A STORED CHARGE MODEL FOR THE SODIUM CHANNEL

ROSALIE C. HOYT *and* JAY D. STRIEB

From the Department of Physics, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010

ABSTRACT A new model is proposed to account for the apparent conductance changes of the sodium, or early, channel in nerve fiber membranes. In this model it is assumed that the channels are gated at the interior side of the membrane and are resistively limited at the exterior side by sodium selective barriers of high resistance to ion flow. Under resting conditions the closed channels accumulate a store of sodium ions, dependent on the exterior sodium concentration. With the application of a depolarizing clamp the interior gates open allowing the stored ions to discharge into the interior low sodium concentration solution. In this model the initial rise in the early current results from the opening of more and more gates in response to the depolarizing clamp. The subsequent fall in the early current results from the "capacitative" discharge of the opened channels, limited by the high resistive barrier at the exterior end. Upon repolarization, the gates reclose and sodium ions reaccumulate in the channels from the high concentration external solution, but at a slow rate determined by the resistive barrier. Preliminary tests of this model, using a number of simplifying assumptions, show that it has the ability to account, at least semiquantitatively, for the major characteristics of the experimental clamp results.

INTRODUCTION

It has become convenient to analyze the transmembrane currents flowing in response to voltage clamps in terms of conductance changes of separate early (sodium) and delayed (potassium) channels. Analyzed in this way, the two channels differ in at least one striking way. The conductance of the early channel rises to a peak and then falls back to a very low value. This is in contrast to the behavior of the delayed channel whose conductance rises monotonically to a steady-state level.[1] In this paper we consider the possibility that the fall, or inactivation, of the early conductance is because of depletion of ions in the early channel rather than to a true conductance change of the channel. To take a simple analogy, consider the circuit of Fig. 1 *a*, in which it is assumed that $R_2 \gg R_1$. Upon closure of the switch S the current I through the ammeter will be as shown in Fig. 1 *b*, starting at a high value V_0/R_1 and decaying exponentially to a low value $V_0/(R_1 + R_2)$. It would be

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[1] Neglecting the very long time constant inactivation of Ehrenstein and Gilbert (1966).

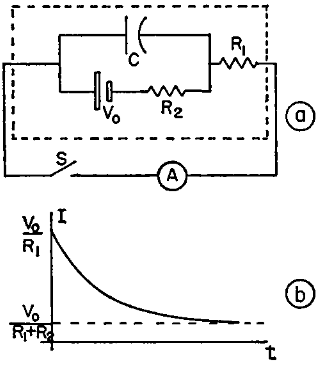

FIGURE 1 A simple circuit analog.

legitimate, though inconvenient, to analyze this circuit in terms of an operationally defined apparent "conductance" $g = I/V_0$, in which case g would be a time-varying characteristic of the system starting at the high value $g_0 = 1/R_1$ and falling exponentially to the low value $g_\infty = 1/(R_1 + R_2)$, with a time constant $\tau = R_1R_2C/(R_1 + R_2)$. Carrying the analogy one step further, consider the results of a "recovery" experiment performed on this circuit (assumed to be enclosed in a "black box," dashed lines in Fig. 1 *a*). Opening the switch S will allow the E.M.F. source V_0 to recharge the capacitor through the high resistance R_2, with a recovery time constant $\tau_R = R_2C$. The magnitude of the initial current obtained upon reclosing the switch a short time later will depend upon how long a recovery time has been allowed. The apparent conductance of the system will thus be found to recover with time from its low g_∞ value back to its original large value g_0.

The behavior shown by the circuit of Fig. 1 is, of course, quantitatively unlike the behavior shown by the early membrane current, but is introduced to show that a time-varying *apparent* conductance need not imply a time-varying "true" conductance but rather the presence of a capacitative element. We are currently investigating the extent to which a model for the early channel can be based on this concept. In this paper we present the results of a preliminary study of the ability of such a model to account for the rise and fall of the early current, for the effect of conditioning preclamps and of test clamps on the magnitude of the peak early current, for recovery from "inactivation" upon reclamping to the holding potential, and for the total charge released.

This study is part of a continuing effort to develop and test alternative plausible models to account for the black box properties of the axon membrane. The validity of the separation into delayed (potassium) and early (sodium) channels has been confirmed both by tetraethylammonium (TEA) and tetrodotoxin (TTX) experiments (Narahashi and Moore, 1968) and by kinetic analysis (Hoyt, 1971). For the early channel, two alternative models have been developed in quantitative detail, the independent factor model of Hodgkin and Huxley (1952) and the coupled model of Hoyt (1963). Recent analyses, Hoyt (1968) and Hoyt and Adelman (1970), have shown that the predictions of the coupled model are in better agreement with the experimental results of inactivation experiments than the independent factor model. Such analyses can make comparative judgments between models but cannot be used to prove the validity of any one model. The new model for the early channel proposed in this paper must be subjected to rigorous comparative experimental testing before it can be adjudged better or less able to explain all the existing experimental results than any of the models developed earlier. Should experiments rule in its favor, the model proposed here will still not have been "proved." A new model may displace it.

DESCRIPTION OF THE MODEL

We consider an axon membrane bathed externally and internally by solutions of high and low sodium concentration, C_e and C_i respectively. The early channels are assumed to be gated at the interior surface by voltage and time-dependent gates. At the exterior surface each channel is assumed to be permanently open, but semiblocked by a region that presents a high but finite resistance barrier to the flow of sodium ions. All anions and all other cations than sodium are assumed to be completely blocked from entering the early channels at the exterior, or barrier, side. The only cation that can enter the early channels through open gates is assumed to be sodium.[2] The assumption that the early channels are gated at the inside of the membrane rather than at the outside is not essential to our argument. Our basic argument could be carried through making the opposite assumption. Which assumption will lead to the best quantitative fit of the model predictions to the results of experiments is a yet-to-be-resolved question. The nature of the gates we leave unspecified at this time. They could be ions acting as plugs, polar ends of phospholipid molecules. side chains of protein molecules, or any of a host of other possibilities.

In Fig. 2 *a* we show, in a highly schematic fashion, a portion of a membrane when it is held at a hyperpolarizing potential. All of the interior gates *G* are closed, and each of the channels (three are shown) contain a total "charge" of sodium ions such that the channels are in equilibrium with the high sodium concentration of the exterior solution. This equilibrium results from the exchange of sodium ions across the resistive barriers *B*, barriers that allow equilibrium to be attained, but only slowly.

In Fig. 2 *b* we diagram the situation to be expected at some time after application of a highly depolarizing voltage clamp. Gate 1 is assumed to have opened at some time earlier, gate 3 to have just opened, and gate 2 has not yet but is about to open.

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[2] We neglect, for simplicity, the evidence of Chandler and Meves (1965) and others that cations other than sodium can sometimes produce an apparent outward positive current flow through the early channels.

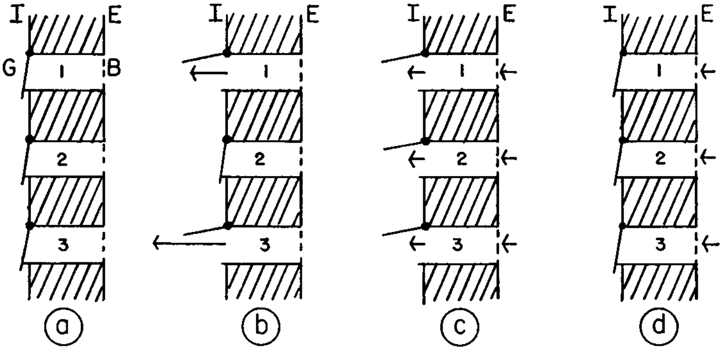

FIGURE 2 Model membrane. The interior and exterior surfaces of the membrane are labeled *I* and *E* respectively. In *a* the gate and barrier of channel 1 are labeled *G* and *B* respectively. (*a*) Hyperpolarized membrane, all gates are closed. (*b*) Depolarized membrane a short time after application of a test clamp. The arrows indicate the magnitude of the channel discharge currents. (*c*) Highly depolarized membrane a long time after application of a test clamp. The arrows indicate the small steady-state currents, limited by the high barrier resistances. (*d*) Hyperpolarized membrane a short time after repolarization. The arrows represent the recovery flow of ions into the channels.

Any one channel, upon the opening of its gate, discharges most of its stored sodium ions into the lower concentration interior solution, until a new equilibrium situation is reached. The fact that the arrow at channel 1 is shorter than that at channel 3 is meant to indicate that channel 1, open for a longer time, has already almost reached its new equilibrium concentration of sodium ions and is therefore discharing at a slow rate, while channel 3, having only just opened, is discharging at a large initial rate.

In Fig. 2 *c* we diagram the situation to be expected at long times after a highly depolarizing clamp. All gates have been open for a long enough time that the channels are now in approximate equilibrium with the low concentration interior solution. A very small, steady current of sodium ions is flowing from the exterior solution across the high resistance barriers *B*. The resistance of these barriers is assumed to be high enough that the steady current across them in Fig. 2 *c* is negligible compared to the initial discharge currents in Fig. 2 *b*.

Finally, in Fig. 2 *d* we diagram the situation to be expected upon repolarization to the hyperpolarizing holding potential. The gates are again all closed and the channels gradually become refilled by the slow passage of sodium ions across the resistive barriers.

As discussed more fully in a later section, in this model the initial rise of the early current results from the opening of more and more gates with time, allowing more and more channels to start discharging their excess sodium ions into the interior solution. The subsequent fall of the early current results from the decrease of sodium ion discharge current with time as the opened channels approach equilibrium with the sodium concentration of the interior solution. The fall of the initial current therefore does not result, in this model, from a true decrease in conductance. Rather, the apparent conductance decrease is analogous to the capacitative effect observed in Fig. 1.

NOTATION AND SIMPLIFYING ASSUMPTIONS

Notation

E_H	Holding potential.
E_m	Potential of the test clamp.
C_e	External sodium concentration.
C_i	Internal sodium concentration.
C_0	A standard reference concentration.
E_e	External "half-cell" potential, $(RT/F) \ln (C_e/C_0)$.
E_i	Internal half-cell potential, $(RT/F) \ln (C_i/C_0)$.
E_{Na}	Nernst equilibrium potential, $E_e - E_i = (RT/F) \ln (C_e/C_i)$.
N	Number of early channels per unit membrane area.
f	Fraction of early channels with open gates.
f_∞	Equilibrium fraction of early channels with open gates.
N_0	Nf, number of early channels with open gates per unit membrane area.
q_c	Charge of sodium ions in a closed channel.
$q_c(E_H)$	Equilibrium charge of sodium ions in a closed channel at the holding potential.
q_0	Charge of sodium ions in an open channel.
$q_0(E_m)$	Equilibrium charge of sodium ions in an open channel at the test potential.
t'	Time at which the gate of a particular channel opens after a depolarizing clamp.
i	Discharge current from a single channel.
I	Combined discharge current of all channels per unit membrane area.
I_p	Polarization surge current from all channels already open.
I_r	Repolarization surge current from all open channels.
g	Apparent conductance, $I/(E_m - E_{Na})$.
C	Stored sodium ion capacitance per channel.
a	Rate constant for the opening and closing of gates.
α	Reciprocal time constant for discharge from an open channel.
β	Reciprocal time constant for recovery across the resistive barriers.

Assumptions

Before the model can be translated into quantitative terms and quantitative predictions drawn from it, assumptions have to be made in answer to a number of questions.

(*a*) How does the equilibrium fraction of gates that are open, f_∞, depend on membrane potential? What are the kinetic laws that determine how f varies with time upon application of a test clamp?

(*b*) For a given value of f_∞, do the open channels remain open and the closed channels remain closed? Or does f_∞ represent merely the average fraction open at any one time, any one channel opening and closing randomly with time?

(*c*) What is the nature of the capacitance that a channel presents for sodium ions? Is the capacitance constant or does it show the nonlinear dependence on bathing concentration and membrane potential to be expected from application of the Poisson-Boltzmann equation to the channel region?

(*d*) Does the discharge current from a channel that suddenly opens have a simple exponential dependence with time, as it would if the resistance to discharge as well as the channel capacitance were both constant, i.e. linear, elements?

Alternative answers to these questions are under investigation in order to determine which assumptions lead to the best quantitative fit with experimental results. The analysis of the present preliminary paper is based on the simplest, quite crude set of assumptions. As will be shown below, even with these simplifying assumptions the predictions from the model are in semiquantitative agreement with the experimental results. The results of refinements of these assumptions will be presented in a later paper.

Assumption 1. The fraction f of open gates depends on the square of a linear variable u,

$$f = u^2, \tag{1}$$

where u is determined by the differential equation

$$\dot{u} = -a\,(u - u_\infty), \tag{2}$$

and u_∞ and a have dependences on membrane potential as shown in Figs. 3 *a* and *b*.

The quadratic assumption of equation 1 has been chosen in order to simplify the preliminary calculations presented in the next section. It will undoubtedly have to be modified, either to a cube law as in the Hodgkin and Huxley (1952) (HH) model, or to a more general monotonic function such as that developed by Hoyt (1963). The functional dependences for u_∞ and a shown in Fig. 3 have been chosen in large part to be similar to the analogous HH variables m_∞ and $1/\tau_m = \alpha_m + \beta_m$.

Assumption 2. In equilibrium at a given membrane potential the gates do not fluctuate open and closed, but any open gate remains open and any closed gate remains closed. This assumption is possibly incorrect, but simplifies the analysis of the next section.

Assumption 3. The channel capacitance C is constant, and the equilibrium values for the stored charges of sodium ions per channel are given by

$$q_c(E_H) = CE_e = (CRT/F)\ln(C_e/C_0), \tag{3}$$

$$q_0(E_m) = C\,(E_i + E_m) = C\,[(RT/F)\ln(C_i/C_0) + E_m]. \tag{4}$$

Note that we assume a negligible dependence of q_c on E_H, but a linear dependence of q_0 on E_m.

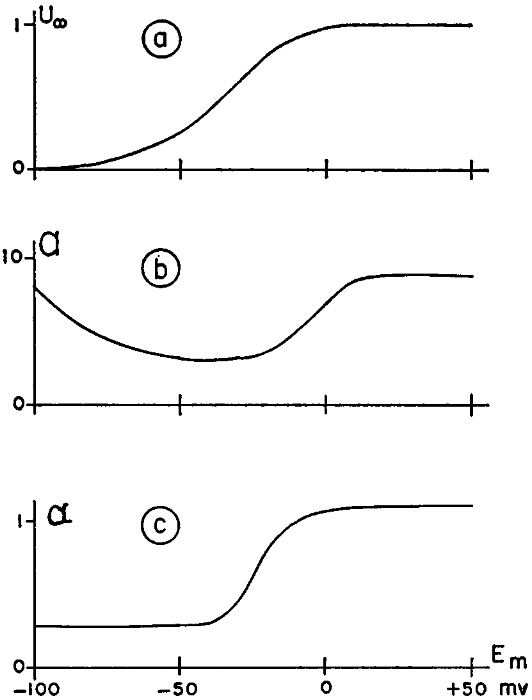

FIGURE 3 Parameter dependences on membrane potential. The a and α ordinate scales are in $(\text{milliseconds})^{-1}$.

The change in stored charge is obviously given, in this linear approximation, by

$$q_c(E_H) - q_0(E_m) = C[RT/F \ln (C_e/C_i) - E_m] = C[E_{\text{Na}} - E_m]. \quad (5')$$

Assumption 4. The time course of discharge from a single channel is exponential, with a time constant τ. The reciprocal time constant ($\alpha = 1/\tau$) has the potential dependence shown in Fig. 3 *c*. Note the similarity between the chosen dependence on E_m of α and that of the analogous HH parameter, $1/\tau_h = \alpha_h + \beta_h$.

For a channel whose gate opens at a time t' after the application of a depolarizing clamp, the charge and current are then given by

$$q_c = q_c(E_H), \qquad t < t',$$

$$q_0 = q_0(E_m) + [q_c(E_H) - q_0(E_m)] \exp [-\alpha(t - t')], \qquad t < t'; \quad (6)$$

and

$$i = 0, \qquad t \geq t',$$

$$i = -\alpha[q_c(E_H) - q_0(E_m)] \exp [-\alpha(t - t')], \qquad t \geq t'. \quad (7)$$

Using the constant capacitance assumption of equation 5 the latter expression may be written

$$i = \alpha C(E_m - E_{\mathrm{Na}}) \exp [-\alpha(t - t')], \qquad t \geq t'.$$

QUANTITATIVE TREATMENT

(*a*) *The Opening of Channels*

Using the simplifying assumptions of the preceding section, the clamp current can easily be derived. The number of channels per unit area opening between t' and $t' + \mathrm{d}t'$ can be written

$$\mathrm{d}N_0 = \frac{\mathrm{d}N_0}{\mathrm{d}t'}\mathrm{d}t' = N\frac{\mathrm{d}f}{\mathrm{d}t'}\mathrm{d}t', \tag{8}$$

and the current density $\mathrm{d}I$ due to this group of channels is

$$\begin{aligned} \mathrm{d}I &= 0 && t < t' \\ \mathrm{d}I &= N\alpha C(E_m - E_{\mathrm{Na}}) \exp - \alpha(t - t') \frac{\mathrm{d}f}{\mathrm{d}t'}\mathrm{d}t' && t \geq t'. \end{aligned} \tag{9}$$

The current density at a time t due to all those channels that have opened between $t = 0$ and $t = t$ is then given by

$$I = \int_0^t \mathrm{d}I = N\alpha C(E_m - E_{\mathrm{Na}}) \exp - (\alpha t) \int_0^t \exp (\alpha t') \frac{\mathrm{d}f}{\mathrm{d}t'}\mathrm{d}t'. \tag{10}$$

The apparent conductance $I/(E_m - E_{\mathrm{Na}})$ therefore can be written

$$g = (N\alpha C) \exp - (\alpha t) \int_0^t \exp (\alpha t') \frac{\mathrm{d}f}{\mathrm{d}t'}\mathrm{d}t'. \tag{11}$$

In this expression for the apparent conductance g we are, of course, neglecting the small leak current across the reistive barriers B of Fig. 2. This approximation is analogous to the often used approximation in the Hodgkin and Huxley (1952) model that h_∞ can be taken as zero for all clamp potentials E_m greater than about -40 mv.

Using the simplified kinetics of assumption 1, equation 11 can now be solved in closed form. Thus, from assumption 1 we can evaluate $\mathrm{d}f/\mathrm{d}t$ as follows:

$$\begin{aligned} u &= u_\infty - (u_\infty - u_0) \exp - (at), \\ \frac{\mathrm{d}f}{\mathrm{d}t} &= 2u\frac{\mathrm{d}u}{\mathrm{d}t} = 2[u_\infty - (u_\infty - u_0) \exp - (at)]a(u_\infty - u_0) \exp - (at). \end{aligned} \tag{12}$$

Inserting equation 12 into equation 10 and integrating yields for g

$$g = A \exp - \alpha t\{B[1 - \exp - (a - \alpha)t] - C[1 - \exp - (2a - \alpha)t]\}, \quad (13)$$

where $A = 2NC\alpha a(u_\infty - u_0)^2$, $B = u_\infty/(u_\infty - u_0)\ (a - \alpha)$, and $C = 1/(2a - \alpha)$.

Curves computed on the basis of equation 13, using the parameter dependences on voltage shown in Fig. 3, are shown in Fig. 4 for two experimental situations, fixed holding potential and different test clamps (Fig. 4 *a*), and fixed test clamp and varying holding potentials (Fig. 4 *b*). The two sets of conductance vs. time curves of Fig. 4 are strikingly similar to those experimentally observed, in spite of the overly simplified assumptions that have been built into equations 11 and 13.

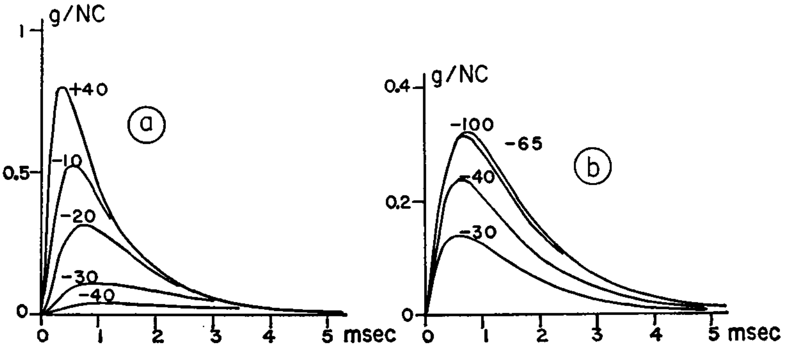

FIGURE 4 Time dependence of apparent conductance of opening channels, calculated from equation 13. The apparent conductances are expressed in units of NC. Curves *a*, holding potential of -100 mv. The numbers associated with each curve give the value of the test potential in millivolts. Curves *b*, test potential of -20 mv. The numbers associated with each curve give the value of the holding potential in millivolts. Note the twofold difference in conductance scale of curves *a* and curves *b*.

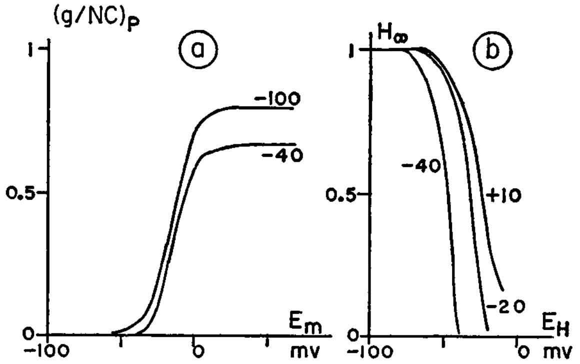

FIGURE 5 Tests of equation 13. (*a*) Peak conductances, in units of NC, as functions of test potential. Two holding potentials were used, -100 mv and -40 mv. (*b*) Steady-state inactivation curves, where $H_\infty = g_P(E_H)/g_P(-100)$. Three test potentials were used: -40 mv, -20 mv, and $+10$ mv.

As further tests of the potential ability of the stored charge model to account for the known experimental behavior of nerve fiber membrane, peak conductances from Fig. 4 *a* and similar calculations are plotted as a function of test clamp potential in Fig. 5 *a*, and the peak conductance ratios obtained from Fig. 4 *b* and other calculations are plotted as a function of holding potential in Fig. 5 *b*. The behaviors shown in Figs. 5 *a* and *b* are in semiquantitative agreement with experimental results obtained from nerve fiber membranes; any quantitative discrepancies can easily be ascribed at this stage to the oversimplifications inherent in assumptions 1–4 inclusive. It is of interest to note that the conditioning curves of Fig. 5 *b* show a shift to the right with increasing test potential, a behavior at variance with the predictions of the HH model, but in agreement with the predictions of a coupled model, Hoyt (1968). Such a shift to the right has been observed experimentally by Hoyt and Adelman (1970).

(*b*) *Polarization and Repolarization Current Surges*

So far we have only considered the apparent conductance changes that result from the opening of new channels. To the current of equation 10 one should add the polarization current surge I_p in those channels that are already open at the particular holding potential used. The charge in an already opened channel will, upon application of a depolarizing clamp, vary exponentially with time

$$q_0 = q_0(E_m) + [q_0(E_H) - q_0(E_m)] \exp - (\alpha t).$$

The polarization current density surge due to the (Nf_0) channels already open at $t = 0$ is therefore given by

$$I_p = -\alpha N f_0 [q_0(E_H) - q_0(E_m)] \exp - (\alpha t).$$

Or, using the linear assumption of equation 4 and the u^2 approximation for f,

$$I_p = -NC\alpha u_0^2 [E_H - E_m] \exp - (\alpha t). \tag{14}$$

In Fig. 6 we show current vs. time curves in which the currents of equations 10 and 14 are shown separately and added to get the total early channel current. In curves *a* there are no polarization surges since at a holding potential of −100 mv all channels are presumed to be closed. In curves *b* the holding potential is −40 mv, and therefore 41% of all channels are open before application of the depolarizing clamps, resulting in sizable initial polarization currents, especially at large test potentials. The initial positive currents predicted by equation 14 and shown in Fig. 6 *b* might be difficult to detect since they would be partially masked by the additional capacitative surge normally present in clamp records. In addition to affecting the initial current, the polarization current of equation 14 is also seen to

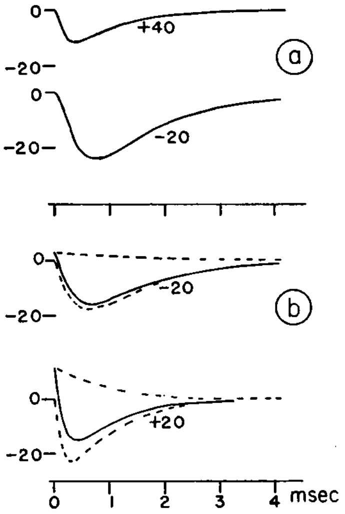

FIGURE 6

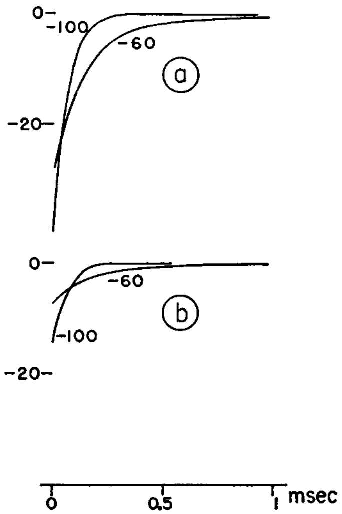

FIGURE 7

FIGURE 6 The effect of polarization currents from open channels. The numbers associated with each curve give the value of the test potential. All currents are given in units of NC. In curves a the holding potential is -100 mv and no polarization currents are observed. In curves b where the holding potential is -40 mv, the upper and lower dashed curves of each set give the polarization and opening channel contributions respectively. The solid curves were obtained by algebraic addition of the dashed curves. $E_{\text{Na}} = 55$ mv.

FIGURE 7 Repolarization currents from open channels in units of NC. The number beside each curve gives the value of the holding potential to which the membrane is repolarized: (a) after a long duration test clamp of $+40$ mv; (b) after a long duration test clamp of -20 mv. *Note:* The current scales are expanded twofold and the time scales fourfold from those in Fig. 6. $E_{\text{Na}} = 55$ mv.

affect the magnitude of the peak negative currents. Using the net currents, solid curves of Fig. 6 *b*, we have recalculated the peak conductances with the result shown in Fig. 8. Inspection of Fig. 8 shows that with depolarizing holding potentials the peak conductances calculated from total currents drop rapidly with increasing positive test potentials, rather than remaining flat as in Fig. 5 *a* where only the opening channel currents were used. The behavior shown in Fig. 8 is in qualitative disagreement with experimental results from nerve fiber membranes; however, it is not obvious how an experimentalist would interpret a current curve such as that of the lower solid line in Fig. 6 *b*. He might judge that a sizable "leakage correction" should be made, and after making such corrections might obtain flat peak conductance curves for test potentials greater than zero.

We have also calculated the repolarization currents to be expected upon reclamping to the holding potential. At the end of a long duration clamp to E_m there will be Nu_0^2 open channels per unit area, each containing q_0 charge, where u_0 and q_0 are those appropriate to the particular value of E_m. As long as any one channel remains open, the charge after repolarization to E_H will be given by

$$q_0 = q_0(E_H) - [q_0(E_H) - q_0(E_m)] \exp - (\alpha t), \tag{15}$$

and its repolarization current by

$$\begin{aligned} i &= \alpha[q_0(E_H) - q_0(E_m)] \exp - (\alpha t), \\ &= \alpha C(E_H - E_m) \exp - (\alpha t). \end{aligned} \tag{16}$$

As soon as a channel closes, its current immediately drops to zero. The total repolarization current is then given by

$$I_r = Nif, \tag{17}$$

where i is given in equation 16 and f is given by

$$f = [u_H - (u_H - u_m) \exp - (at)]^2.$$

Thus,

$$I_r = NC\alpha(E_H - E_m) \exp - (\alpha t)[u_H - (u_H - u_m) \exp - (at)]^2. \tag{18}$$

The values of α and a are those appropriate to the repolarization potential E_H, and u_H and u_m, the equilibrium values of u at E_H and E_m respectively.

Equation 18 has been used to calculate the repolarization currents with the results shown in Fig. 7. As in the case of the initial polarization currents of Fig. 6 *b*, these repolarization currents will be partially masked by the additional capacitative surge normally present in clamp records.

Inspection of Fig. 7 and of equation 18 shows that the initial magnitude of I_r increases with more depolarizing test potentials and more hyperpolarizing holding potentials. Such linear increases result from the equations of assumption 3, and lead, in the limit, to an impossible physical result. Thus, equation 4 would predict that for a sufficiently negative holding potential any channel still remaining open would contain a *negative* charge of sodium ions! The linear capacitance assumption must obviously be discarded at large hyperpolarization potentials, and this in turn will reduce the magnitude of the repolarization currents at these potentials.

(*c*) *The Recovery Process*

Finally we turn to the recovery process after repolarization. Upon closure of its gate after repolarization, a channel will become refilled with sodium ions by the flow of ions across the resistive barrier from the exterior solution. Assuming the same linear approximation used throughout this treatment, this refilling of the

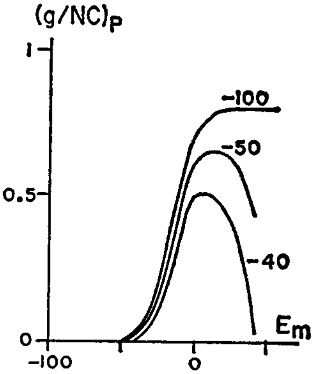

FIGURE 8

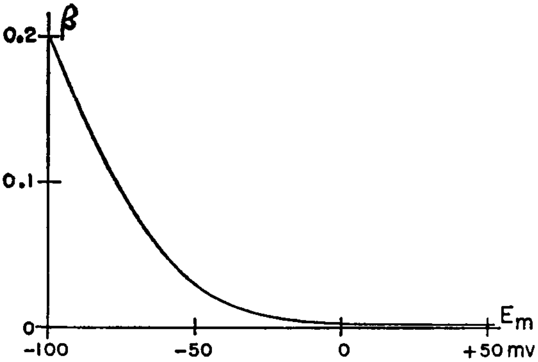

FIGURE 9

FIGURE 8 Peak conductances, in units of NC, calculated from the sum of the currents given in equations 10 and 14. The numbers associated with each curve give the value of the holding potential used.

FIGURE 9 Approximate variation required of the reciprocal time constant for recovery, $\beta = 1/\tau_r$, with membrane potential. The ordinate scale is in (milliseconds)$^{-1}$.

channel will take place exponentially with time, with a recovery time constant τ_r dependent on the potential to which the membrane is repolarized. Assuming as a first approximation that all channels close instantly, the channel charge will recover according to equation 19,

$$q_c = q_c(E_H) - [q_c(E_H) - q_0(E_m)] \exp - \beta t, \tag{19}$$

where $\beta = 1/\tau_r$.

The rate at which the channels refill per unit area is then given by

$$I'_{\text{rec}} = NC\beta f_\infty[E_{\text{Na}} - E_m] \exp - (\beta t), \tag{20}$$

where we have again used the results of assumption 3. The value of f_∞ is that appropriate to the test clamp, while β is that appropriate to the repolarization potential. Under closed gate conditions, the recharging or recovery current (I'_{rec}) does not constitute a current read on an external ammeter since it is balanced by an equal, but opposite, displacement current across the barriers.

The initial value of I'_{rec}, $NC\beta f_\infty(E_{\text{Na}} - E_m)$, should also give the limiting steady-state current under depolarizing clamp conditions. In order that this steady-state current be negligibly small compared to the peak clamp currents of Fig. 6 it is necessary that $\beta \ll \alpha$ for membrane potentials greater than -30 mv. Comparison with Fig. 3 shows that this condition requires that $\beta \ll 0.1$ msec^{-1} for such potentials. On the other hand, the recovery time constant for repolarization to potentials less than -60 mv must be of the order of 5–10 msec. (Note that we are assuming rate constants appropriate to temperatures in the 5–8°C range.) Both requirements can be met if β has a dependence on membrane potential such as that shown in Fig. 9. At hyperpolarizing potentials β is large, implying a small barrier resistance, but the small value of $f_\infty = u_\infty^2$ at hyperpolarizing potentials (Fig. 3 *a*) keeps most channels closed so that the steady-state current is kept negligibly small. At depolarizing potentials β is small, implying a large barrier resistance, so that even though f_∞ becomes large, the steady-state current again is negligibly small.

The simple exponential recovery process of equations 19 and 20 was obtained on the assumption that all gates reclosed instantly upon repolarization. This assumption must be modified for times less than 1 msec after repolarization. During this short time interval the analysis of section *b* must be applied. We shall not present the results of such an analysis here, but instead will indicate the modifications to be expected. In the first place, a short delay in the recovery process is expected as the gates rapidly, but not instantaneously, close with time. A further short delaying effect is expected to result from the repolarization decrease in charge of those channels that close the latest (see equation 15). In fact, this effect might lead to a slight increase in inactivation, or "negative" recovery, during the first few tenths of a millisecond after repolarization. By assuming a nonlinear channel capacitance this effect, like the repolarization currents, can perhaps be made negligible. With appropriate choice of parameters similar delays in the recovery process can also be predicted by the coupled model of Hoyt (1963) but not by the Hodgkin and Huxley (1952) model. Experimental investigation of the initial behavior of recovery curves is therefore of interest.

STORAGE OF IONS IN A CHANNEL

In squid fibers the maximum integrated inward flow of sodium current during a single clamp is typically about 2 μcoul/cm^2. This represents 2×10^{-11} moles of sodium ions released to the interior/cm^2, or 1.2×10^{13} ions/cm^2. If the model described in the earlier sections is to be taken seriously it must be able to account for the storage in the membrane of charges of this magnitude under the resting, closed gate conditions of Fig. 2 *a*.

In order to estimate the ion storage capabilities of the membrane we shall assume channels of large enough cross-section that the Poisson-Boltzmann equation can be applied to them. Consider a channel of length L, containing no fixed charges, of dielectric constant κ_m, and closed at $x = 0$, the interior side. The barrier at $x = L$, the exterior side, is assumed to be permeable only to sodium ions. The concentration $n(x)$ of sodium ions in the channel is then given by Hoyt (1965)

$$n = n_0[1 + \tan^2(\gamma x)], \tag{21}$$

where

$$\gamma = \sqrt{e^2 n_0 / 2\kappa_m \epsilon_0 kT}, \tag{22}$$

and n_0 is the concentration at the closed gate end of the channel. The total number of sodium ions per unit channel area is given by

$$N = \int_0^L n(x)\, \mathrm{d}x = (n_0/\gamma) \tan \gamma L. \tag{23}$$

In order to determine N a value must be obtained for n_0, and this in turn will be dependent on the concentration at $x = L$,

$$n_L = n_0[1 + \tan^2(\gamma L)]. \tag{24}$$

A first crude estimate can be obtained by assuming that n_L is equal to the sodium concentration in the external solution, 450 mM, and that the channel medium is water with $K_m = 80$. Calculations show that under these assumptions a channel of 80 A length would store 2.3×10^{13} sodium ions/cm². Comparison with the 1.2×10^{13} ions/cm² released to the interior per clamp, shows that the involvement of an unreasonably large fraction of the membrane area (50%) in the storage of sodium ions would be required.

Several important factors were omitted from consideration in arriving at the above estimate, and these are listed below.

(*a*) There will be a depletion layer in the exterior solution adjacent to the membrane, reducing the sodium concentration at the membrane surface. This effect may be taken into account by applying the Poisson-Boltzmann equation to the exterior solution as well as applying appropriate boundary conditions at $x = L$ (Hoyt, 1965).

(*b*) The dielectric constant for the channels should be taken as considerably less than 80. In addition to affecting equation 22 a reduction in κ_m will lead to a discontinuous change in electric field strength on the two sides of the barrier. Assuming that the barrier carries no net charge, the electric displacement will be continuous across the boundary at $x = L$, but the electric fields E will be related by the equation

$$\kappa_m E_m = \kappa_s E_s,$$

where the subscript m refers to the membrane side and s refers to the exterior solution side of the barrier.

(*c*) In addition to the effects mentioned under *b*, a reduction of κ_m will lead to a discontinuous change in ion self-energy ΔU on crossing the barrier, the energy being higher on the membrane side than on the solution side. This effect may be estimated either by consideration of the degree of dehydration on crossing the barrier or by a classical calculation of the difference in the self-energy stored in an ion's coulomb field (Parsegian, 1969) when it is in the exterior solution and when it is in the membrane.

(*d*) While each of the three previous effects acts to reduce the total number of sodium ions stored in the membrane, there is one additional effect that can act in the opposite direction, namely, the presence of a dipole layer at the membrane surface. Such a dipole layer will produce a discontinuity of electric potential given by

$$\Delta V = (P/A)\kappa_d \epsilon_0,$$

where P/A is the surface density of dipole moments and κ_d is the average dielectric constant in the immediate region of the dipole layer.

Effects *c* and *d* may be combined to give a net partition coefficient for the ratio of sodium ions immediately adjacent to the two sides of the semipermeable barrier. Thus

$$n(L)_m/n(L)_s = \exp{(\Delta V - \Delta U)e/kT}.$$

Calculations have been carried out taking all four of the above effects into account. In these calculations the following numerical values were chosen:

$$L = 80 \text{ A},$$

$$\kappa_s = 80, \kappa_m = 5, \kappa_d = 18,$$

$$\Delta U = 41 \text{ kcal/mole},$$

$$P/A = 27 \text{ debye units}/(5 \text{ A})^2$$

It is assumed that the dipoles are arranged with their positive ends extending perpendicularly into the exterior solution. Our choice of 27 debye units for the dipole moment of a typical phospholipid molecule agrees with that cited by Goldman (1964) for phosphatidylcholine in its fully extended configuration. The assumption that there is one such dipole every $(5\text{A})^2$ of membrane surface area is not unreasonable.

The above values, together with an assumed sodium concentration in the external solution of 450 mM, lead to a value for N of 1.68×10^{15} stored ions/cm^2. When this value is compared with the experimental value of 1.2×10^{13} sodium ions released/cm^2 per clamp, it is seen that only 1/140 of the membrane area need be involved in the storage of sodium ions. Under these conditions the average concentration of sodium ions in a channel is 3.5 M, considerably larger than that in the bathing solution (0.45 M). This increase results, of course, from the net attractive energy drop produced by the assumed dipole layer. The ions in the channel will, on the average, remain close to the inside surface of the barrier, attracted by the net negative charge in the Debye region just outside the channel.

DISCUSSION

The form of equation 11 for the early channel apparent conductance is easily seen to bear a resemblance to the (ghm^3) conductance of the HH model. NC corresponds to g, the $[\alpha \exp - (\alpha t)]$ term corresponds to the h variable of HH, and the integral term corresponds to the m^3 variable of HH; however, these correspondences cannot be pushed too far. In the HH formulation the h and m^3 terms are independent, while the integral term of equation 11 contains an exponential factor related to the $\exp(-\alpha t)$ term multiplying the integral. This suggests that the present model may have a closer relationship to the coupled model developed by one of us, Hoyt (1963, 1968), than to the Hodgkin and Huxley (1952) model, a suggestion also implied by the shifts with test potentials of the conditioning curves of Fig. 5 *b*. The coupling present in this stored charge model is made evident if one differentiates equation 11 with respect to time. The resulting differential equation for g is

$$\frac{\mathrm{d}g}{\mathrm{d}t} = -\alpha g + N\alpha C \frac{\mathrm{d}f}{\mathrm{d}t}, \tag{25}$$

which shows the coupling of g to f. The nonlinear differential equation for f can be written

$$\frac{\mathrm{d}f}{\mathrm{d}t} = \frac{\mathrm{d}f}{\mathrm{d}u}\frac{\mathrm{d}u}{\mathrm{d}t}, \tag{26}$$

where

$$\frac{\mathrm{d}u}{\mathrm{d}t} = -a(u - u_{\infty}). \tag{27}$$

Once the functional dependence of f on u is chosen, e.g. the $f = u^2$ relationship used in our simplified analysis, equations 25–27 completely specify the time dependence of g (assuming that the potential dependence of the parameters u_{∞}, α, and a are known and also that of the recovery rate constant β).

There are a number of details that remain to be worked out for this model before it can be put to a strict quantitative test. In particular, a number of our simplifying assumptions are probably unrealistic and should be modified, especially assumptions 2 and 3. It is also necessary that this model be tested for its ability to predict membrane action potentials of the correct shape. The preliminary results seem sufficiently encouraging to warrant such further efforts.

Comparative experimental tests of the predictions of this model with those of other models would seem most obviously to lie in analyses of the effects of holding and test potentials on the current surges that occur with depolarization and repolarization. Our model predicts that in addition to the normal capacitative surges to be expected with clamping there are additional surges due to any open early channels, as shown in the curves of Figs. 6 *b* and 7; however, it may be difficult experimentally to separate the normal capacitative surges and step leakage currents from those of Figs. 6 *b* and 7.

In the last section it was shown that it is physically possible for a nerve fiber to have the charge storage capability required for this model; however, if the estimate of 1/140 of the area involved in sodium ion storage is combined with a channel conceived as a narrow, single-file pore of about 30 A^2 cross-section, the presence of one such pore every 4200 A^2 of membrane surface is required, a considerably greater density than that suggested by the TTX bioassay experiments of Moore et al. (1967). It is conceivable that rather than blocking such single-file pores the action of each TTX molecule might be to block an entire "tilelike" membrane unit of the sort suggested by Cole (1968). If the tiles are hexagonal of 200 A width, the required number of sodium storage tiles would be $21/\mu^2$, a number comparable to the TTX results.

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