

Kinetics of the Opening and Closing of Individual Excitability-Inducing Material Channels in a Lipid Bilayer

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ABSTRACT The kinetics of the opening and closing of individual ion-conducting channels in lipid bilayers doped with small amounts of excitability-inducing material (EIM) are determined from discrete fluctuations in ionic current. The kinetics for the approach to steady-state conductance during voltage clamp are determined for lipid bilayers containing many EIM channels. The two sets of measurements are found to be consistent, verifying that the voltage-dependent conductance of the many-channel EIM system arises from the opening and closing of individual EIM channels. The opening and closing of the channels are Poisson processes. Transition rates for these processes vary exponentially with applied potential, implying that the energy difference between the open and closed states of an EIM channel is linearly proportional to the transmembrane electric field. A model incorporating the above properties of the EIM channels predicts the observed voltage dependence of ionic conductance and conductance relaxation time, which are also characteristic of natural electrically excitable membranes.

INTRODUCTION

A lipid bilayer doped with EIM, the "excitability-inducing material" discovered by Mueller and Rudin (1963), conducts ions through discrete channels (Bean et al., 1969; Ehrenstein et al., 1970). In oxidized cholesterol bilayers, the channels have two conductance states, a "closed" state with a conductance of about 0.08 nmho and an "open" state with a conductance of about 0.4 nmho (Ehrenstein et al., 1970). The words "open" and "closed," as used here, are not meant to convey any particular picture of the structural change occurring during the conductance transition, but rather are meant to emphasize that there are two discrete conductance states. EIM channels in

bilayer membranes composed of lipids other than oxidized cholesterol may show three or more conductance states (Bean, 1972). Regardless of the composition of the bilayer, the probability that an EIM channel is in the high conductance state decreases as the membrane potential increases. This property of individual channels leads directly to a shut-off of ionic conductance with increasing membrane potential, producing the negative resistance region in the current-voltage curve of the many-channel membrane. The region of negative resistance, in turn, gives rise to excitability for constant-current stimulation.

The major point of similarity between EIM-induced excitability and that of natural excitable membranes is the sigmoid voltage-dependent steady-state ionic conductance which goes through its full range of variation over an interval of approximately 20–40 mV. In previous work (Ehrenstein et al., 1970; Latorre et al., 1972), we demonstrated the relation between the voltage-dependent conductance and the statistical distribution of open and closed conducting channels, which results from the voltage dependence of the transition rates for conductance change.

In this paper, we examine the kinetics of the EIM conductance transitions in detail. All experiments were performed on oxidized cholesterol bilayers, in order to make use of the well-defined transition between two conductance states. Kinetic measurements were performed in two different ways. In one method, membranes with very few (one to six) EIM channels were used, and the spontaneous discrete fluctuations of conductance at constant voltage were analyzed. In the other method, membranes with 100–1,000 channels were used, and the time for approach to steady state after a step change in membrane voltage was measured.

The object of both methods is to determine the voltage dependence of the transition rates for the change in channel conductance. For membranes with few channels, the two rates (open → close, close → open) can be determined directly and independently from the time-course of spontaneous opening and closing of individual channels. For a membrane with many channels, the individual rates cannot be determined directly, but it is possible to deduce them from experimentally measured values of relative conductance and the relaxation time for the conductance to reach steady state.

METHODS

All experiments reported in this paper were performed on oxidized cholesterol membranes at room temperature (25°C). The procedure for preparing the membranes and for preparing EIM are given in previous papers (Ehrenstein et al., 1970; Latorre et al., 1972).

The circuit for measuring electrical properties of the membrane is also shown in the earlier reference (cf. Latorre et al., 1972). The only departure from the previous circuit was the use of a CAT (Computer of Average Transients, Varian Associates, Instru-

ment Div., Palo Alto, Calif.) for recording the step-function response of the many-channel membranes. With this instrument, several consecutive transient responses were added to enhance the signal-to-noise ratio. Single transient responses could also be recorded and were checked against the averaged responses to insure against long-term drifts in channel properties. Many-channel membranes studied in these experiments had between 10^2 and 10^3 EIM channels.

Membranes with small numbers of EIM channels were obtained by dilution of the stock EIM solution as explained in a previous paper (Latorre et al., 1972). The number of channels (one to six usually) produced in this way is not under control by the experimenter and sometimes new channels enter the membrane during the course of an experiment. Although it is relatively easy to obtain records of conductance fluctuations on a single channel at a given voltage, it is rare that a given membrane can survive long enough to obtain complete records (≈ 25 – 50 jumps) at several different voltages. Accordingly, in some cases we used data for a single membrane corresponding to different numbers of channels. The number of channels prevailing during an actual measurement is listed in Table I.

TABLE I
PARAMETERS DESCRIBING OPENING AND CLOSING OF EIM CHANNELS

Membrane	No. of channels	A	B	V_o	λ^{-1}
		mV^{-1}	mV^{-1}	mV	s
M-142	3, 5	0.057	0.041	66	12.8
M-209	6	0.106	0.038	69	17.2
M-311	2, 4, 6	0.066	0.035	58	5.26
M-338	1	0.036	0.040	43	2.13
M-346	1	0.073	0.080	52	1.54
Rough average for individual channels		0.068 ± 0.026	0.047 ± 0.019	58 ± 11	7.8 ± 6.9
Best fit from many-channel data		0.041 ± 0.015	0.025 ± 0.007	59 ± 9	6.6 ± 0.5

RESULTS

Direct Measurement of Rate Constants for Opening and Closing of Individual Conducting Channels

The rate constants for the opening and closing of individual conducting channels were determined from records of the membrane current at constant voltage for membranes with several (one to six) channels. A typical record of the fluctuating current in a one-channel membrane is shown in Fig. 1. The duration of a single square current fluctuation is the time between successive conductance transitions, which we shall call the dwell time of the channel in one of the two conductance states.

If the transition between the two conductance states is a Poisson process, then the probability of a transition per unit time (say, from the open to the closed state) is a constant, giving rise to an exponential distribution of dwell times. For such a process, the likelihood of a given value of dwell time, t_d , is proportional to $\exp(-\beta t_d)$ where β is the rate constant for the conductance transition.

Fig. 2 shows the distribution of dwell times for both the open and closed states of a single channel. The figure shows the cumulative sum of the number of events of duration longer than t_d plotted as a function of t_d . The dis-

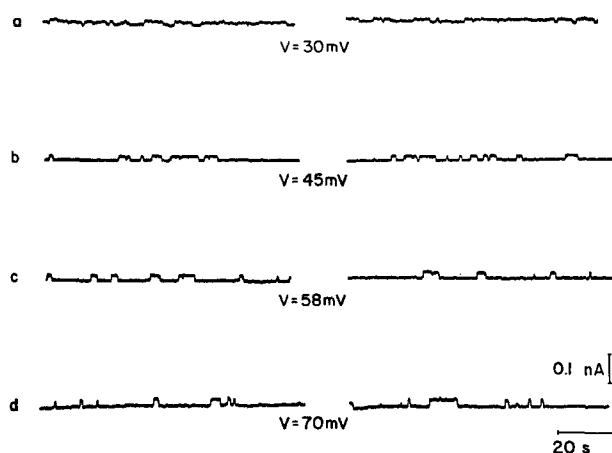


FIGURE 1. Current fluctuations of an individual channel. Two representative segments are shown for each of the following values of potential: (a) 30 mV, (b) 45 mV, (c) 58 mV, (d) 70 mV.

tribution function is seen to be exponential. Thus, the data are consistent with the hypothesis that the opening or closing of a conducting channel is a Poisson process in which the probability of the occurrence of a conductance transition is independent of the time measured from the previous transition. Some insight into the significance of Fig. 2 can be provided by imagining that all the dwell times observed sequentially for a single channel occurred simultaneously for many similar channels. Fig. 2 would then represent the number of channels which had not switched as a function of time, and would be similar to a radioactive decay curve.

Voltage Dependence of the Rates

The average dwell time is equal to the integral of all possible dwell times weighted with the appropriate normalized distribution function. Since the distribution function is exponential,

$$\bar{t}_d = \frac{1}{m} \sum_{i=1}^m t_{di} = \int_0^{\infty} t_d \exp(-\beta t_d) dt_d / \int_0^{\infty} \exp(-\beta t_d) dt_d, \quad (1)$$

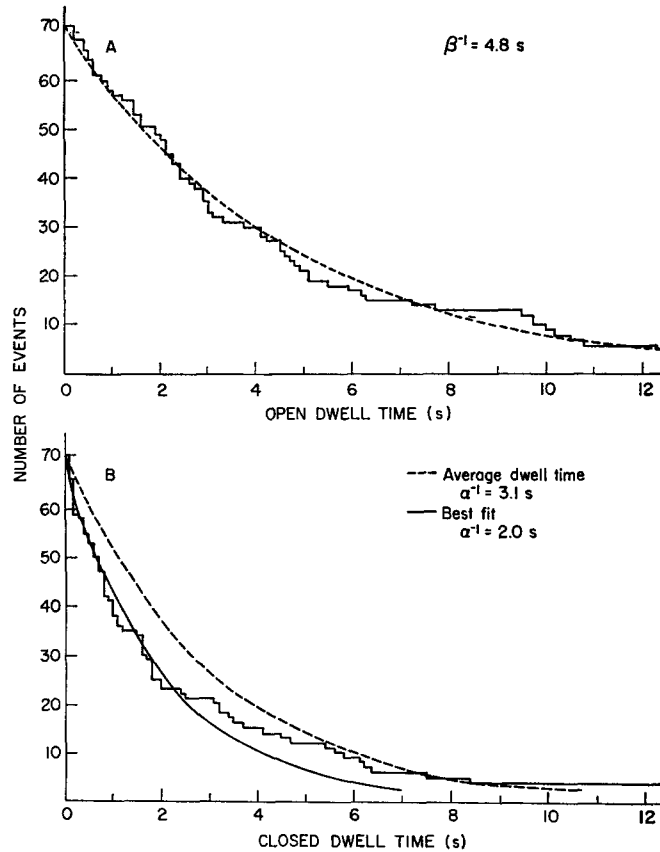


FIGURE 2. Distribution of dwell times for a single channel held at constant potential, 53 mV. In this record, 71 current steps were recorded during 562 s. Graph shows the number of dwell times longer than the time indicated by the abscissa. The dashed curves indicate the best-fit exponential to the distribution. (A) Open dwell times, $\tau = 4.79$ s. (B) Closed dwell times, $\tau = 3.13$ s.

where t_{di} is an individual dwell time, m is the number of jumps in a record, and the integral in the denominator is the normalization factor. Evaluation of the integrals in Eq. 1 leads to:

$$\beta = \frac{1}{t_d} = \frac{m}{\sum t_{di}} \quad (2)$$

Eq. 2 states that the rate constant is simply the reciprocal of the average dwell time.

We shall denote the rate constant for the opening of a closed channel as $\alpha(V)$ and the rate constant for the closing of an open channel as $\beta(V)$, where V is the applied potential. The rates α and β can be determined from the distribution of dwell times. The opening rate, α , is equal to the reciprocal of the

average dwell time in the closed state, and the closing rate, β , is equal to the reciprocal of the average dwell time in the open state.

EIM-doped membranes having a single channel are observed infrequently. More often a membrane doped with minute amounts of EIM will exhibit several channels. In this case too, α and β may be determined from averaging the dwell times, but the averaging process must be more carefully described. For example, since β is equal to the number of closings per second per open channel, the total number of closings observed in any record is equal to β times the total amount of time per channel that channels are in the open state times the number of channels. From a record of current fluctuations, the number of closings can be counted; this number is then divided by the number of channel seconds in the open state to get β . For the case of a single channel, this method reduces to Eq. 2.

The dashed curves in Fig. 2 A and B are exponentials with rate constants determined according to Eq. 2. In Fig. 2 A this method gives a curve that is very close to the best-fit exponential. In Fig. 2 B, however, the best-fit exponential, which is shown by the solid curve, has a rate constant about 50% faster than the rate constant determined from Eq. 2. This is because Fig. 2 B includes a few unusually long dwell times. We have used the average dwell time method for the determination of rate constants, primarily because it applies to records with any number of channels. The best-fit method, on the other hand, cannot be applied to records with more than one channel, because the ambiguity in identifying which channel is opening or closing precludes an accurate determination of all the individual dwell times. This ambiguity does not affect a method based only on averages.

Fig. 3 is a semilogarithmic plot of opening and closing rates as a function of

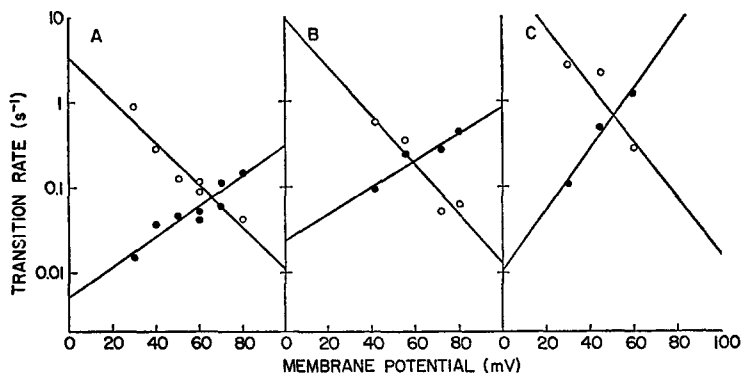


FIGURE 3. Opening and closing rate constants, α (hollow circles) and β (filled circles), as a function of membrane potential from data on three membranes: (A) M-142 (3, 5 channels), (B) M-311 (2, 4, 6 channels), (C) M-346 (1 channel). The straight lines on the semilog scales are least squares fit to Eqs. (3). During the course of an experiment, the number of channels in the membrane sometimes changed. The numbers in parentheses above indicate the numbers of channels prevailing when measurements were made.

membrane potential for three different membranes. At any given voltage, the rate constants vary from membrane to membrane by an order of magnitude, but for each membrane the dependence of the rates upon voltage is similar. Fig. 3 shows that both $\log \alpha$ and $\log \beta$ are linear functions of voltage. Thus, the rates for an individual channel can be represented as exponential functions of membrane potential. For convenience in later discussion, we write these functions in the following manner:

$$\begin{aligned}\alpha(V) &= \lambda e^{-A(V-V_o)} \\ \beta(V) &= \lambda e^{+B(V-V_o)},\end{aligned}\tag{3}$$

where λ is the transition rate measured at the voltage, V_o , for which the rates of opening and closing are equal. Eq. 3 is a convenient representation of the data as obtained experimentally because most jump records are obtained in an interval of about ± 30 mV surrounding the voltage V_o . Table I summarizes the data taken from five membranes having one to six conducting channels. From Table I, we note that the average values of the parameters are:

$$\begin{aligned}A &= 0.07 \pm 0.03 \text{ mV}^{-1} & B &= 0.05 \pm 0.02 \text{ mV}^{-1} \\ V_o &= 58 \pm 11 \text{ mV} & \lambda^{-1} &= 8 \pm 7 \text{ s.}\end{aligned}$$

In view of the small number of events observed, these averages should be taken to be merely suggestive of the mean values of the parameters. Indeed, within experimental accuracy, it is possible that A is equal to B , in which case kinetic relations derived from Eq. 3 take a particularly simple form.

Despite the considerable variation of λ from membrane to membrane indicated in Table I, there is evidence from Fig. 3 that λ does not vary very much from channel to channel in the same membrane. In Fig. 3 A, the points corresponding to voltages of 30, 40, and 50 mV were obtained with three channels, and those corresponding to voltages of 60, 70, and 80 mV were obtained with five channels in the same membrane. In Fig. 3 B, the points corresponding to 71 and 55 mV were obtained with two channels, the point at 41 mV with four channels, and the point at 80 mV with six channels. Despite changes in the number of channels, the points are reasonably consistent, suggesting that λ is approximately constant from channel to channel in the same membrane.

Relaxation of the Voltage-Dependent Conductance for Many-Channel Membranes

When the voltage across a many-channel membrane is suddenly changed, the membrane current quickly changes to a value appropriate to the new driving force and the old conductance. The current then approaches its steady state exponentially. This is shown in Fig. 4 both for a conductance decrease (following an increase in voltage) and for a conductance increase (following a subsequent decrease in voltage). The time-course of conductance change is

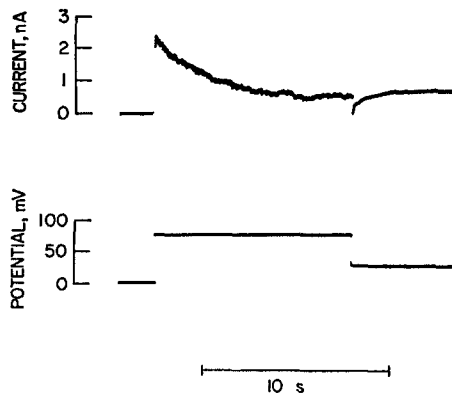


FIGURE 4

FIGURE 4. Response of many-channel membrane to step changes in potential. Lower trace shows the applied steps of potential. Upper trace shows current.

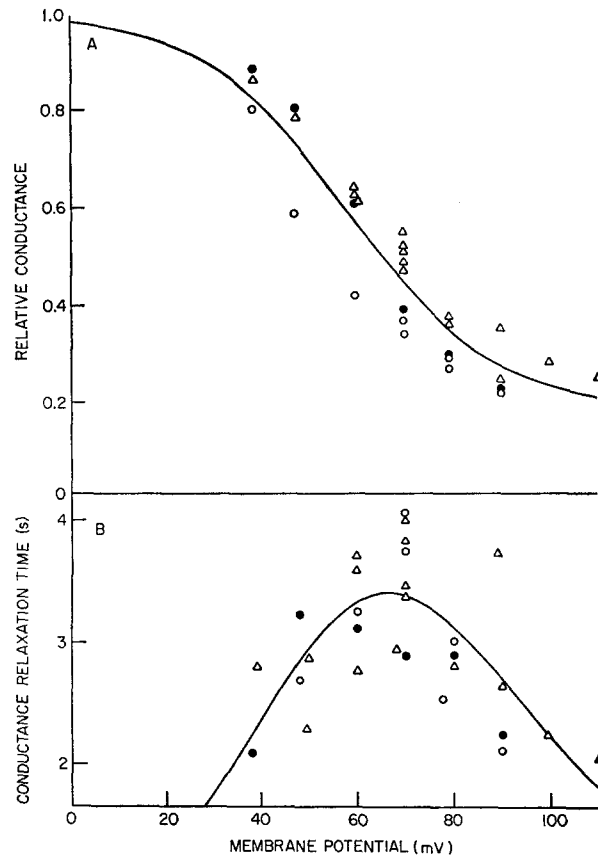


FIGURE 5

FIGURE 5. (A) Steady-state relative conductance as a function of potential for same membranes used in relaxation-time study. (B) Conductance relaxation time as a function of potential. The curves are least squares fits of Eqs. 11 and 12 jointly on the data of A and B. Membranes: ● M-309, △ M-330, ○ M-345.

exponential for all observed step changes of voltage, implying that the EIM channel transitions obey first-order kinetics.

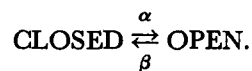
In the actual measurements of conductance and time constant, the initial voltage was maintained at zero, but only one additional pulse was applied. It has previously been determined (Ehrenstein et al., 1970) that at $V = 0$, the channels are all open and the conductance is maximum. Also, the percentage of channels open in steady state at any voltage is given by the same sigmoid function of voltage, independent of the number of EIM channels. Thus, the relative conductance, defined as $g_{rel} = (\text{conductance})/(\text{maximum conductance})$ is simply the ratio of steady-state current to initial current. The steady-state

values of g_{rel} as a function of voltage for several different membranes are shown in Fig. 5 A. As indicated above, the time-course of the approach to steady state can be described by a single time constant. The dependence of this time constant on the final voltage for the case where the initial voltage is zero is shown in Fig. 5 B.

Fig. 5 A and B demonstrates a kinetic relation familiar from the study of natural excitable membranes. The sigmoid voltage-dependent conductance is accompanied by a bell shaped voltage-dependent relaxation time. Both curves are centered at approximately the same voltage, and demonstrate variability over the same voltage range. In the Discussion, we will consider the significance of these relationships in terms of the simplest stochastic picture of the conductance transition.

DISCUSSION

Figs. 2 and 4 demonstrate that the conductance transitions are first-order processes, justifying the use of first-order rate constants for the reaction



The experimental results provide a direct measure of α and β for individual EIM channels. From Fig. 3 and other experiments summarized in Table I, it can be seen that for a given voltage, the absolute magnitude of the rate constants varies from membrane to membrane by an order of magnitude and that the slopes of the variation of the logarithm of rate constant with membrane potential vary by about a factor of 2. Fig. 3 also shows that, despite these variations, for each membrane the rate constants depend exponentially on membrane voltage.

We will now make use of these experimental facts together with previously determined experimental facts about individual EIM channels in oxidized cholesterol to predict the behavior of a many-channel EIM membrane.

Model for EIM Gating

The assumptions of the model are: (a) Each conducting channel has two stable conformational states, each with a unique conductance (demonstrated for individual channels by Ehrenstein et al., 1970). (b) The channels are independent of each other (demonstrated by Latorre et al., 1972). (c) The opening and closing processes are first order with rate constants that depend exponentially on membrane voltage (demonstrated for individual channels in the present paper). (d) All channels are identical with some average value of the parameters λ , A , B , and V_o , which describe the rate constants according to Eq. 3. As shown above, although this assumption is not always true from membrane to membrane, it is a reasonable assumption for a given membrane.

We consider N conducting channels each having two stable conformational states. Let n_o be the number of open channels each with conductance g_o and n_c be the number of closed channels with conductance g_c . Then

$$n_o + n_c = N. \quad (4)$$

From the principle of detailed balance,

$$\bar{n}_o/\bar{n}_c = \alpha(v)/\beta(v), \quad (5)$$

where the bars denote equilibrium values. Substituting the experimentally determined expressions for α and β described by Eq. 3,

$$\bar{n}_o/\bar{n}_c = \exp [-(A + B)(V - V_o)]. \quad (6)$$

Combining Eqs. 4 and 6, we obtain

$$\bar{n}_o/N = (1 + \exp [(A + B)(V - V_o)])^{-1}. \quad (7)$$

Eq. 7 is of the same form as the expression for the average value of the fraction of channels open derived by Ehrenstein et al. (1970) for a Boltzmann distribution with the energy difference between the open and closed states equal to $a(V - V_o)$. The relation between the parameter a and $(A + B)$ is

$$a/kT \equiv (A + B) \quad (8)$$

where k is the Boltzmann constant and T is the absolute temperature.

The steady-state conductance \bar{g} can be expressed in terms of the open and closed channel conductances:

$$\bar{g} = \bar{n}_o g_o + \bar{n}_c g_c. \quad (9)$$

Combining Eqs. 4, 7, and 9, we obtain

$$\bar{g}(V) = N \left(g_c + \frac{g_o - g_c}{1 + \exp[(A + B)(V - V_o)]} \right). \quad (10)$$

The normalized conductance g_{rel} can be obtained from Eq. 10 by dividing by the maximum conductance $N g_o$. The result is:

$$g_{\text{rel}}(V) = (g_c/g_o) + \frac{1 - (g_c/g_o)}{1 + \exp[(A + B)(V - V_o)]}. \quad (11)$$

For a first-order process, the relaxation time τ is simply $1/(\alpha + \beta)$. Making

use of Eqs. (3),

$$\tau(V) = [\alpha(V) + \beta(V)]^{-1} = \lambda^{-1}[\exp[-A(V - V_o)] + \exp[B(V - V_o)]]^{-1}. \quad (12)$$

For the special case where $A = B$, Eq. 12 reduces to a hyperbolic secant function with peak at $V = V_o$. In our experiments, A was somewhat larger than B , resulting in a displacement of the peak from V_o . When $A \neq B$ the displacement of the peak from V_o can be calculated by setting the derivative with respect to V of the right-hand side of Eq. 12 equal to zero. The displacement is

$$V_{\text{peak}} - V_o = \frac{\ln(A/B)}{A + B}. \quad (13)$$

Comparison of Many-Channel Data with Model

We wish to compare the many-channel data for relative conductance (Fig. 5 A) and relaxation time (Fig. 5 B) with Eqs. 11 and 12, which are based on the results of experiments on individual channels. Eq. 11 predicts a sigmoidal curve for relative conductance versus voltage with steepness determined by $(A + B)$ and with the midpoint conductance (midway between minimum and maximum) centered at $V = V_o$. Eq. 12 predicts an asymmetrical bell-shaped curve for relaxation time. Eq. 13 indicates that for the values of A and B shown in Table I, the peak of this curve should be displaced about 7 mV to the right of V_o .

From a least squares fit of Eqs. 11 and 12 jointly on Fig. 5 A and B, the best-fit parameters were found to be:

$$\begin{array}{ll} A = 0.041 \pm 0.01 \text{ mV}^{-1} & B = 0.025 \pm 0.01 \text{ mV}^{-1} \\ V_o = 59 \pm 9 \text{ mV} & \lambda^{-1} = 6.6 \pm 0.5 \text{ s.} \end{array}$$

These parameters were inserted into Eqs. 11 and 12 to give the curves shown in Fig. 5 A and B. There is a reasonable fit to the experimental points. In Table I, the parameters determined from the many-channel experiments are compared with those determined from the individual-channel experiments. As previously indicated, the individual-channel averages are merely suggestive. Nevertheless, the two sets of parameters are in rough agreement. Also, the experimental many-channel data do follow the main qualitative predictions. As indicated in Fig. 5 A and B, the $g_{\text{rel}}(V)$ curve is sigmoidal and the $\tau(V)$ curve is bell shaped, the same A and B parameters fit both curves reasonably well, and the peak of the $\tau(V)$ curve occurs slightly to the right of the voltage corresponding to the midpoint conductance. This agreement shows that the behavior of the many-channel EIM membrane follows directly from a few experimental facts deduced from data on individual EIM channels.

Some Unresolved Questions

The somewhat idealized picture presented above ignores several unresolved questions, which we will now mention. A close examination of records on individual channels reveals occasional jumps whose amplitudes are much smaller than those usually observed. These jumps occur too frequently to be dismissed as extraneous. However, they are not frequent enough to interfere with our statistics. Perhaps these jumps represent rare transitions of the channels to a conductance state different from the two stable states usually observed. The existence of additional conductance states for EIM in bilayers made from other lipids has been suggested by Bean (1972).

Also, our simple model does not take into account a second negative resistance region which occurs in the negative voltage range (Mueller and Rudin, 1963). An extension of the model to include this fact will be given as part of a future paper.

The assumptions in this paper imply that the rate constants depend only upon the actual membrane potential, and are independent of past history. We performed a number of experiments with varying prepulse potentials in order to test this point, but with inconclusive results. We can only state that the question has not been resolved.

Physical Interpretation

In our previous work (Ehrenstein et al., 1970), we demonstrated that the free energy difference between the two stable conductance states of an EIM channel is a linear function of membrane potential. We have now shown that both opening and closing rate constants are exponential functions of membrane potential. According to reaction rate theory (Glasstone et al., 1941), this is equivalent to the statement that the free energy difference between either of the two stable conductance states and the peak of the energy barrier for the transition between them is also a linear function of membrane potential. Furthermore, since A and B (as defined in Eqs. 3) are approximately equal, when the free energy difference corresponding to opening increases, the free energy difference corresponding to closing decreases by an approximately equal amount.

Fig. 6 illustrates this energy barrier picture for the change in EIM configuration which occurs during a channel conductance change. For a membrane voltage of about 60 mV, the energy barriers for both opening and closing processes are equal. For lower voltages, the energy barrier for opening is decreased and the energy barrier for closing is increased, while for higher voltages, the opposite occurs. The exponential dependence of both opening and closing rate constants on membrane voltage indicates that both energy barriers change proportionally to this voltage.

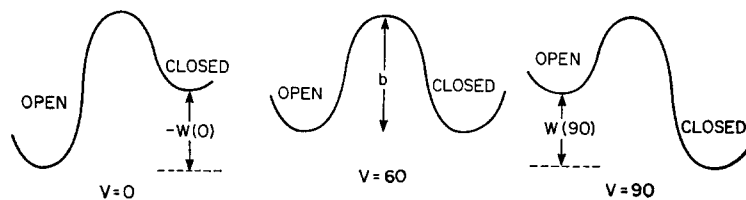


FIGURE 6. Energy barrier profile at three values of membrane potential. At 0 mV, open state has lower energy than closed state. At 60 mV, two states have equal energy. At 90 mV, closed state has lower energy.

A simple physical interpretation of this energy barrier picture can be given. The energy difference between the two stable states at $V = 0$ (labeled $-W(0)$ in Fig. 6) represents the intrinsic configuration energy difference between the two states in the absence of an applied field. When an electric field is applied, the free energies of the two conductance states vary linearly with voltage by approximately equal and opposite amounts. This linear change in free energy implies the interaction of the electric field with charged groups or permanent electric dipoles of the EIM channel structure. The height of the energy barrier (labeled b in Fig. 6 for the special case where the two conformations have equal free energy) apparently varies with the local lipid environment, since the speed of conductance change, which depends upon this barrier height, varies considerably when lipids or solvents are changed (Bean, 1973). As we have shown in this paper, there is even considerable variation in speed of conductance change among EIM channels in different oxidized cholesterol membranes. This suggests that the lipids influence the steric constraints which limit transitions from one conductance state to the other.

Significance for Natural Excitable Membranes

The voltage dependence of the EIM conductance and of the associated relaxation time (Fig. 5 A and B) are similar to the voltage dependences of the comparable parameters for natural electrical excitation. These characteristic shapes seem to be a feature of all electrical excitation processes, such as the potassium-conducting process of the axon membrane (the “ n ” parameter of Hodgkin and Huxley (1952) as described by Cole, 1956), the activation part of the axon sodium conductance (the Hodgkin-Huxley “ m ” parameter), and the analogous ionic permeabilities of muscle cells and electric organs (for references, see Ehrenstein and Lecar, 1972). Thus, it is tempting to suggest that the voltage-dependent ionic conductances and the kinetics of natural excitable membranes are also the result of a statistical distribution of ionic channels capable of switching between states of different conductance.

A difficulty in making this analogy between EIM and the natural systems is the quantitative difference between some of the conductance parameters. For example, the EIM channel conductance (0.4 nmho in 0.1 M salt) is

larger than the conductances estimated for the axon potassium channel (0.01 nmho in 0.1 M salt, Siebenga et al., 1973; 0.001 nmho in 0.4 M salt, Armstrong, 1966), although not much different from the value obtained for the axon sodium channel (Moore et al., 1967) or the chemically activated post-synaptic channel (Katz and Miledi, 1971; Anderson and Stevens, 1973; Sachs and Lecar, 1973). Gating or switching mechanisms, however, may be similar even for channels of widely differing conductance. Similarly, ionic selectivity, which is different for EIM and for the natural channels, is a property of the transport pathway and may have little to do with the switching mechanism.

Another difference between EIM and the natural channels is that EIM in oxidized cholesterol seems to obey first-order kinetics, characterized by an initial linear rise during a conductance increase, whereas the analogous increase of the axon potassium conductance proceeds with a delay. A possible cause of the time delay is that the conductance transitions for axon channels, although similar to that observed for EIM, proceed via one or several intermediate states. In any event, the time-delay phenomenon does not occur in all natural preparations; first-order kinetics has been observed for the potassium process of the barnacle giant muscle fiber (Keynes et al., 1973).

In summary, EIM doping of a lipid bilayer produces ion-conducting channels which change conductance in response to membrane potential by discrete transitions between stable conductance states. The energy difference of these states is a linear function of the transmembrane electric field, and it is this dependence which leads to the voltage-dependent permeability and voltage-dependent relaxation similar to those of natural excitable membranes. The differences between EIM and natural excitable membranes are not serious enough to rule out the possibility that this similarity in properties is based on a similarity in mechanism.

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