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Gating Properties of Inward-Rectifier Potassium Channels: Effects of Permeant Ions

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Abstract. Two inward-rectifier K^+ channels, ROMK2 (Kir1.1b) and IRK1 (Kir2.1), were expressed in *Xenopus* oocytes and their gating properties were studied in cell-attached membrane patches. The gating properties depended strongly on the ion being conducted (K^+, NH_4^+, Rb^+, or Tl^+), suggesting tight coupling between permeation and gating. Mean open times were strongly dependent on the nature of the conducted ion. For ROMK2 the order from the longest to the shortest times was $K^+ > Rb^+ > Tl^+ > NH_4^+$. For IRK1 the sequence was $K^+ > NH_4^+ > Tl^+$. In both cases the open times decreased monotonically as the membrane voltage was hyperpolarized. Both the absolute values and the voltage dependence of closed times were dependent on the conducted species. ROMK2 showed a single closed state whose mean lifetimes were biphasic functions of voltage. The maxima were at various voltages for different ions. IRK1 had at least two closed states whose lifetimes decreased monotonically with K^+, increased monotonically with Tl^+, and were relatively constant with NH_4^+ as the conducted ion. We explain the ion-dependence of gating by assuming that the ions bind to a site within the permeation pathway, resulting in a stable, ion-dependent, closed state of the channel. The patterns of voltage-dependence of closed-state lifetimes, which are specific for different ions, can be explained by variations in the rate at which the bound ions leave the pore toward the inside or the outside of the cell.

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Introduction

Permeation and gating are two of the most fundamental properties of ion channels. Usually they are treated as separate, independent characteristics, especially for voltage-gated ion channels, as in the classical analysis of the electrical properties of the neuronal axon (Hodgkin & Huxley, 1952). Gating of channels is thought to result from conformational changes in the channel protein which can be triggered or influenced by changes in membrane voltage, the presence of specific ligands, mechanical stress or changes in the intracellular pH or Ca^{2+} concentration. A number of recent studies, however, have suggested that the gating of channels can be strongly influenced by permeating ions (*see* Discussion). In studies of the renal K channel ROMK2 (Kir1.1b), we suggested that permeant ions might actually trigger channel closures (Choe et al., 1998). This view was supported by two observations. First, the rate of entry into a kinetically defined closed state was directly dependent on the current through the channel. Second, the rate of leaving this closed state had a biphasic voltage dependence which could be explained if the re-opening were linked to the exit of an ion from the pore in either direction. Because the closed times were in the millisecond range, compared with the microsecond time scale for ion permeation, we suggested that the site of interaction of ions with the channel fluctuates between shallow energy (conducting) and deep energy (blocked) states (Choe et al., 1998).

Most K^+ channels conduct three other monovalent cations: NH_4^+, Rb^+, or Tl^+. In this report we have extended our previous findings to include the effects of these ions on channel gating. We have also studied a second inward-rectifier channel, IRK1 (Kir2.1). The single-channel kinetics of IRK1 (Kir2.1) for inward K^+ current are more complicated than those of ROMK2.

This channel has at least four closed states and one open state (Choe et al., 1999). The findings are interpreted using a kinetic model with a closed state that is stabilized by the binding of a permeant cation within a deep energy well. This model can account for differences in the gating properties of both channels observed when conducting different ions.

Materials and Methods

Expression of Channels

Details were described previously (Choe et al. 2000). Briefly, cRNA was synthesized using mMESSAGE mMACHINE kit (Ambion, Austin, TX) and stored at −70°C before use. Stage V–VI oocytes of female *Xenopus laevis* were obtained by partial ovariectomy and defolliculated. Oocytes were injected with cRNA and incubated at 19°C for 1 to 4 days before measurements were made. Oocytes were subjected to a hypertonic shrinking solution, thereby allowing the vitelline membrane to be easily removed for patch-clamp measurements.

Patch Clamp

Oocytes were bathed in a solution containing (in mM): 110 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES at pH 7.4. Patch-clamp pipettes were pulled from #7052 borosilicate glass (Richland Glass, Richland, NJ) using a three-stage process and were coated with Sylgard. They were filled with solutions containing (in mM): 110 KCl and 5 HEPES with 5 EDTA at pH 7.4. In some cases KCl was substituted with NH_4Cl, RbCl, or $TlNO_3$. When Tl^+ was the major conducted ion, NO_3^- was substituted for Cl^- in the bath solution. Replacing KCl with KNO_3 in the pipette solution did not affect the conduction or gating properties of the channel (Chepilko et al., 1995), suggesting that the effect of $TlNO_3$ is due to the substitution of Tl^+ for K^+. All experiments were performed at room temperature (21–23°C). Pipette resistances were 1 to 3 MΩ. Currents were recorded with a List EPC-7 patch-clamp amplifier and stored, unfiltered, on videotape. For off-line analysis, using an Atari-based data acquisition system (Instrutech, Mineola, NY), current records were replayed from videotape, filtered at 1 kHz, and sampled at 5 kHz. Missed events were corrected as in a previous paper (Choe et al., 1998). For analysis of NH_4^+ data at −250 and −300 mV and Rb^+ data at −300 mV of ROMK2, current records were filtered at 10 kHz and sampled at 50 kHz, because very brief open times with large currents at those voltages distort channel gating kinetics at the standard bandwidths. Construction of open- and closed-time histograms and fitting with exponential distributions were carried using the TAC program (Sigworth & Sine, 1987).

Data Analysis

Missed events correction in ROMK2 channel gating analysis was described previously (Choe et al., 1998).

The closed (τ_c) times were analyzed using the following equation which were developed in the previous study (Choe et al., 1998) with some modifications.

$$\tau_c = \left[\nu \cdot \left\{ \exp\left(-\frac{\Delta U_o + \delta_o \cdot e \cdot V}{k_B \cdot T} \right) + \exp\left(-\frac{\Delta U_i + \delta_i \cdot e \cdot V}{k_B \cdot T} \right) \right\} \right]^{-1} \quad (1)$$

where ΔU_o and ΔU_i represent energy barriers for dissociation of a blocking ion toward the extracellular and cytoplasmic sides of the membrane, respectively (*see* Fig. 2*B*); V is the membrane voltage; δ_o and δ_i are the fractional electrical distances from the blocking site to the energy barriers on the extracellular and cytoplasmic sides, respectively; and ν is a constant "prefactor." The terms: e, V, k_B and T have their usual meanings. The assumed value of ν affects estimates of the energy barrier heights and is difficult to determine independently (Thompson & Begenisich, 2000). A 10-fold increase in the prefactor corresponds to a 2.3 k_BT increase in energy heights. Because we do not intend to estimate absolute values of the barrier heights but compare the relative heights of energy profiles along the pores of ROMK2 and IRK1 with different permeant ions, the ω-value was set to 2.8×10^8 as in our previous paper (Choe et al., 1998). For simplicity, we assume that δ_o and δ_i of ROMK2 and IRK1 are the same. In this analysis, they are each fixed at 30% of the transmembrane electric field, i.e., −0.3 for δ_o and 0.3 for δ_i, following the approximate electrical distances of K^+ in ROMK2 (Choe et al., 1998). Therefore, only the heights of the inner and outer barriers are allowed to vary. This model was used to fit the data in Figs. 2*A* and 4*B–D*, generating the solid lines (*see* figure legends).

Previously we used a complicated equation to analyze the mean open (τ_o) times (Choe et al., 1998). This detailed analysis is not essential in this study, hence we used the following simple equation to fit the data in Figs. 2*C* and 4*E*:

$$\tau_o = \alpha \cdot \exp(\beta \cdot V), \quad (2)$$

where α and β are two free parameters. The α coefficient includes the inverse of the effective concentration of permeant ions in the bath. β is proportional to $1/k_BT$.

The voltage dependence of the open probability (P_o) was fit by the Boltzmann function (Choe et al., 1998):

$$P_o = P_{min} + \frac{1 - P_{min}}{1 + \exp\left(\frac{w - z_g \cdot e \cdot V}{k_B \cdot T} \right)} \quad (3)$$

where P_{min} represents the minimal P_o, w represents the energy increase of the system upon opening the channel in the absence of a membrane potential, and z_g represents gating charge. This equation was used to generate the solid lines of Figs. 2*D* and 4*F*.

All the fitting procedures were done using the "Solver" function, a built-in optimization function of Microsoft Excel. The symbols and the bars in the figures are mean and standard errors, respectively; n represents the number of experiments.

Results

ROMK2 channels exhibited two closed states, long and brief, when conducting K^+ in the absence of divalent cation chelator in the pipette solution (Palmer et al., 1997; Choe et al., 1998). When EDTA, a divalent cation chelator, was added to the pipette solution, a single closed state was observed. This implies that the long closures are due to block by contaminant divalent cat-

ions, possibly Ba^{2+} (Choe et al., 1998). We also have suggested that the short closures are blocks elicited by permeating potassium ions. Here we extend the study to three more conducted ions: NH_4^+, Rb^+ and Tl^+. Figure 1 shows traces of inward currents through single ROMK2 channels in the cell-attached mode when K^+, NH_4^+, Rb^+, or Tl^+ is the major conducted ion. Rb^+ currents showed transient larger conductance levels just after a channel opening, which quickly relaxed to the major conductance state. This phenomenon was not further analyzed in this study. With 5 mM EDTA in the pipette, all recordings showed a single closed state (Fig. 1*B*) and one open state (Figs. 1*C*). The mean lifetimes of both the open and the closed states were different for the various ions.

The voltage dependence of mean closed times of ROMK2 with different conducted ions was complicated (Fig. 2*A*). With K+ (●), ROMK2 had a biphasic voltage dependence, as was shown in the previous study (Choe et al., 1998). With Rb^+ (□) or NH_4^+ (▲) the mean closed times were also biphasic but with distinct absolute values and specific voltages at which the maxima are reached. In the case of Tl^+ (■), the mean closed time increased up to the most negative voltage we inspected (−160 mV). Thus the nature of the permeant ions affects both the rates of channel opening and the influence of voltage on those rates. In our previous study (Choe et al., 1998), we developed a gating model (Fig. 2*B*) that was based on the hypothesis that the short closures of ROMK2 gating involve trapping of potassium ions at or near the selectivity filter. Here we used the same model to analyze the voltage dependence of the closed times with the different conducted ions. According to this scheme, differences in the absolute values of the closed times reflect differences in the binding energies of the ions in the trapped state, whereas differences in the voltage dependence reflect differences in the relative rates at which the ions escape from the trapped state toward the extracellular and cytoplasmic sides of the pore. The lines in Fig. 2*A* are best fits to equation 1 and describe the data reasonably well. The parameters obtained from the fits are given in the figure legend.

The mean open times of ROMK2 with K^+ (●), NH_4^+ (▲), Rb^+ (□), and Tl^+ (■) all decreased monotonically as membrane voltage was made more negative (Fig. 2*C*). Both the absolute value of the open time and the degree of the voltage dependence depended on the ion being conducted. Figure 2*D* shows the voltage dependence of open probability (P_o). ROMK2 had the highest P_o when conducting K^+ (●). With Rb^+ (□), P_o decreased at membrane voltages more negative than −120 mV. When NH_4^+ (▲) or Tl^+ (■) is the major permeant ion, P_o decreased sharply at less negative voltages. The shallow voltage dependence with K^+ as the conducted ion is due in part to the weaker effect of hyperpolarization on mean open times (Fig. 2*C*) and in part to the fact that the mean closed times start to decrease at less negative voltages (Fig. 2*A*).

To see if these interactions were unique to ROMK, we also studied another representative inward rectifier K^+ channel, IRK1 (Kir2.1), which has much more complicated gating kinetics although it is presumed to have the same basic structure and membrane topology as ROMK2. As shown in Fig. 3*A*, we measured K^+, NH_4^+, and Tl^+ currents through IRK1 in the cell-attached mode. Tl^+ currents often relaxed downward after channel opening, similar to Rb^+ currents through ROMK2. Rb^+ currents through IRK1 were too small to permit analysis of the kinetics (Choe et al., 2000). With K^+ as the conducted ion, IRK1 had at least 3 closed states when there was no divalent cation in the pipette solution (Choe et al., 1999). The very few longest (>1 sec) closures (*C*0) with K^+ in Fig. 3*B* are not always observed and may be blocks by divalent cation(s) that are not chelated completely by EDTA (Choe et al., 1999). Only the 3 shortest closed states (*C*1, *C*2, and *C*3) were considered. The channel also had 3 closed states within the frequency range of the system with NH_4^+ and 2 closed states with Tl^+ as the permeant ion (Fig. 3*B*). In all cases there was a single open state (Fig. 3*C*). As with ROMK2, the lifetimes of the open and closed states of IRK1 were strongly dependent on the nature of the conducted ion.

Next we applied the model that was used to explain the gating of ROMK2 to the observations of IRK1. Because IRK1 showed three closed time constants with K^+ and NH_4^+ and two with Tl^+, we assumed that IRK1 can have 2 or more states with "trapped" cations preventing current flow (Fig. 4A). In general, the voltage dependence was similar for each of the closed states for a given ion, consistent with the idea that the different closures reflect similar molecular mechanisms. The closed states did not have the clear biphasic voltage dependence that was seen for ROMK2. However, the log τ *vs.* voltage relationships were clearly nonlinear, suggesting a complex interaction with the electric field. Furthermore, the voltage dependence was opposite for different ions. Closed times decreased with hyperpolarization when K^+ was the conducted ion (Fig. 4*B*), but increased when Tl^+ was conducted (Fig. 4*D*) and had little voltage dependence with NH_4^+ (Fig. 4*C*). These differences can be accounted for by the ion-trapping model if trapped K^+ exits the pore preferentially toward the cytoplasm, while Tl^+ exits more readily back to the outer solution, and NH_4^+ has no preferred direction over this voltage range (−50 to −200 mV).

The mean open times of IRK1 were even more sensitive to the conducted ion than were those of ROMK2, being decreased by >100-fold when Tl^+ (■) rather than K^+ (●) is the cation in the pipette (Fig. 4*E*). They decreased monotonically as the membrane hyperpolarized, similar to those of ROMK2. Figure 4*F* shows the volt-

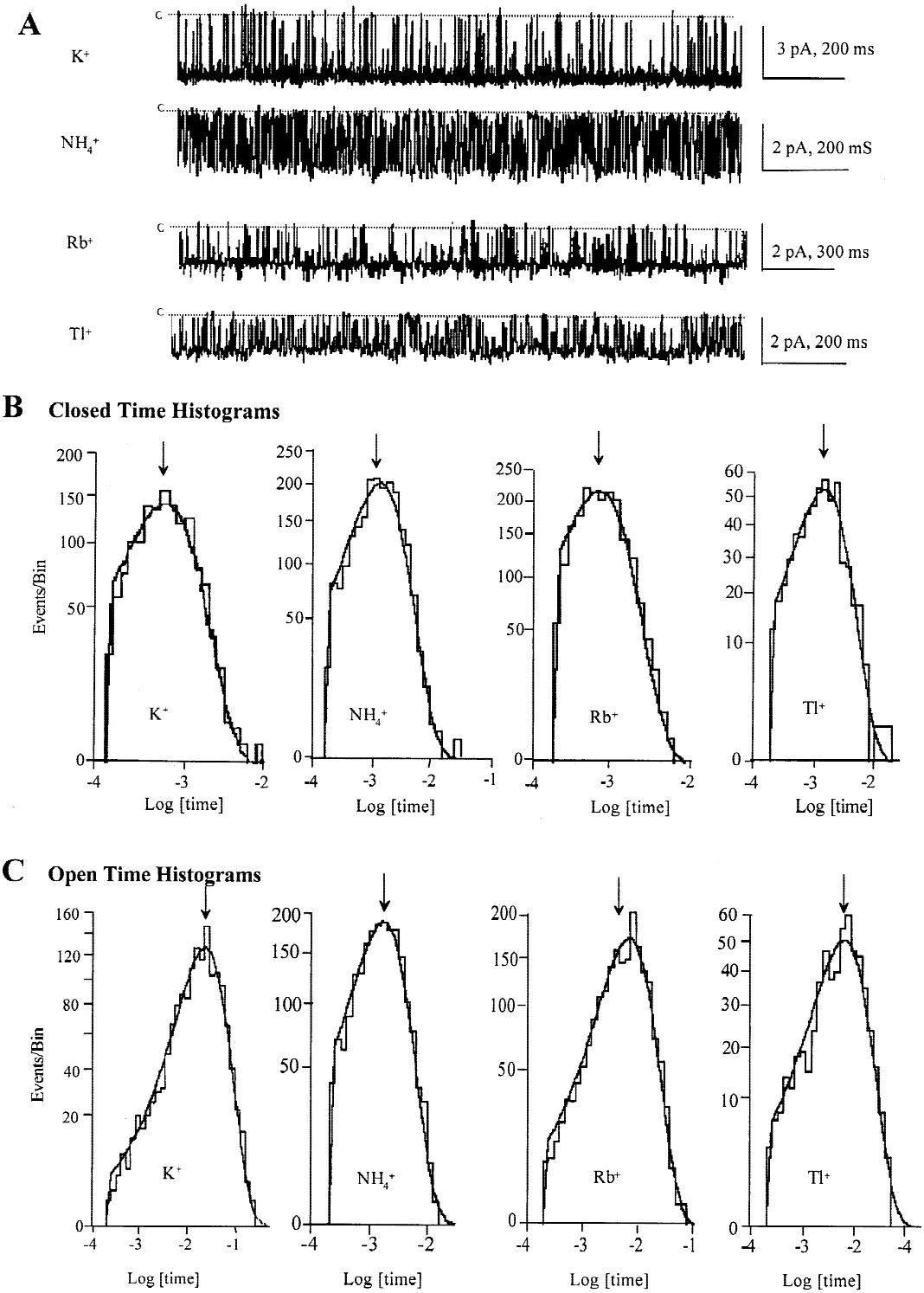


Fig. 1. Single-channel inward currents through ROMK2 channel with different conducted ions in the cell-attached mode. (*A*) Inward current traces with different conducted ions. The pipette solution contained 5 mM EDTA and 5 mM HEPES. Major ions in the pipette solutions were 110 mM KCl, NH_4Cl, RbCl or $TlNO_3$ (from top to bottom). The bath solution contained 110 mM KCl to keep the membrane potential to 0 mV. When Tl^+ was the major ion in the pipette solution, the KCl in the bath solution was replaced with KNO_3. Downward deflections represent inward current. The dotted lines indicate the closed state. The holding potentials are −100 mV, −100 mV, −120 mV, and −60 mV for K^+, NH_4^+, Rb^+, and Tl^+, respectively. (*B*) Closed-time histograms. A single closed state was observed with all four ions. The vertical arrows mark the time constants of the closed states. Mean closed times are for K^+, 1.0 msec at −100 mV; for NH_4^+, 1.4 msec at −100 mV; for Rb^+, 0.7 msec at −120 mV; for Tl^+, 1.5 msec at −60 mV. (*C*) Open-time histograms. All of the open time histograms have single open states. The vertical arrows mark the time constants of the closed states. Mean open times are for K^+, 16 msec at −100 mV; for NH_4^+, 1.6 ms at −100 mV; for Rb^+, 6.2 ms at −120 mV; for Tl^+, 4.3 ms at −60 mV.

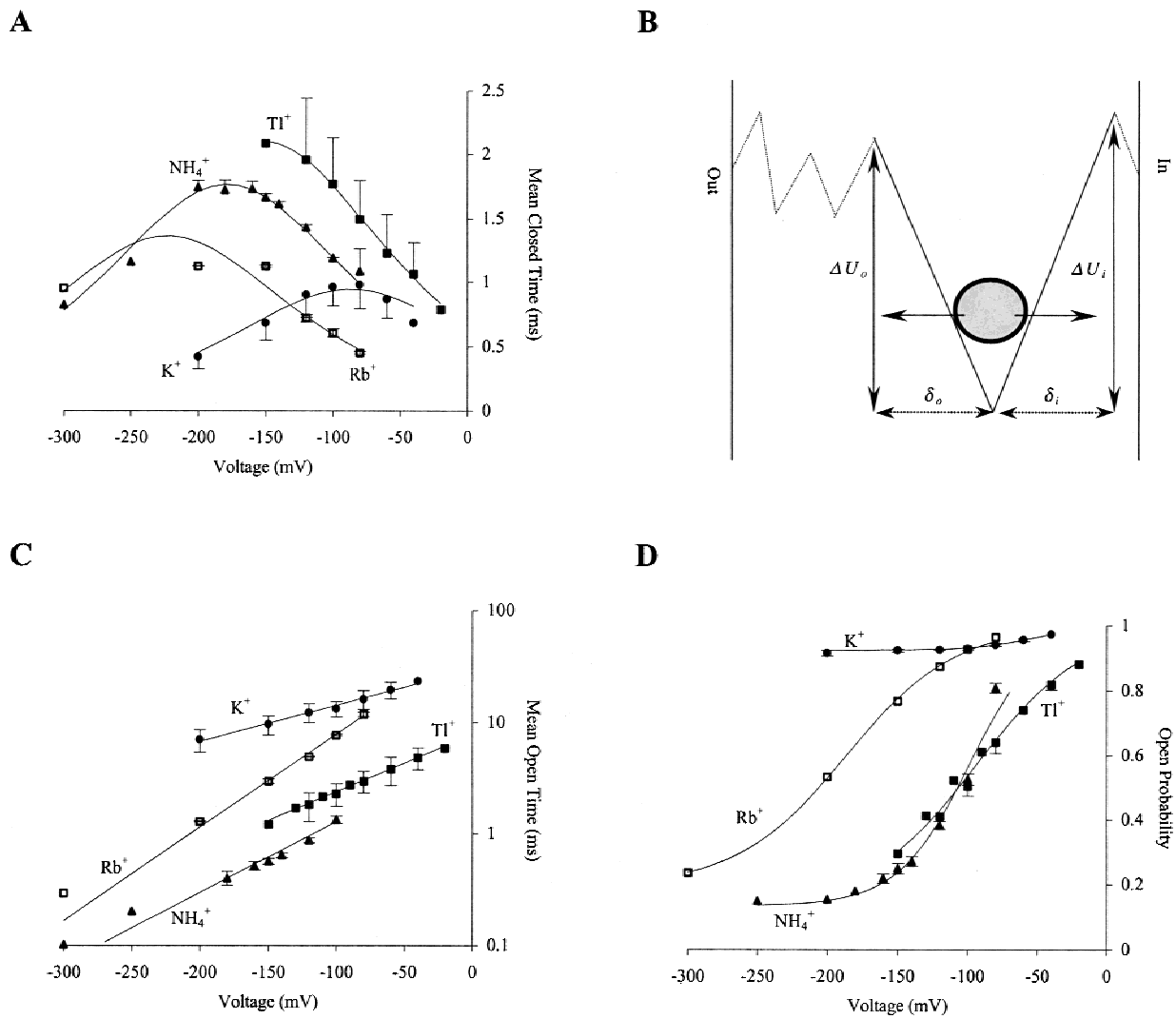
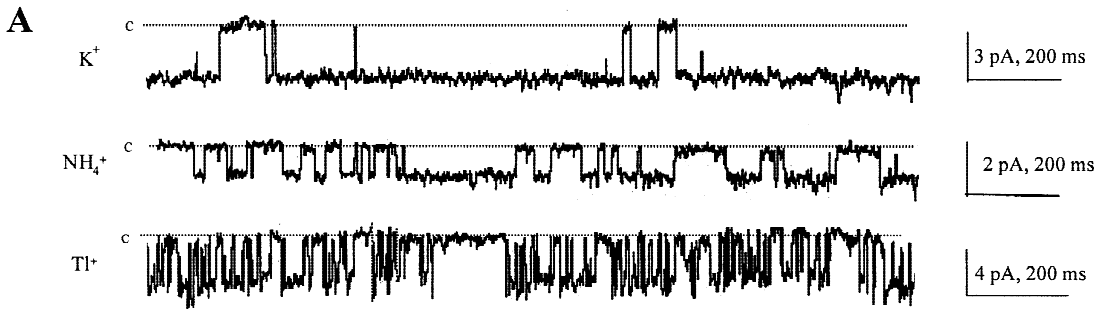


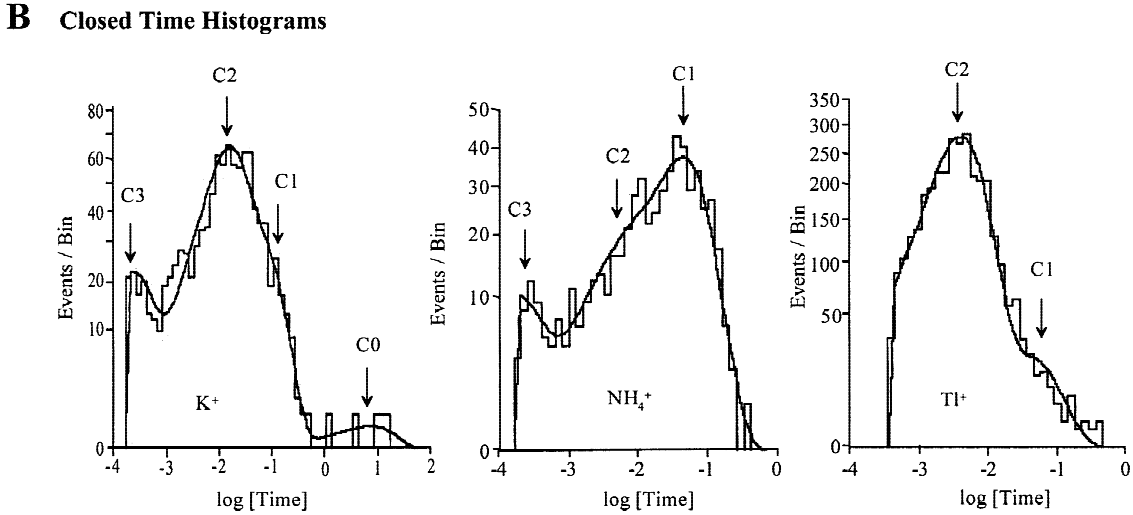
Fig. 2. Voltage dependence of ROMK2 gating. (*A*) Mean closed times of ROMK2 as a function of voltage. With K^+, NH_4^+, and Rb^+ the voltage dependence was biphasic with maxima at −90 mV, −180 mV, and −230 mV, respectively. Mean closed times of Tl^+ increased monotonically as the membrane potential became more negative. The solid lines are drawn using equation *1* (*see* Methods). The best-fit parameters are following: for K^+ (●, $n = 4$), $\triangle U_o = 12.1\ k_BT$ and $\triangle U_i = 14.2\ k_BT$; for NH_4^+ (▲, $n = 5$), $\triangle U_o = 11.7\ k_BT$ and $\triangle U_i = 15.9\ k_BT$; for Rb^+ (□, $n = 3$), $\triangle U_o = 10.7\ k_BT$ and $\triangle U_i = 15.0\ k_BT$; for Tl^+ (■, $n = 3$), $\triangle U_o = 12.2\ k_BT$ and $\triangle U_i = 15.8\ k_BT$. (*B*) The energy profile along the pore for a deep energy-well model representing a closed state. Note that this is different from the shallow energy profile that represents the open conducting state (Choe et al., 2000). When an ion is trapped in the deep energy well, it can escape to either the outside or the inside depending on the heights of the energy barriers and the voltage difference across them. (*C*) Mean open times of ROMK2 as a function of voltage. In all cases there is only a single open state, and the voltage dependence is monotonic with positive slopes. The line is drawn according to equation *2*. The best-fit parameters are for K^+ (●, $n = 4$), $\alpha = 30$, $\beta = 7$; for NH_4^+ (▲, $n = 5$), $\alpha = 6$, $\delta = 15$; for Rb^+ (□, $n = 3$), $\alpha = 54$, $\beta = 19$; for Tl^+ (■, $n = 3$), $\alpha = 8$, $\beta = 12$. (*D*) Open probabilities of ROMK2 as a function of voltage. The line is drawn according to equation *3*. The fit parameters are for K^+ (●, $n = 4$), $P_{min} = 0.92$, $w = -2.33\ k_BT$, $z_g = 1.12$; for NH_4^+ (▲, $n = 5$), $P_{min} = 0.13$, $w = -3.97\ k_BT$, $z_g = 1.01$; for Rb^+ (□, $n = 3$), $P_{min} = 0.20$, $w = -4.82\ k_BT$, $z_g = 0.65$; for Tl^+ (■, $n = 3$), $P_{min} = 0.14$, $w = -2.43\ k_BT$, $z_g = 0.65$.

age dependence of open probabilities of IRK1 with different conducted ions. With K^+ (●), P_o was highest throughout the voltage range tested. With NH_4^+ (▲) and Tl^+ (■), P_o of IRK1 decreased steeply at negative voltages.

Discussion

Although gating and permeation were originally considered to be independent processes, in some channel types these phenomenon seem to be coupled. In many in-





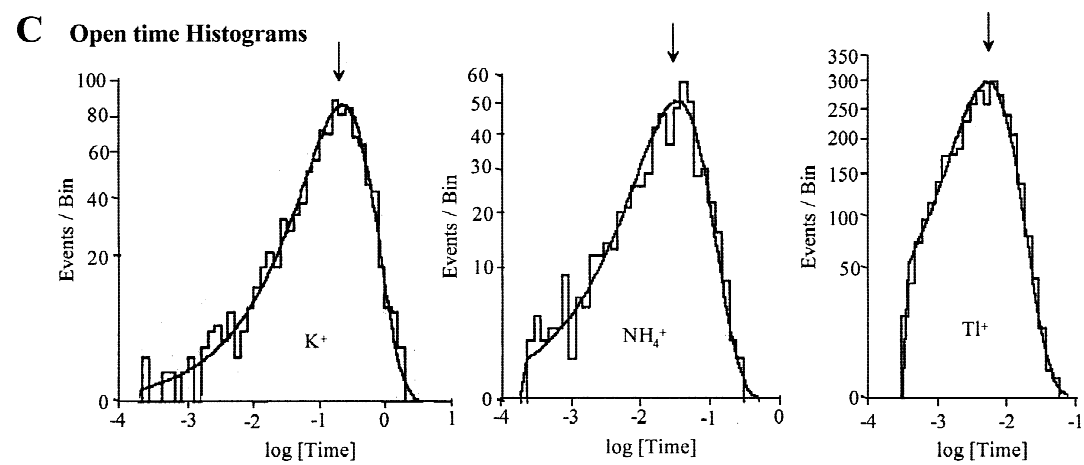


Fig. 3. Single-channel currents through IRK1 in the cell-attached mode. (*A*) Inward current traces with different conducted ions. Conditions were as described in Fig. 1*A*. Downward deflections represent inward current. The dotted lines indicate the closed state. The holding potentials were −100 mV, −120 mV, and −100 mV for K^+, NH_4^+, and Tl^+, respectively. (*B*) Closed-time histograms. In contrast to ROMK2, IRK1 had multiple closed states: 4, 3, and 2 for K^+, NH_4^+, and Tl^+, respectively. The vertical arrows mark the time constants of the closed states. The very few longest closures with K^+ are believed to be blocks by divalent cation(s) that were not chelated completely by EDTA (Choe et al., 1999). Only the 3 shortest closed states were considered for K^+. Mean closed times are for K^+, 0.2 msec (20%), 13 msec (49%), 60 msec (30%), and 7.0 sec (1%) at −100 mV; for NH_4^+, 0.3 msec (13%), 11 msec (24%) and 67 msec (64%) at −120 mV; for Tl^+, 3.9 msec (92%) and 33 msec (8%) at −100 mV. (*C*) Open-time histograms. All of the histograms have single open states. The vertical arrows mark the time constants of the closed states. Mean open times are for K^+, 238 msec at −100 mV; for NH_4^+, 42 msec at −120 mV; for Tl^+, 6 msec at −100 mV.

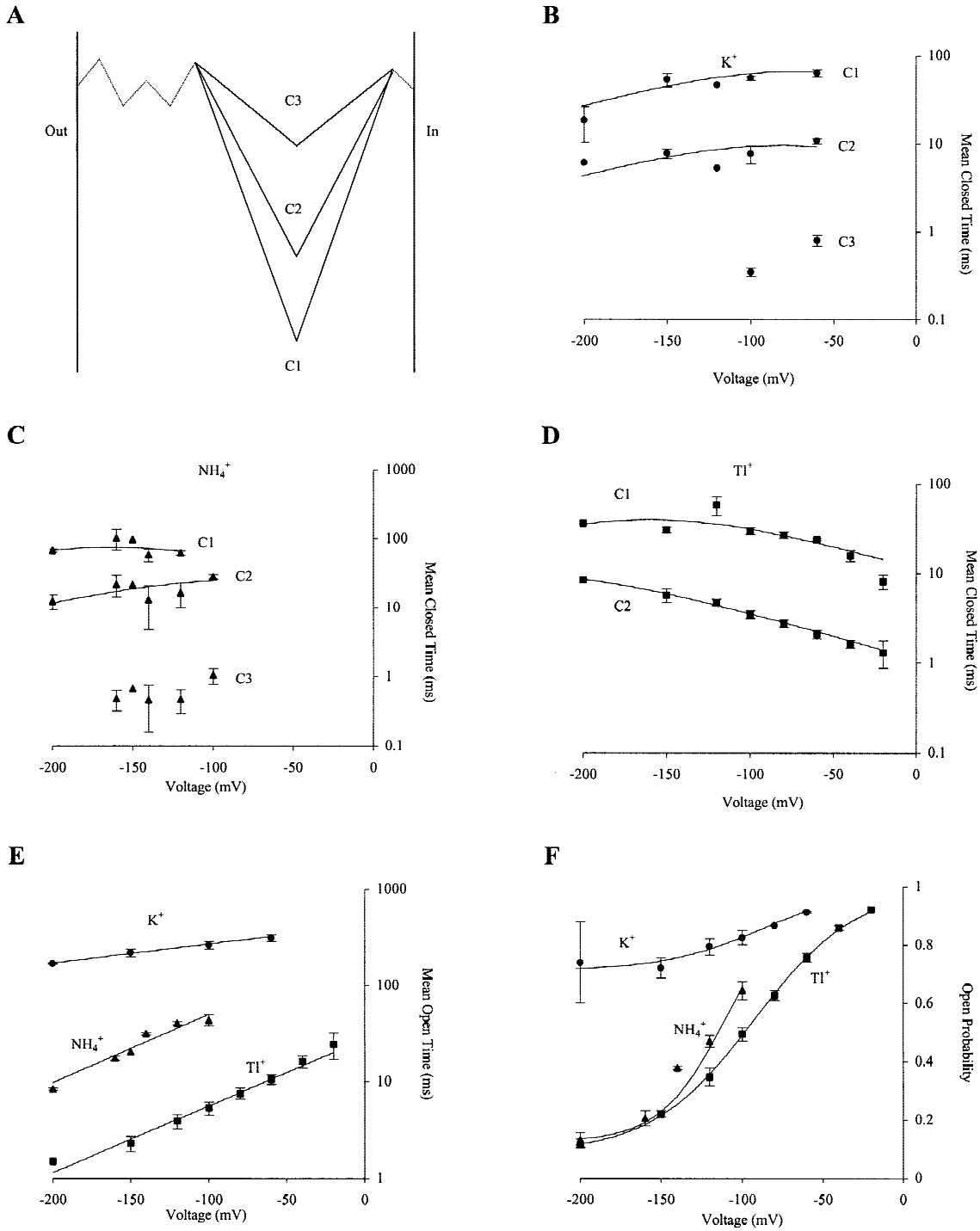


Fig. 4. Voltage dependence of IRK1 gating. (*A*) Because there were at least three closed states with K^+ and NH_4^+ and two closed states with Tl^+, we assumed that IRK1 can have 2 or 3 separate closed conformations with separate energy wells indicated as C1, C2 and C3. (*B*) Voltage dependence of mean closed times with K^+ as conducted ion. There are 3 closed states designated from longest to shortest as C1, C2, and C3. The lines are drawn using equation *1*. The best-fit parameters are ($n = 3$): $\triangle U_{o,C1} = 16.6\ k_BT$, $\triangle U_{i,C1} = 18.3\ k_BT$, $\triangle U_{o,C2} = 14.5\ k_BT$, and $\triangle U_{i,C2} = 16.5\ k_BT$. Mean closed times of C3 were not fit to the equation because there are data at only two voltages. As the membrane hyperpolarizes, closed times of C3 decrease beyond our resolution limit. (*C*) Voltage dependence of mean closed times with NH_4^+ as conducted ion. There are 3 closed states as in *B*. The lines are drawn using equation *1*. The best-fit parameters are ($n = 3$): $\triangle U_{o,C1} = 15.6\ k_BT$, $\triangle U_{i,C1} = 19.5\ k_BT$, $\triangle U_{o,C2} = 15.5\ k_BT$, and $\triangle U_{i,C2} = 17.5\ k_BT$. Mean closed times of C3 were not fit to the equation because they are not well resolved in the histograms. (*D*) Voltage dependence of mean closed time with Tl^+ as conducted ion. There are 2 closed states C1 and C2. The lines are drawn using equation *1*. The vest-fit parameters are ($n = 3$): $\triangle U_{o,C1} = 15.0\ k_BT$, $\triangle U_{i,C1} = 18.8\ k_BT$, $\triangle U_{o,C2} = 12.6\ k_BT$, and $\triangle U_{i,C2} = 18.3\ k_BT$. (*E*) Mean open times of IRK1 as a function of voltage. In all cases, there was only a single open state, and the voltage dependence is monotonic with positive slopes. The line is drawn according to equation *2*. The best-fit parameters are: for K^+ (●, $n = 3$), $\alpha = 427$, $\beta = 5$; for NH_4^+ (▲, $n = 3$), $\alpha = 277$, $\beta = 17$; for Tl^+ (■, $n = 3$), $\alpha = 28$, $\beta = 16$. (*F*) Open probabilities of IRK1 as a function of voltage. The lines are drawn using equation *3*. The best-fit parameters are: for K^+ (●, $n = 3$), $P_{min} = 0.71$, $w = -2.89\ k_BT$, $z_g = 0.84$; for NH_4^+ (▲, $n = 3$), $P_{min} = 0.12$, $w = -5.27\ k_BT$, $z_g = 1.22$; for Tl^+ (■, $n = 3$), $P_{min} = 0.09$, $w = -2.92\ k_BT$, $z_g = 0.80$.

stances this coupling is seen as an inhibition of entry into a closed or inactive state in the presence of a permeant ion, or the acceleration of exit from such a state. This kind of interaction has been observed in voltage-gated K channels (Swenson & Armstrong, 1981; Clay, 1986; Pardo et al., 1992; Baukrowitz & Yellen, 1995; Levy & Deutsch, 1996), Na channels (Townsend, 1997), and Cl channels (Richard & Miller, 1990; Pusch et al., 1995), as well as ligand-gated channels (Van Helden, Hamill & Gage, 1977; Ascher, Marty & Neild, 1978). It can often be thought of as prevention of closure by a "foot-in-the-door" mechanism (Swenson & Armstrong, 1981) or as a "knockout" of a blocking particle.

In the case of ROMK2, K ions moving through the pore appeared to elicit a closed or blocked state (Choe et al., 1998). Entry into the short blocked state was accelerated when the external K concentration was increased. Furthermore, the rate of re-opening of the channel was increased by both hyperpolarization or depolarization, suggesting the removal of a blocking ion from the pore in either direction. The blocking times are much longer than the normal transit time for a K ion through the channel, suggesting that the ions become trapped within the pore. One possible mechanism for this trapping effect is that the presence of the ion could trigger a conformational change in the channel that strengthens the ion-channel interaction, effectively converting the permeant ion into a blocker. Similar schemes were put forward to explain the effects of external Cl on the gating of ClC-0 Cl channels (Chen & Miller, 1996) and the effects of ions on the gating of voltage-dependent K channels (Chapman, Van Dongen & Van Dongen, 1997). Also, a mechanism involving ion-induced conformational changes operating on a more rapid time scale was recently suggested to play a role in determining ion selectivity in K channels (Chapman & VanDongen, 2001). Another possibility is that permeating ions have a limited access to a more stable binding site; in most cases the transiting ions would bypass this site, but occasionally one would fall into it, creating the trapped state.

In the present study we have extended the basic idea of an ion-dependent variable energy well in two ways. First, we examined the effects of different permeant cations on the gating of ROMK2. Second, we extended the analysis to include the strong inward rectifier IRK1.

The mean open times for both ROMK2 and IRK1 were remarkably sensitive to the permeant ion. Replacing K^+ as the major conducted ion by either NH_4^+ or Tl^+ dramatically shortened mean open times (Figs. 2*C* and 4*E*). In our earlier study (Choe et al., 1998), we showed a strong correlation between rate of entering the closed state and the voltage and concentration dependence of single-channel current. This implies that the channel could sense either the rate of ion flow through the pore or, perhaps more likely, the occupancy of a particular site within the pore by an ion. When different permeant ions were compared in the present study, the rate of closure (inverse of open time in our cases) did not depend on the magnitude of the current per se since the sequence for provoking the closures ($NH_4^+ > Tl^+ > Rb^+ > K^+$) was distinct from that for conductance ($NH_4^+ > K^+ > Tl^+ > Rb^+$) or permeability ($Tl^+ > K^+ > Rb^+ >> NH_4^+$; Choe et al., 2000). This suggests either that the occupancy of a crucial site at which the closures are triggered is different for specific ions, or that the triggering mechanism itself depends on the nature of the ion.

The nature of the conducted ion strongly affected both the absolute values and the nature of the voltage dependence of the closed times of ROMK2. The biphasic voltage dependence of the closed times seen with K^+ was also observed for NH_4^+ and for Rb^+, although in the latter case this became evident only at extreme voltages (−300 mV). For Tl^+ permeation, closed times increased monotonically with hyperpolarization between 0 and −150 mV. These patterns, as well as differences in the absolute values of the closed times, can be explained by assuming that (1) all permeant ions can become trapped in the channels, (2) the rates of dissociation depend on the nature of the bound ion and (3) relative rates of dissociation toward the cytoplasm and extracellular space are ion specific. Thus K ions reverse their dominant direction of exit at around −80 mV. Over the voltage range of 0 to −150 mV, the major dissociation route for NH_4^+, Rb^+ and Tl^+ is toward the extracellular space and is therefore slowed by hyperpolarization. Reversal of the voltage dependence for Rb^+ and NH_4^+ is seen at larger negative voltages, at which dissociation toward the cytoplasm becomes dominant. Voltage dependence for Tl^+ may also reverse at these voltages, but our data are incomplete. The difference in the behavior of K^+ compared to the other permeant ions can be explained by a lower (by 1–2 kT) energy barrier for debinding toward the cytoplasm (ΔU_i, Fig. 2B) and a slightly higher (by 0 to 1.5 kT) energy barrier for debinding toward the extracellular space (ΔU_o, Fig. 2B). We should note, however, that energy-well depths and barrier heights were the only parameters allowed to vary. It is possible that changes in the positions of the bound ions within the electric field, which were fixed in our analysis, could also influence the ion-specific kinetics.

In the case of IRK1, the presence of multiple closed states complicates the analysis. However, the characteristic time constants seem to be affected in very similar ways by substitution of permeant ions as well as by changing the membrane voltage. Thus, they may represent distinct closed states but the same basic mechanism of closure. The direction of the voltage dependence for different ions might again reflect predominant routes of ion dissociation. For K^+, dissociation appears to be mainly toward the cytoplasm over the voltage range ex-

amined, whereas for Tl^+, the main direction would be outward toward the extracellular space. The case of NH_4^+ is intermediate. A more quantitative assessment of the rates of escape from the closed states is shown in the figures and described in the figure legends. The combination of decreased open times and increased closed times with Tl^+ leads to a sharp decrease in the open probability with hyperpolarization. This effect may underlie a previous observation that K channels in skeletal muscle are inactivated by hyperpolarization with Tl^+, but not with K^+, as the main conducted ion (Stanfield et al., 1981).

Several differences in the kinetics of ROMK and IRK1 can be readily explained by the model. First, the finding that the closed times of ROMK2 are shorter than those of IRK1 indicates in the latter channel that the ions bind more tightly within the pore in the blocked state. Second, the various voltage-dependencies of IRK1 could result from distinct relative rates of exit of the ions from the pore in the blocked state toward either extracellular space or the cytoplasm. Finally, the multiple closed states for IRK1 could represent different sites of ion trapping or different conformations of the trapped state.

In conclusion, a simple hypothesis of permeant ion block can explain the distinctive gating patterns for conduction of K^+, NH_4^+, Rb^+, and Tl^+ by ROMK2 and IRK1. The various magnitudes and voltage dependencies of closed times with different conducted ions can be accounted for by quantitative differences within the same basic scheme. This idea can explain the coupling between ion permeation and gating observed in these and perhaps other channel types.

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