ABSTRACT We have studied the interactions of Ba ion with K channels. Ba\(^{2+}\) blocks these channels when applied either internally or externally in millimolar concentrations. Periodic depolarizations enhance block with internal Ba\(^{2+}\), but diminish the block caused by external Ba\(^{2+}\). At rest, dissociation of Ba\(^{2+}\) from blocked channels is very slow, as ascertained by infrequent test pulses applied after washing Ba\(^{2+}\) from either inside or outside. The time constant for recovery from internal and external Ba\(^{2+}\) is the same. Frequent pulsing greatly shortens recovery time constant after washing away both Ba\(^{2+}\) and Ba\(^{3+}\). Block by Ba\(^{2+}\) applied internally or externally is voltage dependent. Internal Ba\(^{2+}\) block behaves like a one-step reaction governed by a dissociation constant \((K_d)\) that decreases e-fold/12 mV increase of pulse voltage: block deepens with more positive pulse voltage. For external Ba\(^{2+}\), \(K_d\) decreases e-fold/18 mV as holding potential is made more negative: block deepens with increasing negativity. Millimolar external concentrations of some cations can either lessen (K\(^+\)) or enhance (NH\(_4^+\), Cs\(^+\)) block by external Ba\(^{2+}\). NH\(_4^+\) apparently enhances block by slowing exit of Ba ions from the channels. Rb\(^+\) and Cs\(^+\) also slow clearing of Ba ions from channels. We think that (a) internally applied Ba\(^{2+}\) moves all the way through the channels, entering only when activation gates are open; (b) externally applied Ba\(^{2+}\) moves two-thirds of the way in, entering predominantly when activation gates are closed; (c) at a given voltage, Ba\(^{2+}\) occupies the same position in the channels whether it entered from inside or outside.

INTRODUCTION

Two recent papers have shown that Ba ions are potent blockers of potassium current when applied internally in squid axons (Eaton and Brodwick, 1980; Armstrong and Taylor, 1980). Ba\(^{2+}\), which has nearly the same crystal radius as K\(^+\), enters K channels that have been opened by depolarization and occupies a receptor that is one-half or more of the way through the membrane field, causing the potassium current to decay with time or “inactivate.” When the membrane is repolarized, a fraction of these Ba ions are swept out by inmoving K ions before the channel gate closes. The remainder of the channels

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close with a Ba ion inside them, and remain blocked for as long as 5 min, which suggests that the closed Ba\textsuperscript{2+}-blocked configuration of the channel is very stable (Armstrong and Taylor, 1980). Prolonged residence of a divalent cation in a channel seems surprising, and suggests that the channels contain negatively charged groups. If there were no negative charge, the electrostatic energy of Ba\textsuperscript{2+} in the membrane, a region of low dielectric constant, would be too high to permit long residence.

External Ba\textsuperscript{2+} also blocks K channels, and there are both similarities and differences when compared with the action of internal Ba\textsuperscript{2+} (Arhem, 1980; Brismar, 1979). The major difference is that external Ba\textsuperscript{2+} decreases the potassium current without causing obvious kinetic changes, whereas internal Ba\textsuperscript{2+} causes “inactivation.” This is compatible with the well-established idea that the activation gate protects the channel from internally applied substances when closed, but externally applied substances have access at all times because the gate is at the inner end of the channel.

Both internal and external Ba\textsuperscript{2+} have very slow washout times (Armstrong and Taylor, 1980). An interesting question is whether Ba\textsuperscript{2+} occupies the same position in the channels whether applied internally or externally. In this paper we present results that strongly suggest that it does.

Previous results clearly showed that in the presence of internal Ba\textsuperscript{2+} potassium channels are endowed with a memory of previous activity that far outlasts the memory arising from the normal permeability mechanisms. We examine here some of the consequences of this fact and point out that such a memory could provide a model for short-term (minutes) modification of the behavior of nerve cells and presynaptic terminals.

The blocking effect of Ba\textsuperscript{2+} is profoundly influenced by the ionic composition of the external medium. We have studied the effects of external cations on block caused by both internally and externally applied Ba\textsuperscript{2+}. The results are helpful in defining the number and properties of the ion binding sites within the channels. Particularly interesting is evidence that the outer end of a K\textsuperscript{+} channel can be occupied by an NH\textsubscript{4}, Rb, or Cs ion at the same time that a Ba ion is in the channel.

**METHODS**

Experiments were performed on cleaned, internally perfused giant axons of *Loligo pealei* at the Marine Biological Laboratory, Woods Hole, MA. Perfusion was initiated by a brief treatment of the axon interior with pronase. Pronase treatment was prolonged in some axons in order to remove most of the axoplasm and make possible relatively quick changes of the internal medium. The perfused axons were voltage-clamped using standard techniques (see, for example, Bezanilla and Armstrong, 1977). Ionic current signals were digitized, corrected for linear leakage and capacitative current using the P/4 method (Bezanilla and Armstrong, 1977), and stored on magnetic disks.

The compositions of internal and external solutions are given in Table I. In all experiments except those that examined \( I_{K_\text{Ba}} \), tetrodotoxin (TTX) was added to the external medium to attain a final concentration of \( 2 \times 10^{-7} \) M. pH of internal and external solutions was adjusted to 7.0 ± 0.1.
RESULTS

Long-Term Changes of Potassium Permeability

Repeatedly pulsing an axon that is perfused internally with 1 mM Ba\textsuperscript{2+} progressively decreases the potassium current elicited with each new pulse, as illustrated in Fig. 1A. The trace labeled "0 Ba" is a control trace recorded in the absence of Ba\textsuperscript{2+}. The fiber was left at rest for 75 s after the addition of Ba\textsuperscript{2+} to the internal medium, and then was pulsed to +90 mV once every 8 s. The current from every other pulse is illustrated.

All of the traces have an initial outward transient of current carried by K\textsuperscript{+} moving through the imperfectly selective Na channels (Chandler and Meves, 1965; Hille, 1973). In the control trace, I\textsubscript{K} begins to decline slightly 5 ms after the pulse onset, and this is probably the result of K\textsuperscript{+} accumulation in the confined space near the membrane surface (Frankenhaeuser and Hodgkin, 1956).

The current trace from the first pulse after Ba\textsuperscript{2+} addition (trace 1) superimposes on the control current for the first 2 ms, and then separates and falls below it. I\textsubscript{K} in each of the succeeding pulses is progressively smaller but current through the Na channels remains unchanged.

The interpretation of these traces is that Ba\textsuperscript{2+} enters and blocks a fraction of the channels that are activated by pulsing, and slowly clears out of the channels in the intervals of rest (Armstrong and Taylor, 1980). Clearing is so slow that the blocking effect is cumulative for rapid pulsing. Just before pulse 1 there are no blocked channels, and the initial rise of I\textsubscript{K} is the same as in the control case. Only a small fraction of the blocked channels clears before the next pulse, and at the beginning of pulse 3 about one-third of the channels are still blocked by Ba ions that entered in the preceding pulses. The peak value of I\textsubscript{K} is thus smaller in pulse 3, and dI\textsubscript{K}/dt is smaller because more channels are blocked. These changes are more pronounced with each succeed-

### TABLE I

| SOLUTIONS |
|------------|
| **Internal solution** | Sucrose | K-fluoride | K-glutamate | Trizma 7.0 |
| 275 KFG     | 420     | 50         | 225         | 10         |

| **External solution** | CaCl\textsubscript{2} | NaCl | X-Cl | Trizma 7.0 |
| ASW          | 50      | 440   | 10   |
| K-ASW        | 50      | 440   | 10   |
| Rb-ASW       | 50      | 440   | 10   |
| Cs-ASW       | 50      | 440   | 10   |
| NH\textsubscript{4}-ASW | 50   | 440   | 10   |

External solutions were made by mixing the appropriate ratios of the two stock solutions; e.g., 20 K-ASW = 1:22 of K-ASW and ASW. All external solutions have 200 nM tetrodotoxin added for the measurements of I\textsubscript{K}.
ing pulse until a steady state is reached after ~15 pulses. At this point the number of Ba ions that enter in each pulse is just matched by the number that leave in the interpulse interval. During a long interval of rest in the presence of internal Ba$^{2+}$, the number of available channels slowly increases toward the level in trace 1. The rate and the degree of recovery depend on the ionic content of the external medium, being more complete in the presence of elevated K$^+$ (see below).

When Ba$^{2+}$ is applied externally, the effect of pulsing on current amplitude is precisely the opposite, as shown in Fig. 1B. After recording the control trace

\begin{figure}
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A \hspace{2cm} B
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\caption{Short-term modification of membrane behavior by pulsing in the presence of Ba$^{2+}$. A. Pulsing enhances block by internal Ba$^{2+}$ (1 mM). After recording the 0-Ba$^{2+}$ trace, 1 mM Ba$^{2+}$ was added internally, and 70 s was allowed for equilibration. The fiber was then pulsed to +90 mV once every 8 s, and the current from every second pulse is illustrated. \(I_K\) declines progressively with pulsing, but the early peak of current through the Na channels is unaltered. When pulsing ceased, current amplitude slowly recovered (see text). Experiment AU040A; 8°C; HP -70 mV; ASW/275 K + 1 Ba. B. Relief of external Ba$^{2+}$ block by pulsing. After recording the 0-Ba$^{2+}$ trace (pulse to +80 mV), 3 mM Ba$^{2+}$ was added outside and the fiber allowed to equilibrate for 175 s. Pulsing was then begun at ~0.6 Hz, and currents from the 1st and 25th pulses are shown. Pulsing relieved block, which was slowly reestablished on cessation of pulsing. After 120 s of rest, the current during pulse 28 is somewhat smaller than for pulse 1. Experiment JN199C; 8°C; HP -70 mV; Tris + TTX + 3 Ba/275 K.}
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\end{figure}

(0 Ba), the fiber was bathed in 3 Ba for 450 s, and pulsed to +70 mV once every 75 s. The current labeled “1” was recorded during the last of this series of infrequent pulses. The current is diminished in amplitude, but has about the same time course as the control current, and does not show “inactivation.” Pulse 1 was followed by 24 pulses with an interpulse interval of ~2 s, and the last of this series is labeled “25.” \(I_K\) in this pulse is almost as large as in the control trace. The fiber was then left at rest for ~4 min, and at the end of this interval \(I_K\) had fallen again to the initial low level (28). From these traces, it appears that external Ba$^{2+}$ enters the K channels when the fiber is at rest and is driven out during the pulses, whereas internal Ba$^{2+}$ enters during the pulses and leaves in rest intervals.
Internal $\text{Ba}^{2+}$ Clears Slowly from the Channels

The experiments above suggest that $\text{Ba}^{2+}$ clears very slowly from closed channels (cf. Armstrong and Taylor, 1980). We monitored this directly by blocking the channels with internal $\text{Ba}^{2+}$, then quickly washing away the $\text{Ba}^{2+}$ and recording the current elicited over the next few minutes, pulsing only once every 30–50 s. With this technique there is virtually no $\text{Ba}^{2+}$ present during the recovery period to block channels and thus complicate interpretation. To facilitate rapid washout, most of the axoplasm was removed by pronase treatment in the axons of this series. The time required for changing the internal medium was calibrated by following the recovery of $I_K$ during washout of the internally applied $K$ channel blocker, $\text{TEA}^+$, which does not bind for long in the channels. The fraction of blocked channels, is plotted as a function of time in the semilogarithmic graphs of Fig. 2. At time zero the

![Figure 2](image-url)

**Figure 2.** $K$ channels recover from $\text{Ba}^{2+}$ block much more slowly than from block by $\text{TEA}^+$. At time zero the blocking agent was washed from inside the fiber, and the level of recovery was assayed every 30 s with depolarizations of 12 ms to $+100 \text{ mV}$. Recovery from block by 10 mM $\text{TEA}^+$ or by 1 mM $\text{Ba}^{2+}$ had, respectively, time constants of 32 and 120 s. JN160B; 8°C; HP $-70 \text{ mV}$; ASW + TTX//275 K after 275 K + 10 TEA or 275 K + 1 Ba.
internal medium was changed from 275 K + 10 TEA to 275 K, and the fiber was pulsed once every 30 s to determine the recovery time course.

The TEA⁺ washout curve has a time constant of 32 s, and gives the washout time of a substance that does not bind tightly to the channels. The curve labeled Ba was determined by the same procedure: the channels were blocked by pulsing in the presence of Ba²⁺, which was then washed away beginning at \( t = 0 \) (275 K + 1 Ba²⁺ to 275 K). Recovery in this case is much slower, and has a time constant of 125 s. In a second, similar experiment, the time constants for TEA⁺ and Ba²⁺ washout were 18 and 120 s. Many channels thus seem to remain blocked long after free Ba²⁺ should have been completely removed from the axon interior, as judged by the TEA⁺ washout curve. Under these circumstances the recovery curve is a direct measure of the rate at which Ba²⁺ clears from blocked channels. The very slow observed rate is consistent with the idea that Ba²⁺ forms a relatively stable complex with the closed channel (see below).

**Recovery Rate Is the Same for Internal and External Ba²⁺**

A question of interest is whether Ba²⁺ assumes the same position in the channel whether applied internally or externally. If it does, the recovery rate for internal and external application should be the same. The open circles in Fig. 3A show the recovery of \( I_R \) from block by internal Ba²⁺, after Ba²⁺ had been washed away by the technique described above. In this case the internal perfusion medium contained 3 mM EDTA to chelate the last traces of Ba²⁺. The fiber was pulsed once every 40 s during the recovery period, and the data from every pulse is recorded. Recovery is approximately exponential, and the time constant at this pulse frequency was 210 s.

Recovery from external Ba²⁺ after the removal (at time zero) of 6 mM Ba²⁺ from the medium is illustrated by the open squares in Fig. 3B. One pulse was applied every 40 s, and the current elicited by each pulse is plotted. The time constant was 205 s, almost exactly the same as for recovery from internal Ba²⁺.

Pulsing the fiber after removal of either internal or external Ba²⁺ greatly speeds recovery, as shown by the curves with filled symbols in Figs. 3A and B. In both cases pulsing was begun 80 s after washout, at a frequency ~0.6 Hz, and resulted in a marked shortening of the recovery time constant from ~200 s without pulsing to ~20 s with pulsing.

The same result is shown in different circumstances in Fig. 3C. In this case the external solution contained 10 mM Rb⁺, which, as described below, prolongs the clearing of Ba²⁺ from the channels. The fiber was allowed to recover for 150 s before pulsing was begun at 0.6 Hz (O, Ba²⁺; □, Ba²⁺). Without pulsing, the recovery time constant for both internal and external Ba²⁺ was ~575 s, and it dropped to 35 s with pulsing (filled symbols).

The similarity of the recovery time constants for internal and external Ba²⁺ and the parallel behavior in response to pulsing strongly suggest that Ba²⁺ occupies the same position in the channel whether applied inside or out. Further evidence on this point is given below, in the section describing the modulation of Ba²⁺ block by external cations.
Voltage Sensitivity of Ba\textsuperscript{2+} Block

Results in the literature suggest that Ba ions applied internally travel half (Eaton and Brodwick, 1980) or three-quarters (Armstrong and Taylor, 1980) of the way through the membrane field in moving to their blocking site. We have repeated this experiment, using a method that is more accurate, and in addition we determined the voltage sensitivity of block by external Ba\textsuperscript{2+}.

![Figure 3](image)

**Figure 3.** A and B. Recovery of $I_K$ from Ba\textsuperscript{2+} block has the same time course after internal (A, O, 1.5 mM Ba) or external (B, □, 6 mM Ba\textsuperscript{2+}) application, and is speeded by pulsing. The rate of recovery with slow pulsing (O, □, 0.02 Hz) and the enhanced rate of recovery when the fiber is pulsed at 0.6 Hz (●, ■) are the same whether Ba\textsuperscript{2+} is applied internally (●) or externally (■). Fast pulsing speeded removal of Ba\textsuperscript{2+} from channels, decreasing time constants from 210 and 205 s to 29 and 22 s for internally and externally applied Ba\textsuperscript{2+}. A. Experiment MA201B; 8°C; HP -70 mV; ASW + TTX/275 K + 3 EDTA, after 275 K + 1.5 Ba. B. MA201B; 8°C; HP -70 mV; ASW + TTX/275 K + 3 EDTA, after ASW + TTX + 6 Ba. C. The addition of 10 mM Rb\textsuperscript{+} to the external bath slows recovery of Ba\textsuperscript{2+}-blocked K channels regardless of the side of Ba\textsuperscript{2+} application. The recovery time constant during slow pulsing (0.02 Hz) was more than doubled, to 575 s, by 10 mM external Rb\textsuperscript{+}, for both internal (O) and external (□) Ba\textsuperscript{2+}. Pulsing at 0.6 Hz speeded the removal of both internally (●) and externally (■) applied Ba\textsuperscript{2+}, reducing the time constant to 35 s. JN041A; 8°C; HP -70. □: ASW + TTX + 10 Rb\textsuperscript{+}/275 K, after ASW + TTX + 10 Rb\textsuperscript{+}/275 K, after 275 K + 5 Ba.

A major problem in working with Ba\textsuperscript{2+} is the very long equilibration interval: perturbation of the equilibrium by a pulse has consequences that endure for hundreds of seconds. To get around this problem when using internal Ba\textsuperscript{2+}, we (a) recorded a control current in the absence of Ba\textsuperscript{2+}, (b)
applied Ba$^{2+}$ internally and recorded the current during a single pulse, (c) washed Ba$^{2+}$ away, and again determined the control current. This procedure was repeated for each voltage in order to get a complete current-voltage curve. Rapid change of the internal solution was accomplished by the technique described above.

Typical records are shown in Fig. 4A. The control trace of each pair is the average of currents recorded before Ba$^{2+}$ application and after washing it away. At +40 mV block is about half complete, and increases steeply with voltage. The kinetics of block also speed up markedly with voltage.

The dissociation constant for Ba$^{2+}$ and the channels is proportional to the fraction of channels open divided by the fraction blocked. This ratio is plotted semilogarithmically in Fig. 4B as a function of voltage, for determinations at two external potassium concentrations. In both cases the ratio after applying Ba$^{2+}$ inside the fiber changes e-fold for a 12- or 13-mV change of the membrane voltage during the pulse. The 100-K$^+$ curve is $\sim$12 mV to the right of the 20-K$^+$ curve, presumably because K$^+$ competes with internal Ba$^{2+}$.
for the channels. Evidently membrane voltage influences the probability of finding a Ba ion in the channel. An interpretation of this voltage dependence is discussed below (Discussion), where it is suggested that internal Ba\(^{2+}\) moves all the way through the membrane field to occupy a site near the membranes' outer surface.

The degree of block by external Ba\(^{2+}\) is also voltage sensitive and was measured by determining the severity of block for a fixed Ba\(^{2+}\) concentration at several holding potentials. The equilibration time for external Ba\(^{2+}\) is long, particularly for low concentrations, which makes the experiment tedious. The

![Graph](attachment:image.png)

**Figure 5.** A. \(I_K\) traces are shown for pulses to +80 mV from the indicated holding potential (−60 to −100 mV). Ba\(^{2+}\) was present as labeled, and the higher amplitude traces in each set were recorded before Ba\(^{2+}\) and after washing it away. Block is deeper for more negative holding potentials. B. \(K_d\) (dissociation constant) is plotted as a function of the holding potential. \(K_d\) decreases e-fold/16 mV in this experiment. JN101D; 8°C; HP −60 to −100 mV; ASW + TTX + 1.5 Ba\(^{2+}/275\) K.

procedure we used was to take control traces at several holding potentials, introduce Ba\(^{2+}\) into the external solution and wait for 10 min, take one pulse and immediately change the holding potential, wait 10 min, and pulse again. After three pulses and a total time in Ba\(^{2+}\) of 30 min, Ba\(^{2+}\) was washed away and the control currents were measured again. Typical traces are shown in Fig. 5A, and the numbers give the holding potential. The upper two traces of each group are the controls, taken before applying Ba\(^{2+}\) and after washing it away, and the lower amplitude trace was taken in the presence of 1.5 mM Ba\(^{2+}\). Block is much greater at more negative holding potentials. The record for −100 mV suggests that almost all of the channels are blocked at the onset of the pulse, and the slow upward creep of the trace may mean that Ba\(^{2+}\) is
coming out of some of the blocked channels. If this is so (see below), the fraction of channels blocked is underestimated in proportion to the number of channels that are cleared of Ba during the test pulse. Experiments with smaller test depolarizations, however, suggest this is not a significant source of error.

The dissociation constant from this experiment is plotted semilogarithmically as a function of holding potential in Fig. 5B. \( K_d \) changes e-fold for \( \sim 16 \) mV. Results from other experiments are listed in Table II. The average steepness is e-fold/18.0 mV with a standard deviation of 4.0 mV. The voltage dependence of \( K_d \) for external Ba\(^{2+}\) thus seems significantly less than for internal Ba\(^{2+}\).

A simple explanation for the variation of \( K_d \) with voltage is that external Ba\(^{2+}\) goes about two-thirds of the way through the membrane to reach its blocking site. At first this seems hard to reconcile with the location of the receptor for internal Ba\(^{2+}\), but further discussion is deferred until later.

**Table II**

**DISSOCIATION CONSTANTS, EXTERNAL Ba\(^{2+}\)**

| Experiment | \([\text{Ba}^{2+}]_o\) | \(-60\) mV | \(-80\) mV | \(-100\) mV | e-fold/mV |
|------------|-------------------------|------------|------------|-------------|-----------|
|            | \(mM\)                  |            |            |             |           |
| MA271A     | 5                       | 3.6        | 1.6        | 0.6         | 19.0      |
| JN041B     | 5                       | 4.5        | 1.9        | 0.9         | 22.5      |
| JN101B     | 3                       | —          | 1.5        | —           | 12.5      |
| JN101D     | 1.5                     | 4.8        | 1.3        | 0.5         | 16.0      |
| JN161A     | 2                       | 1.9        | 0.6        | 0.3         | 20.0      |
| \(X \pm SD\) |                      | 3.7 ± 1.3  | 1.4 ± 0.5  | 0.6 ± 0.2   | 18.0 ± 3.9 |

Experiments showed that equilibration with external Ba\(^{2+}\) is faster at more negative holding potentials, and washout is slower. Thus, both association and dissociation rate constants are affected by voltage. We lack sufficient evidence to make a more quantitative statement.

**Ba\(^{2+}\) Stabilizes the Closed Configuration of the Channel**

Ba\(^{2+}\) clears from blocked channels in two phases, a quick one before the channel gates close, and a very slow one afterwards (Armstrong and Taylor, 1980). It has been postulated that the slow recovery phase results because Ba\(^{2+}\) stabilizes the closed configuration of the channel, and the channel gates have a much lower than normal probability of opening during a depolarizing pulse when they are closed and blocked by Ba\(^{2+}\) (Armstrong and Taylor, 1980).

We tested this postulate by the experiment shown in Fig. 6. Ba\(^{2+}\) was introduced into the fiber interior, and after a 150-s wait, successive pulses were applied at intervals of \( \sim 2 \) s. The control current before introducing Ba\(^{2+}\) is appropriately labeled. In pulse 1, the first one in the presence of Ba\(^{2+}\), the initial rise of the current is the same as in the control trace, which indicates
that no channels were \( \text{Ba}^{2+} \) blocked just before pulse 1. The current declines from its peak value to a steady level, which is 2.6% of the control, i.e., 97.4% of the channels are blocked. The fiber was then repolarized, causing an inward tail of current. The tail has a hook: it increases in magnitude for \( \sim 1 \text{ ms} \) as \( \text{Ba}^{2+} \) ions clear out of the channels, and then decreases as the gates close.

The maximum current in pulse 4 is 23% as large as that in pulse 1. Thus,

![Figure 6](image)

**Figure 6.** \( I_K \) declines progressively with pulsing after the introduction of 5 mM \( \text{Ba}^{2+} \) to the internal medium. \( \text{Ba}^{2+} \) was introduced after recording the "no \( \text{Ba}^{2+} \)" trace, and 150 s were allowed for its concentration to equilibrate. The fiber was then pulsed to \( +100 \text{ mV} \) every 2 s, and the results of the pulses 1 and 4 are illustrated. The steady state current at the end of the pulses 1 and 4 is almost identical, but the tail current (on stepping back to \(-70 \text{ mV}\)) is about twice as large after pulse 1. This is because many of the channels are blocked but have open activation gates after pulse 1, whereas after pulse 4 most are blocked and have closed activation gates (see text). With \( \text{Ba}^{2+} \) the tail current has a noticeable hook, produced as \( \text{Ba}^{2+} \) clears from some of the channels before they close. JN091A; 8°C; HP \(-70 \text{ mV}\); 100 K-ASW + TTX//275 K + 5 Ba.

at the beginning of pulse 4, 23% of the channels did not contain \( \text{Ba} \) ions. Since almost all of the channels were blocked at the end of the preceding pulse (not illustrated), 23% of them must have cleared in the interval between pulses. Only slightly more (98.4%) of the channels are blocked at the end of pulse 4 than at the end of pulse 1. The tail current following pulse 4, however, is less than half as large as that following pulse 1. It is clear that the channels
are not in the same state at the end of pulses 1 and 4, although the steady state current is almost the same.

One interpretation is that at the end of pulse 1 most of the channels are Ba\(^{2+}\) blocked and have open activation gates. Only a fraction of the channels clear of Ba\(^{2+}\) before their activation gates close, and the remainder are in a closed-blocked state. By the end of pulse 4 more than half of the channels are in this state, and their activation gates remain closed in spite of the positive membrane voltage. This can be rephrased to say that Ba\(^{2+}\) stabilizes the closed state of the channel.

This interpretation is entirely consistent with the observation of a fast and a slow phase of recovery from Ba\(^{2+}\) block, the fast phase corresponding to clearing of Ba\(^{2+}\)-blocked channels with open activation gates, and the slow one generated by the clearing of closed-blocked channels.

One Ba Ion Blocks One K Channel

Fig. 7 is a plot of all our accumulated data regarding the fraction of channels blocked as a function of Ba\(^{2+}\) concentration in the external medium at a holding potential of \(-70\) mV. The data points are well fitted by the solid curve, which was calculated on the assumptions that only one Ba ion is required to block a channel, and that \(K_d\) for the process is \(3.05\) mM. This \(K_d\) applies only to fibers in artificial seawater (ASW; no K\(^+\)) held at \(-70\) mV. The effect of voltage on \(K_d\) has been examined above, and the next section

\[
\text{FIGURE 7. The logarithmic dose-response relation for external Ba}^{++} \text{is well described by 1:1 stoichiometry. The number of determinations for each concentration is shown below each data point with standard error bars when appropriate. The curve illustrates the predicted response for a model of Ba}^{2+} \text{block of K channels in which one Ba ion blocks one K channel with a dissociation constant of 3.05 mM. Pooled data from many experiments, all at 8°C, HP \(-70,\) in ASW + TTX + Ba//275 K.}
\]
shows that $K_d$ is sensitive to the presence in the external medium of several different cations.

$K_d$ for External Ba$^{2+}$ Is Influenced by the Cation Concentration of the External Medium

The efficacy of external Ba$^{2+}$ as a K channel blocker is decreased by external K ion, as described by Arhem (1980) and suggested by Armstrong and Taylor (1980). A number of other externally applied cations affect the $K_d$ of the Ba$^{2+}$ channel complex, as listed in Table III. $K_d$ (ASW) is the dissociation constant recorded in ASW in each experiment, and $K_d$ (cation) is the dissociation constant from the same experiment when the listed cation replaced Na$^+$ in the external medium. The cations fall into two groups, those that increase $K_d$ (Rb$^+$, K$^+$) and those that decrease it (Cs$^+$, NH$^+_4$). For example, 50 mM K$^+$ increases $K_d$ from 2.8 to 69 mM. Cesium (10 mM) decreases $K_d$ almost fivefold, and 10 mM NH$^+_4$ decreases it about twofold.

Some insight into the mechanism of these effects is provided by measurements of wash-in and washout rates, in the presence and absence of various external cations, as listed in Table IV. Beginning with the clearest of the results, NH$^+_4$ seems to decrease $K_d$ by slowing washout, with relatively little affect on the wash-in rate. The same seems to be the case for Cs$^+$: the washout rate is slowed by a very large factor, and the wash-in rate is modestly increased. In both cases it seems that the cation inhibits clearing of Ba$^{2+}$ from the channels.

Unfortunately, the results for neither K$^+$ nor Rb$^+$ are as clear. They suggest that potassium increases $K_d$ by increasing wash-in time, and perhaps by speeding washout. Rb$^+$ clearly slows washout, and increases wash-in time

| External cation | $K_d$ (ASW) | $[\text{Ba}^{2+}]_o$ | $K_d$ (cation)$_o$ | Fraction of control |
|-----------------|-------------|---------------------|------------------|-------------------|
| 10 Rb$^+$       | 3.9 mM      | 5 mM                | 3.9 (2)‡         | 2.3               |
| 10 K$^+$        | 3.1 mM      | 6 mM                | 3.7 (1)          | 1.2               |
| 10 K$^+$        | 1.6 mM      | 3 mM                | 4.5 (1)          | 2.8               |
| 10 K$^+$        | 3.9 mM      | 5 mM                | 13.9 (3)         | 3.6               |
| 10 K$^+$        | 3.1 mM      | 6 mM                | 6.7 (1)          | 2.2               |
| 50 K$^+$        | 2.8 mM      | 6 mM                | 69.0 (1)         | 23.4              |
| 25 K$^+$        | 75 mM       | 122.0 (1)           | —                | —                 |
| 50 K$^+$        | 30 mM       | 145.0 (1)           | —                | —                 |
| 10 Cs$^+$       | 2.9 mM      | 3 mM                | 0.7 (2)‡         | 0.2               |
| 10 NH$_4^+$     | 1.6 mM      | 3 mM                | 1.0 (1)          | 0.6               |
| 10 NH$_4^+$     | 3.9 mM      | 5 mM                | 2.0 (3)          | 0.5               |

* All measurements at a holding potential of −70 mV.
‡ Numbers in parentheses represent the number of determinations performed. Measurements were always bracketed by controls.
slightly in one case and markedly in another. Increased $K_d$ can be explained if wash-in is slowed more than washout, as seems to be the case in one of the two experiments.

**External Rb⁺ and NH₄⁺ Slow Recovery from Internal and External Ba²⁺ Equally**

If at a given voltage, Ba²⁺ occupies the same position in the channel regardless of whether it is applied inside or out, the slowing effect exerted by Rb⁺ on recovery should be independent of the side of Ba²⁺ application. Fig. 8 shows the results of an experiment to test this. Recovery from internal and external Ba²⁺ was first measured in ASW, i.e., Ba²⁺ was applied internally or externally and the fiber allowed to equilibrate, after which Ba²⁺ was removed and recovery monitored in ASW + TTX//275 KFG. The results for the two

### TABLE IV
TIMES CONSTANTS OF ONSET ($\tau_{on}$) AND RECOVERY ($\tau_{off}$) FROM Ba²⁺ BLOCK

| External cation concentration | [Ba]₀ | $\tau_{on}$, cation (s⁻¹) | $\tau_{on}$, ASW (s⁻¹) | $\tau_{off}$, cation (s⁻¹) | $\tau_{off}$, ASW (s⁻¹) | Experiment |
|------------------------------|-------|--------------------------|------------------------|--------------------------|------------------------|------------|
| mM                           | mM    |                          |                        |                          |                        |            |
| 10 K⁺                        | 3     | 130/110                  |                        |                          |                        | MA111B     |
| 10 K⁺                        | 6     | 330/132                  |                        |                          |                        | MA141A     |
| 50 K⁺                        |       |                          | 116/151                |                          |                        | MA121A     |
| 2 Rb⁺                        | 5     | 1,780/165                |                        |                          |                        | JN101C     |
| 10 Rb⁺                       | 5     | 755/175                  |                        |                          |                        | JN021A     |
| 10 Rb⁺                       | 6     | 217/132                  |                        |                          |                        | MA141A     |
| 19 Rb⁺                       | 5     | 585/88                   | 966/170                |                          |                        | MA281A     |
| 10 Cs⁺                       | 3     | 255/130                  | 1,120/—                |                          |                        | MA301A     |
| 10 NH₄⁺                      | 3     | 110/110                  |                        |                          |                        | MA111B     |
| 10 NH₄⁺                      | 10    | 698/188                  |                        |                          |                        | JN041A     |

* Average of two determinations.
‡ Average of three determinations.
§ Pulse frequency was 0.025 s⁻¹; all other pulse frequencies were 0.02 s⁻¹.

determinations are given by the open symbols in the figure. The curves are very similar and have time constants of 175 (Ba_{in}²⁺, ○) and 190 (Ba_{out}²⁺, □) s. The filled symbols show the time course of recovery when the external solution was ASW with 10 mM Rb⁺ substituted for 10 mM Na⁺, i.e., the procedure was identical except that recovery was in ASW-10 Rb + TTX//275 KFG. Recovery from both internal and external Ba²⁺ in this solution had a time constant of 750 s, and the time course was so nearly identical that a single set of symbols was used for both (○). The similarity of recovery times in the two solutions strongly supports the hypothesis that internal and external Ba²⁺ occupy the same position in the channel once it is closed. Results with NH₄⁺ (not shown) were essentially the same.

**Internal Ba²⁺ and Iₙa**

In Fig. 1A it appears that outward current through Na channels is not affected by internal Ba²⁺. The same is true for an inward current of sodium recorded
at 0 mV, as shown in Fig. 9. Pulses 1 and 11 from Fig. 1A are shown in the upper part of the figure, and illustrate the decline of $I_K$ with successive pulses to +90 mV. The trace labeled "12" (lower) was recorded immediately after trace 11, at 0 mV rather than +90. $I_{Na}$ is the same as in the absence of Ba$^{2+}$ (0 Ba), but $I_K$ in the later part of the trace is depressed, consistent with the upper part of the figure. Thus internal Ba$^{2+}$ at 1 mM affects neither inward nor outward current through normal Na channels.

DISCUSSION

Internal and External Ba$^{2+}$ Occupy the Same Locus in the Channel

Our results suggest very strongly that Ba$^{2+}$ occupies the same position(s) in the channel whether applied internally or externally. Thus, after Ba$^{2+}$ has been washed away, it is impossible to tell during the several hundred seconds required for recovery from which side it was applied. The time course of $I_K$ during a pulse is identical during the recovery period after Ba$^{2+}$ is washed away regardless of the side of application. Pulsing speeds recovery equally for
internally or externally applied $\text{Ba}^{2+}$, and application of small amounts of $\text{NH}_4^+$, $\text{Cs}^+$, or $\text{Rb}^+$ slows it down.

**The Activation Gate Is at the Channel's Inner End**

Internally applied $\text{Ba}^{2+}$ can enter a channel only if the activation gate is open. External $\text{Ba}^{2+}$, on the other hand, seems to have access to the channel regardless of whether the gate is open or not: there is no acceleration of block when the membrane is depolarized in external $\text{Ba}^{2+}$, and in fact $\text{Ba}^{2+}$ clearly comes out during a pulse if the voltage is large. Further, block is greater and the equilibration time is shorter when the holding potential is made more negative. Were $\text{Ba}^{2+}$ entry restricted to the time when the gates are open, a more negative holding potential would be expected to slow equilibration, since the gates are less likely to open at negative voltage. The evidence thus is against the hypothesis advanced previously (Armstrong and Taylor, 1980),

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**Figure 9.** 1 mM internal $\text{Ba}^{2+}$ has no effect on $I_{\text{Na}}$. A. The top panel (taken from Fig. 1) shows that pulsing at 0.12 Hz deepens the block of $I_K$ caused by 1 mM internal $\text{Ba}^{2+}$. The same sequence of pulses had no effect on $I_Na$. The currents were recorded in the order: 0 Ba (before $\text{Ba}^{2+}$ application); trace 1, 1 min after the addition of $\text{Ba}^{2+}$; 11 the last of a series at 0.12 Hz, and 12 immediately after 11. AU040A; 8°C; HP $-70 \text{ mV}$; ASW//275 K $+1 \text{ Ba}^{2+}$. 
that external Ba\textsuperscript{2+} enters only on the infrequent occasions when channels open at the holding potential.

All of the facts summarized support the idea that the activation gate guards the inner end of the channel but does not hinder access to the outer end.

**Ba\textsuperscript{2+} Has More Than One Binding Site in the Channel**

The prolonged residence of Ba\textsuperscript{2+} in the channel suggests the presence of negative charge (Armstrong and Taylor, 1980). Our findings regarding the distance that Ba\textsuperscript{2+} penetrates through the field are hard to reconcile, however, with the idea that there is a discrete charged site for Ba\textsuperscript{2+} binding.

The voltage dependence of Ba\textsuperscript{2+} block, which is the basis for the electrical distance determinations, does not have a unique interpretation. Thus an e-fold/12 mV variation in the steepness of block for internal Ba\textsuperscript{2+} could mean either that in going from the unblocked to the blocked state, Ba\textsuperscript{2+} moves all the way through the membrane, or that Ba\textsuperscript{2+} moves two-thirds of the way and a single K ion moves two-thirds of the way, or that Ba\textsuperscript{2+} moves halfway and two K ions each move halfway, etc. The requirement can be summarized by the equation

\[ 2e = 2e\delta_{Ba} + e\delta_{K_1} + e\delta_{K_2} + \cdots + e\delta_{K_n} \]

in which e is the electronic charge, \( \delta_{Ba} \) is the fraction of the field through which Ba\textsuperscript{2+} moves, and \( \delta_{K_n} \) is the fraction of the field through which the nth K ion moves.

It is likely that K channels are multi-ion pores (Hille and Schwarz, 1978), but the exact arrangement of ions within the pore at this time is unknown. It is simplest and not unreasonable to suppose that internally applied Ba\textsuperscript{2+} moves to the outer edge of the membrane when the voltage is positive. At this position, its electrostatic energy from interaction with the field would be lowest and the image forces arising from the low dielectric constant of the membrane would be least. There is evidence in Fig. 4 to support the idea that K ions do not play a part in determining the voltage sensitivity of Ba\textsuperscript{2+} block. In this figure, raising the external K\textsuperscript{+} concentration from 20 to 100 mM displaced the blocking curve to the right along the voltage axis. This could be interpreted as an occupancy effect: in 100 K\textsuperscript{+} there is a higher probability that the channel contains one or more K ions that compete with Ba\textsuperscript{2+} for the channel. Nonetheless, the voltage dependence of block is the same for both K\textsuperscript{+} concentrations. If there are more K ions in the channel, they do not alter the voltage sensitivity of the blocking reaction.

The same uncertainty with regard to the electrical distance measurements and the number of ions in the channel exists for external Ba\textsuperscript{2+}. These measurements are made at negative voltages, providing a bias for Ba\textsuperscript{2+} to move inward as far as possible. This, in the simple model, is about two-thirds of the way through the field. Unfortunately, we have no measurements of the voltage dependence of Ba\textsuperscript{2+} block in higher K\textsuperscript{+} concentrations. These might provide further evidence regarding the possible role of K ions in the voltage sensitivity of block.
During recovery from Ba\textsuperscript{2+} block, the membrane voltage is negative except during infrequent pulses, and the simple model would predict that Ba\textsuperscript{2+} is about two-thirds of the way toward the inside of the channel whether applied internally or externally. Recovery behavior is thus the same whether application was internal or external.

Whether the simple model is correct or not, it seems clear that Ba\textsuperscript{2+} moves back and forth in the channel as the membrane field dictates and does not bind to a single discrete site. When the voltage is positive, Ba\textsuperscript{2+} travels toward the outer end of the field, where it encounters a barrier to further movement. The origin of this barrier is unknown, but it may have to do with breaking the interactions between Ba\textsuperscript{2+} and the channel walls and rehydrating the ion. The barrier is high but not insurmountable, for Ba\textsuperscript{2+} does come out at an appreciable rate during pulses. When the voltage is negative, Ba\textsuperscript{2+} moves inward, but not all the way through the field. It may be that the gating apparatus at the inner end of the channel provides a barrier to further movement.

In summary, Ba\textsuperscript{2+} seems to have at least two possible positions in the channel, with the probability of occupancy governed by the membrane voltage, and it does not have a single, discrete binding site.

Some External Cations May Stabilize the Closed State in Ba\textsuperscript{2+}-blocked Channels

It was shown that Rb\textsuperscript{+}, NH\textsubscript{4}\textsuperscript{+}, or Cs\textsuperscript{+} in low millimolar concentrations greatly slow recovery from Ba\textsuperscript{2+} block. Concentrations of Rb\textsuperscript{+} as low as 0.1 mM were found to delay recovery from Ba\textsuperscript{2+} block. We think this results from occupation of a site at the external end of the channel, and that the site has a fairly high affinity for these cations. Occupation of the site could slow recovery either by making it less probable that Ba\textsuperscript{2+}-blocked channels open their activation gates on depolarization, or by hindering the movement of Ba\textsuperscript{2+} out of the channels once they are open. Low concentrations of these cations do not interfere with the outward movement of K ions through the channels, which suggests to us that the former possibility is somewhat more likely, i.e., the presence of NH\textsubscript{4}\textsuperscript{+}, Rb\textsuperscript{+}, or Cs\textsuperscript{+} in the postulated external site make it unlikely that activation gates open. These external monovalent cations apparently enhance the stabilization of the closed state that is due to Ba\textsuperscript{2+}. In the absence of Ba\textsuperscript{2+} these cations tend to hold K channels open (Dubois and Bergman, 1977; Swenson and Armstrong, 1981) rather than closed. The interactions of various cations and K channels are evidently complex, and not well understood.

Other Effects of Monovalent Cations

In addition to the slowing of recovery from Ba\textsuperscript{2+} block, monovalent cations have at least two other actions. K\textsuperscript{+} in high concentrations slows the apparent inactivation caused by internal Ba\textsuperscript{2+} (Armstrong and Taylor, 1980); and it also pushes Ba\textsuperscript{2+} out of K channels during a quick phase of recovery, after repolarization but before the gates close. Other cations share one or both of these properties. Although both Rb\textsuperscript{+} and NH\textsubscript{4}\textsuperscript{+} slow recovery from Ba\textsuperscript{2+} block, the two ions have opposite effects on $K_a$, Rb\textsuperscript{+} increasing it and NH\textsubscript{4}\textsuperscript{+} decreasing it.
decreasing it. Monovalent cation effects thus are complex. We are not yet in a position to give a coherent description of these complicated interactions with the K channel.

**Short-Term Modification of Channel Behavior**

The results presented in Fig. 1 show that K⁺ channels in the presence of Ba²⁺ have a short-term memory of preceding activity, as a result of activity dependent block (Fig. 1A) or unblock (Fig. 1B). Ba²⁺ is only one of many substances that cause activity or use dependent channel block. Persistence of block after a burst of activity varies from a few milliseconds (TEA⁺) to seconds (TEA⁺ derivatives) to minutes (Ba²⁺). Na channels also show activity-dependent block with recovery times from seconds to minutes after application of local anesthetics (Strichartz, 1973; Courtney, 1975; Hille, 1977; Lipicky et al., 1978; Cahalan, 1978; Khodorov et. al., 1976; Yeh, 1978). Much longer recovery times are easily conceivable, but might be hard to investigate in axons that have limited lifetimes. With aminopyridines (Yeh et. al., 1976; Kirsch and Narahashi, 1978; Meves and Pichon, 1977; Thompson, 1977), as well as external Ba²⁺, there is activity dependent relief of block, which causes a short-term increase in available channels after activity.

In general, activity dependent block and unblock must be included in any list of the possible bases for short-term modification of membrane behavior.

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