Role of the β1 Subunit in Large-Conductance Ca\(^{2+}\)-activated K\(^{+}\) Channel Gating Energetics

M. Mechanisms of Enhanced Ca\(^{2+}\) Sensitivity

D.H. Cox*‡ and R.W. Aldrich§

From the *Molecular Cardiology Research Institute, New England Medical Center, and ‡Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts 02111; and §Department of Molecular and Cellular Physiology and Howard Hughes Medical Institute, Stanford University, Stanford, California 94305

**Abstract** Over the past few years, it has become clear that an important mechanism by which large-conductance Ca\(^{2+}\)-activated K\(^{+}\) channel (BK\(_{Ca}\)) activity is regulated is the tissue-specific expression of auxiliary β subunits. These first of these to be identified, β1, is expressed predominately in smooth muscle and causes dramatic changes, increasing the apparent affinity of the channel for Ca\(^{2+}\) 10-fold at 0 mV, and shifting the range of voltages over which the channel activates by 80 mV at 9.1 μM Ca\(^{2+}\). With this study, we address the question: which aspects of BK\(_{Ca}\) gating are altered by β1 to bring about these effects? Ca\(^{2+}\) binding, voltage sensing, or the intrinsic energetics of channel opening? The approach we have taken is to express the β1 subunit together with the BK\(_{Ca}\) α subunit in Xenopus oocytes, and then to compare β1’s steady state effects over a wide range of Ca\(^{2+}\) concentrations and membrane voltages to those predicted by allosteric models whose parameters have been altered to mimic changes in the aspects of gating listed above. The results of our analysis suggest that much of β1’s steady state effects can be accounted for by a reduction in the intrinsic energy the channel must overcome to open and a decrease in its voltage sensitivity, with little change in the affinity of the channel for Ca\(^{2+}\) when it is either open or closed. Interestingly, however, the small changes in Ca\(^{2+}\) binding affinity suggested by our analysis (K\(_{b}\) = 7.4 μM → 9.6 μM; K\(_{m}\) = 0.80 μM → 0.65 μM) do appear to be functionally important. We also show that β1 affects the mSlo conductance–voltage relation in the essential absence of Ca\(^{2+}\), shifting it +20 mV and reducing its apparent gating charge 38%, and we develop methods for distinguishing between alterations in Ca\(^{2+}\) binding and other aspects of BK\(_{Ca}\) channel gating that may be of general use.

**Keywords:** BK\(_{Ca}\) channel • mSlo • beta subunit • Ca\(^{2+}\) binding • allosteric model

**Introduction** Several mechanisms have evolved to tune the functional properties of ion channel proteins and thereby the electrical activity of various cell types. Prominent among them is the expression of auxiliary subunits that alter channel gating (Jom et al., 1994; Gurnett and Campbell, 1996; Trimmer, 1998). How auxiliary subunits accomplish this task is in most cases not understood, but finding answers to this question is important both for gaining a better understanding of the relationship between the structure of ion channels and how they function, and for providing information that may be used to design pharmaceuticals that can modulate the many physiological processes in which ion channels play important roles.

One ion channel whose activity is markedly affected by the expression of an auxiliary subunit is the large conductance Ca\(^{2+}\)-activated K\(^{+}\) channel (BK\(_{Ca}\)). In certain tissues, it appears that this channel is composed simply of four identical α subunits surrounding a central pore (Shen et al., 1994; Rothberg and Magleby, 1999). Indeed, cloning and heterologous expression studies have revealed that channels composed of only α subunits display the essential properties of native BK\(_{Ca}\) channels: Ca\(^{2+}\) sensitivity, voltage sensitivity, K\(^{+}\) selectivity, and a large single-channel conductance (Atkinson et al., 1991; Adelman et al., 1992; Butler et al., 1993; Pallanck and Ganetzky, 1994; Tseng-Crank et al., 1994). When BK\(_{Ca}\) channels where purified from bovine smooth muscle, however, two different subunits were shown to be integral membrane proteins. Subsequent cloning and expression of these subunits revealed that while the α subunit forms a functional channel, coexpression of the β subunit increases the sensitivity of the channel to Ca\(^{2+}\) at many membrane voltages (Man et al., 1996; Wallner et al., 1995; Tseng-Crank et al., 1994). The presence of the β subunit therefore appears to explain previous observations that indicated...
that smooth muscle BK_{Ca} channels are more sensitive to Ca^{2+} than are BK_{Ca} channels in many other tissues (McManus, 1991; Tanaka et al., 1997).

Recently, additional BK_{Ca} \beta subunits have been discovered and characterized (Ramanathan et al., 1999; Wallner et al., 1999; Xia et al., 1999; Brenner et al., 2000; Meera et al., 2000; Weiger et al., 2000). Although structurally similar, these novel \beta subunits each alter BK_{Ca} channel gating in distinct ways, and they have different tissue distributions. They thus appear to have evolved to provide tissue-specific tuning of BK_{Ca} channel function, altering such properties as Ca^{2+} sensitivity, voltage-range-of-activation, and activation, deactivation, and inactivation kinetics. In this study, we have analyzed the functional effects of the original \beta subunit, now designated \beta1.

An intriguing property of BK_{Ca} channel gating even in the absence of \beta1 is that in addition to being sensitive to internal Ca^{2+}, these channels are also sensitive to changes in membrane voltage, and there is a synergy between these stimuli. One manifestation of this synergy is a shift in the channel’s conductance-voltage relation toward more negative voltages as internal Ca^{2+} is raised (Marty, 1981; Barrett et al., 1982; Latorre et al., 1982; Methfessel and Boheim, 1982; Wong et al., 1982; Moczydlowski and Latorre, 1983). Another is an apparent increase in the channel’s Ca^{2+} affinity as the membrane voltage is made more positive (Markwardt and Isenberg, 1992; Cui et al., 1997). Recently, we have developed allosteric models that mimic this synergy (Cox et al., 1997a). They do so not by supposing that changes in voltage are directly affecting the binding of Ca^{2+} to the channel in a given conformation, but rather by supposing that the favorable decrease in free energy imparted to the channel’s open conformation by one stimulus lessens the amount of work necessary for the other stimulus to activate the channel. The behavior of these models underscores the possibility that changes in the channel’s apparent Ca^{2+} affinity can be brought about by means other than a direct effect on the channel’s Ca^{2+} binding sites.

How then might the BK_{Ca} \beta1 subunit increase the apparent affinity of the channel for Ca^{2+}? At the single channel level, Nimigean and Magleby (1999, 2000) have demonstrated that \beta1 increases the time the mSlo channel occupies a set of predominately open bursting states, and that simply an increase in all of the channel’s forward Ca^{2+} binding rates cannot account for this behavior. Thus, perhaps something more than just a change in Ca^{2+} binding affinity is occurring. On the other hand, their experiments did not rule out \beta1-induced changes in Ca^{2+} unbinding rates. In fact, Meera et al. (1996) have found that substantial Ca^{2+} (~300 nM) must be present for human \beta1 to shift the hSlo G-V relation toward more negative potentials. This would seem to argue for a direct effect of \beta1 on Ca^{2+} binding or unbinding because when Ca^{2+} is not present, and thus its binding properties are irrelevant, \beta1’s steady state effects are apparently lost.

More generally, we may list at least three mechanisms by which the \beta1 subunit may plausibly increase the BK_{Ca} channel’s apparent Ca^{2+} affinity. It may alter the voltage sensitivity of the channel and thereby indirectly alter the channel’s apparent Ca^{2+} affinity. It may change the energetics of conformational changes that are separate from both voltage sensing and Ca^{2+} binding, or it may in fact change the affinities of Ca^{2+} binding sites. To distinguish between these possibilities, we have examined the gating properties of the mSlo channel (the BK_{Ca} channel cloned from mouse; Butler et al., 1993) in the presence and absence of the \beta1 subunit (bovine) over a wide range of conditions, and analyzed these changes in terms of allosteric models that represent our current understanding of BK_{Ca} channel gating (Cox et al., 1997a; Cui et al., 1997; Horrigan and Aldrich, 1999; Horrigan et al., 1999; Rothberg and Magleby, 1999). The results of this analysis suggest that \beta1 increases the Ca^{2+} sensitivity of the mSlo channel by both altering aspects of gating that do not involve Ca^{2+} binding, and by making subtle but important changes to the channel’s Ca^{2+} binding affinity when it is either open or closed.

**MATERIALS AND METHODS**

**Channel Expression**

The BK_{Ca} \alpha subunit clone (mbr5) was kindly provided by Dr. Larry Salkoff (Butler et al., 1993), and the \beta subunit clone (bovine \beta1) was kindly provided by Dr. Owen McManus (McManus et al., 1995). Both clones were propagated in the Escherichia coli strain Top 10. In vitro transcription was performed with the Message Machine kit with T3 or T7 RNA polymerase (Ambion Inc.). To record macroscopic currents ~0.05–0.5 ng of total cRNA was injected into Xenopus laevis oocytes 2–6 d before recording. \beta1 and \alpha cRNA were mixed in a ratio of 6:1 (\beta1/\alpha) before injection. We found this ratio to be well above that necessary for saturation of \beta1’s effects.

**Electrophysiology**

Electrophysiological recordings were performed essentially as described previously (Cox et al., 1997b). All recordings were done in the inside-out patch clamp configuration (Hamill et al., 1981). Patch pipettes were made of borosilicate glass (VWR Micropipettes). Their tips were coated with wax (Sticky Wax) and fire polished before use. Data were acquired using an Axopatch 200 A patch-clamp amplifier (Axon Instruments, Inc.) in the resistive feedback mode and a Macintosh-based computer system using “Pulse” acquisition software (HEKA Electronik) and the ITC-16 hardware interface (Instrutech). Records were digitized at 20-\mu s intervals (50,000 samples/ s) and low pass filtered at 10 KHz with the Axopatch’s four pole bessel filter. All experiments were carried out at 23°C. Under most conditions, before current records were analyzed and displayed (see, for example, Fig. 6), capacity and leak currents were subtracted using a P/5 leak subtraction.
protocol with a holding potential of −120 mV and voltage steps opposite in polarity to those in the experimental protocol. With the β1 subunit present, there was significant holding current at −120 mV with 39 or 74 μM [Ca²⁺]. Under these conditions, capacity and leak currents were measured by lowering [Ca²⁺] to 0.0005 μM and repeating the experimental protocol. Because of the limited voltage range, no channel activation was observed. The currents recorded with 0.0005 μM [Ca²⁺] were then used for capacity and leak current subtraction. As estimated from the amplitude and decay time constant of capacity currents, the series resistance (Rs) under our experimental conditions was 5–6 MΩ. Typically, 50–90% of Rs was compensated for using the Axopatch 200-A’s Rs compensation circuitry. To increase the signal-to-noise ratio, typically three to six current series were taken consecutively under identical conditions and averaged before analysis and display. Maximum current amplitudes in each patch were typically 1–4 nA. In a series of 45 experiments, 23 without and 22 with the β1 subunit, the means and standard deviations of the current amplitudes recorded with 74 μM [Ca²⁺] at ±150 mV were 2.38 ± 1.19 nA without and 2.44 ± 1.00 nA with the β1 subunit. These means are not statistically significantly different (Students t test, P = 0.84); however, the 95% confidence interval calculated from these data for the difference between mean current amplitudes is 0.06 ± 0.66 nA.

Solutions

Recording solutions were composed of the following (mM): pipette solution: 80 KMeSO₃, 60 N-methyl-glucamine-MeSO₃, 20 HEPES, 2 KCl, 2 MgCl₂, pH 7.20; internal solution: 80 KMeSO₃, 60 N-methyl-glucamine-MeSO₃, 20 HEPES, 2 KCl, 1 HEDTA or 5 EGTA, and CaCl₂ sufficient to give the appropriate free Ca²⁺ concentration, pH 7.20. EGTA (Sigma-Aldrich) was used as the Ca²⁺-buffer for solutions intended to contain <1 μM free Ca²⁺. HEDTA (Sigma-Aldrich) was used as the Ca²⁺-buffer for solutions intended to contain between 1 and 50 μM free Ca²⁺, and no Ca²⁺-chelator was used in solutions intended to contain >50 μM free Ca²⁺. 50 μM (+)-18-crown-6-tetracarboxylic acid (18C6TA) was added to all internal solutions to prevent Ba²⁺ block at high voltages.

The appropriate amount of total Ca²⁺ (100 mM CaCl₂ standard solution; Orion Research Inc.) to add to the base internal solution containing 1 mM HEDTA or 5 mM EGTA to yield the desired free Ca²⁺ concentration was calculated using the program Max Chelator (Bers et al., 1994; available online at www.stanford.edu/~cpton/maxc.html), and the proton and Ca²⁺-binding constants of Bers (supplied with the program) for pH 7.20, T = 23°C, and ionic strength = 0.15. The ability of 18C6TA to chelate Ca²⁺, as well as K⁺ and Ba²⁺, was also considered in these calculations using the following dissociation constants: Ca²⁺ 10⁻⁶ M (Dietrich, 1985), K⁺ 3.3 × 10⁻⁶ M (Dietrich, 1985), and Ba²⁺ 1.6 × 10⁻¹⁰ M (Diaz et al., 1996). For solutions intended to contain 0.5 μM free Ca²⁺ or more, free Ca²⁺ was measured with a Ca²⁺-sensitive electrode (Orion Research Inc.), and the measured value was reported. Free Ca²⁺ measurements were precise to within ~8%. Standard Ca²⁺ solutions for calibration of the Ca²⁺-sensitive electrode were prepared by adding known amounts (1, 10, 100, 1,000, and 10,000 μM) of standard CaCl₂ solution to a solution identical to our base internal solution, except that no chelator was added. Endogenous Ca²⁺ in this solution was estimated from the deviation from linearity of the Ca²⁺-sensitive electrode’s response at 10 μM added Ca²⁺, and was typically ~10 μM. Endogenous Ca²⁺ was then compensated for when making Ca²⁺-buffered solutions. When preparing solutions intended to contain <1 μM free Ca²⁺, the base internal solution was passed through a chelax 100 column before addition of chelator and Ca²⁺. This lowered the endogenous free Ca²⁺ to ~3 μM.

The concentration of Ca²⁺ in the solution bathing the cytoplasmic face of the patch was exchanged using a sewer pipe flow system (DAD 12) purchased from Adams & List Associates, Ltd. To minimize potential systematic variation, all comparisons between mSlo₁ and mSlo₁-β1 currents were done with the same experimental setup and the same solutions. The same batches of oocytes were also often but not always used.

Determination of G-V Curves and Fitting

At [Ca²⁺] = 600 nM, G-V relations were determined from the amplitude of tail currents 200 μs after repolarization to a fixed membrane potential (−80 mV) after voltage steps to the indicated test voltages. Each G-V relation was fitted with a Boltzmann function \[ G = G_{max} / (1 + e^\left(\frac{V - V_{1/2}}{V_t}\right)) \] and normalized to the peak of the fit. At [Ca²⁺] < 600 nM, G-V relations were determined from tail current measurements 200 μs after repolarization to +40 mV. Since these curves did not reach saturation, the percentage of channels active at +200 mV in a given patch was estimated by comparison to current amplitudes recorded from the same patch with 9.1 μM [Ca²⁺], as described in the text (see Fig. 7). Boltzman fits were performed with “Igor Pro” graphing and curve fitting software (WaveMetrics Inc.) using the Levenberg-Marquardt algorithm to perform nonlinear least squares fits.

Model fits to many G-V curves simultaneously were performed with Table-Curve 3D software, which employs an 80-bit Levenberg-Marquardt algorithm (Jandel Scientific) to minimize the sum of squares. When fitting to the 50-state model, several sets of initial parameters were tested. The resulting fits were usually similar, but not always identical. The best fit was then chosen as that which minimized the sum of squares.

Statistical Tests

Unpaired, two-tailed Student’s t tests were used to test for significant differences between the mean V₁/₂ and z values listed in Tables I and II. We tested whether there was a constant shift between mSlo₁ and mSlo₁-β1 mean z*V₁/₂ values over all [Ca²⁺] by fitting the data used to calculate the values plotted in Fig. 5 A (below) with two statistical models. Model 1 assumed that the β1 subunit had a constant effect on z*V₁/₂ regardless of [Ca²⁺]. Model 2 allowed the effects of β1 on z*V₁/₂ to vary with [Ca²⁺]. The two models were fitted to the data using a maximum likelihood criteria. The linear models employed were as follows.

Model 1:(means assumed parallel). For this model, we define the following parameters. A0 represents the expected z*V₁/₂ value in the absence of the β1 subunit at 0.99 μM Ca²⁺. A1 represents the effect of β1 on z*V₁/₂, which in this model is assumed to be the same for all [Ca²⁺]. A2-A6 represent the additional effect of [Ca²⁺] over that observed at 0.99 μM for each measurement depending on the [Ca²⁺] at which a particular measurement was taken. Under this model, the expected value for a particular data point recorded, for example, with the β1 subunit present and 9.1 μM [Ca²⁺], would be A0 (the expected value at 0.99 μM) + A1 (the effect of the β1 subunit) + A4 (the additional effect of 9.1 μM [Ca²⁺] over that caused by 0.99 μM). Using statistical nomenclature, we may write the model then as: expected value at every point i = A0 + A1 I(ab_i) + A2 I(ca_i = 1.86) + A3 I(ca_i = 4.63) + A4 I(ca_i = 9.1) + A5 I(ca_i = 39) + A6 I(ca_j = 74).

Here, as follows from the definitions below, the terms not related to the conditions of a particular measurement evaluate to 0. The following definitions are applied. \( I() \) is the incremental function defined as \( I(x = y) = 1, if x = y \) and \( I(x = y) = 0 \) if \( x \) is not equal to \( y \); \( ab_i = b \) if for the ith observation the β1 subunit was present, \( ab_j = a \) if for the ith observation the β1 subunit was present.
absent, ca_i = 0.99 if for the ith observation [Ca^{2+}] = 0.99, ca_i = 1.86 if for the ith observation [Ca^{2+}] = 1.86, ca_i = 4.63 if for the ith observation [Ca^{2+}] = 4.63, and so on.

Model 2: (means not assumed parallel). The likelihood of each individual observation was calculated from the expected value under the model at the appropriate condition and the estimated variance of the mean at each point. Model 2 may be written as: expected value of p_i \[ 5 \] (ab_i = b) \[ 5 \] A1 (the additional effect of 9.1 \[ 5 \] m) \[ 5 \] M \[ 5 \] [Ca^{2+}] \[ 5 \] M over that observed at 0.99 if for the ith observation [Ca^{2+}] \[ 5 \] b) \[ 5 \] A7–A11 are parameters of the model. The influence that internal Ca^{2+} and membrane voltage have on the steady state open probability of mSlo_{alpha} is often displayed as a series of conductance-voltage curves determined at several internal Ca^{2+} concentrations ([Ca^{2+}]) (Fig. 1 A). The curves in Fig. 1 A correspond to [Ca^{2+}] ranging from \( \sim 1 \) to \( \sim 75 \) \( \mu \)M. As [Ca^{2+}] is raised, the mSlo_{alpha} G-V curve moves leftward along the voltage axis. When the \( \beta \)1 subunit is coexpressed with \( \alpha \), a similar Ca^{2+}-dependent shifting is observed (Fig. 1 B). The G-V curves determined with \( \beta \)1 present, however, differ from those determined with \( \alpha \) alone in that each mSlo_{alpha} + \( \beta \)1 G-V curve lies to the left of the corresponding mSlo_{alpha} curve. Over this [Ca^{2+}] range, at every combination of [Ca^{2+}]...

| [Ca]i | mSlo_{alpha} | n | mSlo_{alpha} + \( \beta \)1 | n |
|-------|--------------|---|----------------|---|
| \( \mu \)M | mV | mV |
| 0.99 | 117.0 ± 11.7 | 10 | 79.3 ± 8.8 | 10^6 |
| 1.9 | 102.0 ± 6.5 | 9 | 24.7 ± 13.0 | 6^3 |
| 4.6 | 65.8 ± 7.6 | 14 | −6.4 ± 12.3 | 11^1 |
| 9.1 | 51.8 ± 8.1 | 15 | −28.1 ± 17.7 | 13^1 |
| 39 | 29.5 ± 8.0 | 12 | −90.0 ± 8.0 | 9^6 |
| 74 | 21.7 ± 8.5 | 12 | −95.9 ± 9.0 | 9^6 |

| z | mSlo_{alpha} | n | mSlo_{alpha} + \( \beta \)1 | n |
|---|--------------|---|----------------|---|
| 1.32 ± 0.129 | 10 | 1.03 ± 0.093 | 10^6 |
| 1.27 ± 0.120 | 9 | 1.29 ± 0.198 | 6 |
| 1.32 ± 0.130 | 14 | 1.15 ± 0.101 | 11^4 |
| 1.24 ± 0.101 | 15 | 1.09 ± 0.184 | 13^4 |
| 1.14 ± 0.090 | 12 | 1.08 ± 0.155 | 9 |
| 1.12 ± 0.129 | 12 | 0.92 ± 0.108 | 9^6 |

*\( n \), number of experiments used to determine each value. \( \dagger \)Values are given as mean ± SD. \( \ddagger \)Statistically significant differences between mSlo_{alpha} and mSlo_{alpha} + \( \beta \)1 means (Student’s t test, \( P < 0.05 \)).

The test was implemented with S-PLUS 2000 software (Mathsoft), and it gave a chi-square value of 38.236 on five degrees of freedom.

**Results**

We will refer to channels composed of \( \alpha \) subunits alone as mSlo_{alpha} and those composed of both \( \alpha \) and \( \beta \)1 subunits as mSlo_{alpha} + \( \beta \)1. The influence that internal Ca^{2+} and membrane voltage have on the steady state open probability of mSlo_{alpha} is often displayed as a series of conductance-voltage curves determined at several internal Ca^{2+} concentrations ([Ca^{2+}]) (Fig. 1 A). The curves in Fig. 1 A correspond to [Ca^{2+}] ranging from \( \sim 1 \) to \( \sim 75 \) \( \mu \)M. As [Ca^{2+}] is raised, the mSlo_{alpha} G-V curve moves leftward along the voltage axis. When the \( \beta \)1 subunit is coexpressed with \( \alpha \), a similar Ca^{2+}-dependent shifting is observed (Fig. 1 B). The G-V curves determined with \( \beta \)1 present, however, differ from those determined with \( \alpha \) alone in that each mSlo_{alpha} + \( \beta \)1 G-V curve lies to the left of the corresponding mSlo_{alpha} curve. Over this [Ca^{2+}] range, at every combination of [Ca^{2+}]...
and voltage, the channels are more active when \( \beta_1 \) is present. This effect was first demonstrated by McManus et al. (1995). Notice also that the mSlo\(_a+\beta_1\) G-V curves are more widely spaced than are the mSlo\(_a\) curves. As shown in Fig. 1 C, when half-maximal-activation-voltage (\( V_{1/2}\)) is plotted as a function of [Ca\(^{2+}\)], the wider spacing of the mSlo\(_a+\beta_1\) curves creates a function with a steeper slope.

Perhaps less apparent, the mSlo\(_a+\beta_1\) G-V curves are also somewhat less steep than are the mSlo\(_a\) curves. In Fig. 1, A and B, each G-V curve represents the average of many experiments (for \( n \) values, see Table I). These curves were fitted with Boltzmann functions (solid curves), and the parameters of these fits are listed in the legend. At each [Ca\(^{2+}\)], the Boltzmann parameter \( z \), which is a measure of the voltage sensitivity of the channel, is smaller for mSlo\(_a+\beta_1\) than it is for mSlo\(_a\) on average by 14%. When mean parameter values from each experiment fitted individually are examined (Table I), a similar decline in voltage sensitivity is observed at all [Ca\(^{2+}\)] except 1.9 \( \mu \)M, although the small decline at 39 \( \mu \)M is not clearly statistically significant. Over all, however, looked at in this way, the average decline in \( z \) is 11%.

The effects of the \( \beta_1 \) subunit can also be seen from the point of view of changing [Ca\(^{2+}\)] at constant voltage (Fig. 2). Looked at in this way, at many voltages \( \beta_1 \) increases the channel’s apparent Ca\(^{2+}\) affinity. For example at +60 mV the apparent Ca\(^{2+}\) affinity of mSlo\(_a\), as judged by the concentration at which the channel’s Ca\(^{2+}\) dose-response curve is half maximal ([Ca\(^{2+}\)\(_{1/2}\)], is 3.8 \( \mu \)M, while for mSlo\(_a+\beta_1\) it is 1.2 \( \mu \)M. At 0 mV, [Ca\(^{2+}\)]\(_{1/2}\) for mSlo\(_a\) is 32.8 \( \mu \)M, while for mSlo\(_a+\beta_1\) it is 3.4 \( \mu \)M. Since the G-V curves in Fig. 1 and the dose-response curves in Fig. 2 represent different ways of looking at the same steady-state gating behavior, the effects of the \( \beta_1 \) subunit illustrated in these figures are manifestations of the same underlying process.

An Interpretation Using a Simple Model

What aspects of gating does the \( \beta_1 \) subunit alter to bring about the effects described above? Because a primary effect of \( \beta_1 \) is to alter the position of the mSlo G-V curve at many [Ca\(^{2+}\)], to answer this question it seems important to understand what determines this curve’s position. In general, the position of the G-V curve of any voltage-gated channel is determined by the free energy difference between open and closed states in the absence of an applied voltage and the degree to which an applied voltage alters this energy difference. This later factor is itself influenced by many factors including: the number of gating charges the channel has and how far each can move through the membrane’s electric field, the degree to which these charges move in concert, and the degree to which the change in energy associated with each moving charge
Regulation of BKCa by its β1 Subunit

affects the energy difference between open and closed. Without some hypothesis about the physical situation, then, it is difficult to relate changes in the channel's G-V relation to changes in specific aspects of gating.

Recently, however, we have shown that models of the form of Scheme I (Fig. 3) can mimic many aspects of mSlo gating including: the leftward shifting of the mSlo G-V curve as \([\text{Ca}^2+]\) is raised, an apparent increase in \([\text{Ca}^2+]\) affinity as the membrane voltage is made more positive, near maximal activation with strong depolarizations at very low \([\text{Ca}^2+]\), and many properties of the kinetics of mSlo macroscopic currents (Cui et al., 1997; Cox et al., 1997a). The dashed lines in Fig. 1 A represent a fit with a model of this form. While Scheme I cannot account for all mSlo gating properties (see below and Cox et al., 1997a; Stefani et al., 1997; Horrigan and Aldrich, 1999; Horrigan et al., 1999; Rothberg and Magleby, 1999), the success of this scheme in many respects suggests that it can be useful, at least initially, as a guide for interpreting the effects of the β1 subunit in terms of changes in specific aspects of mSlo gating. We have discussed the properties of models of the form of Scheme I previously (Cox et al., 1997a). We review them here in so far as they relate to the analysis that follows.

Scheme I supposes the following. The channel exists in two conformations, open and closed. The equilibrium between open and closed conformations is voltage dependent, with depolarization favoring opening. The channel is a homotetramer containing four Ca\(^{2+}\) binding sites, one on each subunit. Ca\(^{2+}\) can bind to both the open and closed conformations of the channel but on average binds better to the open conformation, and thereby promotes opening. The simplest form of Scheme I (wherein in a given conformation each binding site binds Ca\(^{2+}\) equivalently and independently) corresponds to a voltage-dependent version of the well known Monod-Wyman-Changeux (MWC) model of allosteric proteins (Monod et al., 1965). Its open probability as a function of \([\text{Ca}^{2+}]\) and voltage \(V\) is given by:

\[
P_{\text{open}} = \frac{1}{1 + \left(\frac{1 + [\text{Ca}]/K_C}{1 + [\text{Ca}]/K_C} \right) L(0) e^{-QFV/RT}}
\]

where \(L(0)\) represents the open-to-closed equilibrium constant in the absence of an applied voltage, a measure of the intrinsic energetics of channel opening. \(Q\) represents the equivalent gating charge associated with this equilibrium, \(K_C\) the closed-conformation Ca\(^{2+}\) dis-
association constant, and $K_0$ the open-conformation Ca$^{2+}$ dissociation constant (F, R, and T have their usual meanings). Thus, four parameters determine the behavior of the model at equilibrium and, as illustrated in Fig. 4, changes in any of these parameters can affect leftward G-V shifts at many [Ca$^{2+}$]. Of interest to us, however, is which of these changes mimic the effects of the BKCa $\beta 1$ subunit.

The position of the voltage-dependent (VD) MWC model’s G-V relation on the voltage axis, as indexed by its half-maximal activation voltage ($V_{1/2}$) is given by:

$$V_{1/2} = \frac{4RT}{QF} \ln \left( \frac{1 + [Ca]/K_C}{1 + [Ca]/K_O} \right) + \frac{RT}{QF} \ln[L(0)] \tag{2}$$

and the derivative of Eq. 2 with respect to [Ca$^{2+}$] is:

$$\frac{dV_{1/2}}{d[Ca]} = \frac{4(K_C - K_O)RT}{F(([Ca] + K_C)([Ca] + K_O)Q)} \tag{3}$$

Interestingly, this derivative is not a function of $L(0)$. This means that according to the VD-MWC model alterations in the intrinsic energy difference between open and closed states will affect G-V position to the same extent at each [Ca$^{2+}$], and if the $\beta 1$ subunit were only affecting this parameter, there would be no change in the slope of a plot of $V_{1/2}$ vs. [Ca$^{2+}$]. This is illustrated in Fig. 4 B. Here, a simulated decrease in L(0) causes equivalent leftward G-V shifts at each [Ca$^{2+}$] (compare Fig. 4, B with A), with the spacing between the curves maintained. On a plot of $V_{1/2}$ vs. [Ca$^{2+}$], this appears as a downward translation along the $V_{1/2}$ axis with no change in slope (see Fig. 4 F). In contrast to this prediction, we see in Fig. 1 C that $\beta 1$ not only shifts the mSlo channel’s $V_{1/2}$ vs. [Ca$^{2+}$] relation downward, but it also increases its steepness. That is, the magnitude of the $\beta 1$-induced G-V shift increases with increasing [Ca$^{2+}$]. This suggests that the effects of $\beta 1$ are more complicated than just an alteration in the intrinsic energy difference between open and closed. The general downward movement of the channel’s $V_{1/2}$ vs. [Ca$^{2+}$] relation, however, does suggest that this energy difference may be decreased by $\beta 1$ in combination with changes in other aspects of gating.

According to Eq. 3, an increase in the steepness of a plot of $V_{1/2}$ vs. [Ca$^{2+}$] can come about either by
changes in the channel’s \( \text{Ca}^{2+} \) binding constants or by a decrease in its voltage sensitivity. An example of the former possibility is shown in Fig. 4 C. Here, raising \( K_c \) in our simulation produces leftward G-V shifts when \( [\text{Ca}^{2+}] \) is present, with larger shifts at higher \( [\text{Ca}^{2+}] \) (compare Fig. 4, C with A). At many voltages, this would appear as an increase in apparent \( \text{Ca}^{2+} \) affinity. At 0 mV, for example, the model channel is only 28% activated at 10 \( \mu M \) \( [\text{Ca}^{2+}] \) when \( K_c \) equals 10 \( \mu M \), but 78% activated at the same \( [\text{Ca}^{2+}] \) when \( K_c \) equals 100 \( \mu M \). Thus, by decreasing the channel’s true \( \text{Ca}^{2+} \) binding affinity in the closed conformation, we can, in fact, create an apparent increase in \( \text{Ca}^{2+} \) affinity. This underscores the sometimes counterintuitive nature of even simple allosteric systems and highlights the need for a mechanistic and mathematical framework for interpreting alterations in their behavior. In this case, the increase in the model’s apparent \( \text{Ca}^{2+} \) affinity with increasing \( K_c \) arises because as \( K_c \) and \( K_o \) become farther apart, \( \text{Ca}^{2+} \) binding has a more powerful effect on the model’s closed-to-open equilibrium. This can be seen from Eq. 3, where \( \frac{dV_{1/2}}{d[\text{Ca}]} \) always depends on the difference between \( K_c \) and \( K_o \) such that as these constants are separated, larger effects of \( \text{Ca}^{2+} \) are expected. Furthermore, because \( \frac{dV_{1/2}}{d[\text{Ca}]} \) is a function of both \( K_c \) and \( K_o \), any change in either of these constants will affect the steepness of the model’s \( V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) relation.

Eq. 3 also states that the steepness of the model’s \( V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) relation is inversely related to its equivalent gating charge \( Q \). This relation is therefore expected to become steeper as \( Q \) decreases and the model’s G-V curves become more shallow (Cox et al., 1997a; Cui, J., and R.W. Aldrich, manuscript in preparation). This is illustrated in Fig. 4, D and F, where we have decreased \( Q \) in our simulation from 1.4 to 0.7 and observe an increase in \( G-V \) spacing (Fig. 4, D) and thus an increase in \( V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) slope (Fig. 4, F). Thus, some of the increased steepness of the mSlo channel’s \( V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) relation may be due to the general decrease in voltage sensitivity evident upon \( \beta1 \) coexpression (see Fig. 1).

According to Scheme I (Fig. 3), however, we can eliminate changes in voltage sensitivity (and thus G-V steepness) from consideration if we plot \( Q^*V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) rather than \( V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) (Cox et al., 1997a; Cui, J., and R.W. Aldrich, manuscript in preparation). This can be seen by rearranging Eq. 2 to:

\[
QV_{1/2} = \frac{4RT}{F} \ln \left( \frac{(1 + [\text{Ca}]/K_c)}{(1 + [\text{Ca}]/K_o)} \right) + \frac{RT}{F} \ln[L(0)],
\]

and again taking the derivative with respect to \( [\text{Ca}^{2+}] \):

\[
\frac{\partial Q}{\partial [\text{Ca}]} V_{1/2} = \frac{4(K_c - K_o)RT}{F([\text{Ca}] + K_c)([\text{Ca}] + K_o)}.
\]

This derivative is independent of both \( L(0) \) and \( Q \) and depends only on the model’s \( \text{Ca}^{2+} \) binding constants. According to the VD-MWC model, then, a plot of \( Q^*V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) will only change shape upon \( \beta1 \) coexpression, if \( \beta1 \) is actually altering \( \text{Ca}^{2+} \) binding site affinities. This is illustrated in Fig. 4 G, where \( Q^*V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) is plotted for the simulations in Fig. 4, A–E. Furthermore, although we have come to this conclusion based on the mathematics of the VD-MWC model (Eqs. 1–5), it is possible to show that this conclusion holds for all two-tiered models similar in form to Scheme I (Cox et al., 1997a). It therefore does not depend on the simplifying, MWC assumption that in either channel conformation all \( \text{Ca}^{2+} \) binding steps are equivalent. Nor is it necessary to suppose that the channel has any particular number of \( \text{Ca}^{2+} \) binding sites. All that is required is a central voltage-dependent conformational change allosterically modulated by \( \text{Ca}^{2+} \) binding (Cox et al., 1997a).

To apply this test to the effects of the BKCa \( \beta1 \) subunit, we have multiplied each \( V_{1/2} \) value in Table I by its corresponding \( z \) value and plotted \( z^*V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) for both mSlo and mSlo, + \( \beta1 \) in Fig. 5 A. Comparing the resulting relations to those in Fig. 1 C, we see that, in fact, a good deal of the difference in steepness between the mSlo, \( V_{1/2} \) vs. \( [\text{Ca}^{2+}] \) relation and the corresponding mSlo, + \( \beta1 \) relation can be accounted for by \( \beta1 \)–induced changes in voltage sensitivity. That is, the two relations in Fig. 5 A appear close to parallel. This result suggests that much of \( \beta1 \)’s steady state effects can be accounted for by alterations in aspects of gating separate from \( \text{Ca}^{2+} \) binding. In particular, it appears that \( \beta1 \) decreases the intrinsic energy difference between open and closed, thus shifting all G-V curves leftward and, in addition, \( \beta1 \) decreases the channel’s equivalent gating charge, thus expanding their spacing.

To facilitate comparison of the relations plotted in Fig. 5 A, in B we have shifted the mSlo, + \( \beta1 \) relation upward along the \( z^*V_{1/2} \) axis, so that at 9.1 \( \mu M \) \( [\text{Ca}^{2+}] \) the two relations have the same value. Comparing the two relations in this way we see their similar shape. It is also clear, however, that the mSlo, + \( \beta1 \) relation has a somewhat higher value than expected at 0.99 \( \mu M \) \( [\text{Ca}^{2+}] \), if the \( \beta1 \) subunit where to have no effect on \( \text{Ca}^{2+} \) binding, and it has somewhat lower values than expected at 39 and 74 \( \mu M \). Also, as the data are plotted in Fig. 5 B, the standard errors of the mean for the \( z^*V_{1/2} \) values at 0.99 and 39 \( \mu M \) \( [\text{Ca}^{2+}] \) (indicated on the figure) are small compared with the differences between mSlo, and mSlo, + \( \beta1 \) \( z^*V_{1/2} \) values at these \( [\text{Ca}^{2+}] \). Thus, the differences in shape between the two curves in Fig. 5 A may not be due to random variation in our experiments.

To examine this issue more rigorously, we performed a statistical test on the data used to calculate the values plotted in Fig. 5 A. We used a likelihood ratio test and...
and are often smaller than the plot symbols. The dashed lines in this figure represent the least squares best fit. Examining the parameters of this fit, and comparing them with those that best fit the G-V curves in Fig. 1A, we see that to accommodate the changes produced by $\beta_1$ the model, as expected, decreases $L(0)$ (1.263 → 281.3) and $Q$ (1.2 → 1.0). In addition, however, small changes in $K_C(1.24 → 0.82 \mu M)$ and $K_C(6.0 → 9.82 \mu M)$ are also observed, changes that together increase the ratio ($K_C/K_\alpha$) and thereby also tend to expand G-V spacing. Interestingly, similar conclusions are also arrived at when these data are analyzed in terms of a more complex model of BK$_{Ca}$ gating discussed below.

The Effects of the $\beta_1$ Subunit at Subnanomolar [Ca$^{2+}$]

The above analysis suggests that although the $\beta_1$ subunit may alter Ca$^{2+}$ binding-site affinities to a small degree (less than twofold in either conformation), much of its steady state effects are on aspects of gating that are separate from Ca$^{2+}$ binding. If this is in fact the case, then some $\beta_1$ effects should be apparent at subnanomolar [Ca$^{2+}$], where channel gating is Ca$^{2+}$ independent (Meera et al., 1996; Cui et al., 1997). Recently, however, Meera et al. (1996) have found that at subnanomolar [Ca$^{2+}$] there is essentially no change in the hSlo G-V relation upon $\beta_1$ coexpression. They concluded that at very low [Ca$^{2+}$] there is “functional uncoupling” between $\alpha$ and $\beta$ subunits. They called this functional uncoupling a “Ca$^{2+}$ switch.” What this means in a physical sense is unclear. One possibility is that the two subunits become physically disassociated at low [Ca$^{2+}$]. We know this is not the case, however, because the $\beta_1$ subunit has effects on the kinetics of mSlo gating discussed below.

To investigate this issue further, we examined mSlo$_{\alpha}$ and mSlo$_{\alpha+\beta_1}$ currents at subnanomolar [Ca$^{2+}$]. In Fig. 7A is plotted mean I-V relations determined from current families like those shown in Fig. 6 recorded with 0.5 nM [Ca$^{2+}$]. Each current measurement was made at the end of a 50-ms voltage step to the indicated test voltage. Each I-V curve was then normalized to its value at +200 mV, and the resulting curves were averaged. Two properties of these curves are of interest. First,
Regulation of BKCa by its \( \beta_1 \) Subunit

In the presence of \( \beta_1 \), it takes high voltages to activate the mSlo channel, at least \(-80\) mV. And second, the two curves do not superimpose. The mSlo \( \alpha_1 \) I-V curve is more shallow than the mSlo \( \alpha \) curve. If \( \beta_1 \) were having no effect on the steady state activation of mSlo, then we would expect the shapes of their I-V curves to be the same. This is true regardless of the absolute degree to which the channels are activated by \(+200\) mV. Thus, there does appear to be an effect of the \( \beta_1 \) subunit on mSlo steady state gating at subnanomolar Ca\(^{2+}\).

To examine this effect further, we determined mSlo \( \alpha_1 \) and mSlo \( \alpha_1 + \beta_1 \) G-V curves under these low [Ca\(^{2+}\)] conditions. This was done by plotting tail current amplitude vs. test voltage for several current families like those shown in Fig. 6. Each plot was then normalized to its value at \(+200\) mV and then multiplied by the following ratio: the current recorded with 0.5 nM [Ca\(^{2+}\)] at the end of a step to \(+200\) mV, divided by the current recorded with 9.1 \( \mu \)M [Ca\(^{2+}\)] under the same conditions. This procedure estimates the percentage of channels that are active at \(+200\) mV by comparing currents recorded from the same patch at 9.1 \( \mu \)M and 0.5 nM [Ca\(^{2+}\)]. It was necessary because at \(-0.5\) nM [Ca\(^{2+}\)] neither G-V curve reached saturation by \(+200\) mV, and higher voltages often caused the patches to break. Mean G-V curves determined in this way are shown in Fig. 7 B. Most evident is a \( \beta_1 \)-induced reduction in voltage sensitivity. Both curves have been fitted with Boltzmann functions with \( z \) values of 0.98 and 0.61 for mSlo \( \alpha_1 \) and mSlo \( \alpha_1 + \beta_1 \), respectively. Similar to the observations of Meera et al. (1996), however, the \( V_{1/2} \) values of these fits are roughly similar (\(+180\) mV for mSlo \( \alpha_1 \) and \(+200\) mV for mSlo \( \alpha_1 + \beta_1 \)), although, statistically, the observed 20-mV difference is significant (\( P < 0.02 \)).

At first glance, the relatively small and in fact right-shifting effect of the \( \beta_1 \) subunit on mSlo’s half-maximal activation voltage at subnanomolar [Ca\(^{2+}\)] seems to suggest that much of its ability to cause leftward G-V shifts at higher [Ca\(^{2+}\)] is due to its effects on Ca\(^{2+}\) binding, and thus not evident in the essential absence of Ca\(^{2+}\). Upon reflection, however, it becomes clear that the reduction in apparent gating charge induced by \( \beta_1 \)
at subnanomolar \([\text{Ca}^{2+}]\) would, if left unchecked, shift the mSlo\(_{\alpha+b1}\) \(V_{1/2}\) far rightward. Consider, for example, a channel behaving according to Scheme I with a single voltage-dependent step between closed and open. In this case, in the absence of \(\text{Ca}^{2+}\), the system reduces to two states, and we may write:

\[
V_{1/2} = \frac{\Delta G_i}{zF},
\]

where \(\Delta G_i\) represents the intrinsic standard free energy difference between open and closed. Thus, according to Eq. 6, a 50% reduction in \(z\) would be expected to cause a twofold increase in \(V_{1/2}\), and a reduction in \(z\) from 0.98 to 0.61, as is observed upon \(b1\) coexpression, would be expected to shift \(V_{1/2}\) from its mSlo\(_{\alpha}\) value of +180 mV to +294 mV, a difference of 114 mV. We, however, see only a +20-mV rightward shift at subnanomolar \([\text{Ca}^{2+}]\) upon \(b1\) coexpression. This suggests that some compensating effect must be present. In fact, it suggests that in addition to reducing the channel’s voltage sensitivity, \(b1\) decreases \(\Delta G_i\). This would act to oppose the right shift expected to accompany a decrease in voltage sensitivity, and thus it could maintain the mSlo\(_{\alpha+b1}\) \(V_{1/2}\) close to that of mSlo\(_{\alpha}\), while allowing G-V steepness to decrease. Interestingly, however, according to Scheme I, over a range of \([\text{Ca}^{2+}]\), a decrease in \(\Delta G_i\) [which amounts to a decrease in the parameter \(L(0)\) in Scheme I] would be expected to cause equivalent leftward G-V shifts at each \([\text{Ca}^{2+}]\), and a decrease in voltage sensitivity would be expected to expand G-V spacing. Thus, together these effects could in fact produce little change in \(V_{1/2}\) at subnanomolar \([\text{Ca}^{2+}]\), but large leftward G-V shifts at higher \([\text{Ca}^{2+}]\). This is illustrated for the VD-MWC model in Fig. 4 E, and it is qualitatively similar to what is observed in the data (Fig. 1). Thus, our recordings at subnanomolar \([\text{Ca}^{2+}]\) demonstrate an effect of the \(b1\) subunit on the mSlo G-V relation under this condition, and, in fact, they qualitatively support the analysis above based on recordings with higher \([\text{Ca}^{2+}]\).

The \(b1\) Subunit Has Little Effect on the Affinity of the Channel for \(\text{Ca}^{2+}\) when It Is Open

Because \(\text{Ca}^{2+}\) binding promotes BK\(_{Ca}\) channel opening, thermodynamic principles dictate that the average affinity of the channel’s binding sites for \(\text{Ca}^{2+}\) will be higher when it is open than when it is closed. This suggests that if we make the channel’s open probability most energetically sensitive in the absence of \(\text{Ca}^{2+}\) (that is, bring it to \(V_{1/2}\)) and then see how much \(\text{Ca}^{2+}\) it takes to just begin to activate the channels further, this critical \([\text{Ca}^{2+}]\) \([\text{Ca}^{2+}]_{\text{critical}}\) will depend primarily on the affinity of the channel for \(\text{Ca}^{2+}\) when it is open (\(K_o\) in the VD-MWC model). In fact, the mathematics of the VD-MWC model support this conclusion. For the VD-MWC model, the relationship between \(V_{1/2}\) and \([\text{Ca}^{2+}]\) is given by Eq. 2. Because \(K_c\) is necessarily significantly larger than \(K_o\), when \([\text{Ca}^{2+}] < K_c\), Eq. 2 may be approximated by:

\[
V_{1/2} = \frac{\Delta G_i}{zF} 
\]

Figure 7. Effects of the \(b1\) subunit at subnanomolar \([\text{Ca}^{2+}]\). (A) Current–voltage curves for mSlo\(_{\alpha}\) (\(\bullet\), \(n = 11\)) and mSlo\(_{\alpha+b1}\) (\(\bigcirc\), \(n = 8\)) determined from patches superfused with a solution buffered to 0.5 nM \([\text{Ca}^{2+}]\). Each current measurement was made at the end of a 50-ms voltage step to the indicated test voltage. Each curve has been normalized to their values at +200 mV. (B) G-V relations were determined as described in the text from the same data as used to generate the I-V plots in A. Smooth curves represent Boltzmann fits. Parameters of the fits are: mSlo\(_{\alpha}\) \(V_{1/2} = 180\) mV, \(z = 0.98\), mSlo\(_{\alpha+b1}\) \(V_{1/2} = 200\) mV, \(z = 0.61\). (C) In each experiment, data was acquired at both 9.1 \(\mu\)M (squares) and 0.5 nM \([\text{Ca}^{2+}]\) (circles). G-V plots for both concentrations are shown in C. In A–C, error bars indicate SEM.
\[ V_{1/2} = \frac{4RT}{QF} \ln \left\{ \frac{1}{(1 + [Ca]/K_O)} \right\} + \frac{RT}{QF} \ln[L(0)], \quad (7) \]

Eq. 7 states that at [Ca\(^{2+}\)] substantially less than K\(_O\) (less than \(\sim\)5% of K\(_O\), depending on Q), small changes in [Ca\(^{2+}\)] will not affect \(V_{1/2}\). This is in fact what is observed experimentally (see below and Meera et al., 1996). As [Ca\(^{2+}\)] is raised, however, at some point it will become appreciable relative to K\(_O\) and \(V_{1/2}\) will start to move leftward. Furthermore, this [Ca\(^{2+}\)] critical will not depend on L(0), which adds a constant term to \(V_{1/2}\), and it will depend only weakly on Q, which influences the magnitude of the shift but not the ratio of [Ca\(^{2+}\)] to K\(_O\). This is illustrated graphically in Fig. 8. Here we have calculated \(V_{1/2}\) vs. [Ca\(^{2+}\)] plots for the VD-MWC model using parameters similar to those used to fit the mSlo\(_a\) data in Fig. 1 A. In Fig. 8 A, the model's open-conformation Ca\(^{2+}\) dissociation constant (K\(_O\)) is varied and we see large changes in [Ca\(^{2+}\)] critical. In Fig. 8 B, however, when K\(_C\) is varied, we see very little effect on [Ca\(^{2+}\)] critical. Similarly, in Fig. 8 C we also see very little effect on [Ca\(^{2+}\)] critical when L(0) or Q are varied. Thus, the [Ca\(^{2+}\)] at which the VD-MWC model channel's G-V relation just starts to move leftward is determined almost exclusively by its affinity for Ca\(^{2+}\) when it is open. As shown in Fig. 11 (below), we have also found this to be true for a more complex BK\(_{Cs}\) gating model.

While it is difficult to prove this result for all classes of reasonable models, it seems intuitively clear that the concentration at which a ligand begins to allosterically affect a central conformational change will be determined primarily by the affinity of the system when it is most sensitive to ligand given that two requirements are met. (a) In the absence of ligand, the central equilibrium constant is close to 1. For mSlo, we can force this to be true by adjusting the voltage to \(V_{1/2}\). And (b), there is a substantial difference in affinity between active and inactive forms. This requirement, however, is simply necessary for the ligand to have any appreciable effect at all. With the mSlo channel, therefore, if the \(\beta1\) subunit is altering the affinity of the channel for Ca\(^{2+}\) when it is open, we would expect to see this as a change in [Ca\(^{2+}\)] critical.

We have taken two approaches to examining the effects of \(\beta1\) on [Ca\(^{2+}\)] critical. To rapidly determine the effects of [Ca\(^{2+}\)] on channel activity over a range of voltages, we applied voltage ramps from -150 to +150 mV at the following series of [Ca\(^{2+}\)]: 0.5, 2, 10, 50, 100, and 660 nM, and 9.1 \(\mu\)M. Data from a patch presenting mSlo\(_a\) channels are shown in Fig. 9 A. As is evident, there is very little consistent effect of Ca\(^{2+}\) on channel activity at concentrations below 100 nM, a small effect at 100 nM, and then a large effect at 660 nM. The [Ca\(^{2+}\)] critical for mSlo therefore appears to be close to 100 nM. Interestingly, the same experiment produces a very similar pattern with mSlo\(_a + \beta1\) (Fig. 9 B), again no consistent effect below 100 nM, a small effect at 100 nM, and then a large effect at 660 nM. To quantify these results, we measured current amplitudes at +150 mV over the same [Ca\(^{2+}\)] range for a series of mSlo\(_a\) and mSlo\(_a + \beta1\) patches, divided each patch’s amplitudes by that recorded at 9.1 \(\mu\)M [Ca\(^{2+}\)], and then plotted this ratio as a function of [Ca\(^{2+}\)]. The results are shown in Fig. 9 E. For a large number of both mSlo\(_a\) and mSlo\(_a + \beta1\) patches, [Ca\(^{2+}\)] critical appears close to 100 nM, with a large effect of Ca\(^{2+}\) at 660 nM.

To examine more rigorously the effects of the \(\beta1\) sub-
unit on [Ca\textsuperscript{2+}]\textsubscript{critical}, we recorded current families at each [Ca\textsuperscript{2+}] and determined G-V relations as described above with reference to Fig. 7. The results of Boltzmann fits to these data are listed in Table II, and averaged G-V curves are shown in Fig. 9, C and D. Again, as is consistent with published data (Meera et al., 1996), we see very little consistent effect of Ca\textsuperscript{2+} on both mSlo\textsubscript{a} and mSlo\textsubscript{a,\beta1} G-V relations at low nanomolar Ca\textsuperscript{2+}, perhaps a small effect at 100 nM, and then a large effect at 660 nM [Ca\textsuperscript{2+}]. Mean $V_{1/2}$ values from a series of experiments are plotted for both mSlo\textsubscript{a} and mSlo\textsubscript{a,\beta1} in Fig. 9 F. Although at 660 nM [Ca\textsuperscript{2+}] mSlo\textsubscript{a,\beta1} responds with a larger leftward shift than does mSlo\textsubscript{a} (likely reflecting mSlo\textsubscript{a,\beta1}'s weaker voltage dependence), clearly the Ca\textsuperscript{2+} concentrations at which the two channels begin to respond to Ca\textsuperscript{2+} are essentially the same. This suggests that, when open, the affinity of the channel for Ca\textsuperscript{2+} is minimally affected by $\beta1$, or perhaps not affected at all. To estimate $K_0$ for the two channel types, we fit the plots in Fig. 9 F with Eq. 7. These fits yielded $K_0$ estimates of 0.84 and 0.68 $\mu$M for mSlo\textsubscript{a} and mSlo\textsubscript{a,\beta1} channels, respectively. Thus, a small (19%) change in $K_0$ is suggested.

Analysis in Terms of a More Complex Model

Although Scheme I–based models mimic many properties of BK\textsubscript{Ca} channel gating, there are some properties that they do not reproduce. In particular, gating current measurements indicate that the majority of mSlo\textsubscript{a}’s gating charge moves before channel opening rather with the same time course as opening, as is predicted by
Scheme I (Stefani et al., 1997; Horrigan and Aldrich, 1999). Also, mSlo, has a complex relationship between its deactivation time constant and membrane voltage that is inconsistent with this simple scheme (Cox et al., 1997a; Horrigan et al., 1999). Recently, Horrigan et al. (1999) performed a detailed analysis of the macroscopic gating behavior of BK_{ca} channels in the essential absence of Ca^{2+}. Based on their results, they proposed that, rather than there being just a single voltage-dependent step between opened and closed, voltage sensors in each subunit move rapidly in response to changes in membrane voltage regardless of whether the channel is open or closed, and that this movement, in an allosteric manner, favors but is not required for channel opening (Horrigan et al., 1999; Horrigan and Aldrich, 1999). This idea, coupled with the assumption of a weak voltage dependence associated with the central opening conformational change, is represented by Scheme II (Fig. 10 A). Here, horizontal transitions represent voltage sensor movement with an X indicating a voltage sensor in its active conformation, and vertical transitions represent channel opening. Interestingly, Horrigan et al. (1999) showed that Scheme II can account well for the properties of mSlo, macroscopic gating and ionic currents in the essential absence of Ca^{2+}, and they proposed that this scheme, coupled with the allosteric mechanism by which Ca^{2+} affects channel gating in Scheme I, may also account for BK_{ca} gating at higher [Ca^{2+}]. This idea in its simplest form is represented by Scheme III (Fig. 10 B). In Scheme III, the subset of states represented by the 2 × 5 face at the front is equivalent to Scheme I, and the subset of states represented by the 2 × 5 face at the left edge is equivalent to Scheme II. Scheme III has also been recently proposed by Rothberg and Magleby (1999) to account for the gating of single skeletal muscle BK_{ca} channels at high [Ca^{2+}].

Although Scheme III has many states (50), it is based on the straightforward idea that both Ca^{2+} and voltage allosterically influence a central closed-to-open conformational change. The large number of states arises naturally as a property of gating systems regulated by two stimuli. Despite its many states, the equilibrium behavior of Scheme III is governed by only seven parameters: L (0), the open-to-closed equilibrium constant when no voltage sensors are active and no Ca^{2+} binding sites are occupied; Q, the gating charge associated with this equilibrium; V_{h0}, the voltage at which a single voltage sensor is half the time active when the channel is closed; V_{ho}, the same for when the channel is open; Z, the equivalent gating charge associated with each voltage sensor’s movement; and K_{C} and K_{O}, the open and closed Ca^{2+} dissociation constants, respectively. \( P_{\text{open}} \) for Scheme III is given by the following function of [Ca^{2+}] and voltage:

\[
P_{\text{open}} = \text{function of } [\text{Ca}^{2+}] \text{ and voltage}
\]

**Figure 10.** (A) Scheme II, allosteric model of Horrigan et al. (1999). Horizontal transitions represent voltage sensor motion in each of four subunits with \( K_{CV} \) and \( K_{VO} \) representing the forward microscopic equilibrium constants for these transitions for the closed and open channel, respectively. Vertical transitions represent the conformational changes by which the channel opens. All transitions are hypothesized to be voltage dependent. (B) Allosteric model to account for both the Ca^{2+}- and voltage-dependent properties of mSlo gating. This model represents the simplest combination of Schemes I and II. Transitions along the long horizontal axis represent Ca^{2+} binding and unbinding. Transitions along the short horizontal axis represent voltage sensor movement. Transitions from top to bottom represent channel opening. Implicit in this scheme and Eq. B are the assumptions that voltage sensors and Ca^{2+} binding sites in each subunit are identical and act independently, and that voltage-sensor movement does not directly influence Ca^{2+} binding, and vice versa.
\[
P_{\text{open}} = \frac{1}{1 + \left( \frac{1 + [\text{Ca}] / K_C}{1 + [\text{Ca}] / K_O} \right)^4 \left[ \frac{1}{1 + e^{\frac{Zf(V - V_{h,d})}{RT}}} \right]^4 e^{-\frac{QFV}{RT}} L(0) e^{\frac{-QFV}{RT}} .
\]

In keeping with other work (Cox et al., 1997a; Horrigan and Aldrich, 1999), we may also define the ratio of Ca\(^{2+}\) dissociation constants as \(C = K_C / K_O\), and the ratio of voltage sensor equilibrium constants when no voltage is applied as \(D = e^{\frac{Zf(V - V_{h,b})}{RT}} / e^{\frac{Zf(V - V_{h,d})}{RT}}\).

Interestingly, although it is not possible to write \(V_{1/2}\) as an explicit function of \([\text{Ca}^{2+}]\) for Scheme III, simulations indicate that, as with the VD-MWC model, the concentration at which Scheme III starts to respond to Ca\(^{2+}\) is also determined primarily by the affinity of the channel for Ca\(^{2+}\) when it is open. This is illustrated in Fig. 11. Here we have constructed \(V_{1/2}\) vs. \([\text{Ca}^{2+}]\) plots from G-V curves simulated with a model of the form of Scheme III. The parameters used in these simulations were similar to those used below to fit the mSlo\(_o\) data (see below). Varying \(K_O\) (Fig. 11 A) has a large effect on \([\text{Ca}^{2+}]_{\text{critical}}\) for Scheme III, as for Scheme I, depend primarily on the affinity of the mSlo channel when it is open. This is illustrated in Fig. 11. Here we have constructed \(V_{1/2}\) vs. \([\text{Ca}^{2+}]\) plots from G-V curves simulated with a model of the form of Scheme III. The parameters used in these simulations were similar to those used below to fit the mSlo\(_o\) data (see below). Varying \(K_O\) (Fig. 11 A) has a large effect on \([\text{Ca}^{2+}]_{\text{critical}}\) while varying \(K_C\) (B) has very little effect. Thus, according to Schemes I and III, the fact that the mSlo channel’s \([\text{Ca}^{2+}]_{\text{critical}}\) changes very little upon \(\beta 1\) coexpression suggests very little or no effect of \(\beta 1\) on the open channel’s Ca\(^{2+}\) binding affinity.

To further examine the effects of the \(\beta 1\) subunit in terms of Scheme III, we fitted mSlo\(_o\) and mSlo\(_o + \beta 1\) G-V data with Eq. 8. For the mSlo\(_o\) data, we restricted \(L(0), V_{h,d}, D, Q,\) and \(Z\) to a small range of values determined by Horrigan et al. (1999) to reasonably fit mSlo\(_o\) gating and ionic current data in the absence of Ca\(^{2+}\). We also restricted \(K_O\) to be close (within 5%) to the value (0.84 \(\mu M\)) estimated for mSlo\(_o\) from the \([\text{Ca}^{2+}]_{\text{critical}}\) analysis described with reference to Fig. 9. As shown in Fig. 12 A, even with these restrictions, Scheme III can reproduce the shifting of the mSlo\(_o\) G-V relation as a function of \([\text{Ca}^{2+}]\) fairly well. From the parameters of the fit (listed in Fig. 12 A) \(K_C\) for mSlo\(_o\) is estimated to be 7.4 \(\mu M\). When both Ca\(^{2+}\) dissociation constants were free to vary, a very similar best fit was found (not shown) with \(K_C\) equal to 7.3 \(\mu M\) and \(K_O\) equal to 0.72 \(\mu M\).

We next fit the G-V data acquired with the \(\beta 1\) subunit present. In doing so, we constrained only \(K_O\), again keeping it close to (within 5%) the value (0.68 \(\mu M\)) estimated for mSlo\(_o + \beta 1\) from \([\text{Ca}^{2+}]_{\text{critical}}\) analysis. As shown in Fig. 12 B, over the \([\text{Ca}^{2+}]\) range \(\sim 1-75 \mu M\), the 50-state model and the VD-MWC model fits are quite similar (compare with Fig. 1 B, dashed curves). This is perhaps not surprising as the mechanism by which Ca\(^{2+}\) binding affects channel opening is the same for the two schemes. The more complex voltage-dependent gating mechanism of the 50-state model, however, also allows it to account for the prominent change in G-V steepness evident in Fig. 12 B between 0.0005 and 0.99 \(\mu M\) \([\text{Ca}^{2+}]\). This is something that is also evident without \(\beta 1\) coexpression (see Fig. 12 A and Cui et al., 1997), but is most pronounced when \(\beta 1\) is present. Scheme I-based models cannot account for changes in G-V steepness with changing \([\text{Ca}^{2+}]\) because they contain all of their gating charge in the central close-to-open conformational change.

Most interesting, however, is the mechanism by which the 50-state model accounts for the \(\beta 1\) subunit’s steady state effects. As expected based on the preceding analysis, it decreases \(L(0)\), which reflects in part the
Regulation of BK<sub>Ca</sub> by its β1 Subunit

intrinsic free energy difference between open and closed, and it decreases its voltage sensitivity (decreasing its gating charge <i>z</i> and altering <i>V</i><sub>ho</sub> and <i>V</i><sub>hc</sub>), while changing its Ca<sup>2+</sup> dissociation constants very little (<i>K</i><sub>0</sub> moves from 0.8 to 0.65 μM, and <i>K</i><sub>C</sub> moves from 7.4 to 9.6 μM). Also, we found that it was not possible to achieve any sort of reasonable fit to the mSlo<sub>α1β1</sub> G-V data if only <i>K</i><sub>0</sub> and <i>K</i><sub>C</sub> were allowed to vary freely while

<sup>2</sup>The parameter <i>L</i>(0) is defined differently in Schemes I and III (see Figs. 3 and 10B, respectively). In Scheme I, <i>L</i>(0) represents the equilibrium constant between open and closed at 0 mV in the absence of [Ca<sup>2+</sup>]; whereas, in Scheme III, <i>L</i>(0) represents the equilibrium constant between open and closed at 0 mV in the absence of [Ca<sup>2+</sup>] if no voltage sensors are active. For Scheme III, the open-to-closed equilibrium constant in the absence of [Ca<sup>2+</sup>] at 0 mV is given by:

\[
\frac{1 + e^{-\frac{ZFV_{hc}}{RT}}}{1 + e^{-\frac{ZFV_{ho} - \frac{RT}{2}}{RT}}},
\]

which thus depends on <i>Z</i>, <i>V</i><sub>ho</sub>, <i>V</i><sub>hc</sub>, and <i>L</i>(0).

Figure 12. 50-state model analysis. (A) mSlo<sub>α</sub> and (B) mSlo<sub>α1β1</sub> G-V relations over a series of [Ca<sup>2+</sup>]<sub>i</sub> were fit with Eq. 8 as described in the text. The resulting parameters are as indicated on the figure. Those values indicated in parentheses were not parameters of the fit, but rather calculated from the fit parameters. In A, the parameters other than those related to Ca<sup>2+</sup> binding were constrained to be within the following ranges, which might reasonably fit the data of Horrigan et al. (1999) at subnanomolar [Ca<sup>2+</sup>] (Frank Horrigan, personal communication). <i>L</i>(0) 0.25–1 x 10<sup>6</sup>, <i>Q</i> 0.35–0.45, <i>V</i><sub>hc</sub> 135–155, <i>D</i> 10–20, <i>Z</i> 0.51–0.59. <i>K</i><sub>0</sub> was constrained to be within 5% of the value estimated from [Ca<sup>2+</sup>]_{critical} analysis 0.8–0.88 μM and <i>K</i><sub>C</sub> was unconstrained. In B, <i>K</i><sub>0</sub> was constrained to be within 5% of the value estimated from [Ca<sup>2+</sup>]_{critical} analysis 0.65–0.72 μM, all other parameters were free to vary. In C–F, various parameters from the fit in B were restored to their values in A. In C, <i>L</i>(0) was restored. In D, the voltage-sensing parameters <i>Q</i>, <i>Z</i>, <i>V</i><sub>hc</sub>, and <i>D</i> were restored. In E, <i>L</i>(0), <i>Q</i>, <i>Z</i>, <i>V</i><sub>ho</sub>, and <i>D</i> were restored. In F, <i>K</i><sub>0</sub> and <i>K</i><sub>C</sub> were restored. In each panel, G-V relations at the following [Ca<sup>2+</sup>] are shown: from right to left, 0.0005, 0.99, 1.86, 4.63, 9.1, 39, and 74 μM. Error bars indicate SEM.
the parameters not related to Ca$^{2+}$ binding were constrained to those used to fit the mSlo data. Thus, analysis of our data in terms of Scheme III, as with Scheme I, suggests that although the β1 subunit increases the mSlo channel’s apparent affinity for Ca$^{2+}$, much of this effect is via its action on aspects of gating that are separate from, but energetically coupled to, Ca$^{2+}$ binding.

To examine the extent in which changes in various aspects of gating account for β1’s effects, we restored various 50-state model parameters from the mSlo$_{α+β1}$ fit to their value in the mSlo$_α$ fit and recalculated the model G-V curves. We did this without redoing the fitting. The effects of such changes are shown in Fig. 12, C–F. Clearly, restoration of both the model’s L(0) value (Fig. 12 C) and its voltage-sensing parameters (D), as well as both together (E), severely disturbed the original mSlo$_{α+β1}$ fit. In fact, qualitatively, the effects of changing these aspects of gating are similar to what is predicted by Scheme I (Fig. 4). Increasing L(0) shifts all G-V curves rightward (Fig. 12 C), while increasing the model’s voltage sensitivity increases G-V steepness and collapses their spacing (D). Thus, in these respects, Schemes I and III behave similarly.

We also restored just the model’s Ca$^{2+}$ dissociation constants to their values in the mSlo$_α$, fit. Interestingly, this also substantially disturbed the model’s fit to the mSlo$_{α+β1}$ G-V curves (Fig. 12 F). Not at very low [Ca$^{2+}$], but as [Ca$^{2+}$] is increased, the model’s G-V spacing becomes too narrow and the fit becomes very poor. Also, when we did redo the fitting, but restricted $K_C$ and $K_O$ to the values used in the mSlo$_α$, fit, a good fit to the mSlo$_{α+β1}$ G-V data was not found. Thus, while both Schemes I and III suggest only small changes in the channel’s Ca$^{2+}$ dissociation constants with β1 coexpression, the small changes that do occur appear to be important.

It is not clear, however, whether both $K_C$ and $K_O$ need to change to account for β1’s effects, as we were able to find reasonable fits to the mSlo$_{α+β1}$ G-V data when either one, but not both, of these parameters were held at their mSlo$_α$, G-V fit values. What appeared necessary to fit the data, however, was an increase in the ratio $K_C/K_O$ (Fig. 12 C) from ~9.3 for mSlo$_α$ to ~14.8 for mSlo$_{α+β1}$. Also, the standard errors of the fit parameter $K_C$ for the fits shown in Fig. 12, A and B, (0.5 μM for the mSlo$_α$, fit and 0.6 μM for the mSlo$_{α+β1}$, fit, as determined by the curve fitting software) are small, relative to the $K_C$ estimates: <10%. This also suggests that the change in Fig. 12 C that Scheme III uses to account for β1’s effects is required. Interestingly, this change corresponds to a change in the standard free energy difference between open and closed at saturating ligand of only 4.6 kJ/mol.

**DISCUSSION**

BK$_{Ca}$ channels are found in a wide variety of tissues where they are most often thought to provide feedback control over processes that involve membrane depolarization and a rise in intracellular Ca$^{2+}$ (Latorre et al., 1989). One place they do this is at nerve terminals. Here, Ca$^{2+}$ entry through nearby Ca$^{2+}$ channels, in combination with membrane depolarization, results in rapid activation of BK$_{Ca}$ currents that then contribute to the repolarization of the action potential (Robitaille and Charlton, 1992; Robitaille et al., 1993a,b). Thus, neuronal BK$_{Ca}$ channels are likely optimized for speed rather than sensitivity as Ca$^{2+}$ concentrations in close proximity to Ca$^{2+}$ channels are thought to rise as high as 100 μM (Roberts, 1994), a concentration that according to Fig. 1 is sufficient to achieve a $P_{open}$ of ~0.5 at the peak of the action potential (approximately +20 mV). BK$_{Ca}$ channels also play a central role in the control of smooth muscle contractility. In vascular smooth muscle, brief puffs or “sparks” of Ca$^{2+}$ are released periodically from the sarcoplasmic reticulum. These sparks, which are thought to achieve Ca$^{2+}$ concentrations near the plasma membrane in the tens of micromolar (Perez et al., 1999), activate nearby BK$_{Ca}$ channels on the plasma membrane, and the ensuing hyperpolarization leads to muscle relaxation (Nelson et al., 1995; Nelson and Quayle, 1995; Perez et al., 1999). In this tissue, however, the membrane voltage seldom exceeds ~20 mV and typically rests near ~40 mV (Nelson and Quayle, 1995). Thus, for vascular smooth muscle BK$_{Ca}$ channels to be similarly activated, they must be sensitive to Ca$^{2+}$ at lower voltages than their neuronal counterparts.

To achieve this increase in sensitivity, nature apparently has evolved an auxiliary BK$_{Ca}$ β subunit (β1) that shifts the BK$_{Ca}$ channel’s G-V relation ~70 to ~80 mV, into the physiological voltage range at concentrations of 5 or 10 μM. This shift increases the apparent Ca$^{2+}$ affinity of these channels at many voltages. It has been the goal of this study to elucidate in a biophysical sense the mechanism by which the β1 subunit exerts this powerful effect, and in particular to determine whether the apparent increase in Ca$^{2+}$ affinity evident upon β1 coexpression is due to a real increase in Ca$^{2+}$ binding site affinity.

Perhaps the most straightforward approach to this question would be to measure the binding of Ca$^{2+}$ to the channel. The high nonspecific binding associated with attempting to measure $^{45}$Ca$^{2+}$ binding to a low affinity integral membrane protein, however, renders this approach, at present, technically unfeasible. We have therefore relied on electrophysiological measurements. These measurements, however, are necessarily indirect as they measure channel opening rather than Ca$^{2+}$ binding, and therefore require interpretation. Binding measurements, if achieved, however, would also require some mechanistic framework for their interpretation, as such measurements may also be influenced by aspects of gating that are separate from, but linked to, Ca$^{2+}$ binding (Wyman and Gill, 1990).
To aid us in the interpretation of our electrophysiological data, we have relied on two models of BKCa channel gating that have been developed after extensive examination of the macroscopic gating behavior of the mSlo channel. The first is Scheme I and the special case of Scheme I that corresponds to the voltage-dependent MWC model. While, as we have discussed (Cox et al., 1997a; Horrigan and Aldrich, 1999; Horrigan et al., 1999), this system too simple to account for some of the voltage-dependent properties of BKCa channel gating, it can recreate the synergistic relationship between Ca2+ and voltage as they act together to activate the BKCa channel over a wide range of [Ca2+] and voltage. Furthermore, the VD-MWC model is sufficiently simple that it allows one to derive general principles about the ligand-dependent allosteric regulation of voltage-sensitive equilibria. The first of these principles is that an alteration in the intrinsic energy difference between open and closed (ΔGi) can indeed cause large G-V shifts. This is perhaps intuitively clear, and readily understandable as simply due to a change in the energy the voltage-sensing mechanism needs to apply to the central conformational change to affect opening. An important characteristic of these shifts reflected by the VD-MWC model, however, is that as long as the channel’s voltage sensitivity remains constant with [Ca2+] (as is necessarily the case for Scheme I) the magnitude of the shift will not vary with Ca2+. That is, the spacing of the G-V curves will be maintained. Thus, on a V1/2 vs. [Ca2+] plot, a simple V1/2 offset will be observed. We, however, see a change in V1/2 vs. [Ca2+] slope as well as position in Fig. 1 upon β1 coexpression. This suggests that the β1 subunit is doing more than just changing the equilibrium constant between open and closed.

The second general principle we may derive from the VD-MWC model is that the spacing of the BKCa channel’s G-V relation as a function of [Ca2+] will likely change if the model’s Ca2+ dissociation constants in the open or closed configuration are altered. This is evident from Eq. 3, which shows that ΔV1/2/Δ[Ca2+] depends in a complex way on both KC and Ko, and this derivative is an even more complex function of KC and Ko for Scheme III. In either case, however, the complexity of this relationship makes it difficult to make general predictions about how G-V spacing is likely to change in response to changes in Ca2+ binding constants without specifying the exact nature of the change. One important general principle of such ligand-gated voltage-sensitive systems, however, is that the power of a ligand to shift the channel’s G-V relation along the voltage axis (that is, the maximum change in V1/2 that a ligand can produce (ΔV1/2max)], is dependent on the change in affinity that occurs upon channel opening, rather than the absolute affinities of the closed or open channel. In fact, for the VD-MWC model in the limit of saturating [Ca2+], we may write:

$$\Delta V_{1/2}^{\text{max}} = \frac{4RT}{QF} \ln \left[ \frac{K_C}{K_O} \right],$$

(9)

which states that ΔV1/2max varies as the logarithm of the ratio KC/KO. An important result of this fact is that large leftward G-V shifts at high [Ca2+] can occur if either Ko is reduced or KC is increased. In the later case, the channel would in fact bind Ca2+ with lower affinity in the closed conformation and yet affect leftward G-V shifts. Furthermore, such shifts will increase the channel’s Ca2+ sensitivity, particularly under conditions where multiple Ca2+ ions normally need to bind before the channel is appreciably open. Thus, an increase in a channel’s apparent Ca2+ binding affinity can be the result of a true decrease in binding affinity. In fact, analysis of our data suggests that, among other things, the BKCa channel’s KC/KO ratio must change from ~9.3 to ~14.9 to account for β1’s effects, and that simply a small increase in KC may well be responsible. Interestingly, this change in KC/KO corresponds to a change in the standard free energy difference whose shape, for models of the form of Scheme I, is only sensitive to changes in Ca2+ binding. Applying this test to the effects of the β1 subunit, we have found that the z*V1/2 vs. [Ca2+] plots for mSloα and mSloα + β1 are more nearly parallel than are the V1/2 vs. [Ca2+] plots, thus suggesting that some of mSloα + β1’s expanded G-V spacing is due to its general decrease in voltage sensitivity. Analysis of the two curves in Fig. 5, however, indicates that there are differences in shape between the mSloα and mSloα + β1 z*V1/2 vs. [Ca2+] plots, and thus some of the increased spacing of the mSloα + β1 G-V curves is likely due to effects of β1 on Ca2+ binding.

A third principle we may derive from the VD-MWC model is that the spacing of the BKCa channel’s G-V relation as a function of [Ca2+] will likely change if the model’s Ca2+ dissociation constants in the open or closed configuration are altered. This is evident from Eq. 3, which shows that ΔV1/2/Δ[Ca2+] depends in a complex way on both KC and Ko, and this derivative is an even more complex function of KC and Ko for Scheme III. In either case, however, the complexity of this relationship makes it difficult to make general predictions about how G-V spacing is likely to change in response to changes in Ca2+ binding constants without specifying the exact nature of the change. One important general principle of such ligand-gated voltage-sensitive systems, however, is that the power of a ligand to shift the channel’s G-V relation along the voltage axis (that is, the maximum change in V1/2 that a ligand can produce (ΔV1/2max)], is dependent on the change in affinity that occurs upon channel opening, rather than the absolute affinities of the closed or open channel. In fact, for the VD-MWC model in the limit of saturating [Ca2+], we may write:

$$\Delta V_{1/2}^{\text{max}} = \frac{4RT}{QF} \ln \left[ \frac{K_C}{K_O} \right],$$

(9)

which states that ΔV1/2max varies as the logarithm of the ratio KC/KO. An important result of this fact is that large leftward G-V shifts at high [Ca2+] can occur if either Ko is reduced or KC is increased. In the later case, the channel would in fact bind Ca2+ with lower affinity in the closed conformation and yet affect leftward G-V shifts. Furthermore, such shifts will increase the channel’s Ca2+ sensitivity, particularly under conditions where multiple Ca2+ ions normally need to bind before the channel is appreciably open. Thus, an increase in a channel’s apparent Ca2+ binding affinity can be the result of a true decrease in binding affinity. In fact, analysis of our data suggests that, among other things, the BKCa channel’s KC/KO ratio must change from ~9.3 to ~14.9 to account for β1’s effects, and that simply a small increase in KC may well be responsible. Interestingly, this change in KC/KO corresponds to a change in the standard free energy difference whose shape, for models of the form of Scheme I, is only sensitive to changes in Ca2+ binding. Applying this test to the effects of the β1 subunit, we have found that the z*V1/2 vs. [Ca2+] plots for mSloα and mSloα + β1 are more nearly parallel than are the V1/2 vs. [Ca2+] plots, thus suggesting that some of mSloα + β1’s expanded G-V spacing is due to its general decrease in voltage sensitivity. Analysis of the two curves in Fig. 5, however, indicates that there are differences in shape between the mSloα and mSloα + β1 z*V1/2 vs. [Ca2+] plots, and thus some of the increased spacing of the mSloα + β1 G-V curves is likely due to effects of β1 on Ca2+ binding.

A third principle we may derive from the VD-MWC model is that the spacing of the BKCa channel’s G-V relation as a function of [Ca2+] will likely change if the model’s Ca2+ dissociation constants in the open or closed configuration are altered. This is evident from Eq. 3, which shows that ΔV1/2/Δ[Ca2+] depends in a complex way on both KC and Ko, and this derivative is an even more complex function of KC and Ko for Scheme III. In either case, however, the complexity of this relationship makes it difficult to make general predictions about how G-V spacing is likely to change in response to changes in Ca2+ binding constants without specifying the exact nature of the change. One important general principle of such ligand-gated voltage-sensitive systems, however, is that the power of a ligand to shift the channel’s G-V relation along the voltage axis (that is, the maximum change in V1/2 that a ligand can produce (ΔV1/2max)], is dependent on the change in affinity that occurs upon channel opening, rather than the absolute affinities of the closed or open channel. In fact, for the VD-MWC model in the limit of saturating [Ca2+], we may write:

$$\Delta V_{1/2}^{\text{max}} = \frac{4RT}{QF} \ln \left[ \frac{K_C}{K_O} \right],$$

(9)
between open and closed at saturating ligand of only 4.6 kJ/mol, less than the energy of a single hydrogen bond. For a weakly voltage-dependent channel such as mSlo (Q ~ 1.2), however, this small energy change converts to a 40-mV change in ΔV_{1/2,max}. Thus, even quite small changes in the K_c/K_o ratio can have substantial effects on the power of Ca^{2+} to shift the channel’s G-V relation leftward and thus affect an apparent increase in Ca^{2+} affinity.

The general principles just described illustrate the complexity of systems containing binding coupled to conformational changes, and they underscore the futility of interpreting results of structural, modulatory, or pharmacological alterations in such cases without the aide of an appropriate and well-tested mechanistic model.

We have also examined the effects of the β1 subunit on the mSlo G-V relation in the essential absence of Ca^{2+}. A previous report suggested that there is a functional uncoupling between the hSlo channel and the human β1 subunit under these conditions such that β1 has no effect on the channel’s G-V relation (Meera et al., 1996). Similar to this observation, we have found that the ability of β1 to shift the mSlo G-V relation is greatly reduced at subnanomolar [Ca^{2+}]. Inconsistent with this report, however, we have observed a 20-mV rightward G-V shift induced by the β1 subunit at subnanomolar [Ca^{2+}] and a 38% reduction in apparent gating charge. Furthermore, we show that these results can be accounted for if we suppose that coupled with the decrease in voltage sensitivity is a decrease in ΔG^0 (see Fig. 4 E). Since this explanation is consistent with our overall analysis, we greatly favor it over an uncoupling mechanism, particularly as the kinetic effects of β1 remain quite evident at subnanomolar [Ca^{2+}], and effects of β1 on mSlo gating have been observed under this condition at the single channel level (Nimigean and Magleby, 2000). While species differences may account for the differences between our results and those of Meera et al. (1996), it may also be that the limited voltage range used in their study obscured the effects we have observed. The kinetic effects of β1 at subnanomolar [Ca^{2+}], however, were clearly evident in both studies.

One advantage in studying the regulation of BK_{ca} channel gating over other ligand-gated channels is the additional sensitivity of the BK_{ca} channel to voltage. This allows the experimenter to manipulate the equilibrium between open and closed to a point where it is most sensitive to ligand binding, its V_{1/2}, and then determine how much ligand it takes to begin to activate the channels further. We have argued that this critical [Ca^{2+}] is reflective of the affinity of the channel when it is open. To examine [Ca^{2+}]_{critical}, we have looked for changes in BK_{ca} currents at high voltages as we increased [Ca^{2+}], and have found that, consistent with published data (Meera et al., 1996), both mSlo_o and mSlo_o + β1 channels begin to respond to [Ca^{2+}] at the same concentration, close to 100 nM. Thus, the β1 subunit does not appear to substantially alter the mSlo channel’s Ca^{2+} binding affinity when it is open.

In a similar experiment, we have also looked at the point where the mSlo_o and mSlo_o + β1 channels’ G-V relations just start to shift with [Ca^{2+}]. We show, mathematically (Eq. 7) for the VD-MWC model and graphically for the VD-MWC and the 50-state models (see Figs. 8 and 11), that this Ca^{2+} concentration depends almost exclusively on the affinity of the channel for Ca^{2+} when it is open. To be strictly correct, however, it should be stated that the channel’s voltage sensitivity can also affect this critical concentration. This is because the size of the shift observed once the channel does start to respond to Ca^{2+} will be inversely related to voltage sensitivity, and this may influence the ability of the experimenter to detect the shift. We have accounted for changes in voltage sensitivity in our analysis, however, by restricting the value of z in our fits to the channel’s V_{1/2} vs. [Ca^{2+}] plot around [Ca^{2+}]_{critical} (Fig. 9 F) to the value that best fit the G-V at subnanomolar [Ca^{2+}] (the data displayed in Fig. 7 B). An alternative approach would be to look at the point where the channel’s z*V_{1/2} vs. [Ca^{2+}] just starts to respond to [Ca^{2+}]. For the VD-MWC model, this point is independent of Q. Clearly, this type of analysis could also be used to determine whether alterations in BK_{ca} channel function brought about by mutation, phosphorylation, or chemical modulation alter the affinity of the BK_{ca} channel for Ca^{2+} when it is open.

To confirm and extend our analysis based on Scheme I, we have also fit our data with Scheme III. This scheme is identical in general form to that which Rothberg and Magleby (1999) proposed to best describe the Ca^{2+}-dependent properties of single skeletal muscle BK_{ca} channels at +30 mV. It is also a straightforward extension of Scheme II, which, after extensive investigation, Horrigan et al. (1999) found could account well for the gating and ionic current properties of mSlo_o at subnanomolar [Ca^{2+}]. They proposed that combining Scheme II with the mechanism by which Ca^{2+} affects channel gating in the MWC model to produce Scheme III might account also for mSlo_o’s Ca^{2+}-dependent behavior. Remarkably, we have found that we could reproduce the Ca^{2+}-dependent shifting of the mSlo_o G-V relation over a wide range of [Ca^{2+}], and as well a change in G-V slope from very low to moderate [Ca^{2+}], while maintaining the voltage-sensing parameters (Q, Z, V_h, and D) and L(0) of the 50-state model essentially as described by Horrigan et al. (1999). In fact, this was also the case if we restricted K_o to the value estimated from our [Ca^{2+}]_{critical} analysis such that only K_c was free to vary. We consider this confirmation of the general theory that Scheme III represents, al-
though clearly further complexities may occur that are not represented by Eq. 8 and therefore are not included in the form of this scheme. Such complexities might include Ca$^{2+}$ binding steps of differing affinities in either the open or closed conformation, or direct interactions between voltage sensors and Ca$^{2+}$ binding sites. Even without such considerations, however, the added complexity of Scheme III’s voltage-sensing mechanism makes it more difficult to derive general principles for its behavior than it is for Scheme I. Simulated changes in various aspects of gating, however, indicate that this system behaves qualitatively like Scheme I (Fig. 12), thus validating our use of Scheme I in our preliminary analysis. This is perhaps not surprising since, although Scheme III’s voltage-sensing mechanism is more complex than Scheme I’s, Scheme III still represents a voltage-sensitive central conformational change allosterically regulated by ligand binding. Furthermore, we have found that both Schemes I and III suggest the same mechanism by which the $\beta$1 subunit alters the mSlo channel’s steady-state gating behavior: a decrease in $\Delta G^\circ$, a reduction in gating charge, and a subtle but important change in Ca$^{2+}$ binding affinity. Horrigan et al. (1999) performed a detailed experimental analysis of gating and ionic currents at subnanomolar [Ca$^{2+}$], which allowed them to estimate $Q$, $Z$, $V_{h_{\zeta}}$, $D$, and $L(0)$ for the mSlo channel. Without performing a similar analysis, we would not wish to rely on the estimates of these parameters suggested for the mSlo$_{\alpha+1\beta1}$ channel by the fit shown in Fig. 12 B. We do think, however, that the broad [Ca$^{2+}$] range we used in our experiments is sufficient to provide reasonable estimates of the mean Ca$^{2+}$ dissociation constants for both mSlo$_{\alpha}$ and mSlo$_{\alpha+1\beta1}$ under the conditions of our experiments. For mSlo$_{\alpha}$, these estimates are: $K_O = 0.8$ $\mu$M and $K_C = 7.4$ $\mu$M; and, for mSlo$_{\alpha+1\beta1}$, they are: $K_O = 0.65$ $\mu$M and $K_C = 9.6$ $\mu$M.

It should be pointed out, however, that the mean $V_{1/2}$ and z values we report here for mSlo$_{\alpha}$ are somewhat different from those we have published previously at similar [Ca$^{2+}$] (Cui et al., 1997). The mean $V_{1/2}$ values from our current data are on average 5-20 mV right-shifted compared with our previous report, and the mean z values are 10-30% smaller. We do not know the origin of these differences. We believe, however, that they are not due to random sampling, as the large number of experiments included in both studies render the estimates of mean $V_{1/2}$ and z reported here, and previously, precise to a standard error of <5 mV ($V_{1/2}$) and a charge of 0.1 (z). More likely, the differences are systematic, due to differences between the solutions in the two studies (we have reduced the K$^+$ concentration in the internal and external solutions from 142 to 82 mM for the current study), the source or condition of the oocytes employed, or some other unknown aspects of the experimental set up. For the comparisons we have made in the present study, however, we do not believe such systematic variation to be an issue, as the mSlo$_{\alpha}$ and mSlo$_{\alpha+1\beta1}$ data we report here were acquired with the same solutions, using the same experimental setup, in many cases from the same batches of oocytes. Thus, the experiments described here were specifically designed to compare mSlo$_{\alpha}$ and mSlo$_{\alpha+1\beta1}$ currents, and their results are better compared with one another than with previous work.

The first $\beta1$ subunit to be isolated and characterized was bovine (Knaus et al., 1994a), and it is this subunit that we used in our experiments. During the course of this study, however, additional mammalian $\beta1$ subunits were cloned, including those from mouse and human (Dworetzky et al., 1996; Tseng-Crank et al., 1996; Jiang et al., 1999). Mouse and human $\beta1$ are ~83% identical to one another and between 81% (mouse) and 86% (human) identical to bovine $\beta1$. Preliminary experiments we have done with mouse $\beta1$ indicate that its effects on mSlo gating are very similar to bovine $\beta1$ at micromolar [Ca$^{2+}$], and that it produces a somewhat larger rightward G-V shift at subnanomolar [Ca$^{2+}$]. This difference, however, is not likely to affect the main conclusions of this study.

Recently, Nimigean and Magleby (1999, 2000) examined the effects of bovine $\beta1$ on mSlo gating at the single-channel level. They concluded that the $\beta1$ subunit exerts its effects mainly by increasing the time the channel spends in bursting states ~20-fold, and that this effect is evident even in the absence of [Ca$^{2+}$]. If we suppose, as has been suggested by Rothberg and Magleby (1998), that the flickers closed that occur during a burst are to states outside the main gating structure of the channel, then the observations of Nimigean and Magleby (1999, 2000) fit with our observations. In this case, retention of the channel in bursting states could be accounted for by a reduction in $\Delta G_{\zeta}$, as our work suggests. Because plots of $V_{1/2}$ vs. [Ca$^{2+}$] are not parallel for the mSlo$_{\alpha}$ and mSlo$_{\alpha+1\beta1}$ channels, however, our results suggest that the $\beta1$ subunit also affects other aspects of gating.

In conclusion, results of our analysis of the mSlo$_{\alpha}$ and mSlo$_{\alpha+1\beta1}$ G-V relation as a function of [Ca$^{2+}$] suggest that the $\beta1$ subunit exerts its steady-state effects by decreasing the intrinsic energy difference between open and closed conformations, thereby shifting the mSlo G-V curve at all [Ca$^{2+}$] leftward, and by decreasing the channel’s voltage sensitivity, which expands G-V spacing as a function of [Ca$^{2+}$]. In addition, subtle but important changes to one or more of the channels’ Ca$^{2+}$ dissociation constants are required, changes that increase the power of Ca$^{2+}$ to shift the G-V relation upon binding, an effect that is most pronounced at high [Ca$^{2+}$]. This work should help provide a frame-
work by which to interpret experiments designed to determine in a molecular sense how the β1 subunit makes these biophysical changes.

We gratefully acknowledge Dr. Owen McManus for the generous gift of the bovine β1 subunit clone, Dr. Frank Horrigan for helpful comments on the manuscript, Dr. Jianmin Cui for helpful discussions, and Dr. Michael Fay for his statistical analysis of the data in Fig. 5.

This work was supported by a National Institute of Mental Health Silvio Conte Center for Neuroscience Research grant (MH 48108). R.W. Aldrich is an investigator with the Howard Hughes Medical Institute.

Submitted: 7 March 2000
Revised: 25 July 2000
Accepted: 26 July 2000

REFERENCES

Adelman, J.P., K.Z. Shen, M.P. Kavanaugh, R.A. Warren, Y.N. Wu, A. Llagostera, C.T. Bond, and R.A. North. 1992. Calcium-activated potassium channels expressed from cloned complementary DNAs. Neuron. 9:209–216.

Atkinson, N.S., G.A. Robertson, and B. Ganetzky. 1991. A component of calcium-activated potassium channels encoded by the Drosophila slo locus. Science. 253:551–555.

Barrett, J.N., K.L. Magleby, and B.S. Pallotta. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. J. Physiol. 331:211–230.

Bers, D., C. Patton, and R. Nuccitelli. 1994. A practical guide to the measurement of intracellular calcium. Academic Press, San Diego.

Bers, D., C. Patton, and R. Nuccitelli. 1994. A practical guide to the measurement of intracellular calcium. Academic Press, San Diego.

Bers, D., C. Patton, and R. Nuccitelli. 1994. A practical guide to the measurement of intracellular calcium. Academic Press, San Diego.

Bers, D., C. Patton, and R. Nuccitelli. 1994. A practical guide to the measurement of intracellular calcium. Academic Press, San Diego.

Bers, D., C. Patton, and R. Nuccitelli. 1994. A practical guide to the measurement of intracellular calcium. Academic Press, San Diego.
Monod, J., J. Wyman, and J.P. Changeux. 1965. On the nature of allosteric transitions: a plausible model. J. Mol. Biol. 12:88–118.

Nelson, M.T., H. Cheng, M. Rubart, L.F. Santana, A.D. Bonev, H.J. Knot, and W.J. Lederer. 1995. Relaxation of arterial smooth muscle by calcium sparks. Science. 270:633–637.

Nelson, M.T., and J.M. Quayle. 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. Cell Physiol. 268:C799–C822.

Nimigean, C.M., and K.L. Magleby. 1999. The beta subunit increases the Ca\(^{2+}\) sensitivity of large conductance Ca\(^{2+}\)-activated potassium channels by retaining the gating in the bursting states. J. Gen. Physiol. 113:425–440.

Nimigean, C.M., and K.L. Magleby. 2000. Functional coupling of the \(\beta1\) subunit to the large conductance Ca\(^{2+}\)-activated K\(^+\) channel in the absence of Ca\(^{2+}\): increased Ca\(^{2+}\) sensitivity from a Ca\(^{2+}\)-independent mechanism. J. Gen. Physiol. 115:719–736.

Pallanck, L., and B. Ganetzky. 1994. Cloning and characterization of human and mouse homologs of the Drosophila calcium-activated potassium channel gene, slowpoke. Hum. Mol. Genet. 3:1239–1243.

Perez, G.J., A.D. Bonev, J.B. Patlak, and M.T. Nelson. 1999. Functional coupling of ryanodine receptors to KCa channels in smooth muscle cells from rat cerebral arteries. J. Gen. Physiol. 113:229–238.

Ramanathan, K., T.H. Michael, G.J. Jiang, H. Hiel, and P.A. Fuchs. 1999. A molecular mechanism for electrical tuning of cochlear hair cells. Science. 283:215–217.

Roberts, W.M. 1994. Localization of calcium signals by a mobile calcium buffer in frog saccular hair cells. J. Neurosci. 14:3246–3262.

Robitaille, R., E.M. Adler, and M.P. Charlton. 1993a. Calcium channels and calcium-gated potassium channels at the frog neuro muscular junction. J. Physiol. (Paris). 87:15–24.

Robitaille, R., and M.P. Charlton. 1992. Presynaptic calcium signals and transmitter release are modulated by calcium-activated potassium channels. J. Neurosci. 12:297–305.

Robitaille, R., M.L. Garcia, G.J. Kaczorowski, and M.P. Charlton. 1993b. Functional colocalization of calcium and calcium-gated potassium channels in control of transmitter release. Neuron. 11:645–655.

Rothenberg, B.S., and K.L. Magleby. 1998. Kinetic structure of large-conductance Ca\(^{2+}\)-activated K\(^+\) channels suggests that the gating includes transitions through intermediate or secondary states. A mechanism for flickers. J. Gen. Physiol. 111:751–780.

Rothenberg, B.S., and K.L. Magleby. 1999. Gating kinetics of single large-conductance Ca\(^{2+}\)-activated K\(^+\) channels in high Ca\(^{2+}\) suggest a two-tiered allosteric gating mechanism. J. Gen. Physiol. 114:93–124.

Shen, K.Z., A. Lagrutta, N.W. Davies, N.B. Standen, J.P. Adelman, and R.A. North. 1994. Tetraethylammonium block of Slowpoke calcium-activated potassium channels expressed in Xenopus oocytes: evidence for tetrameric channel formation. Pflügers Arch. 426:440–445.

Stefani, E., M. Ottolia, F. Noceti, R. Olcese, M. Wallner, R. Latorre, and L. Toro. 1997. Voltage-controlled gating in a large conductance Ca\(^{2+}\)-sensitive K\(^+\) channel (hslo). Proc. Natl. Acad. Sci. USA. 94:5427–5431.

Tanaka, Y., P. Meera, M. Song, H.G. Knaus, and L. Toro. 1997. Molecular constituents of maxi KCa channels in human coro nary smooth muscle: predominant alpha + beta subunit complexes. J. Physiol. 502:545–557.

Trimmer, J.S. 1998. Regulation of ion channel expression by cytoplasmic subunits. Curr. Opin. Neurobiol. 8:370–374.

Tseng-Crank, J., C.D. Foster, J.D. Krause, R. Mertz, N. Godinot, T.J. DiChiara, and P.H. Reinhart. 1994. Cloning, expression, and distribution of functionally distinct Ca\(^{2+}\)-activated K\(^+\) channel isoforms from human brain. Neuron. 13:1315–1330.

Tseng-Crank, J., N. Godinot, T.E. Johansen, P.K. Ahring, D. Strobaek, R. Mertz, C.D. Foster, S.P. Olesen, and P.H. Reinhart. 1996. Cloning, expression, and distribution of a Ca\(^{2+}\)-activated K\(^+\) channel beta-subunit from human brain. Proc. Natl. Acad. Sci. USA. 93:9200–9205.

Wallner, M., P. Meera, M. Ottolia, G.J. Kaczorowski, R. Latorre, M.L. Garcia, E. Stefani, and L. Toro. 1995. Characterization of the modulation by a beta subunit of a human maxi KCa channel cloned from myometrium. Receptors Channels. 3:185–199.

Wallner, M., P. Meera, and L. Toro. 1999. Molecular basis of fast inactivation in voltage and Ca\(^{2+}\)-activated K\(^+\) channels: a transmembrane beta subunit homolog. Proc. Natl. Acad. Sci. USA. 96:4137–4142.

Weiger, T.M., M.H. Holmqvist, I.B. Levitan, F.T. Clark, S. Sprague, W.J. Huang, P. Ge, C.C. Wang, D. Lawson, M.E. Jurman, et al. 2000. A novel nervous system beta subunit that downregulates human large conductance calcium-dependent potassium channels. J. Neurosci. 20:3563–3570.

Wong, B.S., H. Lecar, and M. Adler. 1982. Single calcium-dependent potassium channels in clonal anterior pituitary cells. J. Gen. Physiol. 79:545–567.