Ion effects on gating of the Ca^{2+}-activated K^+ channel correlate with occupancy of the pore

Susan D. Demo and Gary Yellen

Howard Hughes Medical Institute and the Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205 USA

ABSTRACT We studied the effects of permeant ions on the gating of the large conductance Ca^{2+}-activated K^+ channel from rat skeletal muscle. Rb^+ blockade of inward K^+ current caused an increase in the open probability as though Rb^+ occupancy of the pore interferes with channel closing. In support of this hypothesis, we directly measured the occupancy of the pore by the impermeant ion Cs^+ and found that it strongly correlates with its effect on gating. This is consistent with the "foot-in-the-door" model of gating, which states that channels cannot close with an ion in the pore. However, because Rb^+ and Cs^+ not only slow the closing rate (as predicted by the model), but also speed the opening rate, our results are more consistent with a modified version of the model in which the channel can indeed close while occupied, but the occupancy destabilizes the closed state. Increasing the occupancy of the pore by the addition of other permeant (K^+ and Tl^+) and impermeant (tetraethylammonium) ions did not affect the open probability. To account for this disparity, we used a two-site permeation model in which only one of the sites influenced gating. Occupancy of this "gating site" interferes with channel closing and hastens opening. Ions that directly or indirectly increase the occupancy of this site will increase the open probability.

INTRODUCTION

Ion channels are integral membrane proteins that open and close in response to particular stimuli. There are several well characterized stimuli that have been shown to regulate the gating transitions of different types of ion channels. For example, membrane depolarization causes voltage-dependent Na^+, Ca^{2+}, and K^+ channels to open. Other channels open in response to agonist binding, and still others are opened by mechanical deformation of the membrane.

In the open state, ions cross the membrane through an aqueous pore formed in the protein. Although ion permeation classically was thought to be independent of gating, an increasing body of evidence suggests that channel gating is sensitive to the concentration and species of the permeant ion. Blockade of voltage-dependent Na^+ and K^+ channels by impermeant ions such as tetraethylammonium (TEA), N-methylstrychnine, and pancuronium has been shown to hinder channel closing (Armstrong, 1971; Yeh and Armstrong, 1978; Cahalan and Almers, 1979). Similarly, local anesthetic blockade of the acetylcholine receptor prolongs channel openings (Neher and Steinbach, 1978). Yeh and Armstrong (1971) proposed that the channel blocker acts as a "foot-in-the-door" of the closing gate.

A similar phenomenon has been shown to occur with permeant ions. The lifetime of the open state of the excitatory acetylcholine receptor from *Aplysia*, for example, increases in the presence of permeant ions with high affinity (Ascher et al., 1978; Marchais and Marty, 1979). Similarly, the rate of decay of the acetylcholine-mediated inhibitory postsynaptic potential in *Aplysia* increases with the permeability of the current carrying species (Adams et al., 1982). This behavior suggests that the longer an ion resides in the pore during permeation, the greater the channel's open time, as though the ion restricts channel closing.

Swenson and Armstrong (1981) used the foot-in-the-door model of blocker interaction with gating to describe the effects of permeant ions on the delayed rectifier K^+ channel from squid axon. They suggested that the channel cannot close with an ion in the pore. Moreover, a detailed study of the permeant ion dependence of gating of this channel suggests that there is a particular site in the pore which influences gating (Matteson and Swenson, 1986). Permeant ions alter gating by directly or indirectly changing the occupancy of this "gating site."

A similar "gating site" has been described for the Ca^{2+}-activated K^+ channel (BK channel) (Neyton and Pelleschi, 1991). In contrast to the predictions of the foot-in-the-door model, this channel indeed can close while blocked by a Ba^{2+} (Miller et al., 1987) or Na^+ ion (Neyton and Pelleschi, 1991). The stability of the closed and blocked state is modulated by the occupancy of a "gating site" called the external lock-in site (ELI), which is located external to the site of Ba^{2+} blockade (Neyton and Pelleschi, 1991). Occupancy of this site by ions from the external side of the channel speeds channel opening from the closed and blocked state.

We have studied the gating behavior of the BK

channel from rat skeletal muscle in the presence of different permeant and impermeant ions. In particular, we directly measured the occupancy of the pore by the impermeant ion, Cs^+, and found that it strongly correlates with its effect on the open probability of the channel. This suggests that ions influence gating through their interaction with permeation sites in the pore.

MATERIALS AND METHODS

Bilayer formation and channel incorporation

Plasma membrane vesicles were prepared from rat skeletal muscle as described by Moczydlowski and Latorre (1983). Planar lipid bilayers were formed using the painting method, with a lipid solution composed of 8 mM 1-palmitoyl, 2-oleoyl-phosphatidylethanolamine (POPE), and 2 mM 1-palmitoyl, 2-oleoyl-phosphatidylcholine (POPC) obtained from Avanti Polar Lipids, Inc. (Birmingham, AL) in n-decane. BK channels were inserted into the planar lipid bilayers by the fusion of the plasma membrane vesicles added to the *cis* chamber under an osmotic gradient as previously described (Latorre et al., 1982). After insertion the *cis* chamber was perfused to the recording solution to remove excess membrane vesicles and prevent multiple insertions. All internal solutions contained Ca^{2+} buffered to the noted concentration with nitrilotriacetic acid (NTA) (Sigma Chemical Co., St. Louis, MO); buffers were computed using the stability constants from Martell and Smith (1974). Experiments were performed at room temperature of 21-24°C.

For experiments with the channel carrying inward current, the external solution contained 195 mM KCl, 10 mM 4-morpholinepropanesulfonic acid (MOPS), and 0.5 mM ethylenediaminetetraacetic acid (EDTA) (pH adjusted to 7.2 with KOH). The internal solution contained 5 KCl, 10 MOPS, 390 sorbitol (pH adjusted to 7.2 with KOH), and the noted Ca^{2+} concentration. Test ions were added directly to the external chamber.

For experiments in which the channel was carrying outward current, the external solution contained 195 NaCl, 0.5 EDTA, and 10 MOPS (pH adjusted to 7.2 with NaOH). The internal solution in these experiments contained 195 KCl, 10 MOPS (pH adjusted to 7.2 with KCl), and the noted Ca^{2+} concentration. Test ions were added directly to the internal chamber.

Recording and data acquisition

Currents were measured using standard voltage clamp techniques (Sigworth, 1983). Briefly, the bilayer was voltage clamped and the transmembrane current was measured with a current-to-voltage converter (Alvarez, 1986). A 200-mM salt bridge (usually KCl, except when the external K^+ concentration was zero, in which case NaCl was substituted for KCl) was used to connect the Ag/AgCl electrodes to the recording chambers in order to minimize junction potentials. The current signal was filtered through an eight pole Bessel filter (Frequency Devices, Haverhill, MA) set at 0.5 kHz and sampled at 1 kHz by an Indec PDP 11/73 computer (Sunnyvale, CA). The signal also was fed to a video tape recorder through a PCM recorder for off-line analysis. Physiological sign conventions were used so that the *trans* (or external chamber) was defined as electrical ground.

Since single channels occasionally exhibit sudden or gradual shifts in their open probability, we used data only from those channels that exhibited stable gating over the course of an experiment.

Data analysis

Opening and closing transitions were identified using a 50% threshold criteria. The open probability was calculated by dividing the time spent above threshold by the total observation time. The lifetimes of the open and closed states were determined from log binned duration histograms (Sigworth and Sine, 1987) fit with a simplex search algorithm (Press et al., 1986), using a maximum likelihood estimate (Jackson, 1986; Colquhoun and Sigworth, 1983). Currents recorded in the presence of > 500 μM internal Ca^{2+} showed bursts of activity that were interrupted by nonconducting periods that corresponded to Ca^{2+} blockade (Vergara and Latorre, 1983). We were able to confine our analysis to the bursting activity, avoiding blocking events (nonconducting times ≥ 500 ms were excluded from the analysis). The open and closed times obtained from the duration histograms were corrected for the likelihood of missing events by the methods of Blatz and Magleby (1986), though application of this correction did not significantly alter the estimated time constants. Current-voltage relationships were generated from voltage ramped single channel currents that were analyzed as described by Yellen (1982).

RESULTS

Rb^+ blockade of inward K^+ current causes an increase in the open probability

We tested the effects of external permeant ions under conditions where the channel was carrying inward K^+ current (200 mM external and 10 mM internal [KCl]). Rubidium ions added to the external solution caused a reduction in the single channel current size (Fig. 1 *a*). Eisenman et al. (1986) have previously shown that this reduction in current size occurs because Rb^+ has a higher affinity than K^+ for a binding site in the pore and, therefore, carries less current. They showed that, under these conditions, Rb^+ is in a saturable competition with K^+ for movement through the channel and, because Rb^+ moves through the channel more slowly than K^+, the amount of current observed during a given opening is reduced. These findings are demonstrated in Fig. 1 *b*, which shows a current-voltage (I-V) relationship in the presence and absence of external Rb^+.

The reduction of current with Rb^+ is accompanied by an increase in the open probability (Fig. 1 c). This result is consistent with the foot-in-the-door model described by Swenson and Armstrong (1981). Since Rb^+ has a higher affinity than K^+, it spends more time in the channel, thereby increasing the occupancy of the pore. In the model, the increase in occupancy increases the open probability because the channel cannot close with an ion in the pore.

This model predicts that only the closing rate should be affected by Rb^+. In order to test this hypothesis, we measured the distribution of open and closed durations in the presence and absence of external Rb^+ (Table 1). With our recording conditions, the normal gating mode
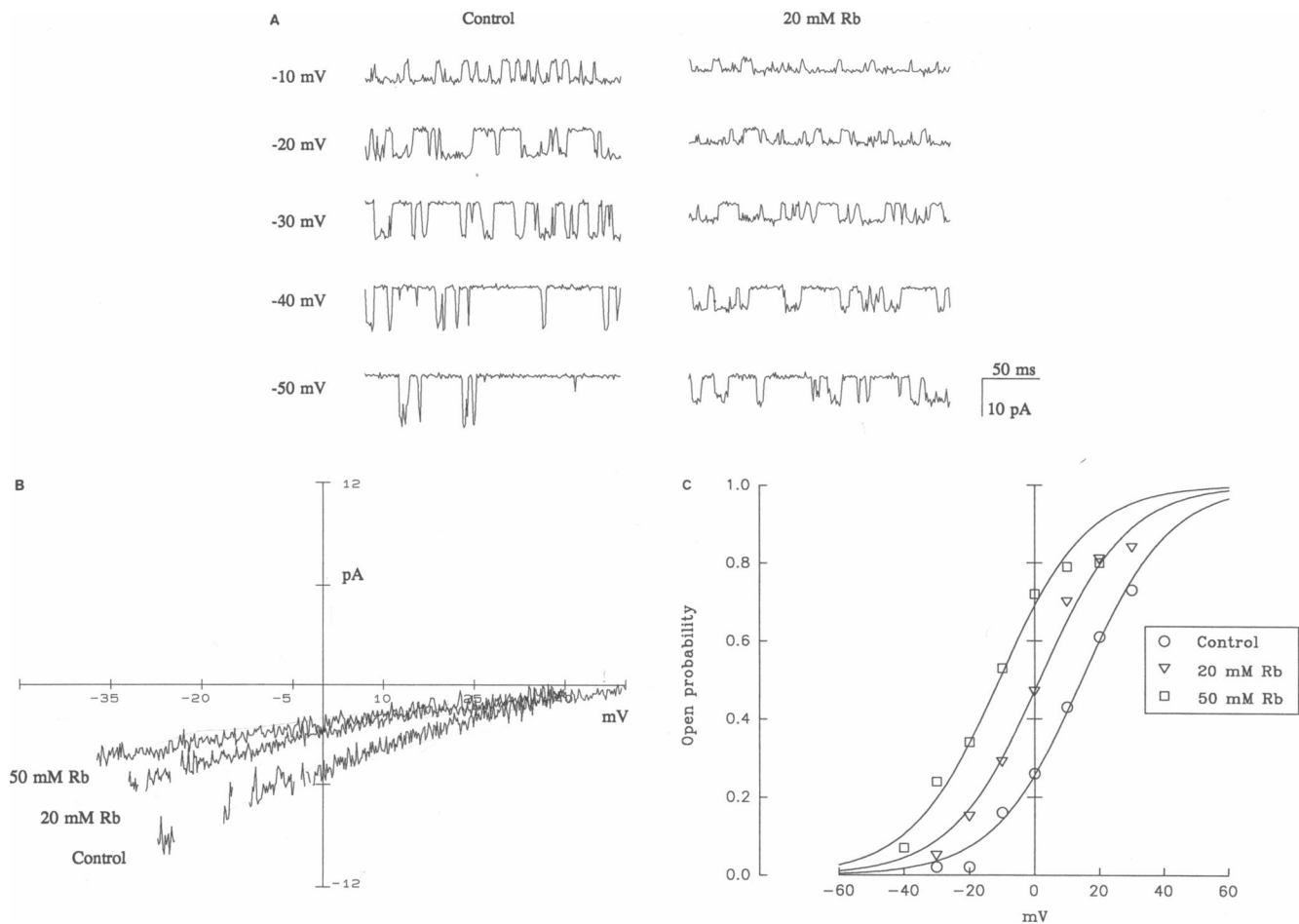
FIGURE 1 Rb⁺ blockade of inward K⁺ current caused an increase in the open probability. (*a*) Single BK channel currents were observed in the presence of 200 mM external K⁺ and 10 mM internal K⁺ at several holding voltages (*left*). The addition of 20 mM Rb⁺ to the external solution reduced the single channel current size (*right*). Channel openings are downward. (*b*) The I-V relationship shows the blockade of inward K⁺ current by the addition of 20 and 50 mM Rb⁺ to the external solution. Curves were determined from single channel currents recorded during ramped voltage pulses. (*c*) The open probability is plotted as a function of voltage in the presence and absence of external Rb⁺. The addition of 20 mM external Rb⁺ shifted the open probability -12 ± 1 mV ($n = 5$). 50 mM Rb⁺ caused a -23 ± 2 mV shift ($n = 5$). Curves represent fits to a Boltzmann distribution where the midpoint of the curve (V_{mid}) for control, 20 mM, and 50 mM Rb⁺ was -15, 2, and -11 mV, respectively; the slope ($z\delta$) was 1.8 for all conditions.

of this channel (excluding periods of Ca^{2+} blockade) had a double exponential distribution of closed times. The open time distribution was single exponential. We found that Rb⁺ not only increased the mean open time, but also decreased the closed times. We determined that the change in the closed times was not attributed to the decreased frequency of missed openings which result from the longer open times (see Materials and Methods). Therefore, in contrast to the predictions of the foot-in-the-door model, Rb⁺ not only slows the closing rate, but it also speeds the opening rate.

To exclude a nonspecific ionic strength effect on gating, we added Na⁺ to the external solution because external Na⁺ is neither a permeant ion nor a blocker of this channel. Under these conditions, Na⁺ had no effect on the open probability, even at concentrations that changed the ionic strength by as much as 50% (data not shown).

External Cs⁺ blockade of inward K⁺ current causes a voltage-dependent increase in the open probability

To test whether permeant ions affect gating by interaction with a site in the pore, we wanted to quantitatively correlate the change in the open probability with the degree of occupancy of the pore by the test ion. For this comparison we used Cs⁺ as the test ion because it is an

		20 mM Rb⁺	50 mM Rb⁺
τ_{open}	Rb⁺/Ctrl	1.6 ± 0.2	2.3 ± 0.2
$\tau_{closed\ fast}$	Rb⁺/Ctrl	0.63 ± 0.1	0.84 ± 0.2
$\tau_{closed\ slow}$	Rb⁺/Ctrl	0.66 ± 0.1	0.56 ± 0.05
Fraction fast	Rb⁺/Ctrl	4.0 ± 1	3.0 ± 1

Single channel parameters are shown as the ratio of that measured in the presence of external Rb⁺ to control. Currents were recorded as in Fig. 1 and parameters were determined as described in Materials and Methods. Data represent the mean ± SEM determined from four different holding potential in two separate experiments. The data are given as ratios because the V_{mid} of the gating curve varied among experiments.

impermeant ion whose occupancy at the blocking site can be directly determined from the extent of blockade.

Because the blocking kinetics for Cs⁺ are too rapid to be resolved by our recording system, Cs⁺ blockade of inward K⁺ current appears as a reduction in the single channel current size (Fig. 2 a). The blockade was voltage dependent; it was much more effective at negative potentials (Fig. 2 b; Cecchi et al., 1981).

Cs⁺ increased the channel open probability more at negative voltages than at positive, consistent with the voltage dependence of blockade (Fig. 2 c). If this is a direct result of Cs⁺ occupancy, then any manipulation that increases occupancy should also produce a corresponding increase in open probability. To test this, we shifted the control gating curve toward more negative voltages by increasing the internal Ca²⁺ concentration. At these negative voltages, external Cs⁺ caused much more blockade and a larger shift in the open probability (Fig. 3 a and b). An equivalent change in the open probability can be produced at more positive voltages by a higher Cs⁺ concentration, which produces more blockade (Fig. 3 c and d). Therefore, over this range of conditions, the Cs⁺ effect on open probability correlates with its occupancy of the pore. As for Rb⁺, single channel kinetic analysis showed that Cs⁺ not only decreased the closing rate, but also increased the opening rate.

Other ions increase the occupancy of the pore without affecting gating

We tested whether increasing the occupancy of the pore by other permeant (K⁺ and Tl⁺) and impermeant (TEA) ions also could increase the open probability. As expected, increasing the external K⁺ concentration in the

external solution increased the conductance. Conversely, Tl⁺ reduced the single channel current size. This occurs because Tl⁺ blocks K⁺ current in a manner similar to that of Rb⁺; it has a higher affinity than K⁺ for a binding site within the pore (Eisenman et al., 1986). TEA also reduced the single channel current size because it blocks K⁺ current with rapid kinetics (Vergara et al., 1984). The voltage dependence of TEA blockade suggests that TEA crosses 30% of the membrane field to get to its blocking site.

None of the ions caused a significant increase in the open probability (Fig. 4). The lack of an effect of increasing external K⁺ concentration was also observed by Neyton and Pelleschi (1991).

Internal Cs⁺ blockade of outward K⁺ current does not affect the open probability

Cs⁺ added to the internal solution blocked outward K⁺ current (data not shown). Blockade from the internal side of the channel required much higher concentrations of Cs⁺ and was much less voltage dependent as observed by Cecchi et al. (1981). Under these conditions, internal Cs⁺ did not affect the open probability. To exclude a nonspecific ionic strength effect on the Ca²⁺ activation sites, we examined the effects of N-methyl-D glucamine (NMG) because it neither permeates nor blocks this channel. Adding 100 mM NMG to the internal side did not affect the open probability.

DISCUSSION

Permeant ion effects on gating are strongly correlated with the occupancy of the pore

We found that permeant ions can affect the gating of the BK channel. Rb⁺ blockade of inward K⁺ current caused an increase in the open probability. This effect of Rb⁺ is similar to that observed in the delayed rectifier K⁺ channel (Århem, 1980; Swenson and Armstrong, 1981; Cahalan and Pappone, 1983; Beam and Donaldson, 1983; Cahalan et al., 1985; Matteson and Swenson, 1986). Substitution of external K⁺ with Rb⁺ slowed the time course of the tail current. Similar kinetic changes in the tail current are observed in the presence of several open channel blockers of voltage-dependent Na⁺ and K⁺ channels (Armstrong, 1971; Cahalan and Almers, 1979; Yeh and Armstrong, 1978). Yeh and Armstrong (1978) proposed that the blockers may hinder the channel's

closing gate by a foot-in-the-door mechanism. This mechanism appears to be fairly common because similar models have been proposed to explain the effects of permeant ions on the gating of several types of ligand-

gated channels (Ascher et al., 1978; Marchais and Marty, 1979; Adams et al., 1982).

In our experiments, Rb^+ blockade increases the occupancy of the pore because it has a higher affinity than K^+ for some binding site in the pore. Under these conditions, the foot-in-the-door model predicts that Rb^+ increases the open probability because it hinders channel closing. Consistent with this model, Rb^+ has been shown in single channel experiments to slow the closing rate of the delayed rectifier K^+ channel from frog skeletal muscle (Spruce et al., 1989) and the "A-current" K^+ channels from toadfish pancreatic islet cells (Matteson and Sala, 1990).

In our experiments, however, the single channel kinetics showed that Rb^+ not only decreased the closing rate, but also increased the opening rate. An effect on the opening rate suggests that Rb^+ is somehow modifying the stability of the closed channel. Therefore, we propose that these results are more consistent with a modified version of the foot-in-the-door model, which states that the channel can close when occupied by an ion, but that the closed and occupied state is less stable than the closed and unoccupied state.

It is interesting that in our single channel analysis Rb^+ blockade causes an increase in the fraction of the fast closing events. This suggests that the Rb^+ occupied channel is more reluctant to enter the prolonged closed state than the brief closed state. This may not be so surprising because these closing events most likely represent different physical processes.

To demonstrate more convincingly that ions act in the pore to affect gating, we sought a quantitative correlation between occupancy of the pore and a change in gating using an impermeant blocker. With an impermeant ion, the level of occupancy can be directly determined from the extent of blockade, whereas permeant ions such as Rb^+ will not only block the current but also contribute to it. The fractional reduction in the current size caused by Rb^+ reflects both of these
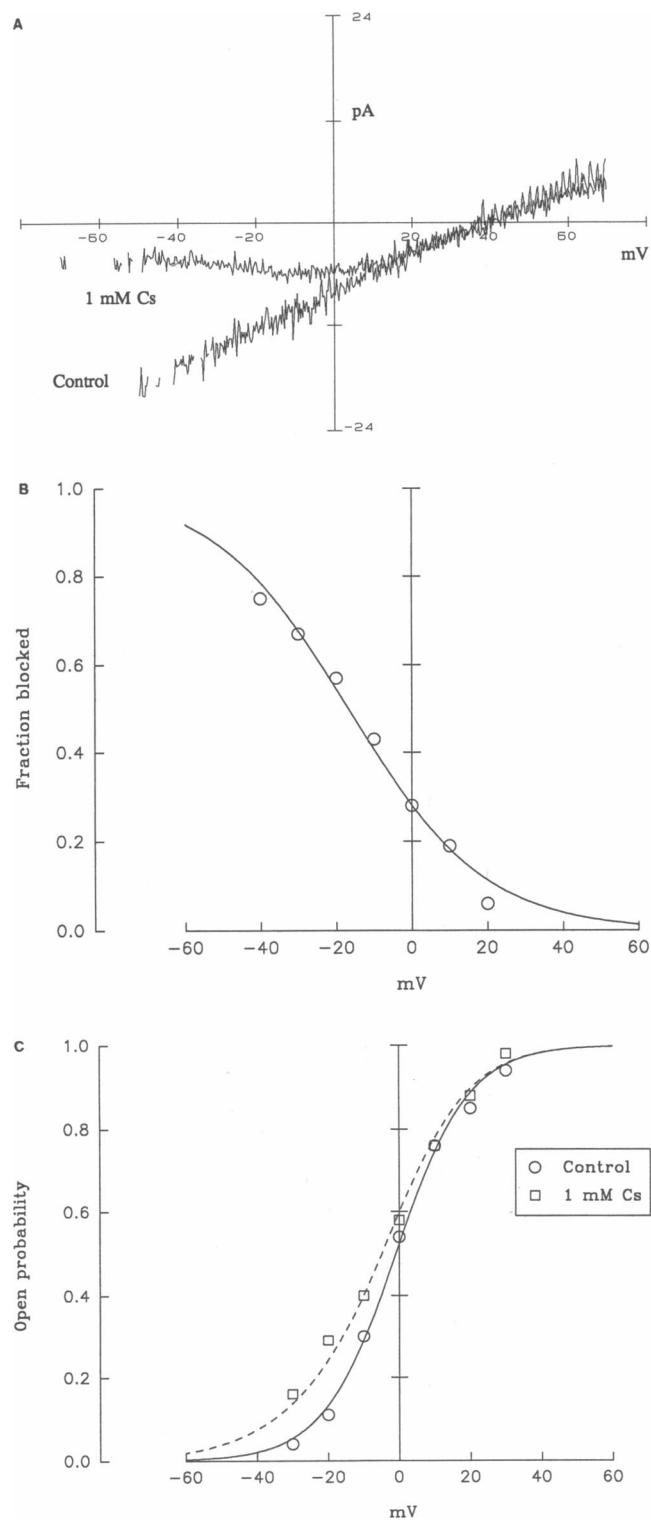
FIGURE 2 Cs^+ blockade of inward K^+ current causes a voltage-dependent increase in the open probability. (a) The I-V relationship shows that the addition of 1 mM Cs^+ to the external solution caused a voltage-dependent blockade of inward K^+ current. Curves were determined from ramped voltage pulses of single channel currents recorded as in Fig. 1 a. (b) The fraction reduction in current is plotted as a function of voltage where fraction blocked equals $1 - i_{Cs^+}/i_{control}$. The curve represents a fit to a Boltzmann distribution where V_{mid} for blockade is -17 mV and $z\delta$ is -1.4. (c) The open probability was determined as a function of voltage before and after the addition of 1 mM external Cs^+. The control curve was fit with $V_{mid} = -1$ and $z\delta = 2.5$. The Cs^+ curve was fit with the model described in the Discussion using the blockade and gating parameters defined above where $\ln\theta = 2.3$.
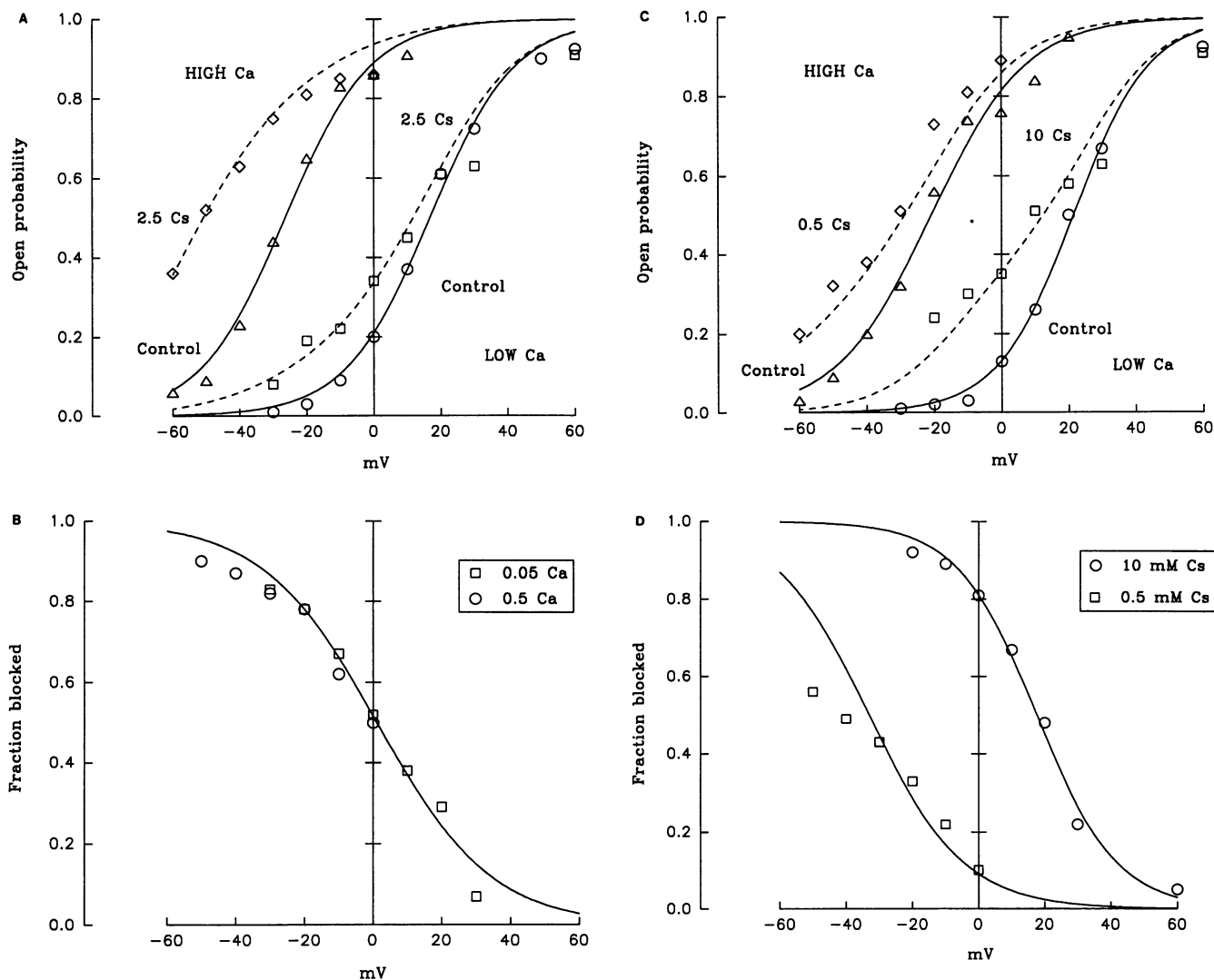
FIGURE 3 The effect of Cs$^+$ on gating strongly correlates with its occupancy of the pore. (*a*) The open probability was determined as a function of voltage from single channel currents recorded as in Fig. 1 at two different internal Ca^{2+} concentrations. With 0.05 mM internal Ca^{2+}, the addition of 2.5 mM external Cs$^+$ caused a slight voltage-dependent increase in the open probability. With 0.05 mM internal Ca^{2+}, the addition of 2.5 mM external Cs$^+$ caused a large increase in the open probability. The control curves were fit with V_{mid} = 17 mV and $z\delta$ = 2.0 (0.05 mM Ca^{2+}), and V_{mid} = -27 mV and $z\delta$ = 2.0 (0.5 mM Ca^{2+}). The Cs$^+$ curves were fit with the model described in the Discussion using the gating parameters given above and the blockade parameters shown in *b*, where lnθ = 2.3. (*b*) The fractional blockade caused by the addition of 2.5 mM external Cs$^+$ is plotted as a function of voltage at 0.05 and 0.5 mM internal Ca^{2+}. The curve was fit with V_{mid} for blockade = 1 mV and $z\delta$ = -1.5. (*c*) The open probability was determined at different voltages in the presence of 0.05 and 0.5 mM internal Ca^{2+} as in Fig. 1 *c*. Control curves were fit with V_{mid} = -21 mV and $z\delta$ = 1.8 (0.5 mM Ca^{2+}), and V_{mid} = 21 mV and $z\delta$ = 2.2 (0.05 mM Ca^{2+}). Cs$^+$ curves were fit with the model using the gating parameters above and the blocking parameters described in *d*, where lnθ = 2.3. (*d*) The fractional blockade caused by 0.5 mM external Cs$^+$ in the presence of 0.5 mM internal Ca^{2+} and 10 mM external Cs$^+$ in the presence of 0.05 internal Ca^{2+} was measured as a function of voltage. The curves were fit with V_{mid} for blockade = -35 mV and $z\delta$ = -1.8 (0.5 mM Ca^{2+}), and V_{mid} for blockade = 18 mV and $z\delta$ = 2.1 (0.05 mM Ca^{2+}).

processes. Therefore, we used the impermeant blocker Cs$^+$.

There is ample evidence that Cs$^+$ blocks K$^+$ current by directly occluding the pore (Cecchi et al., 1981). First, external Cs$^+$ blockade is steeply voltage dependent, and second, it can be relieved by increasing the internal K$^+$

concentration and enhanced by increasing the external K$^+$ concentration. These data suggest that Cs$^+$ competes with K$^+$ for a common binding site deep within the pore.

Cs$^+$ blockade of inward K$^+$ current caused a voltage-dependent increase in the open probability. Furthermore, the voltage dependence matched the voltage
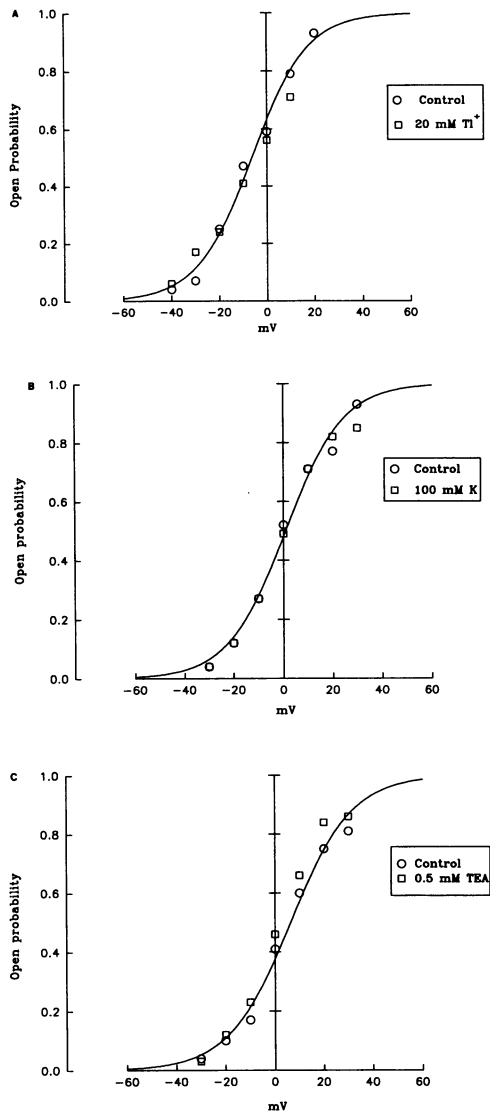
FIGURE 4 Other ions added to the external solution do not affect the open probability. (a) The open probability was determined from single channel currents recorded as in Fig. 1 as a function of voltage in presence and absence of 20 mM external Tl^+. The addition of Tl^+ caused a 35% reduction in the single channel current bud did not affect the open probability. The curve represents a Boltzmann fit to the control data where V_{mid} = −6 mV and $z\delta$ = 2.2. (b) The open probability was measured as in a before and after adding an additional 100 mM K^+ to the external solution. The curve was fit to the control data using V_{mid} = 1 mV and $z\delta$ = 2.2. (c) The open probability is shown as a function of voltage in the presence and absence of 0.5 mM external TEA. The addition of TEA blocked the inward K^+ current with V_{mid} = 70 mV and $z\delta$ = −0.2, but had no effect on the open probability. The curve was fit to the control data with V_{mid} = 6 mV and $z\delta$ = 2.0.

dependence of blockade suggesting that Cs^+ affects gating by binding to its blocking site within the pore. Because the single channel kinetic analysis showed that, as with Rb^+, Cs^+ affects both the closing rate and

opening rate, we can describe the changes in the open probability caused by Cs^+, using the modified version of the foot model shown below.

$$C \underset{}{\overset{K}{\rightleftharpoons}} O$$

$$\Updownarrow \frac{[Cs]}{K_D}$$

$$C \cdot Cs \underset{K\theta}{\rightleftharpoons} O \cdot Cs.$$
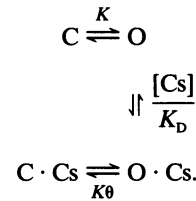
The equilibrium constant K describes the normal equilibrium between the open and closed states. Cs^+ blocks the channel with a dissociation constant K_D. Under these conditions, the channel can close either with or without Cs^+ in the pore. Closing with Cs^+ in the pore shifts the equilibrium by the factor θ. This model was used by Miller et al. (1987) and Neyton and Pelleschi (1991) to describe the gating changes of this BK channel under conditions of Ba^{2+} blockade.

Using the open–closed equilibrium constant measured in the absence of blocker and the fractional reduction in current measured from the I-V relationship, we found that the model predicts the voltage-dependent shifts in the open probability caused by Cs^+. In all cases the value in $\ln(\theta)$ required to fit the data was greater than 1. This finding suggests that Cs^+ occupancy of the pore destabilizes the closed state. Moreover, the mean $\ln(\theta)$ was 2.3 ± 0.18, indicating that Cs^+ occupancy of the pore greatly destabilizes that closed state by ~2.3 kT relative to the unoccupied closed channel. Similarly, Miller et al. (1987) found a 2.5-kT destabilization of the closed state of the BK channel blocked with a Ba^{2+} ion.

It is known that at very negative voltages Cs^+ will permeate through the BK channel (Cecchi et al., 1981). To test for possible complications arising from Cs^+ permeation, we used the two-site permeation model described below. We found that only a slight change in the Cs^+ energy profile was necessary to predict Cs^+ permeation and that the current increased without any significant change in the occupancy of the Cs^+ blockade site. Therefore, this complication is not likely to effect our correlation between Cs^+ occupancy and gating. It probably does account, however, for the deviation of the blockade at negative voltages from the predicted Boltzmann distribution.

Occupancy of a specific "gating site" must change to affect gating

In contrast to the effects of Rb^+ and Cs^+, we found that adding other permeant (K^+ and Tl^+) and impermeant (TEA) ions to the external solution had no effect on gating. This observation is most easily understood by

considering the multi-ion nature of the BK channel. It has been shown to have a minimum of four ion binding sites in the pore (Neyton and Miller, 1988b). An increase in occupancy caused by a particular ion may occur primarily at only one of these sites while the occupancy of the other sites remains constant or decreases. This pattern is probably not the same for every ion because it will depend on the energy profile encountered by the specific ion as it passes through the channel. For example, if Rb^+ has a high affinity for one binding site and Tl^+ prefers another, then occupancy will be increased at different sites by these ions. This explanation can account for the different in gating effects of Rb^+ and Tl^+.

Therefore, we used a model in which the occupancy of one particular site in the pore influences gating. This "gating site" model was also proposed by Matteson and Swenson (1986) to describe the effects of permeant ion on the delayed rectifier K^+ channel. To predict the effects of ion concentration changes on occupancy of the "gating site," we used a two-site model of the permeation pathway. Although there is evidence for more than two sites, this simplified model at least gives a qualitative description of channel occupancy.

In our two-site model, we chose the inner site as the "gating site" because the blocking parameters place Cs^+ binding deep within the pore and our results correlate Cs^+ blockade with the gating effect. We assigned values to the energy profiles of the different ions used in these experiments by requiring that they predict several experimental observations, including bi-ionic permeability ratios, maximal conductance (Eisenman et al., 1986), and blockade of inward K^+ current (Fig. 5). Given a set of reasonable energy values, the model successfully predicts: (a) a voltage-independent effect of Rb^+ on gating; (b) a voltage-dependent effect of Cs^+ on gating; and (c) no effect of elevating K^+ or Tl^+ concentrations.

The model predicts that Rb^+ causes a voltage-independent increase in the occupancy of the inner site because it has a lower well depth than K^+ at this site. Cs^+ causes a voltage-dependent increase because it has a large barrier to exit to the internal side. It is this barrier that restricts Cs^+ permeation. On the other hand, Tl^+ does not affect the occupancy of the inner site because it binds preferentially to the outer site. Increasing the external K^+ concentration causes only a slight increase in the occupancy of the inner site. Because this increase is much less substantial than that caused by Rb^+ and Cs^+, increasing external K^+, as we observe, should not affect gating.

We did not use the permeation model to predict changes in occupancy caused by external TEA and internal Cs^+ because the voltage dependence of blockade by these two ions suggests that they bind to superfi-
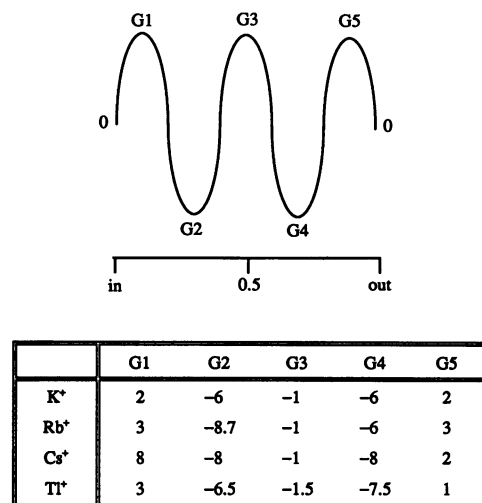

FIGURE 5 A two-site permeation model predicts the occupancy changes at the "gating site." The schematic diagram (*top*) shows the position of the wells and barriers within the membrane field. The exact positions used in terms of the fractional electrical distance from the inside were 0.19, 0.38, 0.5, 0.62, and 0.81 for the inner barrier, inner well, middle barrier, outer well, and the outer barrier, respectively. The barrier and well energies used to predict the permeation properties are given for each ion. The values are expressed in units of kT.

	G1	G2	G3	G4	G5
K^+	2	−6	−1	−6	2
Rb^+	3	−8.7	−1	−6	3
Cs^+	8	−8	−1	−8	2
Tl^+	3	−6.5	−1.5	−7.5	1

cial sites on the channel. Binding superficially is consistent with the inability of these ions to affect gating if we assume that the "gating site" is located deep within the pore.

This model describes the effects of several different ions on gating. It suggests that there is a particular "gating site" in the pore that destabilizes the closed state when it is occupied. We have found it unnecessary to assume that the channel senses the species of the ion occupying the pore and changes its gating solely on this basis. Although a model based on this assumption cannot be dismissed easily, the lack of an effect of internal Cs^+ on gating suggests that if the channel responds to the species of the ion occupying the pore, it must also respond to its location. If the channel were sensitive to only the presence of Cs^+, irrespective of location, then internal and external Cs^+ would have the same effect. This does not appear to be the case, however.

ELI model of permeant ion effects on gating

A similar model has been proposed by Neyton and Pelleschi (1991) to describe the effects of external permeant ions on the Ba^{2+} and Na^+ blocked BK channel. In their model the "gating site" is the external lock-in site (ELI). Occupancy of the ELI by ions coming from

the external solution decreases the exit rate of a blocking Ba^{2+} ion (Neyton and Miller, 1988a) and destabilizes the closed-blocked state in a manner similar to the model proposed here (Neyton and Pelleschi, 1991). Based on the differential effects of external Cs^+ and TEA and their voltage dependence of blockade, we chose the "gating site" to be located toward the inner mouth of the pore. Our data, however, could be interpreted in terms of the ELI model. For example, it is possible that Cs^+ and Rb^+ bind to an inner site and indirectly increase the occupancy of the ELI by "clogging" inward K^+ flow through the channel. Therefore, our observations do not exclude this model.

The general principles of the ELI model and the "gating site" model presented here are quite similar. Some ions, by virtue of their binding location, interfere more than others with the structural changes that occur during channel opening and closing. Because ion binding to any site within the pore affects the occupancy of other sites, it is hard to identify unequivocally which site or sites influence gating. Although the exact mechanism underlying this process is still not entirely clear, our results plainly demonstrate that the effect of permeant ions on gating occurs through their interaction with binding sites within the pore.

REFERENCES

Adams, D. J., P. W. Gage, and O. P. Hamill. 1982. Inhibitory postsynaptic currents at Aplysia cholinergic synapses: effects of permeant anions and depressant drugs. Proc. R. Soc. Lond. 214:335–350.

Alvarez, O. 1986. How to set up a bilayer system. In Ion Channel Reconstitution. C. Miller, editor. Plenum Press, New York. 115–129.

Armstrong, C. M. 1971. Interaction of tetraethylammonium ion derivatives with potassium channels of giant axons. J. Gen. Physiol. 58:413–437.

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

Ascher, P., A. Marty, and T. O. Neild. 1978. Lifetime and elementary conductance of the channels mediating the excitatory effects of acetylocholine in Aplysia neurones. J. Physiol. 278:177–206.

Beam, K. G., and P. L. Donaldson. 1983. Slow components of potassium tail currents in rat skeletal muscle. J. Gen. Physiol. 81:513–530.

Blatz, A. L., and K. L. Magleby. 1986. Correcting single channel data for missed events. Biophys. J. 49:967–980.

Cahalan, M. D., and W. Almers. 1979. Block of sodium conductance and gating current in squid giant axons poisoned with quaternary strychnine. Biophys. J. 27:57–74.

Cahalan, M. D., K. G. Chandy, T. E. Decoursey, and S. Gupta. 1985. A voltage-gated potassium channel in human T lymphocytes. J. Physiol. 358:197–237.

Cahalan, M. D., and P. A. Pappone. 1983. Chemical modification of potassium channel gating in frog myelinated nerve by trinitrobenzene sulphonic acid. J. Physiol. 342:119–143.

Cecchi, X., D. Wolff, O Alvarez, and R. Latorre. 1981. Mechanisms of Cs^+ blockade in a Ca^{2+}-activated K^+ channel from smooth muscle. Biophys. J. 84:1–23.

Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single channel records. In Single Channel Recording. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 191–264.

Eisenman, G., R. Latorre, and C. Miller. 1986. Multi-ion conduction and selectivity in high conductance Ca^{2+}-activated K^+ channel from skeletal muscle. Biophys. J. 50:1025–1034.

Jackson, M. B. 1986. Kinetics of unliganded acetylcholine receptor channel gating. Biophys. J. 49:663–672.

Latorre, R., C. Vergara, and C. Hidalgo. 1983. Reconstitution in planar lipid bilayers of a Ca^{2+}-activated K^+ channel from transverse tubule membranes isolated from rabbit skeletal muscle. Proc. Natl. Acad. Sci. USA. 77:7484–8486.

Marchais, D., and A. Marty. 1979. Interaction of permeant ions with channels activated by acetylcholine in Aplysia neurones. J. Physiol. 297:6–45.

Martell, A. E., and R. M. Smith. 1974. Critical Stability Constants. Plenum Press, London. Vol. 1. p. 269.

Matteson, D. R., and S. Sala. 1990. Rb^+ slows K^+ channel closing by acting at a site in the channel. Biophys. J. 57:509a. (Abstr.)

Matteson, D. R., and R. P. Swenson. 1986. External monovalent cations that impede the closing of K^+ channels. J. Gen. Physiol. 87:795–816.

Miller, C., R. Latorre, and I. Reisin. 1987. Coupling of voltage-dependent gating and Ba^{2+} block in high conductance Ca^{2+}-activated K^+ channel. J. Gen Physiol. 90:427–449.

Moczydlowski, E., and R. Latorre. 1983. Saxitoxin and ouabain binding activity of isolated skeletal muscle membranes as indicators of surface origin and purity. Biochim. Biophys. Acta. 732:412–420.

Neher, E., and J. H. Steinbach. 1978. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. J. Physiol. 277:153–176.

Neyton, J., and C. Miller. 1988a. Potassium block barium permeation through a calcium-activated potassium channel. J. Gen. Physiol. 92:549–567.

Neyton, J., and C. Miller. 1988b. Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high conductance Ca^{2+}-activated K^+ channel. J. Gen. Physiol. 291:427–429.

Neyton, J., and M. Pelleschi. 1991. Multi-ion occupancy alters gating in high conductance, Ca^{2+}-activated K^+ channels. J. Gen. Physiol. 97:641–665.

Press, W. H., B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling. 1986. Numerical Recipes in C. Cambridge University Press, Cambridge, UK. 735 pp.

Sigworth, F. J. Electronic design of the patch clamp. In Single Channel Recording. B. Sakmann and E. Neher, editors. Plenum Press, New York. 3–36.

Sigworth, F. J., and S. M. Sine. 1987. Data transformations for improved display and fitting of single-channel dwell time histograms. *Biophys. J.* 52:1047–1054.

Spruce, A. E., N. B. Standen, and P. R. Stanfield. 1989. Rubidium ions and the gating of the delayed rectifier potassium channels of frog skeletal muscle. *J. Physiol.* 411:597–610.

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

Vergara, C., and R. Latorre. 1983. Kinetics of Ca^{2+}-activated K$^+$ channels from rabbit muscle incorporated into planar bilayers. *J. Gen. Physiol.* 82:543–568.

Vergara, C., E. Moczydlowski, and R. Latorre. 1984. Conduction, blockade, and gating in a Ca^{2+}-activated K$^+$ channel incorporated into planar lipid bilayers. *Biophys. J.* 45:73–76.

Yeh, J. Z., and C. M. Armstrong. 1978. Immobilisation of gating charge by a substance that simulates inactivation. *Nature (Lond.).* 273:387–389.

Yellen, G. 1982. Single Ca^{2+}-activated nonselective cation channels in neuroblastoma. *Nature (Lond.).* 296:357–359.