A KINETIC MODEL FOR THE SODIUM CONDUCTANCE SYSTEM IN SQUID AXON

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ABSTRACT We describe a kinetic reaction sequence for the sodium conductance system in the squid axon. It closely matches the original Hodgkin and Huxley model for voltage clamp experiments but it generates an action potential without a bump on the falling phase. When calcium ions are included in the reaction, this model faithfully reproduces the experimental observations of Frankenhaeuser and Hodgkin on the effects of altered calcium in the medium. The fit to experiment is much better than when a voltage shift in rate constants is assumed. The gating currents recently observed by Armstrong and Bezanilla are not compatible with the Hodgkin and Huxley model but can be reproduced in considerable detail by the kinetic model. Thus it appears that the kinetic model differs from that of Hodgkin and Huxley perhaps in an important and fundamental way that makes it more realistic.

INTRODUCTION AND PERSPECTIVE

It has occurred to a number of investigators that the sigmoid onset and exponential reset of the squid axon membrane conductance changes associated with a depolarizing pulse can be generated by first-order kinetic reaction systems. Goldman (1964, 1965) has proposed a molecular reaction sequence to account for both sodium and potassium conductance transients. Jain et al. (1970) proposed a somewhat different kinetic cycle to accommodate both conductances. Armstrong (1969) has described a kinetic reaction sequence equivalent to the Hodgkin-Huxley (HH) formulation of the potassium conductance in squid axon. Hoyt (1963) has proposed another reaction sequence for the sodium conductance in the squid axon while Moore and Jakobsson (1971) have developed a kinetic reaction scheme for the sodium conductance in frog nodes.

There are two general possibilities for the location of the sites at which permeability to sodium takes place. The currents of sodium may represent merely the statistical occurrence of a process which has a finite, though low, probability of occurring at any point in a homogeneous membrane. Or, there are specialized regions of the membrane which mediate the Na^+ permeability. We can decide between these possibilities on the basis of an experiment (Moore et al., 1967) which demonstrated that, at most, only about 13[1] molecules of tetrodotoxin (TTX) are required to completely block the early

[1]Keynes et al. (1971) have reported a somewhat larger figure of 36 molecules/μm^2 of lobster axon. This confirmation of a very small number of sites strengthens the above argument.

transient conductance in 1 μm^2 of lobster axon membrane. Now it is possible that these TTX molecules could cause some widespread "fracture" phenomena to stop a "statistical" process but it seems unlikely. It seems more likely that some special molecule (or small group of molecules) highly localized and spread very sparsely in the "lipid curtain," acts as a sodium gate in its territory, responsive to voltage changes across the membrane.

The most widely held and seemingly reasonable notion is that this molecule's duty is fulfilled simply by conformational changes, or isomerizations. Alterations in either the shape of the molecule (or its surroundings) may allow passage of sodium (and other cations). These conformational changes would occur in a reversible or cyclical system, so that this molecule would exist in one of a finite number of discrete physicochemical states, but the total number of these molecules per unit area of membrane would not vary with time. Transitions between states would occur with first-order kinetics and one or more of the conformational states would be the "sodium-permeable state." The extremely low number of tetrodotoxin molecules required to block the sodium channel probably means that only an exceedingly small number of such molecules are involved in the sodium conductance mechanism. Hence it is unlikely that the putative molecule can be found by ordinary chemical analyses of membrane components.

We have been trying to fit a variety of types of experimental data into a kinetic model for the early transient of sodium conductance with particular emphasis on the prominent and important role played by external calcium ions. Because the Hodgkin and Huxley (1952) equations provide an excellent fit to a variety of experimental data, they have been widely used as a standard data set for excitable membranes. It seemed feasible and appropriate to first try to fit a kinetic model to this data set for the voltage clamped squid axon. When this had been achieved we tried to see whether calcium could be incorporated into the model in a way to simulate the effects of calcium on the squid axon as described by Frankenhaeuser and Hodgkin (1957). As this work was being completed, "gating" currents were reported by Armstrong and Bezanilla (1973, 1974), Keynes and Rojas (1973, 1974), Bezanilla and Armstrong (1974), and Meves (1974). We immediately checked and found that several of the observations on gating currents were compatible with our kinetic model. The purpose of this paper is to report on the several ways in which the model fits experimental observations. There are also many additional experimental observations which need to be matched but these will be dealt with later.

METHODS

All simulation programs were written in a conversational language, FOCAL,[2] and run on either a PDP-15 or PDP-8 computer. Only a few of the programs required more than the usual minimal size core memory of 4,000 words.

Because most of the simulations were done for the voltage clamp situation, the rate constants were found for the voltage step and then were not changed until another voltage level change

[2]Proprietary to Digital Equipment Corporation (Marlboro, Mass.) computers.

occurred. Therefore the simplest numerical integration method, Euler, was employed, usually with step sizes small enough to reduce deviations from the true integral by only the width of the plotted line.[3]

Output curves were plotted on a Tektronix 4002 Graph Display Terminal and a permanent record obtained with the Tektronix 4601 Hard Copy Unit (Tektronix, Inc., Beaverton, Ore.).

HODGKIN-HUXLEY CONDUCTANCE EQUATIONS

The primary working theory in excitable membrane physiology is the "ionic hypothesis" so successfully employed by Hodgkin and Huxley (1952) in their classic experiments elucidating the role played by specific membrane permeabilities of sodium and potassium in the active phenomena of the membrane. Hodgkin and Huxley (HH) described the sodium conductance system of the squid axon membrane as a product of three terms: (*1*) a scale factor term $\bar{g}_{Na}$, (*2*) a turning-on process m^3, and (*3*) an inactivating process, h.

This conductance gating system might be visualized as being composed of three identical and one different molecular groups which independently respond to the membrane potential changes by assuming either an "open" or "closed" configuration. Taking the sodium conductance to be proportional to the cube of the "open" configuration, m, is equivalent to saying that the conductance turn-on is proportional to the probability that the three identical but independent processes are in the m state. Multiplying m^3 by the first power of the h process is equivalent to stating that the conductance is also proportional to the probability that another single but also independent process is simultaneously in the h or "open" state. Being probabilities, both m and h have values between 0 and 1 and may be represented by the following first order reactions where the left side gives the closed configuration and the right is the open configuration.

$$(1 - m) \underset{\beta_m}{\overset{\alpha_m}{\rightleftharpoons}} m \qquad \text{(Reaction 1)}$$

$$(1 - h) \underset{\beta_h}{\overset{\alpha_h}{\rightleftharpoons}} h \qquad \text{(Reaction 2)}$$

All of the rate constants are voltage dependent (changing instantaneously with any potential change), but those for the much faster m process, are much larger than those for h. At the resting potential, β_m is much larger than α_m, driving the reaction to the left and resulting in a very low value for m; β_h and α_h are of the same order of magnitude but not quite equal so that h at rest is about 0.6. Depolarization causes α_m to become much larger than β_m so that m (starting from a near zero level) increases with a first order lag. Hodgkin and Huxley matched their experimental data displaying a sigmoid onset following a membrane depolarization by this first order lag process raised to the third power. Depolarization also causes β_h to become larger than α_h

[3]For a careful evaluation of numerical integration methods for the Hodgkin-Huxley equations, see Moore and Ramón, 1974.

and a maintained depolarization causes h to gradually decrease in an exponential fashion or "inactivate" the sodium conductance. Restoration of the normal conductance requires that the membrane be repolarized for a relatively long time before h is fully restored to its previous value.

If the membrane potential is stepped from the resting potential (V_r) (or a hyperpolarized level) to a maintained test depolarization, (V_t) the Hodgkin and Huxley formulation can be satisfactorily approximated by the expression

$$G_{Na} \simeq \bar{g}_{Na} m_\infty^3 [1 - e^{-t/\tau_m}]^3 h_o e^{-t/\tau_h}, \tag{1}$$

where $\bar{g}_{Na}$ is a scale factor,

$$m_\infty^3 = [\alpha_m/(\alpha_m + \beta_m)]^3 \tag{2}$$

is the steady-state value of m^3 calculated at V_{test}, and

$$\tau_m = 1/(\alpha_m + \beta_m), \tag{3}$$

$$\tau_h = 1/(\alpha_h + \beta_h), \tag{4}$$

are the time constants calculated at V_{test}, and

$$h_o = \alpha_h/(\alpha_h + \beta_h) \tag{5}$$

is the steady-state value of h calculated at $V_{resting}$.

ALTERNATIVE KINETIC MODELS FOR THE SODIUM CONDUCTANCE

Linear Model

We would like to find a simple reaction cycle which will have kinetics similar to those of the HH equations but which will accommodate calcium ions in such a way as to give a better fit to the Frankenhaeuser and Hodgkin (1957) experimental data.

Let us first consider a linear reaction sequence as a possible kinetic model of distinct states to simulate the condition of a voltage clamp step to a maintained depolarization.

$$P \underset{k_{LP}}{\overset{k_{PL}}{\rightleftharpoons}} L \underset{k_{ML}}{\overset{k_{LM}}{\rightleftharpoons}} M \underset{k_{NM}}{\overset{k_{MN}}{\rightleftharpoons}} N \underset{k_{ON}}{\overset{k_{NO}}{\rightleftharpoons}} O \qquad \text{(Reaction 3)}$$

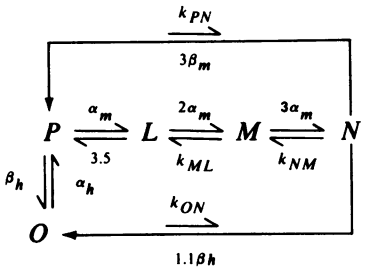
We assume that in the resting or hyperpolarized condition the precursor form P will be the only form present in any significant amount. Upon depolarization, the reaction will proceed to the right causing successive transients through L and M and N, and building up a steady value in O. We will take N to represent the configuration associated with the sodium channel being open. For these boundary conditions, it can be shown analytically (Cox, 1970) that the time course of the concentration in conformation N is identical with the time course of the sodium conductance given in Eq. 1 if the rate constants at the depolarized potential are composed of HH rate constants as follows:

$$k_{PL} = \alpha_m + \beta_m + \alpha_n + \beta_h \qquad k_{LP} = 0$$
$$k_{LM} = 2\alpha_m + 2\beta_m + \alpha_h + \beta_h \qquad k_{ML} = 0$$
$$k_{MN} = 3\alpha_m + 3\beta_m + \alpha_h + \beta_h \qquad k_{NM} = 0$$
$$k_{NO} = \alpha_h + \beta_h \qquad k_{ON} = 0$$

These rates provide the same time course for N as for m^3h but the voltage dependence of the amplitude of the peak N differs from the voltage dependence of the m^3h peak in the Hodgkin and Huxley equations. More disconcerting is the fact that the reaction goes spontaneously at the resting potential in the forward direction (P to N) because of the presence of β_m in the forward rate constants (β_m becomes large upon repolarization to the resting level).

Cyclic Model

A search was made for a better model which would give the best fit to the Hodgkin-Huxley (m^3h) system over the full range of depolarizing voltages. We systematically examined several linear and cyclic reactions and found only one (Reaction 4) which gave satisfactory fits to the HH data set for all of tests applied. When simulation of the calcium effects was undertaken, several alternative schemes, which previously had been considered marginal, performed so poorly that they were dropped from further consideration. Rather than showing and discussing all of the alternative scheme failures, we will proceed to describe in detail the one reaction scheme which remained after our systematic but not totally complete study.



(Reaction 4)

Important rate constants are marked with a value taken from the Hodgkin-Huxley system. Others, labeled with k's, are assigned zero or small constant values.

Computer simulations of this model assisted in finding the best set of rate constants, the investigator selecting the set of rate constants for each run. Excellent fits were found for the full range of physiological voltages as shown in Fig. 1A and the rate constants for Reaction 4 are given in Table I. Examination of these data revealed that there were close parallels between this set of rate constants and some of those used by Hodgkin and Huxley (as may also be seen in Table I). Substitution of these HH expressions[4] into the kinetic model gave fits (see Fig. 1B) which were almost as good as

[4]In the kinetic model we have used the same sign and zero conventions for membrane voltage and the standard temperature of 6.3°C in order to substitute their expressions directly into our model.

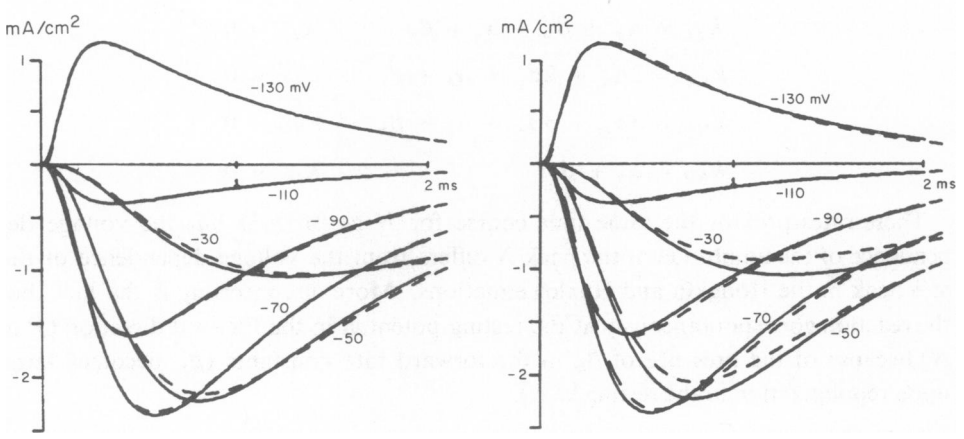


FIGURE 1 Comparison of sodium currents in response to voltage steps calculated from the HH equations (solid lines) and from Reaction 4 (dashed lines). All steps were made from a 30 mV hyperpolarizing holding potential. (*A*) Rate constants in the kinetic model (Reaction 4) chosen to give best fit to the HH system. (*B*) Rate constants in the same kinetic model given by HH expressions. See text for details.

those determined by trial as noted above. It seemed preferable to use the standard HH expressions rather than generate new equations for voltage sensitive rate constants which would be only slightly different from those of Hodgkin and Huxley. This would also simplify correlations and analogies between the kinetic and HH models.

The features of our proposed reaction cycle will now be described in a step-wise fashion.

G_{Na} on Process. Let us for a moment simplify the system and concern ourselves only with matching of the time course of the HH onset or m^3 process with a kinetic model. This can be done by setting the rate constant k_{NO} to zero (eliminating the decay of form *N* to form *O*).

This can be seen to be related to the HH system as follows. Consider that at a hyperpolarized potential, each of the putative three charged particles in the HH scheme is in the nonconducting position ($1 - m$ or $\bar{m}$) but, upon depolarization, the system goes through two intermediate stages before all particles are in the conducting state (m) as follows:

$$\bar{m}\bar{m}\bar{m} \xrightarrow{3\alpha_m} \bar{m}\bar{m}m \xrightarrow{2\alpha_m} \bar{m}mm \xrightarrow{\alpha_m} mmm$$

The rate constants represent the probability of any one of the particles moving from the $\bar{m}$ to m location times the number in the $\bar{m}$ position. From the analytic treatment for Reaction 3, we noted that for the α_m rate constants in the reverse order, a match could also be made to the m^3 process. We further observed that although the intermediates differed, the transient solution for *N* was the same for all permutations of these three rate constants.[5] It is convenient to have such flexibility in the assignment of

[5]We are indebted to Robert Stein for showing that when the differential equation matrix for this system was diagonalized, the diagonal consisted of just the three individual rate constants.

TABLE I

RATE CONSTANTS FOR REACTION 4

Potential	k_{PL}	(α_m)	k_{LM}	($2\alpha_m$)	k_{MN}	($3\alpha_m$)	k_{NO}	(β_h)	k_{NP}	($3\beta_m$)	k_{OP}	(α_h)	k_{PO}	(β_h)	k_{LP}
mV	ms^{-1}		ms^{-1}		ms^{-1}		ms^{-1}		ms^{-1}		ms^{-1}		ms^{-1}		ms^{-1}
−150	13.5	(12.5)	26.0	(25)	38.5	(37.5)	1.0	(1.0)	0	(0)	0	(0)	2	(1.0)	2
−130	11.5	(10.5)	22.0	(21)	32.5	(31.5)	1.0	(1.0)	0	(0.01)	0	(0)	1.8	(1.0)	4
−110	9.5	(8.5)	18.0	(17)	26.5	(25.5)	1.0	(1.0)	0	(0.03)	0	(0)	1.8	(1.0)	4
− 90	7.0	(6.5)	14.1	(13)	20.4	(19.5)	0.99	(1.0)	0	(0.08)	0	(0)	1.65	(1.0)	4
− 70	4.8	(4.55)	10.2	(9.1)	14.7	(13.7)	1.03	(0.98)	0	(0.25)	0	(0)	1.6	(0.98)	3.6
− 50	2.8	(2.72)	6.0	(5.4)	9.0	(8.2)	1.0	(0.88)	0	(0.75)	0	(0)	1.4	(0.88)	3.6
− 30	1.6	(1.27)	2.4	(2.5)	3.2	(3.8)	0.8	(0.50)	0	(2.2)	0	(0.01)	1.2	(0.50)	1.5
0	0	(0.22)	0	(0.44)	0	(0.66)	0	(0.05)	15	(12)	1	(0.07)	0.5	(0.05)	0

Table of rate constants for Reaction 4 for best fit to Hodgkin-Huxley sodium currents in a voltage clamp. The values in parentheses give the HH rates (noted at the top of the columns) which approximate the best fit.

the rate constants at the start because we know that we can rearrange later if the need develops as we attempt to match more data. For the moment, we will continue to use the sequence given in Table I.

In order to fit the HH equations at low amplitudes of depolarization, it was found necessary to introduce a reverse reaction between L and P to slow the onset of sodium conductance. If the rate constant in the forward direction (P to L) is α_m, the best fit to the HH equations at 6.3°C is obtained with a L to P reverse rate constant of 3.5 ms^{-1}. Some variation of this value will give slightly better matching of the kinetic to the HH model at some potentials but, for simplicity, we will take it as voltage independent.

Inactivation. Inactivation following step depolarization can be approximated by setting k_{NO} equal to β_h but a value of 1.1 × β_h gives a somewhat better fit to the HH system. Fig. 1B shows the quality of the match of the kinetic model to the HH system over the full physiological range of depolarized potentials for

$$k_{PL} = \alpha_m \qquad k_{LP} = 3.5\ ms^{-1}$$
$$k_{LM} = 2\alpha_m \qquad k_{ML} = 0^6$$
$$k_{MN} = 3\alpha_m \qquad k_{NM} = 0$$
$$k_{NO} = 1.1 \cdot \beta_h \qquad k_{ON} = 0$$
$$k_{NP} = 3\beta_m \qquad k_{PN} = 0$$

We have also tried a variety of other sets of values for the rate constants k_{LP}, k_{ML}, and k_{NM}. For example, assigning these three rate constants the values of β_m, $2\beta_m$, and $3\beta_m$, respectively, did not fit the HH system very well. Some improvement of fit was found when k_{LP} was taken as $3 + \beta_m$, $k_{ML} = 2\beta_m$ and $k_{NM} = 3\beta_m$, but it was still not as good as found for the above set ($k_{LP} = 3.5$, $k_{ML} = 0$,[6] and $k_{NM} = 0$).

G_{Na} Shut Off and Recovery. Another important feature of the sodium conductance is that following a brief depolarization (just long enough to have the sodium conductance well turned on), an abrupt return of the potential to the resting value results in a rapid nearly exponential decline in conductance. This produces a current pattern frequently referred to as a "sodium tail." In the Hodgkin-Huxley scheme, repolarization makes β_m large and reduces α_m to nearly zero, causing m to revert exponentially to the inactive form ($1 - m$) with a rate constant of approximately β_m. The decay of m^3 is also exponential but three times as fast as the rate of reduction of m. The m^3 decay completely dominates the sodium conductance because the restoration of h by repolarization is very slow.

These experimental observations are incorporated into our kinetic model by the backward reaction taking the N form directly to P with a rate constant of $3\beta_m$.

[6]For theoretical reasons it is preferable to have nonzero reverse reaction rate constants. Some rate constants which need to be small to make the model fit the HH system have been set to zero for convenience and not of necessity. The process of curve fitting does not have high resolution and small variations do not make much difference; for example if one sets $k_{LP} = 3$, $k_{ML} = 0.5$, and $k_{NM} = 0.5$, an almost identical transient occurs.

An additional reaction between O and P is introduced to allow the inactive form O to be restored to the precursor P upon repolarization. This reversible reaction uses the same rate constants which Hodgkin and Huxley originally associated with the variable h (Reaction 2). Therefore the time course of restoration of the precursor should be almost the same as recovery from inactivation in the HH system. The steady-state concentration of the precursor (P_∞) should have a voltage dependence of the same form but not identical with the Hodgkin and Huxley variable h_∞, because of additional reactants.

In the resting or hyperpolarized state, only the forms P and O have any significant concentration and the steady-state distribution between them will be determined by the rate constants α_h and β_h. We have assigned a scaling factor of 120 ($\bar{g}_{Na}$ = 120 mmho/cm^2) to the steady-state sum of $P_\infty + O_\infty$ so that the transient of N in the kinetic model will match the g_{Na} transient in the HH system. Although we are actually modeling the sodium conductance sytem, it seemed useful and appropriate in most cases to express the kinetic model output in terms of the "sodium current" form, i.e.

$$I_{Na} = N(V - V_{Na}), \tag{6}$$

where V and V_{Na} represent the membrane potential and sodium equilibrium potential with the HH convention and usual values. With these conditions and assignments, the N form in the kinetic model will closely simulate the Hodgkin and Huxley sodium conductance and our I_{Na} will approximate their sodium current for the following experimental conditions:

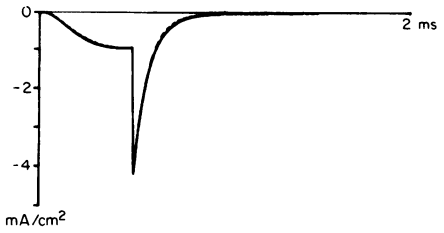
(*a*) *Normal maintained depolarization* for the full range of physiological potentials as illustrated in Fig. 1B. It is possible to achieve better matching by small variations in k_{LP} as a function of voltage (about the best single value of 3.5 ms^{-1}). However the improvement was not judged to be of enough significance to merit writing the additional required rate constant expression. Because the kinetic model transient currents match those of the Hodgkin-Huxley system, the kinetic model also reflects the remarkable steepness of the relation between the peak sodium conductance and the membrane potential (an e-fold conductance increase for a 4–6 mV membrane potential change).

(*b*) *"Tail" currents* associated with repolarization following a brief depolarization are shown in Fig. 2. Those generated by the kinetic model almost superimpose on those of the HH model.

(*c*) *Inactivation* resulting from long conditioning pulses of variable magnitude and measured by a strong depolarizing test pulse is shown in Fig. 3. It compares the steady-state inactivation obtained by simulating such experiments with both the HH and the kinetic model.

(*d*) *Regeneration* of the sodium current to nearly full activation as a function of the duration of repolarization following a depolarization which largely inactivated the sodium conductance. Fig. 4 shows that the regeneration of the peak sodium current is essentially the same in the two models.

(*e*) *Membrane action potentials* generated by substitution of the kinetic model for



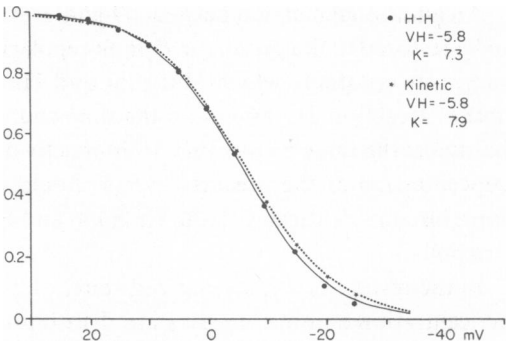
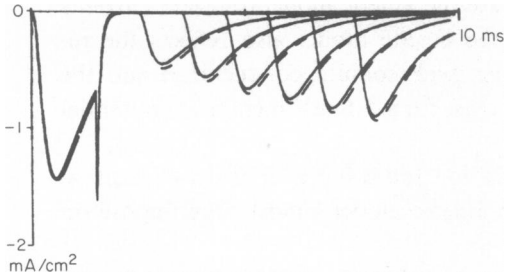


FIGURE 2

FIGURE 3

FIGURE 2 Comparison of "tail" currents associated with repolarization to the resting level following a brief depolarization to −90 mV from rest. The kinetic model response (dashed line) almost superimposes on that of the Hodgkin and Huxley equations.

FIGURE 3 Comparison of the steady-state inactivation curves obtained by simulating the effect of long conditioning pulses on the peak sodium current for a depolarizing test pulse to −70 mV. The points were computer fitted to Eq. 8 by a Davidon-Fletcher-Powell algorithm. The values for V_h and the slope factor, k, are listed for the best fits as drawn.



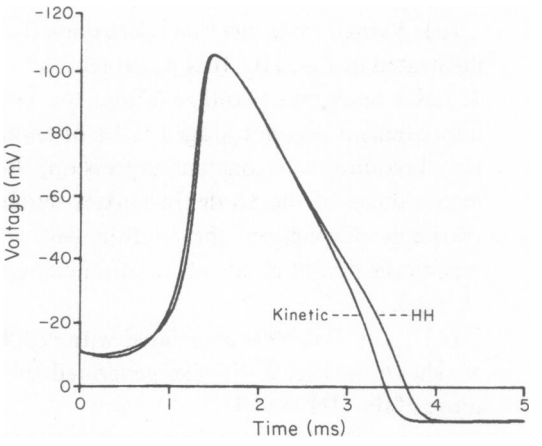


FIGURE 4

FIGURE 5

FIGURE 4 Regeneration of sodium current with repolarization to the resting level following a brief depolarization to −70 mV. The Hodgkin-Huxley system is given by the solid line, the kinetic model by the dashed line.

FIGURE 5 Comparison of membrane action potentials generated by the Hodgkin-Huxley equations and by substitution of our kinetic model for their sodium conductance. The "gratuitous bump" in the action potential generated by the HH equations is barely noticeable in the action potential using the model. The latter looks much more like those observed experimentally.

the sodium system in the Hodgkin-Huxley model are shown in Fig. 5 to overlay those of the complete Hodgkin-Huxley system except that the "gratuitous bump" (Cole, 1968) is largely removed.[7]

EFFECTS OF CALCIUM ON THE SQUID AXON MEMBRANE

In 1957 Frankenhaeuser and Hodgkin published a comprehensive study of the action of calcium on the ionic conductance patterns observed in squid axon. They found that:

(*i*) A reduction in the calcium in the bathing solution, $[Ca]_o$, increased the rate at which the sodium conductance rose under depolarization. In some (but not all) cases the onset of the sodium conductance at five times normal calcium could be made to nearly coincide with that at $\frac{1}{5}$ normal by scaling and shifting the potential by 21–42 mV. This is equivalent to a variable shift of 6.5 to 13 mV per *e*-fold change in $[Ca]_o$ for the three curves where a match was achieved in their Fig. 4.

(*ii*) Reduced $[Ca]_o$ shifted the sodium inactivation curve so that less sodium conductance was available for equal depolarizations from the resting potential. The shift averaged 4 mV per *e*-fold change in $[Ca]_o$ but was quite variable (1–6 mV).

(*iii*) Reduced $[Ca]_o$ caused the curve relating peak sodium conductance to membrane potential to shift along the voltage axis in the hyperpolarizing direction. The shift ranged from 7 to 11 mV per *e*-fold change in calcium with an average of 9.3 mV.

(*iv*) The rate of "shutting-off" the sodium conductance (or "tail" current decline) was slowed by a factor of nearly 5 when the $[Ca]_o$ concentration was reduced nearly fivefold (112–22 mM). For a 25-fold reduction in $[Ca]_o$ (112 to 4.4 mM), the rate of "shutting-off" was reduced by a factor of 10–15, but this statement is not exact because the decline is not truly exponential.

They concluded that these (and other effects on the potassium system) could be summarized by saying that the effects of fivefold reduction of $[Ca]_o$ were similar to those of a depolarization of 10–15 mV (or a 6.2–9.3 mV shift per *e*-fold change). We want to point out here that their summary statement combined what appear to be rather different amounts and kinds of effects into a single average figure and may overly simplify the situation.

Fitting Calcium Effects Into the Hodgkin-Huxley Model

Following the Frankenhaeuser and Hodgkin (1957) summary statement noted above, the effects of variations in $[Ca]_o$ have been frequently simulated with the HH equations by shifting all of the rate constants by 6–9 mV for each *e*-fold of $[Ca]_o$ change. Such modification to the HH equations shift the inactivation curve as well as the peak sodium conductance curve 6–9 mV along the voltage axis, but this shift in the inactivation curve is too much, about twice that observed by Frankenhaeuser and

[7]The reason for this improvement has not been extensively studied but the very small reduction in the sodium current over the range of potentials below −60 mV may be a reflection of the slight misfit of the kinetic model to the HH system shown in Fig. 1B.

Hodgkin. On the other hand, this modification does not provide enough change in the rate of "shutting-off" with $[Ca]_o$ variation. A 7 mV shift assumed for an e-fold increase or decrease in $[Ca]_o$ provides only about one-half the necessary increase or decrease in the "shut-off" rate. Frankenhaeuser and Hodgkin recognized this problem and stated (p. 227), "The effect of reducing the calcium concentration on the shutting off rate is much greater than that expected on the simple principle that a fivefold reduction is equivalent to a depolarization of 10 to 20 millivolts.".

Fitting Calcium Into the Kinetic Model

The simplest way to incorporate calcium into a kinetic scheme is to assume that a single reactant (or conformational state) is a calcium-bound product. Reactions involving transition of that product to another form would release a single calcium ion. In reactions involving formation of that product, the reactant would bind a calcium ion (Reaction 5).

$$A \cdot Ca^{++} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} A + Ca^{++} \qquad \text{(Reaction 5)}$$

The differential equation describing this transition is

$$d[A \cdot Ca] = -k_f[A \cdot Ca^{++}] + k_b[A]\,[Ca^{++}]. \qquad (7A)$$

Assuming the calcium concentration in the medium will not be altered significantly by the few molecules removed or released in these reactions, this expression is reduced to

$$d[A \cdot Ca]/dt = -k_f[A \cdot Ca^{++}] + k_b'[A], \qquad (7B)$$

where $k_b' = k_b[Ca^{++}]$, a first-order rate expression in which the effect of calcium is explicitly seen as a multiplicative factor in the rate constant of calcium binding. It will be shown that, without further assumptions, the choice of P as the calcium-bound product allows matching of diverse phenomena involving changes in calcium concentration.

Reset or "Shutting-off" Following Repolarization. Multiplication of the k_{NP} rate constant ($3\beta_m$) by the ratio of the concentration of calcium in the external medium to its normal concentration provides a rather faithful simulation of external calcium ion effects on the sodium "tail currents." In this important aspect, the kinetic model matches the experimental observations of Frankenhaeuser and Hodgkin much better than do the HH equations with rate constants shifted in voltage.

Inactivation Shift. Fortunately, the choice of P as the calcium-bound form causes the model to be consistent with the Frankenhaeuser and Hodgkin observation that low $[Ca]_o$ decreased the value of h_∞ (roughly analogous to P_∞ in the kinetic model) at or near resting potential. The shift in the steady-state relation between forms P_∞ and O_∞ (in isolation from the rest of the cycle) may be determined analytically and the effect of the external calcium concentration may be predicted.

Replacing Reaction 2 with

$$O \underset{\beta_h}{\overset{\alpha_h}{\rightleftharpoons}} P \qquad \text{(Reaction 6)}$$

and writing the Hodgkin-Huxley expressions for α_h and β_h we have the steady-state relation for P

$$P = \alpha_h/(\alpha_h + \beta_h) = 1/[1 + (\beta_h/\alpha_h)]$$

$$= \frac{1}{1 + \{1/A \exp(aV)[1 + \exp(bV + B)]\}}, \quad (8)$$

where $A = 0.07$, $a = 0.05$, $b = 0.1$, and $B = 3$. Over most of the potential range in which h_∞ varies rapidly, the term $\exp(bV + B)$ is large compared with unity (it is greater than 20 for resting hyperpolarized levels). Thus the expression for P_∞ can be considerably simplified to the form

$$P_\infty = \frac{1}{1 + \{\exp[-(a + b)V - B]/A\}}. \quad (9)$$

Taking V_h as the potential at which P_∞ is one-half of its maximum value, we have

$$0.5 = \frac{1}{1 + \{\exp[-(a + b)V_h - B]/A\}}. \quad (10)$$

From this we can express A as

$$A = \exp[-(a + b)V_h - B], \quad (11)$$

and substitute this into Eq. 9 for P_∞, obtaining an expression similar to that of Hodgkin and Huxley for h_∞.

$$P_\infty = \frac{1}{1 + \exp[(a + b)(V_h - V)]} \cdot \frac{1}{1 + \exp[(V_h - V)/k]} \quad (12)$$

where the slope factor $k = 1/(a + b) = 1/(0.05 + 0.1) = 6.67$ mV. Now, incorporating calcium into the model, we multiply the rate constant α_h by the normalized calcium concentration $[Ca]_o/[Ca]_{sw}$. For an e-fold increase in calcium concentration k_{OP} is given by

$$k_{OP} = A \exp(aV + 1), \quad (13)$$

and the potential for the half-maximum value of P_∞ is now V_h' and approximated by the relation

$$A = \exp[-(a + b)V_h' - 1 - B], \quad (14)$$

Combining this with Eq. 11, we have

$$(a + b)V_h' + 1 = (a + b)V_h \tag{15}$$

and

$$V_h' = V_h - [1/(a + b)] = V_h - k. \tag{16}$$

This predicts that the P_∞ curve should shift along the voltage axis by approximately the magnitude of the slope factor k for each e-fold change in $[Ca]_o$. The actual value for the slope factor found by Hodgkin and Huxley and given by their equations is 7, slightly above the approximate value obtained in Eq. 12 resulting from simplification by dropping the unity term between Eq. 8 and Eq. 9. Steady-state computations for Reaction 6 (identical to those for Reaction 2) at normal $[Ca]_o$ also show a k of 6.9 mV and a parallel shift of the same magnitude (actually 6.85 mV) per e-fold change in $[Ca]_o$. However this is an over-simplified view of the problem because there are other significant paths in Reaction 4.

For a full evaluation of inactivation in our kinetic model it is necessary to simulate the voltage clamp experiments used in experimental determination of inactivation. This has been done and the peak current during the test pulse plotted as a function of the conditioning pulse in Fig. 3 for normal $[Ca]_o$. A computer algorithm (Davidon-Fletcher-Powell) searched for the best fit of Eq. 9 to these points and found a value of -5.8 mV for V_h and 7.9 mV for the slope factor k.

Fivefold increases and decreases in $[Ca]_o$ resulted in inactivation shift of 9.6 mV and 3.8 mV, respectively, or a total shift of 13.4 mV for a 25-fold change in $[Ca]_o$ (or 4.2 mV per e-fold). This represents an excellent fit to the Frankenhaeuser and Hodgkin observation of an average shift of 16 mV for a 25-fold change (between 5 and 0.2 times normal). The kinetic model also happily shows the reduced steepness (increased k) seen in their inactivation curve in low $[Ca]_o$ but the absolute values of V_h and k differed slightly from their observed values.

Onset Transient Changes. Because P has been taken as the calcium-bound form of reactant, calcium will be bound in the reaction L to P and therefore we multiply k_{LP} by the $[Ca]_o/[Ca]_{sw}$ ratio. For a reduced $[Ca]_o$, this will cause a speeding of the onset of sodium conductance and, conversely, an increased $[Ca]_o$ will slow the onset.

That this is consistent with Frankenhaeuser and Hodgkin's observations is shown in Fig. 6 where the time courses of the N form of the kinetic model are compared for five times and $\frac{1}{5}$ the normal $[Ca]_o$ level. The curves are normalized to the same peak amplitudes, so that it can be seen that the onset transients at the low $[Ca]_o$ levels can be made to nearly match those at high levels by shifting the step potential by a variable amount up to 22 mV. Frankenhaeuser and Hodgkin show similar experimental results in their Fig. 4; the potential shift required there was rather variable also.

The involvement of calcium in the reaction from L to P also causes the position of the peak conductance to markedly shift along the voltage axis. Fig. 7 shows the logarithm of the transient peak of N as a function of the depolarizing potential for three concentrations of calcium. The shift seen at the 0.1 max conductance level between 5

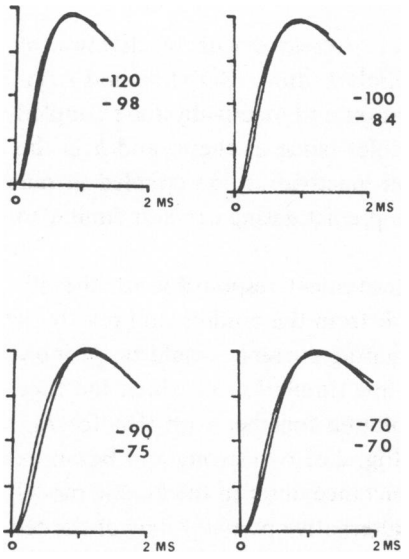


FIGURE 6

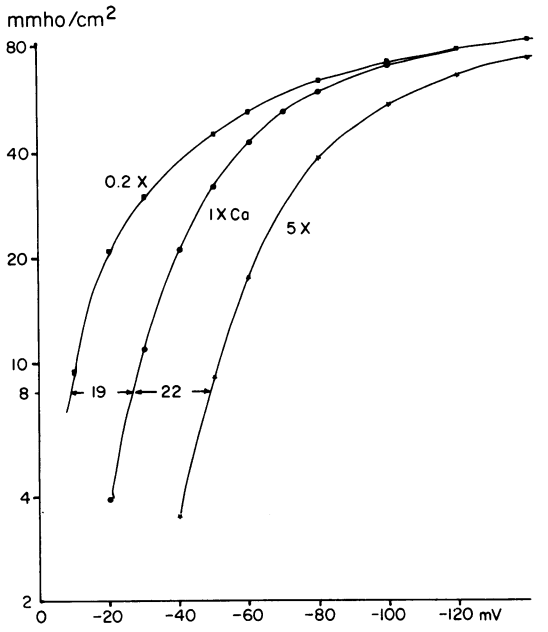


FIGURE 7

FIGURE 6 Shows the effect of $[Ca]_o$ in the kinetic model on the onset transient of the sodium conductance following step depolarization. As in Frankenhaeuser and Hodgkin's Fig. 4, the curves have been normalized to the same peak amplitude for comparison. In each case, the upper figure gives the step potential in five times normal calcium and the lower that in $\frac{1}{5}$ the normal calcium.

FIGURE 7 Shows the effect of $[Ca]_o$ on the peak value of the sodium conductance in the kinetic model as a function of the displacement from a hyperpolarizing holding potential. The shifts in the curves are labeled at the level of $\frac{1}{10}$ the maximum conductance.

and 0.2 times normal $[Ca]_o$ is 41 mV, or a 12.7 mV per *e*-fold change. These results compare very favorably with Fig. 3 and Table 6 of Frankenhaeuser and Hodgkin. For the same $[Ca]_o$ changes, they found a shift of 7.7–11.2 mV per *e*-fold change, with an average of 9.3 mV.

GATING CURRENTS

The "sodium gating currents" first reported by Armstrong and Bezanilla (1973) showed a jump followed by a nearly exponential decay in response to a step depolarization from previous strong hyperpolarization. This seemed compatible with the particle interpretation of the Hodgkin-Huxley equations—that the conductance of each sodium channel may be activated or "turned-on" by the movement of three charged but mutually independent activation particles. For the HH system of equations, the gating currents should be proportional to dm/dt. All three activation particles must be in the "open" position before the channel will conduct and return of any one particle to the "closed" position would close the channel. Thus the sodium current should decay

three times faster than the gating current. Instead, Bezanilla and Armstrong (1974) have recently reported finding that I_{Na} and the gating current "decay with almost the same time course."

Furthermore they observed that the gating currents decreased with inactivation of the sodium conductance as a result of prolonged depolarization. Bezanilla and Armstrong conclude that the sodium conductance activation and inactivation are coupled rather than being associated with independent variables (such as the m and h in the Hodgkin and Huxley formulation). Activation and inactivation are coupled in our proposed kinetic model and furthermore it is able to predict gating currents similar to those observed experimentally.

We have tested two assumptions for the charge movement responsible for the observed "gating currents." The first is that they result from the binding and release of calcium to and from the bound form P. Thus the gating currents would be proportional to dP/dt in the kinetic model. Fig. 8 shows one simulation in which the time course of calcium ion movement in Reaction 4 is plotted together with that for I_{Na}. These patterns appear to be very similar to those in Fig. 2 of Armstrong and Bezanilla (1973). In the discussion of matching sodium conductance onset in the kinetic model to the HH scheme, we noted that there were several alternative permutations of the set of forward (depolarizing) rate constants. Such permutations from the rates shown in Reaction 4 gave a time course of dP/dt which is distinctly different but not as good a fit to the experimental observations. In the kinetic model, restoration of the membrane potential to a hyperpolarized level initiates the restoration of P by binding Ca^{++}. If the depolarization has been brief and a large fraction of the form N remains, there is a large gating current proportional to $3\beta_m$, decaying nearly exponentially at the same rate as the decrease in the sodium conductance (form N in the model). If a depolarization is maintained the reaction will proceed to form O, and a further depolarization

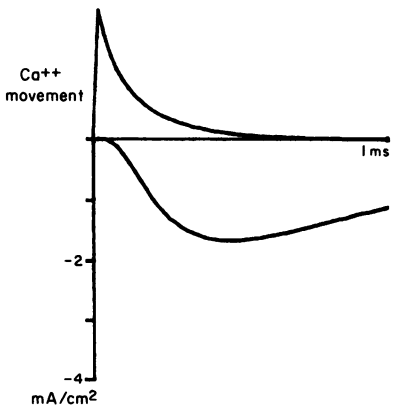


FIGURE 8 Shows the time course of the Ca^{++} movement and the sodium current in the kinetic model following a step depolarization. These patterns appear to be very similar to those of Armstrong and Bezanilla (1973) Fig. 2 showing the time courses of the "gating" current and sodium ion current.

step will give a much smaller gating current. This inactivation of the gating current along with the sodium current is also consistent with the experimental observations of Bezanilla and Armstrong (1974), Armstrong and Bezanilla (1974).

A second assumption for the gating currents is that they result from dipole movement associated with the voltage-sensitive changes in the rate constants. We carried out simulations in which all steps in the reaction contributed to the gating current in proportion to the voltage sensitivity (essentially a charge-distance product) of the rate constant and the net rate of transfer between the associated conformations. In order to have a rapidly decaying gating current most similar to that observed experimentally by Armstrong and Bezanilla (1973), it was found necessary to permute the forward rate constants (from P to N) to $3\alpha_m, 2\alpha_m, \alpha_m$. Then the best fit to the HH conductance data was obtained by setting all of the reverse rate constants equal to a constant of 1 ms^{-1}. The resulting depolarization gating currents show a small steady current similar to that seen in many of the experimental records. The fit of the sodium current in the model to the HH data was almost as good as that shown in Fig 1B. We did not test this set of rate constants against all the experiments previously shown, but, in essence, it reproduced the results shown in Figs. 2, and 4–7 but the inactivation curve was shifted 7 mV and had a slope factor of 13.7 instead of 7.3 mV.

DISCUSSION

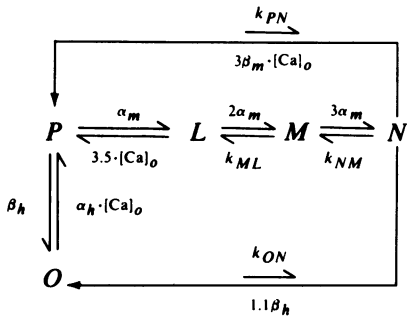
Using the Hodgkin and Huxley expressions as a standard data set for the sodium conductance in squid axon, we have developed a rather unique kinetic reaction scheme which matches the HH equations with precision. By this we mean that the kinetic model fits the HH data set over the full range of physiological potentials for step depolarization onset, for step repolarization reset, and for the recovery associated with the duration of repolarization. For convenience of representation of the kinetic model rate constants, we have substituted some HH expressions for the optimum ones at the cost of a slight degradation of fit to the currents in a voltage clamp but with an improved shape of action potential.

Calcium Effects

The precision fitting of any model to the Frankenhaeuser and Hodgkin data on the effects of $[Ca]_o$ on the squid axon conductances requires more attention to detail than has been given in the past. Frankenhaeuser and Hodgkin noted different magnitudes of effect for each of the types of experiments performed. The summary of their results as a voltage shift of all rate constants represents a compromise, perhaps to achieve a simple statement. Huxley (1959) has shown that, for calculations of membrane action potentials under current control, this simple means to represent the effects of $[Ca]_o$ variations is reasonably adequate. He was able to reproduce several experimental phenomena seen with altered $[Ca]_o$. However the action potential shape is known to be much less sensitive to alterations in conductances than are the currents observed under voltage clamped conditions (e.g., Moore and Ramón, 1974).

A more careful look at the observations of Frankenhaeuser and Hodgkin under more sensitive voltage clamp conditions reveals a number of difficulties. They observed a "shift" in the steady inactivation that was only about one-half that for the peak conductance. On the other hand, the changes in the rate of sodium conductance "shut-off" on repolarization suggest much greater "shifts" than that for the peak conductance. The "shift" to match the onset transient is so variable it cannot be described in a quantitative manner. If one uses the Hodgkin and Huxley model under voltage clamp and shifts all of the rate constants along the voltage axis by 7–9 mV for an e-fold change in $[Ca]_o$, one can match the change in peak conductance fairly well but will predict about twice the observed shift in the inactivation curve and about one-half the observed change in the "shut-off." On the other hand, if one matches the "shut-off" process by multiplying only β_m by the $[Ca]_o$ ratio, the shift in the peak conductance is 50% too large and, more disconcertingly, the change in speed of onset is in the wrong direction.

Having noted these inadequacies of a simple "shift" of rate constants along the voltage axis, the considerably better fit obtained by incorporating calcium into the kinetic reaction may be more fully appreciated. The inclusion of calcium as shown in



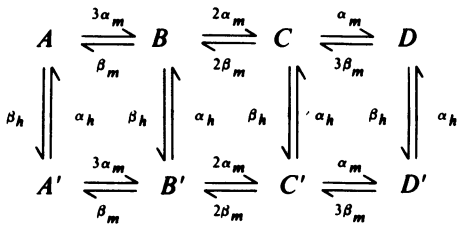
(Reaction 7)

allowed us to match a number of the Frankenhaeuser and Hodgkin (1957) observations in surprising detail as outlined in the results section.

Alternative Kinetic Models

The question of uniqueness of the model shown in Reactions 4 and 7 was raised continually and a number of alternative reactions were tried and compared for quality of fit as the battery of tests was underway. Invariably they gave less satisfactory fits to the HH standard data set. As simulation of the variety of calcium effects was undertaken, several alternate schemes, which had been marginal previously, performed poorly and were dropped from further consideration, leaving Reaction 7 as our putative kinetic model. This is not to say unequivocally that it is the only reaction which could possibly work well. However it is very time consuming, if not almost impossible, to apply the full battery of tests to a wide range of possible rate constants for every reaction which seems at first glance to be potentially useful.

We are indebted to Dr. Terrell Hill for showing us the exact Hodgkin and Huxley sodium conductance system laid out as a kinetic reaction.



(Reaction 8)

The states A–D and A'–D' can be thought of as representing the various possible combinations of the three m units and the single h unit. In the table below the numbers indicate how many of each molecular unit is in the open state:

	A	B	C	D	A'	B'	C'	D'
m	0	1	2	3	0	1	2	3
h	1	1	1	1	0	0	0	0

State D is the only one in which all groups are in the open configuration and the concentration in this state will be proportional to m^3h. There is some similarity of this reaction to our kinetic model and Fig. 1 has demonstrated a close matching of form D (m^3h) and form N. However when the effect of altered concentrations of external calcium $[Ca]_o$ are considered, the differences become apparent. In this (HH) system, multiplication of β_m by the relative calcium concentration causes a shift of the peak conductance in this model in the right direction but the time course is changed in the wrong direction.

As long ago as 1965, D. E. Goldman proposed calcium binding in the kinetic reaction. Some other kinetic models which include calcium have been proposed during the period in which our studies have been underway. For example, both Moore and Jakobsson (1971) and Fishman et al. (1971) proposed linear kinetic schemes for the sodium conductance systems to replace an m^2h (HH-like term) describing observations in frog nodes. These models should show changes in the time course of sodium conductance onset and in the peak conductance-voltage relation[8] when $[Ca]_o$ is altered. We also tried a number of rate constant sets in a linear reaction (Reaction 3) but found that, although it generally behaved in the right way, for step depolarization, the recovery from inactivation by a multistage reaction back through the active form was not very satisfactory. The inclusion of calcium in such linear models always seemed to require reactants with multiple-calcium-bound forms.

To summarize our experience in simulating all of the reaction alternatives, Reaction 7 provides the best fit to all of the experimental data used so far.

Gating Currents

The observation of Bezanilla and Armstrong (1974) that the gating currents are not proportional to dm/dt in the Hodgkin-Huxley model calls for a better model which will

[8] Fishman et al. give an equilibrium analysis of the shift in the peak sodium conductance with calcium binding in a simplified kinetic model in equilibrium.

fit these observations. Their observations of gating currents can be fitted by the kinetic model (Reaction 4) for two assumptions as to the nature of the gating currents. Experimental measurements of gating currents in differing $[Ca]_o$ are needed in order to help distinguish between these alternative hypotheses. For example, if the gating currents result from the binding and release of Ca^{++}, the time course of the gating current associated with sodium tails should be very sensitive to $[Ca]_o$.

The fact that the "gating currents" are observed in a preparation treated with tetrodotoxin (TTX) to block the sodium conductance system indicates that TTX does not disturb the membrane rearrangements resulting from depolarization which lead to the normal sodium conductance increase. This might have been expected from the observation that TTX affects the amplitude but not the *time course* of the sodium conductance transient. A small change in gating current has been noted when an octopus toxin was applied to the squid axon (Gage, Moore, and Westerfield, in preparation). This toxin changes the time course of the sodium conductance as it selectively blocks this conductance. Further studies here should be most helpful in relating gating currents to sodium conductance changes.

Further Tests

To make this kinetic model fully acceptable, it should be subjected to many additional tests for which new experimental data are required. For example, it is important to know with more certainty whether or not $[Ca]_o$ has any effect on the time course of inactivation. If not, this would be another clear indication that a $[Ca]_o$ shift of all rate constants was contra-indicated. Furthermore, the effect of $[Ca]_o$ on the time course of recovery from inactivation has not been fully examined and is needed for comparison with the model predictions. In very limited data on this, Frankenhaeuser and Hodgkin indicate that a change of calcium from 4.4 to 112 mmol increases the rate of reactivation by a factor of 2.3. We have additional unpublished, and not analyzed fully, data which show that following a depolarization in which the sodium system is nearly fully inactivated, recovery under hyperpolarized conditions is faster in axon in a high calcium medium than it is in a low calcium solution. The same effect can be seen in the kinetic model.

Hodgkin and Huxley used two methods of measurement (over different potential ranges) for the time course of the process which they called inactivation. They assumed that only a single process was involved and combined the experimental observations. Recent observations in the transitional voltage range show that there are actually two time courses which differ by a factor of about 3 (Goldman and Schaff, 1972; Moore, Cox, and Arispe, unpublished records). This phenomonon appears to be of fundamental importance in distinguishing between model candidates. Preliminary studies show that our proposed kinetic model can provide the two different time courses but the ratios obtained so far are not as large as those observed experimentally.

Energy Considerations

We are indebted to Dr. Eric Jakobsson for discussions on energetics and setting out the equilibrium condition constraints on the rate constants. Extensive simulations lead us

to the conclusion that no set of rate constants in our kinetic model which approximated the HH standard data set would be energetically conservative or vice versa.

Therefore the cycling in Reaction 7 requires an energy source, with injection of energy at some point in the cycle. It is our view that such energy is probably supplied by the interaction of charged groups of the putative sodium gate molecule with the transmembrane electric field, of the order of 10^5 V/cm. Such energy injection is implicit in the rate equations and voltage dependence of the rate constants. For purposes of this descriptive analysis, the step or steps at which energy injection takes place need not be explicitly located.

It is not certain whether this or any other model can survive careful comparison with the variety of experimental data now available. Nevertheless if one unique kinetic model could survive such an ordeal, it should provide a framework in which a biological chemist could place high confidence and use effectively for screening proposed molecular models. We feel that our proposed kinetic model deserves serious challenge and testing because it fits not only the original data of Hodgkin and Huxley on the voltage sensitivity of squid axons and the important effect of calcium on excitability but also the recently observed gating currents. Any other models which are proposed should be shown to fit these data at least as well or better than our Reaction 7.

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