Sodium and Gating Current Time Shifts Resulting from Changes in Initial Conditions

ROBERT E. TAYLOR and FRANCISCO BEZANILLA

From the Laboratory of Biophysics, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205; the Department of Physiology, Ahmanson Laboratory of Neurobiology and Jerry Lewis Neuromuscular Research Center, University of California, Los Angeles, California 90024; and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543

ABSTRACT   The sodium and gating currents of the squid giant axon elicited by a depolarizing pulse are delayed, with little change in shape, as a result of a hyperpolarizing prepulse. The delays are almost completely saturated, at ~45 μs, for prepulses to -140 mV. At 8°C they develop with time constants of between 60 and 180 μs for prepulses in the -130- to -50-mV range. There is a correlation between the extra charge moved during the gating current and the increase in the time delay of the sodium current as the magnitude of the hyperpolarizing prepulse is increased. These results strengthen the conclusion that the gating current is indeed closely associated with the process of sodium channel opening and provide information concerning the kinetics of the early steps, which are hidden in ionic current measurements. The main features of the gating and sodium current time shifts and the correlation between charge movement and time shifts are duplicated by a sequential six-state model for sodium activation.

INTRODUCTION

Hodgkin and Huxley (1952) dealt with the fact that the sodium and potassium current, after depolarization, increased with a delay but fell at the end of a pulse exponentially by expressing the conductance as being proportional to a power of a variable. For example, the potassium conductance was described by a variable raised to the fourth power. The variable was in turn described by a first-order differential equation. It was pointed out by Richard FitzHugh (see Cole and Moore, 1960) that for such a situation a change in the initial conditions could be exactly compensated for by a shift in the origin of time. This was found to be true for the potassium current by Cole and Moore (1960). They reported that the shift continued to occur for hyperpolarizations of as much as -212 mV, and in order to fit the data it was necessary to raise the power of the variable (n in the Hodgkin-Huxley equations) to 25. Corresponding behavior was not found for the sodium current by Cole and Moore, probably because of the speed of the clamp.

A pronounced lag in the sodium current was reported by Armstrong and Bezanilla (1974) for a step from −130 to −10 mV as compared with a step from −70 mV as was seen by Keynes and Rojas (1976). A similar shift has been reported by Hahin and Goldman (1978) in *Myxicola* axons. We thoroughly confirm this observation and find that the gating current is similarly delayed by a hyperpolarizing prepulse by roughly the same amount as the sodium current. The maximum shift at 8°C from a holding potential of −70 mV is in the neighborhood of 45 μs and saturates for a prepulse to about −150 mV.

A slower component of gating current, not visible at 8°C, is seen at higher temperatures (20°C) and has been correlated with the potassium conductance. This component is delayed by a prepulse in a manner similar to the potassium current (Bezanilla et al., 1982b).

In this paper we present the data for both gating and sodium currents as a function of initial conditions and an attempt is made to correlate our results to a simple sequential activation scheme for the sodium current.

A preliminary report has appeared (Taylor and Bezanilla, 1983).

METHODS

Cleaned segments of giant axons from the squid *Loligo pealei* were internally perfused and voltage-clamped according to the method described previously (Bezanilla et al., 1982a).

Ionic and gating currents were recorded using the P/4 or P/−4 procedure and negative subtracting holding potentials (Bezanilla et al., 1982a) and were subsequently recorded in magnetic discs. The analysis was performed off-line using the procedures already described (Bezanilla et al., 1982a). All traces of ionic and gating currents shown here represent raw data (i.e., baselines have not been subtracted). The time shifts were determined by shifting one experimental trace with respect to the other and recording the amount of shift required to satisfy superposition by visual inspection of the oscilloscope display of the computer.

Solutions used are similar to the ones described in a previous paper (Bezanilla et al., 1982a) and their names and compositions are the following: 200 TMAFG is made of 100 mM tetramethyl ammonium (TMA) fluoride, 100 mM TMA glutamate, and 10 mM Tris and sucrose to an osmolality of 980 mosmol/kg; 200 CsFG is made of 100 mM cesium fluoride, 100 mM cesium glutamate, and 10 mM Tris and sucrose to an osmolality of 980 mosmol/kg; ASW is artificial seawater composed of 440 mM NaCl, 50 mM MgCl$_2$, 10 mM CaCl$_2$, and 10 mM Tris-Cl; Tris TTX is 450 mM Tris-Cl, 50 mM MgCl$_2$, 10 mM CaCl$_2$, and 300 nM tetrodotoxin (TTX); 1/5 NaSW is 362 mM Tris-Cl, 88 mM NaCl, 50 mM MgCl$_2$, and 10 mM CaCl$_2$. The pH of internal solutions was adjusted to 7.2 and external pH to 7.4. The convention followed is external solution//internal solution.

RESULTS

Effect of Long Prepulse on Sodium and Gating Currents

Fig. 1a shows the effect of a 1.2-ms hyperpolarizing prepulse (to −140 mV) and a depolarizing prepulse (to −50 mV) on the total (sodium plus gating) current produced by a pulse to 0 mV from a holding potential of −70 mV.

This is for a squid axon membrane in full (440 mM) external sodium. The hyperpolarizing prepulse has been chosen to maximize the effect, as we shall see later. The sampling interval for these records was 1.2 μs and the curves are relatively noisy because the low-pass filter used had a cutoff at 300 kHz. When the curve with the hyperpolarizing prepulse is shifted to the left (Fig. 1b) by 38 μs and the curve with the depolarizing prepulse is shifted to the right by 42 μs, they coincide rather well over the entire time course (also see Fig. 3b).
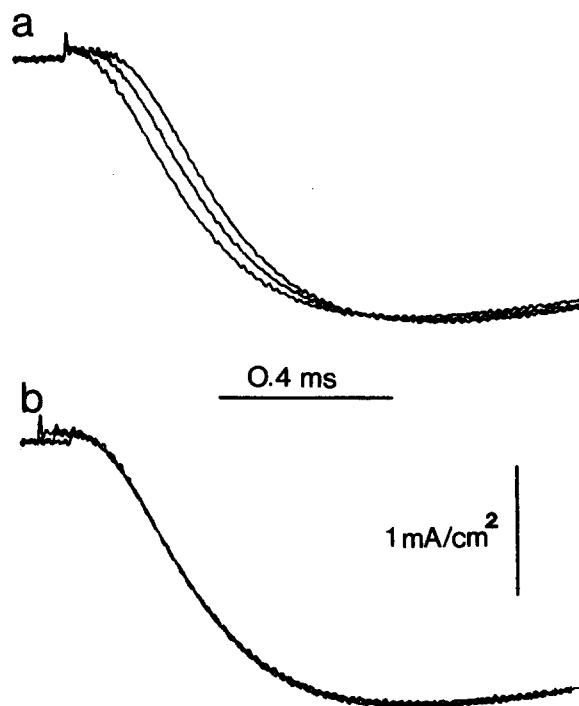

FIGURE 1. (a) Effects of a 1.2-ms prepulse on sodium plus gating current. The left-hand curve is for a prepulse to −50 mV, the center for no prepulse, and the right-hand for a prepulse to −140 mV, all for a holding potential of −70 mV with a pulse to 0.0 mV. Temperature, 7°C. Compensation for 4.6 Ω cm² series resistance. ASW//200 TMAFG. WHMAY162A. (b) As in a but the curve for no prepulse was shifted to the left by 42 μs and for a prepulse to −140 mV it was shifted by 80 μs relative to curve for prepulse to −50 mV.

It is clear from a casual inspection that these curves have the same shape to a remarkable degree, but the gating currents are too small to resolve clearly. By a virtual elimination of permeant ions and the application of tetrodotoxin, it is possible to see the gating currents clearly, as in Fig. 2. We see here that the gating currents are shifted in time in much the same way that the sodium currents were in Fig. 1. In this case the curve with a hyperpolarizing prepulse of 1 ms duration to −110 mV shifts the gating current to the right, and for a depolarizing prepulse to −50 mV the shift is to the left. To a first approxi-

mation the curves are of the same shape. There appear to be minor differences, but with the time resolution we have we will not consider them here.

Fig. 3 shows a similar experiment but includes the sodium current after subtraction of the gating current. The sodium and gating currents are shown in part *a*. In part *b* the sodium currents have been shifted so that the activation phases coincide. When this is done the inactivation phases also coincide. The gating current records in part *b* were then shifted by the same amounts and within the noise they also are superimposable.

If the gating currents in Figs. 2 and 3 were strictly exponential, they could be made to superimpose by simple amplitude scaling and this can be done after ~100 μs, but not before. Horowicz and Schneider (1981) found that in
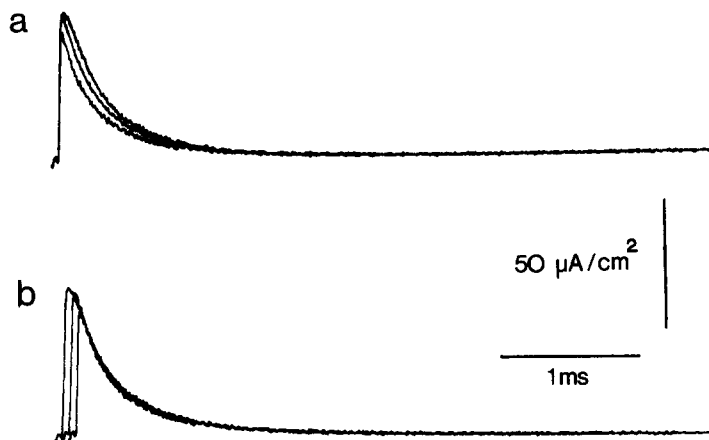

FIGURE 2.   (*a*) Effects of a 1.0-ms prepulse on gating current. The left-hand curve is for a prepulse to −50 mV, the center for no prepulse, and the right-hand for prepulse to −110 mV, all for a holding potential of −70 mV with a pulse to 0.0 mV. Temperature, 8°C. No series resistance compensation. Tris TTX//200 TMAFG. WHMAY301B. (*b*) As in *a* but the curve for no prepulse shifted to the left by 40 μs and for a prepulse to −110 mV it shifted by 84 μs relative to curve for prepulse to −50 mV.

a similar experiment on charge movement in muscle fibers the currents with and without prepulse could not be made to superimpose by amplitude scaling.

Time Course of the Time Shift Development

The important point to be made from these data is that the time shifts for the gating current are about the same as for the sodium current for a 1-ms prepulse. The shift develops rapidly as the duration of the prepulse is increased, as shown in Fig. 4*a* for the sodium current (Δt_{Na}) and in Fig. 4*b* for the gating current (Δt_g). Here a positive value indicates a delay in the time course of the current as compared with no prepulse. In each case the open circles indicate a prepulse to −100 mV and the open triangles indicate a prepulse to −130 mV. The closed circles and triangles are for prepulses to −60 and −50 mV, all

from a holding potential of −70 mV. The dotted curves are exponentials with time constants from 60 to 179 μs, which are not meant to be fitted but are drawn in merely for comparison. The determination of the shift is not considered to be accurate enough to make more than a rough estimate of the
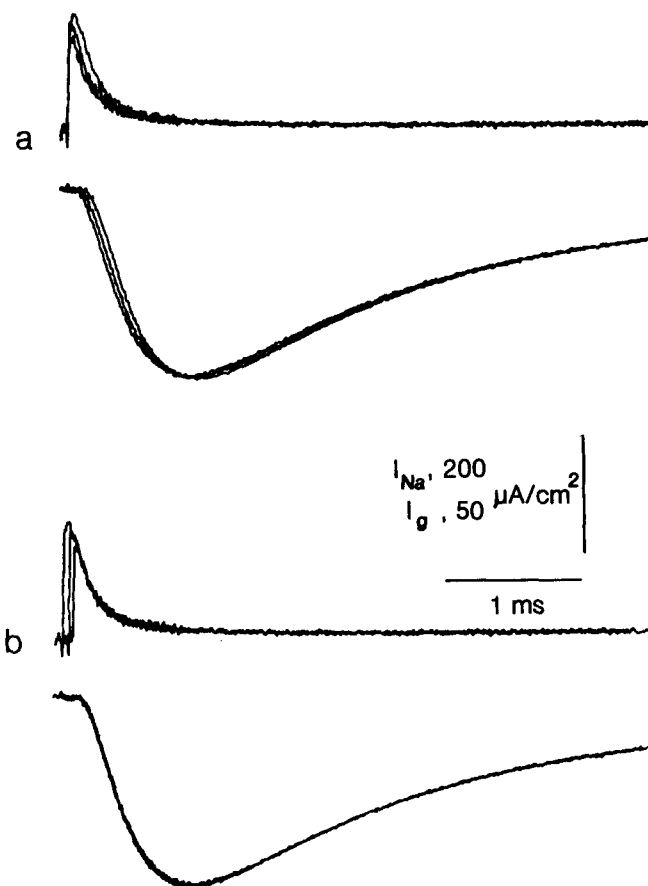

FIGURE 3.   (*a*) Effects of a 1-ms prepulse on sodium and gating currents in 1/5 normal sodium. The left-hand curve is for a prepulse to −150 mV, the center for no prepulse, and the right-hand for a prepulse to −50 mV, all from a holding potential of −70 mV with pulse to 0. No series resistance compensation. Gating current: Tris TTX//200 TMAFG. Sodium current: 1/5 NaSW//200 TMAFG. Temperature, 7°C. WHSEP121A. (*b*) As in *a* but the curves for no prepulse shifted to the left by 32 μs and for a prepulse to −150 mV they shifted by 80 μs relative to curve for prepulse to −50 mV.

time constants. Clearly, the time course as well as the final value of the time shifts are comparable for the sodium and the gating currents. We consider this result to be a further indication that most of the gating current is indeed associated with the opening of the sodium channels. The points for a prepulse
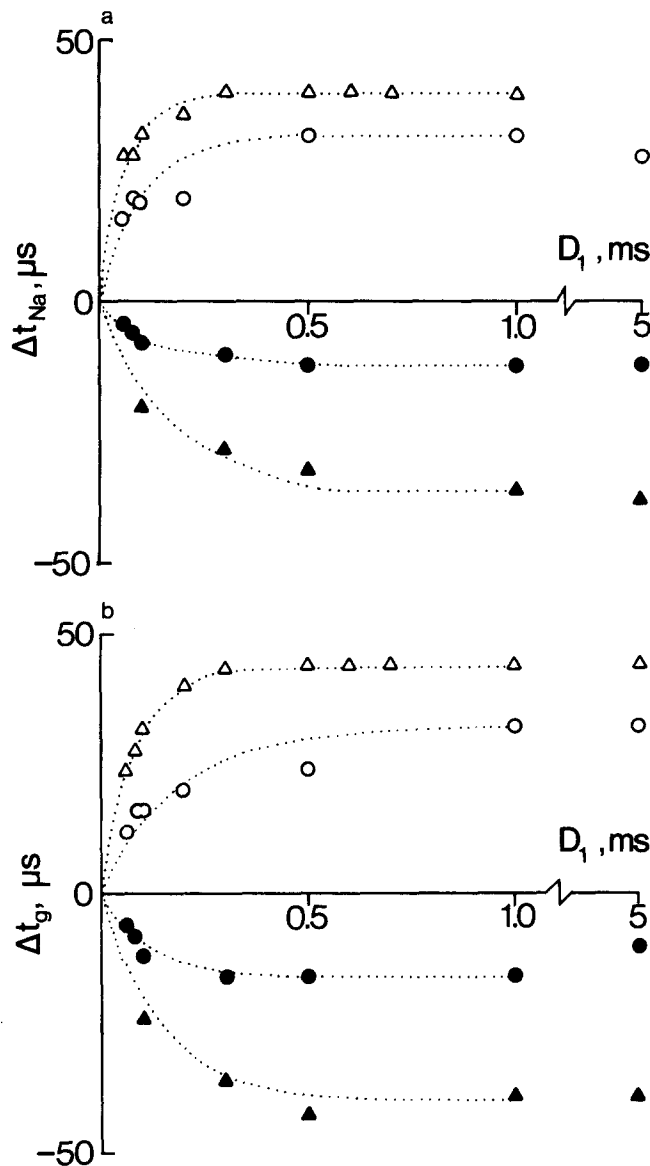
FIGURE 4. (a) Time course of development of time shift of sodium current (Δt_{Na}) for prepulses of different durations (D_1) and magnitudes. Gating currents were subtracted. The dotted curves are exponential for illustrative purposes. Symbols: $\triangle$, P1 to -130 mV, $\tau = 60$ μs; $\bigcirc$, P1 to -100 mV, $\tau = 100$ μs; $\bullet$, P1 to -60 mV, $\tau = 120$ μs; $\blacktriangle$, P1 to -50 mV, $\tau = 166$ μs. All for a holding potential of -70 mV with pulse to zero. No series resistance compensation. 1/5 NaSW/ /200 TMAFG. Temperature, 7°C. WHSEP121A. (b) Time shift for gating currents (Δt_g). Conditions and symbols were as in a except that $\tau = 77$, 179, 124, and 148 μs for P1 $= -130$, -100, -60, and -50 mV. Tris TTX//200 TMAFG.

to −150 mV are not shown. We are reasonably certain that the effect saturates at around −140 mV, but there are difficulties in dealing with pulses of this magnitude associated with the P/−4 procedure (see Methods) and the subtracting holding potentials required. We have included depolarizing prepulses up to −50 mV, but even there some problems with the presence of fast inactivation are present and may influence the results to some extent.

A number of time shifts for sodium current versus those for gating currents are plotted in Fig. 5 under a variety of conditions. Although most of the points are close to the 45° line, in this case, there seems to be a tendency for the gating current time shifts to be somewhat larger than those for sodium
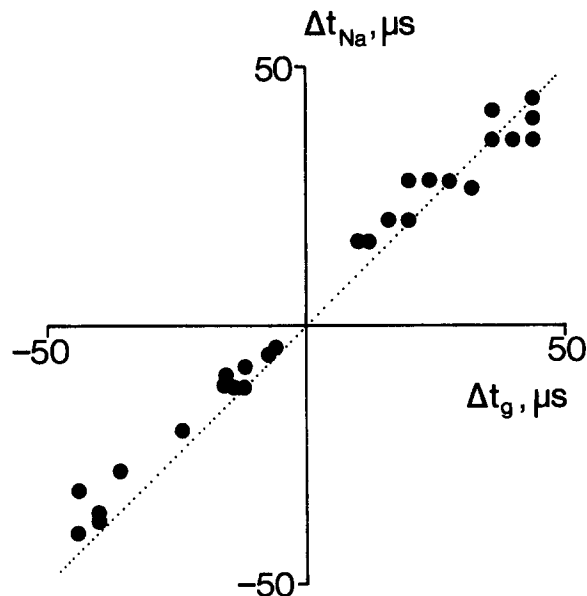

FIGURE 5.   Sodium current time shifts (Δt_{Na}) vs. gating current time shifts (Δt_g) for various prepulse durations and magnitudes from a holding potential of −70 mV with pulse to 0. Same axon as in Fig. 4.

currents. We will not dwell on this result because most of our experiments were done with a time resolution of only 4 μs per point.

In five experiments, at 7 or 8°C the maximum time shift observed for sodium current (Δt_{Na}) with a prepulse to −130 mV was 36.8 ± 1.8 μs and for the gating current (Δt_g) it was 46.6 ± 9.2 μs. In one experiment at 2°C the maximum shift for the sodium current was 70 μs for a prepulse to −130 mV.

Voltage Dependence of the Time Shift and Charge Movement

The shift for the sodium current is shown in Fig. 6 as a function of the voltage during the prepulse of 1 or 5 ms. We integrated the charge of the gating current during the test pulse and it is also shown in Fig. 6. The parallelism

between the charge moved and the time shift is remarkable and is considered in the Discussion.

DISCUSSION

We have seen that if one holds the squid axon membrane at −70 mV and pulses to 0, a hyperpolarizing prepulse results in a delay in the onset of the sodium current with very little change in shape. That the gating current is affected with almost the same delay is another strong indication that the
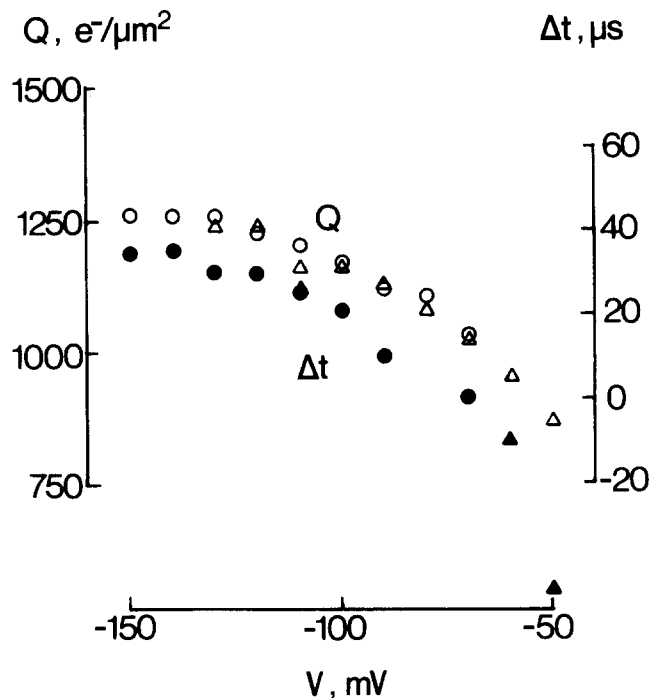

FIGURE 6. Comparison of gating current charge movements (Q) and sodium current time shifts (Δt) vs. the potential during the prepulse, all from a holding potential of −70 mV with pulse to 0. Prepulse duration: 1 ms (O, ●) and 5 ms (Δ, ▲). Temperature, 7°C. No series resistance compensation. 1/5 NaSW//200 CsFG and Tris TTX//200 CsFG. WHSEP111A.

gating current is mostly associated with the opening of the sodium channels. The delay increases with increasing the magnitude of the prepulse until about −140 mV, where the effect appears to level off with a delay of ~45 μs at 8°C. At 2°C the delay was much greater, ~70 μs for a prepulse to −130 mV; this indicates a large Q_{10}, meaning that some process is occurring with large energy barriers involved. The fact that after shifting, the sodium current curves superimpose during the inactivation phase is a further sign that activation and inactivation of the sodium channel gating are coupled, although it is possible that an independent inactivation process has been delayed by the

hyperpolarizing prepulse. We have studied the shifts produced using various test pulses, but our analysis is concentrated using test pulses to 0 mV because the currents are large and the shifts are easier to quantify.

We believe that these results are a direct consequence of a considerable amount of charge movement present for pulses from −70 mV to more hyperpolarized values that level off around −140 mV. In this range there is virtually no sodium conductance, so it is not the effect of the prepulse on the initial sodium conductance that produces the shift, but rather a change in the initial conditions of the charge distribution in earlier steps. Information about these distributions can only be obtained by measurements of gating currents.

Fig. 6 shows the dependence of the time shift of the sodium current (Δt_{Na}), as well as the charge movement (Q) during the test pulse, as functions of the prepulse (V). Clearly, Q is related to the charge movement during the prepulse. As the prepulse becomes more positive, less charge must move in order to result in opening the sodium channels and they open sooner.

For the curves shown in Fig. 4b for the time development of the shifts as a function of duration and magnitude of the prepulse, we measured the amount of charge moved in the gating currents and found a linear relation between the charge moved and the time shift, as shown in Fig. 5, but we did not explore this further because of the uncertainty of the shape of the gating current at short times. Most of our experiments were conducted without series resistance compensation (which does not appear to alter the time shifts) and a recording interval of 4 μs per point, with a low-pass filter that cut off at 33 kHz. Under these conditions, the gating current has a flat or rounded top with the natural result that the time shift will have a linear relation to the amount of charge moved. Further investigation of this point will require more and better experiments.

Many of the results can be duplicated with a fairly simple sequential model with six steps. This model does not include the effects of inactivation, but this is not considered to be a severe problem for hyperpolarizing prepulses or for small depolarizing prepulses. The six steps are used to get the delay in the rising phase of the sodium current. The original scheme of Hodgkin and Huxley (1952) will also produce a delay, but it encounters severe difficulties in other respects and has been abandoned (Armstrong and Bezanilla, 1974). We have not tried to actually fit the data, but Fig. 7 shows one attempt to illustrate the main features.

It is important to realize that this six-state model is an extension of, not an alternative to, the four-state model that we have considered to describe the effects of fast inactivation, or the eight-state model that includes slow inactivation (Bezanilla et al., 1982a; Fernández et al., 1982). Those models are concerned only with the last step in the process of the channel-opening procedure. In the present scheme we have six states involved with the early parts of the process. If inactivation were to be included, we would have 12 or 24 states to consider for our simplified model. This model deals with the linear sequence considered by Armstrong and Bezanilla (1977) and Armstrong and Gilly (1979).

In this model, states 6–2 are closed and state 1 is open. We assume that between any two consecutive states, n and $n + 1$, a charge of magnitude $ez(n)$ (e is the electronic charge and z is the valence), moves across an energy barrier that, in the absence of a field, is $W_A(n)$ for the forward direction and $W_B(n)$ for
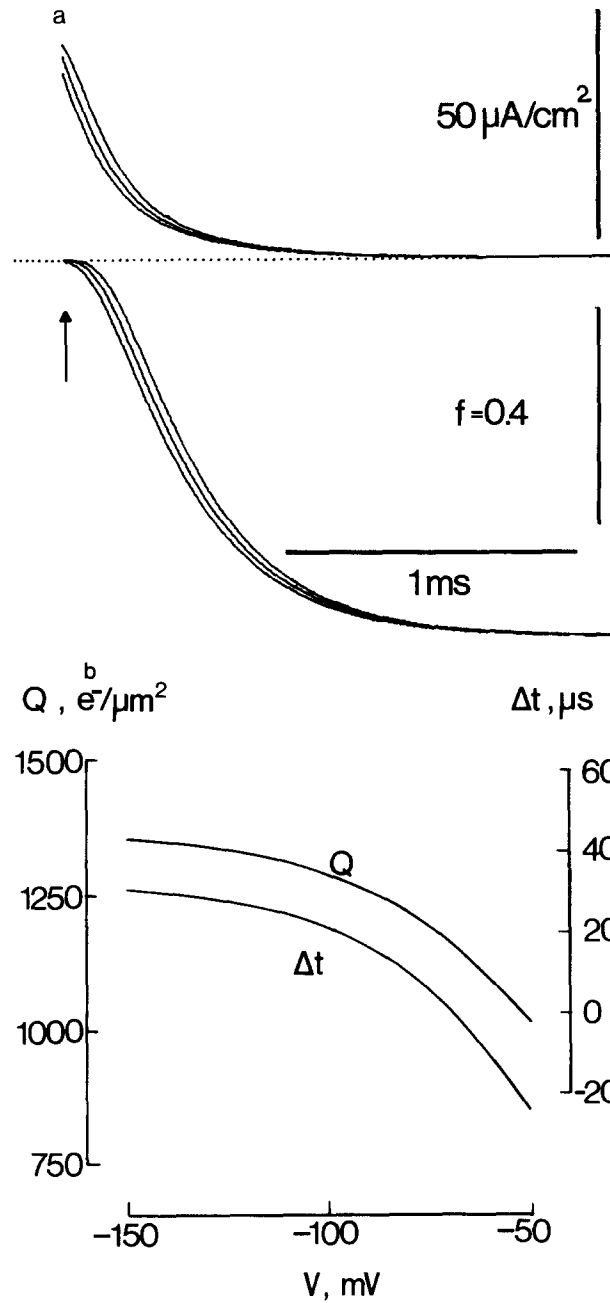
the backward. Using Eyring rate theory, we can write for a voltage V, the forward rate constant $\alpha(n, V)$, and the backward rate constant $\beta(n, V)$:

$$\alpha(n, V) = (kT/h)\exp\{-W_A(n) + [ez(n)V/2kT]\},$$

$$\beta(n, V) = (kT/h)\exp\{-W_B(n) - [ez(n)V/2kT]\},$$

where k and h are the Boltzmann and Planck constants and T is the absolute temperature. We are assuming here that each charge moves through the entire field across the membrane and the barrier is in the middle. Other physical interpretations are possible.

With the values given in the figure legend, the time course of gating current and the fraction of open channels are shown in Fig. 7. For this computer simulation, the prepulses are -150 and -50 mV, for a holding potential of -70 mV and infinite duration. We have not computed the time course of the development of the shift. The computer results shown in Fig. 7 can be compared with the experimental results shown in Figs. 3 and 6. The original estimates for $W_A(n)$, $W_B(n)$, and $z(n)$ were determined in such a way as to produce a reasonable time constant and Q-V curve as well as a fraction of the open channel vs. V curve. Minor modifications were then introduced to get a reasonable shape for the gating current and time shifts resulting from various prepulses. Small changes to the first reaction (between states 6 and 5) are necessary to produce widely different gating current curves for short times, including a rising phase or a sharp, fast-falling phase.

As in Fig. 6 for the experimental results, Fig. 7b shows the time shifts as a function of the prepulse for this model, as well as the charge moved during the test pulse. The agreement of the model with the experimental results is satisfactory, and with some effort exact fits could probably be achieved, but this is part of a greater effort to relate the sodium current to the gating current and the experimental results are the most important part at this time. We believe that to be adequate a model must have at least 24 states, but to implement it fully, many results under different conditions, such as pronase to eliminate fast inactivation, and increasing the time resolution at short times, will be required.

Regardless of the limitations of this model, it is clear that although the sodium conductance is zero in the hyperpolarized region, the charge moving

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Figure 7. (*opposite*) (*a*) Gating currents and fractions of open channels for the six-state model described in text. The gating currents have been calculated on the basis of 1,500 $e^-/\mu m^2$ of total charge available to move. For a pulse to 0.0 mV from a holding potential of -70 mV and prepulses to -150, -70, and -50 mV. Effects of inactivation are not included. Parameters for model; steps 5-2, $z = 1$. Step 1, $z = 1.7$. The barriers in kT units are: $W_A(5) = 19.5$, $W_B(5) = 22.37$, $W_A(4)$ to $W_A(2) = 19.5$, $W_B(4)$ to $W_B(2) = 20.37$, $W_A(1) = 21$, $W_B(1) = 22.39$. (*b*) Comparison of gating current charge movement (Q) and sodium current time shifts (Δt) vs. the potential during an infinitely long prepulse from a holding potential of -70 mV for pulse to 0.0 mV. The parameters for model are as in *a*.

in this region is responsible for the time shifts of the sodium current produced by hyperpolarizing prepulses.

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