AN ASSESSMENT OF A COUPLED THREE-STATE KINETIC MODEL FOR SODIUM CONDUCTANCE CHANGES

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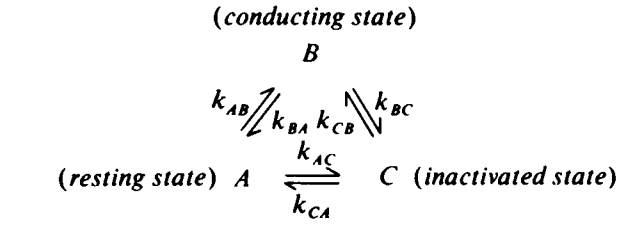
ABSTRACT The behavior of a coupled three-state kinetic scheme is examined to see if it might be a viable model for the conductance changes of sodium channels. It is found that for simulations of experiments which determine the properties of the Hodgkin-Huxley m and h gates, the three-state scheme performs approximately equivalently to the Hodgkin-Huxley model. In particular, the three-state scheme successfully simulates those experiments which the Hodgkin-Huxley model successfully simulates, but fails to simulate those newer voltage clamp experiments which give results anomalous to the H-H model. It is concluded that the three-state scheme is probably as good as the H-H model, but is not a viable successor to it.

In a recent paper in this journal, Goldman (1975) proposed a description of sodium conductance changes in *Myxicola* giant axon in terms of a generalized second-order variable and suggested that this description may be interpreted physically as corresponding to the following kinetic scheme:



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I have done computations on the properties of this scheme which I believe are relevant to Goldman's proposal. In particular, I have fit scheme 1 with numerical values for the rate constants which make it mimic closely the Hodgkin-Huxley model for the squid giant axon and have found that with these rate constants the model does not display either the inactivation shift or the τ_h vs. τ_c separation seen in Goldman's data. Further, I was not able to find any set of rate constants which would make the model display these features.

In addition to the above computations I attempted to calculate numerical values of the rate constants corresponding to Goldman's *Myxicola* data. For practically all

voltages I found that some rate constants would have to be negative in order to match those data.

The above results have led me to believe that scheme 1 is not a viable candidate for the kinetic scheme underlying the sodium conductance changes. The calculations are described below.

The first step is to write equations describing the dynamic and steady-state behavior of scheme 1. A convenient set of three equations describing the model's dynamic behavior is:

$$dB/dt = k_{AB} A - (k_{BA} + k_{BC}) B + k_{CB} C, \tag{2}$$

$$dC/dt = k_{AC} A + k_{BC} B - (k_{CB} + k_{CA}) C, \tag{3}$$

$$A = 1 - B - C. \tag{4}$$

In equation 4, by normalizing $A + B + C$ to unity, we now have each variable A, B, C representing the fraction of the system in the resting, conducting, and inactivated states, respectively. The steady-state values of A, B, and C for any set of rate constants can be calculated from the relationships:

$$A_\infty/B_\infty = k_{BA}/k_{AB}; B_\infty/C_\infty = k_{CB}/k_{BC}; C_\infty/A_\infty = k_{AC}/k_{CA}, \tag{5}$$

together with Eq. 4. These steady-state values are

$$A_\infty = [1 + (k_{AB}/k_{BA}) + (k_{AC}/k_{CA})]^{-1}, \tag{6}$$

$$B_\infty = [1 + (k_{BA}/k_{AB}) + (k_{BC}/k_{CB})]^{-1} \tag{7}$$

$$C_\infty = [1 + (k_{CA}/k_{AC}) + (k_{CB}/k_{BC})]^{-1} \tag{8}$$

After generating Eqs. 2–8 which give a complete description of the dynamic and steady-state properties of scheme 1, the next task is to decide on the best functional relationship between the variable B and the sodium conductance and to evaluate the voltage-dependent rate constants k_{AB}, k_{BA}, k_{AC}, k_{CA}, k_{BC}, and k_{CB}. The evaluation of these functions is partly analytical and partly trial-and-error, with the ultimate standard of verification of course being the closeness of fit to the desired behavior. In this case we start out with the desired behavior being the Hodgkin-Huxley squid axon with standard values for α_m, β_m, α_h, β_h. (Hodgkin and Huxley, 1952).

To get the same type of sigmoidal turn-on of the sodium conductance as for the Hodgkin-Huxley model, it is appropriate to set:

$$g_{Na} = \bar{g}_{Na} B^3. \tag{9}$$

The initial velocity of the turn-on suggests the identification:

$$k_{AB} = \alpha_m. \tag{10}$$

In the Hodgkin-Huxley axon the system turns off under a sustained depolarization primarily through the process with rate constant β_h. In scheme 1 this turn-off is pri-

marily a transition from B to C, suggesting the identification $k_{BC} = \beta_h/3$, where the factor 3 is necessitated by the third-power relationship 9. This equation for k_{BC}, when used in the simulations, turned out to be close but readily improvable; the equation finally settled on was:

$$k_{BC} = 0.333/\{1 + \exp[-(E + 38)/10]\}\ \text{ms}^{-1}, \tag{11}$$

where E is absolute membrane potential in millivolts. The right side of Eq. 11 is just $\beta_h/3$ shifted 8 mv in the depolarizing direction along the voltage axis.

One more defining equation for the rate constants comes from noting that the steady-state conductance in the Hodgkin-Huxley axon is given by

$$g_{\text{Na},\infty} = \bar{g}_{\text{Na}}\, m_\infty^3\, h_\infty. \tag{12}$$

Comparing Eqs. 9 and 12 gives:

$$B_\infty = m_\infty\, h_\infty^{1/3}. \tag{13}$$

Another defining relationship is suggested by deducing from the structure of the model that the peak transient value of B for a depolarizing step to a given voltage will be proportional to the combined population of A and B before the step. This idea suggests that

$$C_\infty = 1 - h_\infty^{1/3}. \tag{14}$$

As will be seen below, numerical simulations verify that Eq. 14 is quite accurate. By difference

$$\begin{aligned} A_\infty &= 1 - B_\infty - C_\infty = 1 - m_\infty h_\infty^{1/3} - 1 + h_\infty^{1/3} \\ &= (1 - m_\infty) h_\infty^{1/3}. \end{aligned} \tag{15}$$

Combining Eqs. 13, 15, 5, and 10 gives

$$\begin{aligned} k_{BA} &= (A_\infty/B_\infty)k_{AB} = [(1 - m_\infty)/m_\infty]\alpha_m = (\beta_m/\alpha_m)\alpha_m, \\ k_{BA} &= \beta_m. \end{aligned} \tag{16}$$

Eq. 16 is a very interesting result, because since the time course of sodium tails on repolarization will be primarily through k_{BA}, it means that the same value of k_{BA} which matches the time course of sodium tails will also match the steady-state sodium conductance and the sodium turn-on kinetics.

The rate constant k_{CB} cannot be reduced to a very simple function of any of the Hodgkin-Huxley α's and β's, but it can be determined. By combining Eqs. 13, 14, 5, and 11, we get:

$$k_{CB} = (B_\infty/C_\infty)k_{BC} = [m_\infty h_\infty^{1/3}/(1 - h_\infty^{1/3})]k_{BC}. \tag{17}$$

So far we have not considered k_{AC} and k_{CA}. It turns out that if we set both of these to zero, the model as defined above gives a good fit for almost all of the squid axon

data except that it takes a much too long time for activation to be restored upon repolarization. This suggests setting the $A \leftrightarrow C$ pathway such that $k_{CA} = \alpha_h$. However this was found to create another problem, to wit: at large depolarizations the flux through the $A \leftrightarrow C$ pathway was so large that the peak simulated sodium conductance was much smaller than the experimental peaks which would be observed at those voltages. The next step was to set k_{CA} such that it asymptoted to α_h in the hyperpolarized region and to zero in the depolarized region. A function that worked fairly well was:

$$k_{CA} = \alpha_h/\{1 + \exp[(E - 4)/8.64]\}.$$

However, this function still gave a somewhat slower time course for removal of inactivation on repolarization than the Hodgkin-Huxley model. An empirically determined function that gave a good fit for the speed of this process was:

$$k_{CA} = \{0.098 + \exp[-(E + 106)/12.6]\}/[1 + \exp(E + 34)]\text{ms}^{-1}, \quad (18)$$

then k_{AC} can be calculated by:

$$k_{AC} = (C_\infty/A_\infty)k_{CA} = [(1 - h_\infty^{1/3})/(1 - m_\infty)h_\infty^{1/3}\} \cdot k_{CA}. \quad (19)$$

Eqs. 9–11, 16–19 give numerical values for the parameters of scheme 1 which mimic the Hodgkin-Huxley model fairly closely. With these parameters the model voltage clamp behavior differed most significantly from the Hodgkin-Huxley model in two respects: (*a*) the time courses of conditioning inactivation in response to a depolarizing step, and of removal of inactivation in response to a hyperpolarizing step, are sigmoidal rather than exponential, and (*b*) the time course of conditioning inactivation in response to a depolarizing step is more rapid than removal of inactivation in response to a hyperpolarizing step to the same voltage, rather than the two being equally rapid. With regard to (*a*) above, the sigmoidal time course of conditioning for depolarizing steps is noted in Goldman's paper. Schauf (1974) has investigated the time course of conditioning for hyperpolarizing steps in *Myxicola*, and has found the sigmoidal shape there also.

The results of the most significant simulations are shown in Figs. 1–5. These figures summarize the ways in which scheme 1 matches and fails to match the Hodgkin-Huxley model. (Although the equations of this model have solutions in closed form for the voltage-clamp case, I found it most convenient to generate the simulations by a numerical analysis program already in use for other purposes. Hence the simulated results are by a 4th order Runge-Kutta integration method with variable step size, available as part of the Continuous Systems Modeling Program on the University of Illinois IBM 360/75 digital computer.) Fig. 1*a* shows peak values of B^3 for scheme 1 and m^3h for the Hodgkin-Huxley model as functions of voltage for depolarizing steps from $E = -90$ mV. Fig. 1*b* shows time-to-peak for these same simulations. The agreement between the two models is fairly good and could probably be made very

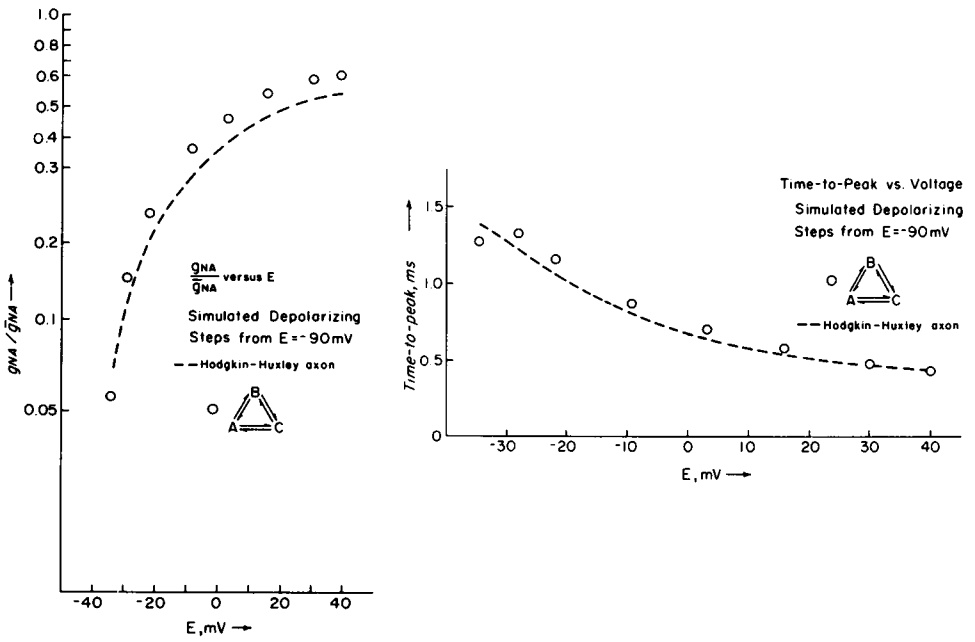
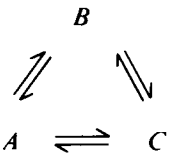


FIGURE 1 Characteristics of rising phase of sodium conductance in simulated voltage step from holding potential of $E = -90$ mV. (*a*) Peak $g_{Na}/\bar{g}_{Na}$ and (*b*) time-to-peak, in milliseconds, for same simulations. +, scheme 1, and - - -, Hodgkin-Huxley axon.

good with more effort at parameter fitting. Fig. 2 shows time constant for sodium turn-off tails on repolarization from a high conductance state, for



and the Hodgkin-Huxley models. Agreement is seen to be very good as would be expected from the values of k_{AB} and k_{BA} used in Eqs. 10 and 16. Figs. 1 and 2 summarize the degree of agreement between the two models for those experiments particularly revealing of the properties of the Hodgkin-Huxley m process. Fig. 3 shows steady-state inactivation as a function of voltage for scheme 1 as determined by $h_\infty = (1 - C_\infty)^3$ and also as determined by simulated test pulses to $E = -28$, -9, and 39 mV. For the simulation, the inactivation was judged to be the peak conductance normalized to the peak conductance as measured by a step from a holding potential of $E = -90$ mV. It can be seen that there is no inactivation shift of the type seen by Goldman even down to a test voltage at which $g_{NA}/g_{Na,max}$ is about 0.23, and that Eq. 14 is a good description of the steady-state inactivation.

It is natural to think of extending Eq. 14 to the time-varying case, that is, to suppose

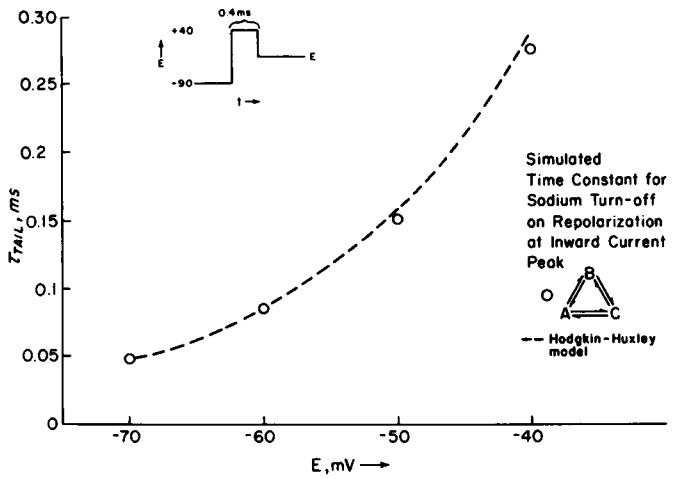
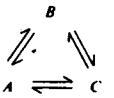


FIGURE 2 Simulated time constant for sodium conductance decay on repolarization after a depolarizing step to a high conductance state. Stimulus pattern (a step of 0.4 ms duration to E = 40 from holding potential of E = −90 mV, followed by repolarization to various levels of E) is shown in insert. Results of simulation represented by + for  model, and - - - for Hodgkin-Huxley model.

$h = (1 - C)^3$ where h and c are varying with time. Fig. 4 shows the results of simulations designed to test this point. The value of $(1 - C)^3$ vs. time was determined for a particular voltage step and then two-step experiments were simulated with a second step of E = 39 mV imposed at various durations of the conditioning step. The inactivation is the ratio of the peak conductance during the test step to the peak conductance achieved by stepping directly to E = 39 mV from a hyperpolarized level. It is seen that the relationship we are testing is only approximately true; i.e.

$$h \simeq (1 - C)^3. \tag{20}$$

Simulations of a range of potential steps show that Eq. 20 is very accurate when the sodium conductance is small and less accurate when the sodium conductance is large. Also, simulations of depolarizing conditioning steps to the plateau region of the $g_{Na}/g_{NA,max}$ curve ($E > \sim -9$ mV) show no current peaks during the test pulse.

Fig. 5 shows simulated inactivation time constants as a function of voltage, measured three ways: (*i*) by the fall-off of sodium conductance during a sustained depolarization, (*ii*) by time course of conditioning from a hyperpolarized holding potential, and (*iii*) by time course of conditioning from a depolarized holding potential. None of these processes are, of course, actually a pure exponential. The time constants are fit by eye to the major portion of the simulated process, using the equation:

$$h = h_\infty + (h_0 - h_\infty)\exp(-t/\tau h). \tag{21}$$

In the case of (*i*) the fit was to the portion of the curve following the peak conductance

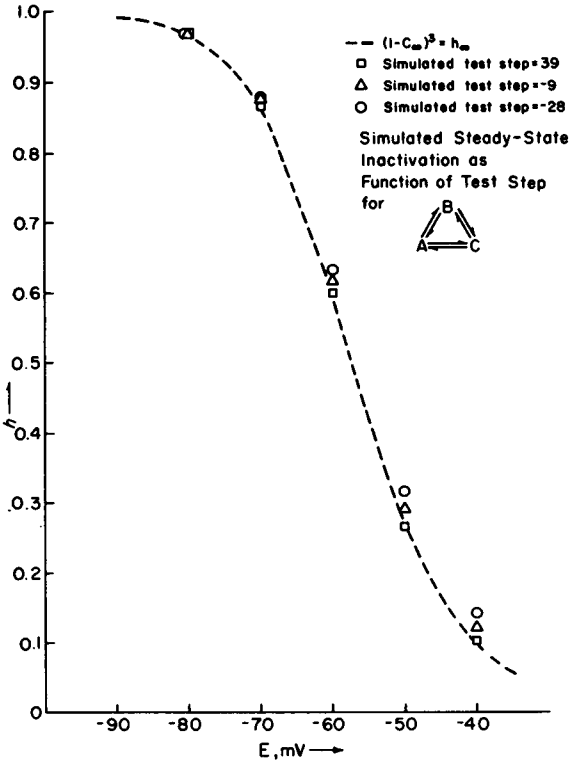


FIGURE 3 Simulation of experiments designed to show "inactivation shift." Dashed line is Hodgkin-Huxley h_∞ and $(1 - C_\infty)^3$ for three-state model. Other symbols are steady-state inactivation from simulated steps to various voltages. It is seen that instead of a pronounced shift to the left with reduced test potential, as in Goldman's data, the model gives a slight shift to the right. The points from the simulations are calculated from the expression 0.992 $g_{Na}/g_{Na}(E_h = -90)$.

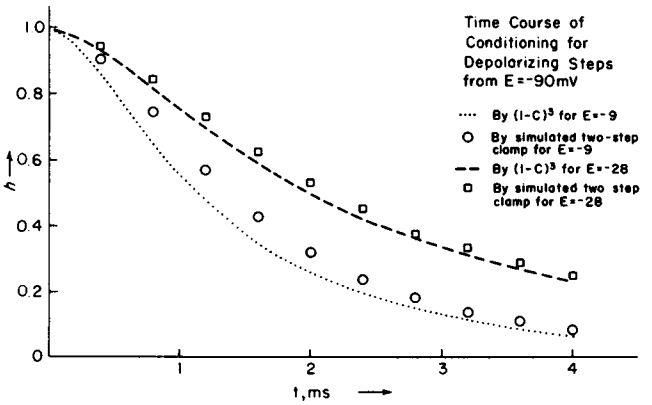


FIGURE 4 Comparison of time course of conditioning as simulated by monitoring $(1 - C)^3$ and by full simulation of two-step experiment. In the two-step simulations the test potential is $E = 39$ mV. The conclusion from this and similar comparisons over a range of voltages is that $h = (1 - C)^3$ is in general a fair approximation and is quite good when the conductance is low.

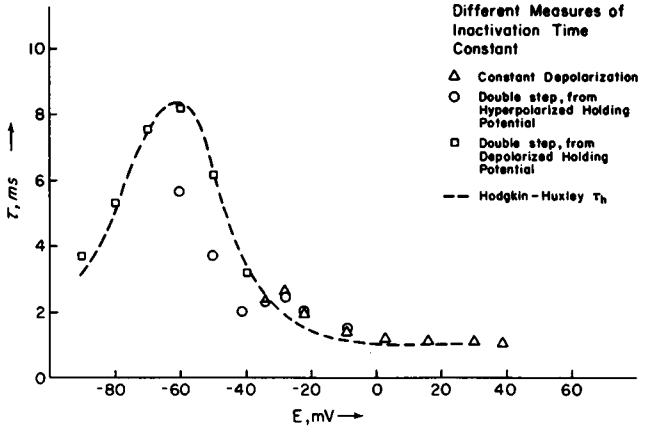


FIGURE 5 Simulated τ_h as a function of voltage determined by three methods, (*i*) fall-off of sodium conductance, (*ii*) time course of conditioning from a hyperpolarized conditioning potential, and (*iii*) time course of conditioning from a depolarized conditioning potential. The simulations show that for voltages where more than one method can be used to determine τ_h, $\tau_{h,i} \simeq \tau_{h,ii}$ and $\tau_{h,ii} < \tau_{h,iii}$. In contrast, experiments of Goldman and others show $\tau_{h,i} < \tau_{h,ii}$.

while in the cases of (*ii*) and (*iii*) it was that portion of the curve following the initial delay. It is readily seen in Fig. 5 that there is no τ_c vs. τ_h separation of the type seen in Goldman's paper. A second point worth noting in Fig. 5 is that the conditioning is faster at a particular potential when the system is stepped from a hyperpolarized holding potential than from a depolarized one. This is the most significant differential prediction that was found between scheme one and the Hodgkin-Huxley model. Figs. 3, 4, and 5 summarize the behavior of scheme 1 in simulations of experiments most revealing of the properties of the Hodgkin-Huxley *h* process.

Clearly these simulations do not behave qualitatively like Goldman's data. One possibility is that these parameters are specific for squid and if one used *Myxicola* data to do the fitting one would get the inactivation shift and the $\tau_c - \tau_h$ separation. This possibility was explored in two ways. One way began with noting that the $A \rightleftharpoons B \rightleftharpoons C$ model, which is just scheme 1 with $k_{AC} = k_{CA} = 0$, does not give these behaviors (also noted by Goldman). Therefore simulations were tried in which all of the other rate constants were held the same and the magnitudes of k_{AC} and k_{CA} were increased, keeping their ratios constant. In this way scheme 1 was made to give a much worse fit to the Hodgkin-Huxley axon in many ways, but an inactivation shift was never induced. As far as the τ_c vs. τ_h separation, it was found that increasing k_{CA} and k_{AC} always made the conditioning time course *faster* relative to the turn-off, rather than vice versa as in Goldman's data. Since scheme 1 with $k_{CA} = k_{AC} = 0$ always gave $\tau_c \simeq \tau_h$, and since increasing k_{CA} and k_{AC} always made conditioning faster relative to turn-off, the results of these computations seemed to suggest that scheme 1 can never give τ_c appreciably slower than τ_h.

A second way of exploring this issue was to attempt to fit scheme 1 with parameters calculated directly from data in Goldman's paper. The necessary data come from the

TABLE I

CALCULATION OF RATE CONSTANTS IN SCHEME 1 FROM DATA OF GOLDMAN (1975) USING EQS. 22, 24, AND 25

For almost the entire voltage range some of the calculated rate constants are negative, but the significance of this result in the far depolarized region may be questionable (see text).

V	a	b	a + b (=sum of k's)	ν_∞	$\dot{\nu}^*(0)$ (=k_{AB})	$\sqrt[5]{h_\infty}$	k_{BA} (from Eq. 24)	$k_{AB}+k_{BA}$	(a + b) $-(k_{AB}+k_{BA})$
-43	1.25	0.123	1.37	0.20	0.802	0.760	2.24	3.04	-1.67
-33	1.69	0.194	1.85	0.25	1.26	0.615	1.84	3.14	-1.29
-23	2.81	0.237	3.05	0.35	2.40	0.483	0.910	3.31	-0.26
-13	3.81	0.219	4.03	0.35	3.49	0.377	0.269	3.76	0.27
-3	4.41	0.253	4.66	0.35	4.20	0.293	-0.685	—	—
7	5.13	0.300	5.43	0.35	4.89	0.227	-1.72	—	—
17	5.97	0.356	6.33	0.35	5.68	0.176	-2.99	—	—
27	6.18	0.409	6.59	0.35	5.86	0.137	-3.56	—	—
37	7.12	0.399	7.52	0.35	6.53	0.106	-4.55	—	—
47	7.68	0.508	8.19	0.35	6.99	0.0826	-5.33	—	—

following parts of Goldman's paper: $\dot{\nu}^*(0)$, ν_∞, a, and b from Table II and Fig. 2, and h_∞ from Fig. 6. The rate constant k_{AB} may be easily calculated as follows:

$$k_{AB} = \dot{\nu}^*(0). \tag{22}$$

Since Goldman set his conductance proportional to B^5, Eq. 14 becomes for this system:

$$C_\infty = 1 - h_\infty^{1/5}. \tag{23}$$

We can solve for k_{BA} in terms of Goldman's ν_∞, $\dot{\nu}^*(0)$, and h_∞ as follows

$$k_{BA} = (A_\infty/B_\infty)k_{AB} = [(1 - C_\infty - \nu_\infty)/\nu_\infty]\cdot\dot{\nu}^*(0) = (h_\infty^{1/5} - \nu_\infty)\dot{\nu}^*(0)/\nu_\infty. \tag{24}$$

It can be shown from relaxation theory (Eigen, 1968) or by simply solving the equations of the system that the sum of the reciprocals of the time constants is equal to the sum of all the rate constants in scheme 1; i.e.

$$a + b = k_{AB} + k_{BA} + k_{AC} + k_{CA} + k_{BC} + k_{CB}. \tag{25}$$

Table I gives the results of using Eqs. 22, 24, and 25 together with Goldman's data to solve for k_{AB}, k_{BA}, and $(k_{AB} + k_{BA} + k_{AC} + k_{CA} + k_{BC} + k_{CB})$. It is seen that only in one small region over the whole voltage range can the scheme possibly have a solution in which all of the rate constants are positive. For $V = -3$ to 47 k_{BA} is negative, and for $V = -43$ to -23 $k_{AB} + k_{BA} > k_{AB} + k_{BA} + k_{AC} + k_{CA} + k_{BC} + k_{CB}$, meaning that one or more of the other rate constants must be negative.

The above calculations are not totally conclusive however, because they neglect experimental error. That is, if one put reasonable error bars on Goldman's data it might be possible somewhere within the range of those error bars to fit scheme 1 with

positive rate constants. This is especially true in the far depolarized voltage region, where the ν_∞ and h_∞ used to calculate k_{BA} by Eq. 24 represent currents that would be almost vanishingly small. Of course in this voltage region, the data are rather well fit by the Hodgkin-Huxley model. On the other hand, in the region of moderate depolarization the errors one would have to postulate in the data, to find $(k_{AB} + k_{BA}) < (a + b)$, seem implausibly large. If the errors were large enough to accommodate the theory, one would begin to suspect the validity of the experiments. In addition, Goldman and Hahin (1975) have made an exhaustive set of full computations on this point for a pair of potentials (one conditioning potential and one test potential). They found no set of positive rate constants which would fit the data to within experimental error.

The above considerations seem to me to weigh heavily against scheme 1 as a viable candidate for the functional subunit of the sodium channel. This is especially true since the τ_c vs. τ_h separation appears to be quite general, having also been seen in giant axon of squid (J. W. Moore, personal communication), giant axon of lobster (Oxford and Pooler, 1975), and axon from blue crab walking leg (J. A. Connor, personal communication). It is interesting to note that the primary reason for ruling out scheme 1 is not that it is not as good as the Hodgkin-Huxley axon but rather that it and the Hodgkin-Huxley axon fail in the same ways to meet the newer voltage clamp data. In the last paper of their classic 1952 series, Hodgkin and Huxley (1952) wrote "First, we might assume that the sodium conductance is determined by a variable which obeys a second-order differential equation. Second, we might suppose that it is determined by two variables, each of which obeys a first-order equation. ... The second alternative was chosen since it was simpler to apply to the experimental results." The results given in this paper seem to me to support the wisdom of these words. Although there are some differences in their behaviors, the two kinds of model appear to fit equally well (and equally imperfectly) the voltage-dependent sodium kinetics in normal bathing solution.

It seems not constructive to write entirely about a model that seems unpromising without pointing out more promising alternatives. With regard to the $\tau_c > \tau_h$ phenomenon, I believe the key point may be to construct a model or models which have more pathways for turning off the conductance from a sustained depolarization than they have for conditioning. The apparent reason that scheme 1 doesn't give this behavior is that under a sustained depolarization there is only one turn-off pathway ($B \rightarrow C$) but two conditioning pathways ($A \rightarrow B \rightarrow C$ and $A \rightarrow C$). There are two models recently proposed for the sodium conductance which have dual turn-off pathways, (Jakobsson and Scudiero, 1975; Moore and Cox, 1975) and which therefore are promising in this respect. Also, a model which involves a transient excited state added to scheme 1 as a precursor to the conducting state has this capability, and will be the subject of a later communication.

Computer time was provided through a grant from the Research Board of the University of Illinois.
Other aspects of the work were supported by U.S. Public Health Service grant no. HL 14125 from the National Heart and Lung Institute and grant no. GB 39946 from the National Science Foundation.

Received for publication 17 March 1975 and in revised form 15 September 1975.

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