Mechanism of αβ4 Subunit Modulation of BK Channels

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INTRODUCTION

BK channels are members of the voltage-dependent potassium channel family that are activated by membrane depolarization and cytoplasmic Ca$^{2+}$. These two stimuli appear to open the channel’s gate via allosteric mechanisms (Horrigan and Aldrich, 1999; Horrigan et al., 1999; Rothberg and Magleby, 1999, 2000; Horrigan and Aldrich, 2002; for review see Rothberg, 2004). Voltage sensor activation and Ca$^{2+}$ binding act independently, in an energetically additive fashion, to stabilize the channel’s open conformation; as [Ca$^{2+}$] is increased, less membrane depolarization is required to open the channel (Cox et al., 1997a; Cui et al., 1997). In different cell types, these channels display diverse functional properties, despite having a pore-forming subunit that is encoded by only a single gene (slo or KCNMA). Numerous mechanisms contribute to BK channel functional diversity, including alternative splicing, channel phosphorylation, and assembly with a family of four accessory subunits, β1–β4 (Stockand and Sansom, 1998; Xie and McCobb, 1998; Fettiplace and Fuchs, 1999; Schubert and Nelson, 2001; Fury et al., 2002; Orio et al., 2002). The accessory β4 subunit is a component of neuronal BK channels and confers properties mediating the so-called type II BK channels. These channels were originally identified in bilayer recordings from synaptosomal membrane preparations from brain (Reinhart et al., 1989, 1991; Reinhart and Levitan, 1995; Behrens et al., 2000; Brenner et al., 2000; Meera et al., 2000; Weiger et al., 2000; Lippiat et al., 2003). Type II BK channels have slow gating kinetics, decreased sensitivity to Ca$^{2+}$, and are insensitive to block by charybdotoxin, consistent with the properties of BK channels coexpressed with the β4 subunit in heterologous cells (Reinhart et al., 1989; Reinhart et al., 1991; Reinhart and Levitan, 1995; Behrens et al., 2000; Brenner et al., 2000; Meera et al., 2000; Weiger et al., 2000; Lippiat et al., 2003). β4 knockout mice display abnormal neuronal firing properties and temporal lobe seizures, indicating that the gating properties conferred by the β4 subunits are essential to normal neuronal function (Brenner et al., 2005).

More detailed analysis of BK channel β4 subunit has been performed by coexpression of the cloned channels in heterologous expression systems. The β4 subunit was proposed to be a “downregulator of BK channels” due to dramatic slowing of activation and a positive voltage...
shift of the conductance–voltage relationship (Weiger et al., 2000). However, although β4 reduces BK channels opening at low Ca\textsuperscript{2+}, it increases channel opening at high Ca\textsuperscript{2+} (Brenner et al., 2000; Ha et al., 2004). In addition, the β4 subunit reduces the voltage dependence (slope) of the macroscopic conductance–voltage (G-V) relationship. These properties are unique among the β subunit family members and the mechanisms underlying these effects are not known.

Here we provide a detailed analysis of the functional properties of BK α+β4 subunit channels. To understand how the β4 subunit modulates BK channels we have employed an allosteric modeling framework for BK channels (Horrigan and Aldrich, 2002), which enables us to ascribe kinetic changes to specific gating transitions. Our results demonstrate that β4 subunit effects can be accounted for by two opposing actions: β4 inhibits BK channel activation mainly by increasing the energetic barrier to opening by decreasing I_{o}. This effect is counteracted by a negative voltage shift in voltage sensor activation for channels in the open state (parameter V_{h}, in the model) and an increase in the allosteric coupling of Ca\textsuperscript{2+} binding to channel opening (parameter C in the model).

MATERIALS AND METHODS

Channel Expression

Experiments were performed with the mouse α subunit cDNA expression vector in pcDNA3 (GenBank/EMBL/DDB accession no. MMU09383), and mouse β4 in the In vitro vector pcDNA3.1Hygro(+). Expression constructs were transfected at a ratio of 1:10 α to β4 subunit using 2–3 μg total DNA and 10 μl lipofectamine reagent per 35 mm dish of HEK293 cells. After 5 h of incubation, the cells were replated on glass coverslips and analyzed by electrophysiology for the following 1–3 d. GFP expression from cotransfection (0.2 μg) of the CLONTECH Laboratories, Inc. EGFP-N1 vector was used to identify channel-expressing cells.

Patch Clamp Recording

Macropatch recordings were made using the excised inside-out patch clamp configuration. To limit series resistance errors, currents 5 nA or less were used for steady-state G-V. For 0 Ca\textsuperscript{2+} experiment determination of limiting P_{o}, larger currents were used but estimates of maximal conductance was determined at high Ca\textsuperscript{2+} and using small tail-current voltage steps. Experiments were performed at 22°C. Data were sampled at 10–30-μs intervals and low-pass filtered at 5.44 kHz using the HEKA EPC8 four-pole bessel filter. Data were analyzed without further filtering. Leak currents were subtracted after the test pulse using P/3 negative pulses from a holding potential of −120 mV. In the presence of β4 at ≥60 μM Ca\textsuperscript{2+}, leak subtraction was not performed. Patch pipettes (borosilicate glass WVR micropipettes) were coated with Sticky Wax (Kerr Corp.) and fire polished to ∼1.5–3 MΩ resistance.

The external recording solution (electrode solution) was composed of 20 mM HEPES, 140 mM KMeSO\textsubscript{4}, 2 mM KCl, 2 mM MgCl\textsubscript{2}, pH 7.2. Internal solutions were composed of a pH 7.2 solution of 20 mM HEPES, 140 mM KMeSO\textsubscript{4}, 2 mM KCl, and buffered with 5 mM HEDTA and CaCl\textsubscript{2} to the appropriate concentrations to give 1.7, 7, and 18.5 μM buffered Ca\textsuperscript{2+} solutions. Higher Ca\textsuperscript{2+} solutions were buffered with 5 mM NTA. Low Ca\textsuperscript{2+} solutions (0.3 μM and 0 Ca\textsuperscript{2+}) were buffered with 5 mM EGTA and Ba\textsuperscript{2+} was chelated with 40 μM (+)-18-crown-6-tetracarboxylic acid (Cox et al., 1997b). Conductance–voltage (G-V) relationships were obtained using a test pulse to positive potentials followed by a step to a negative voltage (−80 at low Ca\textsuperscript{2+}, −120 at high Ca\textsuperscript{2+}), and then measuring instantaneous tail current 200 μs after the test pulse. V_{1/2} and Q values were determined by fitting G-V curves to Boltzmann function (G = G_{max}/[1/(1 + e^{(V - V_{1/2})/Q})]) and then normalized to the maximum of the fit. At 0 and 0.3 μM Ca\textsuperscript{2+}, where maximum conductance could not be obtained in the presence of β4, conductance was normalized to maximal conductance at high Ca\textsuperscript{2+}.

RESULTS

Effects of β4 on Channel Steady-state G-V Relations and P_o

BK channel properties in the absence of the β4 subunit (α alone) or in the presence of saturating amounts of β4 (α+β4) were characterized in transiently transfected HEK293 cells. Currents were recorded in the inside-out configuration over a range of [Ca\textsuperscript{2+}] at the intracellular face of the membrane patch ([Ca\textsuperscript{2+}]i). Fig. 1 A shows representative current traces of α alone and α+β4 recorded at 7 μM [Ca\textsuperscript{2+}]i. In Fig. 1 B, mean steady-state conductance–voltage (G-V) relations are plotted as a function of [Ca\textsuperscript{2+}]i. To better quantify effects of β4 on channel steady-state gating properties, G-V curves of individual recordings were fit with single Boltzmann functions (see MATERIALS AND METHODS) to derive the voltage for half-maximal activation (V_{1/2}) and equivalent gating charge Q (slope of G-V relationship or “voltage dependence”). Mean V_{1/2} and Q values for α alone and α+β4 as a function of [Ca\textsuperscript{2+}]i, are listed in Table I and plotted in Fig. 1 (C and D), respectively. Our data, consistent with previous results obtained with heterologous expression in Xenopus oocytes, show that the β4 subunit affects the steady-state conductance–voltage relationship (Brenner et al., 2000; Ha et al., 2004). At [Ca\textsuperscript{2+}]i <18 μM, V_{1/2} is shifted toward more depolarized voltages in the presence of β4 (Fig. 1 C, inset). However, at [Ca\textsuperscript{2+}]i >18 μM, V_{1/2} is shifted toward more negative...
voltages in the presence of β4. In addition, we also saw that at all [Ca2+]i, β4 has a dramatic effect on the apparent voltage dependence of BK channels (Fig. 1 D). This is particularly prominent at [Ca2+]i < 1.7 μM and becomes incrementally more steep as [Ca2+]i is increased. The relative change in the V1/2 by β4 is between −26 and +90 mV, depending on the calcium concentration (Fig. 1 C, inset). However, the V1/2 may not reflect the relative changes in P0 at physiological voltages. To determine effects on open probability, we measured channel P0 in 300 nM [Ca2+] at the physiological voltages of −100 to +20 mV. The results indicate that at negative voltages, α+β4 channels show a large reduction in P0 as compared with α subunits alone (Fig. 1 E). For example, at −80 mV α+β4 channels have a 4.3-fold reduction in P0 relative to α subunits alone (average P0 α+β4 is 3.5 e−7 ± 1.9 e−7, α alone is 1.5 e−6 ± 3.3 e−7). However, at +20 mV, there is no significant difference in P0 between channel types (Fig. 1 E).

Effects of β4 on Gating through the Low Affinity Ca2+ Binding Site
In considering the calcium-dependent effect of β4 on the V1/2, it is important to note that BK channels are modulated over a wide range of Ca2+, from the submicromolar range up to ~1 mM. The fact that Ca2+ does not appear to saturate channel activation (in the absence of β4) is attributed to the existence of both high and low affinity Ca2+ binding sites (Zhang et al., 2001; Shi et al., 2002). This is apparent in Fig. 1 C (open symbols) as increasing Ca2+ shifts V1/2 strongly at [Ca2+]i < 60 μM, consistent with activation at a high affinity site,

![Figure 1](image_url)

The effects of β4 on the m spatial steady-state G-V relation vary with [Ca2+]. (A) Examples of currents evoked by voltage steps in 7 μM [Ca2+]. α alone is shown in the left panel, α+β4 is shown in the right panel. (B) Mean G-V relations at different [Ca2+] for α alone and α+β4. Each point represents mean data from 8 to 44 experiments. Solid curves represent fits to the Boltzmann function. (C) Mean V1/2 values plotted as a function of [Ca2+]. β4 shifts V1/2 toward more positive voltages <18.5 μM [Ca2+], and toward more negative voltages >18.5 μM [Ca2+]. Inset, α+β4 V1/2 subtracted from mean α alone values. (D) Mean effective gating charge, Q, plotted as a function of [Ca2+]. In the presence of β4 there is a decrease in Q, and a more dramatic increase in Q can be observed as [Ca2+] increases. (E) Mean P0, as a function of voltage in the absence (5–7 patches) and presence (2–6 patches) of β4 in 300 nM Ca2+. Error bars represent SEM.
then shifts the $V_{1/2}$ weakly at $[Ca^{2+}] > 100 \, \mu M$, consistent with activation at a low affinity site.

The $\beta 4$ subunit promotes a negative voltage shift of the G-V curve only at high $[Ca^{2+}]$ (Fig. 1 C, inset). A possible explanation for this is that $\beta 4$ may specifically affect $Ca^{2+}$ binding to low affinity sites to promote activation at high calcium; either its affinity or coupling between $Ca^{2+}$ binding and gating. We examined whether the $\beta 4$ subunits promote activation through low affinity $Ca^{2+}$ binding sites by measuring activation by millimolar concentrations of $Mg^{2+}$, which acts only at low affinity sites (Shi et al., 2002). If the leftward shift in $V_{1/2}$ induced by the $\beta 4$ subunits is due to increased activation at the low affinity site, then addition of $Mg^{2+}$ should produce a larger leftward shift in the presence of $\beta 4$ compared with $\alpha$ alone channels.

Steady-state G-V relations with and without 10 mM $Mg^{2+}$ were obtained in the presence and absence of $\beta 4$. Fig. 2 shows currents activated in 60 $\mu M$ $Ca^{2+}$, (Fig. 2 A, left, and Fig. 2 B, open symbols) and then channels are further activated through low affinity sites with 10 mM $Mg^{2+}$ (Fig. 2 A, right, and Fig. 2 B, closed symbols). $Mg^{2+}$ actually yielded a smaller G-V shift for $\alpha+\beta 4$ channels (by $-69$ mV) compared with $\alpha$ alone (by $-96$ mV), suggesting that $\beta 4$ does not increase gating through $Ca^{2+}$ binding at the low affinity site. A flaw of this interpretation is that inferring effects of $Ca^{2+}$ binding to the low affinity sites using 10 mM $Mg^{2+}$ may be inappropriate in the presence of $Ca^{2+}$. At 60 $\mu M$ $Ca^{2+}$, it is possible that 10 mM $Mg^{2+}$ can compete with $Ca^{2+}$ for the low affinity site and therefore confer some inhibitory effects on gating. To rule out such possibility, effect of $\beta 4$ on G-V shift by 10 mM $Mg^{2+}$ was also examined at 0 $Ca^{2+}$. Again, 10 mM $Mg^{2+}$ produced a smaller shift in $V_{1/2}$ for $\alpha+\beta 4$ channels compared with $\alpha$ alone ($-64$ vs. $-96$ mV, respectively; Fig. 2 C). Together, these results suggest that activation at high $Ca^{2+}$ cannot be explained by changes involving the low-affinity $Ca^{2+}$ binding site.

Understanding Effects of $\beta 4$ on Channel Gating in the Context of an Allosteric Model

BK channel gating can be understood in terms of a dual allosteric model in which activation of a voltage sensor and a $Ca^{2+}$ sensor each facilitate opening of the channel (Scheme 1) (Rothberg and Magleby, 1999; Horrigan and Aldrich, 2002).

TABLE I
Comparing G-V Parameters

| $[Ca^{2+}]$ | $V_{1/2}$ | $Q$ | N | $V_{1/2}$ | $Q$ | N |
|---------|---------|-----|---|---------|-----|---|
| $\mu M$ | mV     | $\mu M$ | mV     | $\mu M$ | mV     | $\mu M$ |
| 0.0005  | 177.6 ± 4.0 | 1.29 ± 0.06 | 12 | 269.1 ± 11.9 | 0.66 ± 0.04 | 17 |
| 0.3     | 128.7 ± 1.9 | 1.58 ± 0.04 | 20 | 181.8 ± 17.7 | 0.85 ± 0.09 | 10 |
| 1.7     | 80.9 ± 1.6 | 1.76 ± 0.05 | 24 | 97.0 ± 2.5 | 1.36 ± 0.05 | 14 |
| 7       | 33.1 ± 1.7 | 1.68 ± 0.06 | 13 | 32.8 ± 2.6 | 1.57 ± 0.06 | 24 |
| 18.5    | -0.6 ± 1.5 | 1.86 ± 0.03 | 44 | -2.0 ± 1.6 | 1.62 ± 0.04 | 26 |
| 60      | -10.6 ± 3.1 | 1.76 ± 0.07 | 8 | -36.5 ± 2.4 | 1.73 ± 0.06 | 17 |
| 100     | -210.1 ± 1.8 | 1.91 ± 0.05 | 30 | -38.9 ± 2.4 | 1.67 ± 0.06 | 26 |
| 300     | -29.6 ± 3.4 | 2.09 ± 0.16 | 14 | -40.4 ± 3.2 | 1.77 ± 0.13 | 13 |
| 1000    | -43.7 ± 2.1 | 2.13 ± 0.17 | 12 | -57.4 ± 3.6 | 1.62 ± 0.15 | 12 |

The values shown are Boltzmann-fit parameters. They indicate mean ± SEM.

TABLE II
70-state Model Gating Parameters (Horrigan and Aldrich, 2002)

| Parameter | Value |
|-----------|-------|
| L         | $L_0\times exp\left(\frac{\alpha_4 V}{kT}\right)$ |
| $\alpha_4$ | $\alpha_4 = \frac{[Ca^{2+}]}{K_c}$ |
| $J_{\alpha}$ | $J_{\alpha} = \frac{[Ca^{2+}]}{K_c}$ |
| $K_c$ | $K_c$ calcium dissociation constant for $Ca^{2+}$ binding |
| $C$ | $C_0$ calcium dissociation constant for $Ca^{2+}$ binding |
| $D$ | $D_0$ calcium dissociation constant for $Ca^{2+}$ binding |
| $E$ | $E_0$ calcium dissociation constant for $Ca^{2+}$ binding |

SCHEME 1

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In this model, channel opening is governed by three equilibrium constants, L (closed-to-open transition), J (voltage sensor activation), K (Ca\(^{2+}\) binding), and three allosteric factors D (coupling of voltage sensor movement to channel opening), C (coupling of Ca\(^{2+}\) binding to opening), and E (interaction between Ca\(^{2+}\) binding and voltage sensor movement). Detailed descriptions of these parameters are listed in Table II. Open probability can be described by Eq. 1:

\[
P_o = \frac{1}{1 + \frac{1}{L(1 + K + J + KJ)}(1 + K + J + KJ)}.
\]  

(1)

To understand the mechanism underlying \(\beta 4\) modulation of the channel, we examined how individual gating parameters are altered by \(\beta 4\). Our experimental approach was to measure \(P_o\) under conditions that reduce the number of occupied states and thereby constrain a number of gating parameters. For example, to observe effects on voltage dependence, we measured \(P_o-N\) relations in the virtual absence of calcium. To observe effects of \(\beta 4\) on calcium dependence, we measured \(P_o-V\) relations at very negative voltages where voltage sensors are in the resting state. These experiments are presented below. The remaining gating parameters were then estimated by fitting all \(P_o[V, Ca^{2+}]\) data using Eq. 1.

The \(\beta 4\) Subunit Shifts Voltage Sensor Activation to Negative Voltages and Increases the Intrinsic Energetic Barrier for Channel Opening

Our first aim was to measure \(\beta 4\) effects on the closed-to-open equilibrium and its voltage dependence (\(L_o\) and \(z_L\),

\[2\]
respectively). Based on the dual allosteric model (Horrigan and Aldrich, 2002) this can be accomplished by measuring \( P_o \) versus \( V \) relations in the virtual absence of \( Ca^{2+} \) (Horrigan and Aldrich, 1999). This effectively reduces the number of occupied states to 10 (Fig. 3 A, Sub-Scheme 1a) and \( P_o \) is determined by the equilibrium of intrinsic gating, \( L \) (where \( L = L_0 \exp(z_L V/kT) \)), voltage sensor activation, \( J \), and the allosteric interaction between them, \( D \) (Horrigan et al., 2002):

\[
P_o = \frac{1}{1 + ((1 + J)^4/L(1 + D)^4)}. \tag{2}
\]

A simulated \( P_o \) versus \( V \) relation predicted by Eq. 2 is shown in Fig. 3 A (right). At very negative membrane potentials, the slope of the \( P_o \) versus \( V \) relation is very shallow (limiting slope), reflecting the weak voltage dependence \( z_L \) of the closed-to-open transition independent of voltage sensor movement. This is followed by a steeper voltage dependence at high voltages where voltage sensors start to activate and contribute to voltage sensor–dependent channel opening. By confining our analysis to very negative voltages, channel occupancy can be further reduced to two states, \( C_o \) and \( O_o \) (Fig. 3 A, Sub-Scheme 1b). Because \( J \) is small \( (J \ll 1/D) \), Eq. 1 reduces to

\[
P_o = \frac{L}{1 + L}. \tag{3}
\]

Where \( P_o \) is also small \( (P_o < 0.01) \), \( L \ll 1 \),

\[
P_o = L = L_0 \exp(z_L V/kT). \tag{4}
\]

Thus parameters describing channel intrinsic gating properties \( (L_0 \) and \( z_L \)) can be estimated by fitting \( \log P_o \) versus \( V \) relation at very negative potentials using Eq. 4. The slope of the fit is a function of voltage dependence of the closed-to-open transition \( (z_L) \), and the 0 mV value of the fit is a measure of the closed-to-open equilibrium of intrinsic channel gating \( (L_0) \).

To evaluate effects of \( \beta 4 \) on channel intrinsic gating parameters \( (i.e., \) independent of \( Ca^{2+} \) or voltage sensor activation) using Eq. 4, BK currents were measured in 0 \( Ca^{2+} \) at negative membrane potentials. Because channel open probabilities are very low under these conditions, recordings were obtained from patches containing hundreds of channels and analyzed using single-channel analysis techniques to estimate \( P_o \) (the number of channels in each patch was estimated by measuring the maximal current amplitude at higher \( Ca^{2+} \) and dividing by the unitary current amplitude, see MATERIALS AND METHODS). Examples of recordings in the absence and presence of \( \beta 4 \) are displayed in Fig. 3 B.

In the presence of \( \beta 4 \), channel openings are less frequent but display longer durations, consistent with previous observations (Ha et al., 2004). Mean \( \log P_o \) versus \( V \) relations (between \(-80 \) and \(+100 \) mV) are displayed in Fig. 3 C. These data demonstrate effects of \( \beta 4 \) on two aspects of channel gating. First, although the slope of \( \log P_o \) versus \( V \) relation shows a clear decrease for the \( \alpha \) alone channels at around \(+30 \) mV, there is little change in slope for the \( \alpha + \beta 4 \) channels over this voltage range (+70 through \(-80 \) mV). Possible explanations are either that (a) for \( \alpha + \beta 4 \) channels, \( z_L \) is increased, and thus comparable to the voltage dependence of opening at higher voltages (which involves voltage sensor activation), or (b) \( \beta 4 \) does not effect \( z_L \), but we could not estimate \( z_L \) because voltage sensor activation occurs at voltages more negative than for \( \alpha \) alone channels. It is difficult to propose a plausible physical mechanism that could account for an increase in \( z_L \) that would not also dramatically alter voltage dependence of \( P_o \) at high voltages. Therefore, we hypothesize that \( \beta 4 \) shifts activation voltage for open-channel voltage sensors \( (V_{ho}) \) to membrane potentials more negative than \(-80 \) mV. Direct measurements of \( z_L \) below \(-80 \) mV at 0 \( Ca^{2+} \) was not feasible because channel openings fell below our level of detection. However, this hypothesis is supported by measurement of \( z_L \) at high \( Ca^{2+} \), as discussed below.

Our data also suggest that \( \beta 4 \) decreases the channel’s intrinsic equilibrium for opening \( (L_0) \). \( L_0 \) value for \( \alpha \) alone channels was estimated by fitting \( \log P_o \) versus \( V \) relations between \(-100 \) and \(-70 \) mV (limiting slope) using Eq. 4 and the estimated \( z_L \) value of 0.3 \( e^0 \). \( L_0 \) for the \( \alpha \) alone channel is estimated to be \( 1.6 \times 10^{-6} \) (Fig. 3 D). In the presence of \( \beta 4 \), we did not reach the membrane potential where contribution of voltage sensors can be ignored. Measuring \( P_o \) below \(-80 \) mV is technically not feasible because channel openings for \( \alpha + \beta 4 \) are too few to get estimates of \( P_o \) (\( P_o < 1 \times 10^{-8} \)). Therefore, we estimated an upper limit for the closed-to-open equilibrium \( (L_0) \) using the mean \( P_o \) value at \(-80 \) mV and \( z_L \) value of 0.3 \( e^0 \) (Fig. 3 D). The estimated upper limit for \( L_0 \) in the presence of \( \beta 4 \) is \( 1.4 \times 10^{-7} \), suggesting that \( \beta 4 \) decreases the equilibrium constant of the closed-to-open transition by at least 11-fold.

**Voltage Dependence of the Closed–Open Transition (\( z_L \)) Is Not Altered by \( \beta 4 \)**

We were able to evaluate effects of \( \beta 4 \) on \( z_L \), the gating parameter that describes the voltage dependence of the closed-to-open transition, in the presence of \( Ca^{2+} \). \( Ca^{2+} \) increases the \( P_o \) even in the absence of voltage sensor activation, which makes it feasible to obtain \( P_o \) versus \( V \) relations at the limiting slope and directly estimate \( z_L \). Based on the dual-allosteric model (Horrigan and Aldrich, 2002), \( Ca^{2+} \) should not change the voltage dependence of the closed-to-open transition. As illustrated in Fig. 4 A (Sub-Scheme 1c), at very low voltages, where voltage sensors remain in resting states, the number of occupied state reduces to 10 and \( P_o \) is described as
When $P_o$ is small ($P_o < 0.01$), $L_o = \frac{(1 + KC)^4}{L(1 + KC)^4 + (1 + K)^4} = L_o \frac{1 + KC}{1 + K}$. Eq. 5 reduces to

$$P_o = \frac{L_o(1 + KC)^4}{L(1 + KC)^4 + (1 + K)^4} = \frac{L_o(1 + KC)^4}{L_o(1 + K)} \exp\left(\frac{z_L V}{kT}\right).$$

$P_o$-V relations predicted by the gating scheme are illustrated by simulated $P_o$-V relations at 0 and 100 $\mu$M $[\text{Ca}^{2+}]$ (Fig. 4 A, right). By using Eq. 6 to fit $P_o$-V at very negative membrane potentials in the presence of $[\text{Ca}^{2+}]$ (Fig. 4 A), $z_L$ can be obtained since the higher $P_o$ makes measurements more feasible.

To estimate $z_L$ in the absence and presence of $\beta_4$, we measured $P_o$ in the presence of 0–100 $\mu$M $[\text{Ca}^{2+}]$ at decreasing membrane potentials. Examples of recordings at 100 $\mu$M $\text{Ca}^{2+}$ at very negative membrane potentials are shown in Fig. 4 B. In a portion of recordings, $\log P_o$-V relation appeared to have reached the “limiting slope”. Fig. 4 C illustrates how $z_L$ was estimated using such recordings. The regions of the log($P_o$)-V relations where $P_o$ is small ($P_o < 0.01$), $L_o = \frac{(1 + KC)^4}{L(1 + KC)^4 + (1 + K)^4} = L_o \frac{1 + KC}{1 + K}$. Eq. 5 reduces to

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voltage dependence of \( P_o \) is clearly reduced were fit with Eq. 6 to estimate \( z_L \). Mean \( z_L \) values for \( \alpha \) subunit alone at different \([Ca^{2+}]\) are summarized in Fig. 4 D. Consistent with the dual allosteric model (Horrigan and Aldrich, 2002), \( z_L \) value estimated at various \([Ca^{2+}]\) appear to be similar. The mean of all estimates of \( z_L \) for the \( \alpha \) alone channels was \( 0.50 \pm 0.02 \, e_0 \) \((n = 26)\). The limiting slope was reached in a much smaller portion of \( \alpha + \beta_4 \) recordings, especially at low \([Ca^{2+}]\) (Fig. 4 D). Estimates of \( z_L \) at 18.5 and 100 \( \mu M \) Ca appeared to be better constrained than at lower \([Ca^{2+}]\). The mean \( z_L \) for \( \alpha + \beta_4 \) channels was \( 0.31 \pm 0.03 \, e_0 \) \((n = 21)\). When only the best constrained data (18.5 and 100 \( \mu M \) Ca were included, the mean \( z_L \) was \( 0.29 \pm 0.05 \, e \) \((n = 10)\). In conclusion, \( \beta_4 \) does not appear to alter \( z_L \), voltage dependence associated with channel’s closed-to-open transition.

**Effect on \([Ca^{2+}]\) Sensitivity**

\( P_o-V \) relations at the limiting slope in the presence of \([Ca^{2+}]\) can be used to assess effects of \( \beta_4 \) on \([Ca^{2+}]-\)dependent gating (Horrigan and Aldrich, 2002). As discussed above, when \( P_o \) is low \((<0.01)\), Eq. 5 reduces to Eq. 6. We can define \( L_0’ \) as the closed-to-open equilibrium with the allosteric contribution of calcium binding (in the absence of voltage sensor activation):

\[
L_0’ = L_0 \left( \frac{1 + KC}{1 + K} \right)^4.
\]

Then \( P_o \) changes in the absence of voltage sensor activation becomes

\[
P_o = (L_0 \left( \frac{1 + KC}{1 + K} \right)^4 \exp(z_{V-L}V/kT)) = L_0’ \exp(z_{V-L}V/kT).
\]

In the presence of \([Ca^{2+}]\), \( P_o-V \) measured at limiting slope can be fitted by Eq. 8 to estimate \( L_0’ \). This is similar to the approach for evaluating \( L_0 \) (Fig. 3 D). \( L_0’-[Ca^{2+}] \) relations can then be fitted by Eq. 7 to estimate \( \beta_4 \) effects on calcium-dependent parameters, \( K_c \) and \( C \).

Mean \( \log P_o-V \) relations for the \( \alpha \) alone and \( \alpha + \beta_4 \) channels are presented in Fig. 5 A. For the \( \alpha \) alone channels, the limiting slopes of the \( \log P_o-V \) relations were reached and fitted with Eq. 8 to estimate \( L_0’ \). Log \( L_0’ \) are plotted as a function of \([Ca^{2+}]\) (Fig. 5 B). Fitting \( \log(L_0’-) \) (from mean data) vs. \([Ca^{2+}]\) with Eq. 7 yielded \( L_0 = 1.7 \times 10^{-6} \pm 5 \times 10^{-7} \), \( K_c = 13 \pm 3 \, \mu M \) and \( C = 10 \pm 1 \). These values are similar to previous estimates of the \( \alpha \) alone channels based on the dual-allosteric model (\( L_0 = 9.8 \times 10^{-7} \), \( K_c = 11 \, \mu M \), and \( C = 8 \); Horrigan and Aldrich, 2002).

For the \( \alpha + \beta_4 \) channels, we again observe a negative shift in the voltage sensor activation (Vh\(_c\)). However, the limiting slope of \( \log P_o-V \) relations were reached in a small percentage of recordings that allow estimation of \( z_L \) (Fig. 4 C), the limiting slope of \( \log P_o-V \) relation was not reached in the majority of the patches (Fig. 4 D and Fig. 5 A). This is reflected in the \( \log P_o-V \) plot in Fig. 5 A (right) where only the foot of the data points show a reduction in voltage dependence. Although \( L_0’ \) could not be obtained directly, nevertheless high limits for \( L_0’ \) at different \([Ca^{2+}]\) were determined by mean \( P_o \) value measured at the lowest membrane potentials using Eq. 8 and the mean \( z_L \) value of 0.3 \( e_0 \). The results show a plot that can be regarded as upper limits of \( L_0’ \) for the \( \alpha + \beta_4 \) channels (Fig. 5 B). Interestingly, \( L_0’ \) values for \( \alpha + \beta_4 \) were smaller than \( L_0’ \) for the \( \alpha \) alone channels, at all \([Ca^{2+}]\). This is an important finding in light of the fact that, at high \([Ca^{2+}]\), \( \beta_4 \) causes an increase in \( P_o \) at higher voltages (negative shift of V\(_{1/2}\) at high calcium, Fig. 1 C). These findings suggest that \( Ca^{2+} \) binding (through high or low affinity sites) alone is insufficient to cause the negative G-V shift conferred by \( \beta_4 \) in high \([Ca^{2+}]\). By default, aspects of \( \beta_4 \) modulation of voltage sensor activation must contribute to the leftward G-V shift at high \([Ca^{2+}]\).

**Effects of \( \beta_4 \) in the Context of an Allosteric Model**

The above analysis directly examined effects of \( \beta_4 \) on several aspects of BK channel gating. Our analysis of open probability at limiting slope suggests that \( \beta_4 \) increases the energetic barrier for channel opening, and causes a negative shift in the activation of voltage sensors for open channels. To understand these effects in a comprehensive framework, and whether other aspects of gating are affected by \( \beta_4 \), families of G-V curves as well \( P_o-V \) relations obtained at low voltages were fit with the dual allosteric model (Scheme 1; Horrigan and Aldrich, 2002).

There are seven free parameters in the allosteric model (Table II). For \( \alpha \) alone channels, four of these parameters were constrained based on analysis of our experimental data. These parameters (and range of values imposed) were \( z_L \) (0.3 \( e_0 \), \( L_0 \) (1.7 \( \times 10^{-6} \)), \( K_c \) (13 \( \mu M \)), and \( K_o \) (1.3 \( \mu M \)). The remaining parameters (\( z_L \), \( V_h \), \( V_h' \), and \( E \)) were allowed to vary freely. Although a range of parameters produce satisfactory fits for the G-V curves, we found only one set of parameters that could also reproduce \( P_o-V \) data measured at very negative voltages. These are shown in Table III. Simulated \( P_o \) with parameters in Table III reproduces reasonably well the \( \alpha \) alone G-V curves over a wide range of \([Ca^{2+}]\) (from nominally 0 through 100 \( \mu M \); Fig. 6 A) as well as \( P_o \) at low voltages (Fig. 6 B), the V\(_{1/2} \) vs. \([Ca^{2+}] \) relation (Fig. 6 G), the Q vs. \([Ca^{2+}] \) relation (Fig. 6 H), and the \([Ca^{2+}]-\)dependent shift in \( P_o \) in the absence of voltage sensor activation (Fig. 6 I). These parameters are similar to previously reported for the \( \alpha \) alone channels (Horrigan and Aldrich, 2002).

To estimate gating parameters in the presence of \( \beta_4 \), we fit G-V data and low voltage \( P_o-V \) relations from \( \alpha + \beta_4 \) channels to the dual allosteric model (Horrigan and Aldrich, 2002). Based on our analysis of \( \alpha + \beta_4 \) channel gating at low \( P_o \), \( z_L \) was constrained to be 0.3 \( e_0 \).
Consistent with the experimental measurements in 0 calcium, the best fit (α+β4, Table III) suggests that major effects of β4 include decrease of L0 (46 fold) and a −75 mV shift of voltage sensor activation (Vh₀) relative to α subunits alone. In addition, the closed channel voltage sensor equilibrium (Vh₀) is shifted to a similar extent (−77 mV), resulting in a relatively small change in voltage-dependent allosteric coupling (D). The fit indicates that there is a threefold decrease in Ca²⁺ binding affinity in the closed channel (Kc) with a smaller reduction in the open channel, resulting in an increase in calcium-dependent allosteric coupling (C). Finally, there is a small decrease in the direct allosteric coupling between Ca²⁺ binding and voltage sensor activation (E).

Figure 4. β4 effects on z₁ in the presence of Ca²⁺. (A, left) According to the dual-allosteric mechanism (Horrigan and Aldrich, 2002), BK channel transitions between closed (C) and open (O) conformation is allosterically regulated by the state of four independent and identical Ca²⁺ binding sites. Sub-Scheme 1c represents BK channel’s gating scheme at very negative voltages, where voltage sensors remain in the resting states. Channel resides in either closed or open conformations, with 0–4 Ca²⁺ binding sites occupied. Equilibrium between the C–O transitions is allosterically regulated by the states of the Ca²⁺ binding sites. In the absence of voltage sensor activation, voltage dependence of the C–O transition is entirely dependent on z₁. (A, right) Illustration of how z₁ can be estimated by logPₒ-V data at high Ca²⁺ and very negative voltages. Curves are simulated logPₒ-V curves in nominally 0 Ca²⁺ and 100 μM Ca²⁺ according to Scheme 1c. Gating parameters used for simulation are as follows: L₀ = 2.5 × 10⁻⁶, z₁ = 0.39 e₀, z₂ = 0.54 e₀, Vh₀ = 173 mV, Vhₙ = 25 mV, Kₐ = 13.9 μM, and Kₐ = 1.4 μM. Dashed lines represent fits for logPₒ-V at limiting slopes using Eq. 8. L₀ and z₁ can be derived from the fits. (B) At 100 μM Ca²⁺, currents were recorded at very negative voltages to determine logPₒ-V relations. Currents were low-pass filtered at 20 kHz. Representative current traces at indicated voltages in the absence and presence of β4, respectively. Traces in B were all obtained from the same patch. Currents were filtered at 5 kHz for display purposes. (C) Representative logPₒ-V relations at various Ca²⁺ where limiting slopes is reached. Upper limits for z₁ were estimated from the apparent limiting slopes (solid lines). (D) Estimates of z₁ plotted as a function of [Ca²⁺] for α subunits alone (open symbols) and α+β4 (closed symbols) for patches where limiting slope was reached. Error bars represent SEM. The number of patches where limiting slope was reached as well as total number of recordings performed (in parenthesis) at each [Ca²⁺] are indicated.
to 0.3 \text{e}_0. Although the best fit using the macroscopic G-V data alone (\(\alpha+\beta_4_b\), Table III) predicts the macroscopic G-V data quite well (Fig. 6, E, G, and H), the parameters poorly predict \(P_0-V\) relations at negative voltage and low calcium (Fig. 6 F), and the predicted log\(L_0'\) values are larger than the measured upper limits in 0 calcium (Fig. 6 I).

The Effects of \(\beta_4\) on \(L_0\) and \(V_{ho}\) Are Robust

Our best fit of the \(\alpha+\beta_4\) data, \(\alpha+\beta_4_a\), indicates that the major effects of \(\beta_4\) are a decrease in \(L_0\) and negative voltage shifts of voltage sensor equilibrium (\(V_{ho}\)). To examine whether the kinetic parameters in \(\alpha+\beta_4_a\) are robust, we fixed \(L_0\) or \(V_{ho}\) at increased or decreased values, and then refit the other parameters to see if compensatory changes could be made in other parameters that might result in an equivalent fit.

To test if the \(L_0\) value is robust, we obtained fits \(\alpha+\beta_4_c\) and \(\alpha+\beta_4_d\) (Table III) by fixing \(L_0\) at values three times larger or smaller, respectively, than that predicted by \(\alpha+\beta_4_a\). When \(L_0\) is three times larger, the fit predicts the G-V data quite well (\(\alpha+\beta_4_c\); Fig. 7 B, left), but does not predict the low voltage \(P_0-V\) relations as well as \(\alpha+\beta_4_a\) (Fig. 7 B, right). When \(L_0\) is three times smaller (\(\alpha+\beta_4_d\)), \(V_{ho}\) is shifted in the negative direction to compensate. The fit to the low voltage \(P_0-V\) relations is improved relative to \(\alpha+\beta_4_c\) (Fig. 7 C), although it is not improved over \(\alpha+\beta_4_a\).

To analyze the impact of shifting \(V_{ho}\) on fitting the \(\alpha+\beta_4\) data, \(V_{ho}\) was fixed to a more positive value, to 0.3 \text{e}_0. Although the best fit using the macroscopic G-V data alone (\(\alpha+\beta_4_b\), Table III) predicts the macroscopic G-V data quite well (Fig. 6, E, G, and H), the parameters poorly predict \(P_0-V\) relations at negative voltage and low calcium (Fig. 6 F), and the predicted log\(L_0'\) values are larger than the measured upper limits in 0 calcium (Fig. 6 I).

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To analyze the impact of shifting \(V_{ho}\) on fitting the \(\alpha+\beta_4\) data, \(V_{ho}\) was fixed to a more positive value,
resulting in $\alpha + \beta_4_e$ (Fig. 7 D). This would correspond to a reduced contribution of voltage sensor activation to channel opening at negative voltages. Table III shows that the major compensatory effect is an increase in L₀ and E, both of which would correspond to increased channel opening at negative voltages. Although the macroscopic G-V data is predicted fairly well (Fig. 7 D, left), the low voltage $P_o$-V relations in low calcium are not predicted well (Fig. 7 D, right). In contrast, shifting $V_{ho}$ to more negative potentials as compared with $\alpha + \beta_4_a$ (Fig. 7 E) allows fits that are comparable to $\alpha + \beta_4_a$. Because the negative shift of $V_{ho}$ has the effect of increasing $P_o$ at negative voltages, this is compensated for by a large decrease in the L₀ value (to 1.6 e⁻⁹; Table III) and reduction in allosteric coupling between voltage sensor movement and calcium binding (E). Interestingly, the best fits ($\alpha + \beta_4_a$, $\alpha + \beta_4_d$, and $\alpha + \beta_4_f$) seem to constrain $V_{ho}$ to around 110 mV (Table III), regardless of a negative shift of the $V_{ho}$ or a reduced L₀. Consistent with this, we observed that a +50 or −50 mV shift in $V_{ho}$ produces fits that deviate significantly from the G-V data (unpublished data).

This analysis demonstrate that low voltage $P_o$-V relations constrain a model in which $\beta_4$ mediates a negative shift of voltage sensor activation ($V_{ho}$), and biases the intrinsic closed to open equilibrium (L₀) toward the
closed state. The data can be fit by L0 that is smaller than our estimates, if there is a corresponding negative shift in Vh0. Independent of changes in these two parameters, this analysis indicates that β4 produces an increase in allosteric coupling to calcium binding (C), a reduction of closed channel calcium binding affinity (Kc), and a negative shift of Vhc.

Understanding Effects of β4 on BK Channels

BK channels α+β4 currents show a positive shift of the G-V relationship at low calcium and a negative shift of the G-V relationship at high calcium. In addition, the β4 subunit causes an apparent reduction in voltage dependence at low calcium. How do the changes in individual gating parameters altered by β4 confer α+β4 properties? To address this question, the α subunit steady-state properties were simulated and compared with simulations where individual β4 gating parameters are used to replace α subunit parameters. These are shown as individual changes in Figs. 8 and 9, and as additive changes in Fig. 10.

Effect on the Closed-to-Open Transition

Independent of voltage sensor movement and Ca2+ binding, BK channel opening is governed by an intrinsic
energetic barrier (described by \( L_0 \)) that has a weak intrinsic voltage dependence (\( z_L \)) (Horrigan and Aldrich, 2002). Data in Fig. 3 D suggest that the equilibrium constant \( L \) is significantly increased (at least by 11-fold) in the presence of \( \beta_4 \). Our best fit indicates that the \( \beta_4 \) subunit decrease (\( L_0 \) from \( 1.7 \times 10^{-7} \) for \( \alpha \) alone to \( 3.7 \times 10^{-8} \) for \( \alpha+\beta_4 \), approximately by 46-fold, indeed lower than our estimated upper limits (Fig. 3 D).

Effects of a 46-fold decrease in \( L_0 \) are illustrated in Fig. 8. We first simulated G-V relations at various \( \text{Ca}^{2+} \) based on gating parameters for \( \alpha \) (Table III) and obtained \( V_{1/2} \)-[\( \text{Ca}^{2+} \)] and \( Q \)-[\( \text{Ca}^{2+} \)] relations by fitting simulated G-V curves with Boltzmann function (Fig. 8, A and B). To see how changes in \( L_0 \) might affect BK channel gating, we simulated G-V curve using \( \alpha \) parameters except for the \( L_0 \), which is replaced by that of \( \beta_4 \) \( L_0 \) (Fig. 8, A and B). As expected, a 46-fold decrease in \( L_0 \) by \( \beta_4 \) creates a positive shift of the \( V_{1/2} \) at all [\( \text{Ca}^{2+} \)] (Fig. 8 A, \( \beta_4 \) \( L_0 \)). Interestingly, the effect of \( L_0 \) also causes a significant decrease in the voltage dependence (Fig. 8 B).

Why does decreasing \( L_0 \) cause a decrease in voltage dependence, particularly at submicromolar [\( \text{Ca}^{2+} \)]? In the dual allosteric gating scheme (Scheme 1), voltage sensors are activated around a voltage range defined by \( V_{h_o} \) for open channels and \( V_{h_c} \) for closed channels. Within this range (between \( V_{h_o} \) and \( V_{h_c} \)), the energetic difference between voltage sensor activation in closed and open channel is greatest, thus allosteric coupling between voltage sensor activation and gating is the strongest, and \( P_o \) is most voltage dependent (large \( Q \)). The effect of \( L_0 \) or calcium positions \( P_o \)-\( V \) curves along the voltage axis relative to \( V_{h_o} \) and \( V_{h_c} \) and therefore affects the voltage dependence. This is illustrated in \( P_o \)-\( V \) relations at two different [\( \text{Ca}^{2+} \)] simulated with the parameter from \( \alpha \) alone channels, in Fig. 8 C. Channel opening at 0 and 7 \( \mu \text{M} \) calcium falls approximately within this voltage range, and G-V curves show high voltage dependence (steep slope). Below and above these ranges, voltage sensors are either in the resting or activated state, respectively, and voltage-dependent channel openings are dependent on the weaker closed-to-open voltage dependence, \( z_L \). In contrast, data simulated using an \( L_0 \) fixed at the value estimated for \( \alpha+\beta_4 \) channels (Fig. 8 D) resulted in channel openings at voltages more positive than \( V_{h_c} \) for the 0 \( \text{Ca}^{2+} \) data. This resulted in a reduced voltage dependence (shallower slope). However, as higher calcium (\( \geq 7 \mu \text{M} \)) contributes significantly to channel gating, openings fall within the ranges where voltage sensors contribute to channel gating and we see a greater apparent voltage dependence.

By examining apparent \( Q \) vs. \( V_{1/2} \) (determined by fitting simulated data with Boltzmann equations; Fig. 8 E), we can see that the \( L_0 \) affects \( Q \) mostly by shifting the \( V_{1/2} \) along the voltage axis. Where \( V_{1/2} \) is similar between \( \alpha \) and \( \beta_4 \) \( L_0 \), the \( Q \) values are similar. At low [\( \text{Ca}^{2+} \)], channel activation occurs at membrane potentials more depolarized than \( V_{h_c} \), causing a decrease in apparent voltage dependence (\( Q \)). This is more dramatic...
in the presence of β4, since the significant decrease in L0 requires much higher membrane potential to open the channels.

**Effect on Ca2+ Dependence**

The fits with Scheme 1 suggest that the α+β4 channels have a threefold reduction in affinity of Ca2+ in the closed state (Kc = 13 μM α alone; 44 μM α+β4) with little change in affinity of the open state (Ko = 1.3 α alone; 1.9 μM α+β4). Thus, the β4 subunit imparts an increase in the strength of allosteric coupling between Ca2+ binding and channel opening (C = 10 for α alone vs. 23 for α+β4). A reduced affinity and greater coupling to Ca2+ binding may contribute to the negative shift in the V1/2 at high Ca2+ (Fig. 9 A). It should be noted however, that the model predicts that effects on Ca2+ sensitivity alone are not sufficient to offset the increased L0, particularly at low Ca2+ (Fig. 9 A, open circles). This is consistent with Ca2+ experiments discussed previously (Fig. 5 B). In these experiments, we found that the contribution of calcium alone in the absence of voltage sensor activation does not impart sufficient energy to shift the V1/2 more negative to α subunit. As discussed below, left shift of voltage sensor activation (Vh0) makes an important contribution to the negative shift of the V1/2.

Interestingly, effects on allosteric coupling to Ca2+ (C) appear to contribute to a slight reduction in apparent voltage sensitivity in high Ca2+ (Fig. 9 B). Increased Ca2+ coupling positions the V1/2 at 100 μM at approximately −60 mV, below the foot of voltage sensor activation (Vh0 is +25 mV for α, see Table III). These effects are predicted to reduce apparent voltage dependence at high calcium, as indeed we see for α+β4 channels (Fig. 1 D).

**Effect on Voltage Dependence**

Although the fits suggest that β4 does not alter z1 (0.56 e0 for α and 0.55 e0 for α+β4), it causes large shifts in the equilibrium of voltage sensor activation in both the open state (Vh0 = 25 mV α alone vs. −50 mV α+β4) and closed state (Vhc = 187 mV α alone vs. 110 mV α+β4). Although coupling between voltage sensor activation and gating (D) is slightly decreased by β4 (35 for α and 32 for α+β4), changing Vh0 and Vhc results in a significant negative shift of the G-V curves at [Ca2+] >7 μM, sufficient to compensate for the increased energetic barrier (L0) conferred by β4 (Fig. 9 C). It should be noted that the effect of changing Vh0, besides shifting the G-V curves to more negative membrane potentials, also positions the G-V at a more optimal position relative to Vh0 and Vhc to increase the apparent voltage dependence (Fig. 9 D). The above results and recent findings by Bao and Cox (2005) illustrate another important prediction of the dual allosteric model: channel gating is regulated not only by the coupling factor D but also by the value of Vh0 and Vhc.

Analysis of the effects of α+β4 currents demonstrates that the change in properties contributed by the β4 subunit offset each other to produce moderate changes in the conductance–voltage relationship. A manner to consider these changes is to simulate the Po[α, Ca2+] using the α subunit parameters, and compare these to simulated data where the α+β4 parameters are incrementally used to replace those of α subunit channels. This is shown in Fig. 10. The effect of β4 on the closed-to-open equilibrium, L0, and coupling between gating and voltage sensor movement, D, have opposing and parallel effects on the V1/2[Ca2+] relations (Fig. 10, A and B). The decrease of L0 shifts the curve to positive potentials, and D has a compensatory shift to negative potentials at [Ca2+] >7 μM. Increased coupling between calcium binding and gating (C) further increases the slope of the V1/2 vs. [Ca2+] curve so that at high [Ca2+] the V1/2 is shifted to more negative membrane potentials relative to α subunits alone (Fig. 10 C). Model fits indicate that the β4 subunit reduces allosteric coupling...
between voltage sensor movement and calcium binding (E), which contributes to a positive shift of the V_{1/2} at high [Ca^{2+}] (Fig. 10 D).

**DISCUSSION**

Our analysis demonstrates that the β4 subunit alters several aspects of BK channel gating. In this respect, β4 is similar to the β1 subunit, which has been shown to modulate BK channels in a complex manner (Cox and Aldrich, 2000; Orio and Latorre, 2005). For the β4 subunit, we show that these are fairly dramatic effects on BK channel gating properties, particularly V_{ho} and L_{0}, that seem to offset one another to produce moderate changes in the conductance–voltage relationship at micromolar [Ca^{2+}]. In many regards, this too is similar to changes observed with β1 subunits. β1 and β4 both mediate a negative voltage shift of open channel voltage sensor activation, V_{ho} (Cox and Aldrich, 2000; Bao and Cox, 2005; Orio and Latorre, 2005). In addition, it appears that β1 and β4 both increase the energetic barrier to opening by reducing L_{0} (Orio and Latorre, 2005). This is somewhat controversial because a more recent study did not see an effect on L_{0} by β1 (Bao and Cox, 2005).

What underlies the negative voltage shift of the G-V relationship at high [Ca^{2+}] that is often described as an “apparent increase in Ca^{2+} sensitivity”? Orio and Latorre attribute the apparent increase in Ca^{2+} sensitivity by β1 to a decrease in z_{f} (Orio and Latorre, 2005). Similar to predictions by Bao and Cox (2005) for the β1 subunit, our simulations indicate that the negative shift in V_{ho} by β4 has the highest contribution to increase channel opening. A very important aspect of the dual-allosteric model is that energetic contributions of voltage sensors are not equivalent over the voltage axis. Although we did not see a dramatic change in voltage-dependent allosteric coupling factor D, the negative shift of voltage sensor activation (V_{ho}) contributed significantly to increase P_{o}. In this, the β4 and β1 are also similar (Bao and Cox, 2005).

Why would evolution alter so many properties of BK channels to produce a net effect on the V_{1/2} that appears relatively moderate, particularly at higher calcium concentrations? For instance, at [Ca^{2+}] between 1.7 and 18 μM, the V_{1/2} is shifted by β4 to positive potentials ~20 mV or less (Table I). At [Ca^{2+}] >18 μM, there is a similar 10–30 mV negative shift. In considering the physiological role for BK α+β4 properties, one must consider the fact that the V_{1/2} value often does not reflect the open probabilities at physiological voltages, particularly at resting global [Ca^{2+}] where the V_{1/2} is >100 mV. Instead, it may be more relevant to consider the changes in open probability in the physiological voltages between −80 and +20 mV. Although P_{o} values can be low, opening of even a relatively few BK channels can nevertheless have profound effects on membrane voltage. For instance in vascular smooth muscle, activation of a cluster of BK channels near a Ca^{2+} spark site can hyperpolarize the membrane by 10–20 mV (Knot et al., 1998). For β4 subunits, which are predominantly expressed in neurons, the larger energetic barrier to opening (L_{0}), would be expected to hold BK channels silent at resting voltages. However, the negative shift of the V_{ho} means that voltage sensors are more easily

Figure 10. Additive effects of β4 gating parameters on BK channel steady-state properties. Simulations with α subunit parameters incrementally replaced by those of α+β4. Panels show experimental data for α (open symbols) and α+β4 (closed symbols) V_{1/2} vs. [Ca^{2+}] relationship. Line shows simulations using α subunit parameters incrementally altered by β4 L_{0} (A), β4 L_{0} + D (B), β4 L_{0} + D +C (C), β4 L_{0} + D +C +E (D).
activated following depolarization. Thus, appropriate with the concept that neurons respond to very transient changes in membrane potential, the opposing properties conferred by β4 subunits, increased energetic barrier to opening (L0) and a negative shift of voltage sensor activation (Vh0), allow BK channels to activate in a switch-like, rather than graded, fashion. For instance, at 1.7 μM calcium and resting membrane voltage of −80 mV, α+β4 L0 confers a >10-fold lower P0 than α subunits alone (P0, α is 1.9 × 10−3, α+β4 1.6 × 10−6). However following depolarization to +20 mV, effects on Vh0 allow α+β4 BK channels to have a similar P0 to that of α subunits alone (P0, α is 4.0 × 10−3, α+β4 3.4 × 10−3). Indeed, recent findings that mutations resulting in gain of function of BK channels lead to seizure phenotypes (Du et al., 2005, Brenner et al., 2005) highlights the importance of holding BK channels silent until necessary.

The current study has focused on steady-state properties conferred by β4 subunits. Yet the β4 subunit has very profound effects on BK channel activation and deactivation kinetics that may be more physiologically important in neurons, where the β4 subunit appears to be enriched (Weiger et al., 2000). In central neurons, BK channels appear to have an important role in membrane repolarization following an action potential (Hu et al., 2001). Yet the β4 subunit slows the activation of BK channels to time scales that are incompatible with a role in shaping individual action potentials (tens to hundreds of milliseconds; Brenner et al., 2000). Further studies are warranted to understand how β4 effects on L0 and Vh0 mediate changes in gating kinetics.

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