Slo1 Tail Domains, but Not the Ca^{2+} Bowl, Are Required for the β1 Subunit to Increase the Apparent Ca^{2+} Sensitivity of BK Channels

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ABSTRACT Functional large-conductance Ca^{2+}- and voltage-activated K^+ (BK) channels can be assembled from four α subunits (Slo1) alone, or together with four auxiliary β1 subunits to greatly increase the apparent Ca^{2+} sensitivity of the channel. We examined the structural features involved in this modulation with two types of experiments. In the first, the tail domain of the α subunit, which includes the RCK2 (regulator of K^+ conductance) domain and Ca^{2+} bowl, was replaced with the tail domain of Slo3, a BK-related channel that lacks both a Ca^{2+} bowl and high affinity Ca^{2+} sensitivity. In the second, the Ca^{2+} bowl was disrupted by mutations that greatly reduce the apparent Ca^{2+} sensitivity. We found that the β1 subunit increased the apparent Ca^{2+} sensitivity of Slo1 channels, independently of whether the α subunits were expressed as separate cores (S0-S8) and tails (S9-S10) or full length, and this increase was still observed after the Ca^{2+} bowl was mutated. In contrast, β1 subunits no longer increased Ca^{2+} sensitivity when Slo1 tails were replaced by Slo3 tails. The β1 subunits were still functionally coupled to channels with Slo3 tails, as DHS-I and 17 β-estradiol activated these channels in the presence of β1 subunits, but not in their absence. These findings indicate that the increase in apparent Ca^{2+} sensitivity induced by the β1 subunit does not require either the Ca^{2+} bowl or the linker between the RCK1 and RCK2 domains, and that Slo3 tails cannot substitute for Slo1 tails. The β1 subunit also induced a decrease in voltage sensitivity that occurred with either Slo1 or Slo3 tails. In contrast, the β1 subunit–induced increase in apparent Ca^{2+} sensitivity required Slo1 tails. This suggests that the allosteric activation pathways for these two types of actions of the β1 subunit may be different.

KEY WORDS: Ca^{2+}-activated K^+ channel • RCK domain • DHS-I • estrogen • Slo3

INTRODUCTION

BK channels are large conductance K^+ channels that are activated in a highly synergistic manner by both intracellular Ca^{2+} (Ca^{2+}_i) and voltage (Pallotta et al., 1981; Marty, 1981; Barrett et al., 1982; Latorre et al., 1982). When activated, K^+ leaves the cells through the open channels, driving the membrane potential more negative, shutting down voltage-dependent Ca^{2+} and Na^+ channels. This dual activation and resulting negative feedback on Ca^{2+}_i and membrane potential allows BK channels to modulate a number of key physiological processes, such as frequency tuning of hair cells (Fettiplace and Fuchs, 1999), neurosecretion (Robitaille et al., 1993), and smooth muscle contraction (Brenner et al., 2000b; Pluger et al., 2000; Petkov et al., 2001). Kinetic studies suggest that the synergistic activation of BK channels occurs through separate allosteric activators for Ca^{2+} and for voltage that act jointly to modulate the opening–closing transitions (Cui et al., 1997; Rothberg and Magleby, 1999, 2000; Horrigan and Aldrich, 1999; Horrigan et al., 1999; Cui and Aldrich, 2000; Magleby, 2001; Magleby and Rothberg, 2001). Consistent with separate allosteric activators, separate conserved regions of the α subunit of the BK channel appear mainly responsible for activation by Ca^{2+} and by voltage (Schreiber and Salkoff, 1997; Schreiber et al., 1999). They are the amino-terminal "core" of the channel (S0-S8), which includes a charged S4 for voltage activation (Diaz et al., 1998; Cui and Aldrich, 2000), and the carboxyl–terminal "tail" (S9-S10), which includes a stretch of five aspartic acid residues (the Ca^{2+} bowl) thought to be involved in Ca^{2+} activation (Schreiber and Salkoff, 1997; Schreiber et al., 1999; Bian et al., 2001; Braun and Sy, 2001; Bao et al., 2002; Xia et al., 2002).

In addition to activation by Ca^{2+}_i and depolarization, BK channels can be modulated by at least four different types of accessory β subunits (McManus et al., 1995; Dworetzky et al., 1996; Tanaka et al., 1997; Chang et al., 1997; Wallner et al., 1999; Jiang et al., 1999; Meera et al., 2000; Ramanathan et al., 2000; Weiger et al., 2000; Xia et al., 2000; Pluger et al., 2000; Brenner et al., 2000a,b; Petkov et al., 2001). When coexpressed with α subunits, the β1 subunit has two different effects. It greatly increases the apparent Ca^{2+} sensitivity (McManus et al., 1995; Meera et al., 1996; Nimigean and Magleby, 1999; Cox and Aldrich, 2000; Nimigean and

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Magleby, 2000; Ramanathan et al., 2000) and decreases the voltage sensitivity (Cox and Aldrich, 2000; Nimigean and Magleby, 2000). The decrease in voltage sensitivity is apparent as a shallower dependence of macroscopic conductance or Po on voltage. The increase in the apparent Ca^{2+} sensitivity is indicated in two ways: 5–10-fold less Ca^{2+}_i is required with the β1 subunit for 50% activation of the channel at a fixed membrane potential, or conversely, 60–100 mV less depolarization is required for 50% activation of the channel at a fixed Ca^{2+}_i. As might be expected from such pronounced effects on the Ca^{2+} sensitivity of BK channels, the β1 subunits are required for proper function in the tissues where they are expressed. For example, knocking out the β1 subunit in smooth muscle of mice leads to chronic hypertension (Pluger et al., 2000; Brenner et al., 2000b) and also decreased frequency of contraction in urinary muscle (Petkov et al., 2001).

This study further examines the mechanism by which the β1 subunit modulates the gating of the BK channel by exploring which structure features of the α subunit are involved in the dual action of the β1 subunit, of increasing the apparent Ca^{2+} sensitivity and decreasing the voltage sensitivity. Attention is directed toward the S9-S10 tail region that includes the Ca^{2+} bowl.

MATERIALS AND METHODS

Clones, Mutagenesis, and Channel Expression in Xenopus Oocytes and HEK 293 Cells

The clones of the wild-type mouse Slo1 α subunit of the BK channel (Butler et al., 1993) and the separate mouse Slo1 core and tail (Wei et al., 1994) were based on the mbr5 cDNA construct (EMBL/GenBank/DDBJ accession no. L16912) (Butler et al., 1993), and were provided by Dr. Lawrence Salkoff. The Slo3 tail construct was also provided by Dr. Lawrence Salkoff, and was derived from the Slo3 channel (Schreiber et al., 1998; EMBL/GenBank/DDBJ accession no. AF039213). Full-length chimeric subunits were provided by Drs. Xiao-Ming Xia and Christopher Lingle and were constructed by joining the core from Slo1 with the tail from Slo3 in the PXMX plasmid, using chimeric junction sites of amino acid 662 for Slo1 and amino acid 663 for Slo3, giving the linkage MRNSPNTS-REQPSLIT. The cDNA was transcribed in vitro using the Ambion mMessage mMachine Kit to obtain cRNA for injection into oocytes. *Xenopus laevis* oocytes were enzymatically separated using collagenase as described previously (Dahl, 1992). Oocytes were microinjected with 0.5–50 ng of the cRNA 2–8 d before recording. When the core and tail of the channel were expressed separately, the Slo1 core was typically mixed at 1:2 to 1:6 molar ratios with the Slo1 tail or Slo3 tail cRNA before injection. If lesser amounts of cRNA for the Slo3 tails were injected, the expressed channels typically showed instability, making sojourns from typical Slo3 channel activity to modes with typical Slo1 channel activity (see also Moss and Magleby, 2001). The cRNA for the β1 subunit was in 4–6 molar excess over that for the α subunit to drive coassembly (McManus et al., 1995).

Using the wild-type construct of the α subunit of the BK channel initially cloned by Pallanck and Ganetzky (1994), as modified (McManus et al., 1995) and provided by Merck Research Laboratories, we made two different mutations to disrupt the Ca^{2+} bowl by either replacing one aspartic acid with asparagine (D965N) changing FDLQDDDDDPD to FDLQNDDDDPD, or by deleting two consecutive aspartic acid residues in the Ca^{2+} bowl: deletion of both D965 and D966 (FLDQDDDDDPD to FLDQ__DDDPD). The indicated residue numbers are those in the EMBL/GenBank/DDBJ under accession no. MMU09383 (Pallanck and Ganetzky, 1994) before modification by Merck. The deletions were made by using the Stratagene QuickChange site-directed mutagenesis kit and checked by sequencing. The human β1 subunit (NM_004137) and bovine β1 subunit (L26101) were kindly provided by Merck Research Laboratories. Unless otherwise indicated, the experiments were performed with the human β1 subunit.

The experiments examining the effect of the bovine β1 subunit on channels formed from mouse α subunits with deletion of D965 and D966 in the Ca^{2+} bowl were performed using human embryonic kidney (HEK) 293 cells. As described in Nimigean and Magleby (1999), these cells were transiently transfected with the above-mentioned constructs together with Plasmid pGreen Lantern-1 (GIBCO BRL) that encodes for a green fluorescent protein as a marker for gene expression.

Electrophysiology and Solutions

Both single-channel and macropatch currents were recorded with the patch clamp technique (Hamill et al., 1981) from patches of membrane excised from *Xenopus* oocytes using an Axopatch 200B amplifier. All experiments were done using inside out patches, except when outside out patches were used for application of 17 β-estradiol to the extracellular surface. For single-channel recording, patches containing a single BK channel were identified by extended recordings at high levels of Ca^{2+} and/or depolarized potentials expected to readily activate the channels. In the absence of injection of cRNA, endogenous BK channels (Krause et al., 1996) were observed so infrequently (less than once in 50 patches) that it is unlikely that any of the single-channel recordings were from endogenous BK channels. In any case, all the results reported were consistently observed, suggesting that they were from expressed channels. For macropatch recording, the expressed channels would far outnumber any endogenous channels. Injection of cRNA for the core or the tail alone does not give rise to measurable currents (Wei et al., 1994; Meera et al., 1997).

The pipette solution contained (mM) KCl 158, TES 5, and usually 10 μM $GdCl_3$ to block the endogenous stretch–activated channels (Yang and Sachs, 1989). The bath solution contained (mM): KCl 158, TES 5, EGTA 1, HEDTA 1, and sufficient added Ca^{2+} to bring the free Ca^{2+} levels to those indicated (Nimigean and Magleby, 1999). (For Fig. 7 only, the KCl was 150 mM.) All solutions were adjusted to pH 7.0. Solutions with no added Ca^{2+} had a calculated free Ca^{2+} of $<10^{-8}$ M. Such solutions will be referred to as 0 Ca^{2+} solutions because Ca^{2+}_i at these concentrations has essentially no effect on the gating of the channel (Meera et al., 1996; Nimigean and Magleby, 2000). Indicated voltages refer to the intracellular potential. Experiments were performed at room temperature (18–22°C).

Dehydrosoyasaponin-I (DHS-I)* was a gift from Merck Research Labs. DHS-I was first dissolved in dimethyl sulfoxide to form a 10 mM stock solution. The stock solution was then diluted to 1 mM with distilled water and added to the experimental solution to make the final concentration of DHS-I of 100 nM (Giangiacomo et al., 1998). Estrogen in the form of 17β-estradiol was first dissolved in 100% ethanol to make a stock solution of 10 mM and then added directly to experimental solutions to make a final concentration of 10 μM.

**Abbreviations used in this paper:* DHS-I, dehydrosoyasaponin I; RCK, regulator of the conductance of potassium.

Data Analysis

Single-channel data were typically filtered at 5–10 kHz and sampled at 200 kHz directly to disk using pClamp7 or pClamp8. Analysis of the digitized records was then performed using custom programs, as described previously (McManus et al., 1987; McManus and Magleby, 1988; Nimigean and Magleby, 1999). Burst analysis was performed using a critical gap calculated from the closed dwell-time distributions to separate bursts, as detailed previously (Magleby and Pallotta, 1983; Nimigean and Magleby, 1999). Macropatch currents were analyzed with Clampfit in pClamp8. Conductance-voltage (G-V) curves were normally constructed from tail currents, but in a few experiments when Ca^{2+} was not present, peak currents were used.

Each G-V curve was fitted with a Boltzmann function (Eq. 1), normalized to the peak current,

$$G(V) = G_{max}/\{1 + \exp[(-V + V_{0.5})/k]\}, \quad (1)$$

where $V_{0.5}$ is the voltage of half-maximal activation of the conductance, and k is the voltage dependence of the activation process in mV per e-fold change. Data are expressed as the mean ± SEM. The *t* test was used to calculate levels of significance.

RESULTS

Fig. 1 presents schematic diagrams of the full-length and chimeric channels studied in this paper to investigate the mechanism by which the β1 subunit increases the apparent Ca^{2+} sensitivity of the BK channel. As shown, the pore-forming α subunit of the BK channel is comprised of S0-S10 hydrophobic domains (Adelman et al., 1992; Pallanck and Ganetzky, 1994; Wei et al., 1994; Wallner et al., 1996). S0-S8 is considered the core of the channel and S9-S10 the tail (Schreiber and Salkoff, 1997). S1-S6 bear homology to the superfamily of voltage-gated K^+ channels. The extracellular NH_2 terminus and S0 segment are required for the modulation of channel activity by the β1 subunit (Wallner et al., 1996). The residues of the α subunit beyond S6 to the end of the COOH terminus are intracellular (Meera et al., 1997; Jiang et al., 2001), and can tentatively be grouped into four or more domains: regulator of K^+ conductance (RCK1), which includes S7 and S8 and runs from just beyond S6 to the beginning of the linker after S8, the linker itself, RCK2, which includes at least S9, and finally, a serine proteinase like domain that includes the Ca^{2+} bowl and S10 (Moss et al., 1996ab; Schreiber and Salkoff, 1997; Meera et al., 1997; Schreiber et al., 1999; Jiang et al., 2001, 2002a,b).

The Ca^{2+} bowl in the tail domain contains five consecutive aspartic acid residues that contribute to the Ca^{2+} sensitivity (Schreiber and Salkoff, 1997; Schreiber et al., 1999; Bian et al., 2001; Braun and Sy, 2001; Moss and Magleby, 2001; Bao et al., 2002; Xia et al., 2002). Fragments of the tail region that include the Ca^{2+} bowl

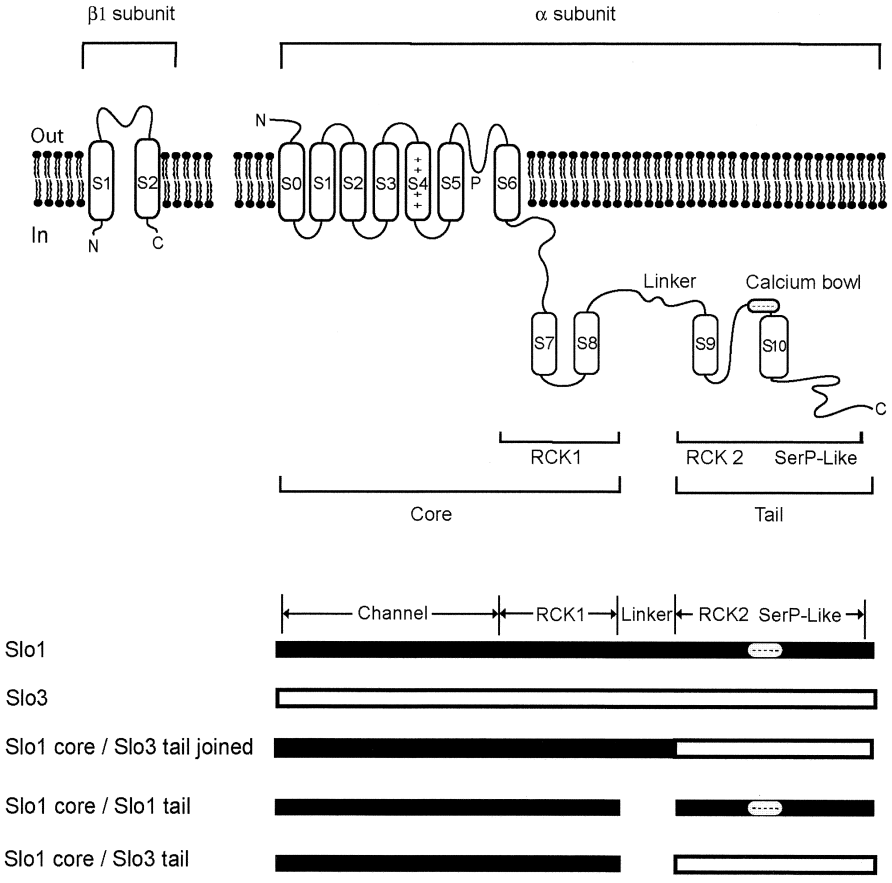

FIGURE 1. Schematic diagram of the membrane topology of the β1 and α subunit of the BK (Slo1) channel is shown in the top panel. The core and tail region of the α subunit are indicated as well as the RCK1, RCK2, and serine proteinase (SerP) like domains. The unconserved linker between S8 and S9 connects the core and tail as well as the two RCK domains. The tail contains the Ca^{2+} bowl with five consecutive aspartic acid residues, implicated with high affinity Ca^{2+} binding. Schematic diagrams of the channels examined in this study are indicated below the topology diagrams. Note that the linker region between the S8-S9 is missing in the Slo1 core/Slo3 tail channel, and is present in the Slo1 core/Slo3 tail joined channel.

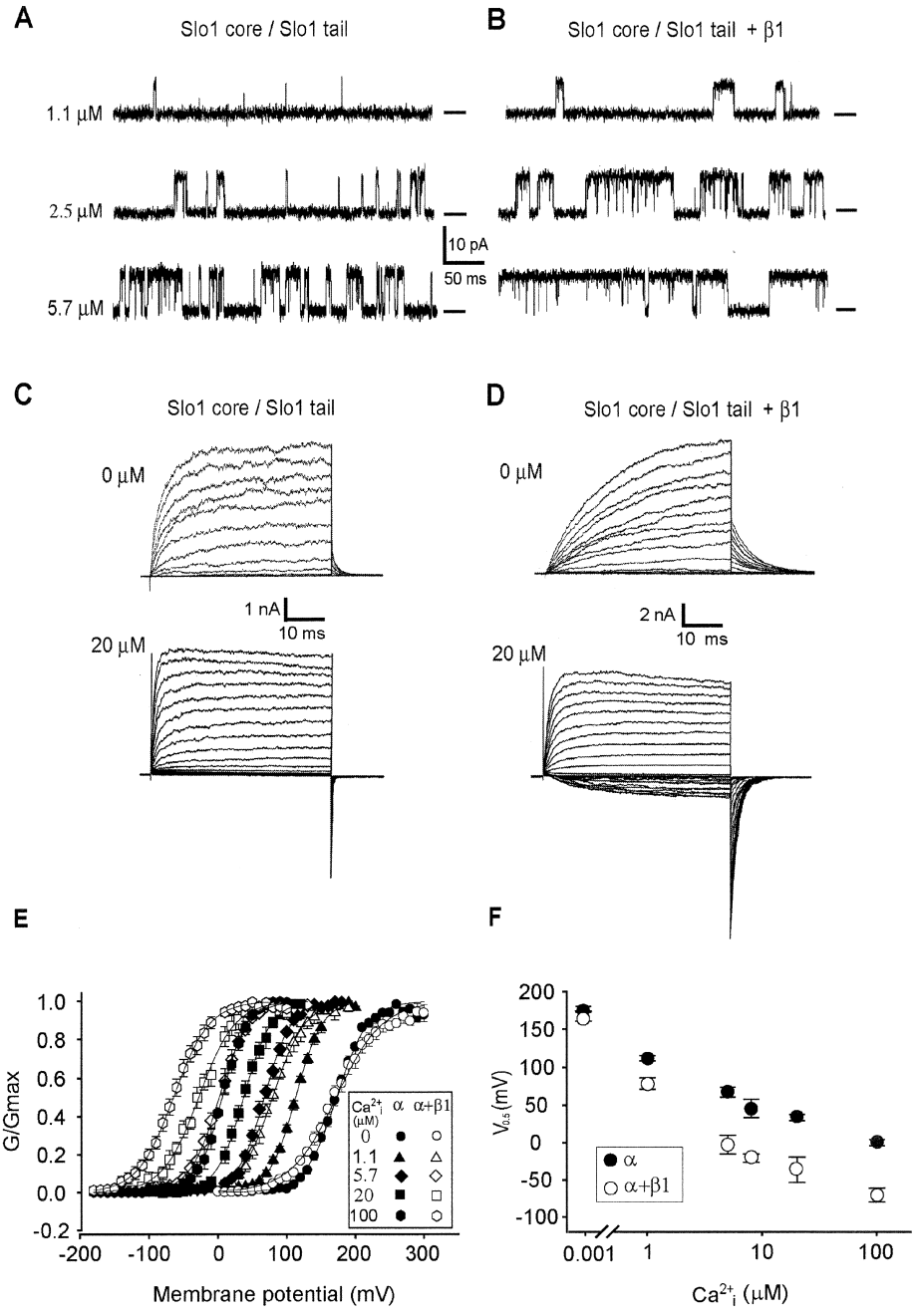

FIGURE 2. The β1 subunit increases the apparent Ca^{2+} sensitivity of the Slo1core/Slo1 tail channels while reducing the voltage sensitivity. (A and B) Representative single-channel current traces recorded from Slo1 core/Slo1 tail channels with and without β1 subunits for 1.1, 2.5, 5.7 μM Ca^{2+}_i, as indicated, at 30 mV. (C and D) Representative macrocurrents recorded for 0 and 20 μM Ca^{2+}_i with and without the β1 subunit. For currents with 0 Ca^{2+}_i, the potential was held at −80 mV, stepped to potentials between 0 and 300 mV in increments of 20 mV, and then stepped to 40 mV to measure tail currents. For currents with 20 μM Ca^{2+}_i, the potential was held at −120 mV, stepped to potentials between −100 to 120 mV in increments of 10 mV and then stepped to −80 mV to measure tail currents. (E) G-V curves derived from tail currents of Slo1core/Slo1 tail channels with and without β1 subunits. Each point plots the mean from >5 different patches. The lines in the GV plots in this and later figures are the fitted Boltzmann function with Eq. 1. The average voltage sensitivity was 20.2 ± 1.0 mV/e-fold without the β1 subunit and 26.3 ± 2.1 mV/e-fold with the β1 (F) Plots of the $V_{0.5}$ versus Ca^{2+}_i for experiments like in (E). $V_{0.5}$ was determined separately for data from each of >5 different patches and then averaged.

bind Ca^{2+} (Bian et al., 2001; Braun and Sy, 2001), and mutation of the Ca^{2+} bowl in these fragments decreases the Ca^{2+} binding (Bian et al., 2001). In contrast, the corresponding region of the Ca^{2+} bowl in the tail of Slo3, a BK like channel that is Ca^{2+} insensitive, contains only two negative charges (Schreiber et al., 1998). There is a 56% amino acid identity between the core domains of Slo1 and Slo3 and a 38% identity between the tail domains.

To examine the contributions of the core and tail to the modulation of the channel by the accessory β1 subunit, we examined three different chimeric constructs. In the first, the tail of Slo3 was joined to the core of Slo1 with the linker between S8 and S9 intact (Slo1 core/Slo3 tail joined channel). In the second, the Slo1 core was coexpressed with the Slo1 tail (Slo1 core/Slo1 tail channel), and in the third the Slo1 core was coexpressed with the Slo3 tail (Slo1 core/Slo3 tail channel). For the channels expressed in two parts, the unconserved linker between S8 and S9 was removed (Wei et al., 1994; Schreiber et al., 1999). Previous experiments have shown that Slo1 core/Slo1 tail channels are functionally expressed and have properties similar to full-length Slo1 channels (Meera et al., 1997; Schreiber and

Salkoff, 1997; Schreiber et al., 1999; Moss and Magleby, 2001), and that the Slo1 core/Slo3 tail channels are functionally expressed and have greatly reduced Ca^{2+} sensitivity (Schreiber et al., 1999; Moss and Magleby, 2001).

The β1 Subunit Increases the Apparent Ca^{2+} Sensitivity of the Slo1 Core/Slo1 Tail Channel While Reducing its Voltage Sensitivity

The β1 subunit increases the apparent Ca^{2+} sensitivity of the BK channel by increasing the open probability Po at a fixed Ca^{2+}_i (McManus et al., 1995; Meera et al., 1996; Cox and Aldrich, 2000; Ramanathan et al., 2000), mainly through an increase in the burst duration (Nimigean and Magleby, 1999, 2000). To explore whether this characteristic action of the β1 subunit was retained when the α subunit of the BK channel was expressed as two separate parts, we coexpressed the Slo1 core and Slo1 tail with and without the β1 subunit. Fig. 2, A and B, which presents representative single-channel records obtained at three different Ca^{2+}_i, show that the presence of the β1 subunit greatly increased Po and the burst duration at all three Ca^{2+}_i. For example, with 2.5 μM Ca^{2+}_i, the Po increased 10-fold from 0.053 ± 0.012 in the absence of the β1 subunit to 0.54 ± 0.08 with the β1 subunit, and the burst duration increased 12-fold, from 4.3 ± 1.0 to 53.5 ± 14.2 ms (30 mV, $n = 5$).

Since BK channels are activated by both depolarization and by Ca^{2+}_i, a second measure of Ca^{2+} sensitivity is to examine the voltage required to activate the channel to half maximum Po ($V_{0.5}$). Fig. 2, C and D, show macro currents recorded from patches typically containing 100–200 Slo1 channels, in the absence and presence of the β1 subunit. The characteristic slowing of the activation and deactivation kinetics with the β1 subunit, and the presence of appreciable currents at more negative potentials are evident (McManus et al., 1995; Meera et al., 1996; Cox and Aldrich, 2000; Ramanathan et al., 2000). Fig. 2, E and F, show that plots of normalized conductance versus voltage obtained from experiments of this type were left-shifted to more negative potentials by the β1 subunit for Ca^{2+}_i ranging from 1 to 100 μM. Thus, in the presence of the β1 subunit, less Ca^{2+}_i was required to activate the channel at a fixed voltage. These characteristic increases in Po, burst duration, and the leftward-shift in voltage induced by the β1 subunit for the two part Slo1 core/Slo1 tail channel were consistently observed, and are similar to those reported previously for the full length wild-type Slo1 channels (McManus et al., 1995; Meera et al., 1996; Cox and Aldrich, 2000; Ramanathan et al., 2000).

Consistent with the observations of Cox and Aldrich (2000) for full-length Slo1, the β1 subunit also decreased the voltage sensitivity of the two part Slo1 core/Slo1 tail channel, from 20.2 ± 1.0 mV/e-fold change to 26.3 ± 2.1 mV/e-fold change ($P < 0.05$; data from 0, 1.1, 5.7, 20, and 100 μM Ca^{2+}_i). This decreased voltage sensitivity was evident as a decrease in the slope of the G/V curves. Thus, expressing Slo1 in two parts did not change the characteristic modulatory effects of the β1 subunit, of increasing the apparent Ca^{2+} sensitivity (expressed as increases in burst duration, Po and the leftward shift in $V_{0.5}$), and of decreasing the voltage sensitivity. Consequently, the ~40 amino acids that comprise the linker between RCK1 and RCK2, that are missing in the Slo1 core/Slo1 tail channel, are not required for the β1 subunit to induce its characteristic effects.

In Fig. 2, E and F, it can be seen that the shift in $V_{0.5}$ induced by the β1 subunit for the two part Slo1 core/Slo1 tail channel becomes progressively less as the Ca^{2+}_i was reduced, with little shift at 0 Ca^{2+}_i, as has been reported previously for full-length Slo1 (Meera et al., 1996; Cox and Aldrich, 2000; Ramanathan et al., 2000). This observation might suggest that the effect of the β1 subunit on increasing Po requires Ca^{2+}_i, but this is not the case. Nimigean and Magleby (2001) found that the β1 subunit still increased burst duration and Po an order of magnitude in 0 Ca^{2+}_i at 30 mV, and the β1 subunit increases currents from macro patch recordings in 0 Ca^{2+}_i for voltages <175 mV (Fig. 2 E; Cox and Aldrich, 2000). The reduced ability of the β1 subunit to shift $V_{0.5}$ at low Ca^{2+}_i arises in large part from the secondary effect of the β1 subunit to decrease the voltage sensitivity of the channel. Large depolarizations are required to half activate the BK channel with 0 Ca^{2+}_i. At such large depolarizations, the decreased voltage dependence of the channel due to the β1 subunit becomes prominent, and consequently, the decreased activation due to the decreased voltage dependence cancels out the primary effects of the β1 subunit to increase burst duration and Po. These interacting effects can be seen in Fig. 4 of Nimigean and Magleby (2000) for 0 Ca^{2+}_i, where the β1 subunit increases burst duration and Po about an order of magnitude at 30 mV, and these β1 subunit induced increases become progressively less as the channel is depolarized.

The β1 Subunit has Little Effect on Mean Burst Duration and Po When the Slo1 Tail Is Replaced with the Slo3 Tail (+30 mV), While Still Reducing the Voltage Sensitivity

The observation that the β1 subunit increases the apparent Ca^{2+} sensitivity while having little effect on the Ca^{2+} affinity (Cox and Aldrich, 2000; Nimigean and Magleby, 2000) suggests that the major action of the β1 subunit may bypass the Ca^{2+} binding site, working on the machinery between the Ca^{2+} binding site and gate. Since the tail of the BK channel contains the Ca^{2+} bowl that is implicated in the Ca^{2+} sensitivity (Schreiber and Salkoff, 1997; Schreiber et al., 1999; Bian et al., 2001; Braun and Sy, 2001; Bao et al., 2002; Xia et al., 2002), then replacing the tail with one from a Ca^{2+}-insensitive

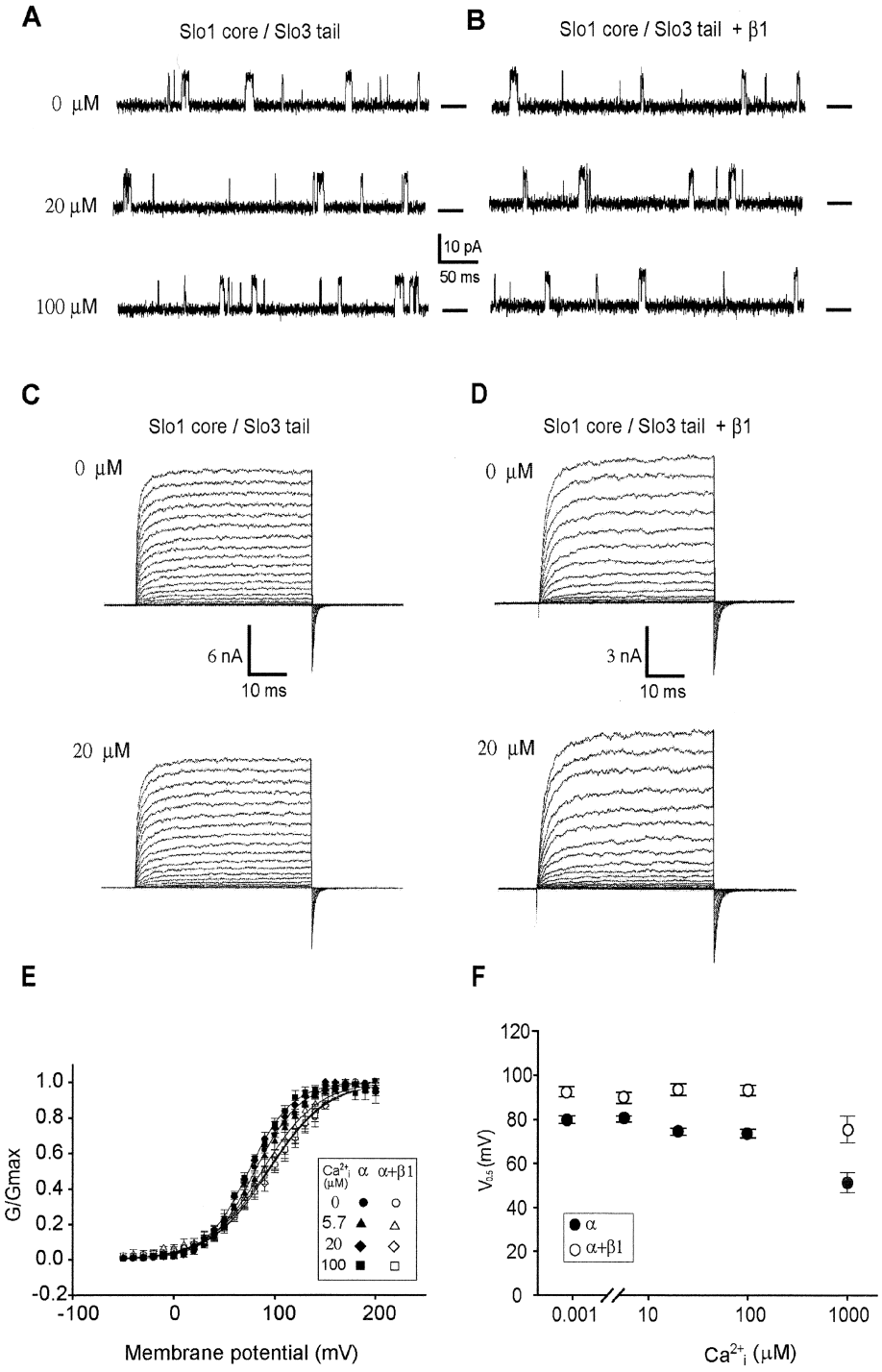

FIGURE 3. The β1 subunit had little effect on burst duration and Po of Slo1 core/Slo3 tail channels at 30 mV, while decreasing the voltage sensitivity. (A and B) Representative single-channel currents recorded from Slo1core/Slo3 tail channels with and without β1 subunits, for 0, 20, and 100 μM Ca^{2+}_i at 30 mV. (C and D) Representative macrocurrents recorded with and without β1 subunits, for either 0 or 20 μM Ca^{2+}_i. Currents were held at −100 mV and then activated by stepping to potentials between −50 to 200 mV in increments of 10 mV, with tail currents measured at −80 mV. (E) G-V curves derived from tail currents of Slo1core/Slo3 tail channels with and without β1 subunits. The average voltage sensitivity was 22.6 ± 0.4 mV/e-fold without β1 subunits and 28.8 ± 0.5 mV/e-fold with β1 subunits. (F) Plots of $V_{0.5}$ for Slo1 core/Slo3 tail channels with and without β1 subunits.

channel, which presumably would not have the machinery associated with the Ca^{2+} binding site in the Slo1 tail, should prevent the action of the β1 subunit. To examine this possibility, we replaced the Slo1 tail with the homologous tail region from Slo3, a BK-like channel with a greatly reduced Ca^{2+} sensitivity (Schreiber et al., 1998). We then examined whether the β1 subunit had its characteristic effects on the gating of the Slo1 core/Slo3 tail channel.

Replacing the Slo1 tail with the Slo3 tail converted the channel from a highly Ca^{2+}-sensitive channel to one with little Ca^{2+} sensitivity, as reported previously (Schreiber et al., 1999; Moss and Magleby, 2001). This is shown in the representative records in Fig. 3, where changing Ca^{2+} from 0 to 100 μM had little effect on either Po or the gating (Fig. 3 A). In contrast to the marked effects of the β1 subunit on the gating of the Slo1 core/Slo1 tail channel (Fig. 2, A and B),

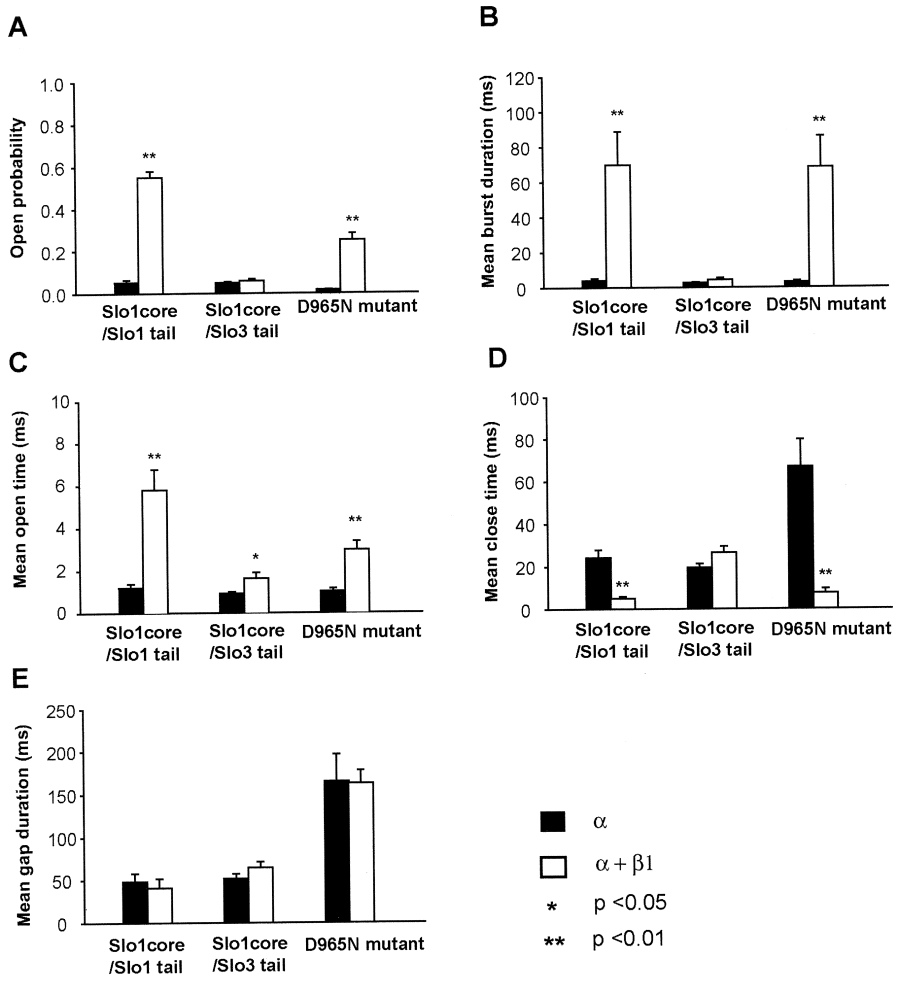

FIGURE 4. Comparison of the single-channel kinetics for various channel constructs with and without β1 subunits. Slo1 core/Slo1 tail channels: 2.5 μM Ca^{2+}_i. Slo1 core/Slo3 tail channels: 0 μM Ca^{2+}_i. Slo1 with D965N mutation: 20 μM Ca^{2+}_i. Average of 5–9 patches in each case, except 3 patches for burst duration and gap duration of D965N.

the β1 subunit had little effect on the gating of the Slo1 core/Slo3 tail channel (Fig. 3, A and B). The results are summarized in Fig. 4. The β1 subunit greatly increased Po, mean burst duration, and mean open time, and decreased mean closed time for channels with the Slo1 tail, while having little effect on these parameters for channels with the Slo3 tails. In some experiments, channels with Slo3 tails could enter a mode of activity with longer bursts than those in Fig. 3, A and B, somewhat similar to the bursting activity seen with channels with Slo1 tails, as in Fig. 2, A and B. Data from this mode were excluded from the analysis.

As a further test of whether the correct tail of the BK channel is needed for the β1 subunit to have its characteristic effect, we examined whether the β1 subunit shifted $V_{0.5}$ to more negative potentials for channels with Slo3 tails as it did for channels with Slo1 tails. In contrast to the marked leftward shifts in $V_{0.5}$ for channels with Slo1 tails (Fig. 2, E and F), the β1 subunit induced a small rightward shift in $V_{0.5}$ for Slo1 core/Slo3 tail channels (Fig. 3, C–F). The lack of a leftward shift indicated that the β1 subunit did not increase Po (Fig. 4 A). The small rightward shift resulted because the β1 subunit decreased the slope of the G/V curves, so that more depolarization was required to half activate the channel. The voltage sensitivity decreased from 22.6 ± 0.4 mV/e-fold change in Po without the β1 subunit to 28.8 ± 0.5 mV/e-fold change in Po with the β1 subunit (Fig. 3 E; $P < 0.01$).

The results in Figs. 2 and 3 show that the β1 subunit decreased the voltage sensitivity, independently of whether the tail of the channel was the Slo1 tail (Fig. 2 E) or the Slo3 tail (Fig. 3 E). With Slo1 tails, the increase in burst duration and Po induced by the β1 subunit dominated the decrease in voltage sensitivity and $V_{0.5}$ was shifted to the left. With Slo3 tails, Po was not increased by the β1 subunit so the decreased voltage dependence was apparent, shifting $V_{0.5}$ to the right. The results in Figs. 2 and 3 show, then, that the effect of the β1 subunit on decreasing the voltage sensitivity was independent of whether the channels had Slo1 tails or Slo3 tails, but that the Slo1 rather than the Slo3 tail was required for the β1 subunit to induce its characteristic effects of increasing Po through increases in mean burst duration.

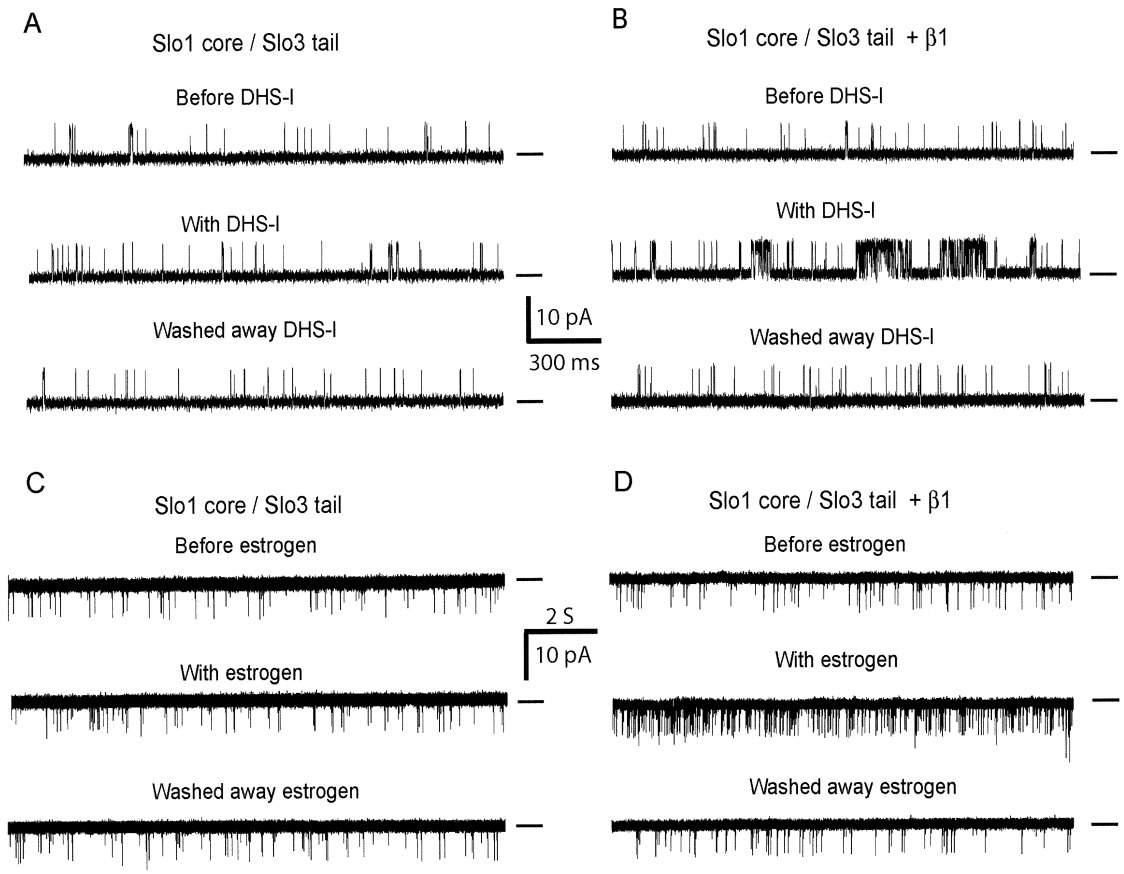

FIGURE 5. DHS-I and estrogen activate Slo1 core/Slo3 tail channels only in the presence of the β1 subunit, indicating that the β1 subunit is functionally associated with Slo1 core/Slo3 tail channels. (A and B) Representative single-channel currents from Slo1core/Slo3 tail channels with and without β1 subunits and 100 nM intracellular DHS-I, as indicated. (C and D) Representative single-channel currents from Slo1core/Slo3 tail channels with and without β1 subunits and 10 μM extracellular 17β-estradiol, as indicated.

As observed by Moss and Magleby (2001), we also observed that Slo1 core/Slo3 tail channels had less voltage sensitivity than Slo1 core/Slo1 tail channels (20.2 ± 1.0 mV/e-fold change in Po versus 22.6 ± 0.4 mV/e-fold change in Po), although this difference was not significant in this current study. The values for the voltage sensitivity observed in this current study using macro patches, which average data from many channels, were less than in the study of Moss and Magleby (2001) using analysis of data from one channel at a time. An explanation for this is that the natural variation in the Po among BK channels (McManus and Magleby, 1991) flattens the I/V curves for data averaged from multiple channels (Matthews, 1998; Ruiz et al., 1999).

The β1 Subunit Is Functionally Associated with Both Two Part and Full-length Slo1 Core/Slo3 Tail Channels

It is clear from the Figs. 3 and 4 that the β1 subunit does not increase burst duration and Po when the Slo1 tail is replaced by the Slo3 tail. This lack of effect may arise because: (a) β1 subunits are not appropriately expressed in the presence of the Slo3 tails, (b) β1 subunits are expressed but do not functionally associate with the Slo1 core/Slo3 tail channel, or (c) β1 subunits are expressed and associate, but the proper machinery in the Slo3 tail is missing for the β1 subunit to carry out its modulating effects.

The observation in Fig. 3 E that the β1 subunit decreases the voltage sensitivity of Slo1 core/Slo3 tail channels, shifting $V_{0.5}$ 10–20 mV positive (Fig. 3 F), suggests that the β1 subunit is expressed and that it does associate in some manner with the Slo1 core/Slo3 tail channels. Nevertheless, the type of association for a decrease in voltage sensitivity may be different than for the increase in burst duration and Po. Consequently, as a further test of functional association, we took advantage of the BK channel opening agonists DHS-I and estradiol as probes. Previous studies have shown that DHS-I (McManus et al., 1995; Giangiacomo et al., 1998; Brenner et al., 2000b) and estradiol (Valverde et al., 1999; Dick and Sanders, 2001) activate BK channels only in the presence of the β1 subunit. If DHS-I and es-

tradiol also activate Slo1 core/Slo3 tail channels only in the presence of the β1 subunit, then this would suggest that the β1 subunit is also functionally associated with Slo1 core/Slo3 tail channels.

Representative results for testing this hypothesis are shown in Fig. 5. In the absence of the β1 subunit, DHS-I had no effect on the gating of the Slo1 core/Slo3 tail channel (Fig. 5 A), while in the presence of the β1 subunit, DHS-I dramatically increased Po by increasing the burst duration (Fig. 5 B). The effects of DHS-I were readily reversible. Similar results were observed in four additional experiments of this type, where DHSI had no effect on Slo1 core/Slo3 tail channels in the absence of the β1 subunit, while increasing burst duration from 1.7 ± 0.1 ms to 6.2 ± 0.8 ms and Po from 0.024 ± 0.006 to 0.13 ± 0.004 in the presence of the β1 subunit (30 mV). The DHS-I–induced increase in burst duration and Po for Slo1 core/Slo3 tail channels is similar to what we observed for the Slo1 core/Slo1 tail channels (unpublished data) and to what has been reported previously for the Slo1 (full length) channel (McManus et al., 1995; Giangiacomo et al., 1998). Our results with DHS-I suggest that the β1 subunit is functionally associated with the Slo1 core/Slo3 tail channel. Otherwise, DHS-I should have no effect.

This activating effect of DHS-I in the presence of the β1 subunit on Slo1 core/Slo3 tail channels was observed for all Ca^{2+}_i examined, from 0 to 100 μM (30 mV). This is in contrast to Slo1 channels with the β1 subunit, where DHS-I does not activate at 0 Ca^{2+}_i when $V < 40$ mV (McManus et al., 1993; Giangiacomo et al., 1998). This difference may result because DHS-1 preferentially binds to the open state (Giangiacomo et al., 1998), and Slo1 core/Slo3 tail channels typically have higher activity than Slo1 channels with 0 Ca^{2+}_i (Moss and Magleby, 2001).

To test further whether the β1 subunit functionally associates with the Slo1 core/Slo3 tail channel, we examined whether estrogen (17 β-estradiol) would activate the channel in the presence of the β1 subunit. Estrogen had no effect on Slo1 core/Slo3 tail channels in the absence of the β1 subunit (Fig. 5 C), but reversibly activated the Slo1 core/Slo3 tail channels in the presence of the β1 subunit (Fig. 5 D). Similar results were observed in three additional experiments of this type. Estrogen had no effect on Slo1 core/Slo3 tail channels in the absence of the β1 subunit, while greatly increasing channel activity and Po fivefold, from 0.0015 ± 0.0006 to 0.007 ± 0.0004, in the presence of the β1 subunit (−30 mV). These effects of estrogen on Slo1 core/Slo3 tail channels are similar to those reported previously for Slo1 channels (Valverde et al., 1999). Consistent with the single-channel results, recordings from macro patches showed that both DHS-I and 17 β-estradiol introduced (leftward) shifts in $V_{0.5}$ of −30.4 ± 3.2 mV and −33.3 ± 3.7 mV, respectively, in the presence of the β1 subunit, but not in its absence (unpublished data).

The observations that the two channel opening agents, DHS-I and estrogen, that require the presence of the β1 subunit to activate BK channels, also activate Slo1 core/Slo3 tail channels in the presence of the β1 subunit, but not in its absence, suggest that the β1 subunit is functionally associated with Slo1 core/Slo3 tail channels. Hence, the inability of the β1 subunit to increase burst duration of Slo1 core/Slo3 tail channels, even though the β1 subunit is functionally associated with the channel, suggests that either the Slo1 tail (or some part of the Slo1 tail) is required for the β1 subunit to have its characteristic effects, or that the Slo3 tail blocks the effects of the β1 subunit acting at a site separate from the tail.

Slo1 core/Slo3 tail channels can exhibit wanderlust kinetics, as observed for dSlo (Silberberg et al., 1996), if insufficient Slo3 tail cRNA is injected (unpublished data). Consequently, we examined the effects of the β1 subunit, DHS-I, and 17 β-estradiol on joined Slo1 core/Slo3 tail channels. We found that the joined Slo1 core/Slo3 tail channel typically had more stable kinetics than two part channels. Using these more stable joined Slo1 core/Slo3 tail channels we have observed similar results with the β1 subunit, DHS-I, and 17 β-estradiol to those observed for the two part Slo1 core/Slo3 tail channels (unpublished data). Hence, the S8-S9 linker between the two RCK domains, which was present in the joined Slo1 core/Slo3 tail channel and not in the two part Slo1 core/Slo3 tail channel, is not required for the effects of the β1 subunit, DHS-I, or estrogen on the Slo1 core/Slo3 tail channel, but may influence the stability of the gating and Po, perhaps by assuring that there is one Slo3 tail for each Slo1 core.

A Functional Ca^{2+} Bowl Is Not Required for the β1 Subunit to Have its Characteristic Effects on the BK Channel

As shown in a previous section, the β1 subunit no longer increased burst duration and Po after the Slo1 tail was replaced by the Slo3 tail. A major difference between the Slo1 tail and Slo3 tail is that the Slo3 tail does not contain a Ca^{2+} bowl, thought to be a high affinity Ca^{2+} binding site (Wei et al., 1994; Schreiber and Salkoff, 1997; Schreiber et al., 1999; Bian et al., 2001; Braun and Sy, 2001; Bao et al., 2002). Hence, perhaps the inability of the β1 subunit to increase burst duration and Po in the presence of the Slo3 tail is due solely to the disruption of the Ca^{2+} bowl. To test this possibility, the effects of the β1 subunit on Slo1 channels with disrupted Ca^{2+} bowls were examined. Based on the work of Schreiber and Salkoff (1997), a point mutation (D965N, see MATERIALS AND METHODS) was made in

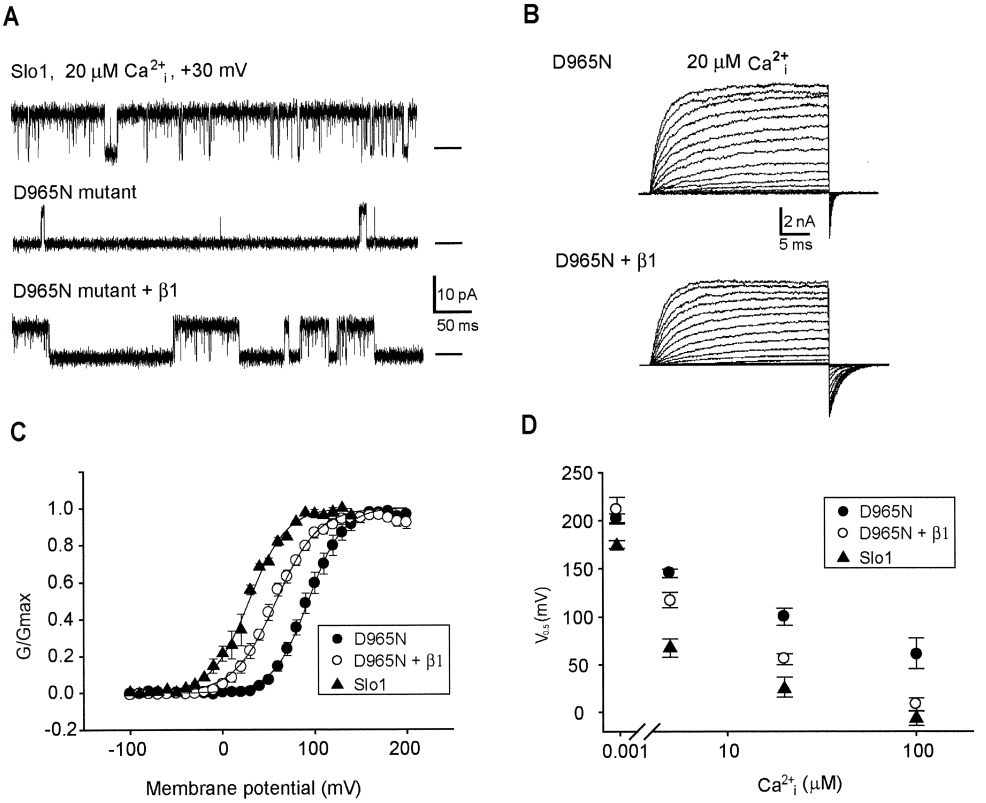

FIGURE 6. The β1 subunit greatly increases burst duration and Po, and leftward shifts $V_{0.5}$ after the Ca^{2+} bowl mutation D965N to the Slo1 channel. (A) Representative single-channel currents recorded from Slo1 channels, and Slo1 channels with the Ca^{2+} bowl mutation D965N without, and with β1 subunits. 20 μM Ca^{2+}_i, 30 mV. (B) Representative macrocurrents recorded from the D965N mutant Slo1 channels with and without β1 subunits. The potential was held at −120 mV, stepped to potentials between −100 to 200 mV in increments of 10 mV, and then stepped to −80 mV to measure tail currents. (C) G-V curves derived from tail currents of Slo1 channels, D965N mutant Slo1 channels, and D965N mutant Slo1 channels with β1 subunits (20 μM Ca^{2+}_i). The average voltage sensitivity was 19.1 ± 0.6 mV/e-fold without the β1 subunit and 23.1 ± 1.2 mV/e-fold with the β1 ($n = 5$ patches in each case except 3 patches for Slo1). (D) Plots of $V_{0.5}$ for Slo1 channels, and D965N mutant Slo1 channels without and with β1 subunits.

the Ca^{2+} bowl of Slo1 (full length) to essentially remove the high affinity Ca^{2+} sensitivity of the channel. Both single-channel and macroscopic currents were then recorded from the channel in the presence and absence of the β1 subunit to see if an intact Ca^{2+} bowl is required for the β1 subunit to have its characteristic effects of increasing burst duration and Po.

Representative results are presented in Fig. 6 A, which shows single-channel records from Slo1, Slo1 with a mutated Ca^{2+} bowl (D965N), and Slo1 with the mutated Ca^{2+} bowl in the presence of the β1 subunit. As expected (Schreiber and Salkoff, 1997), the Ca^{2+} bowl mutation dramatically decreased the Ca^{2+} sensitivity. With 20 μM Ca^{2+}_i, the Po for the wild-type channel was typically > 0.7 (top trace), compared with only 0.04 for the mutated channel (middle trace). In spite of the greatly reduced Ca^{2+} sensitivity of Slo1 with a mutated Ca^{2+} bowl, the β1 subunit still had its characteristic effects of increasing burst duration and Po (Fig. 6 A, bottom trace). Similar results were observed in six additional experiments of this type. With 20 μM Ca^{2+}_i, the β1 subunit increased the burst duration of D965N 21-fold, from 3.2 ± 0.8 to 68.5 ± 17.5 ms and Po 16-fold, from 0.016 ± 0.002 to 0.25 ± 0.03. Results are summarized in Fig. 4.

As might be expected from the β1-induced increase in burst duration and Po for the single-channel records from D965N channels, the β1 subunit also shifted the G-V curve to the left for these mutated channels (Fig. 6, B and C). In the absence of the β1 subunit, mutating the Ca^{2+} bowl shifted the G-V curve 65 mV to the right from that of the wild-type channel, so that more depolarization was required to activate the channel. This rightward shift reflects a decreased Ca^{2+} sensitivity after the Ca^{2+} bowl mutation. The presence of the β1 subunit then shifted the G-V curve back 35 mV to the left, so that less depolarization was required to half activate the channel. Fig. 6 D presents results from experiments of this type over a range of Ca^{2+}. The β1 subunit–induced the greatest leftward shift in $V_{0.5}$ at the highest Ca^{2+}_i examined (100 μM). The magnitude of this leftward shift decreased as the Ca^{2+}_i was decreased, with no significant effect of the β1 subunit on $V_{0.5}$ with 0 Ca^{2+}_i, as was observed in Fig. 2 F for the Slo1 core/Slo1 tail channel without a Ca^{2+} bowl mutation. The β1 subunit also decreased the voltage sensitivity of D965N, from 19.1 ± 0.6 mV/e-fold change in Po without the β1 subunit, to 23.1 ± 1.2 mV/e-fold change with the β1 subunit ($P < 0.05$, $n = 5$ in both cases).

It should be noted that the values of the β1 subunit–induced leftward shifts were less for the mutated channel than for the wild-type channel at the same level of Ca^{2+}_i. However, if one adjusts the data for the differ-

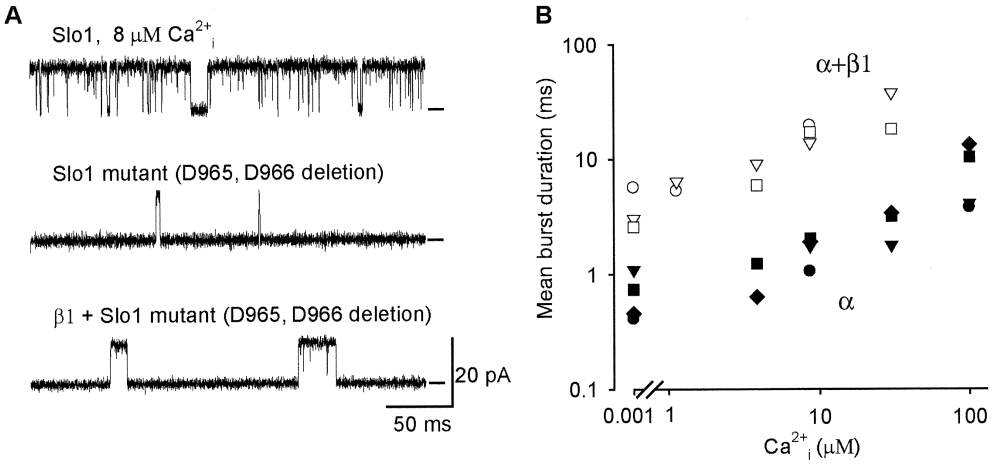

FIGURE 7. The β1 subunit greatly increases burst duration and Po after a Ca^{2+} bowl mutation that deletes D965 and D966 of Slo1. (A) Representative single-channel currents recorded from Slo1 channels, and Slo1 channels with the Ca^{2+} bowl mutation that deletes D965 and D966 without and with β1 subunits. 8 μM Ca^{2+}_i, 50 mV. (B) Plots of mean burst duration versus Ca^{2+}_i for three mutant channels and four mutant channels with the β1 subunit.

ences in Po, then the shifts with and without the β1 subunit were the same for the two types of channel. For example, D965N with 20 μM Ca^{2+}_i had a Po of 0.016, which was similar to the Po of the wild type channel with 1–2 μM Ca^{2+}_i. In both these cases the β1 subunit–induced leftward shift in $V_{0.5}$ was ~35 mV. It will be recalled from an earlier section that the β1 subunit–induced leftward shift becomes less when the Po is less, because the high positive voltages required to half-activate the channel for low initial Po emphasize the decreased voltage sensitivity induced by the β1 subunit, which cancels out the facilitatory effects of the β1 subunit. Similar effects of the β1 subunit on channels with and without a functional Ca^{2+} bowl suggests that the Ca^{2+} bowl is not required for the β1 subunit to decrease either the voltage sensitivity or increase the apparent Ca^{2+} sensitivity.

As a further test of whether the β1 subunit still had it characteristic effects after disrupting the Ca^{2+} bowl, we also examined a deletion mutation to the Ca^{2+} bowl (deletion of D965 and D966), which had the greatest effect of reducing the Ca^{2+} sensitivity of all the mutations to the Ca^{2+} bowl examined by Schreiber and Salkoff (1997). Representative single-channel records from Slo1, the deletion mutation of Slo1, and the deletion mutation of Slo1 plus the β1 subunit are shown in Fig. 7 for 8 μM Ca^{2+}_i (50 mV). As expected, the deletion mutation greatly decreased Po, and coexpression of the mutant with the β1 subunit then increased Po by increasing the burst duration (Fig. 7 A). Results from four mutated channels without the β1 subunit and three mutated channels in the presence of the β1 subunit are shown in Fig. 7 B. The β1 subunit increased burst duration ~10-fold, independent of Ca^{2+}_i for changes in Ca^{2+}_i >3 orders of magnitude, including 0 Ca^{2+}_i. The retention of the characteristic effects of the β1 subunit on channels with disrupted Ca^{2+} bowls for both the oocyte expression system with the human β1 subunit (Fig. 6) and in the HEK cell expression system with the bovine β1 subunit (Fig. 7), indicates that the β1 subunit still induces its characteristic effects when the Ca^{2+} sensitivity is greatly reduced due to mutations in the Ca^{2+} bowl, and that these observations are independent of the expression system and the species of the β1 subunit.

From Figs. 6 D and 7 B it can be seen that, although the Ca^{2+} bowl mutations greatly reduce the Ca^{2+} sensitivity of Slo1 channels, the channels still retain Ca^{2+} sensitivity. This is consistent with previous studies that suggest that mutations to the Ca^{2+} bowl do not remove all the Ca^{2+} binding sites (Schreiber and Salkoff, 1997; Schreiber et al., 1999; Bian et al., 2001; Braun and Sy, 2001; Shi and Cui, 2001; Zhang et al., 2001; Bao et al., 2002; Xia et al., 2002).

DISCUSSION

The β1 accessory subunit for BK (Slo1) channels has two different and concurrent modulatory effects on channel activity: to greatly increase the apparent Ca^{2+} sensitivity by increasing burst duration and Po (Nimigean and Magleby, 1999), and to decrease the voltage sensitivity, indicated as a shallower slope of the G-V curve (Cox and Aldrich, 2000) or Po-V curve (Nimigean and Magleby, 2000). This study explores which structural features of the α subunit are involved in mediating these two different effects of the β1 subunit. We address this question by examining the effect of the β1 subunit after either replacing the tail of Slo1 with the tail from Slo3, a BK like channel without a Ca^{2+} bowl and little Ca^{2+} sensitivity (Schreiber et al., 1998), or by mutating the Ca^{2+} bowl to greatly reduce the Ca^{2+} sensitivity of the channel (Schreiber and Salkoff, 1997). Our results suggest that these two actions of the β1 subunit are mediated through separate functional domains of the BK channel.

Consistent with previous observations, we found in the absence of the β1 subunit, that replacing the Slo1 tail with the Slo3 tail (Slo1 core/Slo3 tail channel) removed most of the Ca^{2+} sensitivity for Ca^{2+} < 100 μM

(Fig. 3 and Schreiber et al., 1999; Moss and Magleby, 2001), and mutating the Ca^{2+} bowl greatly decreased the Ca^{2+} sensitivity, such that levels of Ca^{2+}_i that gave Pos > 0.7 before the mutations gave Pos < 0.05 after mutation (Figs. 6 A and 7 A, and Schreiber and Salkoff, 1997; Schreiber et al., 1999; Bian et al., 2001; Braun and Sy, 2001; Bao et al., 2002; Xia et al., 2002).

We found that the β1 subunit still had its characteristic effects of increasing the apparent Ca^{2+} sensitivity (through increases in burst duration and Po) and decreasing the voltage sensitivity when the Slo1 channels were expressed in two parts, from separate S0-S8 cores and S9-S10 tails (Slo1 core/Slo1 tail channel), rather than from full-length α subunits (Fig. 2). This indicates that the missing 40-amino acid S8-S9 linker is not required for the β1 subunit to exert either of its two different effects.

When the Slo3 tail replaced the Slo1 tail, the β1 subunit no longer increased burst duration and Po, but still decreased the voltage sensitivity. The lack of effect of the β1 subunit on burst duration and Po for channels with Slo3 tails was not due to a lack of functional association between the β1 subunit and the chimeric channel, as the specific channel openers DHS-I and estrogen that only activate BK channels in the presence of the β1 subunit, also only activated the channels with Slo3 tails in the presence of the β1 subunit. The observation that the β1 subunit decreased the voltage sensitivity of the channels with Slo3 tails provides additional support that the β1 subunit was functionally associated with the channels with Slo3 tails. Since the β1 subunit functionally associates with channels with either Slo1 tails or Slo3 tails, then some difference of the Slo3 tail from the Slo1 tail must prevent the β1 subunit from increasing burst duration and Po for channels with Slo3 tails. In contrast, the β1 subunit decreased voltage sensitivity for channels with either Slo1 tails or Slo3 tails. These differential effects of the β1 subunit on channels with different tails suggests that the β1 subunit–induced increase in burst duration and Po (apparent Ca^{2+} sensitivity) is mediated through the tail domain of the channel, while the β1 subunit induced decrease in voltage sensitivity may be mediated through the core domain.

One difference between Slo1 tails and Slo3 tails is the virtual absence of the Ca^{2+} bowl in Slo3 tails. However, we found that disrupting the Ca^{2+} bowl in the tails of Slo1 channels by mutation did not prevent the β1 subunit from increasing the apparent Ca^{2+} sensitivity of Slo1 channels. This finding, together with the previous observations, suggests that one or more structural features of the Slo1 tail may be required for the β1 subunit to exert its characteristic effects of increasing apparent Ca^{2+} sensitivity through increases in burst duration and Po, but the Ca^{2+} bowl is not one of them.

Could sites other than the Ca^{2+} bowl contribute to the apparent increased Ca^{2+} sensitivity observed with the β1 subunit? In addition to the Ca^{2+} bowl, BK channels may have one or more additional high affinity Ca^{2+} sites (Braun and Sy, 2001; Bao et al., 2002; Xia et al., 2002), as well as a low affinity site activated by mM Ca^{2+}_i or Mg^{2+}_i (Shi and Cui, 2001; Zhang et al., 2001). The low affinity site has been shown to be functional for channels with either Slo1 tails or Slo3 tails, consistent with its location on the core of the channel (Shi and Cui, 2001; Xia et al., 2002). Thus, our observation that the β1 subunit had little effect on burst duration and Po for channels with Slo3 tails when compared with the pronounced effects on channels with Slo1 tails, suggests that a functional low affinity site is not a major factor contributing to the actions of the β1 subunit on these parameters. If it were, then the β1 subunit should have increased burst duration and Po for channels with Slo3 tails. It also seems unlikely that the β1 subunit is acting through major changes in the affinities of any additional high affinity sites, as previous studies have shown that the critical level of Ca^{2+}_i that first starts to increase channel activity is little changed by the β1 subunit (Cox and Aldrich, 2000; Nimigean and Magleby, 2000).

Together, all of these observations suggest that neither the Ca^{2+} bowl nor the low affinity site nor other high affinity sites are required for the β1 subunit to exert its major action on increasing apparent Ca^{2+} sensitivity through increases in burst duration and Po. These conclusions are consistent with the observations that the β1 subunit still exerts its major effect on burst duration and Po in the absence of Ca^{2+}_i (Nimigean and Magleby, 2000). These conclusions do not exclude that the β1 subunit may act on the allosteric machinery downstream from Ca^{2+} binding sites. Even though the major actions of the β1 subunit appear to be through Ca^{2+}-independent mechanisms, the β1 subunit does have some Ca^{2+} dependent effects on the gaps between bursts (Nimigean and Magleby, 2000) and the apparent Ca^{2+} affinity (Cox and Aldrich, 2000), which will not be considered here.

If the major actions of the β1 subunit on increasing Po and burst duration are not through action on Ca^{2+} binding sites, as discussed above, then the absence of the Ca^{2+} bowl or other high affinity sites on the Slo3 tail is not the explanation for why the β1 subunit does not increase burst duration and Po when the Slo1 tail is replaced with the Slo3 tail. Possible explanations are that there are specific structural features (other than Ca^{2+} binding sites) on the Slo1 tail that are not present on the Slo3 tail, that are required for the action of the β1 subunit, or that the Slo3 tail, either directly or allosterically, prevents the action of the β1 subunit. It is unlikely that the Slo3 tail blocks the action of the β1 subunit, as two channel opening agents (DHS-I and estrogen) that activate Slo1 channels only in the presence of the β1 subunit, also activated channels with Slo3 tails only in the

presence of the β1 subunit (Fig. 5), indicating that β1 subunit is not blocking the gating of the channel when the Slo3 tail is present. If anything, the channel is typically more active with the Slo3 tail (Schreiber et al., 1999; Moss and Magleby, 2001). Perhaps the β1 subunit and the Slo3 tail are increasing Po by acting through a common mechanism, such that once the mechanism is employed by the Slo3 tail, it becomes saturated so that the β1 subunit can contribute no further effect. This possibility seems unlikely, however, since, for comparisons at the same Po, the Slo3 tail typically decreases mean burst duration, mean open time, and the mean duration of gaps between bursts (Moss and Magleby, 2001), whereas the β1 subunit has the opposite effect on these parameters (Nimigean and Magleby, 1999).

Studies by Jiang et al. (2002a,b) on the structure of a bacterial Ca^{2+}–gated potassium channel, MethK, suggest that the Ca^{2+}-dependent gating of MethK is controlled by eight RCK domains (regulators of the conductance of K^+). Four of these arise from a COOH terminus domain attached to each of the four α subunits (RCK1) and four are assembled separately from solution as soluble proteins (RCK2). The eight RCK domains assemble to produce four fixed and four flexible interfaces to form a gating ring that hangs beneath the channel on the intracellular side. Jiang et al. (2002a) propose that the binding of two Ca^{2+} at each of the four flexible interfaces changes the structure of the gating ring so that each RCK1 domain pulls on a flexible linker attached to the intracellular end of each inner helix (S6 equivalent of Slo1), opening the channel.

By analogy to MethK, RCK1 in BK channels would include S7 and S8, and RCK2 would include at least S9. Whether the Ca^{2+} bowl and S10 should be functionally considered as part of RCK2 or as an additional attachment, since they are contained in a serine proteinase-like domain (Moczydlowski et al., 1992; Moss et al., 1996a,b), is unclear (see Fig. 1). With four α subunits, each with a sequential RCK1 and RCK2 domains, BK channels would also have eight RCK domains like MethK. The Ca^{2+} coordinating sites in MethK, D184, E210, and E212, appear to be replaced with L, Q, and L, respectively, in RCK1 of BK channels (see alignment in Jiang et al., 2002a), suggesting that the Ca^{2+} sites in BK channels are located in different places than in MethK. Nevertheless, the idea that a Ca^{2+}-induced movement at flexible interfaces between RCK domains leads to gating of the BK channel can serve as starting point for discussion of mechanism.

Our observation that the unconserved linker between S8 and S9 in BK channels was not required for the β1 subunit to have its characteristic effects would suggest that this linker has little function except to attach RCK2 to RCK1 (see Fig. 1). This conclusion is consistent with the observation (see above) that the linker is missing altogether in MethK channels, where the RCK2 equivalent domain is a separate protein. Based on the model of Jiang et al. (2002a), replacing the Slo1 tail with the Slo3 tail in our experiments would replace the four native RCK2 domains of Slo1 with four foreign RCK2 domains from Slo3, a BK like channel with low Ca^{2+} sensitivity. This substitution appears to decrease the energy barriers for the open-closed transition, as channels with Slo3 tails have increased activity and Po in 0 $Ca^{2+}{}_i$ together with decreased mean open and closed times at the same Po (Schreiber et al., 1999; Moss and Magleby, 2001).

The simplest interpretation of the actions of the β1 subunit to increase the apparent Ca^{2+} sensitivity in light of the model of Jiang et al. (2002a), is that the β1 subunit alters some energy barriers for the movement of the gating ring to favor reentry into the open conformation and to decrease the rate constants for closing in order to increase both the numbers of openings per burst and the mean open times. The proper RCK2 domain (Slo1 tail rather than Slo3 tail) is required for the β1 subunit to induce these changes in apparent Ca^{2+} sensitivity, while having little effect on the β1 subunit–induced decrease in voltage sensitivity, which may arise from other areas of the channel.

Current allosteric models for the activation of BK channels suggest that voltage and Ca^{2+} sensors act relatively independently of one another to modulate the open-closing transitions (Horrigan et al., 1999; Horrigan and Aldrich, 1999; Cox and Aldrich, 2000; Cui and Aldrich, 2000; Zhang et al., 2001; Niu and Magleby, 2002). Independent allosteric activators could provide a means for the β1 subunit to exert its differential effects on channel activity, of increasing Po through increases in burst duration and decreasing Po through decreases in the voltage sensitivity. Contact of the β1 subunit with at least two regions of the channel, each associated with a different allosteric activator could provide these differential effects.

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