shown in Fig. 2, A–C). Examining −40 mV for example, the membrane potential of typical smooth muscle myocyte, 100 μM Ca$^{2+}$ shifts the BKCa ΔG0–C–V curve downward such that ΔG0–C (−40 mV) = 0.96 kcal/mol, which corresponds to a Popen of 0.164 (Fig. 11 E, gray line). With β1 present, the downward shift with 100 μM Ca$^{2+}$ makes ΔG0–C (−40 mV) = −1.34 kcal/mol, which corresponds to a Popen of 0.907. Thus, Ca$^{2+}$ more effectively opens the channel at low voltages because it shifts the BKCa–β1 channel’s ΔG0–C–V curve deeper into the gating box when β1 is present. Part of this is due to β1’s effects on voltage sensing, in this case 41%, as if β1 only affecting voltage sensing, Popen at −40 mV and 100 μM Ca$^{2+}$ would be 0.467, and part is due to its effects on Ca$^{2+}$ binding, here 59%. At higher voltages, however, +100 mV for example, 100 μM Ca$^{2+}$ is able to lower both ΔG0–C–V curves enough to fully activate both channels, and thus no difference in maximum Popen is expected. But because at all voltages on average fewer bound Ca$^{2+}$ are required to maximally activate the channel when β1 is present, at this voltage, as well as almost all others (between −200 and +200 mV), the β1 subunit is expected to increase the apparent affinity of the channel for Ca$^{2+}$.

Our Results Relative to Previous Work
Studies by Nimigean and Magleby (1999, 2000) at the single channel level have indicated that the β1 subunit shifts the BKCa channel’s Ca$^{2+}$ dose–response curve leftward at +30 mV from an apparent affinity of 9.2 to 2.6 μM (Nimigean and Magleby, 1999 [Fig. 2 A], 2000 [Fig. 2 B]). The models of the BKCa and BKCa–β1 channels we have produced predict almost an identical shift (9.3 to 2.3 μM). Furthermore, Nimigean and Magleby estimated that 80% of the shift was due to β1’s effects on aspects of gating separate from Ca$^{2+}$ binding, while 20% were due to a Ca$^{2+}$-dependent effect. Our models suggest a similar ratio (87% voltage sensing, 13% Ca$^{2+}$ binding). Also they found that at +30 mV, in the absence of Ca$^{2+}$, β1 increases Popen approximately seven-fold (Nimigean and Magleby, 2000). Our models predict a 12-fold increase. Thus, on the whole, our results
are remarkably consistent with those of Nimigean and Magleby. Furthermore, they found that $\beta_1$ increases $P_{\text{open}}$ by increasing the occupancy of bursting states, which, when interpreted in terms of our results, suggests that voltage sensor activation gives rise to longer bursts of openings.

In a previous study (Cox and Aldrich, 2000) we concluded, based on macroscopic ionic current recordings, that $\beta_1$ shifted $V_{h}$ and $V_{m}$ leftward, qualitatively, as we have observed, but as well probably increased $L$ from $3.4 \times 10^{-6}$ to $17.9 \times 10^{-6}$ and decreased $z_J$ from 0.51 to 0.4. Having now directly measured the movement of the channel’s gating charge, however, we no longer think that $z_J$ changes upon $\beta_1$ coexpression, and if there are changes in $L$ they are smaller than we first supposed. We also concluded in this study that $\beta_1$ reduces the affinity of the channel for Ca$^{2+}$ when it is closed, but has little or no effect on the affinity of the channel when it is open. As we have discussed, we still think this is correct.

Recently, Orio and Latorre (2005) concluded that the effects of $\beta_1$ on Ca$^{2+}$ sensing can be explained entirely by a reduction in $z_J$ from $\sim0.5e$ to $\sim0.3e$, with no effect on Ca$^{2+}$ binding and no change in $V_{h}$. Clearly, this idea is not consistent with our results, as with gating currents, we have directly determined the value of $z_J$ to be 0.57$e$ to 0.58$e$ and unchanged by $\beta_1$ coexpression, and we have found $V_{h}$ to be shifted 71 mV leftward by $\beta_1$. Why their conclusions differ from ours is not clear, and it could be due in part to species differences between $\beta_1$ subunits, as we have used bovine and they used human $\beta_1$. Perhaps more important, however, in the absence of gating currents they relied in their analysis on the notion that the peak of the BK$_\alpha$ channel’s $\tau_{\text{relaxation}}$-$V$ relation could not remain at or near the same voltage, if $V_{h}$ were changing appreciably. But, as can be seen by our fits in Fig. 7 C, this statement is not correct. While it is true that changes in $V_{h}$ will tend to alter the position of the peak of this curve, it is also true that changes in $V_{h}$ can, and apparently do, counteract this effect. Also, as evidence that $z_J$ is changing with $\beta_1$ coexpression, Orio and Latorre (2005) sited a change in the maximum slope of the channel’s $\ln(P_{\text{open}})$ vs. voltage relation upon $\beta_1$ coexpression; however, as can be seen in Fig. 8 B (compare solid line to dashed line), we do not observe a clear change in the maximum slope of this relation upon $\beta_1$ coexpression. Where our two studies agree, however, is in the finding, based in both studies on limiting-$P_{\text{open}}$ measurements, that the voltage dependence of the central conformational changed ($z_J$) is unaltered by $\beta_1$.

A Structural Hypothesis

While our data do not bear directly on the issue of what physical interactions between $\alpha$ and $\beta_1$ are required for $\beta_1$ to stabilize the activated voltage sensor, considerable evidence indicates that the extracellular loop common to all BK$_\alpha$ $\beta$s can extend over the $\alpha$ subunit as far as the channel’s pore. The extracellular loop of $\beta_3$ enters and blocks the pore in a voltage-dependent manner (Zeng et al., 2003), and the extracellular loop of $\beta_4$ prevents charybdotoxin, a pore-blocking peptide, from interacting with the channel (Meera et al., 2000). Thus, it seems reasonable to suppose that the extracellular loop of $\beta_1$ may interact with the external face of the $\alpha$ subunit as well. Indeed, perhaps it interacts with the voltage sensor at or near the top of S4. If this interaction were specific for the activated voltage sensor, than it would stabilize this position of the voltage sensor and create leftward Q–V shifts as we have observed. How much energy would it take to create such an effect? As it turns out, not very much. The 71-mV leftward $V_{h}$ shift we have observed with $\beta_1$ coexpression corresponds to a stabilization of the active voltage sensor by only 0.9 kcal/mol, the energy of a very weak hydrogen bond or a hydrophobic interaction. Thus from an energetic point of view this idea is plausible.

Of course this hypothesis doesn’t explain the effects of $\beta_1$ on Ca$^{2+}$ binding, and at this point we have none but the most general hypothesis to offer. As the physical nature of the BK$_\alpha$ channel’s Ca$^{2+}$ binding sites becomes more clear, however, perhaps how $\beta_1$ influences one or more of the channel’s Ca$^{2+}$-binding sites will become more clear as well.

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