

Kinetic Characteristics of the Excitability-Inducing Material Channel in Oxidized Cholesterol and Brain Lipid Bilayer Membranes

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ABSTRACT The kinetic characteristics of the opening and closing of the excitability-inducing material (EIM) channel in oxidized cholesterol and in brain lipid bilayers are compared. The kinetics of the opening and closing of individual ion-conducting channels in bilayers doped with small amounts of EIM are determined from discrete fluctuations in ionic current. The kinetics for approach to steady-state conductance are determined for lipid bilayers containing many channels. Steady-state and kinetic characteristics for the EIM channel incorporated in brain lipid bilayers can be accounted for by the model developed for the EIM channel incorporated in oxidized cholesterol membranes. Relaxation time, calculated from rate constants of single-channel membranes or directly measured in many-channel membranes is strongly temperature dependent, and is always shorter in brain lipid membranes. Changes in temperature do not affect the interaction of the electric field and the open channel, but the open configuration of the EIM channel in brain lipid bilayers is stabilized with increasing temperature. The configurational energy difference between the open and closed channel, calculated from temperature studies, is larger in brain lipid bilayers. The energy barrier which separates the two configurations of the channel is larger in oxidized cholesterol bilayers.

INTRODUCTION

Bimolecular lipid membranes treated with excitability-inducing material (EIM) exhibit voltage-dependent ion conductance (Mueller and Rudin, 1963). When EIM is added to the solution bathing the membrane, the con-

ductance develops in discrete steps (Bean et al., 1969). The steps arise from the formation of individual ionic channels. In oxidized cholesterol bilayers, the channels present a bistable pattern with two conductance states, a high conductance state (open channel) and a low conductance state (closed channel) (Ehrenstein et al., 1970). When added to bilayers of other lipids, EIM may show more than two conductance states (Bean, 1972). In oxidized cholesterol membranes, the fraction of time a single channel is open is a function of the membrane potential. This function is the same as that which describes the voltage dependence of the conductance of many-channel membranes (Ehrenstein et al., 1970; Latorre et al., 1972). The conductance transitions of the EIM channel in oxidized cholesterol membranes are first-order processes (Ehrenstein et al., 1974), where the opening and closing of the channel are Poisson processes. Transition rates vary exponentially with the applied potential, implying that the energy difference between the open and closed states of the EIM channel is linearly proportional to the transmembrane electric field. In addition to EIM, alamethicin (Mueller and Rudin, 1968; Gordon and Haydon, 1972; Eisenberg et al., 1973) and monazomycin (Muller and Finkelstein, 1972) show strong voltage-dependent conductances. For alamethicin, the connection between channel formation and the voltage-dependent conductance observed at high conductance levels has been clearly demonstrated by Eisenberg et al. (1973), but it appears that the gating process is substantially different from that of EIM. These substances provide models for a better understanding of the voltage-dependent permeabilities which are also characteristic of natural excitable membranes.

In this paper, we examine the effect of temperature on the kinetics of the EIM conductance transitions. The purpose of this study is to determine the energy barrier that separates the two conductance states and to see whether temperature can modify the interaction between the EIM channel and the applied external field. The second goal of the present experiments is to compare the kinetics of the opening and closing of the EIM channel in oxidized cholesterol membranes and brain lipid bilayers. Kinetic studies of the same ionophore in different lipid moieties could give us some insight into the fluidity of the membrane matrix.

METHODS

Experiments reported in this paper were performed on oxidized cholesterol or brain lipid membranes in 0.1 M KCl, pH 7, at temperatures in the range 20–50°C. Brain lipid extract and bilayer were prepared according to Mueller and Rudin (1969). The procedure for preparing oxidized cholesterol membranes and for preparing EIM was taken from Ehrenstein et al. (1970), and Latorre et al. (1972).

Temperature was controlled by placing the membrane chamber in the cavity of a brass block, which was heated with circulating water. Temperature was monitored by means of a thermistor thermometer.

A block diagram of the system for measuring electrical properties of the mem-

brane is shown in Fig. 1. The circuit of the front end current transducer (FET) is shown in Fig. 2. The sensitivity of this circuit can be varied from 10 pA to 10 nA per volt in decades; the rise time is 0.5 ms, given by the capacitor in the feedback loop of the FET amplifier. The input impedance ranges from 1 to 1,000 Ω , according to

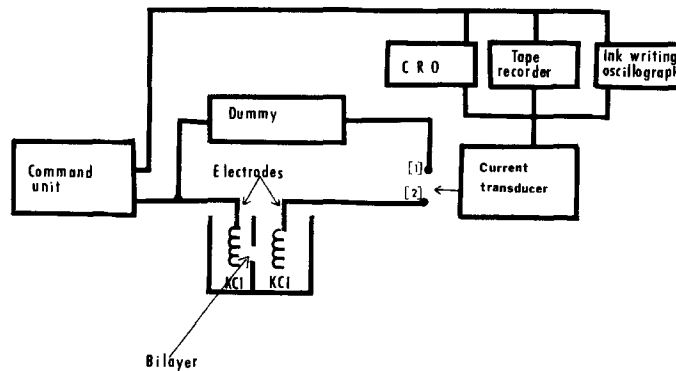


FIGURE 1 Block diagram of the experimental setup. The command unit generates DC or triangular potential functions, which are applied to the membrane. Current flowing through the membrane is transduced to a potential in the current transducer amplifier. Membrane dummy consists of a 2-nF capacitor connected in parallel with a $10^9 \Omega$ resistor.

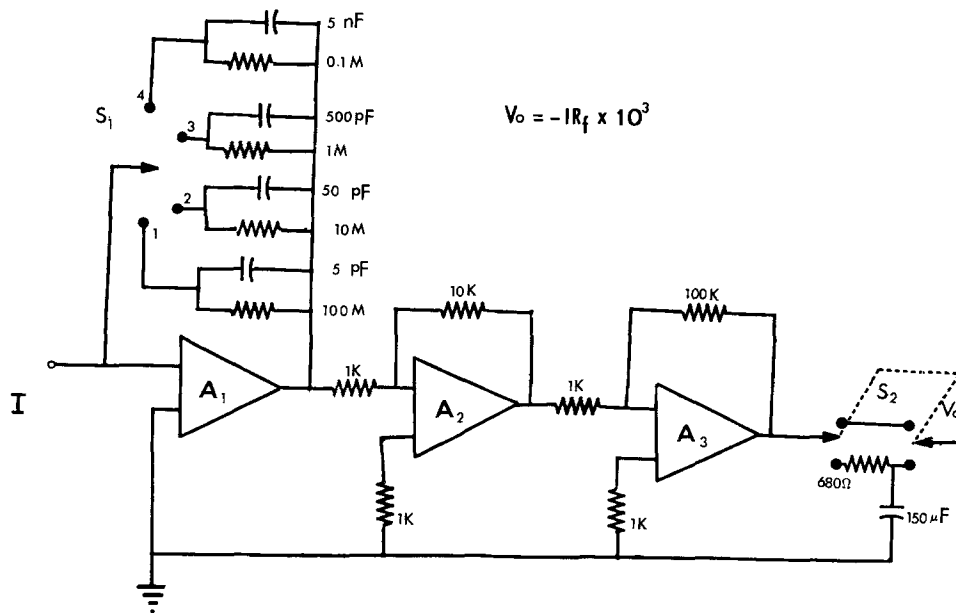


FIGURE 2 Current transducer amplifier. The current flowing into the input, I , is transduced to a potential, V , according to the equation $V = -IR_f$, where R_f is the feedback resistor of A_1 . The amplifiers A_2 and A_3 scale up the potential V by a factor of 1,000. The switch S_2 can connect a 100-ms RC filter which is used when steady-state conductance is measured.

the sensitivity used. This impedance is very low compared with the membrane resistance; therefore, there is a negligible voltage at the input, and the membrane potential difference is equal to the output voltage of the control unit.

Current records of membranes with small numbers of channels were stored in an FM magnetic tape recorder-reproducer. The total dwell time in each conductance state was measured with an electronic clock switched by the discriminator circuit shown in Fig. 3. The number of current transitions was counted from records of an

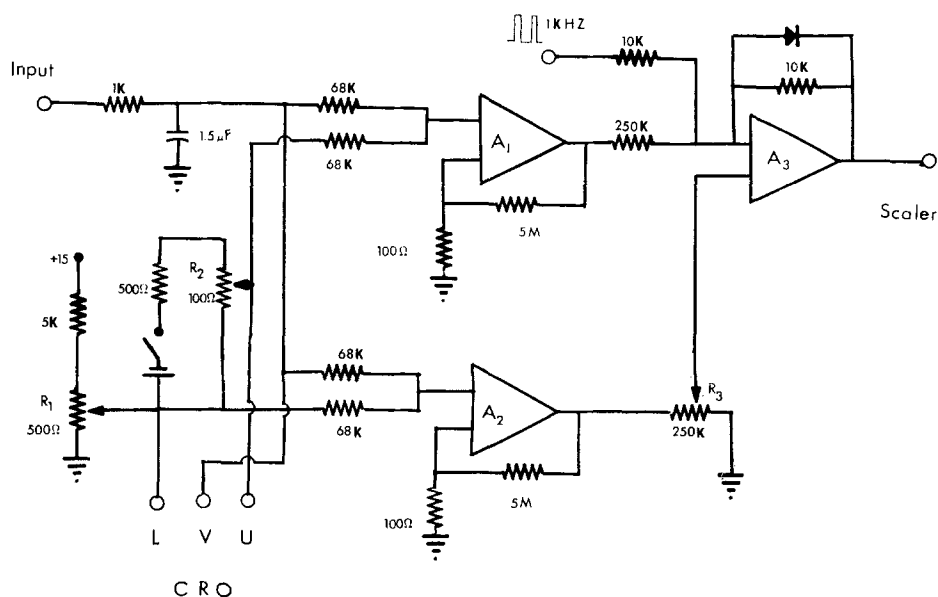


FIGURE 3 This unit is a switch controlled by the potential applied to its input. If the potential, V , is in a selected interval, a 1-kHz square wave appears on the scaler output. The switch consists of two voltage comparators: A_1 is set at the lower limit of the interval, L ; and A_2 is set at the upper limit, U . The output of the comparators is applied to a differential amplifier A_3 . When $V > U$ the output of A_3 is zero; when $V < L$ the output of A_3 is also zero. When $U > V > L$ the output of A_3 is $1.2V$. This potential is modulated by the 1-kHz signal. The diode in the feedback loop of A_3 avoids the appearance of negative potentials at the output, therefore, a square wave appears only if $U > V > L$.

ink-writing oscillograph. Having the total dwell time and the number of current transition, we could calculate the rate constants by the relations (Ehrenstein et al., 1974):

$$\alpha(V) = \frac{\text{number of transitions}}{\text{accumulated dwell time in the closed state}},$$

$$\beta(V) = \frac{\text{number of transitions}}{\text{accumulated dwell time in the open state}},$$

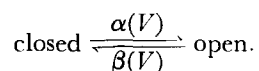
where $\alpha(V)$ denotes the rate constant for the opening of a closed channel and $\beta(V)$ denotes the rate constant for the closing of an open channel, V being the applied potential.

The step function response of many-channel membranes was recorded from the oscilloscope on photographic film. Exponential function parameters were obtained by comparison of the experimental curves with analog computer-generated functions. Curve fitting with a digital computer gave essentially the same results. In some instances, more than one exponential term was needed to fit the data. In these cases, readings were taken from enlarged records and plotted on semilog paper.

RESULTS

Effect of Temperature on EIM Kinetics in Oxidized Cholesterol Membranes

Ehrenstein et al. (1974) have shown that in EIM-doped membranes the steady-state conductance can be described by the equilibrium between the number of open and closed channels; this equilibrium is characterized by two first-order rate constants $\alpha(V)$ and $\beta(V)$:



Since these rate constants are exponential functions of the membrane potential (Ehrenstein et al., 1974), we will write these functions in the following manner:

$$\alpha(V) = \lambda \exp(-A(V - V_o)), \quad (1)$$

$$\beta(V) = \lambda \exp(+B(V - V_o)), \quad (2)$$

where V is the membrane potential and λ the rate constant when $V = V_o$. To examine the effect of temperature on these parameters, experiments were performed to measure α and β in membranes with only one channel at 37°C. These data were compared with the values at 26°C reported by Ehrenstein et al. (1974). In Table I, the best fit of Eqs. 1 and 2 for these experiments and for the data of Ehrenstein et al. (1974) is given. It can be seen that λ increases with temperature by a factor of ca. 10 and the other parameters seem to be little affected.

To study further the effect of temperature, a series of experiments with membranes with many channels at different temperatures was performed. The number of EIM channels incorporated into a membrane greatly increased with temperature. Since too few channels gave noisy current transients and too many channels made the membrane unstable, the amount of EIM added was carefully controlled. The higher the temperature, the smaller the amount of EIM. Fig. 4 shows a current response to a step function of

TABLE I
PARAMETERS DESCRIBING OPENING AND CLOSING OF EIM CHANNELS
IN OXIDIZED CHOLESTEROL MEMBRANES

Temperature	λ	A	B	V_o
°C	s^{-1}	V^{-1}	V^{-1}	mV
26	0.125 ± 0.09	70 ± 30	50 ± 20	58 ± 11
37	1.1 ± 0.2	43 ± 14	67 ± 14	70 ± 12

Summary of the constants describing the voltage-dependent rate constants for the opening and closing of the EIM channels in oxidized cholesterol membranes at 26 and 37°C. The parameters are obtained from the least square fit of the equations $\ln \alpha = \ln \lambda - A(V - V_o)$, and $\ln \beta = \ln \lambda + B(V - V_o)$, where α and β are the rate constants, V_o is the membrane potential at which both rate constants are equal, and λ is the rate constant at $V = V_o$. Dispersion measures are standard error of the estimated parameters using the least square fitting method. Data at 26°C were taken from Ehrenstein et al. (1974).

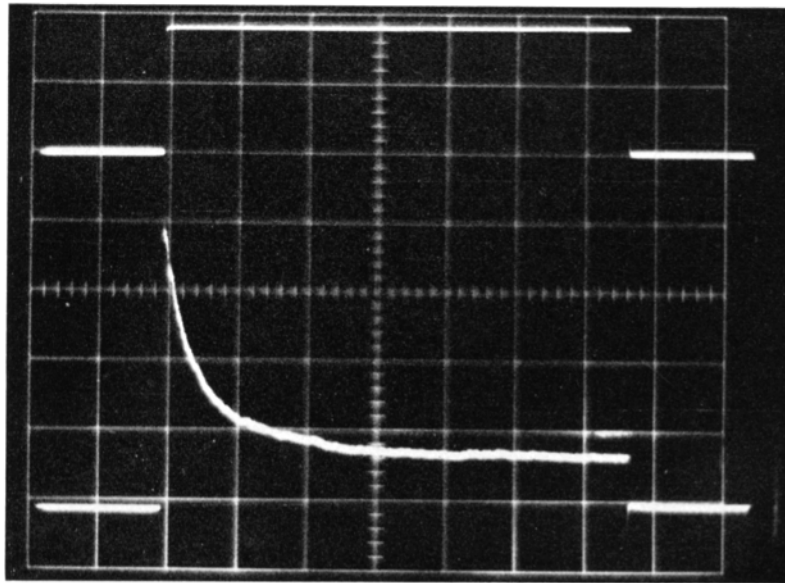


FIGURE 4 Response of a many-channel membrane to a step change in potential from 0 to 90 mV. Upper trace shows the applied step of potential. Lower trace shows current. Oxidized cholesterol membrane. Temperature, 45°C. Calibration: vertical 2 nA/division or 50 mV/division, horizontal 200 ms/division.

potential. The relative conductance (G_{rel}) of a membrane is

$$G_{rel} = G_{ss}/NG_o,$$

where G_{ss} is the steady-state conductance, G_o is the conductance of the open channel, and N is the number of channels.

Since the steady-state conductance can be expressed in terms of the open and closed channel conductances, and the fraction of open channels at any given potential is $\alpha(V)/[\beta(V) + \alpha(V)]$, (Ehrenstein et al., 1974),

$$G_{rel} = G_c/G_o + (1 - G_c/G_o)/(1 + \exp(a(V - V_o)/kT)), \quad (3)$$

where G_c is the conductance of the closed channel, k the Boltzman constant, T the absolute temperature, and $a = (A + B)/kT$ is a measure of the strength of interaction of the electric field and the channel. The parameters a and V_o were calculated from the semilog plot of $[(1 - G_c/G_o)/(G_{rel} - G_c/G_o)] - 1$ vs. potential. The slope of the straight line is a/kT and V_o is the potential at which the line crosses the potential axis. The value of G_c/G_o was calculated in each experiment. This value varied from membrane to membrane and was temperature dependent (Latorre et al., 1974).

In Table II the values for the parameters a and V_o at different temperatures are shown. a and V_o seem to be independent of temperature. The relaxation time, τ_o , on the other hand, decreased with increasing temperature. At any temperature, τ turned out to be a bell-shaped function of membrane potential, whose maximum, τ_o , occurred at V_o . Since the conductance transitions are first-order processes, τ equals $1/(\alpha + \beta)$. Using this relationship, we can compare the data of these experiments with the data from single-channel membranes. At 37°C, τ_o calculated from the individual rate constant was 0.45 ± 0.08 s. The relaxation time directly measured in many channel membranes was 0.55 ± 0.06 s. At 26°C, τ_o calculated from the value of λ was 4 ± 3 s; directly measured, 3.3 ± 0.2 s. Calculation of a from single-channel data gave 2.9 ± 0.9 for 26°C and 3.0 ± 0.5 electronic charges for 37°C. The average value of the many-channel data was 2.3 ± 0.3 electronic charges.

TABLE II
KINETIC PARAMETERS OF OXIDIZED CHOLESTEROL MEMBRANES
WITH MANY EIM CHANNELS

Temperature	a (electronic charges)	V_o	τ_o	N
°C		mV	s	
25-26	2.0 ± 0.3	69 ± 3	3.3 ± 0.2	3
27-28	2.7 ± 0.4	67 ± 5	2.1 ± 0.7	2
35-35.5	2.3 ± 0.1	63 ± 6	0.58 ± 0.07	3
36-37	2.2 ± 0.2	67 ± 2	0.55 ± 0.06	7
44-45	2.5 ± 0.1	58 ± 2	0.17 ± 0.01	5

Parameters describing the voltage-dependent ion conductance of oxidized cholesterol in many-channel membranes. Dispersion measurements are the standard error of the mean of N observations.

These comparisons demonstrate the consistency of the data obtained from the two types of experiments.

From the transition theory of reaction rates (Frost and Pearson, 1961), $\lambda = K \exp(-b/kT)$, where b is the activation barrier and K is a constant. Since τ_o is the reciprocal of 2λ , it is expected that its temperature dependence has the form $\tau_o = (1/2K) \exp(+b/kT)$. Fig. 5 shows a plot of the values of relaxation time measured at several temperatures. The temperature dependence seemed to be of the expected type, and the activation energy was 30 kcal/mol.

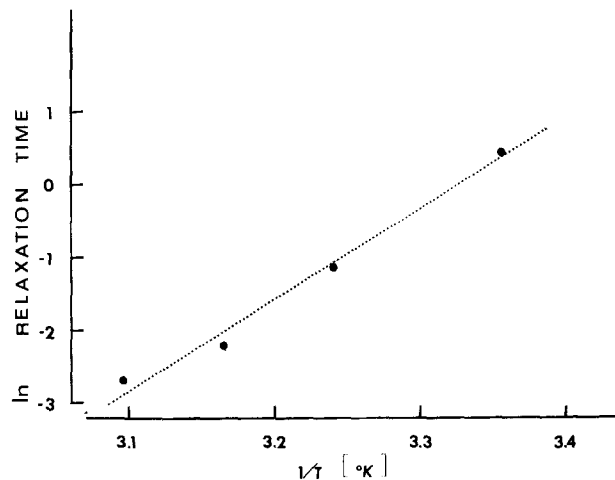


FIGURE 5 Arrhenius plot of the relaxation time measured at $V = V_o$. Oxidized cholesterol membrane with many EIM channels. The activation energy is 30 ± 2 kcal/mol.

Kinetics of EIM in Brain Lipids

The theory of the kinetics of the opening and closing to the EIM channel was developed to describe the properties of EIM in oxidized cholesterol membranes. A series of experiments was performed on single-channel brain lipid membranes to investigate the applicability of that theory to the EIM channels incorporated in brain lipid membranes.

Fig. 6 contains segments of records from a membrane with only one channel. The first transition of this experiment appeared when a potential of 50 mV was applied across the membrane. It can be seen in the figure that the formation jump, i.e. the first current transition, was equal in amplitude to an on-off jump. Therefore, the conductance of the closed channel was very small. Transitions with only a fraction of the full channel amplitude were

seldom seen. These small transitions can be seen twice at the beginning and in the middle of the 60-mV record. The complete record of this experiment showed only four small transitions over the entire voltage range tested. Some full size current steps of short duration appear less than full size on the chart recorder because of the recorder's limited bandwidth. These transitions appeared equal to the full channel amplitude when current was observed in the oscilloscope. In Fig. 6 it can also be seen that as in oxidized cholesterol membranes, the fraction of time a channel stayed in its open state decreased with the membrane potential.

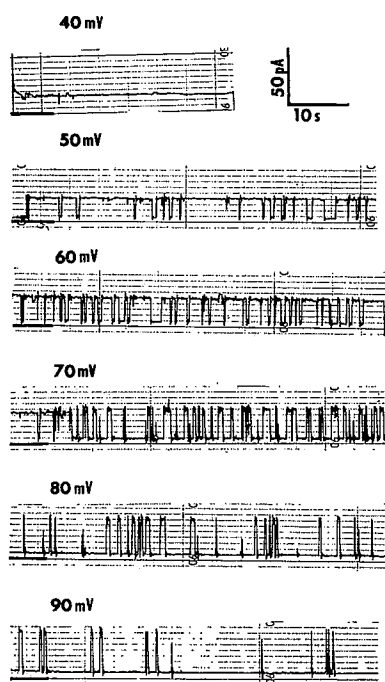


FIGURE 6

FIGURE 6 Current fluctuations of a brain lipid membrane with only one channel at 26°C. The main features are: (a) transition amplitude is proportional to the membrane potential. For very small dwell times the amplitude appears lower due to the slow writing speed of the recorder; (b) the fraction of time that the channel dwells in the high conductance state decreases with increasing membrane potential; (c) comparison of the formation jump observed at the 50-mV record with the subsequent on-off jumps allows the estimation of the conductance of the closed channel, which is ca. 1% of the open channel conductance; (d) very small conductance jumps are seen in the 60-mV record.

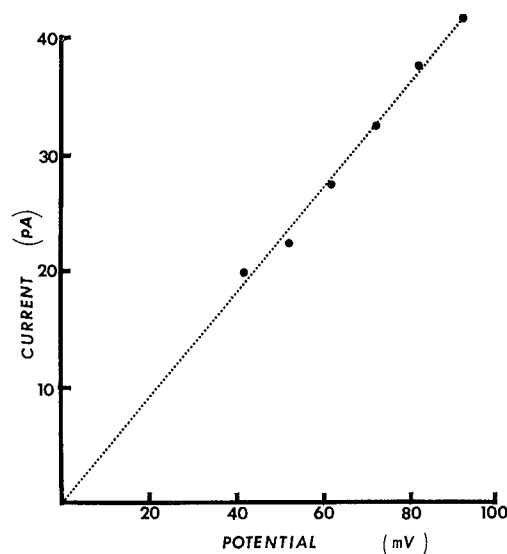


FIGURE 7

FIGURE 7 Current passing through an open channel. The linear relationship demonstrates that the open EIM channel in brain lipid bilayers behaves like an ohmic resistor. The conductance of the open channel is 4.7×10^{-10} mho. Brain lipid membrane, 26°C.

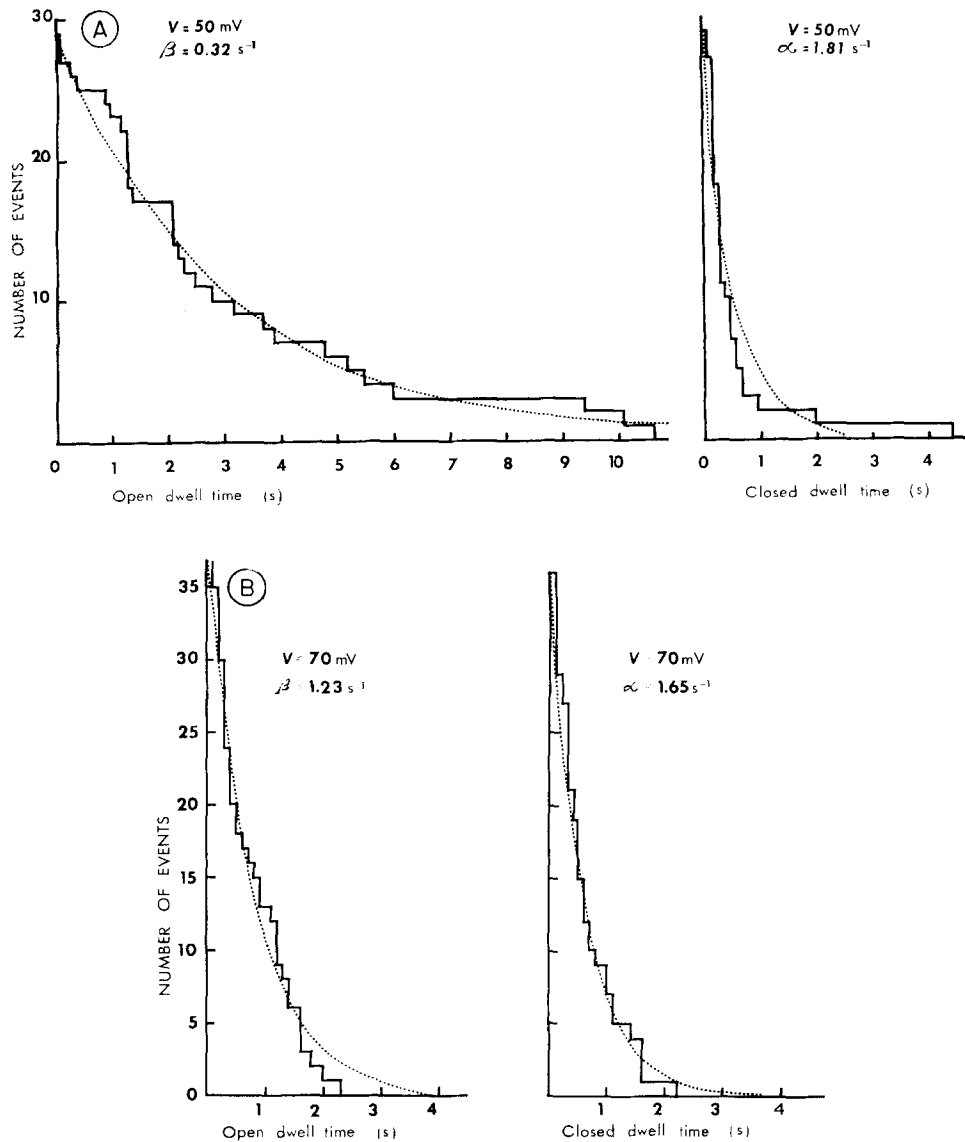


Fig. 7 is a plot of the jump height at several membrane potentials. The linear relationship demonstrates that the open EIM channel behaved as an ohmic resistor, as it did in oxidized cholesterol membranes. The conductance of the open channel in this membrane was 4.7×10^{-10} mho.

Fig. 8 shows the distribution of dwell times measured from records obtained from playback of the experiments from the tape unit at reduced tape speed (to have the best time resolution). The distribution of dwell times was

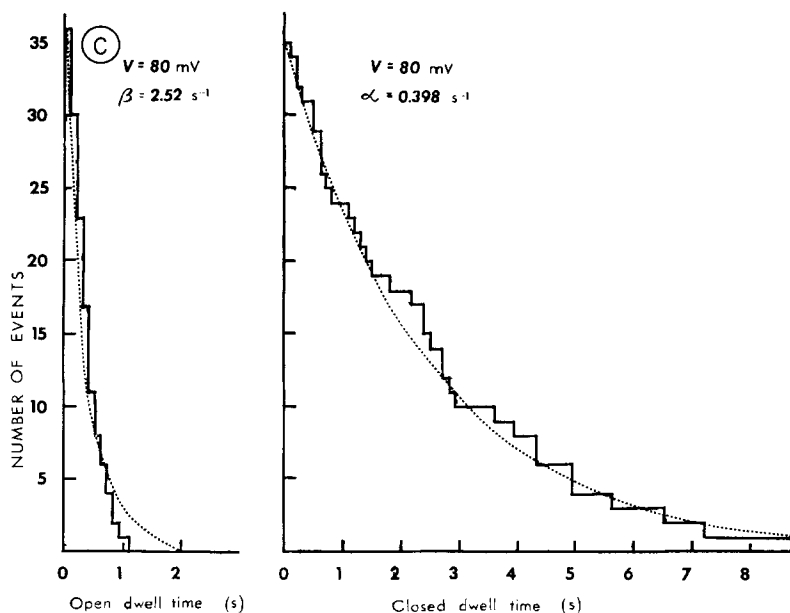


FIGURE 8 Accumulated distribution of dwell times. These plots show the number of individual dwell times which are longer than the time indicated by the abscissa. The dashed line is the exponential distribution expected, assuming that the rate constant is equal to the reciprocal of the average dwell time.

exponential; therefore the rate constants could be calculated either from curve fitting of the dwell-time distribution or from the average dwell time (Ehrenstein et al., 1974). Table III shows values of rate constants calculated using both methods. The agreement is reasonable and indicates that the average dwell time is a good estimate of the rate constants. Fig. 9 demonstrates that the rate constants are exponential functions of the membrane potential, as they are in oxidized cholesterol membranes.

Effect of Temperature on the Kinetics of the EIM Channel in Brain Lipid Membranes

Having demonstrated that all the characteristics of the EIM channel in brain lipid membranes are the same as in oxidized cholesterol membranes, we used the same theory to describe its kinetics. The parameters describing the opening and closing of EIM channels in five brain lipid membranes are shown in Table IV. Rate constants were larger at 37°C than at 26°C. The parameters A , B , and V_0 seemed to be little affected by temperature. As in oxidized cholesterol membranes, A and B , the slopes of the curves $\ln\alpha$ and $\ln\beta$ vs. potential, seemed to be equal. In only one membrane was there a significant

TABLE III
COMPARISON OF THE RATE CONSTANTS CALCULATED FROM THE
AVERAGE DWELL TIME AND FROM EXPONENTIAL CURVE FITTING
OF THE DWELL TIME DISTRIBUTION

mV	β		α	
	Average dwell time	Exponential fitting	Average dwell time	Exponential fitting
	s^{-1}		s^{-1}	
50	0.32	0.35	1.8	2.8
70	1.35	1.35	1.65	1.55
80	3.50	2.52	0.40	0.40

These values correspond to the curves of the experiment shown in Fig. 8.

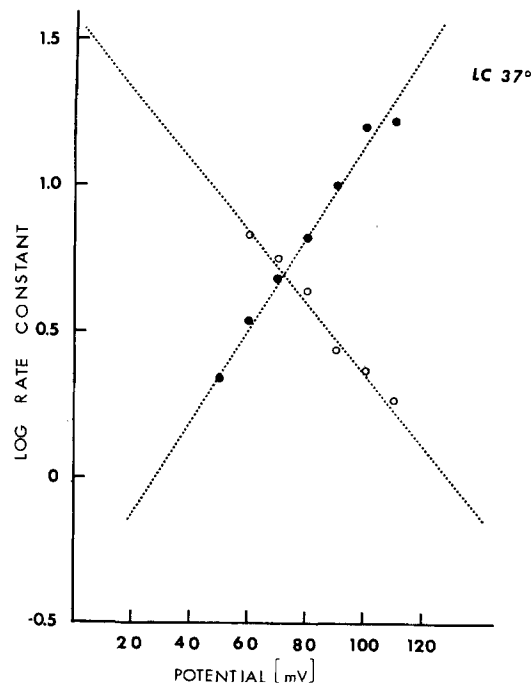


FIGURE 9 Voltage dependence of rate constants. This plot shows the effect of membrane potential on single-channel rate constants. The linear relationship demonstrates the validity of the expressions $\alpha = \lambda \exp - A(V - V_0)$ and $\beta = \lambda \exp + B(V - V_0)$. The coordinates of the intersection point are at λ, V_0 . Brain lipid membrane at 37°C.

difference. Rate constants in brain lipid membranes were larger than in oxidized cholesterol membranes. For example, at 26°C the rate constant was $0.1 s^{-1}$ for oxidized cholesterol membranes and $1.2 s^{-1}$ for brain lipid membranes.

TABLE IV
PARAMETERS DESCRIBING OPENING AND CLOSING OF EIM CHANNELS
IN BRAIN LIPID MEMBRANES

Temperature	λ	A	B	V_o
°C	s^{-1}	V^{-1}	V^{-1}	mV
26	1.8 ± 0.3	62 ± 7	60 ± 12	68 ± 10
26	0.7 ± 0.1	70 ± 10	53 ± 7	92 ± 13
37	4.7 ± 0.3	58 ± 7	69 ± 2	60 ± 5
37	3.9 ± 0.4	44 ± 9	71 ± 9	78 ± 9
37	5.0 ± 0.6	28 ± 2	37 ± 5	72 ± 8

Summary of the constants describing the voltage-dependent rate constants for the opening and closing of the EIM channels in brain lipid membranes. Parameters as in Table I.

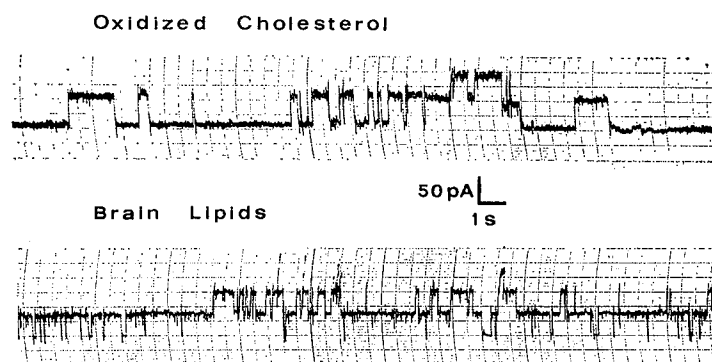


FIGURE 10 Comparison of the channel-switching frequency in membranes of oxidized cholesterol and brain lipids. Both membranes have three channels and are at a membrane potential of 70 mV and at 37°C.

In Fig. 10 records of the channel activity in both types of membranes are compared. The frequency of transitions was higher in the brain lipid membrane, as expected from the rate constants.

To study further the effect of temperature on kinetic parameters, a series of experiments with many-channel membranes was performed. A typical relaxation curve is shown in Fig. 11. The transient response to the step function of potential showed an exponential decay to a current intensity which decreased slowly with time. This contribution to the current was subtracted before the exponential was analyzed. In Table V a summary of the results is shown. The major effect of temperature is on the relaxation time, with a 20-fold change for a 25°C change in temperature. V_o also changed with temperature, as previously observed by Bean and Chan (1969). The parameter a seemed to be independent of temperature.

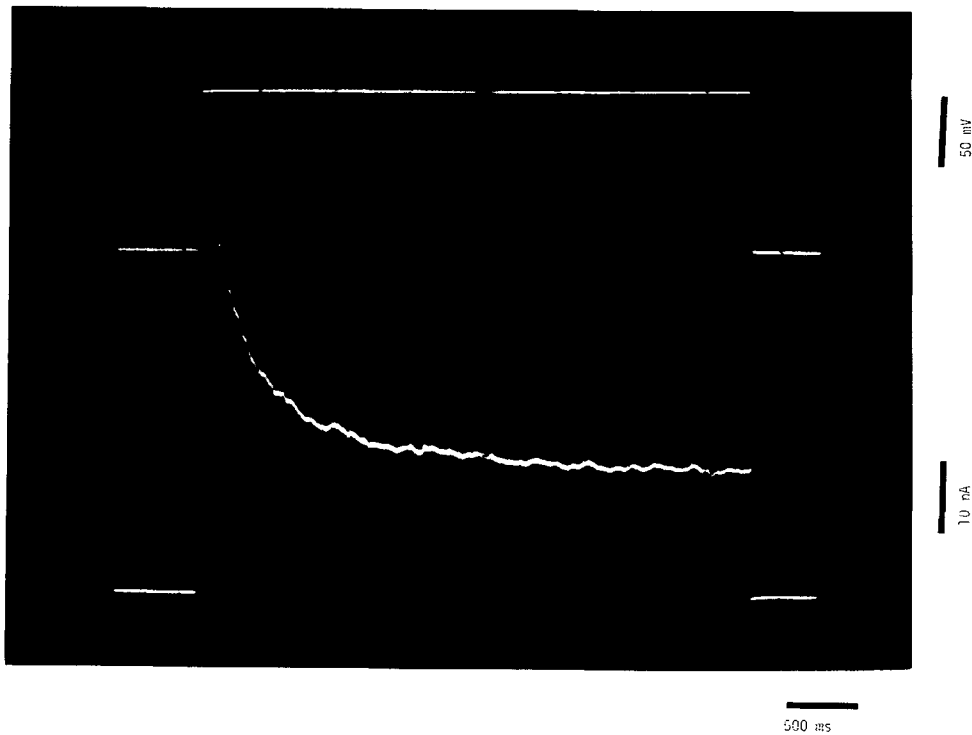


FIGURE 11 Current response to a potential step function in a brain lipid membrane with many EIM channels. Membrane potential is 110 mV and the temperature is 37°C.

TABLE V
KINETIC PARAMETERS OF BRAIN LIPID MEMBRANES WITH MANY
EIM CHANNELS

Temperature	a (electronic charges)	V_o	τ_o	N
°C		mV	s	
25	1.6	114	1.61	1
34-35	2.6±0.3	102±2	0.32±0.5	4
43	2.2±0.4	102±3	0.11±0.1	3
50	2.1	84	0.069	1

All parameters as in Table II.

Relaxation times calculated from single-channel experiments were 0.3 and 0.7 s for two membranes at 26°C, and averaged 0.11 s at 37°C. The average values of a from single-channel data were 3.0 and 2.8 electronic charges for 26 and 37°C, respectively. These results were consistent with the relaxation curve data. Values for V_o , on the other hand, were lower for single-channel experiments.

The effect of temperature on the relaxation time could be described by an exponential function as seen in Fig. 12. The activation energy of this process was 24 kcal/mol.

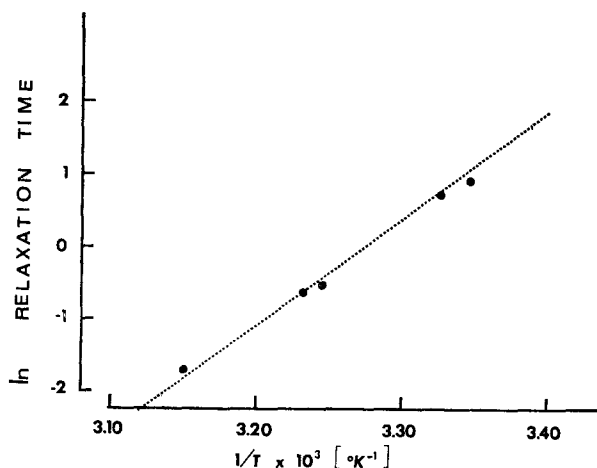


FIGURE 12 Arrhenius plot of the relaxation time measured at $V = V_0$ in brain lipid membranes. The temperature dependence is exponential, and the activation energy calculated from the slope of the graph is 24 ± 2 kcal/mol.

DISCUSSION

The voltage-dependent ion conductance induced by EIM has been the subject of several studies (Mueller and Rudin, 1969). Kinetic properties had received attention only in many-channel membranes (Bean et al., 1971; Bean, 1973). Mueller and Rudin (1963) proposed a model of two-state channels to account for the properties of EIM-doped bilayers. That the channels do in fact exist has been demonstrated by Bean et al. (1969), and by Ehrenstein et al. (1970). Although in oxidized cholesterol and brain lipids the channel fluctuates mostly between two conductance states, a clear definition of the conductance of the unit channel is complicated in other lipids by the existence of several conductance levels for each channel (Bean, 1972).

Ehrenstein et al. (1974) developed a kinetic theory for the opening and closing of the channels and were able to account for the kinetic properties of many-channel membranes. We will apply this theory to the results obtained in the present paper.

According to Ehrenstein et al. (1970), the difference of potential energy $W(V)$ of the open and closed channel is a function of the transmembrane electric field:

$$W(V) = a(V - V_0), \quad (4)$$

where V is the membrane potential, V_0 is the potential at which both states of the channel have the same potential energy and a is a measure of the strength of interaction of the electric field and the channel. aV_0 is then a measurement of the difference of configurational energy of the open and closed states of the channel in the absence of an electric field. When $V = V_0$ both

states, open and closed, have the same potential energy and the rate constants of the opening and closing are equal to λ . Applying the classical rate constant theory, λ can be expressed as:

$$\lambda = K \exp(-b/kT), \quad (5)$$

where b is the activation barrier when $V = V_o$, K is a constant and k and T have their usual meaning.

When $V \neq V_o$, the potential energy of the two configurations of the channel is different; if we assume that both states of the channel change in energy to the same extent, we can write for the rate constants:

$$\alpha(V) = K \exp(-b/kT) \exp(-W(V)/2kT), \quad (6)$$

$$\beta(V) = K \exp(-b/kT) \exp(+W(V)/2kT). \quad (7)$$

Comparison of Eqs. 6 and 7 with Eqs. 1 and 2 implies that $A = B$. In Tables I and IV it can be seen that indeed $A = B$. The experiments reported in this paper give the necessary information to calculate all the energies that appear in Eqs. 6 and 7.

Fig. 13 shows a schematic representation of these energies in oxidized cholesterol and brain lipid membranes at several temperatures. Here b was calculated from the temperature dependence of the relaxation time measured at $V = V_o$. Since $\tau_o = 1/2\lambda$, b can be easily obtained from Eq. 5.

Phase transitions of the bilayer can have a strong effect on the relaxation constants. The conductance of valinomycin-treated bilayers changes by several orders of magnitude when the bilayer goes from liquid to solid state (Krasne et al., 1971) as a consequence of a big change of membrane viscosity that slows down the carrier movement. The relaxation time-temperature relationship in EIM-doped brain lipids or oxidized cholesterol membranes does not present discontinuities, suggesting that no phase transitions occur in the temperature range explored. Experiments made with valinomycin in the same way as those of Krasne et al., (1971) showed a continuous change of conductance with temperature which demonstrates that there actually were no phase transitions. For oxidized cholesterol membranes, the energy barrier for the transition from open to closed is 1.25 ± 0.08 eV and for the same process in brain lipid it is 1.0 ± 0.08 eV, implying that the energy barrier b depends on the membrane matrix.

If we assume that the channel must change its external shape when changing from the closed to the open state, we expect changes in the spatial distribution of the bilayer components around the channel during this process. If we have a rigid bilayer, the channel will be constrained, assuming a configuration of a high energy content during the transition; therefore, the energy barrier will be high. On the other hand, if the bilayer components are

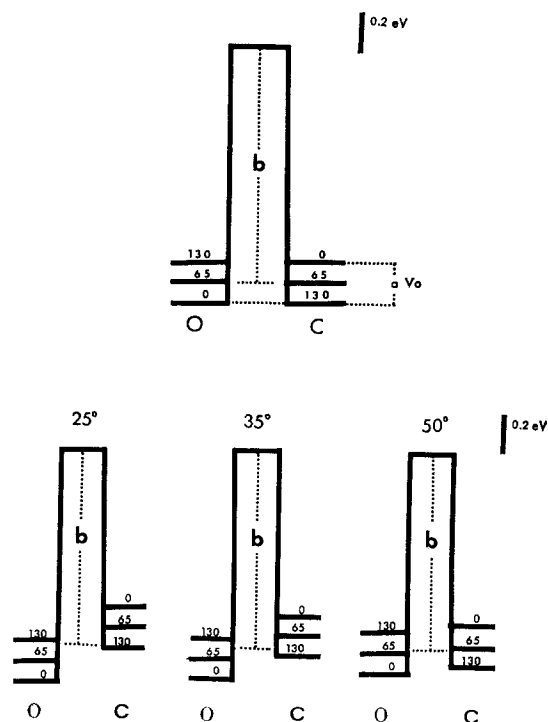


FIGURE 13 Potential energy representation of the EIM gating process in oxidized cholesterol (upper part) and in brain lipid membranes (lower part). The energy b is the energy barrier which separates the open and closed states of the channel at $V = V_o$. The energy difference of the open and closed channel is aV_o . This difference changes with the membrane potential, and is zero when $V = V_o$. In brain lipid membranes aV_o is larger than in oxidized cholesterol membranes; this difference decreases with increasing temperature.

allowed to “move freely,” the energy barrier will be lower. Our results suggest that oxidized cholesterol membranes, at least in the channel environment, are more rigid than those made of brain lipid. Bean (1973) showed that speed of conductance change, which depends on the height of the energy barrier, may vary by a factor of more than 20-fold as a result of changing the plasticizing agent from tocopherol to various esters, indicating that the membrane composition is an important factor in determining the transition rates for the processes of opening and closing of the EIM channel.

In oxidized cholesterol membranes the EIM channel parameters a and V_o are independent of temperature, and the difference of configurational energy, aV_o , between the open and the closed channel is 0.14 eV. In brain lipid membranes, on the other hand, a is independent but V_o changes with temperature. The energy difference between the two conductance states of the EIM channel is 0.26, 0.23, and 0.19 eV, for 25, 35, and 50°C, respectively.

The V_o shift reflects a stabilization of the open-channel configuration, probably due to weak chemical interactions between the open channel and the membrane lipids, which are lost at high temperature.

One of the major differences of the behavior of EIM in the two lipids used in the present work is the conductance of the closed channel. In oxidized cholesterol this conductance can be as high as 10^{-10} mho at 26°C, whereas in brain lipid membranes the conductance is almost zero. We have shown that in oxidized cholesterol membranes the conductance of the closed EIM channel decreases with increasing temperature, being almost zero at 45°C (Latorre et al., 1974). This result indicates that in oxidized cholesterol membranes the conductance of the closed channel represents some structure that is lost as consequence of increasing temperature, and is not present in brain lipid membranes. It can be speculated that in oxidized cholesterol membranes the EIM channel cannot be fully closed due to membrane constraints which are not present in the more fluid brain lipid membranes. At higher temperatures the channel can be fully closed in oxidized cholesterol membranes presumably because the lipid bilayer becomes more fluid and the constraints vanish.

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