Commentary

Kinetic Gating Mechanisms for BK Channels: When Complexity Leads to Simplicity

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Large conductance calcium-activated K^+ channels, also referred to as "BK" or "maxi K" channels because of their big single-channel conductance (~300 pS in symmetrical 150 mM KCl) are widely distributed in many different tissues (Latorre, 1994; Kaczorowski et al., 1996). A signature feature of BK channels, in addition to their high K^+ selectivity and conductance, is that they are activated by both intracellular calcium ion (Ca^{2+}_i) and depolarization in a highly synergistic manner (Marty, 1981; Barrett et al., 1982). It is this synergistic interaction that elevates BK channels to be guardians of calcium-driven processes and protectors of cellular integrity. Although Ca^{2+}_i serves as a key messenger to trigger processes such as muscle contraction and secretion of transmitters, prolonged elevated Ca^{2+}_i can be detrimental to the processes and deadly to cells. How then do BK channels serve as guardians? Consider the case in which the initial elevation of Ca^{2+}_i arises from depolarization-induced opening of voltage-dependent calcium channels. The BK channels would sense both the level of Ca^{2+}_i and the degree of depolarization. If both are high, then the synergistic action would readily open BK channels, which restores the resting membrane potential, turning off the voltage-dependent calcium channels to prevent Ca^{2+}_i overload. If the Ca^{2+}_i is less or the degree of depolarization is less, then the response of the BK channels would be less, allowing further Ca^{2+}_i influx. In addition to protective and regulatory actions, such a positive feedback system based on BK channels has been used to facilitate oscillations in membrane potential, as occurs in cochlear hair cells for frequency tuning (Hudspeth and Lewis, 1988; Wu et al., 1995). As expected, the underlying mechanisms are complex, and it has taken over 20 years of study to develop kinetic gating mechanisms that can account for the synergistic actions of voltage and Ca^{2+}_i activation of BK channels over wide ranges of experimental conditions (see references in Cui and Aldrich, 2000; Rothberg and Magleby, 2000).

In addition to their activation by Ca^{2+}_i, it has been known for some time that BK channels also are modulated by Mg^{2+}_i (Golowasch et al., 1986; Oberhauser et al., 1988), but the mechanism of this modulation has remained obscure. This has now all changed, not in 20 years but essentially overnight, with the publication of two papers in this issue of the *Journal of General Physiology*. These landmark studies raise our knowledge about Mg^{2+}_i action on BK channels from some very provocative observations to complete mechanistic descriptions that can account for the action of Mg^{2+}_i over wide ranges of Ca^{2+}_i and voltage. The work comes from the laboratories of Jianmin Cui (Shi and Cui, 2001) and Chris Lingle (Zhang et al., 2001).

How is it possible that so much progress has been made in just a few years of study, rather than decades? Enter the ability of the kinetic model to explain complex phenomena and the power of molecular biology to dissect the physical basis of mechanism. However, before the new findings on Mg^{2+} can be discussed, it is first necessary to briefly review the structure and gating mechanism of BK channels. The four pore forming α subunits of BK channels (Slo) show homology with the pore-forming subunits of the six transmembrane superfamily of voltage-dependent K^+ channels, including an S4 voltage sensor (Atkinson et al., 1991; Adelman et al., 1992; Butler et al., 1993; Diaz et al., 1998; Cui and Aldrich, 2000). BK channels have an additional S0 transmembrane segment that places the NH_2 terminus extracellular (Meera et al., 1997). The COOH terminus of BK channels exceeds the length of region S0–S6, being much longer than in typical K^+ channels, and contains a high affinity Ca^{2+} binding site termed the calcium bowl (Wei et al., 1994; Schreiber and Salkoff, 1997; Schreiber et al., 1999; Bian et al., 2001).

A gating mechanism that can account for the Ca^{2+}_i and voltage activation of BK channels from 0 Ca^{2+}_i to high Ca^{2+}_i (1 mM) over a wide range of voltages is shown in Scheme I (Horrigan and Aldrich, 1999; Rothberg and Magleby, 1999, 2000; Cui and Aldrich, 2000; Cox and Aldrich, 2000). This two-tiered gating mechanism consists of 25 closed states on the upper tier and 25 open states on the lower tier. The tetrameric structure of BK channels (Shen et al., 1994) is reflected in the four subunits comprising each state of the channel. In Scheme I, Ca^{2+}_i binding to the calcium bowl is indicated by shading, and the depolarization-induced move-

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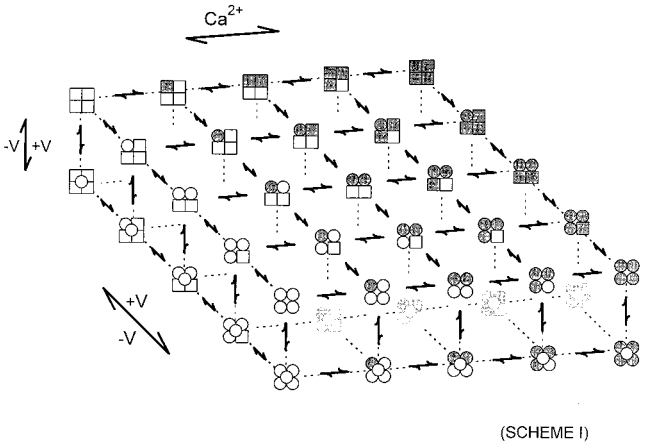

(SCHEME I)

ment of each S4 voltage sensor is indicated by a transition from a square to a circle. It is the four potential configurations of each of the four subunits that leads to the large numbers of states on each tier. Since the theoretical basis for such large numbers of states was pointed out more than 30 years ago by Eigen (1968), why has it taken so many years to arrive at Scheme I. One reason is that gating mechanisms are constructed in a stepwise manner, adding additional states only as necessary to account for the experimental data. Initial models for gating of BK channels were based on the relatively simple 10-state Monod-Wyman-Changeux (MWC) model (Monod et al., 1965), and provided reasonable descriptions of the gating over relatively wide ranges of experimental conditions (McManus and Magleby, 1991; Wu et al., 1995; Cox et al., 1997; Cui et al., 1997).

It was only when improved analysis methods were used to investigate the extremes of gating at large, depolarized voltages and also at zero and high Ca^{2+}_i that it became obvious that the gating was far more complex than the 10-state MWC model (Horrigan and Aldrich, 1999; Horrigan et al., 1999; Rothberg and Magleby, 1999; Nimigean and Magleby, 2000; Talukder and Aldrich, 2000). Not only would the theoretical states proposed by Eigen (1968) be needed, but the Eigen model would have to be expanded with an entire second tier to provide both the required numbers of states and also multiple transition pathways between the open and closed states at the extremes of gating at zero and high Ca^{2+}_i, as shown in Scheme I. The actual gating mechanism most likely is more complex than Scheme I (a third tier may be required to fully account for the brief closings), and there is the potential for up to 55 states per tier (Cox et al., 1997), but Scheme I will be sufficient to discuss the new findings on Mg^{2+}.

Just as Eigen (1968) predicted the potential for large numbers of states many years ago from subunit structure, perhaps we can predict a gating mechanism to include the effects of Mg^{2+}_i before looking in detail at the new findings. The previous observations by Golowasch et al. (1986) and Oberhauser et al. (1988) that 1–10 mM Mg^{2+}_i increases the Hill coefficient for Ca^{2+} activation of BK channels from ~2 to >4 in a dose-dependent manner, were an early indication that BK channels may contain low affinity Mg^{2+} binding sites that facilitate activation.

Given Scheme I, and assuming that each one of the four subunits comprising the channel also has a separate Mg^{2+} binding site, what would the predicted model be? As each one of the 50 states in Scheme I now could bind either zero, one, two, three, or four Mg^{2+} ions, Scheme I would be expanded to 250 states. This is just the type of model that Shi and Cui (2001) and Zhang et al. (2001) have found consistent with the complex interactions between voltage, Ca^{2+}, and Mg^{2+}. Although a 250-state model may appear complex at first inspection, the basis for such a model is simplicity itself, following directly from four subunits, each with a voltage sensor, a high affinity Ca^{2+} binding site, a low affinity Mg^{2+} (divalent) binding site, and an additional (concerted) conformational change associated with channel opening.

What then are the effects of Mg^{2+}_i on BK channels reported in these new studies? One effect, the reduction of single-channel current amplitude by millimolar concentrations of intracellular divalent cations due to voltage-dependent fast block has been known for some time (Ferguson, 1991), and will not be considered here. To discuss the other effects of Mg^{2+}_i, first, it is necessary to explain the assay method typically used to characterization the Ca^{2+} activation of BK channels. For a fixed Ca^{2+}_i, the holding potential is changed and the membrane conductance or open probability (Po) of the BK channel or channels is measured. This produces a sigmoid curve, with normalized conductance (or Po) changing from zero to one as the voltage moves in the depolarizing direction. Because of the synergistic interaction between voltage and Ca^{2+}_i, the voltage for half activation ($V_{0.5}$) shifts to the left for higher Ca^{2+}_i (i.e., with higher Ca^{2+}_i, less depolarization is needed for the same level of activation). Alternatively, if some factor such as Mg^{2+}_i facilitates the activation of the channel, then this would be indicated by a leftward shift in $V_{0.5}$ for a fixed Ca^{2+}_i, as less depolarization would be required for the same level of activation.

Using this assay, Zhang et al. (2001) found a continuous monotonic leftward shift in $V_{0.5}$ as Ca was increased more than four orders of magnitude, from $<10^{-6}$ to $>10^{-2}$ M. A series of exhaustive experiments over wide ranges of Ca^{2+}_i, Mg^{2+}_i, and voltage then suggested two binding sites with different properties. For divalent cation concentrations, <300 μM, the leftward shift and altered kinetics of activation were specific for Ca^{2+}_i, suggesting a high affinity Ca^{2+} binding site. Once the high affinity site was saturated with Ca^{2+}, the addition of

1–100 mM Mg^{2+}_i was just as effective as the addition of 1–100 mM Ca^{2+}_i in continuing the leftward shift and altering the kinetics, suggesting a low affinity nonspecific divalent site. Ca^{2+}_i was not required for the facilitating effect of Mg^{2+}_i, as 10 mM Mg^{2+}_i gave similar leftward shifts in the presence or absence of 4, 10, 100, and 300 μM Ca^{2+}_i. Increases in Ca^{2+} at the high affinity site greatly increased the rate of activation, whereas increases in Ca^{2+} and/or Mg^{2+} at the low affinity site gave only small increases in the rate of activation, while greatly slowing deactivation. Zhang et al. (2001) suggested that the differential effects on the kinetics of low and high concentrations of cations indicated separate sites of action and separate regulatory mechanisms. Zhang et al. (2001) found that the interactions between Ca^{2+}_i, Mg^{2+}_i, and voltage were consistent with Scheme I expanded to 250 states to include four independent low affinity Ca^{2+}/Mg^{2+} binding sites in addition to the four independent high affinity Ca^{2+} binding sites and the four voltage sensors per channel. In their model Mg^{2+}_i increases Po by binding more tightly to the open states once they have been activated, slowing the rate at which channels leave the open states. For both the experimental data and the theoretical predictions of the 250 state model, the Hill coefficient for activation by Ca^{2+}_i increased with Mg^{2+}_i and also with depolarization. These effects of Mg^{2+}_i (Golowasch et al., 1986; Oberhauser et al., 1988) and depolarization (Cox et al., 1997) are consistent with previous findings.

Whereas Zhang et al. (2001) coaxed the secrets of Mg^{2+}_i modulation from the channel with a heavy dose of kinetics applied over a wide range of Ca^{2+}_i, Mg^{2+}_i, and voltage, Shi and Cui (2001) examined fewer conditions, but selected them to maximize the effects and also applied the power of molecular biology to dissect the binding sites. Consistent with the findings of Zhang et al. (2001), Shi and Cui (2001) found that the Mg^{2+}_i induced leftward shift in $V_{0.5}$ first became significant as Mg^{2+}_i approach 1 mM, and that the leftward shift then continued up to the examined concentration of 100 mM Mg^{2+}_i. A compelling superimposed plot showed that the leftward shift was independent of whether Ca^{2+}_i was either 0 or 110 μM, suggesting independent mechanisms of action for the high affinity Ca^{2+} site and the low affinity Mg^{2+}_i site.

To locate the low affinity site, Shi and Cui (2001) examined the effect of Mg^{2+}_i on Slo3, a pH- sensitive K^+ channel that is insensitive to Ca^{2+}_i and does not have a functional calcium bowl (Schreiber et al., 1998, 1999). For the Slo3 channel, $V_{0.5}$ was unaffected by 10 mM Mg^{2+}_i, indicating the absence of a functional low affinity site. Replacing the core (S0–S8) of the Slo3 channel with the core (S0–S8) of the Slo1 (BK) channel to form a Slo1 core/Slo3 tail channel then restored the leftward shift in $V_{0.5}$ induced by 10 mM Mg^{2+}_i. Thus, the low affinity Mg^{2+} site is located on the core (S0–S8) of the BK channel. Since the Slo1 core/Slo3 tail channel does not contain a high affinity site (calcium bowl), these results clearly establish that the low and high affinity sites are different. Shi and Cui were able to account for their observations using the equivalent of a 250-state model in which each subunit of the channel had a separate high affinity Ca^{2+} binding site and a low affinity Mg^{2+} binding site. Shi and Cui also suggested that high concentrations of Mg^{2+}_i could compete for Ca^{2+} at the high affinity site, reducing its expected effect. Sharing of information between the two groups when the papers were in review alerted Zhang et al. (2001) to the possibility of Mg^{2+}_i competition at the high affinity Ca^{2+} site, allowing them to further refine their analysis before publication. (One cannot help but wonder how much faster science might progress if there were more sharing of information before publication.)

The findings of Zhang et al. (2001) and Shi and Cui (2001) were in close agreement, with estimates of the various equilibrium constants within a factor of two of each other. One minor difference is that Zhang et al. argue that the unusual behavior of the Hill coefficients for Ca^{2+}_i action arises from the differential contribution of the high and low affinity sites to the Hill plots at different voltages and Mg^{2+}_i. On the other hand, Shi and Cui (2001) argue that the inhibitory effect of Mg^{2+}_i on the high affinity site together with the activation effects on the low affinity sites contribute to the increased Hill coefficients in the presence of Mg^{2+}_i. Often when there are differences in model-dependent conclusions of this type, the differences may reflect that the underlying models are still too simple, so that the conclusions become sensitive to the data being analyzed.

What then is the mechanism of action of Mg^{2+}_i? These studies suggest that Mg^{2+}_i activates BK channels independently of voltage and Ca^{2+}_i by binding to the open configuration of the channel. The low affinity Mg^{2+}_i sites do not participate in the Ca^{2+}-dependent steps that influence the rate of activation. Thus, there are three separate factors that act to shift the equilibrium from the closed to the open states for BK channels: depolarization, Ca^{2+}_i acting at high affinity sites, and Mg^{2+}_i acting at separate low affinity sites. Under physiological conditions, Ca^{2+}_i tends to be low compared with the millimolar concentrations of Mg^{2+}_i, so that Mg^{2+}_i would be the main ion bound to the low affinity sites. However, for BK channels close to active Ca^{2+} channels, it is possible that Ca^{2+}_i could increase activity by binding to both the high and the low affinity sites.

These papers answer the fundamental question of how Ca^{2+}_i over a range spanning more than four orders of magnitude can shift $V_{0.5}$ in a monotonic fashion with pCa. The conclusions in this and previous work that multiple allosteric regulators (voltage, Ca^{2+}_i, and Mg^{2+}_i)

can separately and independently regulate gating is of interest to how channels can be modulated. The fact that the low and high affinity sites map to different parts of the channel suggests that perhaps such allosteric regulatory domains are modular in nature. Finally, these results provide an explanation for the observation in these and previous studies (Golowasch et al., 1986; Oberhauser et al., 1988) that $Mg^{2+}{}_i$ increases the Hill coefficients for activation of the channel by $Ca^{2+}{}_i$. The interactions between the various allosteric modulators are sufficient to account for the observations, so that it is not necessary to propose a $Ca^{2+}{}_i$-induced change in the numbers of apparent Ca^{2+} binding sites.

What are the future directions of research in this area? Since kinetic models are seldom complete, they just get closer and closer to the underlying processes, it would be worthwhile to examine the effects of $Mg^{2+}{}_i$ using single-channel recording to determine if the proposed models for $Mg^{2+}{}_i$ actions can capture the detailed single-channel kinetics. If not, then further analysis would be needed. It would also be useful to obtain rate constants for the various steps in the gating mechanism to supplement the equilibrium constants obtained in these studies. Models that have been developed to account for the gating of BK channels often assume that the various allosteric modulators are independent of one another, acting on a common step of open-closed equilibrium. Although these assumptions have proven successful in describing the data and are especially useful for simplifying the analysis, it would be worthwhile to determine to what extent these assumptions are valid using more detailed analysis of the kinetics of the single-channel data and macroscopic currents.

The present studies were concerned with $Ca^{2+}{}_i$ and $Mg^{2+}{}_i$, whereas Oberhauser et al. (1988) have shown that many different divalent cations can activate and/or modulate the activity of BK channels. It would be of interest to extend the present studies to include additional divalent cations to further characterize the sites and their actions. More precise location of the low affinity site could give insight into its mechanism of action, and it is not entirely clear whether the calcium bowl accounts for all of the effects of the high affinity site (Braun and Sy, 2001). Finally, it will be necessary to see how many additional allosteric modulating sites and/or mechanisms are involved in the gating of BK channels, leading to further expansion of the kinetic models.

The 250-state kinetic models for the voltage- and Ca^{2+}-dependent gating of BK channels and their modulation by $Mg^{2+}{}_i$ appear complex due to the large numbers of states, but it needs to be remembered that these models follow directly from the simple underlying assumptions of a tetrameric channel with three allosteric activators (voltage, Ca^{2+}, and Mg^{2+}) that bias the closed-open transitions. These complex models provide a means, perhaps the only means, to describe the combined actions of the allosteric activators on the gating. How then does complexity lead to simplicity? Once one has the complex model, it is a simple matter to predict the intricate effects of the allosteric activators on the gating of BK channels.

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