INTERACTION OF BARIUM IONS WITH POTASSIUM CHANNELS IN SQUID GIANT AXONS

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ABSTRACT Blocking of potassium channels by internally and externally applied barium ions has been studied in squid giant axons. Internal Ba (3–5 mM) causes rapid decay or "inactivation" of potassium current (I_K). The kinetics and degree of block are strongly voltage-dependent. Large positive voltages speed blocking and make it more profound. Raising the external potassium concentration (K_o) from 0 to 250 mM has the opposite effect: block is made slower and less severe. In contrast, for positive voltages block by the tetraethylammonium derivative 3-phenylpropyltriethylammonium ion is almost independent of K_o and voltage. Recovery from block by internal Ba has a rapid phase lasting a few milliseconds and a slow phase lasting ~5 min. Internal Ba causes a "hook" in the I_K tails recorded on repolarizing the fiber in high potassium external medium. External Ba, on the other hand, blocks without much altering I_K time-course. K_D (the dissociation constant) for block by external Ba is a few millimolar, and depends on the internal potassium concentration, the holding potential, and other factors. A reaction scheme for Ba and K channels is presented, postulating that internal and external Ba reach the same point in the channel. Once there, Ba blocks and also stabilizes the closed conformation of the channel. The extreme stability of the Ba channel complex implies the existence of negative charge within the channel.

INTRODUCTION

Experiments of the last 10 years have led to a rather concrete picture of the pathway by which potassium ions cross excitable membranes. This pathway is a pore, pictured in squid giant axons as having a dilatation at either end and a relatively narrow selective portion in the middle (Armstrong, 1975). The inner end of the pore is very unselective, and large enough to accommodate tetraethylammonium (TEA)[1] and its various derivatives. This suggests that the inner mouth is at least 8 Å in diameter. Many other ions are also capable of occupying the inner mouth and blocking K^+ permeation. Access to the inner mouth of the pore is controlled by a voltage-sensitive gate, the K^+ activation gate of the channel. The outer mouth of the channel in myelinated frog fibers is also large enough to accept a TEA ion which blocks it (Hille, 1967; Armstrong and Hille, 1972). However, the outer mouth of K^+ channels in squid axons is quite insensitive to TEA, but can be blocked by Cs ions (Adelman and French, 1978; Bezanilla and Armstrong, 1972).

Selectivity appears to be a function of the narrow central portion of the pore, which is probably ~3 Å in diameter (Hille, 1973; Bezanilla and Armstrong, 1972). The conductance of

[1] *Abbreviations used in this paper:* ϕC3, 3-phenylpropyltriethylammonium bromide; TEA, tetraethylammonium; TTX, tetrodotoxin.

a K^+ pore is probably a few picosiemens (Armstrong, 1966, 1975; Conti et al., 1975; Begenisich and Stevens, 1975). There is evidence that an open K^+ pore usually contains one or more K ions (Hodgkin and Keynes, 1955; Horowicz et al., 1968; Armstrong, 1975; Hille and Schwarz, 1978).

One ion that has not been reconciled with this picture is barium ion, which was shown to block K^+ conductance of muscle fibers when applied externally (Werman and Grundfest, 1961; Sperelakis et al., 1967). In muscle, Ba suppresses not only the "delayed rectifier" K^+ conductance (Hagiwara and Kiddokoro, 1971) but also the inward or anomalous rectifier (Standen and Stanfield, 1978). We became interested in the effects of Ba on squid giant axons after the interesting report (and additional personal communications) by Eaton and Brodwick (1975) regarding the blocking actions of internally applied Ba on K pores. We report here results on the blocking action of both external and internal Ba in squid giant axons. As Eaton and Brodwick have described, internal Ba evidently has access to its blocking site only when the channel gate is open. We find, however, that there are significant differences between block by Ba and by the TEA derivatives (Armstrong, 1971). These differences lead us to believe that the primary blocking site for Ba is deep inside the pore, and that Ba can penetrate into the pore because its crystal radius (1.34 Å) is very similar to that of the K ion (1.33 Å). The combination of Ba with its receptors is evidently extremely stable under some circumstances (presumably when the channel gate is closed), and the complex decays with a time constant of more than 200 s. TEA derivatives, on the other hand, bind relatively loosely and at a different site; they occupy the inner mouth of the pore and cannot penetrate further because of their large diameter.

METHODS

Experiments were performed on thoroughly cleaned internally perfused giant axons at the Marine Biological Laboratory, Woods Hole, Mass. Perfusion was usually initiated by a very brief treatment of the axon interior with pronase. The perfused axons were then voltage clamped using standard technique (see for example, Bezanilla and Armstrong, 1977). Survival in experimental conditions was generally several hours, and for this period the electrical properties of the fibers were almost completely stable. The ionic current signal from the fibers was digitized (10–100 μs/sample rate) and stored on the tape unit of a PDP8 computer.

The compositions of internal and external solutions are given in Table I. 2×10^{-7} M tetrodotoxin was added to the external solution in all experiments save those in which I_{Na} was examined. Ba ion activity in the internal solution was probably lower than the stated concentration because of formation of a weak complex with glutamate (Lumb and Martel, 1953).

In some experiments it was important to know the time required for changing the external solution in the chamber. This was determined by following the time-course of disappearance of I_{Na} on replacing external Na ion with the impermeant cation Tris. Washout was 95% complete in 20 s.

RESULTS

Internal Barium

Normal potassium currents from a tetrodotoxin (TTX)-poisoned fiber in 100 K Tris TTX//275 KFG (external solution//internal solution) are shown in Fig. 1*a*. Membrane voltage (V_m) was held at -70 mV and stepped to the voltage indicated next to each trace, with an interval between steps of ~3 s. Potassium current (I_K) rises during the first few

TABLE I
SOLUTIONS*

Name	K^+	F^-	Glutamate$^-$	PO^-	Trizma 7.0‡	Sucrose
	mM	*mM*	*mM*		*mM*	*mM*
Internal						
138 KFG	138	50	88		15	600
275 KFG	275	50	225		15	420
550 KFG	550	50	500		15	
SISA	417	50	320	30		230

	Trizma 7.0	KCl	$CaCl_2$
	mM	*mM*	*mM*
External			
0 K Tris TTX§	480		50
440 K SW		440	50

20, 50, 150, and 200 m*M* K Tris TTX were appropriate mixtures of these two solutions. Ba was simply added to the external or internal solution to the concentration indicated in the text (in millimolars).
*Osmolality of all solutions was between 950 and 1,050 mosmol/kg. pH was between 7.0 and 7.5.
‡Tris premixed crystals. Sigma Chemical Co. (St. Louis, Mo.).
§TTX, 200 nM.

milliseconds as the activation gates of the K channels open, and then remains approximately constant for the remainder of the step. At −30 mV I_K is inward because of the high external K^+. Current droops a bit at large positive voltages as K^+ accumulates in the Frankenhaeuser-Hodgkin space (Frankenhaeuser and Hodgkin, 1956).

When Ba is added to the internal perfusion medium its effects are hard to detect until voltage exceeds 30–50 mV (Fig. 1 *b*). Above this voltage the current rises normally but then decays, presumably as Ba ions enter and block open pores. The block is very long-lasting, and we found it necessary to wait 300 s between steps, which made the experiments somewhat tedious.

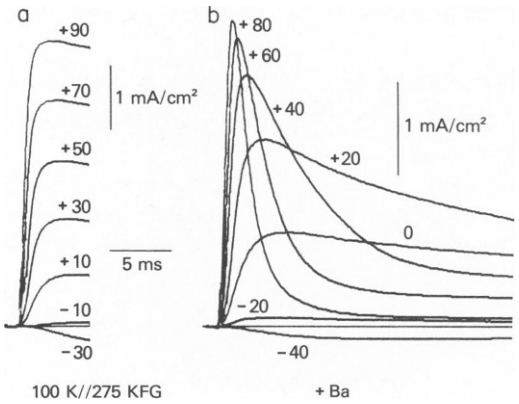

FIGURE 1 I_K and internal Ba^{2+}. (*a*) Potassium currents, recorded in 100 mM K Tris TTX//275 KFG at the voltage indicated. (*b*) Traces were recorded after adding 5 mM Ba^{2+} to the internal medium. Exp. AU317A, 15°C. Holding potential: −70 mV.

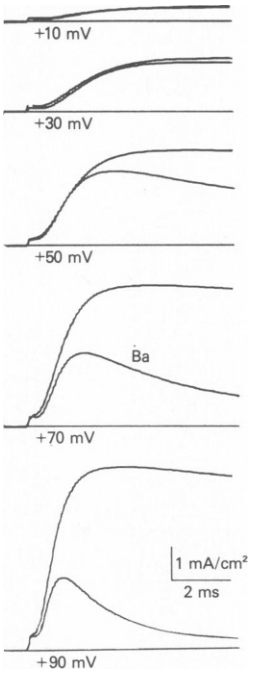

FIGURE 2 The voltage dependence of block by internal Ba. I_K was recorded at the voltages indicated with and without 4 mM internal Ba. Block is evident for voltages positive to +30 mV. 50 mM K Tris TTX//275 KFG + 4 mM Ba. Exp. AU317C, 12°C.

The rate of decay of the current and its final level are strongly voltage-dependent, as can be seen for two different fibers in Figs. 1 and 2. I_K in the presence and absence of internal Ba is superimposed in Fig. 2. Above 50 mV the decay rate of the current increases with voltage, and the final level diminishes. The early time-course of I_K is unaffected in some traces, but at +30, +70, and +90 mV the current in Ba is smaller from the outset of the step. We are not sure whether this is a significant result or simply reflects a failure to wait long enough between steps.

The dependence of the rate and degree of block on V_m was quantified for the fiber of Fig. 1 by assuming that Ba combines with a site S in the pore to block (a more complete model is given in the Discussion):

$$\mathrm{Ba} + S \underset{b_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{Ba}S,$$

for which

$$\tau_{dcy} = \frac{1}{a_1[\mathrm{Ba}] + b_1}; \quad \begin{matrix}\text{fraction blocked at} \\ \text{equilibrium}\end{matrix} = \frac{a[\mathrm{Ba}]}{a_1[\mathrm{Ba}] + b_1}; K_{eq} = \frac{a_1}{b_1},$$

τ_{dcy} is the time constant of decay, and K_{eq} is the equilibrium constant. Fig. 3 gives estimates of $a_1[\mathrm{Ba}]$, b_1 and $K_{eq} \times \mathrm{Ba}$ at several voltages. The lines are least-squares fits to the points, and

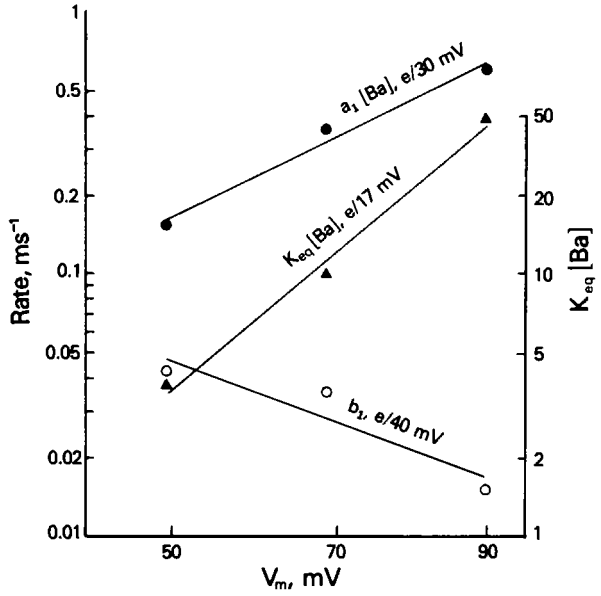

FIGURE 3 Voltage dependence of the equilibrium and rate constants for block by internal Ba. The reaction scheme Ba + S = BaS was assumed. Because of uncertainty about the Ba activity, the forward rate is expressed as a_1[Ba], and K_{eq}[Ba] is plotted rather than K_{eq}. The lines are least-squares fits. The voltage dependence of the three quantities plotted is expressed as the number of millivolts change in V_m that is required for an e-fold change. Same experiment as Fig. 2.

next to each line is its steepness, expressed as the number of millivolts by which V_m must be changed to cause and e-fold increase or decrease of the quantity plotted. The steepness of these curves will be used in the Discussion to estimate how far Ba penetrates into the membrane field.

The voltage dependence of Ba block can be strikingly demonstrated by use of a different pulse protocol. In Fig. 4 the channels were first activated by a 10-ms pulse to 30 mV, sufficient to open most of the K channels but below the voltage range where Ba blocks effectively. The voltage was then changed to 110 mV. In the absence of Ba (Fig. 4 *a*) the current jumps instantaneously as a result of the increased driving force, and then increases slowly as

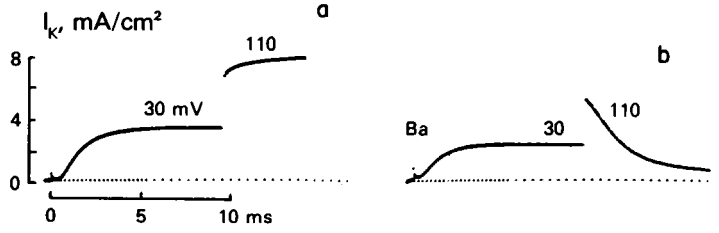

FIGURE 4 The voltage dependence of block by internal Ba^{2+}. V_m was stepped from −70 to +30 mV for 10 ms, and then to 110 mV. In the absence of Ba (*a*), I_K increases following the step to 110 mV. With 4 mM Ba in the internal medium (*b*), there was little effect at +30, but at 110 mV I_K decays almost exponentially to a low value. 100 mM K Tris TTX//275 KFG ± 4 mM Ba. Exp. AU218A, 12°C.

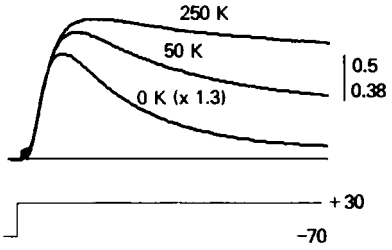

FIGURE 5 External K reduces block by internal Ba^{2+}. I_K in the presence of 5 mM internal Ba was recorded at +30 mV with the three external K concentrations indicated. For ease of comparison, the 0 mM K trace was scaled up by a factor of 1.3. The vertical bar represents 0.5 mA/cm^2 for the upper two traces, 0.38 mA/cm^2 for the lower. 0, 50, or 250 mM K Tris TTX//275 KFG + 5 mM Ba. Exp. AU317C, 12°C. Holding potential: −70 mV.

additional channels open. In the presence of Ba (Fig. 4 *b*) current in the first pulse is not much changed, but the current decays rapidly during the second pulse, as Ba ions block the channels. Other traces (not illustrated) showed that decay rate and the degree of block are voltage-dependent, as has been described.

INTERNAL BA IS ANTAGONIZED BY EXTERNAL K Raising the external K^+ concentration (K_o) slows and diminishes block caused by internal Ba, as illustrated in Fig. 5. The three traces are I_K recorded at +30 mV in 0, 50, and 250 mM K_o. The 0 mM trace has been scaled

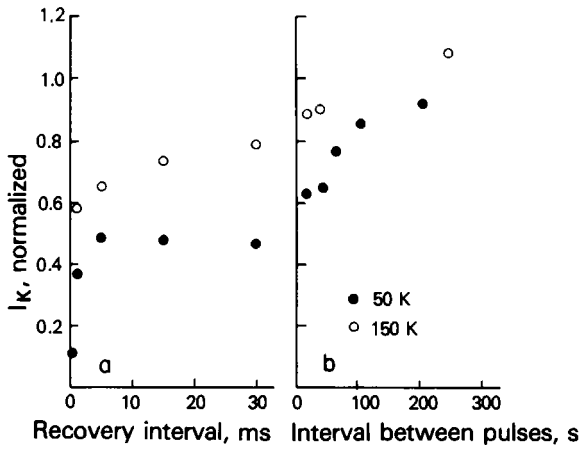

FIGURE 6 Two phases of recovery from block by internal Ba. (*a*) the abcissa is the interval between a 20-ms prepulse (to 90 mV) and a test pulse to the same voltage. The ordinate is I_K measured in the test pulse. There was a 300-s interval between each prepulse test pair. The graph shows the fast phase of recovery from Ba block, which has the same time-course as the I_K tails shown in Fig. 7. Recovery in the fast phase is more complete in 150 than in 50 mM K. (*b*) The slow phase of recovery. I_K was measured during a series of pulses with progressively longer interval between a pulse and its predecessor. This interval is given on the abcissa. The graph gives a rough measure of the time-course of slow recovery, which requires ~300 s for completion. 50 or 150 mM K Tris TTX//275 KFG. Exp. AU267A, 12°C. Holding potential: −60 mV.

up by a factor of 1.3 to facilitate comparison. Both the rate and the final level of block are lessened by raising K_o, which apparently hinders the entry of Ba into the channels.

DISSOCIATION OF BA FROM THE CHANNELS ON REPOLARIZATION Internally applied Ba clears out of the channels in two phases, as shown in Fig. 6. The legend of the figure describes the pulse protocol. The fast phase of recovery (Fig. 6 *a*) is over in a few milliseconds, and has the same time-course as the I_K tails to be described in Fig. 7. The fraction of the channels that are cleared of Ba at the end of the fast phase depends on K_o: ~50% in 50 mM K_o, 70% in 150 mM K_o.

The slow phase of recovery is shown in Fig. 6 *b*. Because of the very long wait required between pulses, this part of the experiment used a different protocol (see figure legend) and is not an ideal measure of recovery time-course. It shows nonetheless that full recovery required ~300 s in 50 mM K_o. The effect of external K ion on the slow phase was not studied carefully, but it seems likely from other experiments that raising K_o makes it faster. In the absence of external K recovery is not complete even after 300 s, and consequently the maximum amplitude of I_K is less in 0 mM K than in 50 or 150 mM K.

Another manifestation of the fast phase of recovery is shown in Fig. 7, which has superimposed traces of current flowing during and after five pulses of varying lengths from −70 to +100 mV and back to −70 mV. At the end of a 2-ms pulse few of the channels are blocked by Ba. The change in driving force on repolarization causes the current to reverse from outward to inward, and the unblocked channels close with normal exponential kinetics. After a 5-ms pulse, the initial amplitude of the inward current is smaller because some of the

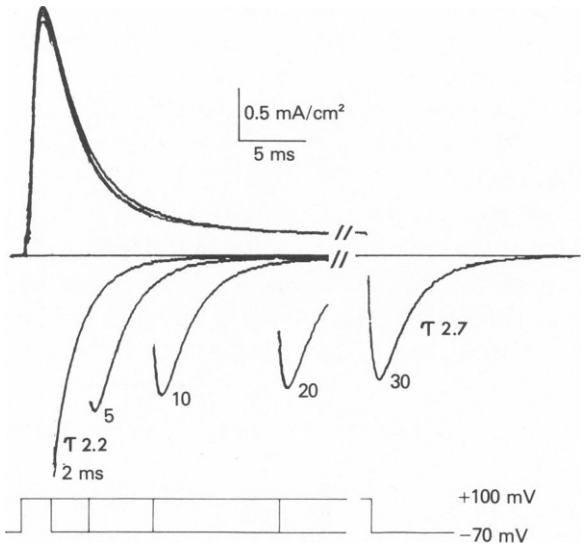

FIGURE 7 Ba rapidly leaves some of the channels on repolarization. After a pulse (to 100 mV) long enough to block most of the channels with Ba, there is a prominent hook in the repolarization tail, as Ba ions come out of their blocking sites. Current magnitude then decays as the K activation gates close. After a short pulse in which few of the channels are blocked, there is no hook. Exp. AU267A, 12°C. Holding potential: −80 mV.

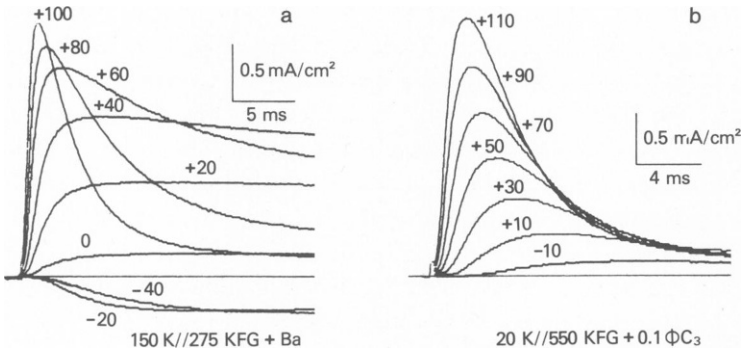

FIGURE 8 Comparison of block by Ba^{2+} and ϕC3. I_K was recorded in the presence of 4 mM internal Ba^{2+} (left) or 0.1 mM ϕC3. The rate and degree of block by Ba^{2+} is much more strongly voltage-dependent than is ϕC3 block. (*a*) 150 mM K Tris TTX//275 KFG + 3 mM Ba. Exp. AU267A, 12°C. Holding potential: −70 mV. (*b*) 20 mM K Tris TTX//550 mM KFG + 0.1 mM ϕC3. Exp. Se097A, 15°C. Holding potential: −70 mV.

channels are Ba blocked, and a hook appears in the tail; i.e., the current magnitude increases for ~0.5 ms before the normal closing process prevails and the current begins to decrease. The hook presumably means that Ba clears rapidly from some of the channels, and this outweighs for a short-time channel closing. As step duration increases, a steadily larger fraction of the channels are blocked by Ba, making the initial amplitude of the tail smaller and the hook more prominent. The hook in Ba was first observed by Brodwick and Eaton (Personal communication).

Figs. 6 and 7 thus show a quick and a slow component of recovery from Ba. There are two possible explanations for these phases. First, there may be two categories of sites within the channel with different dissociation rate constants for Ba. Or the slow phase may mean that the gates of some channels have closed and trapped Ba in its blocking site. These possibilities will be considered in the Discussion.

DIFFERENCES BETWEEN BLOCK BY BA AND BY TEA DERIVATIVES Some of the phenomena reported above resemble the block produced by TEA derivatives (Armstrong, 1971), but there are important differences as well. TEA derivatives combine with open pores, with a forward rate constant κ and dissociation rate constant λ. λ is voltage and K_o-dependent, but κ is almost independent of both factors (Armstrong, 1971). The contrast with Ba is

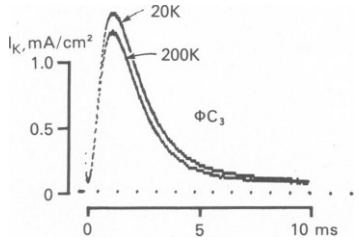

FIGURE 9 The rate of block with ϕC3 is independent of K_o. I_K was recorded at +10 mV in 20 and 200 mM K Tris TTX//138 mM KFG + 0.1 mM ϕC3. Unlike Ba^{2+} (Fig. 5), the rate of block is unaffected by the external potassium concentration. Exp. Se097A, 15°C. Holding potential −70 mV.

illustrated in Fig. 8. From examining the figure it is clear that block with the TEA derivative 3-phenylpropyltriethylammonium bromide (ϕC3) is evident when V_m is only +10 mV, and is not very voltage-dependent above +10 mV. Block by Ba is scarcely detectable even at +40 mV, and increases steeply as voltage is made more positive. Also, in contrast to Ba, the rate of block with ϕC3 is almost independent of V_m.

Other differences are (*a*) unlike Ba (Figs. 1 and 2) the rate of block with ϕC3 is independent of K_o. Fig. 9 illustrates this point for a ϕC3-perfused fiber in 20 and 200 mM K_o. Exponentials fitted to the decay of the current at 110 mV had time constants of 3.0 ms (20 mM K) and 2.8 ms (200 mM K), probably not significantly different, and if anything showing a reversed dependence on K_o relative to Ba. (*b*) the TEA derivative C9 reduces and greatly prolongs the decay of I_K on repolarization (Armstrong, 1971). Ba does not much affect the decay rate (Fig. 7).

External Barium

EXTERNALLY APPLIED BA BLOCKS K CHANNELS Fig. 10 shows two families of I_K currents, the right one in the presence of 10 mM Ba. External Ba has little effect on the time-course of I_K, but it does diminish its amplitude. There are three other points of interest in the figure, all of which have a somewhat doubtful interpretation. (*a*) The spacing between traces in the presence of Ba is uneven, suggesting that at higher voltages Ba may be expelled from the channels. This could however be an effect of frequent pulsing (see below). (*b*) In Ba, the trace at +90 mV creeps steadily upward, unlike the control trace. This also may reflect expulsion of Ba from the channels. (*c*) After the control trace at +10 mV, there is an inward tail of current, the result of K accumulation in the Frankenhaeuser-Hodgkin space. In the Ba trace to +90 mV the current is slightly larger, but there is no tail after the pulse. This suggests that on return to −70 mV Ba quickly equilibrates with a site that blocks the current tails.

EXTERNAL BA EQUILIBRATES SLOWLY WITH K CHANNELS Both the onset of block with external Ba and recovery after Ba is washed out are very slow in comparison to the time required to wash out external Na. As judged from the amplitude of I_{Na}, sodium washout was 95% complete in 20 s. Fig. 11 *a* is a semilog plot of the decay and recovery of I_K during, respectively, application and washout of 8 mM external Ba (see figure legend for details). The time constant for block on application of Ba, τ_B, was 72 s, and the time constant for washout,

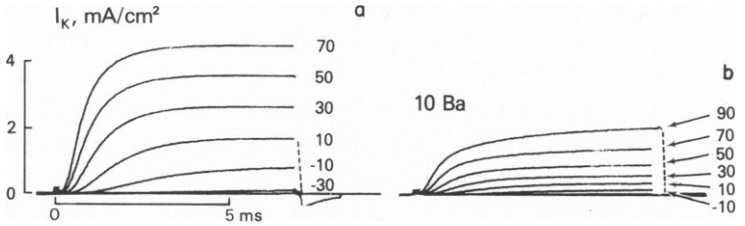

FIGURE 10 External Ba reduces I_K. (*a*) I_K recorded at the voltages shown, in 0 mM K Tris TTX//SISA. The tail follows the pulse to +10 mV. (*b*) I_K after the addition of 10 mM Ba (10 mM Ba 0 mM K Tris TTX//SISA). The recording is continued after the pulse to +90 mV, and there is no inward tail of current. Pulse interval was irregular, roughly one pulse per 5 s, and the traces were taken in the order −10 → +90. Exp. AU087B, 12°C.

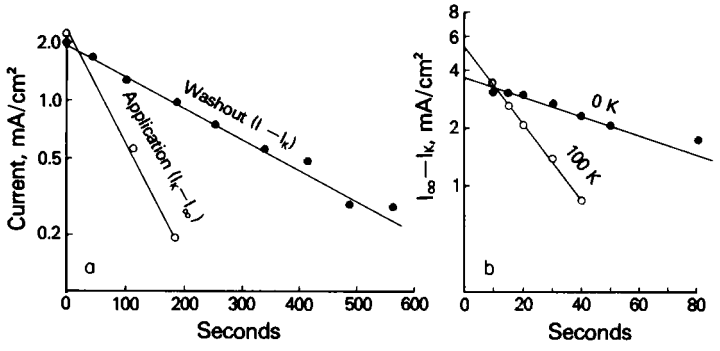

FIGURE 11 (*a*) Time-course of block and recovery after application and washout of external Ba^{2+}. After 3 mM Ba Tris TTX was applied, I_K was measured during infrequent pulses to 30 mV (all measurements are plotted). It decayed with a time constant of 72 s. The time constant for recovery of I_K when 3 mM Ba Tris TTX was replaced by Tris TTX was 265 s. Internal solution SISA. Exp. AU057A, 12°C. Holding potential: −70 mV. (*b*) High external K speeds recovery from block by external Ba. At time zero, the external solution was changed to 0 or 100 mM K Tris TTX, and I_K was measured at the times shown by a pulse to +30 mV. Internal solution: SISA. Exp. AU087B, 12°C. Holding potential: −70 mV.

τ_W, was 265 s. (As will be shown, both time constants probably depend on pulse frequency.) If it is assumed that reaction of external Ba with a channel is a one step reaction:

$$\mathrm{Ba} + S \underset{b_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{Ba}S$$

then the following relations hold:

$$\tau_B = 1/(a_2[\mathrm{Ba}] + b_2);\ \tau_W = 1/b_2;\ \text{Fraction blocked} = 1 - \tau_B/\tau_W.$$

The block calculated from these relations was 72%, which agreed well with the observed block, 71%.

WASHOUT TIME DECREASES WITH EXTERNAL K CONCENTRATION Fig. 11*b* compares the recovery from block when a fiber in 15 mM Ba was placed at time zero in either 0 K Tris TTX or 100 K Tris TTX. All of the pulses applied to the fiber during washout are recorded. τ_W in 0 K was 88 s, and in 100 K it was only 22 s. Raising K_o thus increases recovery rate. τ_W in 0 K was substantially lower in this experiment than in the one of Figure 11 *a*, possibly because of the higher pulse frequency. The effect of pulse frequency on equilibration time was not studied systematically.

K_D FOR EXTERNAL BA DEPENDS ON INTERNAL K, V_H, AND OTHER FACTORS The dissociation constant for the blocking of K channels by external Ba was calculated from the formula

$$K_D = \frac{[\mathrm{Ba}] \times \text{fraction open}}{\text{fraction blocked}}.$$

The first entries in Table II (JL147A) show that Ba block at three Ba concentrations can be adequately described by a single dissociation constant of 2.8 mM (the average of K_D at the

TABLE II
K_D FOR EXTERNAL Ba*

Experiment	Solution External//Internal	V_H	Pulse frequency	K_D
	mM	*mV*		*mM*
JL147A	2.5 Ba Tris//SISA	−70	1/50 s	2.9
	5.0 Ba Tris//SISA	−70		2.8
	15.0 Ba Tris//SISA	−70		2.7
JL217A	2.5 Ba Tris//138 KFG	−70	1/50 s	0.7
	" //275 KFG			2.7
	" //550 KFG			5.8
JL267B	2.5 Ba Tris//138 KFG	−70	1/100 s	1.13
	" //275 KFG			1.86
	" //550 KFG			2.93
JL137B	5 Ba Tris//SISA	−55	Irregular	13
		−70	Irregular	4.7
		−100		0.6

*In all cases, I_K was measured at +30 mV.

three concentrations). Thus the data are consistent with the view that one Ba ion interacts with one channel, and follows the usual saturation isotherm.

The next two experiments show that K_D increases with internal K concentration. Both the absolute values of K_D and the dependence on K_{in} are somewhat different in the two experiments, but in both cases K_D significantly increases as K_{in} is increased from 138 to 275 to 550 mM. In the Discussion this effect is interpreted as meaning that internal K ions push Ba ions from the channel in the outward direction.

The last experiment in Table II shows that block by Ba increases steeply as the holding potential is made negative. Unfortunately the experiment was performed before the importance of pulse frequency was realized, and this variable was not carefully controlled. But it seems clear that there is a very large effect of V_H on the blocking potency of Ba, which can be summarized by saying that external Ba is drawn into the channels by internal negativity just as internal Ba is drawn into the channels by external negativity (Figs. 1 and 2).

The effect of external K concentration on K_D was not studied. Based on the experiment of Fig. 11 *b*, which shows that washout time decreases with K_o, we predict that K_D increases with K_o.

RAPID PULSING DECREASES BLOCK BY EXTERNAL BA The slow increase of I_K for the Ba-treated fiber in Fig. 10 suggests that Ba is expelled from the channels by strong depolarization. We tested this by applying a large number of depolarizations in quick sequence, and comparing I_K in the first pulse to that in the last of the sequence. In the one experiment on this question a fiber in 2.5 Ba Tris//275 KFG was pulsed from −70 to +30 mV (6-ms pulses) at 2.5 Hz. In the first pulse a maximum of 43% of the channels were conducting and 69% were conducting in the last of 30 pulses. This is consistent with the hypothesis that Ba is expelled by depolarization.

DO EXTERNAL AND INTERNAL BA ACT AT THE SAME SITE? At first glance the experiment of Fig. 12 suggests that they do not. It will be recalled that channels blocked by

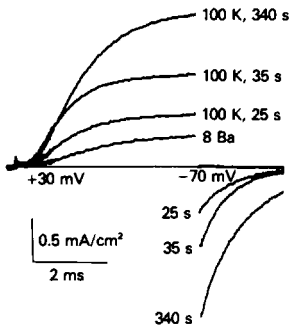

FIGURE 12 I_K during recovery from block by external Ba. The fiber was equilibrated in 8 mM Ba Tris TTX//SISA. At time zero the external solution was changed to 100 mM K Tris TTX, and I_K was measured at the times indicated, at +30 and −70 mV. The tails at −70 mV have no hook. Exp. AU087B, 12°C. Holding potential: −70 mV.

internal Ba have a rapid phase of clearing on repolarization, and that I_K tails have a hook which reflects this (Figs. 6 and 7). Channels blocked by external Ba behave differently: there is no rapid phase of clearing and no hook. The fiber in Fig. 12 was first equilibrated in 8 mM Ba 0 mM K, and the trace labeled 8 mM Ba was recorded. There is no inward tail of I_K because K_o was zero, and the small current caused little accumulation at the membrane surface. The external medium was then quickly changed to 100 mM K with no Ba. The trace recorded 25 s later (100 mM K, 25 s) has an inward current tail and no hook, even though a majority of the channels are still Ba blocked, as judged from the amplitude of the outward current. The tails recorded later (35 and 340 s) also do not have a hook.

The time-course of recovery for this fiber after Ba removal has been plotted in Fig. 11 *b* and there is no rapid phase of clearing (cf., Figs. 6 and 11 *b*).

This experiment has two possible interpretations. (*a*) Internal and external Ba act at different sites. (*b*) A Ba-occluded channel closes readily, trapping Ba in the channel. The "closed Ba-occluded" configuration is very stable, and as a result few of the occluded channels open their activation gates when V_m is pulsed to a positive value. In Fig. 12 then, the tails would have no hooks because the activation gates of the occluded channels remain closed.

DISCUSSION

The evidence that internal Ba enters K channels is (*a*) the membrane field affects the degree and rate of block, which suggests that Ba must enter the channels, and (*b*) external K ion antagonizes block by internal Ba. That is, a K ion passing inward through a channel pushes Ba into the cytoplasm.

Access to the narrow, selective, part of the K channel can best be explained on the basis of crystal radius (Hille, 1973; Bezanilla and Armstrong, 1972), suggesting that ions enter this part of the channel in at least a partially dehydrated state. Ba has a crystal radius quite close to K, and its actions conform well to this picture of the channel. Partial dehydration is presumably catalyzed and made possible by an energetically favorable interaction with the walls of the pore (Mullins, 1959; Bezanilla and Armstrong, 1972).

Our results show that Ba and the channel can form a stable complex that decays with a very slow time-course, and this gives some insight into channel structure. One possible model for the channel, discussed by Parsegian (1969), holds that the pore is a narrow electrically neutral tube with walls composed of material possessing a high dielectric constant. Parsegian shows that such a structure could satisfactorily catalyze the passage of a monovalent cation through the membrane, but for electrostatic reasons the interior of the pore is an extremely hostile environment for divalent cations. Our results clearly speak against such a model, and are more compatible with a pore which is part of a large protein or a negatively charged structure, and not hostile to doubly charged cations.

How far Ba ions go into the pore when applied from the inside can be estimated from Fig. 3. The estimate depends on the model assumed (see below), for some of the voltage dependence could arise from interaction between Ba and K ions within the pore, as it does with TEA derivatives (Armstrong, 1971; Hille and Schwarz, 1978). The blocking rate for the TEA derivative ϕC3, which is thought not to penetrate further than the pore mouth, is almost independent of voltage. Ba blocking rate, on the other hand, is very voltage dependent: the forward rate constant for Ba changes e-fold in ~17 mV. This suggests that Ba ions, unlike ϕC3, penetrate well into the pore and pass over a dominant energy barrier that is almost halfway through the membrane field, to occupy a site about three-quarters of the way through.

The evidence that external Ba enters the pore when it blocks is (*a*) both internal and external K ions antagonize its blocking action (Table II). (*b*) Raising external K speeds the clearing of the channels, as it does also when Ba is applied from the inside. (*c*) Pulsing the channels partially relieves block by external Ba, presumably because the outward traffic through the channels forces Ba ions out. (*d*) There is suggestive evidence that external Ba equilibrates much more rapidly with the channels when they are open (Fig. 10).

A major question that remains unanswered is whether external and internal Ba act at the same site. A different site would imply the existence in the channel of an absolute barrier that prevented external Ba from reaching the binding site of internal Ba and vice versa. This seems a rather unattractive idea, since from the electrical evidence, internal Ba seems to penetrate more than halfway through the membrane field. Further, Ba clears out of the closed channels extremely slowly whether applied from outside or inside, which suggests there is only one site of action. It is possible to account for all of the phenomena reported in the paper by the model given in the next section, which assumes that Ba ions can move all the way through the channel, and that a stable binding site for both internally and externally applied Ba exists near the outer end of the pore.

A Model

Fig. 13 is a proposed reaction diagram for Ba interacting with a K channel. The dashed line divides the states in which the K activation gate is closed (below) from those with open activation gate. The conducting states were marked by an asterisk. Opening and closing of the activation gate is a multistep process, and this is indicated by the dashed arrows. All other reactions are one step. The reactions with Ba, which are all above the line, cannot occur when the activation gate is closed.

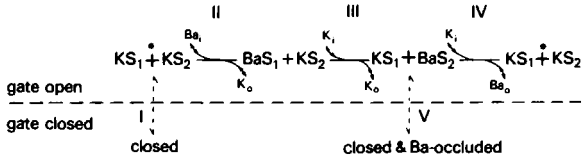

FIGURE 13 A state diagram for K channels in the presence of Ba. The activation gate of the channel is open above the line, closed below. Conducting states are marked with an asterisk. Ba can enter or leave the pore only when the activation gate is open.

The pore has two sites which can be occupied by either K or Ba. S_1 is the inner mouth of the channel, has a low affinity for Ba, and equilibrates with Ba too rapidly to detect when the gate is open. The postulate of S_1 is useful chiefly in explaining the dependence of Ba's blocking rate on K_o as described below. S_2 is the high affinity site, and is deep in the channel. Going from left to right in the diagram, a Ba ion enters the channel mouth in reaction II, and displaces a K ion from S_1 to S_2. This K ion in turn displaces another from S_2 to the outside. The reaction is reversed if a K ion enters from outside. If, instead, a K ion enters from inside, reaction III occurs, and the Ba ion moves to S_2, displacing the ion there to the outside. In this reaction the divalent Ba moves more than halfway through the membrane field, so the equilibrium and the rates of the reaction are strongly voltage dependent. Finally, reaction IV represents the exchange of a Ba ion between S_2 and the outside.

For simplicity some possible reactions have been omitted. For example, it may be that internal Ba most frequently enters S_1 when this site is empty. As written, the reaction is voltage-dependent, for it involves transfer of a K ion part way through the membrane.

This model accounts for the phenomena reported in the paper as follows.

(*a*) Voltage-dependent block by internal Ba. In reaction III Ba surmounts a barrier that is roughly halfway through the membrane field, and occupies a site about one-quarter of the way in from the outer surface.

(*b*) Antagonism of external K. The rate at which Ba ions enter S_2 from inside is proportional to BaS_1 (the fraction of the S_1 sites that are occupied by Ba). Raising K_o drives reaction II to the left, and decreases BaS_1.

(*c*) Clearing of internal Ba from the channels on repolarization. At the end of a strong depolarization the S_2 site in most of the channels is occupied by Ba. On repolarization there are two possible reaction paths: III, II, I, leading to the normal closed state, or V leading to the state in which the activation gate is closed and a Ba ion remains in S_2. The distribution of the channels between the two paths depends among other things on K_o. If K_o is high, reactions III and II are driven strongly to the left, and a large fraction of the channels end in the normal closed state, ready to conduct after activation by a depolarizing pulse. If K_o is low, most of the channels end in the closed, Ba occluded state. The fraction of the channels that are cleared of Ba in the fast phase (Fig. 6) thus depends on K_o. It is postulated that the closed Ba occluded state is extremely stable, and that the activation gates of channels in this state open very reluctantly on depolarization.

(*d*) Slow equilibration of channels with external Ba (Fig. 9). External Ba enters S_2 and blocks the channel by reaction IV. This reaction can occur only when the activation gate of the

channel is open, a small fraction of the time at −70 mV. Washout is slow for the same reason.

(*e*) K_o speeds up the washout of external Ba. When the activation gate of a Ba occluded channel opens, the chance that the Ba ion will be swept out is larger if K_o is high, for this drives reactions III and II to the left. K_{in} might speed clearing by driving IV to the right, but there is no data on this.

(*f*) K_D for external Ba increases with K_{in}. K_{in} drives reaction IV to the right, tending to clear the channels, and this increases K_D for external Ba.

(*g*) Near normal I_K kinetics with Ba_o (Fig. 10). Ba does not affect reaction I, which determines the time-course of I_K. The Ba occluded channels that open by reaction V do not conduct, and therefore do not affect I_K time-course. The strong outward driving force on K at 90 mV drives reaction IV to the right, causing a slow upward creep of the trace as Ba ions are cleared from S_2. The slowness of this process results not from a long time constant for reaction IV, but instead from the extreme stability of the closed, Ba-occluded state, and the slow rate of reaction V in the upward direction.

(*h*) No fast phase of clearing with external Ba. In Fig. 11 *b* and 12 the fiber was equilibrated in 0 K 8 Ba. Washout was measured after changing to a solution that contained no Ba and 0 or 100 mM K. It is postulated that the closed Ba occluded configuration of the channel is very stable, and the activation gates of these channels do not readily reopen. Thus on depolarization in the washout solution the occluded channels do not open their activation gates, and there is no opportunity for reactions III and II to occur.

The model thus accounts for all the phenomena reported in the results section, if it is hypothesized that the closed Ba occluded state of the channel is extremely stable. At present there is no independent evidence for or against this hypothesis. It does not seem unreasonable, however, to think that a divalent cation in a site near the membrane's outer surface would bias the gating machinery toward the closed state. A similar effect of external Ca on the Na channels is well known (Frankenhaeuser and Hodgkin, 1957).

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