Competitive Mg\(^{2+}\) Block of a Large-Conductance, Ca\(^{2+}\)-activated K\(^{+}\) Channel in Rat Skeletal Muscle

\textit{Ca}^{2+}, \textit{Sr}^{2+}, \textit{and Ni}^{2+} Also Block

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\textbf{ABSTRACT} The patch-clamp technique was used to investigate the effect of intracellular Mg\(^{2+}\) (Mg\(^{2+}\)) on the conductance of the large-conductance, Ca\(^{2+}\)-activated K\(^{+}\) channel in cultured rat skeletal muscle. Measurements of single-channel current amplitudes indicated that Mg\(^{2+}\) decreased the K\(^{+}\) currents in a concentration-dependent manner. Increasing Mg\(^{2+}\) from 0 to 5, 10, 20, and 50 mM decreased channel currents by 34\%, 44\%, 56\%, and 73\%, respectively, at +50 mV. The magnitude of the Mg\(^{2+}\) block increased with depolarization. For membrane potentials of -50, +50, and +90 mV, 20 mM Mg\(^{2+}\) reduced the currents 22\%, 56\%, and 70\%, respectively. Mg\(^{2+}\) did not change the reversal potential, indicating that Mg\(^{2+}\) does not permeate the channel. The magnitude of the Mg\(^{2+}\) block decreased as the concentration of K\(^{+}\) was increased. At a membrane potential of +50 mV, 20 mM Mg\(^{2+}\) reduced the currents 71\%, 56\%, and 25\% for K\(^{+}\) of 75, 150, and 500 mM. These effects of Mg\(^{2+}\), voltage, and K\(^{+}\) were totally reversible. Although the Woodhull blocking model could approximate the voltage and concentration effects of the Mg\(^{2+}\) block ($K_0 \sim 30$ mM with 150 mM symmetrical K\(^{+}\); electrical distance $\sim 0.22$ from the inner surface), the Woodhull model could not account for the effects of K\(^{+}\). Double reciprocal plots of 1/single channel current vs. 1/[K\(^{+}\)] in the presence and absence of Mg\(^{2+}\), indicated that the Mg\(^{2+}\) block is consistent with apparent competitive inhibition between Mg\(^{2+}\) and K\(^{+}\). Ca\(^{2+}\), Ni\(^{2+}\), and Sr\(^{2+}\) were found to have concentration- and voltage-dependent blocking effects similar, but not identical, to those of Mg\(^{2+}\). These observations suggest the blocking by Mg\(^{2+}\) of the large-conductance, Ca\(^{2+}\)-activated K\(^{+}\) channel is mainly nonspecific, competitive with K\(^{+}\), and at least partially electrostatic in nature.

\textbf{INTRODUCTION}

Mg\(^{2+}\), which is the predominant inorganic divalent cation in cytoplasm, reduces (blocks) the conductance of many different ion channels. Reduction of single-channel currents by intracellular Mg\(^{2+}\) (Mg\(^{2+}\)) has been observed for agonist-gated channels,
such as the N-methyl-D-aspartate receptor channel (Nowak et al., 1984) and the
muscarnic receptor channel (Horie and Irisawa, 1987, 1989), as well as for the
voltage-gated Na⁺ channel (Pusch et al., 1989). Various K⁺ channels are also blocked
by Mg²⁺, such as the inward rectifier channel (Matsuda et al., 1987; Vandenburg,
1987; Matsuda, 1988), and the ATP modulated channel (Findlay, 1987a, b; Horie et
al., 1987).

The purpose of this article is to examine the effect of intracellular Mg²⁺ and other
divalent cations on the conductance of the large-conductance Ca²⁺-activated K⁺ (BK)
channel in primary cultures of rat skeletal muscle. The study is restricted to the
effects of inorganic divalent cations at the intracellular surface, since the effects of
similar ions at the extracellular surface have been studied previously (MacKinnon
et al., 1989). Single-channel currents (Hamill et al., 1981) are studied so that any effects
of Mg²⁺ on conductance can be clearly separated from effects on kinetics (Golowasch
et al., 1986; Oberhauser et al., 1988). The BK channel is ideal for studies on
conductance for several reasons. First, the channel does not inactivate so that large
amounts of data over a range of solutions and membrane potentials can be collected
easily. Secondly, the relative absence of sub-conductance states means that any effects
on conductance are easily observable. Mg²⁺ is found to reduce the conductance of the
channel in a concentration-dependent manner. This blocking is enhanced at more
positive membrane potentials and is reduced by increasing K⁺. Ca²⁺, Sr²⁺, and Ni²⁺
have blocking effects similar (within a factor of 2 or 3) to those of Mg²⁺. All the
observed blocking effects are totally reversible. Mg²⁺ and K⁺ appear to compete and
the apparent competition may arise from the screening of negative charges at the
inner surface of the channel protein. The blocking effects of Mg²⁺ would be present
under physiological concentrations of this ion. A preliminary report of some of these
results has appeared (Ferguson and Magleby, 1989).

M E T H O D S

Preparation
Large-conductance, Ca-activated K⁺ channels obtained from the surface membrane of primary
cultures of rat skeletal muscle were studied using the single-channel recording technique
(Hamill et al., 1981). Tissue was obtained from fore- and hindlimbs of 19–20 d-old embryos or
from 1–2-d-old newborn Sprague-Dawley rats. The skinned and deboned muscle was finely
minced then treated for 1 h in 10 ml of Dulbecco’s modified Eagle’s medium (DMEM) with 1
mg of trypsin and 5 mg of collagenase added. This solution was acidified by aerating with 100cA
CO₂. After being stirred every 10 min for 1 h, the solution was centrifuged for 10 min. The
pellet was then resuspended in DMEM plus 6% fetal bovine serum and plated on 35-mm
culture dishes (Falcon Labware, Oxnard, CA). The cultures were maintained at 36°C in an
atmosphere containing 6% CO₂. After 24 h the media was removed and replaced with fresh
DMEM plus 6% fetal bovine serum. Fusion into myotubes usually occurred after 3 d, and the
cells were satisfactory for experiments for up to 2 wk in culture.

Solutions
The pipette solution, which bathed the extracellular side of the membrane, contained 10 mM
morpholinopropanesulfuric acid (MOPS) buffer and either 75, 100, or 150 mM KCl as
indicated. The solution bathing the intracellular side of the membrane contained 10 mM
MOPS buffer; 10 μM added CaCl₂, and either 75, 100, 150, 500, or 1,000 mM KCl as
indicated. All solutions were titrated to pH 7.2 with KOH. Solutions containing millimolar
concentrations of divalent cations were made by adding the appropriate amount of 2 M stock solution to the control solution. This addition changed the K⁺ concentration <3% and shifted the reversal potential <1 mV for the solutions examined. Changes of this magnitude would have negligible effects on the experiments reported here. The pipette with the patch of membrane was placed into a microperfusion chamber, as described by Barrett et al. (1982), for rapid solution changes.

Activities of some of the ions in the solutions were checked using ion-selective electrodes. This was done to determine if the observed effects could be due to changes in activities. A Ca²⁺-selective electrode (model 476041, Coming Medical, Medfield, MA) was used to examine the effects of increasing concentrations of Ca²⁺ (range 5–150 mM) and K⁺ (range 75–1,000 mM) on Ca²⁺ activity. Tables in Robinson and Stokes (1959) suggested that the results from this determination should be applicable to the other examined divalent cations. A K⁺-selective electrode (model 476132, Corning Medical) was used to examine the effects of increasing concentrations of Mg²⁺ (range 5–50 mM) on K⁺ activity for different concentrations of K⁺ (range 75–1,000 mM).

**Recording and Analysis**

All experiments were performed with excised inside-out patches of membrane. Even though the patches were excised, the terms intracellular and extracellular will be used to refer to the membrane surfaces as if the cell were intact. Membrane potentials are reported as the voltage at the normal intracellular side minus the voltage at the normal extracellular side. Outward currents are displayed as upward or positive deflections. Currents were recorded on a frequency-modulated tape recorder (frequency response 0–40 kHz).

The large-conductance, Ca²⁺-activated K⁺ channel (BK) was identified by its conductance and voltage sensitivity, as described by Barrett et al. (1982). Currents from the BK channel were the most commonly observed under the conditions of these experiments. Contributions from other membrane channels were seldom observed because appropriate permeant ions were not included in the bathing solution, or because holding potentials were such that other channels, such as delayed rectifier and Na⁺ channels, would be inactive. When other channel currents were observed, particularly in solutions with high [KCl], currents from the BK channel were several times larger, and could easily be distinguished for analysis.

For analysis, the recorded current records were sampled every 50 μs with a DEC 11/73 computer (Digital Equipment Corp., Marlboro, MA). Single-channel current amplitudes were then obtained from the digitized records by two methods: by visually fitting cursor lines to closed and open single-channel current levels on a computer displayed record and measuring the distance between the peaks, or by plotting histograms of number of observations versus current amplitude and measuring the distance between the peaks, which represented the closed and open current levels. Both methods gave similar results. Experiments were typically performed on membrane patches with only one or two BK channels.

The data were used to evaluate several models as discussed in the Results and Discussion. Parameters for the evaluated models were obtained using mathematical algorithms to minimize the squared differences between the predictions of the model and the data. The fits were always performed with several different initial values and step sizes to ensure that the optimal solution was found.

Experiments were performed at room temperature (21–24°C).

**RESULTS**

**Magnesium Blocks Single BK Channels**

Fig. 1A presents currents from a single BK channel in symmetrical 150 mM KCl and a membrane potential of +50 mV. Increasing Mg²⁺ from 0 to 5 mM decreases the
Figure 1. Mg$^{2+}$ reduces (blocks) the current through single large-conductance Ca$^{2+}$-activated K$^+$ (BK) channels. (A) Single-channel currents are shown with 0, 5, and 50 mM Mg$^{2+}$ and 150 mM symmetrical K$^+$. Upward steps indicate channel opening and outward currents. Membrane potential: +50 mV. (B) Single-channel current amplitudes are plotted against membrane potential for 0, 5, 10, 20, and 50 mM Mg$^{2+}$. Each symbol is the average of the data obtained from 13 different patches. The bars plot the standard deviation of the mean and are less than the symbol size for most points. The conductance with 0 Mg$^{2+}$, determined from the slope of the straight line, is 292 ± 10 pS. The lines connect the data points at a given [Mg$^{2+}$].

single-channel current from 14 to 10 pA (29% reduction). A further increase in Mg$^{2+}$ to 50 mM reduces the current to 3.5 pA (75% reduction of the original current). These effects of Mg$^{2+}$ were totally reversible, and the time of onset or offset was similar to that expected for the solution change.
Fig. 1 B plots single channel current amplitude versus membrane potential for five different [Mg\^2\+]. The reduction of the current becomes greater as Mg\^2\+ increases and as the membrane potential is made more positive, indicating that the reduction in current by Mg\^2\+ is both concentration and voltage dependent. Fig. 1 B also shows that the reversal potential of 0 mV in the symmetrical K\(^+\) is unchanged by Mg\^2\+, indicating that Mg\^2\+ does not permeate the channel.

It is apparent in Fig. 1 B that the current–voltage relationship is linear for the control solution (150 symmetrical KCl, 0 Mg\^2\+) indicating ohmic behavior. Such ohmic behavior in the absence of Mg\^2\+ was observed for all experiments with symmetrical KCl for membrane potentials ranging from -60 to 100 mV. For potentials > 100 mV the slope decreases (not shown). Consequently, membrane potentials > 100 mV were not examined in this study because the decreased conductance would complicate the interpretation of the Mg\^2\+ effects. Data at potentials negative to -60 mV were difficult to obtain in the 10 \(\mu\)M Ca\(^{2+}\) used to activate the channel, because the BK channel becomes less active at negative membrane potentials (Barrett et al., 1982). However, with elevated Mg\(^{2+}\), data were more easily obtained at potentials negative to -60 mV owing to the increased probability of the channel opening caused by Mg\(^{2+}\), as previously reported (Golowasch et al., 1986; Oberhauser et al., 1988).

To test whether the Mg\(^{2+}\)-induced reduction of current shown in Fig. 1 might be due to changes in osmolarity resulting from the added Mg\(^{2+}\), mannitol was added to the control solution to bring the concentration to 60 mM (approximately equivalent in osmolarity to 20 mM MgCl\(_2\)) and washed in. This solution had little effect (< 5% decrease in conductance) over the tested range of 20–100 mV (not shown). Since any effects of osmolarity were negligible compared to the large effect of Mg\(^{2+}\) shown in Fig. 1, the reduced current with Mg\(^{2+}\) was not due to any osmolarity changes.

Another possible explanation for the reduction of currents by Mg\(^{2+}\) in Fig. 1 is that Mg\(^{2+}\) might be having its effect by reducing the activity of the K\(^+\). This possibility was examined with a K\(^+\)-selective electrode (see Methods). Changes in activity of K\(^+\) with the addition of Mg\(^{2+}\) were far too small (< 5%) to account for the pronounced decrease in the currents with the addition of Mg\(^{2+}\).

This reduction of current will be referred to as Mg\(^{2+}\) block, without implying a specific mechanism. Since the block is graded, and there is no obvious increase in the open channel noise (not flickery) in the presence of the blocker, the block is a fast block as defined by Yellen (1984). The basis of this fast block by intracellular Mg\(^{2+}\) may be due to Mg\(^{2+}\) physically blocking the channel pore, allosteric interactions affecting the conduction pathway, or some other mechanism.

The Woodhull Model Can Approximate the Mg\(^{2+}\) Block for a Fixed Concentration of K\(^+\)

One possible mechanism for the concentration- and voltage-dependent block of the BK channel by Mg\(^{2+}\) is the blocking model proposed by Woodhull (1973). This model was developed to describe the blocking effect of H\(^+\) on whole-cell Na\(^+\) currents, and has been used to describe the blocking effects of many ions on the BK channel (Blatz and Magleby, 1984; Yellen, 1984; Vergara et al., 1984; Villarroel et al., 1988). The
Woodhull model can be summarized by:

\[
\frac{i_o}{i_B} = 1 + \left( \frac{[B]}{K_d(0)} \right) \exp \left( \frac{z d V F}{RT} \right)
\]

where \(\frac{i_o}{i_B}\) is the ratio of the single-channel currents in the absence and the presence of blocker, \([B]\) is the concentration of the blocking ion, \(K_d(0)\) is the dissociation constant of the blocking ion at 0 mV, \(z\) is the valence of the blocking ion, \(V\) is the voltage drop across the membrane, and \(d\) is the fraction of the voltage influencing the ion at the binding site as measured from the intracellular side of the membrane. \(RT/F\) is 25.4 mV at 22°C.

The best fit of the Woodhull model for all the data in Fig. 1 B, as determined by least squares (see Methods), is shown in Fig. 2 A. The Woodhull model can approximate the form of the concentration- and voltage-dependent block of the BK channel by Mg\(^{2+}\), but at positive potentials, where the blocking effect is largest, it underestimates the block with 5 mM Mg\(^{2+}\) and overestimates the block with 20 and 50 mM Mg\(^{2+}\). Measurements with an ion-selective electrode (see Methods) indicated that the activity coefficient of Mg\(^{2+}\) over the range of concentrations used in Fig. 2 A with 150 mM KCl, would be expected to change < 10%. The change in the activity coefficient, although in the correct direction, would account for < 30% of the difference between the predictions of the Woodhull model and the experimental observations. (This same argument still holds if it is assumed that the concentration of Mg\(^{2+}\) at its site of action within the membrane field is tripled at 100 mV.) Thus, the inability of the Woodhull model to better describe the Mg\(^{2+}\) block at positive potentials would still be present if the estimated (small) corrections were made for the concentration-dependent changes in the activity coefficient for Mg\(^{2+}\).

In contrast to the approximate fit at positive potentials, the Woodhull model describes the smaller blocking effect of Mg\(^{2+}\) at negative voltages, as shown in Fig. 2 A. This Mg\(^{2+}\) block at negative potentials is clearly seen for the BK channel, and contrasts to the reported lack of Mg\(^{2+}\) block of inward currents of the ATP-sensitive K\(^+\) channel (Horie et al., 1987; Findlay, 1987a, b), muscarinic K\(^+\) channel (Horie and Irisawa, 1987, 1989), and the inwardly rectifying K\(^+\) channel (Matsuda et al., 1987; Matsuda, 1988).

If the fit of the Woodhull model is restricted to a single concentration of Mg\(^{2+}\), then the Woodhull model gives excellent descriptions of the data, as shown in Fig. 2 B, which presents the fits carried out independently for each concentration of Mg\(^{2+}\) presented in Fig. 2 A. For the individual fits, the \(K_d\)'s range from 20.0 mM in 5 mM Mg\(^{2+}\) to 36.9 mM in 50 mM Mg\(^{2+}\), with a blocking distance, \(d\), of ~0.2 for all Mg\(^{2+}\) concentrations. The approximate fits shown in Fig. 2 A and the progressive change for \(K_d\) calculated from the individual fits in Fig. 2 B suggest that the Mg\(^{2+}\) block of the BK channel is more complicated than can be described by the Woodhull model.

**Decreasing the Intracellular K\(^+\) Enhances the Mg\(^{2+}\) Block**

To better understand the block of the BK channel by Mg\(^{2+}\), the effects of changing the concentration of K\(^+\) were investigated. Manipulating the concentration of K\(^+\) may give insight into which of the two general classes of blocking mechanism, competitive or noncompetitive, is most consistent with the Mg\(^{2+}\) block of the BK channel. Furthermore, these experiments will give some insight into the validity of the
Woodhull model to describe the Mg\textsuperscript{2+} block of the BK channel, since the Woodhull model does not explicitly account for the effects of changing K\textsuperscript{+}. Fig. 3A shows the effect of changing the concentration of K\textsuperscript{+} on single-channel currents in the presence and absence of 20 mM Mg\textsuperscript{2+} for a fixed membrane potential of +50 mV. When Mg\textsuperscript{2+} is increased from 0 to 20 mM, the current is decreased from 14.0 to 6.8 pA, in symmetrical 150 mM KCl. When the KCl is reduced to symmetrical 75 mM KCl, the reduction of the current is from 12.6 to 4.0 pA for the same change in Mg\textsuperscript{2+}. Thus, the percent reduction in current by 20 mM Mg\textsuperscript{2+} is greater in 75 mM K\textsuperscript{+} (68%) than in 150 mM K\textsuperscript{+} (51%).
FIGURE 3. Decreasing [K⁺] enhances Mg²⁺ block. (A) Currents through a single BK channel. The top traces were obtained with 0 mM Mg²⁺ and the bottom traces with 20 mM Mg²⁺. The left traces were obtained from one experiment with symmetrical 150 mM K⁺, and the right traces from another experiment with symmetrical 75 mM K⁺. Note that in both experiments the noise was decreased in the presence of Mg²⁺. This reduced noise with Mg²⁺ arises from an increase in the seal resistance. Applied potential: +50 mV. (B) The ratio of single channel current amplitude with i(Mg) and without i(0) 20 mM Mg²⁺ over a wide range of potentials and for 75, 100, 150, 500, and 1,000 mM K⁺. The K⁺ was symmetrical for the lower three curves and was 150 mM at the extracellular surface (in the pipette) for the upper two curves. The lines were calculated using the Woodhull model (Eq. 1) with the data from each line fit separately. The fitted parameters for Eq. 1 are 75 mM K⁺—K₀ = 17.7 mM and d = 0.18; 100 mM K⁺—K₀ = 25.2 mM and d = 0.19; 150 mM K⁺—K₀ = 33.4 mM and d = 0.19; 500 mM K⁺—K₀ = 181.0
and $d = 0.24$; and 1,000 mM K$^+$—$K_d = 587.0$ and $d = 0.26$. (C) The Woodhull model (Eq. 1) is fit to the Mg$^{2+}$ block in symmetrical 100 mM K$^+$, similar to Fig. 2 A. The fitted parameters for all the data shown are $K_d = 22.9$ mM and $d = 0.24$. (D) The Woodhull model fit to the Mg$^{2+}$ block in symmetrical 75 mM K$^+$. The fitted parameters to all the data shown are $K_d = 14.4$ mM and $d = 0.24$. 
The same type of experiment shown in Fig. 3 A was also repeated for 100 mM symmetrical K⁺ and for asymmetrical solutions of 150 mM extracellular K⁺ (Kₒ) with either 500 or 1,000 mM intracellular K⁺. Data were obtained over a range of membrane potentials for each condition, and the results are plotted in Fig. 3 B as the ratio of currents, \( \frac{i(Mg)}{i(0)} \), with and without 20 mM Mg²⁺. It is apparent from Fig. 3 that the blocking effect of Mg²⁺ is enhanced as [K⁺] is decreased, and this is clearly the case for both symmetrical and asymmetrical solutions. Fig. 3 B presents data for a single concentration of Mg²⁺. Similar effects of K⁺ were found for four different concentrations of Mg²⁺, as shown in Fig. 3, C and D, and Fig. 2 B.

In contrast to the K⁺-dependent effects in Fig. 3, the Woodhull model predicts that K⁺ would have no effect on either the currents or the \( K_d \)’s for the Mg²⁺ block. The inability of the Woodhull model to account for the effect of K⁺ can be estimated by calculating separately for each concentration of K⁺ the \( K_d \) for the Mg²⁺ block. The \( K_d \)'s, from the fit of the Woodhull model, for the Mg²⁺ block ranged from 17.7 to 537 mM, as K⁺ was changed from 75 mM to 1,000 mM for the data in Fig. 3 B. (The lines in Fig. 3 B indicate the separate fitting for each K⁺.) The \( K_d \)'s for the Mg²⁺ block ranged from 14.4 to 30.5 mM, as K⁺ was changed from 75 to 150 mM for the data in Figs. 3 D and 2 A.

**Competitive Interaction between Mg²⁺ and K⁺**

Having observed that K⁺ affects the ability of Mg²⁺ to block the channel, this data can be used to investigate whether the interaction between Mg²⁺ and K⁺ might be consistent with either a competitive or noncompetitive blocking model. To investigate this interaction an approach from enzyme kinetics (Stryer, 1981) has been used. In enzyme kinetics a simple test of competitive vs. noncompetitive inhibition is to plot 1/velocity vs. 1/[substrate]. For the BK channel the velocity and substrate can be represented by current, \( i \), and [K⁺], respectively. Fig. 4 A shows theoretical results as double reciprocal plots for a competitive and a noncompetitive block. For a noncompetitive block the results with (N) and without (0) blocker intersect on the x-axis (infinite current). More importantly, it is obvious that the block by a noncompetitive blocker cannot be overcome, even at infinite K⁺ (which occurs at \( x = 0 \)). Whereas for a competitive block the results with (C) and without (0) blocker intersect on the y-axis, indicating that at infinite K⁺ the blocker (Mg²⁺) has no effect.

Experimental results for the BK channel are presented in Fig. 4 B, which plots 1/\( i \) vs. 1/[K⁺] with and without 20 mM Mg²⁺ at +80 mV membrane potential. The intersection of the lines near the y-axis is consistent with a competitive interaction between Mg²⁺ and K⁺ (compare with Fig. 4 A). The fact that \( i_{max} \) is unaltered can be seen in Fig. 4 B where the lines intersect at \( \sim 3 \) M K⁺; at this point 20 mM Mg²⁺ would theoretically have no effect on the current. A competitive block also suggests that the ions interact with each other by changing the apparent \( K_d \) for the other ion. The finding of apparent competitive inhibition between Mg²⁺ and K⁺ for the BK channel may explain why Villarroel et al. (1988), using the Woodhull model (a noncompetitive model), found different \( K_d \)'s for organic blockers when the concentration of K⁺ was changed.

All the plotted points in Fig. 4 are consistent with a competitive model even though the data with 500 and 1,000 mM intracellular K⁺ were obtained with asymmetrical...
solutions (150 mM extracellular K\(^+\)). It is possible to include the data obtained with asymmetrical solutions by correcting for the increased driving force on K\(^+\). The correction for asymmetrical solutions was made by subtracting the current at 0 mV from the current at the specified applied voltage, which was 80 mV for the data in Fig. 4. (This correction is equivalent to correcting for the increased driving force by
multiplying the observed current by the applied potential divided by the applied potential minus the reversal potential.)

It is important to note that a conclusion of competitive inhibition would still be obtained if the plots were restricted to data obtained with only symmetrical solutions, since the data points with symmetrical and asymmetrical solutions fell on the same line (Fig. 4 B). Thus, differences in the permeation process that may result from differences between symmetrical and asymmetrical solutions seem to have little effect on the apparent competitive block for these experimental conditions. Results similar to Fig. 4 B were seen at three additional voltages (50, 30, and -60 mV) with 20 mM...
Mg\(^{2+}\), as well as with 5, 10, and 50 mM Mg\(^{2+}\). In all cases the y-intercept was not statistically different for data obtained with and without blocker. Thus, the results in Fig. 4 and the six other experiments suggest that models for Mg\(^{2+}\) block should be based on mechanisms which can give an apparent competitive type of block, rather than noncompetitive block.

**Ca\(^{2+}\), Sr\(^{2+}\), and Ni\(^{2+}\) Also Block the BK Channel**

After finding that the block by Mg\(^{2+}\) is competitive in nature, the next step was to examine the specificity of the block. For these experiments the effects of Ca\(^{2+}\), Sr\(^{2+}\), and Ni\(^{2+}\) were used. Fig. 5 shows the results of experiments testing the effects of 0.5, 5, and 20 mM Ca\(^{2+}\), Sr\(^{2+}\), Mg\(^{2+}\), and Ni\(^{2+}\). Each part of Fig. 5, A–C, presents an experiment where all four divalent cations (Mg\(^{2+}\), Ca\(^{2+}\), Sr\(^{2+}\), Ni\(^{2+}\)) were tested on the intracellular side of the same patch at the given concentration.

There are several points to be made about Fig. 5. First, all four divalent cations block in a similar concentration- and voltage-dependent manner that is preserved across the examined 40-fold change in blocker concentration, suggesting similar blocking mechanisms. Furthermore, the observation that all four divalent cations have estimated K\(_d\)'s within two- to threefold of each other, and that the K\(_d\)'s of Ca\(^{2+}\) and Sr\(^{2+}\) are almost identical, suggests that the binding at the blocking site is not very specific (apparent K\(_d\)'s in figure legend). This nonspecificity of binding would be expected if the underlying competitive mechanism were electrostatic in nature. However, the fact that the blocking by all four divalent cations is not identical, with a blocking order of Ni\(^{2+}\) > Mg\(^{2+}\) > Ca\(^{2+}\) ~ Sr\(^{2+}\), indicates that the mechanism of
blocking is more complicated than the mere screening of negative charges or dipoles. Some of these complicating factors will be presented in the Discussion.

**Resolving Some Paradoxes**

Fig. 5 shows that Ca$^{2+}$ (as well as other divalent cations) blocks the BK channel. In contrast to the Ca$^{2+}$ block in Fig. 5, Barrett et al. (1982) observed that 1 mM Ca$^{2+}$ had no effect on single-channel conductance, and Marty (1983a) also reported no effect, attributing a possible Ca$^{2+}$ block in an earlier study (1981) to a change in K$^+$. Interestingly, Barrett et al. (1982) and Marty (1981) had 2 mM Mg$^{2+}$ in their solutions. To test the possibility that the Mg$^{2+}$ may have masked any obvious blocking effects of Ca$^{2+}$, single-channel conductance was examined in the presence and absence of 1 mM Ca$^{2+}$ and 2 mM Mg$^{2+}$. Results are shown in Fig. 6. With 2 mM Mg$^{2+}$, the additional blocking effect of 1 mM Ca$^{2+}$ would be difficult to detect.

A variety of conductances have been reported for the BK channel from the same preparation (primary rat muscle). For example, Blatz and Magleby (1984) found a conductance of 307 ± 36 pS in 140 mM KCl with no added Mg$^{2+}$, whereas Barrett et al. (1982) found a conductance of approximately 218 ± 6 pS in 144 mM KCl with 2 mM Mg$^{2+}$. Both results were obtained at 21–24°C. Fig. 6 shows that 2 mM Mg$^{2+}$ reduces single-channel conductance from 280 pS (continuous line) to 232 pS (dashed line). Thus, at least part of the difference in channel conductance in these (and other) experiments may arise from divalent ions. The solutions of Barrett et al. (1982) also contained 16 mM Na$^+$, which would further reduce single-channel conductance (Marty, 1983b; Yellen, 1984), possibly to the observed value of 218 pS. The fact that 2 mM Mg$^{2+}$ is in the lower range of the reported physiological concentrations of Mg$^{2+}$ (Hess et al., 1982; Gupta et al., 1983; Blatter and McGuigan, 1986; Blatter et al., 1989) suggests that the Mg$^{2+}$ blocking reported here is of physiological importance.
DISCUSSION

The results of this study show that intracellular Mg\(^{2+}\) reversibly reduces (blocks), in a concentration-dependent manner, the single-channel current through the large-conductance, Ca\(^{2+}\)-activated K\(^{+}\) (BK) channel in cultured rat skeletal muscle. The block is a fast block as defined by Yellen (1984), in that there is a graded decrease in the single-channel current level without an apparent increase in the open-channel noise. Mg\(^{2+}\) does not shift the reversal potential, indicating that Mg\(^{2+}\) is not permeable through the BK channel. The Mg\(^{2+}\) block is present at negative potentials and increases in magnitude as the voltage is made more positive (Fig. 1). The observation that Mg\(^{2+}\) also blocks inward currents as well as outward currents is in contrast to the observations of other K\(^{+}\) channels where Mg\(^{2+}\) has no apparent effect on inward currents, even though it blocks outward currents (Horie et al., 1987; Findlay, 1987a; Horie and Irisawa, 1987, 1989; Matsuda et al., 1987; Matsuda, 1988).

Block of ion channels is a common phenomenon and a number of different mechanisms have been proposed to describe it. One of the first blocking models was presented by Woodhull (1973). Her elegantly simple model, which has found wide applicability, involves a single blocking ion entering the membrane potential field a distance d to a binding (blocking) site. The blocking site is often thought to be directly in the path of the permeating ion, but other possibilities, such as allosteric interactions, have also been considered. The Woodhull model can describe the block of BK channels by Na\(^{+}\) (Yellen, 1984), tetraethylammonium (Blatz and Magleby, 1984; Vergara et al., 1984), and other quaternary ammonium ions (Villarroel et al., 1988). Fig. 2 and Fig. 3, C and D, show that the Woodhull model approximates the Mg\(^{2+}\) block or a fixed concentration of K\(^{+}\). However, decreasing K\(^{+}\) enhances the Mg\(^{2+}\) block of the channel (Fig. 3), and the Woodhull model does not explicitly account for this K\(^{+}\) effect on the Mg\(^{2+}\) block. Furthermore, the Woodhull model may have some theoretical limitations, as considered by Tang et al. (1989).

To further understand the effects of K\(^{+}\) on the Mg\(^{2+}\) block, plots of the inverse of the current amplitude vs. the inverse of the K\(^{+}\) concentration were used. This type of plot (Fig. 4 A) is borrowed from enzyme kinetics where it is used to distinguish competitive blockers from noncompetitive blockers. This borrowing from enzymology seems quite natural since current (charges per second) for an ion channel is analogous to velocity (products per second) for an enzyme, and concentration of permeant ion for ion channels is analogous to concentration of substrate for an enzyme (Hille, 1975; Eisenberg, 1990). If a blocker such as Mg\(^{2+}\) is competitive, then increasing the concentration of the substrate K\(^{+}\) should relieve the block. This is exactly what was observed (Fig. 4 B), suggesting a competitive type of interaction between Mg\(^{2+}\) and K\(^{+}\).

Since the Woodhull model does not account for the competition between Mg\(^{2+}\) and the K\(^{+}\) another model is needed. The first such model examined was derived based on the competitive model used in enzyme kinetics. The Michaelis-Menten equation for competitive inhibition was modified by multiplying the concentration terms by a Boltzmann function to describe the effect of voltage on the concentrations of K\(^{+}\) and Mg\(^{2+}\). (Similar modification of the Michaelis-Menten equation for non-competitive
inhibition leads to an equation identical to the Woodhull model.) This competitive model could account for some, but not all, of the effect of K⁺ relieving the Mg²⁺ block, and hence, has not been presented in the Results. The inaccuracies of this model may arise from the simplification of the K⁺ permeation process, which in the Michaelis-Menton competitive equation is modelled by a simple saturating function. Such functions are, at best, a simplification of a complex process (Moczydlowski et al., 1985; Neyton and Miller, 1988).

Another model examined to account for the interaction between Mg²⁺ and K⁺ was the one used by Pusch et al. (1989) to describe the block of the Na⁺ channel by Mg²⁺. Their model is based on the Goldman-Hodgkin-Katz equation with binding sites for the blocking. The model of Pusch et al. (1989) gave a poorer description of the Mg²⁺ block than the model derived from Michaelis-Menton competitive inhibition, and hence, has not been presented in the Results.

A more mechanistic representation of how Mg²⁺ reduces single-channel current and competes with K⁺ may be that it acts to screen negative surface charge rather than binding to a single specific site (Dani, 1986; Jordan, 1987). In a landmark study of acetylcholine (ACh) receptor channels using site-directed mutagenesis, Imoto et al. (1988) detailed the importance of negatively charged amino acid residues on channel conductance. The negative charges involved are located in inner and outer vestibules which form the transition space from the bulk solution to the narrow permeation pore. (Toyoshima and Unwin [1988] have characterized the structure of the permeation pathway for the ACh receptor channel.) Imoto et al. (1988) found that eliminating negative charges from the intracellular vestibule reduced the outward flow of current more than the inward flow of current. Furthermore, the Mg²⁺ block of current in the ACh channel was decreased when negative charges were removed (Imoto et al., 1988), suggesting that negative surface charges are at least partially responsible for the blocking effects of Mg²⁺ on the ACh receptor channel. Thus, a possible explanation of the Mg²⁺ block that is observed for the BK channel may be that Mg²⁺ is screening negative changes. The reduction of current would then be due to the reduction of the local concentration of K⁺ that is accumulated by the presence of negative charge. Negative charge has been measured at the intracellular face by Villarroel and Eisenman (1989) and at the extracellular face by MacKinnon et al. (1989) in large-conductance, Ca²⁺-activated K⁺ channels from rat T tubules inserted into planar lipid bilayers.

To test if surface charge is involved in the block of the BK channel by Mg²⁺, the possible blocking effects of three additional divalent cations: Ca²⁺, Sr²⁺, and Ni²⁺ were examined. If surface charge is involved in the Mg²⁺ block, then all these divalent cations should have similar blocking effects because of their similar charge densities. Ca²⁺, Sr²⁺, and Ni²⁺ were found to have blocking actions similar to Mg²⁺ (Fig. 6), suggesting that screening of surface charge may play a role in the block by these divalent cations. However the Kᵢ's varied two- to threefold, suggesting that the blocking mechanism may be more complex than simple screening of charges. This complexity may arise from some weak binding as discussed by Dani (1986).

Screening of charge at an inner vestibule surface by Mg²⁺ and the accumulation of Mg²⁺ in a vestibule by the membrane potential could reduce the conductance of the BK channel. This reduction of conductance may be through a local reduction in the
concentration of the $K^+$. However, surface charge could act through a different mechanism. MacKinnon and Miller (1989) found that chemically removing extracellular negative charges on the BK channel led to symmetrical reduction in single-channel conductance, in contrast to the asymmetrical reduction found by Imoto et al. (1988) for the removal of surface charge in the ACh receptor channel. MacKinnon et al. (1989) suggested that the potential from the negative surface charge could alter the "energy profile" of the permeating ion, making it easier for the ion to permeate the channel. Indeed, both of these or some other mechanism could contribute independently or simultaneously to the effects produced by surface charge.

The surface charge model presented in MacKinnon et al. (1989) was not applied to the data presented in this present paper, inasmuch as their model does not account for the effects of membrane potential on blocking and is only applicable to conditions with unidirectional fluxes. Furthermore, it is not a straightforward problem to incorporate the effects of both negative charges and membrane potential, as both surface charge and membrane potential change the local concentration of ions and therefore the double-layer potential in an interactive manner (Dani, 1986). The modeling of the voltage-dependent $Mg^{2+}$ block with surface charge models will require knowing the structure of the channel and the location of the charge, and this information is not yet available.

In conclusion, the $Mg^{2+}$ block of the BK channel can be approximated by the Woodhull model (Eq. 1) at a single $K^+$ concentration. However this model fails to account for the apparent competitive interaction between $K^+$ and $Mg^{2+}$. This competitive inhibition could arise from $Mg^{2+}$ displacing $K^+$ from the entrance to the pore, possibly in a vestibule similar to that found for the ACh receptor channel (Toyoshima and Unwin, 1988). The decrease of the permeant ion $K^+$ concentration near the pore would then decrease the single-channel current. Increasing the concentration of $K^+$ then displaces $Mg^{2+}$, relieving its blocking effect, as expected for a competitive type inhibition. Though there is some evidence for a displacement mechanism of this type, a quantitative test of this model must await detailed knowledge of the three-dimensional structure and charge distribution for the BK channel.

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