The Role of Calcium Ions in
the Closing of K Channels

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ABSTRACT   The effects of external Ca ion on K channel properties were studied in squid giant axons. Increasing the Ca concentration from 20 to 100 mM slowed K channel opening, and was kinetically equivalent to decreasing the depolarizing step by ~25 mV. The same Ca increase had a much smaller effect on closing kinetics, equivalent to making the membrane potential more negative by ~4 mV. With regard to the conductance-voltage curve, this Ca increase was about equivalent to decreasing the depolarizing step by ~10 mV. The presence of K or Rb in the bath slowed closing kinetics and made the time course more complex: there were pronounced slow components in Rb and, to a lesser extent, in K. Increasing the Ca concentration strongly antagonized the slowing caused by Rb or K. Thus, Ca has a strong effect on closing kinetics only in the presence of these monovalent cations. Rb and K do not significantly alter opening kinetics, nor do they alter Ca's ability to slow opening kinetics. High Ca slightly affects the instantaneous I-V curve by selectively depressing inward current at negative voltages. The results imply that Ca has two actions on K channels, and in only one, the action on closing, does it compete with monovalent cations. We propose (a) that opening kinetics are slowed by binding of Ca to negatively charged parts of the gating apparatus that are at the external surface of the channel protein when the channel is closed; monovalent cations do not compete effectively in this action; (b) Ca (or possibly Mg) normally occupies closed channels and has a latching effect. External K or Rb competes with Ca for channel occupancy. Channels close sluggishly when occupied by a monovalent cation and tend to reopen. Thus, slow closing results from occupancy by K or Rb instead of Ca. The data are well fit by a model based on these ideas.

INTRODUCTION

This paper addresses the question, Do K channels contain a Ca ion when they close? The question is approached by studying the interactions of divalent cations with the K channel and its gating apparatus. Divalent cations have a strong and physiologically significant action on the properties of electrically excitable channels. With regard to the K channel, the literature suggests that divalents act by

two separate mechanisms, a gating action and a blocking action. The gating action was described first, and was attributed to alteration of fixed membrane surface charge, e.g., on phospholipids. Frankenhaeuser and Hodgkin (1957) observed that raising the Ca concentration 10-fold had effects on kinetics and conductances similar to adding a steady hyperpolarizing bias of 15 mV. They postulated that Ca binds to, or electrostatically screens (McLaughlin et al., 1971), negative charge at the membrane's external surface, thereby altering the surface potential. This in turn alters the field perceived by the voltage-sensing apparatus of the channels, wihout necessarily altering the measured membrane potential. Later experiments with Zn^{2+} (Gilly and Armstrong, 1982a, b) and with H^+ (Shrager, 1974; Schauf, 1983) suggested an alternative hypothesis, that divalent cations and protons interact directly with the charged structures that control the channel gates.

Divalent cations also permeate Na and K channels, and in the process may impede current flow. Blocking has clearly been demonstrated for Ca ions acting on Na channels (Taylor et al., 1976; Yamamoto et al., 1984) and for Ba and Sr ions acting on K channels (Eaton and Brodwick, 1980; Armstrong and Taylor, 1980; Armstrong et al., 1982). In addition, there is good evidence that Ca can carry significant current through K channels (Inoue, 1980, 1981).

Of particular interest here is evidence that K channels can close when occupied by a Ba ion. The closed Ba-occupied state is particularly stable, and for this reason recovery from Ba block requires several minutes: Ba occupancy makes reopening of the channel less likely (Armstrong et al., 1982). The stability of the Ba–closed channel complex suggests that the channel contains negative charge in its wall. It also suggests that a K channel normally closes with a divalent cation in it, albeit one that binds less tightly than Ba. It seems clear that there must be a preferred state of occupancy for a closed channel, monovalent- or divalent-occupied, or empty, and that the energy of the channel in the preferred state is lower than in the others. The fact that K channels can close very securely when they contain a Ba ion strongly suggests that the divalent-occupied state is the preferred one. This leads one to suspect that permeant external monovalent cations slow closing not because they occupy a channel that must be empty to close (as previously suggested by Swenson and Armstrong, 1981), but because they prevent or delay the occupancy by a divalent cation that is essential for rapid and secure closing. In physiological solutions, the obvious candidates for occupying the closed channel are Ca and Mg, the dominant divalent cations in squid extracellular fluid. Thus, one might suggest that the voltage-dependent gating machinery of the channel closes the channel gate, but the presence of an appropriate divalent cation in the channel is a necessary part of the process, and latches the channel in closed conformation.

The experiments reported here were initiated to test these possibilities regarding Ca action. We conclude that Ca has more than one site of action on K channels, and we provide evidence that a K channel normally closes with a Ca ion in it. Monovalent cations compete with Ca for a site in the channel, and slow the closing process (Matteson and Swenson, 1986) by preventing or delaying Ca occupancy of the site. The effects of Ca on closing kinetics and the instantaneous

I-V curve are well fit by a model that invokes these ideas of monovalent-divalent competition.

A preliminary report of these results has appeared (Armstrong and Matteson, 1984).

METHODS

Experiments were performed on cleaned segments of giant axon from the squid *Loligo pealei*, obtained at the Marine Biological Laboratory, Woods Hole, MA. The axons were internally perfused and voltage-clamped using standard techniques for this laboratory (Bezanilla and Armstrong, 1977). Experiments were controlled by a PDP 11/23 computer, which communicated with the clamp electronics via an interface designed by the authors in collaboration with Dr. Richard Bookman. The interface controls sampling and pulsing, and provides for data display. The computer-interface combination made the performance of the experiments very rapid, so there was virtually no rundown of the axons during the experiments. The computer automatically subtracted linear leakage and capacitance currents from the records, using a P/4 procedure (Bezanilla and Armstrong, 1977).

TABLE I

External Solutions

Name	Ca	Na	K	Rb	Tris	Cl
			mmol/liter			
20 Ca	20	485	—	—	10	525
100 Ca	100	365	—	—	10	525
120 Ca	120	335	—	—	10	525
150 Ca	150	290	—	—	10	525
20 Ca, 75 Rb	20	410	—	75	10	525
100 Ca, 75 Rb	100	290	—	75	10	525
20 Ca, 75 K	20	410	75	—	10	525
100 Ca, 75 K	100	260	75	—	10	525

The external solutions used are given in Table I. The internal solution (called 275 K) contained 275 mM K^+, 50 mM F^+, 225 mM glutamate, 10 mM Tris-Cl, and 400 mM sucrose. All solutions were adjusted to pH 7.0–7.1. In the text, solutions are designated external//internal. The Ca concentration was limited to the range between 20 and 150 mM, which are the approximate limits for maintaining axons in good condition. All experiments were performed at 8°C.

RESULTS

Calcium and K Channel Opening Kinetics

Increasing the external Ca concentration slows K channel opening, as illustrated in Fig. 1 A. Ca effects on K channels (see below) are not equivalent to a simple change of membrane voltage. Nonetheless, for purposes of quantitation, it is convenient to match the kinetics of a K current trace in 100 mM Ca to a 20 mM Ca trace at lower voltage. In Fig. 1 B, the trace at +30 mV in 20 mM Ca has almost exactly the same time course as the +60 mV, 100 mM Ca trace. (The 20 mM Ca trace has been scaled as described in the figure legend.) Kinetically,
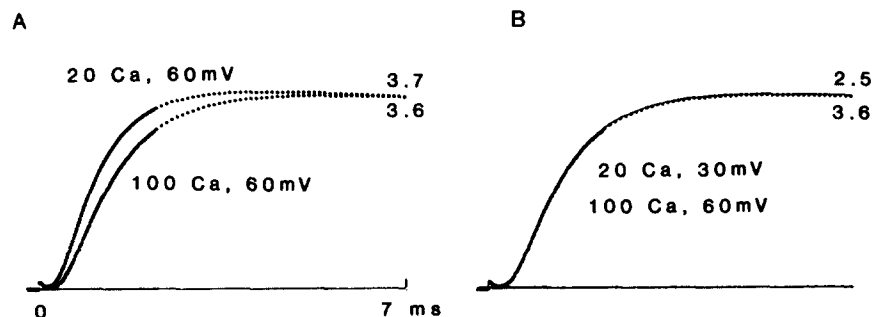
A   B

FIGURE 1.   High Ca slows K channel opening. (A) K current (I_K) recorded at 60 mV in 20 and 100 Ca. The 100 Ca trace has been scaled up by a factor of 1.03. Final current amplitudes were 3.7 (20 Ca) and 3.6 (100 Ca) mA/cm^2. (B) Two almost-superimposed traces are plotted, recorded at 60 mV in 100 Ca and 30 mV in 20 Ca. Final amplitudes were 2.5 (20 Ca) and 3.6 (100 Ca) mA/cm^2. Experiment SE212C.CAR. Solutions: 20 or 100 Ca//275 K. Temperature, 8°C. HP, −70 mV.

raising Ca from 20 to 100 mM has about the same effect as decreasing the depolarizing step from +60 to +30 mV.

The voltage shift equivalent to a Ca change is almost the same for any large depolarization. For example, if the comparison is made using the +20-mV trace in 100 mM Ca, the best-matching trace in 20 mM Ca is for a depolarization to −5 mV, a displacement of 25 mV, which is only slightly smaller than the 30-mV displacement noted above.

The effect of Ca^{2+} is extremely reproducible, as can be seen in Table II, which gives ΔV_{eq}, the voltage change equivalent to increasing Ca from 20 to 90–150 mM, for all our experiments on opening kinetics.

TABLE II

Voltage Changes That Have the Same Effect on Opening Kinetics
as the Stated Ca Concentration Increases

Experiment	Low Ca	High Ca	ΔV_{eq}
	mM	mM	mV
AU302R	20	90	−30
SE082R	20	100	−25
SE212C	20	100	−25
SE212R	20	100	−30
MA163C	20	100	−20
MA163D	20	100	−23
MA173C	20	100	−25
MA183C	20	100	−25
MA193C	20	100	−25
JN013C	20	100	−25
AU243C	20	150	−30
AU253C	20	120	−30
AU303C	20	100	−25

Effect of Ca on Closing Kinetics Is Small After Short Activating Pulses, But Increases with Step Length

Closing kinetics were studied using the double-pulse procedure described by Matteson and Swenson (1986). The fraction of still-open channels is plotted as a function of the closing interval in Fig. 2, for 20 and 100 mM Ca at −70 and −90 mV. The closing curves at the two concentrations almost superimpose at both voltages: under these conditions, raising Ca had only a small effect on closing
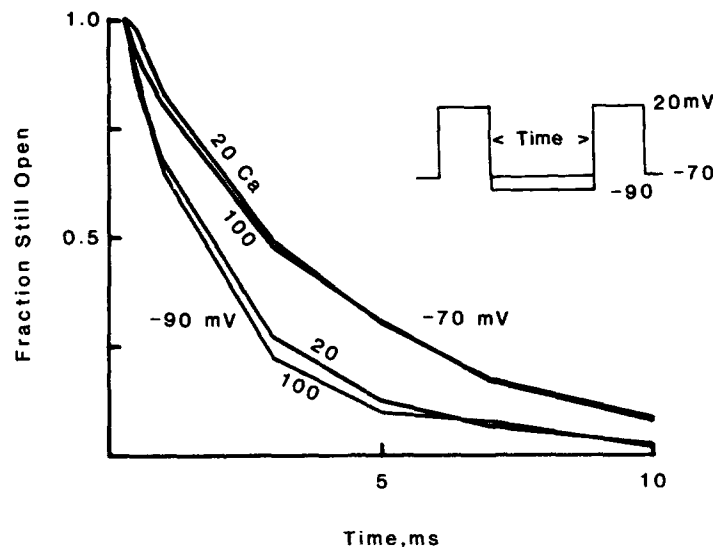

FIGURE 2.   Ca has little effect on K channel closing kinetics after short activating pulses. Channels were activated by a 1.5-ms step to 20 mV. V_m was then returned to −70 or −90 mV for the time given on the abscissa. The fraction of K channels still open was assayed by measuring I_K shortly after applying a second step to 20 mV. The 20 Ca curve is the average of two determinations, one before and one after exposure to 100 Ca. Experiment MA163C.CAK. Solutions: 20 or 100 Ca// 275 K. Temperature, 8°C. HP, −70 mV.

kinetics. For purposes of rough quantitation, single exponentials were fit to the closing curves at several voltages, and the time constants are plotted semilogarithmically as a function of V_m in Fig. 3. To a reasonable approximation, the time constant varies exponentially as a function of V_m. The curves for 20 and 100 Ca almost superimpose, which indicates that in this experiment, raising Ca altered the closing kinetics only slightly. The same change of Ca concentration shifted the opening kinetics by 20 mV in this fiber.

The results from all of our experiments are compiled in Table III, which gives the time constant of channel closing in high and low Ca. The table also gives ΔV_{eq}, the voltage change equivalent to the Ca increase. To obtain ΔV_{eq}, the time constant was measured at two or more voltages in each experiment, and the voltage dependence of the time constant was determined. In general, the slope

was e-fold for a 25–30-mV change of V_m. For each table entry, ΔV_{eq} was determined twice in 20 Ca, before and after 100 Ca, and the before and after results were averaged. Raising Ca from 20 to 100 mM had the same effect as hyperpolarizing by ~5 mV, which is much smaller than the effect on opening kinetics.

After activating steps of longer duration, 8 or 10 ms, the closing of K channels was slower than after short ones, as shown in Table III. In experiment SE212C, for example, the time constant of closing was 3.3 ms after a 1.5-ms step, and 4.0
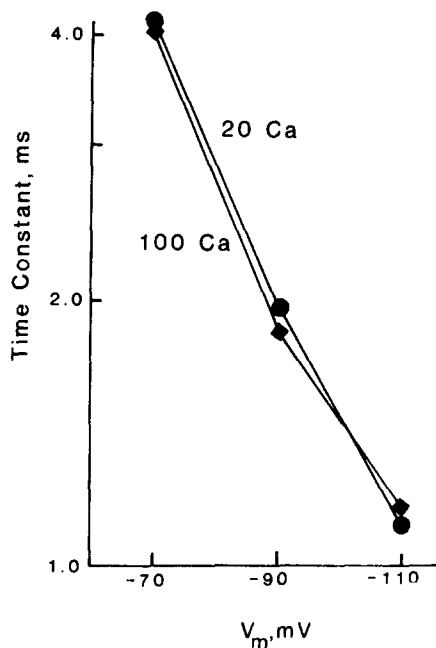

FIGURE 3.   Time constant of K channel closing as a function of V_m in 20 and 100 Ca. The effect of raising the Ca concentration is quite small, and, from −70 to −90 mV, is equivalent to adding −3 mV to the membrane potential. Experiment MA163C.CAK. Solutions: 20 or 100 Ca//275 K. Temperature, 8°C. HP, −70 mV.

after 8 ms in 20 mM Ca. Raising external K slowed channel closing, as shown in the accompanying paper (Matteson and Swenson, 1986) and below. During long steps, the K ion concentration in the Schwann cell space can rise to 50 or 100 mM, as judged from the reversal potential, and the slower closing after long steps is probably a result of such a rise (see below).

The acceleration of closing kinetics by high Ca is much more pronounced with longer steps, as can be seen in Table III. In experiment SE212C, after an 8-ms step, the time constant was decreased from 4 to 2.8 ms on raising Ca, vs. 3.3–2.9 ms for the short step. From these numbers, one would say that K accumulation during a long depolarization tends to slow closing, and increased Ca tends to restore it to normal speed. Thus, in Table III, the closing time constant was not much affected by the step duration in high Ca. The effect of Ca on closing

after long steps is also more prominent when one considers the equivalent voltage change, ΔV_{eq}, which was -3.5 mV after the short step and -9.3 mV after the long one. (A small part of this acceleration may be due to the fact that Ca slightly depresses I_K and slows its time course, thus slightly reducing K accumulation. This can be shown to be a small effect.)

Ca and the g_K-V Curve

In three experiments, the g_K-V curve was determined in 20 and 100 mM Ca. In order to minimize the effects of K accumulation, the method for determining g_K

TABLE III

Voltage Changes That Have the Same Effect on Closing Kinetics as the Stated Ca Concentration Increases

Experiment	Solution	τ, step length	ΔV_{eq}	τ, step length	ΔV_{eq}
		ms	*mV*	*ms*	*mV*
SE212C	20 Ca	3.3, 1.5		4.0, 8	
	100 Ca	2.9, 1.5	−3.5	2.8, 8	−9.3
MA163C	20 Ca	4.1, 1.2			
	100 Ca	4.0, 1.2	−0.7		
MA163D	20 Ca	3.2, 2			
	100 Ca	2.5, 2	−6.0		
MA173C	20 Ca	2.9, 1.2			
	100 Ca	2.4, 1.2	−6.2		
MA183C	20 Ca	4.5, 1.2			
	100 Ca	3.6, 1.2	−5.3		
MA193C	20 Ca	2.9, 1		3.1, 3	
	100 Ca	2.4, 1	−5.0	2.4, 3	−8.5
	20 Ca	3.0, 2		3.6, 10	
	100 Ca	2.4, 2	−7.1	2.7, 10	−10.7
JN013C	20 Ca	2.0, 1		2.5, 3	
	100 Ca	1.9, 1	−2.2	2.3, 3	−2.8
	20 Ca	2.3, 2		2.8, 10	
	100 Ca	2.2, 2	−2.0	2.3, 10	−6.0

was somewhat complex. g_K was calculated from $\Delta I/\Delta V$, where I was measured at 0 and 50 mV. Over this voltage range, g_K is not very sensitive to the external K concentration. Each g_K measurement required two experimental traces. In one, the voltage was stepped from the holding potential to the activating voltage for which g_K was to be determined, and held there long enough to reach a steady state for activation (14 ms for small depolarizations, 6 ms for large ones). A second step then took V_m to 0 mV. The other trace was the same, except that V_m in the second step was 50 mV. The two traces thus gave current at two voltages after identical activating steps, which made possible the calculation of g_K.

The resulting g_K curves are plotted in Fig. 4, with a before and after curve in 20 mM Ca. The g_K-V relation is sigmoid and similar in shape in the two solutions, but is shifted along the voltage axis by ~12 mV. The average value for the three experiments, measured at the half-point of the activation curves, was 10 mV.

Experiments with K^+ or Rb^+ Outside

In a previous section, it was shown that closing kinetics vary with step length. This dependence was attributed to K accumulation in the periaxonal space during long activating pulses, but could also arise from an inherent property of the gating machinery. To settle this question, we performed the experiments described in the next sections, in which K^+ or Rb^+ was added to the external medium and was therefore present at significant concentration even after a short step. For uniformity, we first present the effects of these cations on opening kinetics.
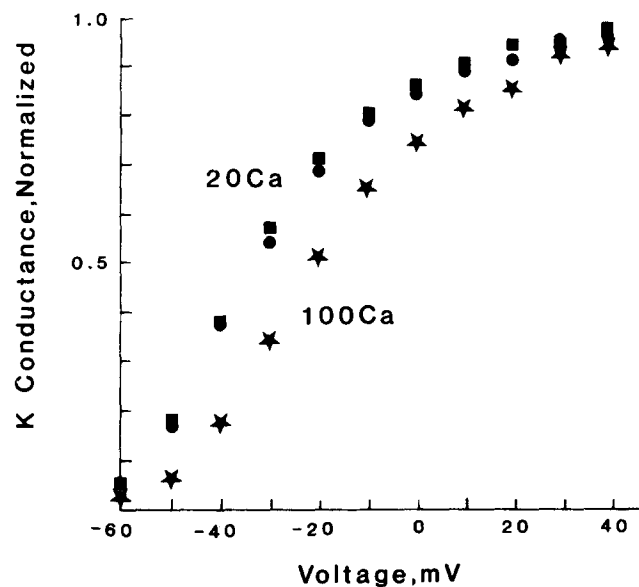

FIGURE 4. Raising Ca shifts the conductance-voltage curve to the right on the voltage axis. The shift is about half as big as for opening kinetics, but larger than for closing kinetics. Experiment AU303C.CAR. Solutions: 20 (■, before; ●, after) or 100 (★) Ca//275 K. Temperature, 8°C. HP, −70 mV.

K^+ and Rb^+ do not affect opening kinetics. Opening kinetics at two Ca concentrations with and without external K^+ are shown in Fig. 5, *A* and *B*. K did not affect opening kinetics at either Ca concentration (cf. Matteson and Swenson, 1986). It is thus clear that K^+ does not alter Ca's ability to slow opening kinetics. Similar results were obtained for external Rb.

Ca has a strong effect on closing in Rb^+ or K^+. Ca had little effect on closing kinetics after short steps (above), but when Rb was present, raising Ca from 20 to 100 mM sped the closing of channels, even after short pulses (Fig. 6, curves labeled 20 Ca, 75 Rb and 100 Ca, 75 Rb). Ca thus antagonizes the slowing caused by Rb (Matteson and Swenson, 1986), even though it has only a small effect in the absence of Rb (Fig. 6, curves labeled 20 Ca and 100 Ca; Table III). A strong effect of Ca on closing kinetics was also seen with K added to the external medium.
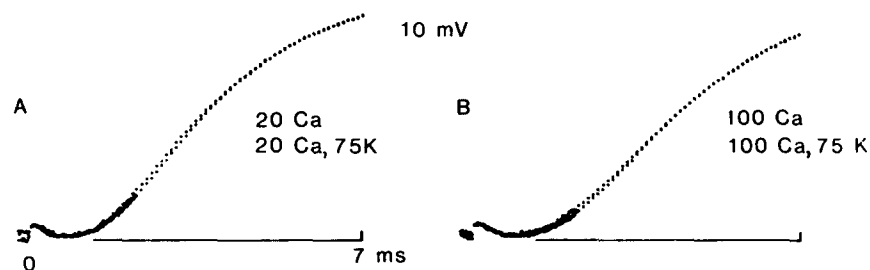
FIGURE 5.   K added externally does not alter channel opening kinetics. Currents are for steps from −110 to 20 mV in the solutions indicated. Experiment MA163C.CAK. Solutions: 20 Ca or 20 Ca, 75 K or 100 Ca or 100 Ca, 75 K//275 K. Temperature, 8°C. HP, −70 mV.

As an empirical tool for quantifying the initial closing rate, an exponential was fit to the first few points of each curve in Fig. 6, and to a closing curve determined at one other voltage. ΔV_{eq}, the voltage displacement that is equivalent to raising Ca, was determined as described above and is given in Table IV for a number of experiments in K^+ or Rb^+. Data from the same experiments but in artificial seawater (ASW) are given in Table III. Examination of the tables shows two interesting points. (a) After short steps in 75 K, ΔV_{eq} was much larger in magnitude than in K-free ASW. Also, ΔV_{eq} was about the same for short and for long steps in high K^+. We conclude that in ASW (no K), accumulation of K^+ is an element in the pronounced acceleration of closing kinetics by Ca after long steps. The accumulated K^+ slows closing, and Ca antagonizes this effect. (b)
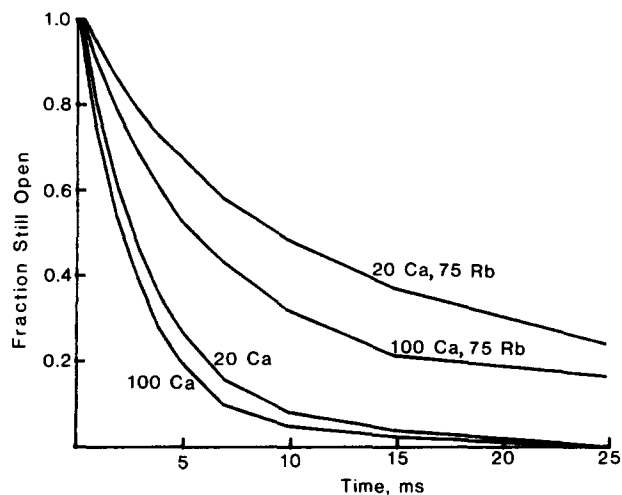

FIGURE 6.   Ca speeds channel closing in the presence of Rb. Channels were activated by a 1.5-ms pulse to 20 mV, and closing kinetics were determined (at −70 mV) by the procedure of Fig. 2. The effect of raising calcium on closing kinetics is much stronger in the presence of Rb. Experiment SE212C.CAR. Solutions: 20 Ca or 20 Ca, 75 Rb, or 100 Ca or 100 Ca, 75 Rb//275 K. Temperature, 8°C. HP, −70 mV.

TABLE IV

Ca-Voltage Equivalence in K or Rb

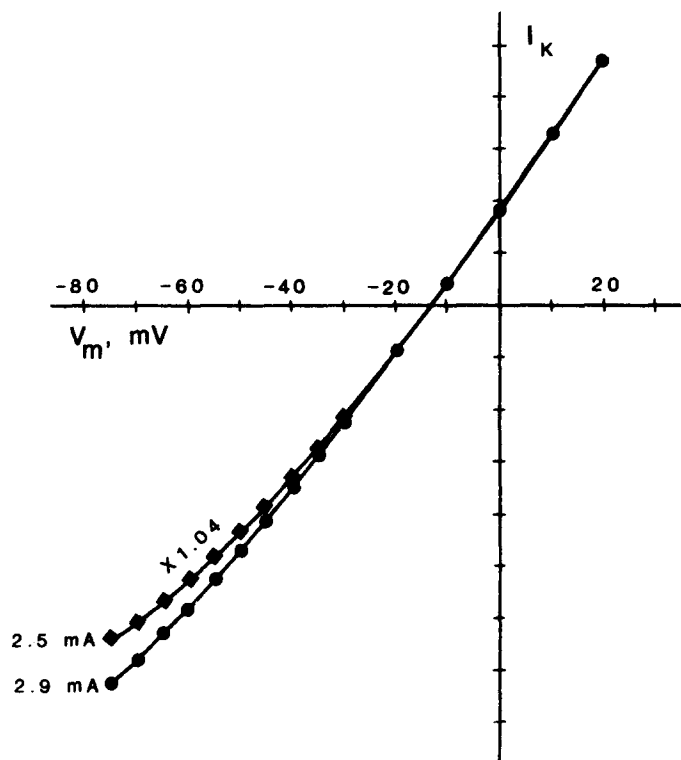| Experiment	Solution	τ, step length	ΔV_{eq}	τ, step length	ΔV_{eq}
		ms	mV	ms	mV
SE212C	20 Ca, 75 Rb	11.5, 1.5		9.6, 8	
	100 Ca, 75 Rb	6.8, 1.5	−10.3	5.7, 8	−11.9
MA163 C	20 Ca, 75 K	8.2, 1.2			
	100 Ca, 75 K	5.4, 1.2	−12.3		
MA163D	20 Ca, 75 K	5.0, 1.2			
	100 Ca, 75 K	2.0, 2.0	−29.4		
MA173C	20 Ca, 75 Rb	13.0, 1.2			
	100 Ca, 75 Rb	8.8, 1.2	−9		
MA183C	20 Ca, 75 Rb	13.0, 1.2			
	100 Ca, 75 Rb	7.0, 1.2	−12.4		

FIGURE 7.   Instantaneous I_K-V_m curves in two Ca concentrations. The channels were activated by a 2- (20 Ca) or 3- (100 Ca) ms step to 40 mV. Voltage was then stepped to the value given on the abscissa, and the instantaneous current was measured 40 μs later. The 100 Ca trace has been scaled up by a factor of 1.04. At higher Ca, there is a small, selective depression of current at negative V_m. Experiment AU253C.CAK. Solutions: 20 Ca, 75 K or 120 Ca, 75 K//275 K. Temperature, 8°C. HP, −90 mV.

External Rb (75 mM) slowed the initial closing by a factor of ~3 or 4 in 20 mM Ca^{2+}. Increasing Ca in the presence of Rb accelerated closing by an average factor of 1.7.

In general, Ca speeds channel closing if it has been slowed by addition or accumulation of K or Rb in the external medium. This suggests that, during closing, Ca competes for occupancy of the pore with permeant monovalent cations in the external medium, or in some way impedes the entry of monovalent cations into the pore.

Ca Has Little Effect on K^+ Entry into the Channel

To test the possibility that high Ca impedes the inward movement of K^+, we examined the instantaneous I_K-V curve in high and low Ca. The fiber was bathed in 75 mM K with 20 or 100 mM Ca. The K channels were activated by depolarization, and after 2 ms, voltage was stepped to a new value. Current was measured immediately after the second step. Because the shape of the instantaneous I-V curve depends on the external K concentration, the duration of the step in 100 Ca was slightly longer than in 20 Ca, to allow the same degree of K accumulation, as judged by the reversal potential. Instantaneous curves at the two Ca^{2+} concentrations are shown in Fig. 7. The 100 Ca curve has been scaled by a factor of 1.04 to facilitate comparison of shapes. Ca had a slight inhibitory action on current flow at negative voltages, which was reduced by a factor of 0.89 at −70 mV. The inhibition was small, and we conclude that Ca does not impede the entry of K^+ into the channels sufficiently to explain its twofold effect on closing in high K (Table IV).

DISCUSSION

Ca slows K channel opening, independently of the external monovalent cation composition (Figs. 1 and 5). In contrast, Ca affects channel closing only slightly when there is no K or Rb in the external medium (Fig. 2 and Table III). This asymmetric effect, slowed opening with almost unchanged closing, is the subject of the first section of the Discussion. It is attributed to a direct interaction between Ca and unpaired gating charge, which we postulate to be negative and located at the outer membrane surface at rest.

The addition of K^+ or Rb^+ externally does not affect channel opening, but K channels close more slowly (Matteson and Swenson, 1986), and Ca^{2+} in the presence of these ions acquires a strong effect on closing kinetics. This second, and we think separate, action of Ca is the subject of the second part of the Discussion. A model is presented, based on Ca-K competition for the occupancy of closing channels, that accounts for all of our major findings on channel closing.

Ca^{2+} in the Absence of External Permeant Monovalent Cations

An asymmetric effect on opening and closing has previously been observed with external Zn ion acting on K channels (Gilly and Armstrong, 1982a). A direct attraction between Zn^{2+} and gating charge was proposed to account for this effect. In the transitions that open a K channel, the equivalent of approximately six electronic charges are transferred across the membrane. Because the interior of the membrane is a region of low dielectric constant and is hostile to the

presence of free charge, the gating charge (or the unpaired part of it) probably resides most of the time at one of the membrane surfaces. At the surface, it would interact with ions in the medium and attract counterions of the opposite sign. The attraction would be particularly strong for di- or multivalent counterions. This direct interaction hypothesis predicts that an external Ca increase would slow opening kinetics, shift the g_K-V curve, and not affect closing kinetics, all in agreement with the data (for more details, see Gilly and Armstrong, 1982a).
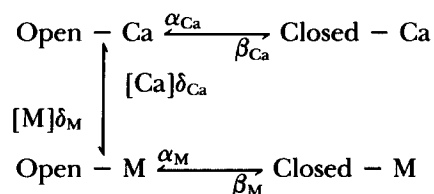
Ca^{2+} in the Presence of Permeant Monovalent Cations Externally

The following arguments suggest that during channel closing, Ca^{2+} competes with permeant monovalents for channel occupancy, and that channels containing monovalent cations rather than Ca^{2+} close slowly. First, the slowing action of the monovalents is exerted within the pore. The evidence is that the selectivity requirements for monovalent cations that slow channel closing are the same as for entry in the pore (Matteson and Swenson, 1986). Further, slowing is correlated with an ion's tendency to bind in the pore, as judged by the shape of the instantaneous I-V curve (Matteson and Swenson, 1986). Thus, slowing is most pronounced with Rb^+, which enters the pore readily and binds relatively tightly compared with other permeant monovalent cations.

In antagonizing the slowing action, Ca^{2+} could either prevent the monovalent cations from entering the pore, by, e.g., binding near the channel mouth, or it could compete for pore occupancy. In the latter case, monovalents would slow closing by decreasing the likelihood of occupancy by a calcium ion. To decide between these possibilities, we note that Ca^{2+} alters the shape of the instantaneous I-V curve only slightly, reducing I_K at -70 mV by a factor of 0.89 (Fig. 7). Its influence on K^+ entry in the pore is thus detectable, but is not large enough to explain its twofold or more speeding of closing (Table IV). By exclusion, then, direct competition for pore occupancy seems the more likely mechanism.

To account for the data, we make the following proposals. (a) During closing, Ca^{2+} competes for channel occupancy. Evidence that Ca can enter K channels is cited in the Introduction. The slight suppression of inward current at negative voltages (Fig. 7) is also compatible with Ca^{2+} entry. (b) In the absence of other external permeant cations, Ca^{2+} occupies all channels without competition during closing, even at low Ca^{2+} concentration. Thus, Ca has little effect on closing kinetics unless K or Rb is present. Ca-occupied closed channels are securely "latched." (c) Pores close slowly when occupied by a monovalent cation rather than Ca^{2+}, and tend to reopen quickly.

To test these propositions, we devised a simplified gating model that accounts for the effects of Ca on K channel closing almost quantitatively.

$$\text{Open} - \text{Ca} \underset{\beta_{Ca}}{\overset{\alpha_{Ca}}{\longrightarrow}} \text{Closed} - \text{Ca}$$

$$[M]\delta_M \uparrow \quad \downarrow [Ca]\delta_{Ca}$$

$$\text{Open} - \text{M} \underset{\beta_M}{\overset{\alpha_M}{\longrightarrow}} \text{Closed} - \text{M}$$

The model postulates that open channels are occupied by either monovalent ions (Open $-$ M) or calcium ions (Open $-$ Ca), and the channels can close when occupied by either (although, as it turns out, not very well when occupied by K^+ or Rb^+). A state might have been included for unoccupied channels, both open and closed, but for our purposes it was adequate to assume that the channel is always occupied by Ca^{2+} or a monovalent ion. It is known that channels have a number of closed conformations, but these also proved unnecessary. Finally, there are probably several ion binding sites in each channel, but we assume here that channels are occupied by a single K, Rb, or Ca ion.

In the model, M-occupied channels close at a rate β_M and open at a rate α_M. Ca-occupied channels close at rate β_{Ca} and open at rate α_{Ca}. M^+ and Ca^{2+} displace each other from open channels at rates proportional to their concentrations. For Ca^{2+} displacing M^+, this rate is $[Ca]\delta_{Ca}$, and for M^+ displacing Ca^{2+}, the rate is $[M]\delta_M$.

By adjusting the rate constants, it was possible to generate curves that closely simulate the closing of K channels with 20 and 100 mM Ca, in the presence and absence of Rb. At the beginning of the calculation, all of the channels are assumed to be occupied by M ions, and Ca occupation is negligible because the field in the membrane during large depolarizations makes it difficult for Ca ions to enter the pores. For similar reasons, it was assumed that at -70 mV, few ions from inside move into the pores. Both of these occupancy arguments are rather crude and could be refined in a more sophisticated model.

The predictions of the model are shown in Fig. 8, and the rate constants are given in the figure legend. In the absence of Rb, increasing Ca from 20 to 100 mM has only a small influence on closing kinetics (cf. Figs. 2 and 6), because there are no other external ions to compete with calcium for the pores. In 20 Ca, 75 Rb, Rb ions compete very effectively for the pores, many of which remain open and Rb-occupied for the 25-ms duration of the calculation. Rb^+-occupied channels close only sluggishly (β_M is 0.08, compared with 0.31 for a Ca^{2+}-occupied channel), and reopen fairly readily (α_M is 0.04 ms^{-1}). Thus, many of the channels remain Rb^+-occupied for a substantial time before they capture a Ca ion and close securely. Raising the Ca concentration from 20 to 100 mM increases the rate of capture of Ca ions, and thus hastens closing (curve 100 Ca, 75 Rb). All of the curves in Fig. 8 have a strong resemblance to the experimental curves in Fig. 6.

The model predicts that at -70 mV, current magnitude is decreased by a factor of 0.88 when Ca is raised from 20 to 100 mM. (Although all channels are open at the beginning of the theoretical voltage step, some become Ca-occupied in the 40 μs required to make the experimental measurement, and this is reflected in the calculation.) This is in excellent agreement with the data of Fig. 7, where current at -70 mV was decreased by a factor of 0.89.

Tight Closing of Ba^{2+}-occupied K Channels

Ba-occupied channels close very securely and have a low probability of opening even during strong depolarization (Armstrong et al., 1982; Introduction). In terms of the preceding paragraph, Ba latches the channel closed in the same way

that Ca does, but more strongly. Tighter latching with Ba^{2+} may occur for reasons similar to those invoked to explain K-Na selectivity (Mullins, 1959; Bezanilla and Armstrong, 1972; Hille, 1973). One may postulate that coulombic attraction makes the channel contract dynamically about an ion that is occupying it or passing through. Because of conformational constraints, the channel has only a limited ability to accomodate to the ion, and there is a potential energy minimum for ions with radius near 1.33 Å (the crystal radius of K^+). Ba^{2+} (crystal radius, 1.34 Å) thus is a good fit, and the Ba–channel complex is relatively stable, both because of the good fit and the large coulombic forces generated by Ba's
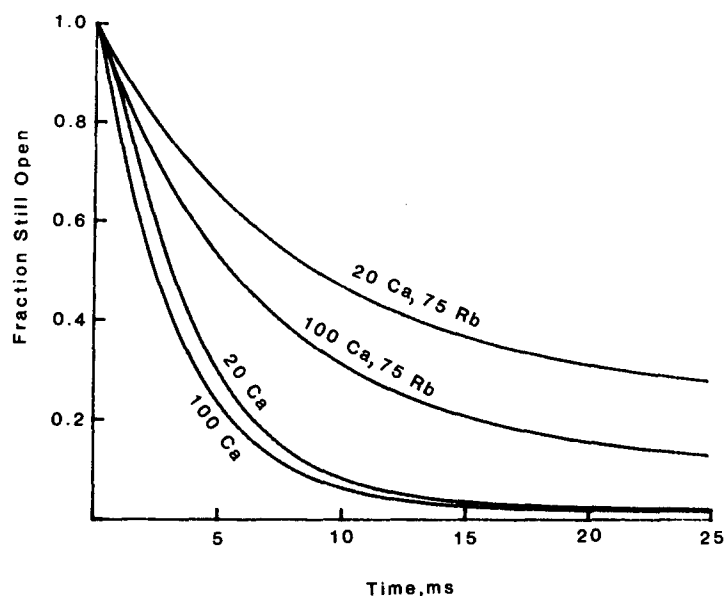

FIGURE 8. Closing of K channels, predicted by the model in the text. The rate constants for generating the curves were as follows. $\alpha_{Ca} = 0.005$; $\beta_{Ca} = 0.31$; $\alpha_M = 0.04$; $\beta_M = 0.08$; $\delta_{Ca} = 0.05$; $\delta_M = 0.20$. The calculated curves closely match Fig. 6.

double charge. Ca^{2+} (0.99 Å) is a poorer fit, making the Ca–closed channel complex higher in energy and sufficiently unstable that the channel can open promptly on depolarization. A K^+-occupied closed channel is still less stable, because of reduced coulombic attraction.

Comparison with the Literature

Our results agree with those of Frankenhaeuser and Hodgkin (1957), who found that raising Ca slows the rise of I_K, and that a fivefold change in Ca shifts the g_K-V relation by 10–15 mV. Gilbert and Ehrenstein (1969) studied the steady state K current of squid axons in high external K and found that the midpoint of the I-V curve shifted by 13 mV when Ca was increased from 10 to 160 mM (in the presence of 50 mM Mg). Hille (1968) saw no change of opening kinetics and a small g_K-V shift in myelinated fibers, working with no external K. On the other

hand, Mozhayeva and Naumov (1970), with myelinated fibers in 50 mM external K, observed that a 10-fold Ca change shifted the g_K-V curve by 15 mV. In light of our results, which show that the action of Ca changes when there is external K, it may be significant that these authors found a larger shift than did Hille in the absence of external K. Our prediction is that the g_K-V shift should be larger in the presence of K. In summary, three of four groups have reported a g_K-V shift of ~10–15 mV per 10-fold Ca change, and slowing of the rise of I_K by Ca has been reported in squid axon but not in myelinated fibers. We are aware of no report prior to ours regarding the effects of Ca on closing kinetics.

Frankenhaeuser and Hodgkin (1957) have pointed out the difficulties of working on squid axons in zero Ca. Nonetheless, they and Gilbert and Ehrenstein (1969) provide evidence that K channels retain voltage-dependent gating in the absence of external Ca and Mg. In both reports, there is an indication that the K channels do not close securely without Ca. Gilbert and Ehrenstein, for example, found that "relative conductance" in the absence of Ca had a minimum value of 0.3, while in the presence of Ca, it declined to 0.1. This seems entirely consistent with the latching hypothesis.

Conclusions

In conclusion, we postulate that secure closing of a K channel is accomplished by the gating machinery of the channel in cooperation with Ca ion. It may seem strange that the fundamental action of Ca^{2+} on K channels that is postulated here can have escaped attention so long, and, of course, it did not do so entirely. Frankenhaeuser and Hodgkin (1957) considered a similar hypothesis for Na channels. They found it inadequate as a complete explanation of gating, but noted that "the general possibility that depolarization acts by removing Ca^{2+} from combination with a sodium carrier seems sufficiently plausible to keep in mind." The idea that Ca^{2+} might occupy and help close channels as a part of the gating process subsequently fell into disfavor. One reason is that the action of Ca on K channels is not very large, particularly in the absence of external K^+ or Rb^+. Further, it seems clear from the literature that, in squid axons, channels can open and close to some degree in the absence of Ca, and the high "leakage" current seen in zero Ca (Frankenhaeuser and Hodgkin, 1957) makes it difficult to analyze these experiments closely. It may be that opening of K (and Na?) channels contributes to the high conductance of the membrane in these circumstances. Clearly such questions could profitably be pursued at the single channel level.

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