ON THE THEORY OF ION TRANSPORT ACROSS THE NERVE MEMBRANE

V. Two Models for the Cole-Moore K^+ Hyperpolarization Delay

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ABSTRACT Two illustrative molecular models, designed to explain the Cole-Moore K^+ hyperpolarization delay, are proposed and analyzed. Both introduce a process supplementary to the usual Hodgkin-Huxley (HH) one for a K^+ channel. In both cases the new process becomes involved as a consequence of the conditioning hyperpolarization of the membrane and would account for the observed delay time in the K^+ current after depolarization to near E_{Na}. The first model uses adsorption or desorption of phospholipid molecules on the surface of the assumed protein K^+ channel or gate. The second model involves the translocation of the charged subunits of the channel in the hyperpolarizing electric field.

INTRODUCTION

The well-known hyperpolarization delay found by Cole and Moore (1) presents an interesting puzzle which is without an adequate explanation at the molecular level. We describe two possible molecular models here. These are meant primarily as contrasting examples of the kinds of processes that might be involved.

Experimental Observations

Cole and Moore (1) found that the potassium current vs. time curves, for a family of voltage clamp depolarizations to +60 mv (approximately E_{Na}) starting from various potentials between −52 mv (approximately the resting potential) and −212 mv, were essentially identical except for a displacement along the time axis. That is, the only effect on $I_K(t)$ of hyperpolarization before depolarization was a delay in the start of the curve. Fig. 1 shows $\Delta = t_{1/2} - t_{1/2}^{(r)}$ (time delay) plotted against $-V$, the negative of the initial membrane potential where $t_{1/2}^{(r)}$ is the time to $I_K = I_K(\infty)/2$ starting from $V_r = -52$ mv, and $t_{1/2}$ is the time to $I_K(\infty)/2$ starting from V. Estimates of $I_K(\infty)$, $t_{1/2}^{(r)}$, and $t_{1/2}$ were made from a considerable photographic enlargement of Cole and Moore's Fig. 5.

This is the only example of $\Delta(V)$ data known to us. It would obviously be very

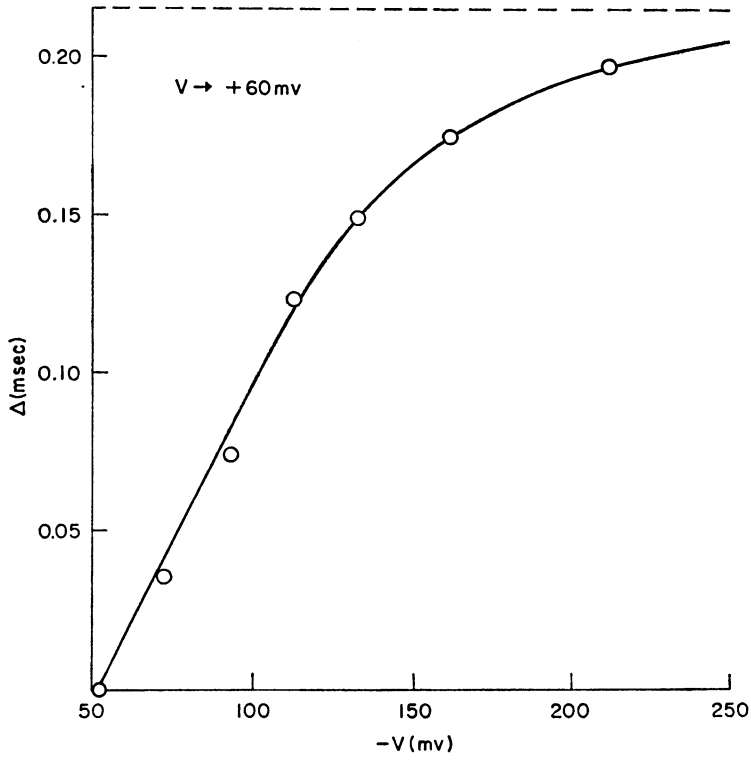

FIGURE 1 Delay time Δ (defined in text) as a function of conditioning potential −V. The limiting value of Δ is estimated as $\Delta_m = 0.22$ msec.

helpful in trying to assign a molecular mechanism to this effect to have available $\Delta(V)$ curves for various final potentials in addition to E_{Na}. In fact, even the qualitative feature of time displacement as the only effect of hyperpolarization has to be confirmed for final potentials other than near E_{Na}.

In the hope of stimulating further experimental work on this problem, we present in this paper two quite different illustrative models which might explain the behavior shown in Fig. 1. In both cases we assume (2–6) that the K^+ channel or gate is a protein complex of x independent (noninteracting) subunits and that a conformational change $i \rightarrow ii$ must take place in all x subunits in order for the channel to be open. Also, because the models are necessarily speculative, we give in both cases only the simplest first-order theory.

Required Properties of a Model

In depolarizations to near E_{Na} starting from $V \geq V_r$, we require that essentially HH kinetics be obeyed.[1] Also, when $V < V_r$ in depolarizations to near E_{Na}, there

[1] If we apply the usual HH ($x = 4$) kinetics to the Cole-Moore $V_r = -52$ mv curve, the value of the slope (see equation 9 below) at $t_{1/2}^{(\tau)}$ requires that $\tau = 0.180$ msec. If we take the HH $n_0 = 0$, we

is to be a delay followed by the same kinetics to within experimental error. This suggests that hyperpolarization of the membrane introduces, or renders important, a state or condition of the K^+ channels which is absent or relatively unimportant at $V \geq V_r$ and which, in depolarizations from $V < V_r$, must be reversed or restored to "normal" before the usual HH conformational change $i \rightarrow ii$ in the subunits can take place. This temporal separation between the previous reversal process and the HH process, however, presumably need not be absolute. But if the two processes were to occur substantially simultaneously, no delay would result, as required.

Another necessary feature of a model, as seen from Fig. 1, is that there must be "saturation" of the delay Δ as $-V$ becomes very large. Cole and Moore (1) suggested that the delays they observed might be accounted for by taking x of order 25 in otherwise "conventional" HH kinetics (see also FitzHugh [7]). In effect, we are simply generalizing the Cole–Moore proposal by use of two successive processes, one of which is HH with, say, $x = 4$, rather than only one with very large x. Since a channel or gate will have a length of order of, for example, 20–100 A, it is physically unrealistic to expect it to contain a large number of independent (i.e., noninteracting) subunits of whatever kind (4–6). We therefore feel that some kind of a two-state model is much more likely to correspond to molecular reality.

MODEL 1

Adsorption or Desorption of a Large Ion

Suppose, to be specific, that under normal conditions ($V \geq V_r$) there is a good probability that each protein subunit of a complex (channel) will have bound to it a maximum number z of, say, phosphatidylserine (negative) ions and that the conformational change $i \rightarrow ii$ practically does not occur at any V without all of these z ions bound. Suppose further that hyperpolarizing potentials $V < V_r$ reduce the equilibrium binding of the ion so that a given hyperpolarized subunit may very well have less than z ions bound. Then, on depolarization to E_{Na}, a subunit with submaximal binding must first acquire the full complement of z bound ions before $i \rightarrow ii$ is possible. Hence the reversal process in this case, responsible for the time delay Δ, is the binding of a large negative ion.

Having briefly outlined the model, a number of further qualitative comments are in order:

(*a*) We shall assume that the reduced binding, as a result of hyperpolarization, follows from a change in the electrochemical potential of the negative ion at the protein complex (see details below). The reservoir of negative ions for the binding equilibrium is considered to be in the membrane itself (e.g., a layer of the phospholipid bilayer [8]). An alternative assumption, which we shall not pursue here, is that

find the largest allowable HH $t_{1/2}$, namely, 0.33 msec. The experimental value $t_{1/2}^{(r)}$ is 0.44 msec. Thus, if $x = 4$, some other process is contributing a delay even at V_r. Incidentally, if we take, say, $x = 8$, we find $\tau = 0.172$ msec and $t_{1/2} = 0.43$ msec.

binding is reduced by the effect of the electric field on the intrinsic binding constant (second Wien effect [9]).

(*b*) Phosphatidylserine is mentioned above only as a likely example (10). Other negative ions (e.g., phosphatidylinositol) are possible, or a positive ion. Since Δ is "large," of order 10^{-4} sec, Ca^{++} is probably excluded (also for another reason, see below), but if A^- is, for example, a negative phospholipid ion, CaA^+ would be a possible positive ion.

(*c*) We want to avoid unjustified refinements and keep only essential ideas in the model. We therefore assume that all z sites are identical, that the transitions $i \rightleftarrows ii$ are important only when all z ions are bound, and that, in depolarizations to the neighborhood of E_{Na}, desorption of the ion occurs at a negligible rate because equilibrium binding is taken to be largely completed even at V_r. Furthermore, we start off by assuming that the binding sites are independent of each other, but this is merely the first step in an iteration process which we shall outline later but not carry out. As we shall see, the data in Fig. 1 indicate that, if we accept the present model, the bound ions do in fact interact with each other.

Mathematical Formulation

Fig. 2 shows the notation used for the different states, as well as probabilities and rate constants, for a *single subunit* at equilibrium at $t < 0$ and V, the hyperpolarizing membrane potential. Fig. 3 gives the corresponding notation for $t > 0$ and the final potential, E_{Na}; i.e., the depolarization $V \rightarrow E_{Na}$ occurs at $t = 0$. As already mentioned, $\eta \gg \theta$ at E_{Na}. The sum of the $p_j(t)$ is unity. The probability that a subunit is in the state ii_z at t, $t > 0$, is $p(t) \equiv p_z(t)n(t)$, where $n(t)$ is the HH n. The probability that a given K^+ channel (x subunits) is open at t is then $p_K(t) = p(t)^x$. At $t = \infty$, $p_z(\infty) = 1$ and $p(\infty) = n_\infty = \alpha/(\alpha + \beta)$. Schematically, we are generalizing the HH model from $(i \rightleftarrows ii)^x$ to $(i_0 \rightleftarrows \cdots i_z \rightleftarrows ii_z)^x$. The states $ii_0, \cdots, ii_{z-1}$

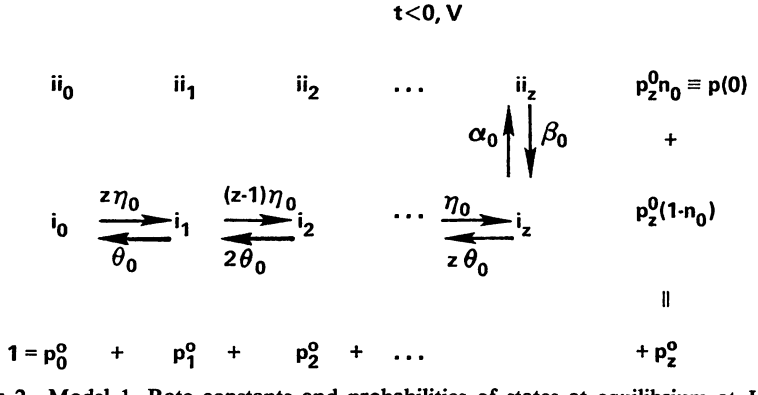

FIGURE 2 Model 1. Rate constants and probabilities of states at equilibrium at V and $t < 0$. α_0 and β_0 are HH rate constants.

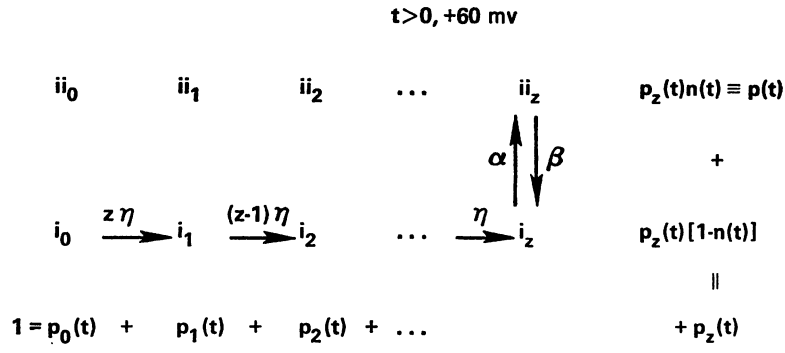

FIGURE 3 Model 1. Rate constants and time dependent probabilities of states at +60 mv and $t > 0$. α and β are HH rate constants.

are assumed to be unimportant here because of the sensitivity of the $i \rightleftarrows ii$ equilibrium constant to the bound ion, but it is not difficult, as we have done, to include these states in a more general formulation. In this case, however, the dominant kinetic pathway must still be that shown in Fig. 3 in order to produce a substantial delay time.

At $t < 0$ (equilibrium), from Fig. 2, we have

$$n_0 = \alpha_0/(\alpha_0 + \beta_0), \qquad \nu_0 \equiv \eta_0/(\eta_0 + \theta_0), \tag{1}$$

$$p_j^0 = \frac{z!\,\nu_0{}^j(1 - \nu_0)^{z-j}(1 - n_0)}{j!(z - j)!N}, \qquad j = 0, 1, \cdots, z - 1, \tag{2}$$

$$p_z^0 = \nu_0^z/N, \qquad N \equiv 1 - n_0 + \nu_0^z n_0, \qquad p(0) = \nu_0^z n_0/N.$$

The physical significance of ν_0 is the fraction of the z sites in subunits of conformation i (i.e., i_0 to i_z) occupied at equilibrium ($t < 0$). Incidentally, the separate rate constants η_0 and θ_0 are not needed here, only ν_0.

For $t > 0$, from Fig. 3, the differential equation in $p(t)$ is

$$\frac{dp}{dt} = \alpha p_z(t)[1 - n(t)] - \beta p(t),$$

or

$$\frac{dp}{dt} + (\alpha + \beta)p(t) = \alpha p_z(t). \tag{3}$$

With the boundary condition $p = p(0)$ at $t = 0$, the solution of equation 3 can be written

$$\frac{p(t)}{p(\infty)} = \frac{p(0)}{p(\infty)}\, e^{-t/\tau} + \frac{e^{-t/\tau}}{\tau} \int_0^t p_z(t')e^{t'/\tau}\, dt', \tag{4}$$

where $p(0)$ is given by equation 2, $p(\infty) = \alpha\tau$, and $\tau = 1/(\alpha + \beta)$. The function

$p_z(t)$, for use in equation 4, is easily found from equation 2 and Fig. 3 to be

$$p_z(t) = \left[1 - \left(1 - \frac{\nu_0}{N^{1/z}}\right) e^{-\eta t}\right]^z. \tag{5}$$

Term-by-term or numerical integration of equation 4 is then straightforward. Finally,

$$p_K(t)/p_K(\infty) = [p(t)/p(\infty)]^x. \tag{6}$$

Because of the proportionality between $I_K(t)$ and $p_K(t)$, that is,

$$I_K(t) = g_K p_K(t)(E_{Na} - E_K), \tag{7}$$

$t_{1/2}$ is determined by

$$p_K(t_{1/2})/p_K(\infty) = \tfrac{1}{2}. \tag{8}$$

The asymptotic or maximum value of Δ, Δ_m, is estimated from Fig. 1 to be Δ_m = 0.22 msec. Also, we estimate $t_{1/2}^{(\tau)}$ to be 0.44 msec so that $t_{1/2}^{(m)}$ = 0.66 msec. The slope at $t_{1/2}$ is essentially the same for all curves (all choices of V) in Fig. 5 of Cole and Moore. Our estimate of this value from the photographic enlargement is

$$\left(\frac{dp_K/p_K(\infty)}{dt}\right)_{t_{1/2}} = 2.10 \text{ msec}^{-1}. \tag{9}$$

The maximum delay case $V \to -\infty$ is characterized by $n_0 = 0$, $\nu_0 = 0$, and $p(0) = 0$. The remaining parameters in equation 4 are τ and η. These are properties of E_{Na}, not V. For given choices of x and z, we may then, by a straightforward numerical procedure which we shall not describe, use the above experimental values of $t_{1/2}^{(m)}$ and the slope to solve equation 4 for τ and η, provided that a solution exists (the $t_{1/2}^{(m)}$ value can always be realized, but not necessarily with sufficient slope). Some of these solutions are given in Table I. Dashes indicate that a solution does not

TABLE I

RATE CONSTANTS FROM MAXIMUM HYPERPOLARIZATION

	τ	η	$p_z(t_{1/2}^{(m)})$
	msec	*msec*$^{-1}$	
$x = 3, z = 10$	0.1304	7.673	0.9386
$z = 8$	—	—	—
$x = 4, z = 8$	0.1470	8.481	0 9707
$z = 6$	0.1198	7.121	0.9466
$z = 4$	—	—	—
$x = 6, z = 6$	0.1614	9.940	0.9915
$z = 4$	0.1460	8.173	0.9819

exist. It is interesting but not surprising that borderline acceptable values of the product xz are in the neighborhood of the Cole and Moore exponent 25. Note that τ and η^{-1} have similar magnitudes. The values of $p_z(t_{1/2}^{(m)})$ near unity are reassuring because one would expect this to be necessary in order for the model to provide the observed "superposition," a family of curves for different V values practically identical except for displacement along the time axis. Thus, over the principal part of the rising $p_K(t)$ curve, the binding process in this model has been substantially completed ($p_z \to 1$) and the channels are essentially in a simple HH kinetic regime ($i_z \rightleftarrows ii_z$). As we shall see, excellent superposition in calculated curves is in fact found.

Calculations

Let us concentrate on the case $x = 4$, $z = 6$ since this is the HH value of x and z is as small as possible in Table I. We have made some calculations on other cases with similar results. We assume that n_0 is rather small at the rest potential and approaches zero fairly rapidly for $V < V_r$ (2, 6). Above we took $n_0 = 0$ when $\nu_0 = 0$ but it is also safe to take $n_0 = 0$ up to, say, $\nu_0 = 0.6$. For $\nu_0 > 0.6$ we would have to consider $n_0 > 0$. Table II gives values of Δ calculated from equation 4 and the $x = 4$, $z = 6$ case in Table I, using $t_{1/2}^{(r)} = 0.44$ msec. From the smooth curve in Fig. 1, we can convert these Δ values into V values (Table II) and then test the self-consistency of our model by examining ν_0 as a function of V. To do this we need the theoretical relation between ν_0 and V implicit in the model.

We are concerned here with the equilibrium between ions bound on the subunits in conformation i (see the definition of ν_0) of a complex (c) and unbound ions in a nearby pool (p) or reservoir, also in the membrane (see above). Let the electrostatic potential ψ at the complex be $\alpha_c V$ and in the pool, $\alpha_p V$, where the α's are constants. In the simplest (but unlikely) case, ψ would drop linearly when $V < 0$ from $\psi = 0$ at the outside surface of the membrane to $\psi = V$ at the inside surface so that the α's would be fractional distances across the membrane in the direction outside →

TABLE II

TIME DELAYS IN $x = 4$, $z = 6$ CASE

n_0	ν_0	Δ	$-V$
		msec	*mv*
0.0	0.0	0.220	∞
0.0	0.1	0.205	247
0.0	0.2	0.189	189
0.0	0.3	0.170	155
0.0	0.4	0.148	132.5
0.0	0.5	0.123	114.5
0.0	0.6	0.091	97.5

inside. In any case, they would have approximately this significance. At equilibrium, the two electrochemical potentials are equal, and we have (11)

$$\frac{\mu_p}{kT} + \frac{\epsilon Z \alpha_p V}{kT} = \ln \frac{\nu_0}{(1 - \nu_0)q} + \frac{\epsilon Z \alpha_c V}{kT}, \qquad (10)$$

where μ_p is the chemical potential of the ions in the pool (a constant), ϵ is the charge on a proton, Z is the charge number (e.g., $Z = +2$ for Ca^{++}), and q is the partition function for binding an ion on a subunit in conformation i. The z binding sites are assumed to be equivalent and independent in equation 10. We then have

$$\ln \frac{\nu_0}{1 - \nu_0} = \text{const} + a(-V), \qquad (11)$$

where $a = \epsilon Z(\alpha_c - \alpha_p)/kT$. A plot of $\ln [\nu_0/(1 - \nu_0)]$ against $-V$ should give a straight line. The upper curve in Fig. 4 shows such a plot using the data in Table II. The line is not straight. The curvature suggests, however, that there are interactions between the bound ions as one would expect, since (*a*) the ions are presumably large and would form a compact monolayer on the surface of a subunit, and (*b*) they are charged.

The simplest way to include interactions is to use the Bragg-Williams (BW) ap-

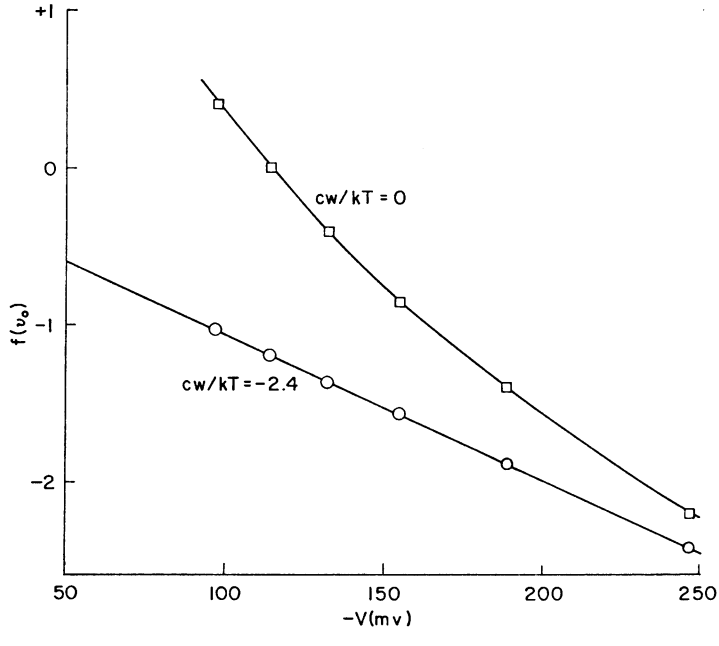

FIGURE 4 Model 1. $f(\nu_0)$, defined in equation 12, as a function of $-V$ for two values of cw/kT.

proximation (11), though with z so small an exact treatment of the interactions would be easy, given the arrangement of sites. With this approximation equation 11 becomes

$$f(\nu_0) \equiv \ln \frac{\nu_0}{1 - \nu_0} + \left(\frac{wc}{kT}\right) \nu_0 = \text{const} + a(-V), \qquad (12)$$

where w is the nearest neighbor interaction energy between ions and c is the number of sites nearest neighbor to a given site. We find that $cw/kT \cong -2.4$ makes $f(\nu_0)$ a linear function of $-V$ (straight line in Fig. 4) as required by equation 12. Since w is negative, the van der Waals and other attractive forces between bound ions (e.g., phosphatidylserine) must exceed the coulombic repulsion, which is not unreasonable, but $w < 0$ excludes Ca^{++}. If we take $c = 4$, for example, we find $w \cong 0.35$ kcal mole^{-1}; if $c = 2$, $w \cong 0.7$ kcal mole^{-1}. These are modest energies.

The straight line in Fig. 4 has a slope $a = -0.0092$ mv^{-1}. Since a is negative, we can have Z negative and $\alpha_c > \alpha_p$, or Z positive and $\alpha_p > \alpha_c$. If the pool (p) and complex (c) are on the same side of the membrane as seems likely (12), Fig. 5 *a* shows the four possibilities for the spatial location of p and c relative to each other (normal to the plane of the membrane). If $|Z| = 1$, $a = -0.0092$ mv^{-1} leads to $|\alpha_c - \alpha_p| = 0.23$. If ψ has the simple linear behavior described above and the membrane is 70 A thick, then c and p are separated by about 16 A, which is a reasonable order of magnitude.

So far we have considered that desorption ($\nu_0 \rightarrow 0$) of an ion from subunits occurs on hyperpolarization (ν_0 is the fraction of sites occupied in subunits of conformation i), but the model (Figs. 2 and 3) also applies when hyperpolarization causes adsorption, also $\nu_0 \rightarrow 0$, of an ion to subunits of type i, redefining ν_0 as the fraction of sites empty. In this case the conformational change ($i_z \rightleftarrows ii_z$) takes place only when all z sites are empty. The changes in equations 10–12 are straightforward

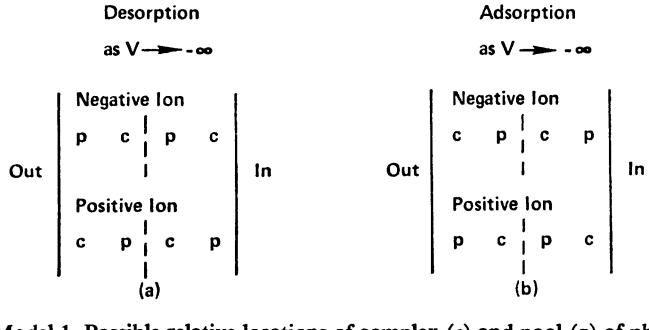

FIGURE 5 Model 1. Possible relative locations of complex (c) and pool (p) of phospholipid ions in the membrane. Dashed line is the middle of the membrane. On hyperpolarization, positive (phospholipid) ions tend to move to the right in these diagrams and negative ions to the left.

and will be omitted. We find from the data in Table II that $cw/kT \cong -2.4$ (net attraction between bound ions) as before but that $a = +0.0092$ mv^{-1}. Hence Z and $\alpha_c - \alpha_p$ have the same sign, as illustrated by the four possibilities in Fig. 5 *b*.

We return now to the original model. At the rest potential $V_r = -52$ mv, we would have $n_0 > 0$. What value of n_0 will give a point at $-V = 52$ mv on the straight line in Fig. 4? From $f(\nu_0) = -0.62$ (on the line) we get $\nu_0 = 0.77_7$. We then find from solutions of equation 4 that $n_0 = 0.15$ (with $\nu_0 = 0.77_7$) gives $t_{1/2}^{(r)} = 0.44$ msec, as required. In these solutions we have used $p(\infty) = n_\infty = \alpha\tau = 0.9742$ from the HH $n_\infty(V)$ function at $E_{Na} + 3$ mv, which is what we estimate the final Cole-Moore potential to be. The value $n_0 = 0.15$ happens to be the same as that arrived at in part IV (6), but this exact agreement is certainly fortuitous (for one thing, n_0 is quite sensitive to ν_0).

Fig 6 *a* illustrates time delay and superposition for $x = 4$, $z = 6$ with three simulated depolarizations to $E_{Na} + 3$ mv, according to equation 4. The left-hand curve, *a*, is from the rest potential while the right-hand curve is from a hypothetical $V = -\infty$. Sliding the curves horizontally confirms that the superposition is perfect for all practical purposes, though it is of course not mathematically exact because these are no longer one-parameter curves (1, 4). Because we are using parameters previously chosen to accomplish this, $t_{1/2}^{(r)}$, $t_{1/2}^{(m)}$, and the slope at $t_{1/2}$ necessarily have the experimental values. Fig. 6 *b* gives the corresponding $p_z(t)$ curves. These indicate the fractional completion of the prior binding process (see Fig. 3). Very similar families of curves with "perfect" superposition, have also been computed for the cases $x = 4$, $z = 8$ and $x = 6$, $z = 6$.

Iteration Procedure

The calculations in Table I, Table II, and Fig. 6 assume no interactions between bound ions ($w = 0$), but equations 10–12 and Fig. 4 show that in the BW approximation, $cw/kT \cong 2.4$. Strictly speaking, all of these results have to be considered as just a first step in an iteration procedure. In the second step, we have to modify the equilibrium binomial probability distribution for $t < 0$ in equation 2 by incorporating (13) the BW approximation with $cw/kT = -2.4$. The result is to broaden the distribution somewhat around the mean value (13). The rate constant scheme in Fig. 3, however, would be unaltered in the second iteration step because the effect of attractive interactions between bound ions on the kinetics would undoubtedly be contained entirely in the desorption rate constants which are negligible, in any case, near E_{Na}. Since the only consequence of $cw/kT = -2.4$ in the second step is the rather minor one of a broadened initial probability distribution, we anticipate that the second step would give results differing very little from the first step. Incidentally, in the second step, equation 5 is no longer applicable. In any event, the model is sufficiently speculative at present that application of this iteration procedure would seem to be an unjustified refinement.

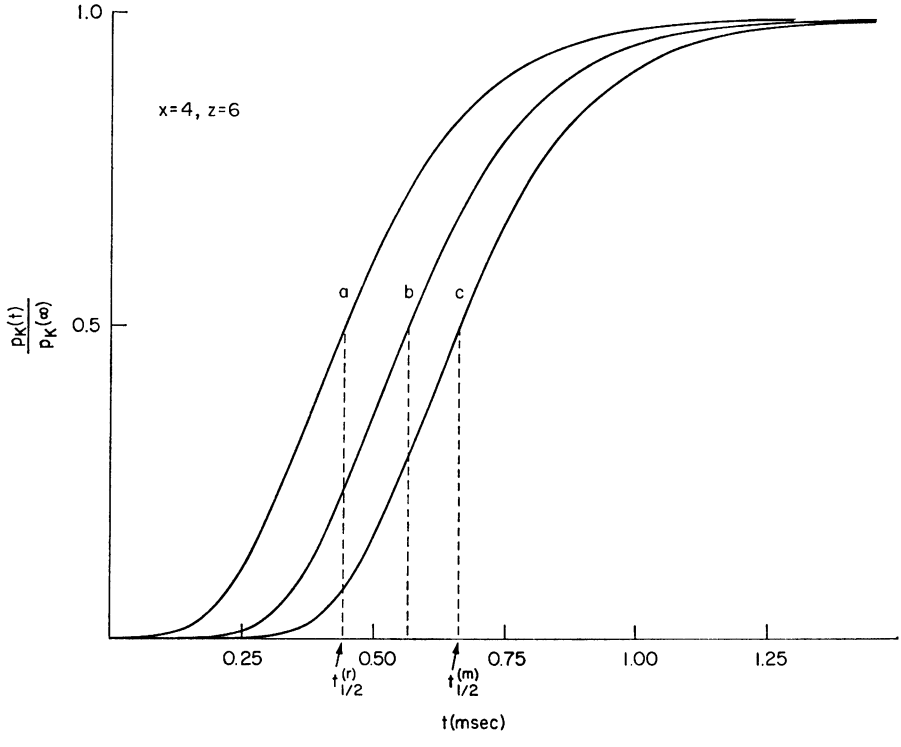

FIGURE 6 *a*

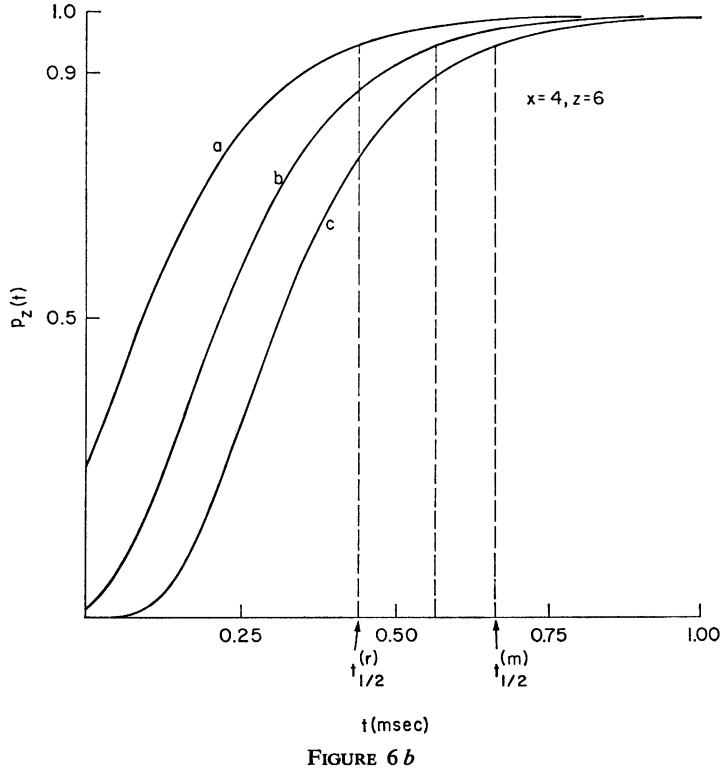

FIGURE 6 *b*

Conclusion

Adsorption or desorption of, say, phospholipid ions onto protein subunits of a K^+ channel appears to be a possible process, prior to the HH (conformational change) process, which could explain the hyperpolarization time delays and superposition found by Cole and Moore. Of course, quite different prior processes which have similar formal kinetics would also be possibilities.

MODEL 2

Field-Dependent Translocation of Channel

In this quite different model we assume that a hyperpolarizing potential V pulls the charged complex as a whole out of position within the membrane along a line (y axis) normal to the plane of the membrane, and that the time delay on subsequent depolarization to near E_{Na} can be attributed to the necessary return time under a restoring force. This possibility occurred to us[2] on recalling that the sliding filament velocity in muscle and common velocities of proteins in microtubules (14) are of the same order of magnitude as needed here, namely, several microns per second. For example, if the complex moved 10 A in 0.2 msec on its return, the average velocity would be 5 μ/sec. It should be noted that this velocity is very small, by a factor of 10^3 or 10^4, compared with that which would be predicted from protein electrophoretic mobilities in aqueous solutions and a field strength of about 10^5 v cm^{-1}, as in a membrane. Thus the complex in this model must move in the membrane against very considerable frictional resistance, which would not be surprising if, for example, the protein is or becomes, on hyperpolarization, entangled or enmeshed in phospholipid molecules. We turn now to a number of details which will make the model more explicit.

(*a*) The arrows in Fig. 7 show the direction in which the complex would be pulled on imposing a hyperpolarizing potential (inside more negative), assuming again somewhat arbitrarily, as in Fig. 5, that the motion is confined to only one side of the membrane.[3] There are four possibilities shown. For any of these cases, we take $y = 0$ as the equilibrium position of the center of mass of the complex at V_r, with y increasing in the direction of the arrow. The equilibrium position for an arbitrary $V < V_r$ is denoted by $y_0 > 0$. The delay time $\Delta(V) = t_{1/2} - t_{1/2}^{(r)}$, on depolarization, is the time required for the complex to move between y_0 (V) and $y = 0$.

[2] An alternative translocation model was suggested independently by Dr. Robert Blumenthal (private communication).

[3] Actually, pulling the complex from one phospholipid layer to the other might help account (12) for the small velocity referred to above. Also, if complex = channel (not gate), the complex would extend across the membrane.

FIGURE 6 Model 1. Depolarizations to +60 mv. $p_K(t)$ = fraction of K^+ channels open; $p_s(t)$ = total probability of states i_s and ii_s (see Fig. 3). (*a*) Depolarization from $V_r = -52$ mv ($n_0 = 0.15$, $\nu_0 = 0.777$). (*b*) Depolarization from $V = -114.5$ mv ($n_0 = 0$, $\nu_0 = 0.50$). (c) Depolarization from $V = -\infty$ ($n_0 = 0$, $\nu_0 = 0$).

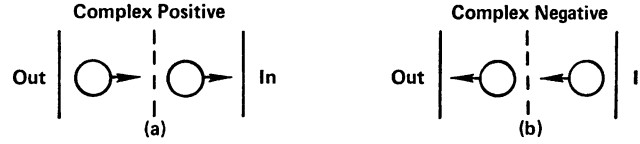

FIGURE 7 Model 2. Direction of translocation of charged complex within membrane on hyperpolarization. Dashed line is the middle of the membrane.

This, we assume, would be brownian motion under a restoring force (details below) through an inhibiting medium with a large frictional coefficient f (see above).

(*b*) As pointed out in footnote 1, if we take $x = 4$, the second process contributes a delay of 0.11 msec even in the V_r case. Hence, in the present model the "operating" position for the HH process of the complex is at or around some value $y = y'$ such that $y' < 0$. The experimental values of Δ (Fig. 1) will give us information about forces acting on the complex for $y > 0$ but not for $y' < y < 0$. On depolarizing from $V < V_r$, the complex starts at $y_0(V)$ and passes through $y = 0$ on its way to $y = y'$.

(*c*) At equilibrium, with $V \leq V_r$ and $y_0 \geq 0$, all x subunits are in state i. The complex has been pulled by the electric field out of its normal position at y' and forced into an "abnormal" environment. We have to assume that, on depolarization to near E_{Na} at $t = 0$, the conformational change $i \rightarrow ii$ in the subunits cannot take place until the complex reaches y' (from y_0). This could be a consequence of the complex being under compressional stress when $y > y'$, with the effect of inhibiting the structural change from i to ii. Without some such inhibition of $i \rightarrow ii$, the observed simple time delay with superposition would not be accounted for. Superposition would be automatic (exact) in the present model. The above-described absolute temporal separation between the two processes, translocation and HH, simplifies the theory but could be relaxed somewhat.

The prior adsorption process in model 1 is here replaced by translational diffusion in a force field, but the diffusion involves the complex as a whole whereas in model 1 adsorption occurs independently on each subunit. Hence the formal kinetics are somewhat different in the two cases.

(*d*) Let Z be the charge number on the protein complex i^z. For definiteness, we take Z to be positive, but only trivial changes are necessary in the equations below if Z is negative. Z could be of order 10 or 20. In the simple first-order theory presented here, we ignore the fact that Z would be somewhat V dependent. We also assume that the magnitude of the electric field strength is $|V|/d$, independent of y, where d is the thickness of the membrane. Then the force acting on the complex in the y direction owing to the hyperpolarizing field is $-Z \epsilon V/d$, a positive quantity. Let $U(y)$ be the potential energy of the complex in the inhomogeneous membrane material, including all contributions[4] other than $Z \epsilon \psi(y)$, ψ being the electrostatic

[4] To be more precise, $U(y)$ is the potential of the average force on the complex at y. $U(y)$ would include contributions from the membrane environment perturbed by the presence of the complex (15). In a more sophisticated treatment, $U(y)$ would itself be somewhat V dependent.

potential. The force resisting the pull of the field then is $-dU/dy$, the derivative being positive. We do not know the function $U(y)$, but if we accept the model, we can use the experimental $\Delta(V)$ to obtain information about this function.

At equilibrium ($t < 0$), the net force on the complex is zero. Thus the value of y which satisfies the equation

$$dU/dy = -Z \epsilon V/d, \tag{13}$$

is $y_0(V)$. At V_r,

$$(dU/dy)_{y=0} = -Z \epsilon V_r/d. \tag{14}$$

At $t = 0$, V is switched to V_f, $E_{Na} + 3$ mv in the Cole-Moore experiment. Thus the total force on the complex at y, for $t \geq 0$, is

$$K(y) \equiv -(Z \epsilon V_f/d) - dU/dy. \tag{15}$$

Both contributions to $K(y)$ are negative. This is the "restoring force."

(*e*) The brownian equation of motion for $t > 0$ is (16)

$$m \frac{d^2y}{dt^2} = -f \frac{dy}{dt} + K(y) + F(t) \tag{16}$$

where m is the mass of complex, f is the frictional coefficient, and $F(t)$ is the fluctuating force. We are interested here only in the mean position $\bar{y}$, not the dispersion. Hence we can drop $F(t)$ sbove and understand y to signify, strictly, $\bar{y}$. A numerical examination of m, f (for a protein like hemoglobin in a very resistant medium, see above), and the linear force constant in the second approximation to $K(y)$, see below, show that we are concerned here with the strongly overdamped case (16). In this situation, we have

$$f \frac{dy}{dt} = K(y), \tag{17}$$

and

$$\Delta(y_0) = f \int_{y_0}^{0} dy/K(y). \tag{18}$$

The velocity $v = dy/dt$ (a negative quantity) is a function of y only, and Δ is determined solely by the motion between $y = y_0$ and $y = 0$; i.e., the motion between $y = 0$ and $y = y'$ is the same for all starting positions $y_0 \geq 0$.

Iteration Procedure

Since $U(y)$ is not known, an iteration procedure is called for. If we start with an assumed first approximation $K_1(y)$, equation 18 gives $\Delta_1(y_0)$. This, with the experimental $\Delta(V)$, leads to $V_1(y_0)$; equation 13 then provides dU_1/dy. Use of this in equation 15 gives $K_2(y)$, and the process can be repeated. We limit ourselves here to the first set of steps just outlined, starting with $K_1 =$ constant.

Calculations

If we take K = negative constant as a first approximation, the return velocity $v = K/f$ is a constant, the same for all starting positions y_0. Thus $\Delta(y_0) = -y_0/v$; that is, Δ is simply proportional to y_0. Fig. 1 in this case may be regarded as a plot of $-y_0/v$ as a function of $-V$, i.e., the equilibrium position of the complex in the membrane as a function of the hyperpolarizing potential. From equation 13, Fig 1 is then also a plot of $(d/Z\epsilon)\mathrm{d}U/\mathrm{d}y$ (abscissa) against $-y/v$ (ordinate). This is also shown as the lower curve in Fig. 8. Numerical integration of this curve gives a first approximation to $U(y) - U(0)$, as shown in Fig. 9. To obtain a feeling for the orders of magnitude in Fig. 9, consider the point on the curve at $-y/v = 0.2$ msec. If we take $-v = 5\ \mu$/sec, then $y = 10$ A. If we also use $d = 70$ A, then $U(10\text{ A}) - U(0)$ is 0.36 Z kcal mole^{-1}, which seems reasonable if $Z = O$ (10 or 20).

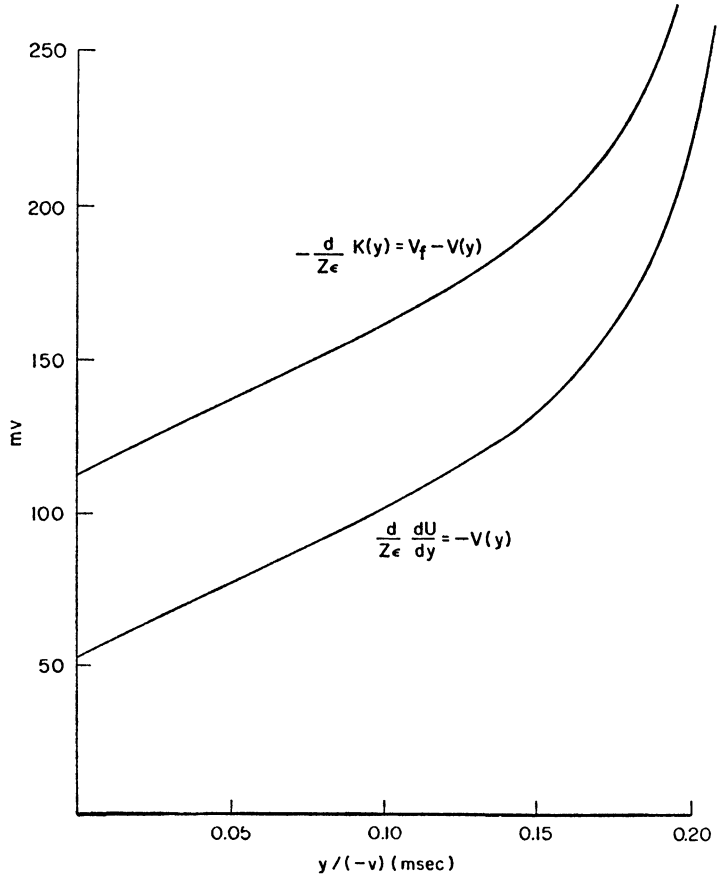

FIGURE 8 Model 2. Force functions. Lower curve: first approximation to $\mathrm{d}U/\mathrm{d}y$. Upper curve: second approximation to $-K(y)$. See text for definitions of $U(y)$ and $K(y)$.

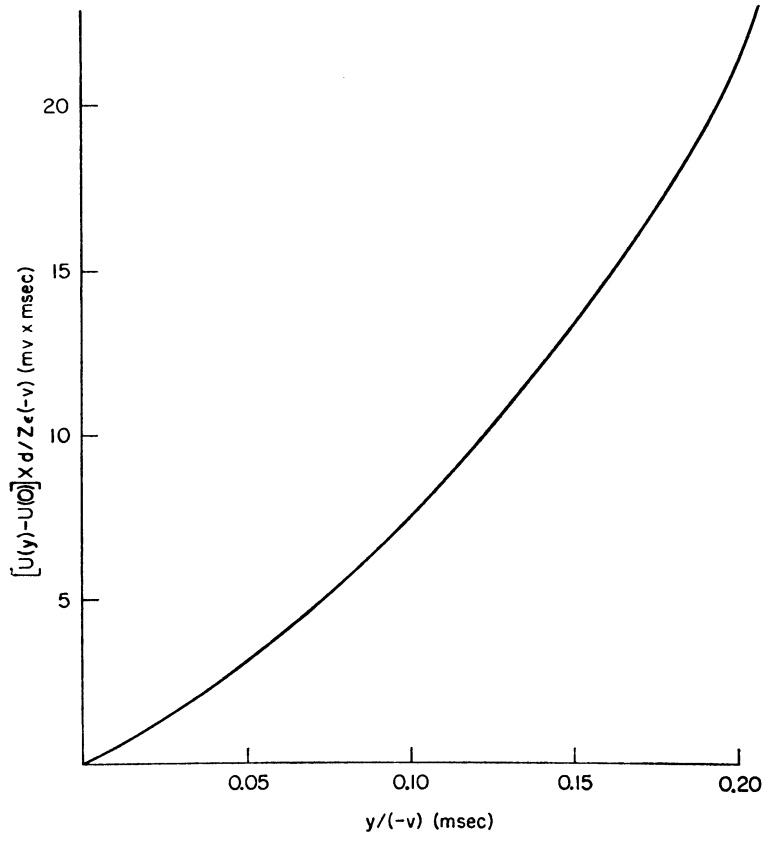

FIGURE 9 Model 2. Potential energy. First approximation to $U(y) - U(0)$ (see text for details).

The upper curve in Fig. 8 shows (see equations 13 and 15) the second approximation to the total restoring force $K(y)$, using $V_f = +60$ mv, as in Cole and Moore. Since V_f is constant, the two curves in Fig. 8 have the same shape (also seen in Fig. 1). The force $K(y)$ is linear (harmonic) in part of the interval shown.

Conclusion

This model also seems to check out rather well numerically and hence appears to be a possibility. Perhaps the most suspect point is the very low mobility of the complex in the membrane. In effect, we have used the experimental delay curve $\Delta(V)$ to deduce something about the restoring force and potential required to make the model work.

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REFERENCES

1. COLE, K. S., and J. W. MOORE. 1960. *Biophys. J.* **1**:1.
2. HODGKIN, A. L., and A. F. HUXLEY. 1952. *J. Physiol. (Lond.).* **117**:500.
3. HILL, T. L. 1972. *In* Perspectives in Membrane Biophysics. D. Agin, editor. Gordon and Breach, Science Publishers, Inc., New York. In press.
4. HILL, T. L., and Y. CHEN. 1971. *Proc. Natl. Acad. Sci. U. S. A.* **68**:1711.
5. HILL, T. L., and Y. CHEN. 1971. *Proc. Natl. Acad. Sci. U. S. A.* **68**:2488.
6. HILL T. L., and Y. CHEN. 1971. *Biophys. J.* **12**:948.
7. FITZHUGH, R. 1965. *J. Cell. Comp. Physiol.* **66**(2):111.
8. KORNBERG, R. D., and H. M. MCCONNELL. 1971. *Proc. Natl. Acad. Sci. U. S. A.* **68**:2564.
9. ONSAGER, L. 1934. *J. Chem. Phys.* **2**:599.
10. TRIGGLE, D. J. 1971. *In* Neurotransmitter-Receptor Interactions. Academic Press, Inc., New York. Chapter 2.
11. HILL, T. L. 1960. Statistical Thermodynamics. Addison-Wesley Publishing Co., Inc., Reading, Mass.
12. KORNBERG, R. D., and H. M. MCCONNELL. 1971. *Biochemistry.* **10**:1111.
13. HILL, T. L. 1963. *In* Thermodynamics of Small Systems, part I. W. A. Benjamin, Inc., Menlo Park, Calif. 147.
14. SCHMITT, F. O. 1968. *Neurosci. Res. Program Bull.* **6**:119.
15. HILL, T. L. 1956. *In* Statistical Mechanics. McGraw Hill Book Company, New York. Chapter 6.
16. UHLENBECK, G. E., and L. S. ORNSTEIN. 1930. *Phys. Rev.* **36**:823.