Ion Conductance of the Ca^{2+}-Activated Maxi-K^+ Channel from the Embryonic Rat Brain

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ABSTRACT By using single-channel recording techniques, we measured the conductance (g_K) of the Ca^{2+}-activated Maxi-K^+ channel from the embryonic rat brain, and examined its dependence on K^+ ions present in equimolar concentrations on both sides of the membrane patch. With ionic strength maintained constant by substitution of *N*-methyl-D-glucamine for K^+, g_K has a sigmoidal dependence upon $[K^+]$. This result has been obscured in previous work by variations in ionic strength, which has a marked effect on single-channel conductance, especially in the limit for which this variable approaches zero. The g_K versus $[K^+]$ relationship is described, theoretically, by a three-barrier, two-binding-site model in which the barrier that an ion must cross to leave the channel is decreased as $[K^+]$ is increased.

INTRODUCTION

The permeability characteristics of large-conductance K^+-selective channels have been a topic of considerable interest since these channels were first discovered in the mammalian sarcoplasmic reticulum (SR; Coronado et al., 1980). The SR channel and the Ca^{2+}-activated Maxi-K^+ (BK) channel (Marty, 1981; Pallotta et al., 1981) have two seemingly contradictory properties; namely, high conductance and high selectivity (Latorre and Miller, 1983). A multi-ion pore model was proposed by Latorre and Miller (1983) as a possible solution to this problem. Subsequent experiments have clearly demonstrated this property for the BK channel (Eisenman et al., 1986).

A related issue concerns the known effect of ionic strength on single-channel conductance (Green et al., 1987; Kell and DeFelice, 1988; MacKinnon et al., 1989). One useful way to obtain information on the electrical properties of an ion channel is to measure single-channel conductance for conditions in which the permeant ion concentration is the same on both sides of the membrane. Conductance as a function of concentration can be used to infer binding and saturation properties of the ion within the channel. This experiment has apparently been carried out only for ion channels incorporated in planar lipid bilayers (Coronado et al., 1980; Bell and Miller, 1984; Eisenman et al., 1986; Green et al., 1987; MacKinnon et al., 1989). One significant aspect of these results is an apparent finite conductance in the limit of zero permeant ion concentration, an effect that has been attributed to a surface charge either on the channel itself or on the membrane lipids (Bell and Miller, 1984; Moczydlowski et al., 1985; Green et al., 1987; MacKinnon et al., 1989). In all of these reports the ionic strength was not maintained constant. Rather, the permeant salt concentration was raised on both sides of the membrane.

The aim of our experiments was to determine the conductance versus concentration (g_K versus $[K^+]$) relation for the BK channel in conditions where ionic strength was maintained constant while $[K^+]$ was altered on both sides of the membrane. The embryonic (E) rat telencephalon is well suited for this experiment because BK channels are relatively abundant at early (E13-14) stages and inside-out patches can be obtained in a relatively routine manner directly from the cells lining the ventricular surface of the brain (Mienville, 1994; Mienville and Clay, 1996). Moreover, the conductance of the channel for a given set of conditions was found to be remarkably consistent from patch to patch. Consequently, we were able to obviate the technical difficulty of exchanging pipette contents "on-line," and simply pooled results from several different patches in each experimental condition.

Perhaps the most significant result from our experiments was the finding that g_K has a sigmoidal dependence upon $[K^+]$, a result that may ultimately have bearing on the issue raised above—namely, why is it that this channel has such a large conductance for physiologic levels of K^+?

MATERIALS AND METHODS

In situ patch-clamp experiments were carried out as previously described (Mienville, 1994). Briefly, cerebral vesicles were dissected from E13-14 rat embryos and placed ventricular side up in a 150-μl recording chamber perfused continuously with a solution consisting of (in mM): 120 NaCl, 5 KCl, 1.25 NaH_2PO_4, 4 $MgCl_2$, 26 $NaHCO_3$, 1 sodium pyruvate, 10 dextrose, and 1 EGTA (pH = 7.4 when gassed with 95% O_2, 5% CO_2), and supplemented with essential amino acids and minimum essential medium vitamins (Gibco). The absence of Ca^{2+} favored inside-out patch formation (Hamill et al., 1981). As described previously (Mienville, 1994; Mienville and Clay, 1996), most BK channels in this preparation exhibit a buzz mode gating that can be converted to normal mode gating by a brief exposure to a low concentration of trypsin without altering the fundamental properties of the channels. Measurements of the g_K versus $[K^+]$ relation were carried out with the same solution in the patch pipette and in the bath. For

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experiments in which the ionic strength was maintained constant (311 mM salt), the bath and pipette solution contained x (KCl + KOH) and 300-x *N*-methyl-D-glucamine (NMG; Sigma), where x was 20, 50, 100, 225, or 300 mM. NMG was initially prepared as a 2 M stock solution titrated to pH $\approx$ 7.2 with HCl. Recording solutions also contained 10 HEPES, 0.28 $CaCl_2$, and 1 *N*-hydroxyethyl-ethylenediamine-triacetic acid (HEDTA) to yield a final pH of 7.2 and a pCa = 6. Experiments were also carried out with 600 K (0 NMG) and 225 K + 175 NMG. NMG was omitted in another series of experiments (20, 150, and 225 K) for which ionic strength was specifically not maintained constant but osmolarity compensated by appropriate additions of sucrose.

The current signal was filtered and sampled at 2 and 10 kHz, respectively. Sampling and analysis were performed with PAT 7, the single-channel program from SES software (courtesy of J. Dempster, Strathclyde University, U.K.). Single-channel current (i) was measured either from the peaks of amplitude distributions or directly from a readout cursor. Conductance was estimated from linear regression of i/V plots. Due to slight inward rectification of the channel, the least-squares fit was limited to $V = \pm 60$ mV, except for the 20 K + 280 NMG condition, for which data throughout the $V = \pm 100$ mV range were used.

RESULTS

Recordings of the embryonic rat brain BK channel for various ionic conditions are illustrated in Fig. 1. The results in the top and bottom panels of Fig. 1 *A* were obtained with 20 mM K$^+$ (20 K) and 0 NMG, or 20 K and 280 NMG, respectively, on both sides of the membrane. A different patch was used for each condition. These recordings illustrate a large reduction of single-channel conductance with an increase in ionic strength, similar to results reported for the rat skeletal muscle BK channel incorporated in planar lipid bilayers (MacKinnon et al., 1989). The consistency of the data from different patches for the ionic conditions used in Fig. 1 *A* is illustrated in Fig. 1 *B*, with current-voltage relations plotted from six and four different experiments in 20 K and 20 K + 280 NMG, respectively. Similar results for 225 K with 0, 75, or 175 NMG are shown in Fig. 1 *C* and *D*.

Pooled single-channel conductances in various conditions are illustrated in Fig. 2. The three results noted by empty circles, as well as the 300 K and 600 K results, correspond to conditions in which the ionic strength was not maintained constant. The data describe a curve that appears to intersect the conductance axis at a finite value with [K$^+$] = 0, similar to the bilayer results of MacKinnon et al. (1989). In con-

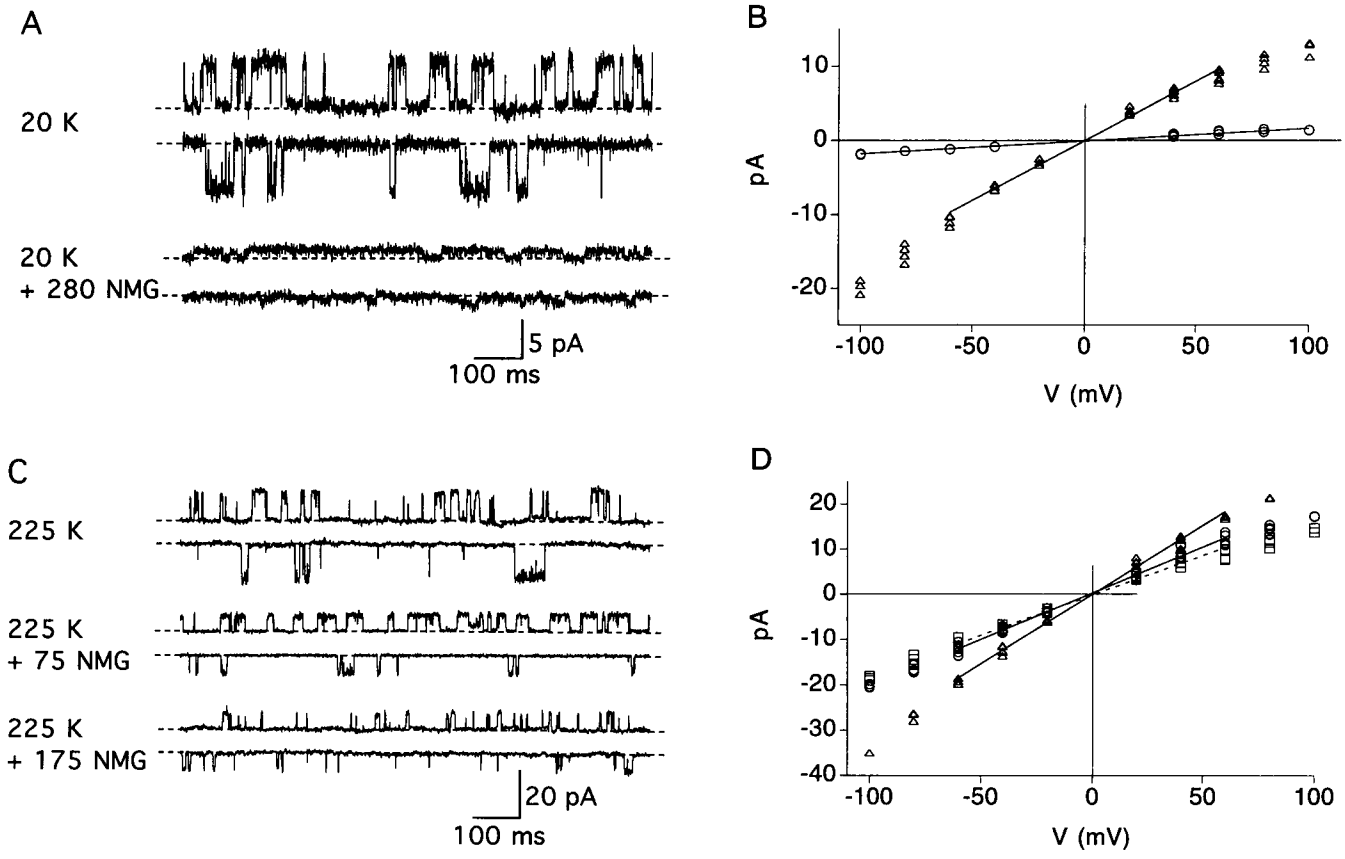

FIGURE 1 Effects of ionic strength on single-channel conductance of the BK channel. (*A* and *C*) Recordings from single BK channels for the conditions given to the left of each panel. The top and bottom recording for each case was obtained with a holding potential of either +40 or −40 mV, respectively. A different patch was used for each condition with the solution indicated to the left in both the patch pipette and the bath (see Methods). (*B*) Current-voltage (*i/V*) relations from patches recorded in 20 K or 20 K + 280 NMG (*triangles* and *circles*, respectively). (*D*) *i/V* relations in 225 K (*triangles*), 225 K + 75 NMG (*circles*), and 225 K + 175 NMG (*squares*). Straight lines are least-squares fits to each set of data (*dashed line* is for 225 K + 175 NMG). As indicated, only the points from −60 to +60 mV were used in this analysis, except for the 20 K + 280 NMG results, for which all points were used.

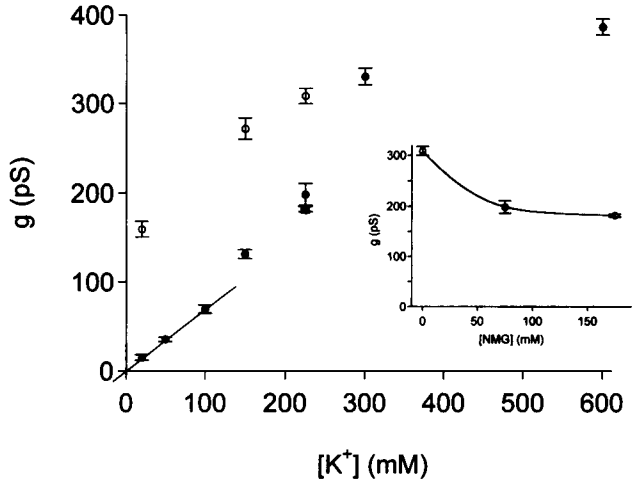

FIGURE 2 Summary of the dependence of single-channel conductance on symmetrical K^+ concentration for all conditions used. Open symbols correspond to 0 NMG while filled circles indicate conditions in which ionic strength was maintained at 311 mM, save for the 600-mM result. The single square corresponds to 225 K + 175 NMG. In the inset, the points corresponding to 225 K are plotted against NMG concentration. The smooth curve through these points has no particular significance. Each symbol and error bar represents the mean and standard deviation of the mean ($4 \leq n \leq 9$).

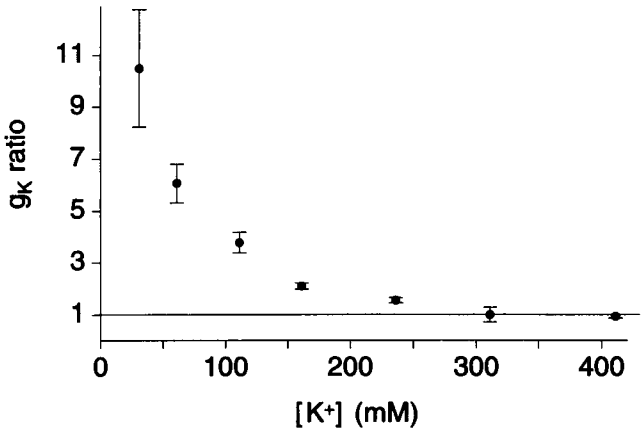

FIGURE 3 Effect of ionic strength on single-channel conductance. These results represent the ratio of g_K obtained under conditions of constant ionic strength to that obtained in 0 NMG (ionic strength not maintained constant), except for the 411-mM point, which represents the ratio 225 K + 175 NMG/225 K + 75 NMG. Each symbol represents the ratio, r, of the means for the two conditions. The error bars correspond to $r[(\delta g_1/g_1)^2+(\delta g_2/g_2)]^{1/2}$, where g_1 and g_2, and δg_1 and δg_2 are the respective means and standard deviations for the two conditions. The results with 0 NMG and 50 or 100 K were taken from Fig. 2 of MacKinnon et al. (1989).

trast, conductances obtained at constant ionic strength (*filled circles*) clearly extrapolate to zero for $[K^+] = 0$. Moreover, the results at constant ionic strength are clearly superlinear for $[K^+] > 100$ mM and approach a saturating value for $[K^+] = 600$ mM, so that the entire g_K versus $[K^+]$ relation has a sigmoidal shape. Fig. 2 also illustrates g_K for 225 K + 175 NMG (*filled square*). This data point along with those for 225 K + either 0 or 75 NMG are also plotted in the inset as a function of NMG concentration. The conductance appears to reach a constant, finite value for NMG = 175 mM, which is consistent with its reduction being attributable to an ionic strength effect rather than channel blockade (see Discussion).

The effect of ionic strength on g_K is further illustrated by Fig. 3, which was obtained by taking the ratio of g_K with 0 NMG ($[K^+]$ = 20, 150, and 225 mM in Fig. 2) and the corresponding results with NMG present on both sides of the membrane (total salt concentration, i.e., K^+, NMG, and buffer = 311 mM). (The 61- and 111-mM points in Fig. 3 were obtained with the aid of results in Fig. 2 of MacKinnon et al., 1989). Fig. 3 also contains the 300 K point from Fig. 2 normalized to unity as well as the 225 K + 175 NMG result normalized relative to the g_K value obtained with 225 K + 75 NMG. These results demonstrate that a reduction in ionic strength from the physiologic level of 150 mM has an increasingly large effect on g_K, an effect which possibly approaches infinity as ionic strength approaches zero. Moreover, an increase in ionic strength from the physiologic level has a relatively modest effect on g_K, which reaches a saturating level at ~300 mM salt concentration. Consequently, the relative difference between the 300 K and 600 K results in Fig. 2 does not require correction for the difference in ionic strength between these two solutions.

A barrier model of the g_K versus $[K^+]$ relationship

The g_K versus $[K^+]$ results for which ionic strength was maintained constant (along with the 600 K result) are reproduced in Fig. 4. A three-barrier, two-binding-site model similar to that used by Cecchi et al. (1986) is also illustrated, schematically, in the upper panel. The model is a standard treatment of ion channel permeation (Hille and Schwarz, 1978) with one exception: the probability that an ion exits the channel is dependent upon $[K^+]$. That is, the barriers adjacent to the external and internal surface of the membrane are decreased as $[K^+]$ is increased (cf. legend of Fig. 4), allowing ions to exit at a faster rate. (A related dependence of barrier geometry on $[K^+]$ was proposed by Waggoner and Oxford (1987) for the delayed rectifier K^+ channel.) As can be seen from the energy profile, a decrease in the barriers to exit is equivalent to a decrease in well depth. The model that best described the data involved a change in well depth that was an exponential function of $[K^+]$ (as depicted in the *inset*), according to the equation:

$$\text{Well depth} = (\text{depth}_{max} - \text{depth}_{min}) \times \exp(-[K^+]/k) + \text{depth}_{min},$$

where k is a constant that can be used to calculate the concentration of K^+ for half-maximal effect ($C_{[1/2]}$) according to the relation $C_{[1/2]} = k \times \ln 2 = 79$ mM.

Overall, the model qualitatively mimics the experimental g_K versus $[K^+]$ relation, as shown by the sigmoidal curve in Fig. 4.

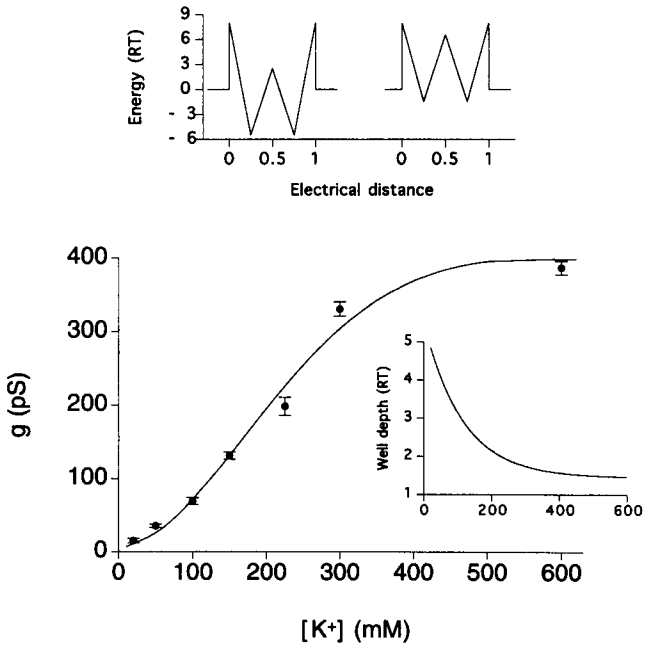

FIGURE 4 A model of the g_K versus $[K^+]$ relationship. Constant ionic strength data were taken from Fig. 2. The theoretical line was obtained from the three-barrier, two-binding-site model illustrated, schematically, by the free energy diagrams at the top of the figure. Barrier heights were chosen arbitrarily so as to account for the high ionic selectivity of the BK channel, resulting in an entry rate equal to $2.05 \times 10^9\ s^{-1}\ M^{-1}$. Well depth was determined so as to fit experimental data by eye. The left and right profiles correspond to the low and high ends of the K^+ concentration range, respectively. The model was implemented with a program written in QBasic (Hess and Tsien, 1984). From the relationship between well depth and $[K^+]$ (*inset*), the K_D for K^+ (exp $-\Delta G$) ranges from 4 to 236 mM, and exit rate (k_{out}) corresponds to $8.4 \times 10^6 \leq k_{out} \leq 4.8 \times 10^8$ at $V = 0$.

DISCUSSION

Relatively few studies have appeared that describe ion conductance as a function of permeant ion concentration with equimolar ion concentrations on each side of the membrane. For K^+ channels, the only reports, other than bilayer results, appear to be the macroscopic measurements of the delayed rectifier current, I_K, from intracellularly perfused squid giant axons by Waggoner and Oxford (1987) and Clay (1991). Our study represents the first time this experiment has been carried out at the single-channel level for K^+ channels in biological membranes. Similar experiments have been performed with ion channels incorporated in bilayers, most notably the work of MacKinnon et al. (1989) for the BK channel from rat skeletal muscle. Our results with 0 NMG in Fig. 2 are essentially identical to those obtained by these authors in the absence of a constant ionic strength (see MacKinnon et al., 1989, Fig. 2). Controlling the latter variable, we found a sigmoidal conductance versus concentration relationship, a result that has not been previously reported.

The primary concern in this study is that the ion substitute for potassium does not interact with the channel in some manner, such as by blocking it or altering its kinetics (Bell and Miller, 1984; Waggoner and Oxford, 1987). *N*-methyl-D-glucamine (NMG) appears to meet these criteria (Demo and Yellen, 1992). Ionic blockade may be associated with rapid flicker of the channel current, as with Na^+ block of BK channels (Yellen, 1984). In neither this nor our previous study (Mienville and Clay, 1996) was this type of effect observed with NMG. Alternatively, very fast block rates can be recorded as a reduction in channel conductance, as with TEA block of BK channel (Yellen, 1984). Our data also tend to rule out such an effect for NMG: Fig. 2 shows that the g_K values obtained with a more than twofold change in NMG are very nearly the same. If g_K reduction with 225 K + 75 NMG relative to 225 K + 0 NMG were due to NMG block, then a much larger effect should occur with 225 K + 175 NMG. Furthermore, the inset of Fig. 2 shows that the g_K/[NMG] curve does not tend to zero as would be expected if NMG blocked the channel. Thus, the difference between the high- and low-ionic-strength results appears to be due to a nonspecific reduction of single-channel conductance by an increase in ionic strength. Moreover, this effect saturates at ~300 mM salt concentration, as illustrated in Fig. 3. Another argument against a channel block by NMG is that if this were the case, such an effect might be expected to exhibit some voltage dependence. Fig. 1 *D* shows that the *i/V* relationships have the same shape in 0, 75, or 175 NMG.

As noted by Green and Andersen (1991), the effects of ionic strength on channel gating have been extensively documented, whereas the influence of this variable on single-channel conductance has been investigated in relatively few reports. The bilayer experiments in which conductance versus permeant ion concentration was measured with varying ionic strength mask, in our view, the specific relationship between the permeant ion and the ion channel because of a nonspecific interaction of ionic strength with surface charge residing either on the channel or its lipid environment. This issue has been addressed in previous reports, either by incorporating channels without apparent surface charge, such as the SR K^+ channel, in a neutral lipid bilayer (Bell and Miller, 1984), or by using trimethyloxonium to remove the surface charge on the channel, as in the case of the BK channel (MacKinnon and Miller, 1989). In those studies, the g_K versus $[K^+]$ relationship does appear to extrapolate to zero, or at least close to zero, in the limit of $[K^+] = 0$. However, effects of ionic strength still cannot be ruled out, since this variable was not controlled in those experiments.

A finite conductance in the limit of zero permeant ion concentration is, in our view, paradoxical. The paradox may appear to be solved by surface charge theory, which in some formulations predicts such a result (MacKinnon et al., 1989), although difficulties with this interpretation have been noted by Cai and Jordan (1990). With respect to the BK channel, this paradox is eliminated in our study. In its place is a surprising, novel result, namely a sigmoidal g_K versus $[K^+]$ relationship. Such a relationship is reminiscent of the enzyme-substrate cooperativity modeled by Monod et al. (1965). (In that context, it is interesting to note that

unscreened surface charges may appear to play the same role as enzyme activators in leading from a sigmoidal to a hyperbolic relation.) Cooperativity in multi-ion channels might be achieved through permeant ion interactions. Based on the original work of Hille and Schwarz (1978), Latorre and Miller (1983) suggested that multiple occupancy may, through electrostatic repulsion, effectively decrease energy barriers and speed up ion exit rate. In line with this hypothesis, we account for our results with a model consistent with Eyring rate theory and postulating an increased exit rate of K^+ following higher occupancy at larger concentrations of the ion. It will be of interest to see if other channels also follow a sigmoidal conductance-concentration relation when ionic strength is not allowed to be a variable of the analysis.

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