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A Simplified Cooperative Model of Excitable Membranes

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Summary. A cooperative model of an excitable membrane is constructed and discussed. The model has at most two stable and one unstable steady states. It is closely related to the model of Blumenthal, Changeux and Lefever (*J. Membrane Biol.* **2**:351, 1970), but it is simplified by the absence of equilibration layers. Transitions between the steady states can be brought about by changes in pH or by the Wien field-dissociation effect.

The ideas of the school of Tasaki (1968), concerning neural membrane excitation in terms of a cooperative phase transition, have recently been embodied in a detailed specific model (Blumenthal, Changeux & Lefever, 1970; Glansdorff & Prigogine, 1971). The membrane is considered to contain a set of protomers each capable of two states, combined with permeant molecules in four ways; cooperativity enters the model through the exponential dependence of some of the rate constants, controlling the interconversion of the eight combinations, upon the proportion of protomers in one of their states.

Their model embodies also the ideas of the school of Tasaki concerning the essential role of the *equilibration layers* separating the membrane from the two bulk solutions. Indeed, the authors assert that no nontrivial unstable states of the protomer-permeant system could exist without equilibration layers (Blumenthal *et al.*, p. 363). It is to be noted that the equilibration layers are physical entities postulated (though never observed as yet) to have physical properties different from both the membrane and the bulk solution for *any concentration distribution* of the permeant. For if they were merely convenient reference values taken in the transition regions between the interior of the membrane and the bulk solutions, we could calculate everything without them and subsequently obtain, in particular, the course of events in the defined region. Thus, the influence of the parameters of the

equilibration layers upon the model corresponds to that of independently variable *hidden* physical parameters.

Without wishing to deny the possible physical reality and significance of the equilibration layers, we shall show that a cooperative model of this type has the essential phase-transition features obtained by Blumenthal *et al.*, *even in the absence* of physically significant equilibration layers.

Hill and Chen (1971) have shown that cooperativity, when used to modify Hodgkin-Huxley kinetics, does not improve the fitting of $g_K(t)$ of squid axon under voltage clamp. The time-dependence of the transition composed of transitions of many elements (Tasaki, 1968) is a complicated problem (not treated here) for which no unique model exists. The general requirements for cooperative phenomena in membrane permeability continue to be of interest for a variety of natural and artificial membranes.

The Simplified Model and its Steady States

We consider only two states of the protomers; the fraction of protomers in the "open" state, permitting transfer of permeant through the membrane, is x; the fraction in the "closed" state is $1-x$. The time-dependence of the interconversion is described by

$$\dot{x} = -k_c' x + k_c(1-x) \tag{1}$$

where the equilibrium constant has the cooperativity property (Blumenthal *et al.*)

$$\frac{k_c'}{k_c} = e^{(\varepsilon - \eta x)\, kT} \tag{2}$$

where $\varepsilon > 0$ and η are free energies, k is Boltzmann's constant and T is the absolute temperature. The distribution of the x-dependence between the absolute rate-constants is unimportant in the present context, so long as Eq. (2) holds for the ratio. The essential cooperative feature is the change in the barrier with x. The flow of the permeant associated with x is

$$j = -hx(C_{+\infty} - C_{-\infty}) \tag{3a}$$

in the case of diffusion of uncharged permeant between bulk solutions of concentration $C_{+\infty}$, $C_{-\infty}$, or

$$j = gx(V - V_e) \tag{3b}$$

for ions with equilibrium potential V_e between the bulk solutions; h and g are characteristic positive constants. For completeness we could replace x by $x+L$, $0 < L \ll 1$, in (3a, b) to allow for leakage at $x=0$.

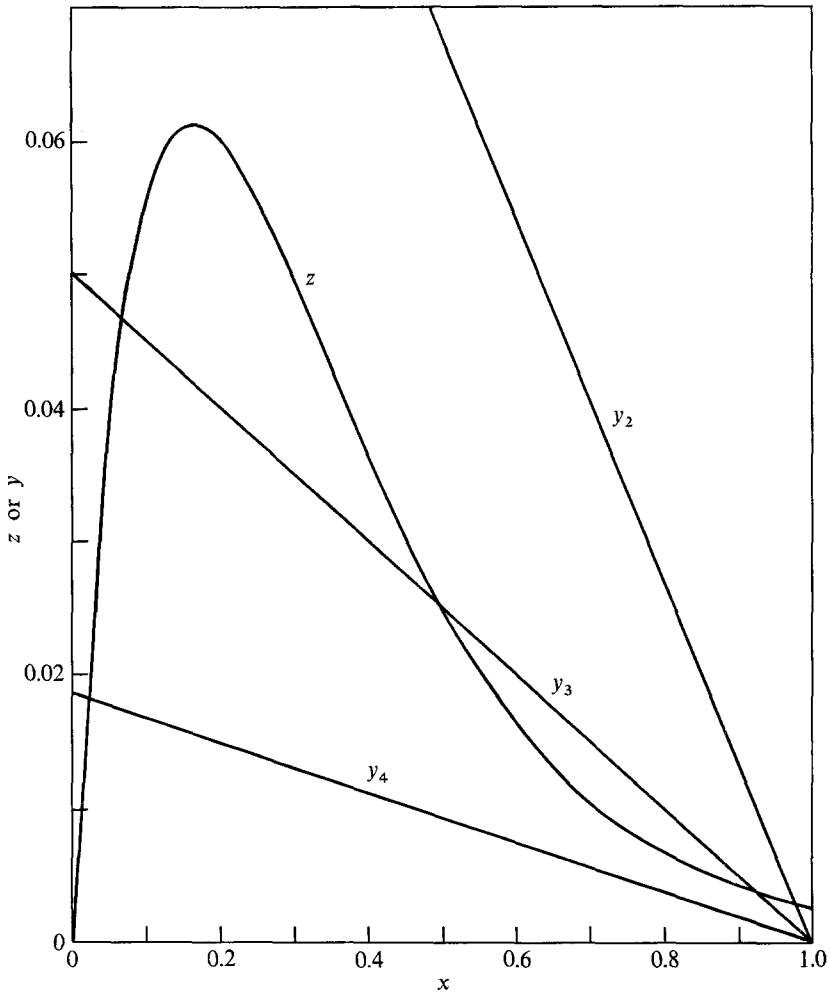

Fig. 1. Graphical representation of the regimes of the cooperative membrane model for the case of a constant cooperativity parameter $\eta = 6\,kT$, and three different values of ε, the free energy of the reaction for independent protomers. The lines $y_2(\varepsilon = 2\,kT)$ and $y_4(\varepsilon = 4\,kT)$ yield only a single stable state, while the line $y_3(\varepsilon = 3\,kT)$ arising from an intermediate value of the free energy represents a regime of two stable and one unstable steady states

To examine the steady states, we write (Fig. 1)

$$y = e^{-\frac{\varepsilon}{kT}}(1-x)$$
$$z = x e^{-\frac{\eta}{kT}x} \tag{4}$$

and seek intersections $y=z$ (corresponding to $\dot{x}=0$). While the constant slope of y is $-e^{-\varepsilon/kT}$, the coordinates of the maximum of z are $\left(\frac{kT}{\eta}, \frac{kT}{\eta}e^{-1}\right)$ and of its inflexion, $\left(\frac{2kT}{\eta}, \frac{2kT}{\eta}e^{-2}\right)$; the slope at the inflexion is $-e^{-2}$. It is apparent that there is always at least one steady state; there are three if the inflexion is in the interval $0<x<1$, i.e.,

$$\eta > 2kT; \tag{5}$$

if also at the inflexion z falls more steeply than y, $e^{-2} > e^{-\frac{\varepsilon}{kT}}$, i.e.,

$$\varepsilon > 2kT; \tag{5'}$$

and if furthermore y passes sufficiently close to the point of inflexion (line y_3):

$$\frac{\eta}{2kT} \simeq 1 + e^{\frac{\varepsilon}{kT}-2} > 2. \tag{5''}$$

(We shall not give the straightforward analysis of the relevant interval $\Delta\eta = \Delta\eta(\varepsilon)$ within which y is of the type y_3). Consider varying η at a fixed ε: for all negative η and for positive small η the inflexion is outside the relevant interval, and all y are of the type y_4; for larger η there can be three intersections (type y_3); for still larger η the concave part of z lies below y (type y_2) and there is again only one steady state (of high x). When ε is varied at a sufficiently large fixed η, small and large ε's give single states, and intermediate ε's give three states (Fig. 1).

The *stability* character of the steady-state solutions follows from a theorem (Bass & Moore, 1971) concerning equations of the type

$$f(x)\dot{x} = -x + g(x)(1-x), \quad g>0,\ 0 \leqq x \leqq 1.$$

We note first that the form of $f(x)$ does not affect stability so long as f does not change sign ($f = 1/k_c' > 0$ in our case): for small deviations from steady states, $\dot{x}$ is of first order of smallness, so $f(x)$ is replaced by its steady-state value. Next, if the roots of $x = g(x)(1-x)$ are labeled, $x_1 < x_2 < x_3 \ldots x_m \ldots$, then x_{2m+1} give stable and x_{2m} give unstable states. Hence in the present model the single root is always stable, while of three roots the middle is unstable and the other two are stable.

Transitions Between States

In the model of Blumenthal *et al.*, changes in the concentration of the permeant can induce transitions between states of the membrane via changes in the hidden variables pertaining to the unstirred layers. In the absence of

unstirred layers, as in our model, changes in the state of the membrane can be induced via changes in the relation (5″) between η and ε, which defines the regime having three states.

The very general cooperativity postulate Eq. (2) is consistent with a variety of mechanisms. Of these, the most interesting are those that couple the cooperative states to the electrical polarization of and pH effects upon the membrane.

By way of example, let us consider that one of the protomers can exist in either a protonated or a nonprotonated form, only the latter being able to undergo the conformational change that allows transfer of permeant through the membrane. For instance,

$$R\mathrm{NH}^+ \underset{k}{\overset{kK}{\rightleftharpoons}} R\mathrm{N} + \mathrm{H}^+$$

where $R\mathrm{NH}^+$ represents an imidazole residue in a membrane protein (Clark & Strickholm, 1971; Bass & Moore, 1973). If the proton equilibrium is achieved rapidly compared to the rate of the conformational change, Eq. (1) becomes

$$\dot{x} = -k_c' x + \frac{k_c}{1+(H/K)}(1-x) \tag{1'}$$

where x is again the fraction of protomer in open conformation, and H is the concentration of protons. To apply the earlier analysis directly to the modified system, we incorporate the factor $[1+(H/K)]^{-1}$ into starred rate constants,

$$\frac{k_c'^*}{k_c^*} = \exp\left[\varepsilon - kT \ln\left(1+\frac{H}{K}\right) - \eta x\right] \Big/ kT \tag{2'}$$

so that Eq. (1′) becomes Eq. (1) in terms of $k_c'^*$ and k_c^*. Now H may be varied by changes in the buffer medium, or by the effect of polarization changes on the dissociation constant of ambient neutral acids, which thus become transient sources or sinks of protons (Bass & Moore, 1968; Neumcke, Walz & Lauger, 1970). Thus, if initially both Eqs. (5) and (5″) are satisfied, yielding three states, it is apparent that moderate alterations of H or K within the physiological range can lead to a change of regime to a single state through a violation of (5″), or vice versa.

Alternatively, there is a class of models in which changes of state may be engendered by the dependence of the cooperativity parameter η on the pH in the membrane (Kirkwood & Shumaker, 1952*a*, *b*). This membrane pH in its turn can depend on the electric field in the membrane (Bass & Moore, 1968).

The general class of Wien-effect mechanisms for electrical excitation of membranes (noncooperative as well as cooperative) requires that the membrane conductance be a function of the absolute value of the electric field $|E|$. Gilbert and Ehrenstein (1969) observed that the steady state K^+ conductance g_K of squid giant axons became symmetrical about $E=0$ when the fixed negative charges were at about the same density on each side of the membrane. Because of rapid inactivation, the corresponding situation for g_{Na} remains unresolved. Thus in the case of g_K, at least, the ionic conductance is a function of $|E|$. As pointed out by Hill (1972) it may not be easy to find mechanisms apart from the Wien effect or induced dipole polarization that display this independence of the field direction.

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