External Monovalent Cations That Impede the Closing of K Channels

D. R. MATTESON and R. P. SWENSON, JR.

From the Departments of Neurosurgery and Physiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT We have examined the effects of a variety of monovalent cations on K channel gating in squid giant axons. The addition of the permeant cations K, Rb, or Cs to the external medium decreases the channel closing rate and causes a negative shift of the conductance-voltage relationship. Both of these effects are larger in Rb than in K. The opening kinetics of the K channel are, on the other hand, unaffected by these monovalent cations. Other permeant species, like NH_4 and Tl, slightly increase the closing rate, whereas the relatively impermeant cations Na, Li, and Tris have little or no effect on K channel gating. The permeant cations have different effects on the reversal potential and the shape of the instantaneous current-voltage relationship. These effects give information about entry and binding of the cations in K channels. Rb, for example, enters the pore readily (large shift of the reversal potential), but binds tightly to the channel interior, inhibiting current flow. We find a correlation between the occupancy of the channel by a monovalent cation and the closing rate, and conclude that the presence of a monovalent cation in the pore inhibits channel closing, and thereby causes a leftward shift in the activation-voltage curve. In causing these effects, the cations appear to bind near the inner surface of the membrane.

INTRODUCTION

Until recently, the gating of ionic channels was reported to be unaffected by changes in the type or concentration of the permeant cation present, and gating was therefore thought to be independent of ion permeation. Hodgkin and Huxley (1952), for example, found that a large change in the external Na^+ concentration had no significant effect on the time course of Na currents. Chandler and Meves (1965), also working on the Na channel, reported that activation and inactivation kinetics were unaltered when external Na was replaced by K, which permeates Na channels to some degree. In both Na and K channels, the currents carried by NH_4^+ had approximately the same kinetics as those carried by Na^+ or K^+ (Binstock and Lecar, 1969). From these early experiments, it appeared that

Address reprint requests to Dr. D. R. Matteson, Dept. of Physiology, G4, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/86/05/0795/22 $1.00

Volume 87 May 1986 795–816

channel activation kinetics were independent of the charged species flowing through the pore.

More recent investigations have indicated that some aspects of channel gating can be modulated by variations in the type or concentration of ions present in the channel. This was first demonstrated for the acetylcholine (ACh) channel, where it was found that the channel open time varied with the species of permeant cation present (Van Helden et al., 1977; Ascher et al., 1978). Gage and Van Helden (1979) reported a reciprocal relationship between current amplitude and channel lifetime, and suggested that a site in the channel might modulate both the permeability and the open time. In a similar model, Marchais and Marty (1979) proposed that variations in the open time were mediated by changes in the occupancy of a cation binding site located in the pore, and they hypothesized that the channel is prevented from closing when a cation is bound at the site. We will refer to this idea as the "occupancy hypothesis." Interactions between monovalent cations and Na channel gating have also been described recently (Oxford and Yeh, 1985).

Swenson and Armstrong (1981) proposed a model similar to that of Marchais and Marty to explain the slowing of the K channel closing rate in squid axons, by external K^+ or Rb^+. They found that Rb^+, which seems to bind more tightly in the channel, has a larger effect on the closing rate than the more loosely binding K^+. This result is consistent with the occupancy hypothesis, and it prompted us to test for possible effects of other permeant monovalent cations on K channel gating and conductance. A major goal of this work was to use the instantaneous current-voltage (*I-V*) relationship as a tool for estimating channel occupancy under a variety of ionic conditions, and then to test for a correlation between the closing rate and occupancy. Our results are consistent with the idea that the presence of monovalent cations at a site within the channel inhibits or prevents the channel from closing. The following paper (Armstrong and Matteson, 1986) presents evidence that the channel prefers to close with a calcium ion at the site. A preliminary account of this work has appeared elsewhere (Matteson and Swenson, 1982).

METHODS

Experiments were performed at the Marine Biological Laboratory, Woods Hole, MA, on internally perfused giant axons from *Loligo pealei*. Our voltage-clamp technique was essentially the same as that described in detail elsewhere (Bezanilla and Armstrong, 1977). In most experiments, we compensated for 3 $\Omega \cdot cm^2$ of series resistance. The linear components of total currents were subtracted out with a P/4 procedure (Armstrong and Bezanilla, 1974), with control pulses starting from −120 mV. Pulses were generated from an LSI 11/23 computer, which was interfaced to the voltage clamp. The computer was used to acquire, store, and analyze the data.

Solutions

The composition of the internal solution used throughout was 225 mM K-glutamate, 50 mM KF, 420 mM sucrose, and 10 mM Tris, pH 7.0. All external solutions contained 200 nM tetrodotoxin (TTX) to block Na channels. Our control external solution was an artifical seawater (ASW) containing 440 mM NaCl, 50 mM $CaCl_2$, and 5 mM Tris, pH

7.0. Other external solutions were made by substituting the appropriate amount of the chloride salt of the test cation (e.g., RbCl) for an equimolar amount of NaCl in ASW, and the solution is named for the test cation (e.g., 200 Rb). To examine the effect of Na on the K channel, we substituted 440 mM Tris for 440 mM Na in ASW. Finally, since TlCl has a low solubility, nitrate salts were used for the Tl experiments. Control experiments showed that replacement of all the Cl^- in ASW with nitrate had no significant effect on K current kinetics. Junction potential differences between control and test solutions were <2 mV and were neglected.

Barrier Modeling

Fits of instantaneous *I-V* relationships were obtained by calculating current from a three-barrier, two-site model using a matrix method like that described by Begenisich and Cahalan (1980). The rate constants governing ion movements were calculated using absolute reaction rate theory (Glasstone et al., 1941). A frequency factor value of 10^{11} mol^{-1} s^{-1} was used for rate constants involving ion movements from bulk solution to a site in the channel, and a value of 10^{13} s^{-1} was used for all other rate constants (cf. Lewis and Stevens, 1979). Where appropriate, the rate constants include ionic activities. Our experimental protocol for measuring instantaneous current uses a large activating prepulse (cf. Fig. 8), which causes K^+ accumulation in the Schwann cell space. Thus, the external K activity is unknown, and therefore was estimated from the observed reversal potential in ASW or symmetrical K^+ solutions. The steady state flux of each ionic species through the channel was calculated as the net flux across the central barrier. Total net flux was converted to current, which was scaled in magnitude (by a constant proportional to the number of K channels) to fit the measured instantaneous current.

RESULTS

Closing Kinetics of K Channels Are Altered by Permeant Cations

The closing rate of K channels was monitored by recording the tail currents that flow through activated channels after a repolarizing voltage step. After the K channels were opened with a 4-ms step to +60 mV, they were closed by stepping to −120 mV, where the tail currents illustrated in Fig. 1 were recorded. The current during the activating pulse is not shown in the figure. In each tail current pair, the solid trace is the control in ASW and the dotted trace was recorded after substitution of 200 mM test cation for 200 mM Na^+ in the external solution. For comparison of kinetics, each experimental trace was scaled so that its initial amplitude matched that of the control trace. Three types of results were obtained with the various monovalent cations tested: (*a*) Rb^+, Cs^+, and K^+ (not shown) slow channel closing kinetics; (*b*) NH_4^+ and Tl^+ speed closing; and (*c*) substitution of Li^+ or Tris for Na^+ has relatively little effect. In each instance, the change produced by the test cation was reversed after returning to ASW.

Tail current kinetics provide a reasonable qualitative assessment of channel closing kinetics, but in low external K^+ (as in our ASW solution) they are not a good quantitative measure because their time course is affected by the depletion of K ions that accumulate in the Schwann cell space during the activating pulse (cf. Dubois and Bergman, 1975). A more accurate measure of the K channel closing rate can be obtained with the double-pulse procedure illustrated in Fig. 2. The membrane was first depolarized to open K channels, and was then

repolarized for a variable period of time (Δt) to allow channels to close. The jump in current (labeled ΔI_K in Fig. 2) elicited by a second pulse is proportional to the number of K channels still open. As shown in the figure, ΔI_K declined as a function of interpulse interval, and the time course of this decline reflects the rate at which K channels close. The rationale of the method is that outward K currents (at large positive potentials) are less sensitive to changes in external K^+

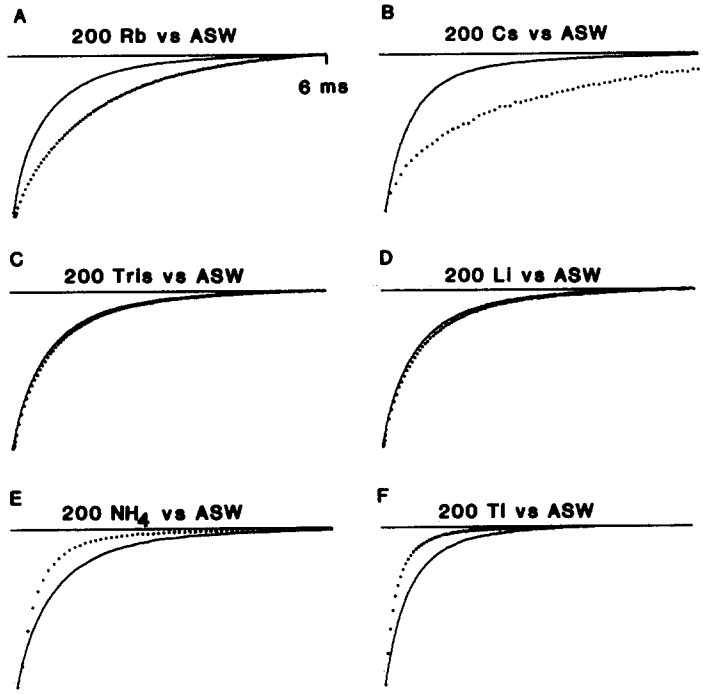

FIGURE 1. Monovalent cations alter tail current kinetics. In each panel, the solid trace was recorded in ASW and the dotted trace was obtained after substituting 200 mM of the test cation for 200 mM Na in ASW. The tail currents in each panel were scaled to have the same initial amplitude in order to compare time courses. The holding potential (HP) was −70 mV and the tails were recorded at −120 mV. The temperature was 8°C, except in *F*, which was done at 15°C. Experiments: SE251Y (*A*), SE221Y (*B* and *E*), SE231Y (*C* and *D*), and OC011A (*F*).

than are inward currents, a property expected for pores that conform to the independence principle over the concentration range in question. This has been demonstrated experimentally (Clay, 1984; also see Fig. 11*A*).

Results obtained with the double-pulse protocol agree qualitatively with the tail current experiments. The data from four experiments are shown in Fig. 3, where the fraction of open channels is plotted on a semilog scale as a function of the interpulse interval. The straight lines drawn through the data were obtained from least-squares fits of a single exponential to the first six to eight points. These fits provide an estimate of the initial closing time constant (τ_c). In ASW at

−70 mV and 8°C, τ_c had an average value of 2.6 ± 0.65 ms (mean ± SD, n = 9). In Fig. 3*A*, 200 mM external Rb^+ increased τ_c by a factor of 2.6 and 200 mM K^+ increased it 2.0 times relative to ASW. In another axon, 200 mM Cs^+ increased τ_c 2.7 times, and 200 mM NH_4^+ decreased it 0.61 times (Fig. 3*B*). This figure also illustrates that 200 mM Tl^+ decreased τ_c 0.66 times (Fig. 3*C*) and 200 mM Li^+ had no effect relative to ASW (Fig. 3*D*).

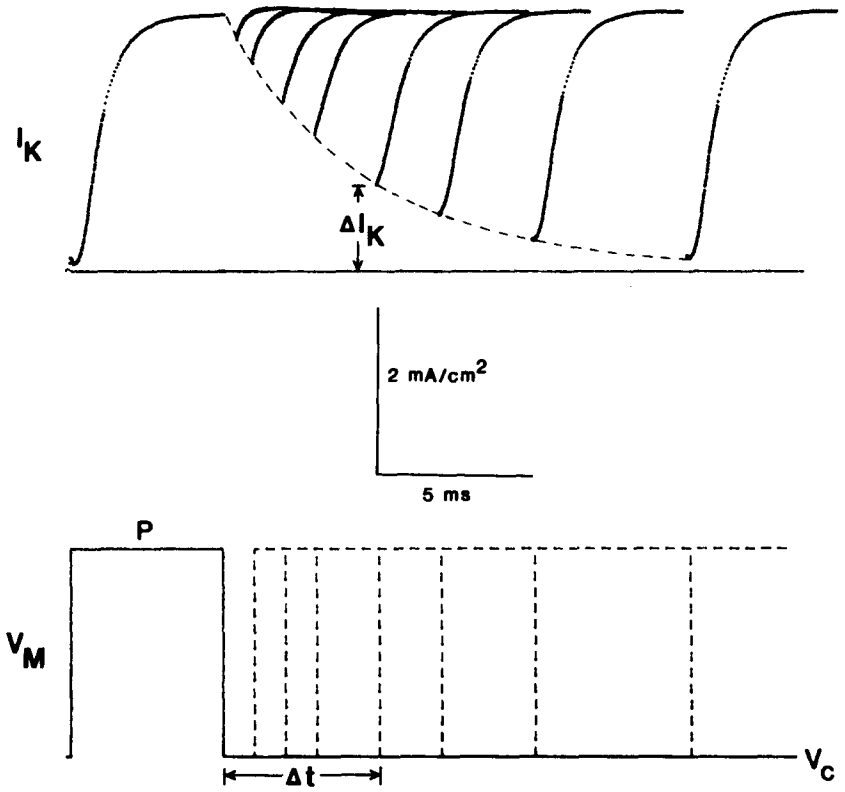

FIGURE 2. Double-pulse procedure used to measure K channel closing kinetics. A prepulse (P, bottom trace) was used to open the K channels. The channels were then allowed to close for a short time (Δt) by repolarizing to V_C. The number of channels still open at this time was assayed by delivering a test pulse and measuring the jump in K current (ΔI_K). The envelope of the current jumps (dashed line) reflects the rate at which K channels close at V_C. Currents were recorded in ASW.

The alterations of closing rate at −70 mV produced by 200-mM concentrations of test cation are summarized in Table I. The second column in the table gives the average ratio of τ_c recorded in the presence of the test cation to the τ_c of control (in ASW). The relative potencies of the cations that slow closing are $Cs^+ > Rb^+ > K^+$, and they slow by factors of 2.53, 2.31, and 1.70, respectively. Tl^+ decreased the closing time constant by a factor of 0.63 and NH_4^+ decreased it by a factor of 0.86.

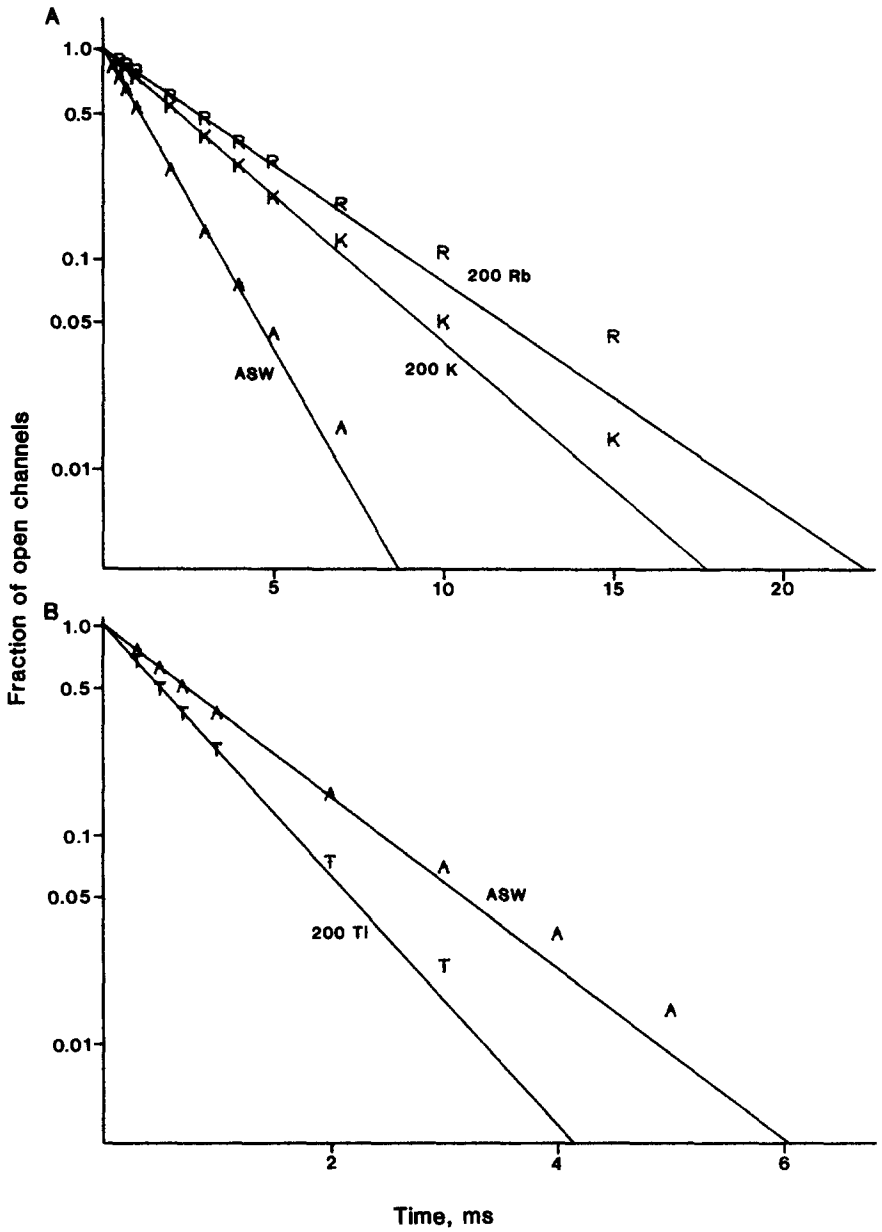

FIGURE 3. K channel closing kinetics at −70 mV in a variety of external solutions. The double-pulse procedure illustrated in Fig. 2 was used to estimate the fraction of open channels, which is plotted on semilogarithmic coordinates as a function of the closing interval at −70 mV. The straight lines are fits of a single exponential to the first six to eight points and the time constants are indicated in the table on the opposite page.

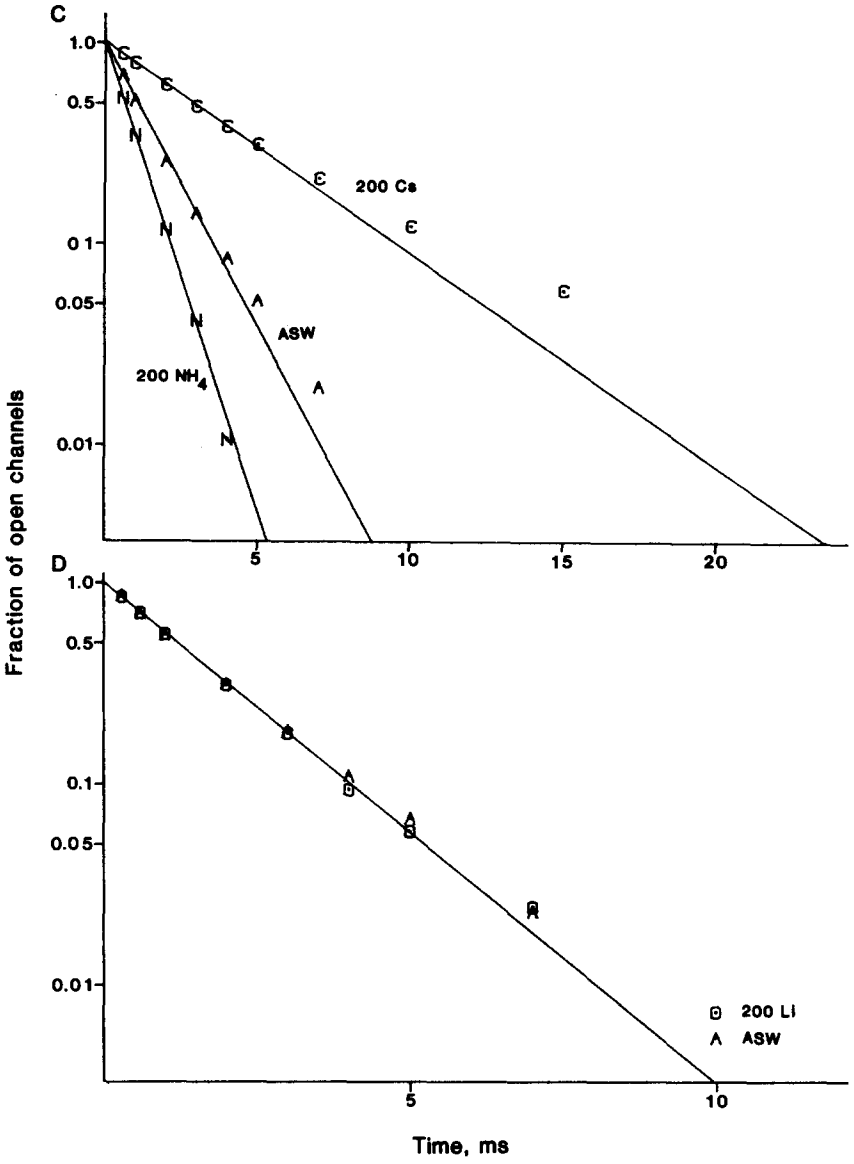

Figure	Experiment	Temperature (°C)	HP	Solution	τ
A	AU122S	8	−80	ASW	1.52
				200 K	3.11
				200 Rb	3.93
B	JN222R	15	−70	ASW	1.06
				200 Tl	0.73
C	SE221Y	8	−70	ASW	1.53
				200 NH_4	0.93
				200 Cs	4.13
D	SE231Y	8	−70	ASW	1.75
				200 Li	1.71

It is interesting to note that Na^+, Li^+, and Tris do not readily enter the K channel, whereas all of the other cations we tested can do so. Rb^+, NH_4^+, Tl^+, and K^+ are all permeant, as indicated by their ability both to carry current and to shift the reversal potential of the channel (Oxford and Adams, 1981; Hille, 1973). Cs^+, on the other hand, blocks K channels in a voltage-dependent manner (Bezanilla and Armstrong, 1972; Adelman and French, 1978). Thus, only the monovalent cations that enter the pore can alter the channel closing rate, which strongly suggests that these cations exert their action at a site within the channel.

Concentration Dependence of Slowing by Rb

A dose-response curve for the slowing effect of external Rb^+ on K channel closing is shown in Fig. 4. In this experiment, as little as 0.4 mM Rb^+ had a reversible effect on the closing rate, slowing it by a factor of 1.1. The slowing

TABLE I

Effect of Monovalent Cations on Closing Rate at −70 mV

Solution	τ_{cation}/τ_{ASW}	n
200 K	1.70±0.43*	7
200 Rb	2.31±0.45	8
200 Cs	2.53±0.61	3
200 Tl‡	0.63±0.08	4
200 NH_4	0.86±0.19	4
200 Tris	0.91	1
200 Li	0.96	1

* Ratios were taken from individual axons and then averaged. These numbers are means ± standard deviation.

‡ Measured at 15°C.

effect saturated at ~50 mM Rb. Fits of these data to the Hill equation produced a Hill coefficient of 1.1, which is consistent with the idea that only a single Rb ion is required to slow channel closing. A fit of an adsorption isotherm to the dose-response data (solid line in Fig. 4) yielded a value of 5.4 mM for the dissociation constant of Rb with its site of action.

Opening Kinetics

The monovalent cations we have tested had little or no effect on K channel opening kinetics, and an example from an Rb experiment is illustrated in Fig. 5. The currents in Fig. 5*A* were recorded at +100 mV in ASW (larger trace) and in 200 mM Rb^+. To facilitate a comparison of time course, the time-varying portion of the Rb^+ current was scaled in amplitude to match the control current (Fig. 5*B*). From the scaled records, it is clear that Rb^+ did not have a significant effect on K channel activation. The following paper (Armstrong and Matteson, 1986) shows that, like Rb^+, external K^+ had no effect on opening kinetics.

In Fig. 5*A*, there is a large jump in current at the onset of the depolarization in 200 mM Rb^+. As shown below, this jump is due to an increase in the number of K channels open at the holding potential.

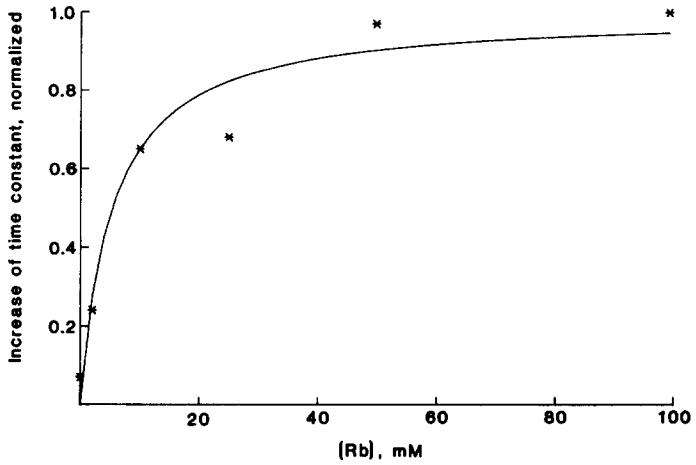

FIGURE 4. The increase in closing time constant (τ_c) as a function of Rb concentration. τ_c was measured with a double-pulse procedure, using a 4-ms activating prepulse to 70 mV. Closing kinetics were determined in ASW before and after a determination in Rb. The ASW values were averaged and the normalized difference between τ_c in Rb and τ_c in ASW is plotted in this figure. The smooth curve is a least-squares fit of the linear form of the equation $y = 1/(1 + K_d/\text{Rb})$, which yielded a K_d estimate of 5.4 mM. Experiment JN162R. HP, −70 mV. Temperature, 8°C.

Voltage Dependence of Closing

If permeant cations influence K channel gating by acting at a site within the channel (as in the occupancy hypothesis), changes in membrane voltage could alter the closing kinetics in more than one way. In addition to directly influencing the state of the K channel gating structures, the membrane potential might indirectly influence the closing kinetics by altering the probability that a monovalent cation is bound to the site. Thus, the voltage dependence of the closing

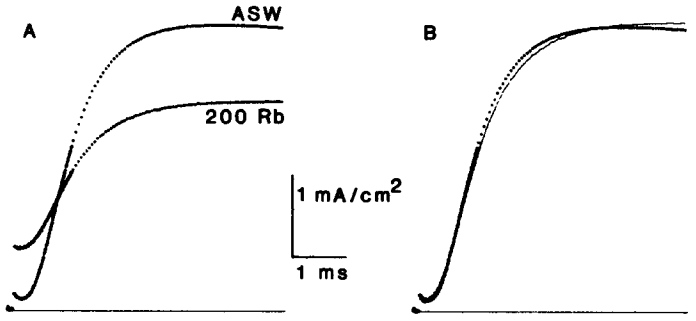

FIGURE 5. External Rb has little or no effect on K channel opening kinetics. (*A*) Currents were recorded during 5-ms steps to 60 mV in ASW or 200 mM Rb^+. (*B*) The time-varying portion of the trace in 200 Rb was scaled in magnitude (thin line) to match the ASW record (dotted trace), in order to facilitate a comparison of kinetics. Experiment AU092R. HP, − 70 mV. Temperature, 8°C.

rate might be different in high and low concentrations of external permeant cations.

The initial closing rate of the K channel was estimated as the reciprocal of the exponential time constant measured with the double-pulse protocol ($1/\tau_c$). Semilog plots of $1/\tau_c$ as a function of voltage are shown in Fig. 6. The relationship

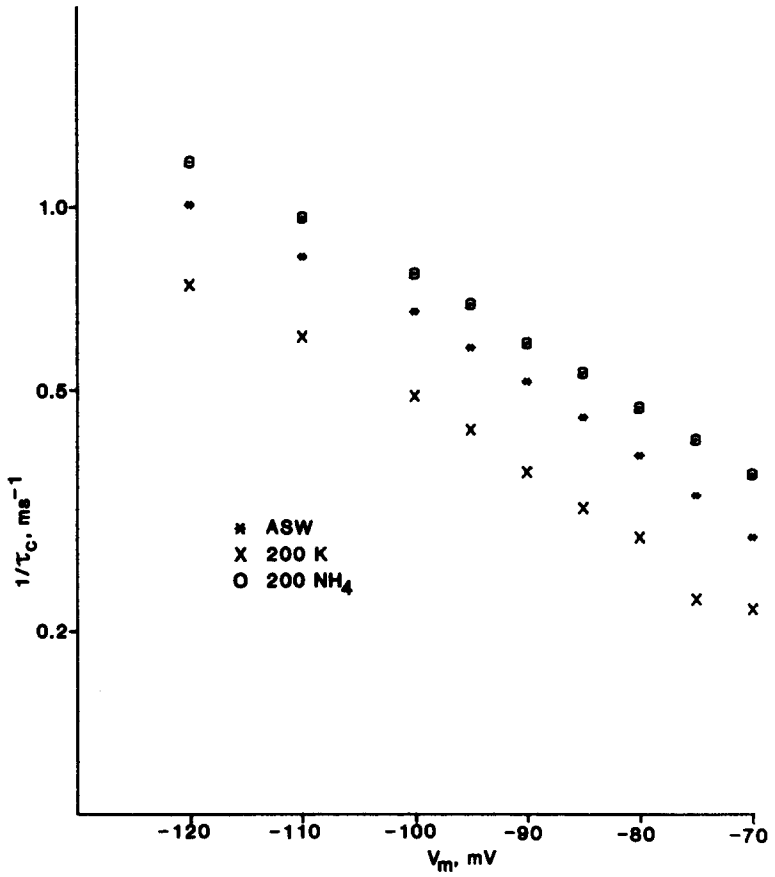

FIGURE 6. Voltage dependence of closing rate. The closing time constant (τ_c) was measured with a double-pulse procedure and its reciprocal is plotted semilogarithmically as a function of the closing voltage (V_C of Fig. 2). In this fiber, closings were measured in ASW (*), 200 K (×), and 200 NH_4 (○). Experiment JN222S. HP, − 70 mV. Temperature, 8°C.

between the closing rate and voltage is approximately exponential over the range −70 to −110 mV. In this experiment, the closing rate increased about e-fold for 38 mV of hyperpolarization when measured in ASW (asterisks in Fig. 6). The substitution of 200 mM NH_4^+ increased the closing rate and 200 mM K^+ decreased it. These ionic changes, however, had no significant effect on the steepness of the $1/\tau_c$-voltage curve. Thus, external cations may simply produce a shift of

closing kinetics along the voltage axis. The substitution of 200 mM K^+ resulted in an apparent shift of the $1/\tau_c$-voltage relationship of about −12 mV.

Steady State K Conductance-Voltage Relationship

The steady state K conductance at −70 mV appears to be larger in external Rb than it is in ASW, as evidenced by the large current jump that occurs upon depolarization in Rb^+. The conductance can be reduced by lowering the holding potential to −100 mV (Fig. 7), which suggests that the steady state conductance-voltage (g_K-V) relationship might be shifted in the negative direction by Rb^+. This was confirmed by the following experiments. K conductance was estimated from a linear region of the instantaneous I-V relationship, as shown in Fig. 8. An initial depolarization was used to activate g_K to a steady state level (cf. inset of Fig. 8). The voltage was then stepped to a new level (0–50 mV), and the

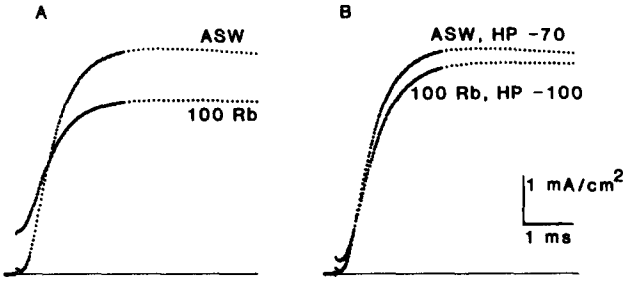

FIGURE 7. External Rb^+ induces a large conductance at −70 mV, which is reduced by hyperpolarization. (*A*) The large-magnitude trace was recorded in ASW and the smaller trace in 100 mM Rb^+, during steps to 90 mV from a holding potential of −70 mV. In 100 mM Rb, there was a large instantaneous jump in current after depolarization. (*B*) Both traces were recorded at 90 mV. In ASW the holding potential was −70 mV, and in 100 Rb it was −100 mV. By holding at −100 mV, the initial current jump in Rb was greatly reduced. Experiment AU102U. Temperature, 8°C.

instantaneous current (labeled I_i) was measured. The instantaneous I-V curves, for activating steps to the indicated voltages, are plotted in Fig. 8. The straight line drawn through each set of points is a least-squares fit that provides an estimate of g_K at each voltage.

Conductance-voltage relationships generated by this procedure are shown in Fig. 9. The control g_K-V curve (recorded in ASW) has a sigmoid shape, with half-maximal conductance at about −25 mV. In 200 mM Rb^+, the curve is shifted in the hyperpolarizing direction by ~15 mV (measured at the midpoint). 200 mM K^+ produced a smaller shift, which at negative voltages is roughly comparable to the shift in closing kinetics noted in Fig. 6. In addition to the shift, the shape of the g_K-V curve was changed by both Rb^+ and K^+. At relatively negative voltages, the conductance was larger than one would expect from a simple shift of the control curve.

The voltage dependence of g_K is the second property of the channel gating apparatus, which we have shown to be sensitive to external permeant cations. In

the same fiber used in Fig. 9, 200 mM Rb^+ slowed the initial closing rate 2.5 times, and 200 mM K^+ slowed it by a factor of 1.8. Thus, Rb and K have a similar potency sequence for their effect on the closing rate and the voltage dependence of g_K.

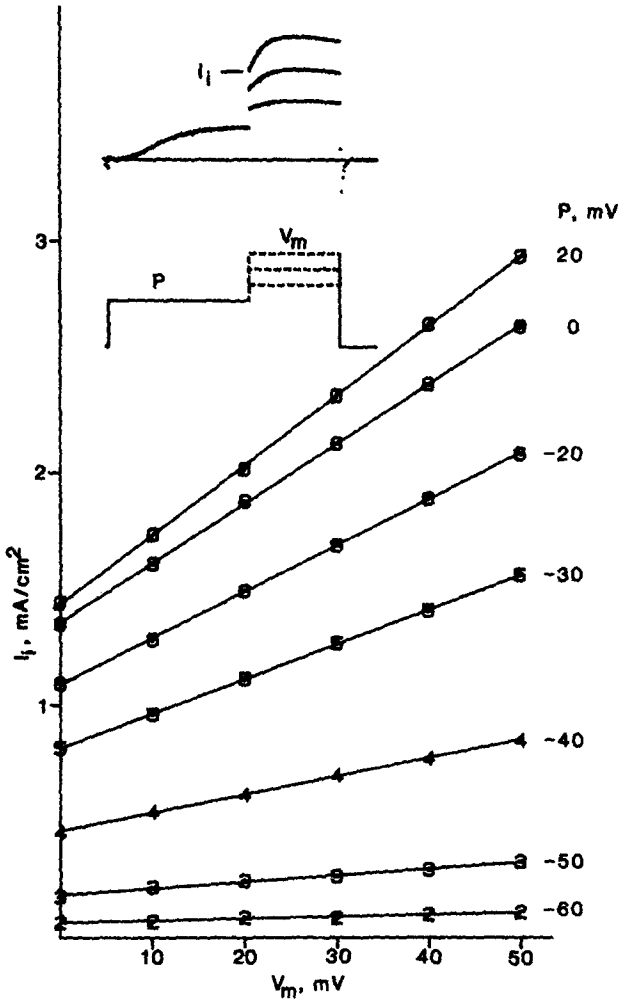

FIGURE 8. Method for determining the steady state g_K-V relationship. The pulse procedure shown in the inset was used to measure the instantaneous I-V relationship, which reflects the conductance of open K channels. A prepulse (P) was used to activate the channels to a steady level. The instantaneous change in current (I_i) was then measured after a step to a new potential (V_m), which was in the range 0–50 mV (dotted lines). Each set of data points is the instantaneous I-V curve measured at the indicated (prepulse) voltage (P). These curves are linear and therefore the slope provides an estimate of g_K. The external solution was ASW. Experiment AU122S. HP, −80 mV. Temperature, 8°C.

External Monovalent Cations and the Instantaneous I-V Curve

In the Introduction, we outlined the occupancy hypothesis, which proposes that a K channel is inhibited from closing when occupied by a monovalent cation. Information about the entry and binding of ions in channels can be obtained from reversal potentials and the instantaneous *I-V* curves. We have used an energy barrier model of permeation to calculate current and to fit the instantaneous *I-V* relationship. The parameters of the fit were then used to estimate

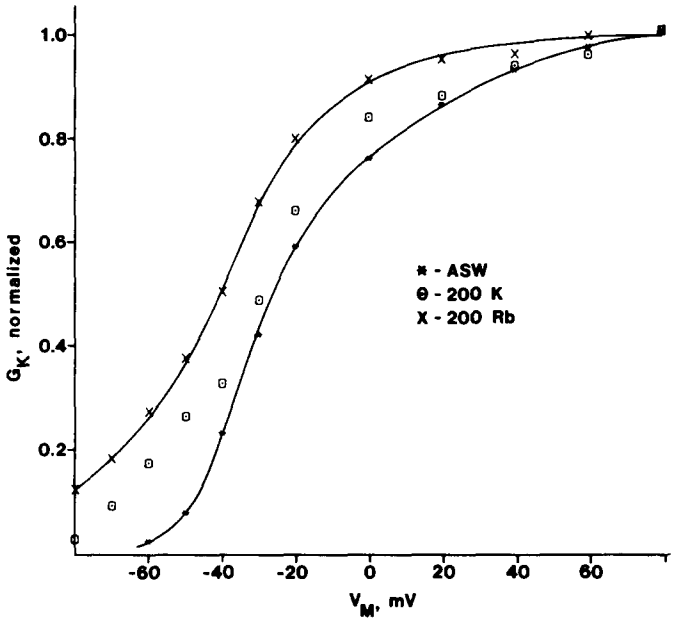

FIGURE 9. The g_K-V curve is shifted in the negative direction by Rb^+ and K^+. K channel conductance (g_K) was taken as the slope of the instantaneous *I-V* curve, as shown in Fig. 8. All data were obtained from a single fiber, with solutions changed in the following order: ASW, 200 Rb, ASW, 200 K, ASW. The shift of the g_K-V curve produced by Rb^+ or K^+ was reversed on switching back to ASW. Therefore, the three determinations in ASW were averaged. The maximum K conductances were (in mS): ASW, 35.4; 200 Rb^+, 38.8; 200 K^+, 41.3. Experiment AU122S. HP, −80 mV. Temperature, 15°C.

channel occupancy (cf. Hille and Schwarz, 1978; Begenisich and Cahalan, 1980). The instantaneous *I-V* curves are presented in this section, and the details of the barrier model are described in the Discussion.

The instantaneous *I-V* curves shown in Figs. 10 and 11 were obtained using a pulse protocol like that shown in Fig. 8. In ASW (Fig. 10*A*), with zero added K^+, the instantaneous *I-V* curve was nonlinear, with lower conductance at negative potentials. The inward current in this case was carried by K^+, which accumulated in the Schwann cell space during the depolarizing pulse. Using the Nernst equation, we can estimate from the reversal potential of −50 mV that the

external K^+ concentration was 35 mM. With higher external K, the instantaneous *I-V* curve was nearly linear, as shown by the 200 mM K curve in Fig. 10*A*. These instantaneous *I-V* relationships, with K as the only permeant species present,

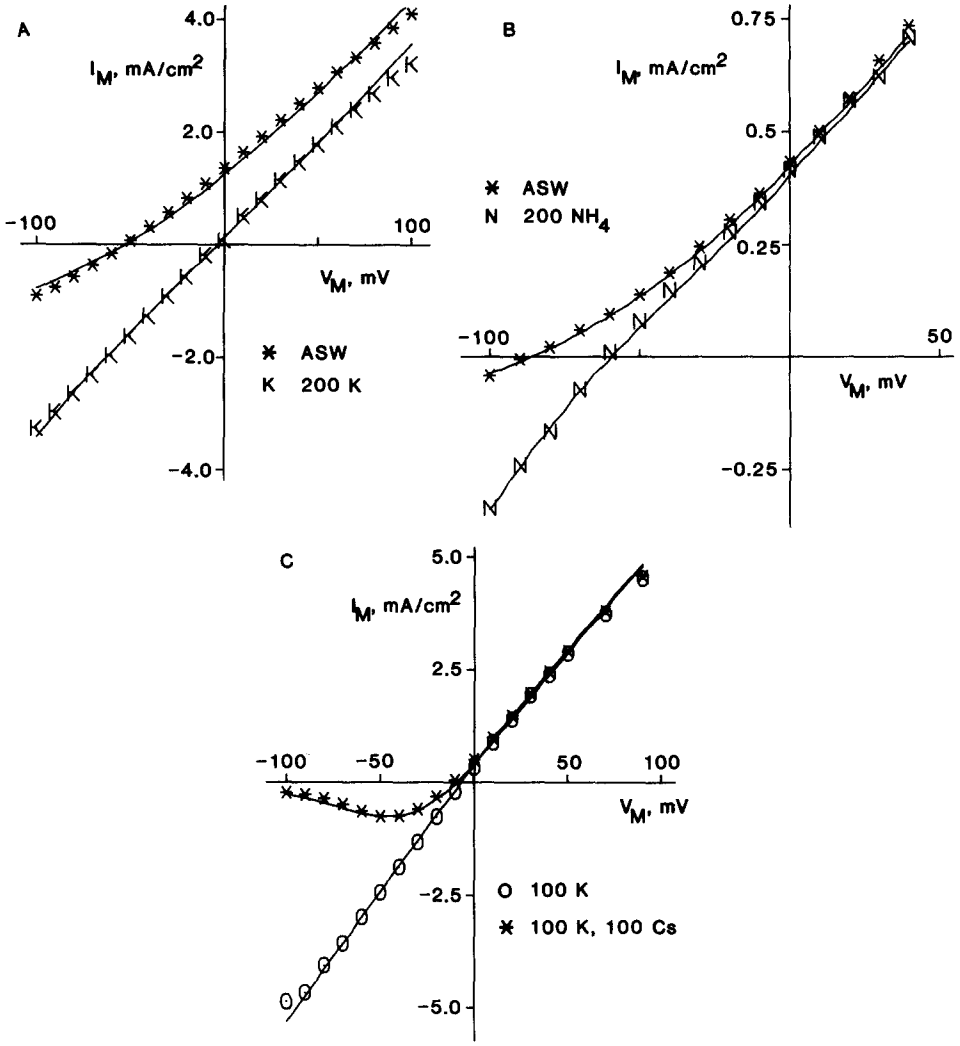

FIGURE 10. External K, NH_4, and Cs change the shape of the K channel instantaneous *I-V* relationship. The symbols indicate the data points, which were obtained using a pulse protocol similar to that shown in Fig. 8. The smooth curves are fits produced by a three-barrier, two-site model as described in the Discussion. (*A*) The activating prepulse was to 80 mV for 3 ms. Records were taken in ASW (*) and 200 K (K) in the same axon. Experiment JN222S. HP, −70 mV. Temperature, 8°C. (*B*) Prepulse to 20 mV for 1 ms. The instantaneous *I-V* data were taken in ASW (*) and 200 NH_4 (N). Experiment SE063C. HP, −80 mV. Temperature, 8°C. (*C*) Prepulse to 80 mV for 10 ms. In this axon, the instantaneous *I-V* data were taken in 100 K (*) and 100 K plus 100 Cs (O). Experiment SE050C. HP, −70 mV. Temperature, 8°C.

provide a baseline for assessing the effects of other monovalent cations on K channel conductance. The smooth curves through the data points were generated by a three-barrier, two-site model as described in the Discussion.

The substitution of 200 mM NH_4^+ for 200 mM Na^+ had a small but significant effect on the instantaneous *I-V* curve (Fig. 10*B*). This effect was best demonstrated in the near absence of external K^+, a condition achieved by using a short (1 ms) activating pulse to minimize K^+ accumulation. The reversal potential was increased by ~29 mV on switching from ASW to 200 mM NH_4. Assuming that the external K^+ activity was the same in both conditions, use of the Goldman-Hodgkin-Katz equation gives a P_{NH_4}/P_K ratio of 0.09, which suggests that NH_4^+

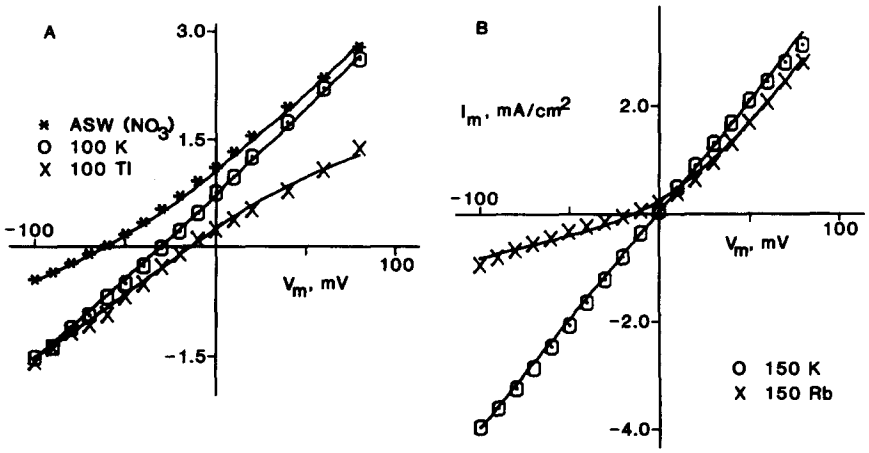

FIGURE 11. Instantaneous *I-V* curves in external Tl and Rb. (*A*) Activating prepulse was to 100 mV for 2 ms. Nitrate salts were used for all external solutions. The instantaneous *I-V* curve was measured in ASW (*), 100 K (O), and 100 Tl (×) in this axon. Experiment JL030A. HP, −60 mV. Temperature, 15°C. (*B*) Prepulse to 90 mV for 3 ms. External solutions were 150 K (O) or 150 Rb (×). Experiment AU260A. HP, −70 mV. Temperature, 10°C.

does not enter the channel readily. The inward current was significantly larger in 200 NH_4^+ than in ASW, which indicates that NH_4^+ can carry current (see also Binstock and Lecar, 1969). Thus, in spite of its low entry rate, NH_4^+ is able to pass through the channel readily.

External Cs ions have a high probability of occupying K channels at negative voltages, as indicated by their ability to block inward K^+ current in a voltage-dependent manner (Bezanilla and Armstrong, 1972; Adelman and French, 1978). Instantaneous *I-V* relationships in 100 mM K^+, with and without 100 mM Cs^+, are shown in Fig. 10*C*. External Cs^+ had no effect on the outward current, but progressively reduced the inward current as voltage was decreased from −10 to −100 mV. Thus, at negative membrane potentials in the presence of external Cs^+, there is a relatively high probability that a K channel will be occupied by a Cs ion, which inhibits current flow.

External Tl shifts the reversal potential more than an equal concentration of K, which suggests that it enters the channel more readily than K (Fig. 11*A*). In

the voltage range from −100 to −20 mV, the current was slightly larger in 100 mM Tl^+ than in 100 mM K^+, which indicates that Tl is an effective carrier of inward current. On the other hand, the outward current was dramatically reduced by external Tl^+. This effect of Tl can best be explained by assuming that it binds tightly to a site located close to the outer surface of the membrane, and thereby inhibits outward K movement. Increasing the membrane potential had little effect on Tl^+ removal from the site, because of the proximity of the site to the membrane surface.

Swenson and Armstrong (1981) showed that external Rb shifts the K channel reversal potential almost as much as K, but that it carries much less current. Instantaneous *I-V* curves exhibiting these effects are shown in Fig. 11*B*. In 150 mM Rb^+, the reversal potential was about −12 mV and in 150 mM K it was ~0 mV. The inward current in 150 mM Rb was much smaller than in 150 mM K. Thus, Rb^+ enters the K channel almost as readily as K^+ does (shifts the reversal potential almost as much), but it carries less current because it binds more tightly to the channel interior.

DISCUSSION

The changes in K channel gating produced by external monovalent cations can be summarized as follows. (*a*) The only cations that alter K channel gating are those that are able to enter the pore. (*b*) Opening kinetics are affected little, if at all, by permeant external cations. (*c*) The *I-V* relationship is shifted in the hyperpolarizing direction by external Rb^+ or K^+.

In agreement with these results, it has previously been noted that external Rb slows K channel closing in myelinated nerve (Arhem, 1980) and in squid axon (Swenson and Armstrong, 1981). With respect to activation, Dubois and Bergman (1977) found that K channels in myelinated nerve opened faster when external K was raised from 0 to 2.5 mM. Under the conditions of our experiments in squid axon, there was always some external K present because of accumulation in the Schwann cell space. Thus, a possible effect of very low K concentrations on channel opening cannot be excluded, but over the range we tested (0–200 mM added K), there was no effect.

Cations Modulate Gating at a Site within the Channel

Monovalent cations affect K channel gating by acting at a site located within the pore. The major evidence is that only cations that can enter the pore are able to change the closing rate. Further, as shown in the next paragraph, the extent to which cations occupy K channels correlates with the magnitude of their effect on closing kinetics. Na and Li ions are nearly impermeant in delayed rectifier channels of nerve (Hille, 1973; Reuter and Stevens, 1980; Oxford and Adams, 1981), and changes in the external concentration of these cations have little or no effect on gating (Figs. 1 and 3; Table I). Rb is almost as permeant as K (Moore et al., 1966; Hille, 1973), and both of these cations decrease the channel closing rate and shift the g_K-*V* curve in the negative direction (Figs. 3 and 9). Cs ions have a potent slowing effect, and Cs entry into K channels from outside, producing block, is well documented (Bezanilla and Armstrong, 1972; Adelman and French, 1978). Tl and NH_4 are permeant (Hille 1973; Oxford and Adams,

1981; Binstock and Lecar, 1969), but these cations speed closing, with Tl exhibiting a larger effect than NH_4.

With the exception of Tl, which is a special case, there is a correlation between the degree of channel occupancy by monovalent cations and their effect on the closing rate. Rb binds more tightly than K in the channel, and produces a more potent slowing action (Swenson and Armstrong, 1981). External Cs ions have a high probability of occupying the channel at negative voltages, as evidenced by their blocking action. Cs ions block by entering the pore and getting stuck, thereby preventing the flux of K^+. Thus, in the presence of external Cs, the K channel would be more likely to contain a cation than it would in external K, because K can pass through the channel and Cs cannot. This correlation between occupancy and closing kinetics lends further support to the idea that monovalent cations alter gating by acting at a site within the pore.

The following paper (Armstrong and Matteson, 1986) presents evidence that Ca^{2+} competes with monovalents for pore occupancy, and that Ca^{2+}-occupied channels close more rapidly and more securely than monovalent-occupied channels.

Occupancy Hypothesis

In other channels, gating is also affected by the monovalent cation composition (gramicidin channel: Kolb and Bamberg, 1977; ACh channel: Van Helden et al., 1977; Ascher et al., 1978; Gage and Van Helden, 1979; Ca channel: Nelson et al., 1984). It has been suggested that the cations exert their action on gating by binding to sites located in the channel, and that the occupied sites in some way inhibit channel closing (Ascher et al., 1978; Marchais and Marty, 1979). The channel open time would then be proportional to the occupancy of the channel binding sites. In an attempt to test the applicability of this model to the action of external cations in the squid K channel, we have estimated the probability of site occupancy under various ionic conditions and compared it with the K channel closing rate.

K channels behave as multi-ion pores. The best evidence, with respect to the squid K channel, is the fact that influx and efflux are coupled and that the flux ratio exponent is >1 (Hodgkin and Keynes, 1955; Begenisich and De Weer, 1980). These flux data indicate that the channel contains at least two and possibly three or more sites. To characterize the occupancy of each site, we have used an energy barrier model of the K channel, as described in the next section.

Barrier Models of the K Channel

It has been useful to model multisite channels as a series of energy barriers and wells, to explain why K channels deviate from the theoretical behavior of free-diffusion pores (Woodbury, 1971; Hille and Schwarz, 1978; Begenisich and Cahalan, 1980). The energy wells correspond to ion binding sites, and ions move through the channel by jumping from one site to another across the intervening barrier. The rate constants for jumping are exponentially related to the barrier heights (cf. Glasstone et al., 1941). The use of this model has allowed us to estimate the probability that each site contains a monovalent cation.

A three-barrier, two-site pore model proved adequate for our analysis. The

positions and magnitudes of the barriers and wells were estimated by fitting the model to the instantaneous *I-V* curves. We allowed both sites to be occupied simultaneously and included an ionic repulsion factor, as described by Hille and Schwarz (1978), which reduces the entry rate to one site (and increases the exit rate) by a factor of 2 when the other site is occupied. Current was calculated from the model using a matrix method like that described by Begenisich and Cahalan (1980) (see Methods). These calculations give the distribution of channels among the nine states that are possible with two permeant species present.

The energy profile for K ions was obtained first by estimating the parameters that best fit the instantaneous *I-V* curves recorded at two different external K^+

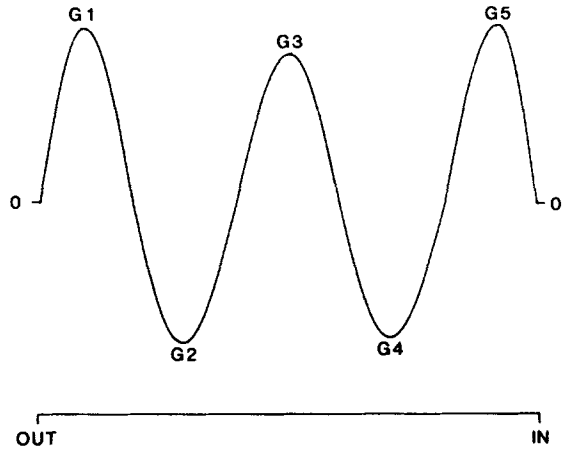

FIGURE 12. Average energy profile of the K channel for K^+ at 0 mV. The energy levels, relative to bulk solution, in units of *RT* are: G1, 9.4; G2, −7.4; G3, 8.1; G4, −7.1; G5, 9.9. The abscissa represents the electrical distance through the membrane. Energy profiles were obtained from six axons by fitting instantaneous *I-V* relationships measured in the presence of K^+ only. The illustrated profile is the average of the six fits.

concentrations. As a first approximation, the outer barriers were placed close to the membrane surface and the middle barrier about halfway through the membrane; the energy wells were evenly spaced between the barriers. Barrier positions and heights were then fine-tuned (by eye) to yield the very good fits illustrated in Figs. 10 and 11.

The average ($n = 6$) energy profile for K^+ (at 0 mV) is shown in Fig. 12. The energy levels are similar, in some respects, to those in the four-barrier, three-site model described by Begenisch and Smith (1984). In their model, the outer- and innermost barriers were 10 *RT*, whereas in our model they were 9.4 and 9.9 *RT*, respectively. Their middle barriers were much lower, and their wells were somewhat deeper, on the average, than ours. The energy profile shown in Fig. 12 provides a baseline for comparing the effects of other cations on channel conductance and site occupancy.

After obtaining the barrier profile for K ions, energy profiles were determined for the test cations. The barriers and wells were assumed to be at the same

TABLE II

Energy Profiles for Permeant Cations

Ion	G1*	G2	G3	G4	G5
K‡	9.4§	−7.4	8.1	−7.1	9.9
Rb	9.4	−8.4	8.2	−7.9	11.5
Cs	10.7	−5.7	7.2	−7.3	17.4
Tl	9.9	−10.3	5.8	−5.2	5.2
NH_4	11.1	−4.9	4.0	0	13.6

* G1–G5, as illustrated in Fig. 12, are, in the following order, the outer barrier, the outer well, the middle barrier, the inner well, and the inner barrier.

‡ For K, this is the average profile illustrated in Fig. 12.

§ Units of *RT*.

electrical distance from the surface for both K^+ and the test cation, so the only free parameters were the barrier heights and well depths for the test cation.

The heights and depths for the instantaneous *I-V* curves in Figs. 10 and 11 are given in Table II. As expected, the energy wells for Rb^+ were deeper than those for K^+. Thus, although Rb can enter the channel almost as readily as K (the outer barrier is the same for the two ions), it binds more tightly (deeper wells) and therefore carries less current (cf. Fig. 11*B*).

To fit the shape of the instantaneous *I-V* curve in Cs with this two-site model, the innermost barrier had to be high, to prevent Cs exit to the inside. The instantaneous Cs *I-V* curve could not be fit accurately using a high middle barrier: this made the voltage dependence of block too small. The outer well for Cs had to be relatively shallow, because with a deeper well the model predicted some block of outward current by external Cs, and this was not observed experimentally (cf. Fig. 10*C*). At negative membrane potentials, block of inward current occurs as the field drives Cs to the inner site, where it occludes the channel.

The most interesting feature of the Tl profile is the deep outer well, which was required to fit the block of outward K current by external Tl (Fig. 11*A*). Negative membrane potentials enhance the exit of Tl from the site toward the cell interior, and the absence of large barriers correlates with the ability of Tl to carry inward current.

For NH_4, the outer barriers were necessarily high to prevent NH_4 entry into the channel. This feature reflects the fact that NH_4 shifts the reversal potential less (relative to ASW) than an equivalent concentration of K (cf. Hille, 1973; Oxford and Adams, 1981). The energy wells had to be shallow to allow NH_4

TABLE III

Probability of Site Occupancy by Monovalent Cations at −70 mV

External solution	Outer site	Inner site
20 K	0.237	0.444
120 K	0.428	0.542
20 K, 100 Rb	0.687	0.754
20 K, 100 Cs	0.266	0.895
20 K, 100 Tl	0.436	0.377
20 K, 100 NH_4	0.316	0.425

passage through the channel, which reflects its known ability to carry current (Fig. 10*B*; Binstock and Lecar, 1969).

Probability of Site Occupancy

We have used the model described in the previous section to calculate the probability that each site contains a monovalent cation under various ionic conditions. The results of the calculations (Table III), when compared with effects of the cations on channel closing rate, are consistent with the idea that closing is inhibited when the inner site contains a cation. For example, raising the external K concentration from 20 to 120 mM resulted in an increase in the probability of inner site occupancy from 0.444 to 0.542 (Table III), and, as we have shown, a similar increase in the K concentration would slow the channel closing rate. Rb and Cs increased inner site occupancy, and both of these ions had a larger effect on occupancy than K. The model predicts that extracellular Tl would decrease the probability of cation binding at the inner site. This effect is due to tight binding of Tl at the outer site (a result of the deep outer well), which would decrease cation occupancy at the inner site by ionic repulsion. Finally, NH_4 is also predicted to decrease inner site occupancy slightly. Thus, the ionic interventions that slow closing increase inner site occupancy, and those that speed closing decrease occupancy. Qualitatively, the potency sequence for cation action on closing kinetics is remarkably similar to the sequence describing changes in cation binding at the inner site.

We would very much like to thank Dr. Clay M. Armstrong for providing invaluable advice and guidance during the course of these experiments. This study was largely conducted while the authors were postdoctoral fellows in Dr. Armstrong's lab.

Original version received 17 June 1985 and accepted version received 31 January 1986.

REFERENCES

Adelman, W. J., Jr., and R. J. French. 1978. Blocking of the squid axon potassium channel by external caesium ions. *Journal of Physiology.* 276:13–25.

Arhem, P. 1980. Effects of rubidium, caesium, strontium, barium and lanthanum on ionic currents in myelinated nerve fibres from *Xenopus laevis*. *Acta Physiologica Scandinavica.* 108:7–16.

Armstrong, C. M., and F. Bezanilla. 1974. Charge movement associated with the opening and closing of the activation gates of the Na channels. *Journal of General Physiology.* 63:533–552.

Armstrong, C. M., and D. R. Matteson. 1986. The role of calcium ions in the closing of K channels. *Journal of General Physiology.* 87:817–832.

Ascher, P., A. Marty, and T. O. Neild. 1978. Life time and elementary conductance of the channels mediating the excitatory effects of acetylcholine in *Aplysia* neurones. *Journal of Physiology.* 278:177–206.

Begenisich, T. B., and M. D. Cahalan. 1980. Sodium channel permeation in squid axons. I. Reversal potential experiments. *Journal of Physiology.* 307:217–242.

Begenisich, T. B., and P. De Weer. 1980. Potassium flux ratio in voltage-clamped squid giant axons. *Journal of General Physiology.* 76:83–98.

Begenisich, T. B., and C. Smith. 1984. Multi-ion nature of potassium channels in squid axons. *Current Topics in Membranes and Transport.* 22:353–369.

Bezanilla, F., and C. M. Armstrong. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *Journal of General Physiology.* 60:588–608.

Bezanilla, F., and C. M. Armstrong. 1977. Inactivation of the sodium channel. I. Sodium current experiments. *Journal of General Physiology.* 70:549–566.

Binstock, L., and H. Lecar. 1969. Ammonium ion currents in the squid giant axon. *Journal of General Physiology.* 53:342–361.

Chandler, W. K., and H. Meves. 1965. Voltage clamp experiments on internally perfused giant axons. *Journal of General Physiology.* 180:788–820.

Clay, J. R. 1984. Potassium channel kinetics in squid axons with elevated levels of external potassium concentration. *Biophysical Journal.* 45:481–485.

Dubois, J. M., and C. Bergman. 1975. Potassium accumulation in the perinodal space of frog myelinated axons. *Pflügers Archiv European Journal of Physiology.* 358:111–124.

Dubois, J. M., and C. Bergman. 1977. The steady-state potassium conductance of the Ranvier node at various external K-concentrations. *Pflügers Archiv European Journal of Physiology.* 370:185–194.

Gage, P. W., and D. Van Helden. 1979. Effects of permeant monovalent cations on end-plate channels. *Journal of Physiology.* 288:509–528.

Glasstone, S., K. J. Laidler, and H. Eyring. 1941. The Theory of Rate Processes. McGraw-Hill, New York. 611 pp.

Hille, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. *Journal of General Physiology.* 61:669–686.

Hille, B., and W. Schwarz. 1978. Potassium channels as multi-ion single-file pores. *Journal of General Physiology.* 72:409–442.

Hodgkin, A. L., and A. F. Huxley. 1952. Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *Journal of Physiology.* 116:449–472.

Hodgkin, A. L., and R. D. Keynes. 1955. The potassium permeability of a giant nerve fibre. *Journal of Physiology.* 128:61–88.

Kolb, H. A., and E. Bamberg. 1977. Influence of membrane thickness and ion concentration on the properties of the gramicidin channel. Autocorrelation, spectral power density, relaxation and single channel studies. *Biochemica et Biophysica Acta.* 464:127–141.

Lewis, C. A., and C. F. Stevens. 1979. Mechanism of ion permeation through channels in a postsynaptic membrane. Membrane Transport Processes. C. F. Stevens and R. W. Tsien, editors. Raven Press, New York. 3:133–151.

Marchais, D., and A. Marty. 1979. Interaction of permeant ions with channels activated by acetylcholine in *Aplysia* neurones. *Journal of Physiology.* 297:9–45.

Matteson, D. R., and R. P. Swenson, Jr. 1982. Permeant cations alter closing rates of K channels. *Biophysical Journal.* 37:17*a*. (Abstr.)

Moore, J. W., N. Anderson, M. Blaustein, M. Takata, J. Y. Lettvin, W. F. Pickard, T. Bernstein, and J. Pooler. 1966. Alkali cation selectivity of squid axon membrane. *Annals of the New York Academy of Sciences.* 137:818–829.

Nelson, M. T., R. J. French, and B. K. Krueger. 1984. Voltage-dependent calcium channels from brain incorporated into planar lipid bilayers. *Nature.* 308:77–80.

Oxford, G. S., and D. J. Adams. 1981. Permeant cations alter K channel kinetics and permeability. *Biophysical Journal.* 33:70*a*. (Abstr.)

Oxford, G. S., and J. Z. Yeh. 1985. Interactions of monovalent cations with sodium channels in squid axon. I. Modification of physiological inactivation gating. *Journal of General Physiology.* 85:583–602.

Reuter, H., and C. F. Stevens. 1980. Ion conductance and ion selectivity of potassium channels in snail neurones. *Journal of Membrane Biology.* 57:103–118.

Swenson, R. P., and C. M. Armstrong. 1981. K^+ channels close more slowly in the presence of external K^+ and Rb^+. *Nature.* 291:427–429.

Van Helden, D., O. P. Hamill, and P. W. Gage. 1977. Permeant ions alter endplate channel characteristics. *Nature.* 269:711–712.

Woodbury, J. W. 1971. Eyring rate theory model of the current-voltage relationships of ion channels in excitable membranes. *In* Chemical Dynamics: Papers in Honor of Henry Eyring. J. O. Hirschfelder, editor. John Wiley & Sons, New York. 601–617.