Activation of Squid Axon K⁺ Channels

*Ionic and Gating Current Studies*

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**ABSTRACT** We have used data obtained from measurements of ionic and gating currents to study the process of K⁺ channel activation in squid giant axons. A marked improvement in the recording of K⁺ channel gating currents (Ig) was obtained by total replacement of Cl⁻ in the external solution by NO₃⁻, which eliminates ~50% of the Na⁺ channel gating current with no effect on Ig. The midpoint of the steady state charge-voltage (Qₑ - V) relationship is ~40 mV hyperpolarized to that of the steady state activation (fₒ - V) curve, which is an indication that the channel has many nonconducting states. Ionic and gating currents have similar time constants for both ON and OFF pulses. This eliminates any Hodgkin-Huxley scheme for K⁺ channel activation. An interrupted pulse paradigm shows that the last step in the activation process is not rate limiting. Ig shows a nonartifactual rising phase, which indicates that the first step is either the slowest step in the activation sequence or is voltage independent. These data are consistent with the following general scheme for K⁺ channel activation:

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slowest   slow    fast
C₁ → C₂ → ... → Cₙ₋₁ → Cₙ → O
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**INTRODUCTION**

The opening of an ion channel involves a series of conformational changes that result in the formation of an ion-conducting pore. These conformational changes can be driven by voltage (e.g., the Na⁺ and K⁺ channels of nerve [Hodgkin and Huxley, 1952]), the binding of a ligand (e.g., the acetylcholine receptor channel [del Castillo and Katz, 1955]), or both (e.g., the Ca²⁺-activated K⁺ channel [Barrett et al., 1982]). There are three types of measurements one can make in the study of ion channel activation: macroscopic ionic currents (Hodgkin and Huxley, 1952; Nass et al., 1978), microscopic ionic or single channel currents (Aldrich et al., 1983; Leibowitz and Dionne, 1984), and, for voltage-gated channels, gating currents (Armstrong and Bezanilla, 1977). Each type of mea-
surement has its advantages and disadvantages and ideally one should combine information from each in the same preparation to determine the gating process.

This paper is concerned with the activation of the delayed K⁺ channel of squid giant axon. We have measured both macroscopic ionic and gating currents in response to various pulse protocols. When combined with steady state activation data, several features of the gating process become apparent. We use this information to construct a model that qualitatively describes the kinetic and equilibrium behavior of K⁺ channels.

Preliminary accounts of this work have already appeared (Bezanilla et al., 1982b; White et al., 1983).

**METHODS**

All experiments were performed at the Marine Biological Laboratory, Woods Hole, MA, using internally perfused, voltage-clamped giant axons of *Loligo pealei*. The voltage clamp, perfusion chamber, pulse generator, and data acquisition system used were identical to those described by Bezanilla et al. (1982a). Unless noted otherwise, temperature was maintained at 20.0 ± 0.3°C by means of a negative feedback circuit connected to a Peltier device mounted in the chamber.

Ionic and gating currents were recorded with a bandwidth of 50 kHz and a sampling rate of 10 μs/point and occasionally 5 μs/point using the P/−4 or P/4 procedure (Bezanilla et al., 1982a) to remove linear leakage and capacitive currents and stored on magnetic media for subsequent off-line analysis. Unless otherwise noted, all test pulses were made from a holding potential (HP) of −60 mV and the subtracting pulses were made from a subtracting holding potential (SHP) of −140 mV.

For measurement of gating currents, the internal solution was 166 mM N-methylglucamine (NMG)-glutamate, 33 mM NMG-F, and 10 mM Tris-Cl (NMG-FG); the external solution was either 520 mM Tris-methanesulfonate (MS), 50 mM Ca(MS)₂, 300 mM tetrodotoxin (TTX), and 0.25 mM dibucaine (Tris-MS-TTX), or 520 mM Tris-NO₃, 50 mM Ca(NO₃)₂, 300 mM TTX, and 0.25 mM dibucaine (Tris-NO₃-TTX). Each trace represents the average of 20–40 sweeps. For measurements of ionic currents, the internal solution was 140 K-glutamate, 60 mM KF, and 10 mM Tris-Cl (KFG), and the external solution was Tris-NO₃-TTX containing 2 mM KNO₃. For measurements of Na⁺ currents, the external solution was either 88 mM NaCl, 352 mM Tris-MS, 2 mM KCl, and 50 mM Ca(MS)₂ (1/5 Na-SW), or 104 mM NaNO₃, 416 mM Tris-NO₃, 50 mM Ca(NO₃)₂, and 2 mM KNO₃ (1/5 Na-NO₃-SW). Each trace represents a single sweep. All solutions were adjusted to 970 mosmol/kg using sorbitol and to pH 7.6–7.7 using the appropriate acid. Membrane potentials for gating currents were corrected for a junction potential of −7 mV for NMG-FG relative to KFG. The potential in KFG solutions was not corrected for junction potentials.

**RESULTS**

*Improvements in the Measurement of K⁺ Channel Gating Currents*

The measurement of K⁺ channel gating currents is complicated by the presence of the charge movement associated with Na⁺ channel activation. We have previously reported (Bezanilla et al., 1982b) that up to 50% of the Na⁺ channel gating current ($I_{Na}^{N}$) can be eliminated by the combination of shifting the holding potential from −70 to −60 mV (which slow-inactivates some of the Na⁺ channels)
and by the addition of 0.25 mM dibucaine (Gilly and Armstrong, 1980) to the external solution. Neither of these conditions affects the steady state or kinetic properties of K\(^+\) activation. Although one can clearly see the K\(^+\) channel gating currents \((I_g^K)\) (the slow component of the recorded gating current [Bezanilla et al., 1982b]), the early phase of \(I_g^K\) is still masked by \(I_g^{Na}\).

In a search for an anion for the external solution that would decrease the small nonlinear leakage currents present, we found that total replacement of MS\(^-\) or Cl\(^-\) by NO\(^-\) decreased both \(I_{Na}\) and \(I_g^{Na}\) by \(-50\%\) with no effect on either \(I_K\) or 

\[ \text{FIGURE 1. NO}^-\text{selectively inhibits Na}^+\text{ channel ionic and gating currents. Upper panel: the left-hand trace shows gating current in response to a pulse from } -60 \text{ to } +20 \text{ mV recorded from an axon bathed in Tris-MS-TTX. The external solution was then changed to Tris-NO}_3\text{-TTX and gating current was recorded in response to the same pulse. Note that the fast component (I"Na") is decreased while the slow (I"g") is not. Lower panel: ionic current (I}_K + I_{Na}\text{) was recorded from a different axon in response to the same pulse pattern as above. The left-hand panel shows the currents recorded when the axon was bathed in 1/5 Na-SW. The external solution was changed to 1/5 Na-NO}_3\text{-SW and the current shown in the right panel was recorded. Note that I}_{Na}\text{ is decreased while } I_K \text{ is not. The temperature for the ionic current measurements was } 6^\circ\text{C.} \]

\(I_g^K\). Fig. 1 shows gating and total \((I_k + I_{Na})\) ionic currents recorded from the same axon before and after the external solution was changed from either Tris-MS-TTX to Tris-NO\(_3\)-TTX (upper traces) or 1/5 Na-SW to 1/5 Na-NO\(_3\)-SW (lower traces). The effect is rapid (within the exchange time of the chamber) and reversible. What \(I_g^{Na}\) and \(I_{Na}\) remains has a time course similar to that recorded before the solution change. All subsequent data were taken using Tris-NO\(_3\)-TTX as the external solution. On the basis of the reduction in the peak of the \(I_g\) transient, we estimate that only \(~25\%\) of \(I_g^{Na}\) (relative to that found in axons held at \(-70\) mV in dibucaine- and NO\(_3\)-free solutions) is detectable under these conditions.
Fig. 2 shows $K^+$ ionic and gating currents in response to a pulse from $-60$ to $0$ mV (ON) and from $0$ to $-60$ mV (OFF). Note that $I_K$ and $I_{K^+}^*$ have similar time courses for OFF currents and the final approach to steady state for ON currents is similar. This will be described in greater detail in a later section.

Steady State Properties

The voltage dependence of $Q_{rel}$, the normalized charge movement, was determined in several ways. (a) $I_{K^+}^*$ in response to a test pulse from $-60$ mV was extrapolated to zero time using a single-exponential fit and the fitted curve was integrated. (b) The test pulse was preceded by a large hyperpolarizing prepulse to induce a delay in $K^+$ channel activation (Cole and Moore, 1960; Bezanilla et al., 1982b). This delay gives a clear breakpoint between $I_{K^+}^*$ and $I_{Na}$. $I_{K^+}^*$ was integrated from this breakpoint on. (c) The charge carried in $I_{K^+}^*$ tail currents at $-60$ mV was determined in response to a test pulse of given amplitude. At 20°C, about one half of $I_{Na}^*$ at $-60$ mV is over after 25 μs (determined in separate experiments with data taken at a sampling rate of 4 μs/point) and the contamination of $I_{K^+}^*$ by $I_{Na}^*$ is negligible. The bulk of the data were obtained using the third method, but all three gave similar results (Fig. 3A). Fig. 3B illustrates the advantage of the third method of determining $Q_{rel}$. The figure shows the gating current recorded in response to a pulse from $-110$ to $0$ mV, followed by a return to $-60$ mV. The ON gating current shows a clear breakpoint between the
FIGURE 3. (A) Comparison of methods of determining the $Q_{\text{rel}} - V$ relationship. $Q_{\text{rel}}$ was determined using the three methods described in the text: on gating currents (method a, triangles), on gating currents with a prepulse (method b, solid circles), and off gating currents (method c, open circles). See text for details. Each set of points represents a single axon. The data for methods b and c were obtained from the same axon. (B) $I_g^k$ tail currents are well separated from $I_g^{Na}$. Gating current was recorded in response to a pulse to 0 mV preceded by a 5-ms prepulse to −110 mV and followed by a return to −60 mV. The arrow indicates the small amount of $I_g^{Na}$ present in the tail current.
fast $I_{Na}^f$ and the slower $I_{K}$. However, $I_{Na}^f$ is large and its time constant (as well as that of $I_{K}$) will vary with the test voltage, so the possibility of contamination of $I_{K}$ at some potentials is quite real. The use of OFF gating currents, on the other hand, avoids this problem. $I_{Na}^f$ is very fast at $-60$ mV; it is indicated by the arrow in the figure. Since the measurement is made at the same potential for each test pulse, the time constants for $I_{Na}^f$ and $I_{K}$ are the same for each determination. At $-60$ mV, the time constants are $\sim 40$ ms for $I_{Na}^f$ and $\sim 400$ ms (Fig. 5) for $I_{K}$, which makes kinetic contamination of $I_{K}$ by $I_{Na}^f$ highly unlikely.

The voltage dependence of $f_o$, the relative fraction of open channels, was obtained from the conductance-voltage relationship determined as described by Gilly and Armstrong (1982), which minimizes complications caused by accumulation of $K^+$ in the periaxonal space since it does not require knowledge of the $K^+$ reversal potential. Once the conductance-voltage relationship has been determined, a correction must be made for the nonlinearity of the single channel conductance. The macroscopic $K^+$ conductance at a given voltage, $g(V)$, is given by

$$g(V) = N\gamma(V)f_o;$$

$$g(V_{max}) = N\gamma(V_{max}),$$

where $N$ is the total number of channels, $\gamma(V)$ is the (weakly) voltage-dependent single channel conductance, and $V_{max}$ is the voltage at which $f_o = 1$. To convert from the normalized conductance $g(V)/g(V_{max})$ to $f_o$, we use

$$f_o(V) = g(V)/g(V_{max}) \cdot \phi(V),$$

where $\phi(V) = \gamma(V_{max})/\gamma(V)$. The term $\phi(V)$ is obtained from the instantaneous $I-V$ curve, which reflects the single channel behavior. Although this correction is small [$\phi(V)$ increases smoothly from 0.83 at $-70$ mV to 1.00 at $+50$ mV], nonetheless it must be included to obtain the correct $f_o - V$ relation. As a verification of this method, we have obtained similar $f_o - V$ curves using instantaneous $I-V$ curves measured at the end of each test pulse to obtain $\phi(V)$.

Fig. 4 shows that both $Q_{rel}$ and $f_o$ have a sigmoid voltage dependence. The midpoint of the curves is $-35$ mV for $Q_{rel}$ and $+5$ mV for $f_o$. The maximum amount of charge moved, $Q_{max}$, is $490.3 \pm 37.9$ e$^-$/\mu m$^2$ (mean $\pm$ SEM of six separate axons), and the maximum conductance, $g_{max}$, was $45.3 \pm 2.1$ mS/cm$^2$ (mean $\pm$ SEM of four separate axons). Using published estimates of $K^+$ channel density in squid axons ($\sim 70$/\mu m$^2$; Armstrong, 1966) and our value for $Q_{max}$, we obtain a value of $\sim 7$ e$^-$/channel. Given the uncertainty in the determination of the channel density, this value agrees well with the 5–6 e$^-$/channel expected from macroscopic current measurements (data of Fig. 4 for $f_o \leq 0.07$ and Almers and Armstrong, 1980). The displacement of the $Q_{rel} - V$ curve relative to the $f_o - V$ curve indicates that an appreciable amount of charge moves during transitions among nonconducting states of the channel.

**Gating and Ionic Currents Have Similar Time Courses**

Fig. 5 shows the time constants of activation ($\tau_{on}^f$, $\tau_{on}^K$) and deactivation ($\tau_{off}^f$, $\tau_{off}^K$) as functions of voltage. The ionic and gating tail currents could be fit as single-
exponential decays after the first 50 μs. The time constant for ionic current activation was operationally defined as the time constant obtained by fitting the ionic current after the initial lag period to a single-exponential process (see Fig. 2). $\tau^{\text{on}}_k$ was obtained by fitting the late phase of $I_k^{\text{on}}$ to a single exponential. In all cases, a single exponential was sufficient for a period of at least three time constants. The figure shows that the time constants of both $I_k$ and $I_k^{\text{on}}$ for both ON and OFF currents are identical over an extended voltage range.

The Last Step in the Activation Process Is Not Rate Limiting

As a first step in the elucidation of the activation mechanism, one can ask which step in the process is rate determining. The interrupted-pulse protocol described by Oxford (1981) was used to isolate the last step in the activation process. Channels were opened with a 10-ms prepulse to 0 mV and the voltage was returned to −60 mV. After a variable amount of time, $D$, the voltage was stepped back to 0 mV and the resulting ionic current was recorded. During the interpulse duration $D$, the channels begin to close and relax through the various closed states toward the state(s) favored at −60 mV. If $D$ is very long, the channels will relax all the way to those states and the current elicited by the test pulse will be identical to that from the conditioning pulse. As $D$ becomes shorter and shorter, the channels relax through fewer and fewer of the closed states. If $D$ is short enough, only the first transition, the actual closing of the channel, is made, and the kinetics upon depolarization should reflect only the actual opening of the channel. If this step is rate-determining, the measured time constant of activation, $\tau^{\text{on}}_k$, will not depend upon the length of the interpulse duration, $D$. 

![Figure 4. Steady state properties of K⁺ channel activation. The fraction open channels, $f_o$ (solid circles), and the relative charge moved, $Q_{rel}$ (open circles), were determined as described in the text. The curves were drawn by eye and each point represents the mean ± SEM of four ($f_o$) or six ($Q_{rel}$) axons. The midpoints of the $f_o - V$ and $Q_{rel} - V$ curves are +5 and −35 mV, respectively.](image-url)
Fig. 5. Ionic and gating currents have similar time constants. The solid circles represent the measured time constant for ionic currents in response pulses from -60 (ON) or 0 mV (OFF) to the indicated voltage, while the open circles are the time constants for gating currents in response to the same pulses. Each set of points represents single determinations on one axon.

Fig. 6 shows that this is not the case. As the interpulse duration becomes shorter and shorter, the shape of the current changes and the currents do not superpose with a time delay. \( \tau_{on} \) becomes smaller and smaller; when \( D = 10 \text{ ms} \), \( \tau_{on} = 600 \mu\text{s} \), and when \( D = 60 \mu\text{s} \), \( \tau_{on} = 260 \mu\text{s} \). This result clearly demonstrates that the actual opening of the channel is not rate limiting.

Fig. 6. The last step in the activation process is not rate limiting. (A) Ionic currents were measured in response to the pulse protocol shown in the bottom of the figure. The arrow marks the start of data acquisition. The three traces correspond to values of \( D \) (from left to right) of 0.3, 1.0, and 10.0 ms. (B) The time constant for the final exponential phase of the ionic current was measured for values of \( D \) ranging from 60 \( \mu\text{s} \) to 10 ms. Note that \( \tau_{on} \) depends upon \( D \).
**$I_g^K$ Shows a Rising Phase**

The shape of the gating current at the start of the voltage step can provide information concerning the early steps in the activation process that cannot be obtained from either macroscopic or microscopic ionic current measurements. In our earlier experiments (Bezanilla et al., 1982b), the initial shape of $I_g^K$ was masked by $I_g^Na$ and little could be said about the early stages of $K^+$ channel activation. The use of NO₃ in the external solution eliminates enough $I_g^Na$ to make the early phase of $I_g^K$ visible. Fig. 7 shows a gating current in response to a step to 0 mV preceded by a 5-ms prepulse to −120 mV. The effect of the hyperpolarizing prepulse is to delay the activation of $K^+$ channels, which results in a lag in both the ionic (Cole and Moore, 1960) and gating currents (Bezanilla et al., 1982b). A rising phase with a time to peak of ~400 μs is clearly visible. The more negative the prepulse, the more pronounced the rising phase becomes.

**Figure 7.** $I_g^K$ shows a pronounced rising phase. The gating current was recorded in response to a pulse to 0 mV preceded by a 5-ms prepulse to −120 mV. The fast spike is all that remains of $I_g^Na$.

In addition, the time constant of the declining phase of $I_g^K$ is not changed by the prepulse (data not shown).

**Discussion**

The experiments described in this paper were designed to examine different facets of $K^+$ channel activation. There are many different types of measurements one can make (steady state $Q_{rel} - V$ relationship, ionic tail currents, etc.), and each type provides information concerning the activation process. In practice, no single measurement provides all of the information necessary to construct a kinetic model that can adequately describe channel gating under various experimental conditions. It is only after amalgamation of the interpretation of the various experimental results that a viable picture of the gating process emerges.

Almers and Armstrong (1980) have reported that $K^+$ channel ionic currents
are irreversibly abolished after perfusion of the axon with solutions lacking permeant cations (i.e., TMA-based solutions). We have found this to be the case when axons are either perfused or dialyzed with either NMG- or TEA-based solutions. At 20°C, the K+ currents “die” within 30 min. We tried to record \( I_g^k \) in axons perfused with Cs+, which should protect K+ channels (Almers and Armstrong, 1980), but were unable to do so because Cs+ currents through K+ channels are clearly visible at 20°C and are comparable in size to \( I_g^k \). Why do we continue to record what we consider to be \( I_g^k \) in the absence of “functional” channels? We can think of two possible reasons. First, the absence of permeant cations could destroy the channel’s permeation process without affecting the gating. Alternatively, the lack of permeant ions could eliminate one or more steps in the gating process. We could not detect any change in \( I_g^k \) as the ionic currents expired. This suggests that the first possibility may be true. However, model simulations show that if the last step in the activation process (the actual opening of the channel) is the one removed, the gating currents may not be appreciably affected. In this case, it would be possible to measure \( I_g^k \) in the absence of conducting channels. We are unable to decide between these two possibilities.

Gilly and Armstrong (1980) have described a component of gating current at 6°C that disappears after the above-mentioned “destruction” of K+ channels. They suggest that this fraction may be part or all of \( I_g^k \). However, no data concerning the voltage dependence of either the charge moved or the kinetics of the charge movement were presented. In the absence of this information, the relation between this component and K+ channel activation must be considered tentative.

**There Are Many More Than Two Kinetic States**

The displacement of the \( Q_{rel} - V \) curve relative to the \( f_e - V \) curve (Fig. 4) indicates that there is more than one closed state. If there is more than one closed state, charge movement could take place between the nonconducting states without channel opening and the \( Q_{rel} - V \) would be shifted to the left. In general, the more closed states there are, the greater the displacement of the curves. However, it is not possible to determine the number of closed states from the displacement, as this depends on the particular kinetic scheme (Bezanilla, 1982). The use of gating tail currents to determine \( Q_{rel} \) (method c; Fig. 3B) makes it highly unlikely that our measured \( Q_{rel} \) contains charge carried by the Na+ channel gating process. The displacement, although large, appears to be genuine.

**The Hodgkin-Huxley Scheme Does Not Adequately Describe the Data**

The Hodgkin-Huxley (1952) \( n^4 \) scheme makes strong predictions concerning the kinetics of both ON and OFF gating currents. First, the ON gating currents should follow a single exponential with a time constant \( \tau_n \). The rising phase seen in Fig. 7 shows that this is not the case. Second, the model predicts that the time constant of the gating tail current, \( \tau_{g^{off}} \), will be four times that of the ionic tail current, \( \tau_{ik} \). Fig. 5 shows that \( \tau_{g^{off}} = \tau_{ik} \) over an extended voltage range. Both behaviors eliminate not only the \( n^4 \) model, but any model based on the movement
of one or more identical \( n \)-type particles. It should be noted that Gilly and Armstrong (1982) eliminated both the \( n^4 \) and \( n^6 \) schemes on the basis of poor fits to ionic currents.

**The Last Step Is Not Rate Limiting**

The interrupted pulse protocol shown in Fig. 6 has been used by several workers to study the last step in channel activation. Using this procedure, Oxford (1981) concluded that the last step in Na\(^+\) channel activation is the slowest. Gilly and Armstrong (1982) applied this to the study of K\(^+\) channel activation and concluded that the last step in the activation sequence is not rate limiting. Our data support this conclusion. It must be pointed out that the interrupted pulse paradigm can tell us nothing about the last step if that step is not rate determining; at best it can put an upper limit on the transitional time constant of the last step (the reciprocal of the sum of the forward and backward rate constants).

Conti and Neher's (1980) records of single channel fluctuations from K\(^+\) channels in squid giant axons show that once a channel opens, it rapidly flickers between an open and closed state for a brief period and then closes for a longer period. This implies that the last step in the activation sequence is faster than the preceding one. Llano's (1983) studies of single channel fluctuations and the bursting activity of squid axon K\(^+\) channels support this conclusion. Our observations are not at odds with this notion.

**The First Step in the Activation Process Is Slow or Voltage Independent**

The clear rising phase in \( I_K \) shown in Fig. 7 tells us much about the first step in the activation sequence. The rising phase is genuine; it is not modified by series resistance compensation and in any case is too slow to be attributed to a series resistance effect. It is not an artifact of the P/\(-4\) procedure as we have varied the subtracting holding potential between -140 and -110 mV with no effect on the rising phase.

There are two conditions that can produce a nonartifactual rising phase (Bezanilla and Taylor, 1982). If the first step is either voltage independent or the slowest in the sequence, the charge movement will show a delay that translates into a rising phase in its time derivative, the gating current. Simulations show that the first step need only be approximately half as fast as the next slowest step to give a rising phase, and that the rising phase becomes more pronounced as the first state becomes more populated with hyperpolarization.

Gilly and Armstrong (1982) have described a model for K\(^+\) channel activation in which the early steps in the activation sequence are equally fast and the next-to-last step is the slowest in the sequence. The assignment of the slow step was based on model simulations that produced a gating current similar to that described by those authors in a previous report (Gilly and Armstrong, 1980), which has already been discussed.

**A Model for \( K^+ \) Channel Activation**

Any kinetic model for K\(^+\) channel activation should reproduce the following behavior: (a) the shapes and positions of the \( f_c - V \) and \( Q_{in} - V \) curves; (b) similar time constants for ionic and gating currents; (c) the decrease in \( r_{in} \) as \( D \) decreases
in an interrupted-pulse experiment; (d) the Cole-Moore shift seen for both ionic and gating currents; (e) flickering single channels.

We have chosen to model the activation as a linear sequential model with a single open state preceded by \( n - 1 \) closed states. The interpretations of the data presented earlier suggest that the following is appropriate:

\[
\begin{align*}
\text{slowest} & \quad C_1 \xrightarrow{} C_2 \xrightarrow{} \ldots \xrightarrow{} C_{n-2} \xrightarrow{} C_{n-1} \xrightarrow{} O \\
\text{slow} & \quad C_2 \xrightarrow{} \ldots \xrightarrow{} C_{n-2} \xrightarrow{} C_{n-1} \xrightarrow{} O
\end{align*}
\] (Scheme 1)

The first step in the sequence is the slowest; this produces the rising phase in the gating current that increases with hyperpolarization. We have incorporated Conti and Neher's (1980) conclusion that the last step is faster than the preceding one in order to obtain flickering single channels. We have no information concerning the intermediate steps other than the fact that they must be faster than the first and that there are many of them (this is due to the large displacement of the \( f_o - V \) curve relative to the \( Q_{rel} - V \) curve).

We have been unable to produce a model that quantitatively predicts channel gating behavior under a wide range of conditions. We have, however, been able to qualitatively reproduce the gating behavior (see Appendix). Fig. 8 shows the
predictions from this model. The ionic and gating currents show a Cole-Moore shift in response to a hyperpolarizing prepulse to \(-120\) mV and the gating currents show a pronounced rising phase when the test pulse is preceded by the prepulse. The single channels flicker and, in addition, show bursting activity similar to that described by Llano and Bezanilla (1983) (not shown).

Although the generated gating currents are comparable to the experimental ones, the ionic currents are not. We have been unable to obtain the long lag followed by the sharp rise seen in the ionic currents of Fig. 2 and still obtain acceptable gating currents and maintain the correct shapes and positions of the \(f_0 - V\) and \(Q_{rel} - V\) curves. Taylor and Bezanilla (1983) had similar problems in modeling Na\(^+\) currents. Correction of current records for accumulation of K\(^+\) in the periaxonals space using model I of Frankenhaeuser and Hodgkin (1956) improves the situation slightly, but by no means solves the problem.

Our failure to obtain a model that quantitatively predicts K\(^+\) channel gating behavior is due to one of three possible causes. First, not all the charge movement that we call \(I_g\) is actually due to the gating of K\(^+\) channels. The agreement between the time course of \(I_g^o\) and \(I_K\) (Fig. 5), the correlation of \(Q_{max}\) with the steepness of the \(f_0 - V\) curve (Fig. 4), and the fact that \(I_g^o\) and \(I_K\) show similar Cole-Moore shifts (Bezanilla et al., 1982b) lead us to conclude that if there is contamination of K\(^+\) channel gating currents by charge movement associated with other voltage-dependent processes, it is negligible.

Second, the combination of our untested assumptions concerning the Eyring modeling and a lack of herculean perseverance may have caused us to miss the correct parameters. Although uninteresting, this remains a very real possibility. Third, a linear sequential model may not adequately describe the gating process. We have tried variations of the box models proposed for Na\(^+\) channel activation (Armstrong and Bezanilla, 1977; Bezanilla and Taylor, 1982) and they offer no improvement. The aggregation model of Baumann and Easton (1980) is eliminated on theoretical grounds as it cannot produce gating currents with nonartifactual rising phases (Baumann and Easton, 1982). The aggregation model introduced by Muller and Peskin (1981) to describe monazomycin conductance kinetics can produce gating currents with rising phases, but those authors were unable to fit their model to K\(^+\) channel ionic currents. In addition, the model cannot produce the shape change of the ionic currents in response to the triple-pulse protocol seen in Fig. 6. The diffusional model of Neumcke et al. (1979) can be considered the same as the Hodgkin-Huxley model in the limit \(n \rightarrow \infty\), and is therefore eliminated for the same reasons as the Hodgkin-Huxley schemes.

We are unable to distinguish between these last two possibilities. Measurement of single K\(^+\) channel fluctuations (Conti and Neher, 1980; Llano, 1983; Torres et al., 1984) will enable one to measure directly the rate constant for channel closing as a function of voltage in order to test the validity of our assumptions concerning the shape of at least one of the energy barriers for the conformational changes involved in channel activation. Despite the failure of the models to quantitatively describe K\(^+\) channel activation, we feel that the basic picture of the activation process shown in Scheme 1 holds.
APPENDIX

Linear Sequential Model for $K^+$ Channel Activation

We have chosen to model $K^+$ channel activation as a linear sequential process with a single open state preceded by $n - 1$ closed states. The conformational changes involved in the activation sequence are modeled as Eyring processes. We assume that during the transition between any two consecutive states, $x_i$ and $x_{i+1}$, a charge $z_i$ moves across an energy barrier with zero-voltage free energies $W_{A,i}$ and $W_{B,i}$ for the forward and backward directions, respectively. We further assume that each charge moves through the entire field and that the energy barriers are symmetrical. (Although we have no reason to believe this to be true, it reduces the number of parameters needed per transition.) We can use Eyring rate theory to write the forward, $\alpha_i(V)$, and backward, $\beta_i(V)$, rate constants for each transition as functions of voltage:

$$\alpha_i(V) = \frac{kT}{h} \exp \left( -\frac{W_{A,i} + \frac{zeV}{2kT}}{2kT} \right)$$

$$\beta_i(V) = \frac{kT}{h} \exp \left( -\frac{W_{B,i} - \frac{zeV}{2kT}}{2kT} \right)$$

where $e$ is the electronic charge, and $k$, $h$, and $T$ have their usual meanings. $W_{A,i}$ and $W_{B,i}$ are given in units of $kT$. A total of $3(n - 1)$ parameters ($z_i$, $W_{A,i}$, and $W_{B,i}$ for each transition) are needed to generate the $f_o - V$ and $Q_{tot} - V$ curves and the ionic and gating currents in response to any pulse protocol. Models were constructed with the goal of predicting all behaviors at all potentials. Parameters were chosen by trial and error guided by intuition gained through the examination of the behavior of simpler models. Models were judged by eye.

We have assigned a total of 5 $e^-$/channel in our model in order to obtain the correct steepness for the foot of the $f_o - V$ curve, e-fold/4–5 mV (Fig. 4 and Almers and Armstrong, 1980). Table A1 presents the parameters used to generate the traces shown in Fig. 8. The model consists of 15 closed and 1 open state. As described in the Discussion, the data indicate that the first step must be the slowest and that the next-to-last step must be slower than the last. We have no information concerning the intermediate steps other than that they must be faster than the first, so we have arbitrarily made them identical in order to reduce the number of parameters needed for the model. The model is more complicated than the six-state model proposed by Gilly and Armstrong (1982) because we must take into account the gating current data, which were not available to those authors.

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| $i$ | $z_i$ | $W_{A,i}$ | $W_{B,i}$ |
|-----|------|----------|----------|
| 1   | 0.350| 22.1     | 21.7     |
| 2–13| 0.303| 18.7     | 19.3     |
| 14  | 0.350| 21.1     | 22.1     |
| 15  | 0.660| 19.1     | 20.1     |
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