Effects of Intracellular K^+ and Rb^+ on Gating of Embryonic Rat Telencephalon Ca^{2+}-Activated K^+ Channels

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ABSTRACT We have investigated the effects of intracellular K^+ and Rb^+ on single-channel currents recorded from the large-conductance Ca^{+2}-activated K^+ (BK) channel of the embryonic rat telencephalon using the inside-out patch-clamp technique. Our novel observation concerns the effects of these ions on rapid flickering of channel openings. Specifically, flicker gating was voltage dependent, i.e., it was reduced by depolarization in the −60 to −10 mV range with equimolar concentrations of K^+ ions (150 K_o^+/150 K_i^+). Removal of K_i^+ resulted in significant flickering at all potentials in this voltage range. In other words, the voltage dependence of flicker gating was effectively eliminated by the removal of K_i^+. This suggests that a K^+ ion entering the channel from the intracellular medium binds, in a voltage-dependent manner, at a site that locks the flicker gate in its open position. No effects of changes in K_i^+ were observed on the primary, voltage-dependent gate of the channel. The change in flickering did not cause a change in the mean burst duration, which indicates that the primary gate is stochastically independent of the flicker gate. Intracellular Rb^+ can substitute for—and is even more effective than—K_i^+ with regard to suppression of flickering. Substitution of Rb_i^+ for K_i^+ also increased the mean burst duration for $V \geq -30$ mV. Both effects of Rb_i^+ were removed by membrane hyperpolarization.

INTRODUCTION

The original work on squid giant axons by Hodgkin and Huxley (1952) and Chandler and Meves (1965) suggested that ion channel gating was independent of ion permeation through channels. An alternative view has been provided by a continuing series of reports, especially for K^+ channels, which suggest an interaction between gating and permeation, specifically a slowing of channel deactivation, most notably with K^+ and Rb^+ (Arhem, 1980; Swenson and Armstrong, 1981; Cahalan et al., 1985; Clay, 1986; Matteson and Swenson, 1986; Spruce et al., 1989; Sala and Matteson, 1991; Demo and Yellen, 1992). Most of these reports pertain to the effects of *extracellular* cations. In contrast, surprisingly little information is available concerning effects on gating of *intracellular* cations, except for the work of Sala and Matteson (1991) on whole-cell recordings of K^+ current from toadfish pancreatic islet cells in the presence of Rb_i^+, and the single-channel recordings from the frog skeletal muscle delayed rectifier of Spruce et al. (1989). To our knowledge no information on this topic has been reported for the large conductance, Ca^{2+}-activated K^+ (BK) channel. In their work on the selectivity of the BK channel from rat skeletal muscle, Blatz and Magleby (1984) briefly noted that intracellular ion substitution affected channel kinetics, but they did not further investigate this issue.

The single-channel recording technique has unique advantages for a study concerning the effects of ions on gating, because channel closings and openings can be investigated separately. Indeed, Neher and Steinbach (1978) first used this approach to obtain information pertaining to the effects of local anesthetics on the ACh-activated channel in the frog skeletal muscle and found a transient, flicker block of the open state of the channel by these drugs. Similarly, we have found a substantial increase in rapid openings and closings, or flickers, of the BK channel from the embryonic rat telencephalon after a reduction in $[K_i^+]$. The nature of this result provides insight concerning the mechanism of flicker gating in this channel. Our results with Rb_i^+ are consistent with whole-cell (Sala and Matteson, 1991) and single-channel (Spruce et al., 1989) data reported for different K^+ channel species, which suggests structural similarities within the K^+ channel family concerning the interaction between channels and permeant ions. Moreover, the single-channel approach provides additional information concerning the mechanism of the effects of Rb_i^+ on gating.

A preliminary report of some of these observations has appeared (Mienville and Clay, 1995).

MATERIALS AND METHODS

Tissue preparation and recording technique

The methods we used have been described elsewhere (Mienville, 1994). Briefly, experiments were performed with the in situ patch-clamp technique, mainly in the inside-out configuration (Edwards et al., 1989). The telencephalon was dissected from 12–14-gestational day-old rat embryos and placed ventricular side up in a 150-μl recording chamber perfused continuously with a solution gassed with carbogen (95% O_2, 5% CO_2) and consisting of (in mM): 125 NaCl, 5 KCl, 1.25 NaH_2PO_4, 4 $MgCl_2$, 26 $NaHCO_3$, 10 dextrose, and 1 EGTA (pH = 7.4). The absence of Ca^{2+} favored inside-out patch formation. As described recently (Mienville,

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1994), most BK channels in this preparation exhibit buzz mode gating, which 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. Moreover, the effects of K_i^+ removal reported below (Results) were found both in trypsin-treated channels and in untreated channels that were not initially in the buzz mode. The patch pipette solution ("extracellular" solution in the inside-out mode) consisted of (in mM): 135.2 potassium gluconate, 10 KCl, 10 HEPES, and 4.8 KOH (pH = 7.4). After patch excision, the bath saline was switched to an "intracellular" solution consisting of either 150, 5, or 0 K^+ in the K_i^+ experiments with, respectively, 0, 145, or 150 *N*-methyl-D-glucamine (NMG); or, for the Rb_i^+ results, either 150 Rb^+, or 75 K^+ and 75 Rb^+, plus 10 HEPES, 2.8 $CaCl_2$ and 10 *N*-hydroxyethyl-ethylenediamine-triacetic acid (HEDTA, Sigma; pH 7.2). We investigated any potential effects of NMG in our observations (Fig. 2) by using the 150 K_i^+ solution as control and a test solution consisting of either 100 KCl, 100 NMG, 10 HEPES, 10 HEDTA and 2.8 $CaCl_2$ (100 K_i^+ + 100 NMG), or 5 KOH, 5 HEPES, 1 HEDTA, and 0.28 $CaCl_2$ with 250 sucrose added to maintain constant osmolarity (310 mOsm; 5 K_i^+ + 250 Suc). The pCa throughout these experiments was 6, save for the results in Fig. 4, for which $[Ca^{2+}]$ was elevated to pCa 5 by substitution of 8 mM $CaCl_2$ to the intracellular solution. Membrane currents were recorded in the steady state at potentials calculated as the negative of the pipette potential plus a junction potential of 10 mV, and referred to as *V*. Patch currents were amplified via an Axopatch-1C (Axon Instruments), filtered at 2 kHz (8-pole Bessel; Frequency Devices), and stored on videotape with a VR-100A digital recorder (Instrutech; bandwidth of DC to 37 kHz).

Data analysis

The data were analyzed with the PAT.7 program of the Strathclyde Electrophysiology Software (courtesy of J. Dempster). The signal was sampled at 20 KHz with a Lab-PC interface (National Instruments). Open-channel current amplitude was determined from gaussian fitting of all-point distributions. Open-closed transitions were then determined from linear interpolation of sample points according to a 50% amplitude threshold protocol, and dwell times were log-binned at 16 bins/decade, corresponding to a bin width ~14.5% of bin midtime. These techniques obviate both sampling and binning promotion errors (McManus et al., 1987). The resulting distributions were displayed with a square-root ordinate (Sigworth and Sine, 1987) and fitted with exponential probability density functions (PDF) using nonlinear, least-squares methods after exclusion of data less than twice the dead time of the recording system. A major concern in our observations was the existence of a large proportion of missed events, an issue that technically is not easily addressed. Because the fastest time constant of closed-time distributions (τ_{cf}) was in the dead time range, many fast closures were not detected, which resulted in an overestimation of mean open times. This, however, does not invalidate the relative comparison of the various experimental conditions used here. A more serious concern was the possibility that the decrease in mean open time seen in 5 K_i^+ might be accounted for by the concomitant increase in τ_{cf} (Fig. 6 *B*) through an increase in the number of detected events. This hypothesis can be rejected for the following reasons: taking $\tau_{cf[150K]} = 0.1$ ms in 150 K_i^+ and $\tau_{cf[5K]} = 0.2$ ms in 5 K_i^+ yields a percentage of captured events in each condition equal to ~37% and ~61%, respectively (Blatz and Magleby, 1986). This means that for every 3 closures in 150 K_i^+, only ~2 more closures would be observed in 5 K_i^+, which clearly is not reflected in the experimental data (Fig. 1). Furthermore, based on the above hypothesis, mean open time in 5 K_i^+ can be approximated as

$$MOT_{[5K]} = 3/5(MOT_{[150K]} + \tau_{cf[150K]}) - \tau_{cf[5K]}$$

Thus, a $MOT_{[150\ K]}$ of 3 ms would only be 1.8 times greater than $MOT_{[5K]}$, which does not compare well with the >3-fold difference in this parameter at $V \geq -30$ mV (cf. Fig. 3 *B*). Finally, hyperpolarization in 150 K_i^+ reduces mean open time close to the 5 K_i^+ value without changing τ_{cf}.

The significance of the number of dwell-time distribution exponentials was statistically evaluated by comparing the ratio of the sums of squared errors of the different models to the value with the corresponding degrees of freedom in an F-distribution table. The intermediate (τ_{ci}) and slow (τ_{cs}) time constants of closed-time distributions allowed for the determination of gaps between bursts (t_g) according to the equation: $1 - \exp(-t_g/\tau_{ci}) = \exp(-t_g/\tau_{cs})$ (Colquhoun and Sakmann, 1985). Mean burst length was determined as the slow component of burst distributions (τ_{burst}), and mean open time as total open time/number of openings. Data are expressed as mean ± SE.

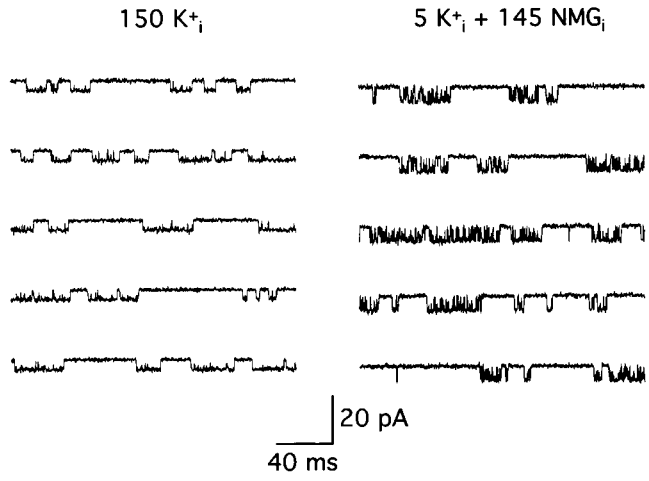

FIGURE 1 Effects of K_i^+ reduction on inward currents (downward deflections; $V = -20$ mV) through a BK channel recorded in an inside-out patch from rat embryonic telencephalon. Each panel shows continuous traces recorded in the condition indicated.

RESULTS

Effects of changes in K_i^+ on BK channel kinetics

The primary observation in this study is illustrated in Fig. 1. The results labeled 150 K_i^+ (150 K_o^+) represent continuous traces at $V = -20$ mV. Channel openings are in the downward or inward current direction, because −20 mV is negative to the reversal potential (0 mV). The channel exhibited relatively long duration openings or "bursts," which were occasionally interrupted by closings of brief duration ("flickers"), as originally demonstrated for BK channels under similar conditions (Pallotta et al., 1981). Removal of most of the charge carrier from the internal face of the channel resulted in a marked increase in flickering at −20 mV, as indicated in the right-hand panel of Fig. 1. Similar results were obtained with 0 K_i^+ (150 NMG_i; not shown). We did not observe an effect on gating after a change in K_i^+ during outside-out patch recording, which is consistent with the observations of Demo and Yellen (1992).

One obvious concern in the interpretation of the results in Fig. 1 is that the decrease of mean open time with 5 K_i^+ could be attributable to a "flicker" block of the BK channel by NMG. We tested for this effect in the experiments illustrated in Fig. 2 (Materials and Methods; 150 K_i^+ for all recordings). In particular, the degree of flicker was, by eye, the same at either −30 or +30 mV with 100 K_i^+ + 100 NMG as in 150 K_i^+ (Fig. 2 *A*, *middle*), which demonstrates that NMG is not responsible for the effect shown in Fig. 1.

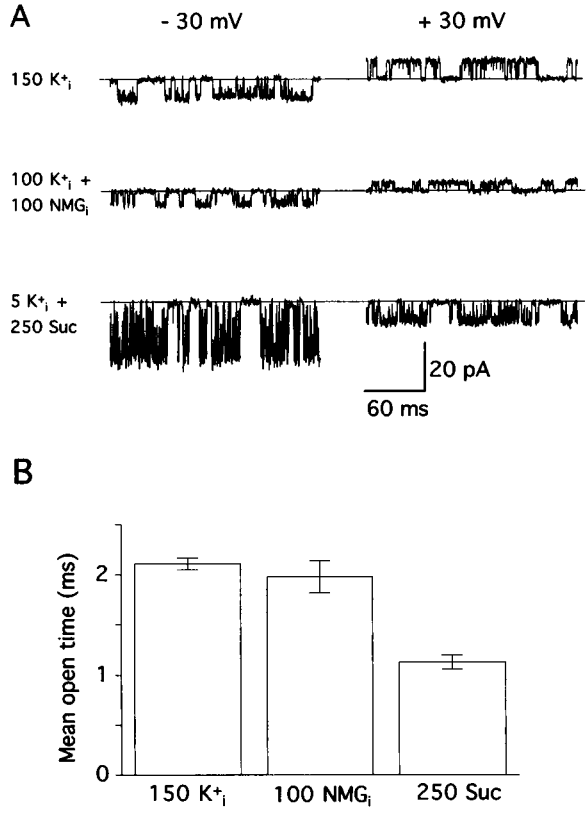

FIGURE 2 Effects of intracellular NMG and sucrose substitution for K_i^+ on rat BK channel. (*A*) The traces are from the same inside-out patch held at two different voltages and exposed to various "intracellular" solutions as indicated. The continuous lines indicate closed state. (*B*) Mean open time for $V = -30$ mV in the same three conditions. Mean open time was not different in 100 NMG versus control (150 K_i^+) conditions ($p = 0.34$; ANOVA followed by Fisher's *posthoc*), and was reduced ($p < 0.0001$) in 250 mM sucrose.

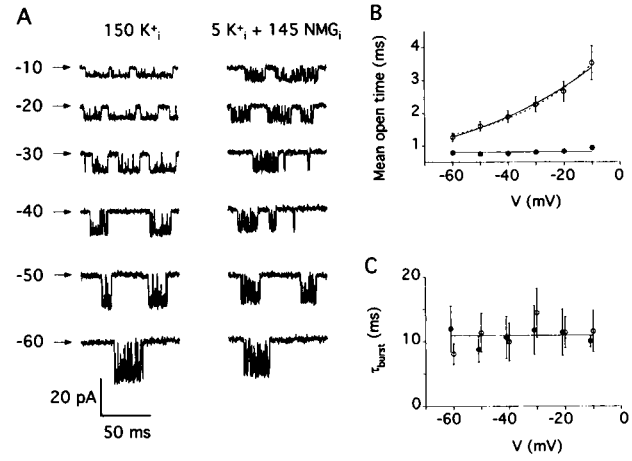

FIGURE 3 Voltage dependence for BK channel activity recorded in inside-out patches bathed in either 150 K_i^+ or 5 K_i^+ + 145 NMG. (*A*) Inward currents from one patch are shown for each condition. Membrane potential is indicated on the left of the traces and the arrows indicate closed state. (*B*) Mean open time in 150 K_i^+ (*open circles*) and 5 K_i^+ (*filled circles*) is plotted against membrane voltage (*V*). Error bar is smaller than symbol for the 5 K_i^+ condition. The data for 150 K_i^+ are fitted with a single exponential (*solid line*) and with Eq. 1 (*dashed line*; see Discussion). (*C*) Mean burst length (τ_{burst}) is fitted with a regression line of slope = 0 (symbols have the same meaning as in *B*).

(The slight decrease in open channel current at -30 mV in 100 K_i^+ + 100 NMG relative to 150 K_i^+ is probably attributable to the change in ionic strength.) Moreover, a significant increase in flickering was observed in 5 K_i^+ + 250 sucrose, which further demonstrates that the results in Fig. 1 are not attributable to NMG. (The large increase in open channel current in 5 K_i^+ + 250 sucrose in Fig. 2 *A* is, again, attributable to a change in ionic strength). The lack of effect of NMG on mean open time and the reduction of this parameter in 5 K_i^+ without NMG are quantified in Fig. 2 *B* ($n = 7$; both results obtained at -30 mV). Based on these results, we used NMG substitution for K_i^+ throughout the remainder of this analysis.

Further results concerning the effect of K_i^+ removal on flickering are described in Fig. 3. The records in Fig. 3 *A* show, once again, that flickering was much more clearly evident in 5 K_i^+ as compared to 150 K_i, especially at potentials depolarized from rest. The difference in flickering between the two conditions was not as marked at the more negative potentials illustrated in Fig. 3 *A*. This result is quantified in Fig. 3 *B*. Specifically, mean open time was clearly voltage dependent in 150 K_i^+, increasing *e*-fold per 60 mV (*solid curve* in Fig. 3 *B*), whereas it was voltage independent in 5 K_i^+. (The dashed curve describing the 150 K_i^+ results was obtained from the model given below.) The traces in Fig. 3 *A* also show a voltage-dependent change in the duration of the clearly resolved, relatively long lasting closed times between channel bursts. Finally, we note that burst duration does not appear to be significantly voltage dependent over the -60 to -10 mV range (Fig. 3 *C*), although this parameter was increased with larger depolarizations (see Fig. 8 *B*, *right*). The important point here is that burst duration was independent of K_i^+ (Fig. 3 *C*), which is of particular significance for mechanisms of flicker gating, as described below (cf. Discussion).

The effect of K_i^+ on flickering appears to be independent of Ca^{2+} activation of the channel, as shown in Fig. 4. The third trace of Fig. 4 *A*, which corresponds to 5 K_i^+ and pCa 5, clearly shows a change in probability of channel opening relative to pCa 6. However, the flicker process does not appear to be altered, as shown by the lower three traces in Fig. 4 *A*, which were reproduced from the upper three respective records on an expanded time scale. Specifically, flickering is essentially absent from the channel opening shown for 150 K_i^+, whereas it is clearly evident in 5 K_i^+ with either pCa 5 or 6. Pooled results of the mean open time from several experiments of this type ($n = 6$) are shown in Fig. 4 *B*. Results similar to those in Fig. 4 were originally reported by Christensen and Zeuthen (1987) for $K_i^+ = 112$ mM. The results given in Fig. 4 extend this observation to $K_i^+ = 5$ mM, conditions for which flickering is much more pronounced than with 112 K_i^+.

The distributions of open- and closed-time intervals are illustrated in Fig. 5 for $K_i^+ = 5$ and 150 mM (same

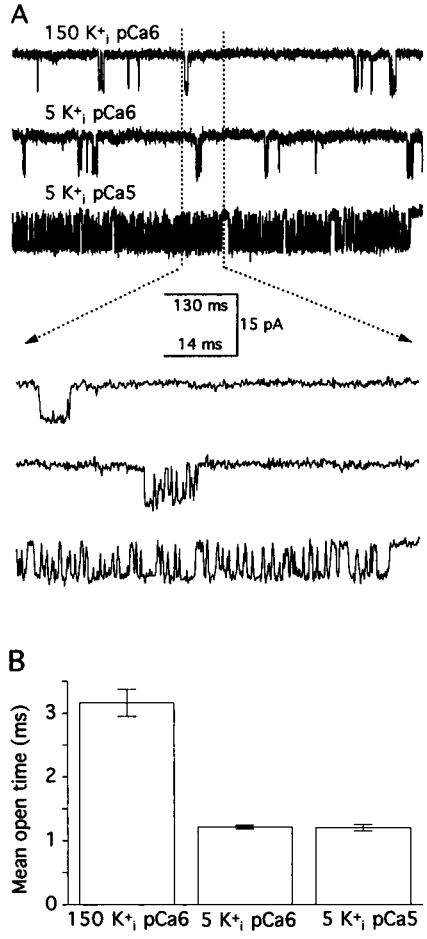

FIGURE 4 Effects of K_i^+ reduction (145 NMG_i) on BK channel activity recorded at two different pCa's ($V = -30$ mV). (*A*) The same channel was recorded in the three different conditions indicated. Portions of the traces (*between dotted lines*) are shown on an expanded time scale in the lower panel. (*B*) Summary of the effect of K_i^+ reduction on channel mean open time at pCa 6 and pCa 5.

experiment as in Fig. 1, $V = -20$ mV). The open-time distributions are well described by a single exponential for 150 and 5 K_i^+ (Fig. 5, *A* and *B*, respectively), but the former condition exhibited a statistically better fit with two components, with the fast component occupying only a minor proportion of the fit (area = 10.2 ± 1.9%, $n = 15$). These results clearly confirm the reduction of mean open time after a reduction in K_i^+. The closed-time distributions in Fig. 5, *C* and *D*, require three exponentials for both levels of K_i^+, which indicates at least three closed states of the channel. The effect of a change in K_i^+ was to principally alter the fast time constant of the closed-time distribution, τ_{cf}, which appears to be related to the flicker process. The voltage dependencies of the various time constants obtained from the analysis illustrated in Fig. 5 are shown in Fig. 6 from several preparations ($n = 15$). Fig. 6 *A* illustrates this result for the two time constants obtained from the open-time distribution in 150 K_i^+ and the single time constant obtained with 5 K_i^+. The corresponding results for τ_{cf} and τ_{ci} are shown in Fig. 6, *B* and *C*, respectively. Neither of the

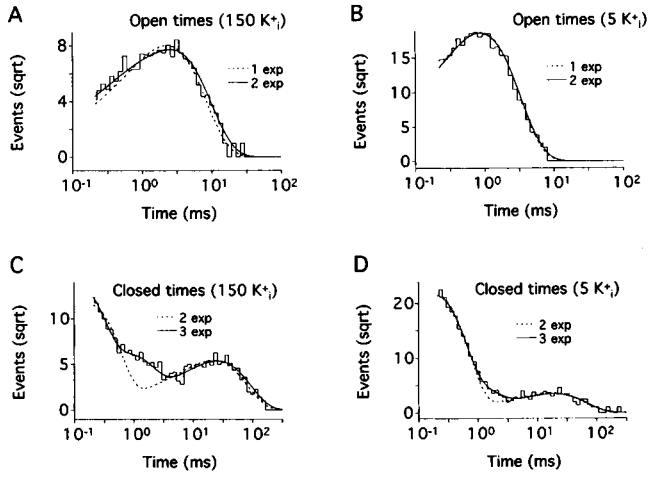

FIGURE 5 Dwell-time distributions for ~50 s records from the same channel as that displayed in Fig. 1. For open-time distribution from 150 K_i^+ data (*A*), double exponential fitting with a fast (τ_f = 0.48 ms, area = 15.9%) and a slow (τ_s = 2.83 ms, area = 84.1%) component provides a statistically more accurate description of the data than monoexponential fitting ($p < 0.01$), whereas a single component (τ = 0.89 ms) can accurately describe the open-time distribution from 5 K_i^+ data (*B*). For closed times, the parameters in 150 K_i^+ (*C*) were: τ_{cf} = 0.11 ms, area = 73.3%; τ_{ci} = 0.81 ms, area = 14.6%; τ_{cs} = 26.7 ms, area = 12.1%. These parameters were changed in 5 K_i^+ (*D*) to the following values: 0.20 ms, 92.9%; 0.77 ms, 4.6%; 19.8 ms, 2.5%. Binding and fitting procedures, as well as the test of comparison between fits, are described in Materials and Methods. The experimental conditions were as described in Fig. 1.

latter parameters was significantly dependent upon membrane potential. Moreover, τ_{ci} was not affected by a change in K_i^+, whereas τ_{cf} was increased by a factor of 2 after the reduction of K_i^+ from 150 to 5 mM. The longest closed-time

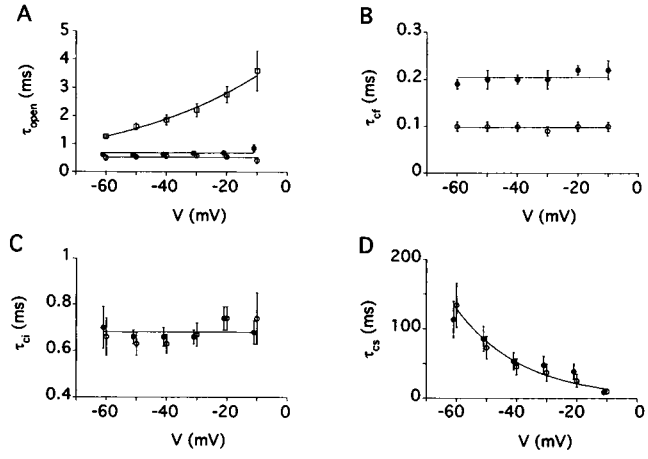

FIGURE 6 Voltage dependence of dwell-time parameters for BK channels from inside-out patches bathed in 150 K_i^+ (*open symbols*) versus 5 K_i^+ (*closed symbols*). (*A*) Fast (*open circles*) and slow (*squares*) open time constants (τ) for the 150 K_i^+ condition, as well as the single τ (*filled circles*) for the 5 K_i^+ condition, are plotted against membrane voltage (V). Error bar is smaller than symbol for some points. (*B* and *C*) Fast (τ_{cf}) and intermediate (τ_{ci}) closed time constants are fitted with regression lines of slope = 0. (*D*) Slow closed time constants are fitted with a single exponential.

constant obtained from the analysis in Fig. 5, τ_{cs}, did appear to be voltage dependent, decreasing e-fold per ~22 mV. Moreover, τ_{cs} was not significantly altered by a change in K_i^+.

Effects of Rb_i^+ on BK channel kinetics

The effects of substitution of 150 K_i^+ by 150 Rb_i^+ are illustrated in Fig. 7 *A* for $V = +30$ and -30 mV. We did not observe a clear outward current at +30 mV with 150 Rb_i^+. Consequently, this part of the analysis was focused on inward currents. The effects of 150 Rb_i^+ were markedly different from those obtained with NMG replacement for K_i^+. Flickering was not noticeably altered, by eye, whereas the mean burst length was significantly increased. These qualitative observations are amplified by the analysis in Fig. 7 *B*. The mean open time as a function of membrane potential for the two conditions illustrated in Fig. 7 *A* is shown in Fig. 7 *B1*. There was a statistically significant increase in this parameter with 150 Rb_i^+ at -30 mV ($p = 0.04$, two-tail paired t-test), and this effect was removed with hyperpolarization. The results in Fig. 7 *B2* reinforce the visual impression in Fig. 7 *A* of a large increase in mean burst length with 150 Rb_i^+ at -30 and -20 mV relative to the results with 150 K_i^+ ($p = 0.04$ and 0.05, respectively). Once again, this effect was removed with hyperpolarization. That is, burst duration did not appear to be voltage dependent in 150 K_i^+ ($p = 0.24$ for the values at -60 and -20 mV), whereas some voltage dependency was apparent in 150 Rb_i^+ ($p = 0.05$ for the corresponding data). As previously noted, Fig. 8 *B* indicates that mean burst length in 150 K_i^+ is voltage dependent when considering a broader voltage range (see below). The time constants of channel closing did not appear to be altered by Rb_i^+, as illustrated in Fig. 7, *B3* and *B4*.

To investigate the effects of Rb_i^+ on outward current we used an equimolar mixture of K_i^+ and Rb_i^+ (75 K_i^+ + 75 Rb_i^+). Outward currents obtained under these conditions and in control at +50 mV are shown in Fig. 8 *A*. The single-channel amplitude was clearly reduced in 75 Rb_i^+, whereas mean open time and mean burst length both increased, which is illustrated quantitatively in Fig. 8, *B* and *C*, respectively. No difference was observed at -40 mV under these conditions of a lower [Rb_i^+] than in the previous experiment, which further supports the observations from

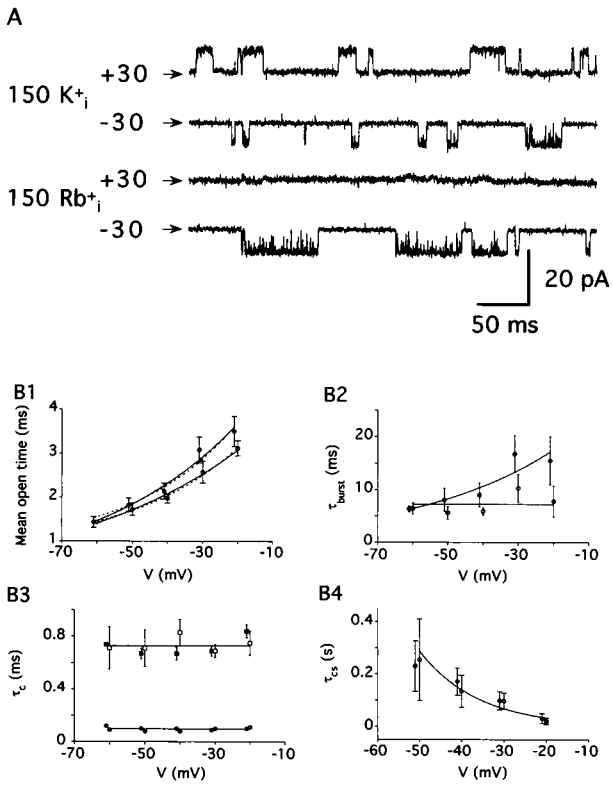

FIGURE 7 Effects of intracellular Rb^+ on BK channel kinetics. (*A*) BK channel activity was recorded at two different membrane potentials in the presence of either 150 K_i^+ or 150 Rb_i^+ (0 K^+). Note the slight upward deflections at +30 mV in 150 Rb_i^+. (*B*) Mean open time (*B1*), mean burst length (*B2*), and closed time constants (*B3* and *B4*) were compared in 150 K_i^+ (*open symbols*) versus 150 Rb_i^+ (*closed symbols*) [$n = 5$]. In *B1*, mean open times are fitted with a single exponential (*solid lines*) and with Eq. 1 (*dashed lines*; see Discussion). In *B2*, mean burst length is fitted with a regression line of slope = 0 for 150 K_i^+, and with a single exponential for Rb_i^+. All data in *B3* are fitted with regression lines of slope = 0 (error is smaller than symbol for τ_{cf}), and τ_{cs} in *B4* are fitted with a single exponential.

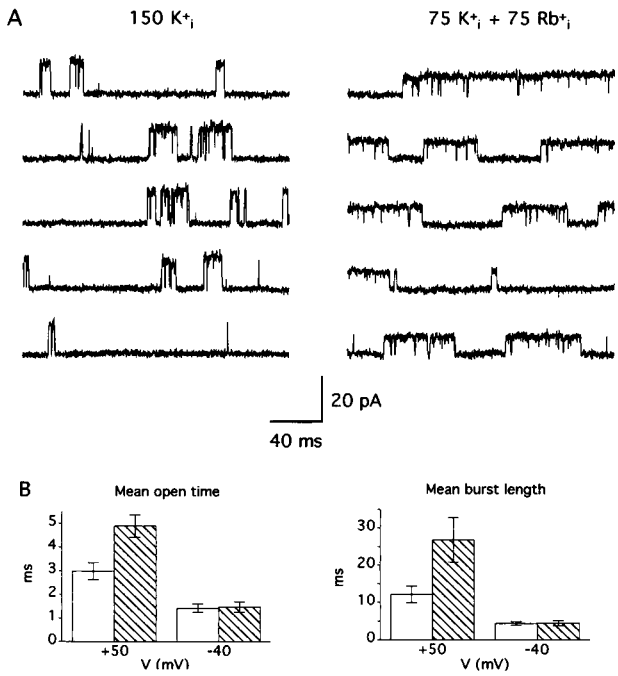

FIGURE 8 Effects of 75 mM intracellular Rb^+ on BK channel outward currents. (*A*) Continuous traces of BK channel activity were recorded from an inside-out patch held at a membrane voltage of +50 mV and alternately exposed to either 150 K_i^+ or 75 Rb_i^+ + 75 K_i^+. (*B*) Mean open time (*left*) and mean burst length (*right*) were estimated from six inside-out patches for the 150 K_i^+ (*empty bars*) versus 75 Rb_i^+ + 75 K_i^+ (*hatched bars*) conditions. Data at a membrane potential (V) of -40 mV are also given for comparison.

Fig. 7 that the effects of Rb_i^+ are voltage dependent. Furthermore, it is clear from the results depicted in Fig. 8 *B* that the voltage dependency of mean burst length is enhanced by Rb^+ ions. The reduction in current amplitude and the corresponding increase in mean burst length shown for 75 Rb_i^+/75 K_i^+ relative to control are consistent with similar results reported by Demo and Yellen (1992) for extracellular Rb^+.

DISCUSSION

A model for flicker gating

The effect of a removal of K_i^+ from the BK channel is reminiscent, in some ways, of results with QX-222 and the ACh-activated channel originally reported by Neher and Steinbach (1978). The removal of K_i^+ appears to add a rapid gating process to the channel, especially for $V > -60$ mV, which is similar to the rapid flickering induced in the ACh-activated channel by local anesthetics. However, QX-222 significantly increased the mean burst length of the latter preparation, whereas mean burst length of the BK channel was not altered by an increase in flickering, as noted above (Fig. 3). This observation appears to rule out mechanisms for the flicker gate that have been suggested previously. For example, one possibility is that the channel must open before flickering can occur, which is represented by the following scheme (Christensen and Zeuthen, 1987):

$$[C_s] \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} [O] \underset{k_{f-1}}{\overset{k_{f1}}{\rightleftharpoons}} [C_f]$$

in which $[C_s]$ is the long-lived closed state of the channel, [O] is the open state, and $[C_f]$ is the flicker state. Bursts in this scheme correspond to transitions between [O] and $[C_f]$. Consequently, the mean burst duration is given by $\beta^{-1}[(k_{f1} + k_{f-1})/k_{f-1}]$ (Colquhoun and Hawkes, 1981). The removal of K_i^+ appears to correspond to a significant increase in transitions between [O] and $[C_f]$, which should correspond to a significant increase in mean burst duration, as with the effects of QX-222 on the ACh-activated channel (Neher and Steinbach, 1978). This prediction is at odds with our results. A related model for flickering is given by

$$[C_s] \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} [C_f] \underset{k_{f1}}{\overset{k_{f-1}}{\rightleftharpoons}} [O]$$

In this scheme the channel must pass from the closed state through the flicker state before it can open. This model is consistent with a simplified account of the findings of Ferguson et al. (1993), which suggest that both flickers and long closings transit through a common subconductance state of brief duration. Here, the mean burst duration, given by $\beta^{-1}[(k_{f1} + k_{f-1})/k_{f1}]$, should be reduced after K_i^+ removal because the channel spends more time in $[C_f]$ than in control. Consequently, the probability for a transition to $[C_s]$ and, hence, a termination of the burst, should increase, which is also at odds with our observations.

The simplest alternative interpretation of our results is that flickering is independent of transitions between $[C_s]$ and [O], which is represented by

$$\begin{array}{ccc} [O_s; C_f] & \underset{k_{f-1}}{\overset{k_{f1}}{\rightleftharpoons}} & [O_s; O_f] \\ {\scriptstyle\alpha}\downarrow\uparrow{\scriptstyle\beta} & & {\scriptstyle\alpha}\downarrow\uparrow{\scriptstyle\beta} \\ [C_s; C_f] & \underset{k_{f-1}}{\overset{k_{f1}}{\rightleftharpoons}} & [C_s; O_f] \end{array}$$

in which C_s and C_f are the closed states of the slow and fast (flicker) gates, respectively, and O_s and O_f are their open states. Both gates must be open for the channel to be conducting. Consequently, the open state in the above scheme is $[O_s;O_f]$. The other three states represent closed states of the channel. However, given that the flicker process has two time constants related to channel closing (Fig. 5, *C* and *D*), a more likely scheme is

$$\begin{array}{ccccc} [O_s; C_i] & \underset{k_{i-1}}{\overset{k_{i1}}{\rightleftharpoons}} & [O_s; O_f] & \underset{k_{f-1}}{\overset{k_{f1}}{\rightleftharpoons}} & [O_s; O_f] \\ {\scriptstyle\alpha}\downarrow\uparrow{\scriptstyle\beta} & & {\scriptstyle\alpha}\downarrow\uparrow{\scriptstyle\beta} & & {\scriptstyle\alpha}\downarrow\uparrow{\scriptstyle\beta} \\ [C_s; C_i] & \underset{k_{i-1}}{\overset{k_{i1}}{\rightleftharpoons}} & [C_s; O_f] & \underset{k_{f-1}}{\overset{k_{f1}}{\rightleftharpoons}} & [C_s; O_f] \end{array}$$

That is, the flicker process is represented by $[C_i] \rightleftharpoons [C_f] \rightleftharpoons [O_f]$, i.e., two closed-state configurations and one open state, and the slow, or primary gate is represented by $[C_s] \rightleftharpoons [O_s]$. The kinetics of either gate can be considered to be independent of each other. The results given above provide some information on the various rate constants in this scheme. For example, α is given by the inverse of the closed time, τ_{cs}, in Fig. 6 *D*. This result can be approximated by $\alpha = 0.11 \exp(-0.04\ V)\ ms^{-1}$ for both levels of K_i^+. Similarly, β corresponds to the inverse of the mean burst length (Fig. 3 *C*), which is approximately given by $0.09\ ms^{-1}$ for both levels of K_i^+ and all potentials in the -60 to -10 mV range. The macroscopic current kinetics are given by $(\alpha + \beta)^{-1}$, because the rapid flicker events are essentially filtered by the membrane capacitance. Consequently, the voltage dependence of this BK channel in the range of potentials we investigated appears to be attributable, primarily, to the forward rate constant for channel opening, α. Both α and β are independent of changes in K_i^+, so that the mean burst duration in the above scheme, which is given by β^{-1}, is also predicted to be independent of K_i^+. The mean open time of the model is given by $(\beta + k_{f-1})^{-1}$. (We are assuming that the channel has only one open state for all levels of K_i^+.) This expression corresponds to the results in Fig. 3 *B*. Because $k_{f-1} \gg \beta$ for $K_i^+ = 5$ mM and for $-60 > V > -10$ mV in 150 K_i^+, the mean open time essentially provides a direct determination of k_{f-1} (this approximation does not hold true for $V > 0$ mV). We then assume that k_{f-1} is given by $k'_{f-1}(1 - p_o)$, where $k'_{f-1} = k_{f-1}$ in the absence of K_i^+, and p_o is the probability that a particular site within the permeation pathway that affects k_{f-1} is occupied by a K^+ ion originating from the intracel-

lular side of the membrane. Therefore, $k_{f-1} = k'_{f-1}\, p_{no}$, where p_{no} is the probability that this site is not occupied by a K^+ ion of intracellular origin. We have used the Woodhull (1973) approach to modeling p_{no}. That is,

$$k_{f-1} = \frac{k'_{f-1}}{1 + [K_i^+]/K_D \exp(dqV/kT)} \quad (1)$$

where K_D is the dissociation constant for a K^+ binding site located within the channel at an electrical distance d from the intracellular face of the membrane. The constants in Eq. 1, i.e., q, k, and T, have their usual meaning, with $kT/q \approx 25$ mV at room temperature. The results in Fig. 3 *B* with 5 K_i^+ give $k'_{f-1} = 1.23\ ms^{-1}$, assuming that $K_D \gg 5$ mM. A best fit of Eq. 1 to the results in Fig. 3 *B* and 7B1 for 150 K_i^+ gives $d = 0.8$ and $K_D = 30$ mM. A similar analysis for mean open time with 150 Rb_i^+ (Fig. 7 B1) gives $d = 0.8$ and $K_D = 20$ mM.

Physical interpretation of the model

The physical interpretation of the model is that the channel has two gates: a primary gate whose opening is voltage dependent and which determines macroscopic kinetics, and a flicker gate which, when $K_i^+ = 0$, appears to be voltage independent in the -60 to -10 mV range. However, because single-channel currents in 5 K_i^+ and for $V > 0$ are not amenable to a detailed kinetic analysis, we cannot exclude the possibility of a weak voltage dependence for the intrinsic gating of the flicker gate. Thus, the primary gate would be located somewhere within the channel and the flicker gate would be outside the electric field—or nearly so. The observation that the gates are stochastically independent of one another is consistent with the prediction that they are physically separated within the channel. Occupancy of a site within the channel by a K^+ ion approximately 80% of the way across the electric field from the intracellular surface of the membrane interferes with closing of the flicker gate, thereby conferring on it an apparent voltage dependency. These considerations are consistent with a location for the flicker gate near the extracellular face of the channel. The effects of Rb_i^+ in Fig. 7 *B1* are in agreement with the model with regard to flickering. Indeed, flicker is depressed in 150 Rb_i^+ even more than in 150 K_i^+, which is consistent with the idea that Rb_i^+ remains in the channel longer for $V \approx 0$ mV than K_i^+. The effects of K_i^+ on τ_{cf} are not easily interpretable and will need further study.

The effects of K_i^+ are reminiscent of the "occupancy" hypothesis of Swenson and Armstrong (1981) and Matteson and Swenson (1986). However, the nature of our results in this regard is novel for the following reasons: 1) We have found that gating appears to be altered by occupancy of a site within the permeation pathway, although the primary gate within the electric field does not appear to be the target for this effect. The sine qua non of the occupancy hypothesis would appear to be that the voltage-dependent process that underlies macroscopic gating is affected by the primary permeant ion that passes through the channel. This observation does not appear to apply to BK channels. 2) The occupancy hypothesis would suggest that flicker should be depressed with 150 K_o^+ (the conditions of these experiments) for $V \approx 0$ mV, even when $K_i^+ = 0$. That is, the appearance of flicker in the vicinity of $V = 0$ mV should actually occur only in the absence of K^+ ions from both sides of the membrane. One way around this paradox may be that K^+ ions that originate from the extracellular medium differ in their interaction with the channel compared to ions that originate from the intracellular medium, possibly because of differences in the ion hydration layer (Clay and Kowtha, 1991). Alternatively, the barriers to ion permeation within the channel might be altered by a change in K_i^+, so that the interaction between ions and the channel with regard to gating effects would also be altered (Waggoner and Oxford, 1987). The effects of Rb_i^+ on flickering are similar to those of K_i^+, as noted above. Rb_i^+ has an additional effect concerning the closing rate constant, β. Consistent with the results of Spruce et al. (1989) on the frog delayed rectifier, β is reduced by 150 Rb_i^+ relative to control, and the effect is diminished by hyperpolarization. That is, Rb_i^+ has two distinct effects on gating, which is consistent with the multi-ion nature of the BK channel pore (Eisenman et al., 1986). According to this principle, both K^+ and Rb^+ may affect the flicker gate by binding to the same site in the channel, whereas only Rb^+ seems able to bind to the site that mediates its effects on the voltage-dependent gate.

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