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TEMPERATURE CHARACTERIZATION OF THE CONDUCTANCE OF THE EXCITABILITY INDUCING MATERIAL CHANNEL IN OXIDIZED CHOLESTEROL MEMBRANES

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SUMMARY

The temperature dependence of the voltage-dependent excitability inducing material channel has been studied in thin lipid membranes containing only one channel. It is shown that the conductance of the open configuration has a temperature dependence that is just that expected from the temperature dependence of the bathing electrolyte. The conductance of the closed channel decreases increasing temperature.

INTRODUCTION

In 1969 Bean [1] showed that the conductance of a black lipid membrane increases in discrete steps when excitability inducing material is added to the electrolyte medium surrounding the membrane, each step having a conductance of about $4 \cdot 10^{-10}\ \Omega^{-1}$. Afterwards, Eherenstein et al. [2] were able to show that the negative resistance that appears when an oxidized cholesterol membrane is treated with excitability inducing material can be explained in terms of a variation of the fraction of time that the excitability inducing material channel dwells in its maximum conductance state (open channel), or in its minimum conductance state (closed channel), when the applied potential is changed. Accordingly, the conductance of an excitability inducing material-doped membrane can be fully described by the equation:

$$g_T = g_B + Ng_C + \bar{n}(V)g_\Delta$$

Where g_T is the total membrane conductance, g_B is the conductance of the black lipid film, g_C is the conductance of a closed channel, g_Δ is the differential conductance of the open and closed channel, N the total number of channel and $\bar{n}(V)$ is the number of open channels and is the only voltage-dependent term.

In order to have a better understanding of the functioning of these channels, the ionic selectivity [3], the kinetic properties [4] and the effect of the lipid moiety [5] have been studied. These type of substances provide models for a better understanding of the voltage-dependent permeabilities which are also characteristic of natural excitable membranes.

This paper deals with the effect of temperature on the conductance of the closed and open channel.

METHODS

Black lipid membranes were made according to the Mueller and Rudin [6] brushing techniques. Oxidized cholesterol [7] 4 % in decane was used to form the membranes. Membranes were formed across a 0.5-mm diameter hole in a teflon cup which is immersed in a 0.1 M KCl solution at pH 7. Reduction of the diameter of the hole from the 1 mm previously used [3], improves the signal to noise ratio and decreases the basal membrane conductance for reasons discussed elsewhere [8]. Small holes increase also the probability for maintain only one channel in the membrane over long periods of time. Excitability inducing material was prepared according to Kushnir [9] and was added to the KCl solution contained in the cup; only membranes containing one excitability inducing material channel are considered in this study. Membrane conductance was determined by measuring the current as a function of imposed potential difference using a voltage clamp. A temperature controller under the membrane chamber provided the desired temperature to within $\pm$ 0.25 °C.

RESULTS AND DISCUSSION

The upper part of Fig. 1 shows the time course of the current when different potential test pulses were applied across an oxidized cholesterol membrane. The lower part of Fig. 1 shows the conductance of lipid membranes as a function of temperature. Each point represents the average of at least three membranes. Conductance changes from $10^{-11}\ \Omega^{-1}$ at 20 °C to $10^{-10}\ \Omega^{-1}$ at 50 °C.

The upper part of Fig. 2 shows a record of one channel membrane using high current resolution. By one channel membrane, we mean that the current is fluctuating between two states only [2], and do not intend to imply any particular physical picture. Since both the open and the closed states of the channel are ohmic [2], the conductance of the two states were determined from the current measured at different voltages. The lower conductance state is $g_B + g_C$ and the upper is $g_B - g_C + g_A$. Since g_B was already measured in the same membrane before exitability inducing material was added, g_C and $g_C + g_A = g_0$ (where g_0 is the conductance of the open channel) can be easily obtained.

The lower part of Fig. 2 shows the effect of temperature on these conductances The conductance of the closed channel decreases with increasing temperature. At 20 °C it is $8.5 \cdot 10^{-11}\ \Omega^{-1}$ and decreases to $2.5 \cdot 10^{-11}\ \Omega^{-1}$ at 40 °C. At higher temperatures the conductance is so low that it cannot be measured. The conductance of the open channel increases with increasing temperature. The broken line represents the temperature dependence of the limiting K^+ equivalent conductance, λ_0 [10]. Assuming the channel conducts like a cylindrical pore filled with electrolyte solution, the equation that relates the conductance of the open channel and the K^+-equivalent conductance is then:

$$g_0 = (\pi r^2 C/1000\, l)\lambda_0 \qquad (1)$$

where r and l are the radius and the length of the pore, C is the concentration of the

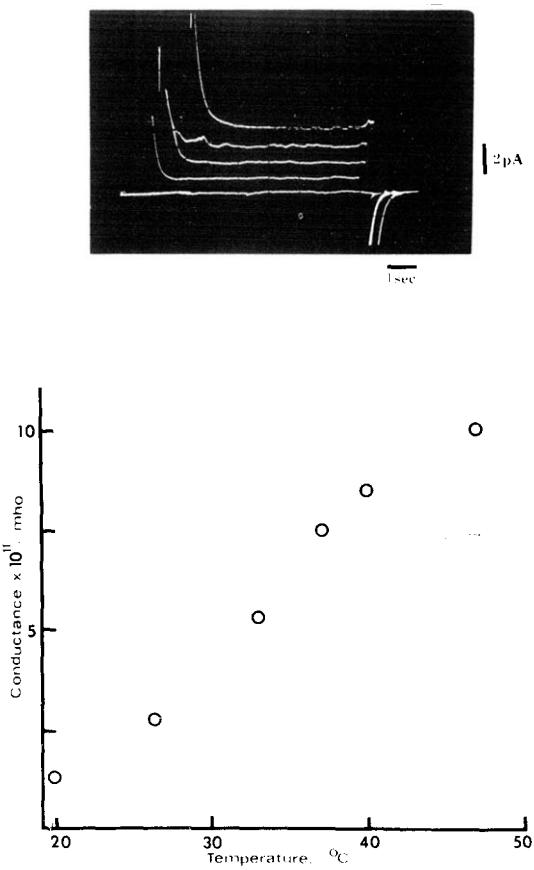

Fig. 1. Upper part: Currents measured in one oxidized cholesterol membrane at 37 °C. Applied potential 0, 20, 40, 60 and 80 mV. The initial currents represent the capacitative transient, long transients are due to the high time constant of the electrical system of measurements (100 ms). Increasing time constant allowed high current resolution. Lower part: Oxidized cholesterol membrane conductance at different temperatures. Conductance measurements were obtained from records similar to that shown in the upper part of the figure. Each point represents the average conductance of at least three membranes.

solution it contains, which was assumed to be 0.1 M K^+.

Oxidized cholesterol at 25 °C in contrast with other lipids, shows a high conductance state of the closed channel [2, 5]. This can explain the weak voltage-dependent conductance in oxidized cholesterol membranes with many channels. Strongly voltage-dependent conductances are found when excitability inducing material is included in other lipids [6] and this coincides with a very low conductance of the closed channel in the same lipids [5].

In this paper, we show that the excitability inducing material channel, when included in oxidized cholesterol membranes, can be fully closed with increasing temperature, resembling the excitability inducing material channel in sphingomyelin, egg lecithin and dipalmityl lecithin [5]. Preliminary experiments in oxidized cholesterol

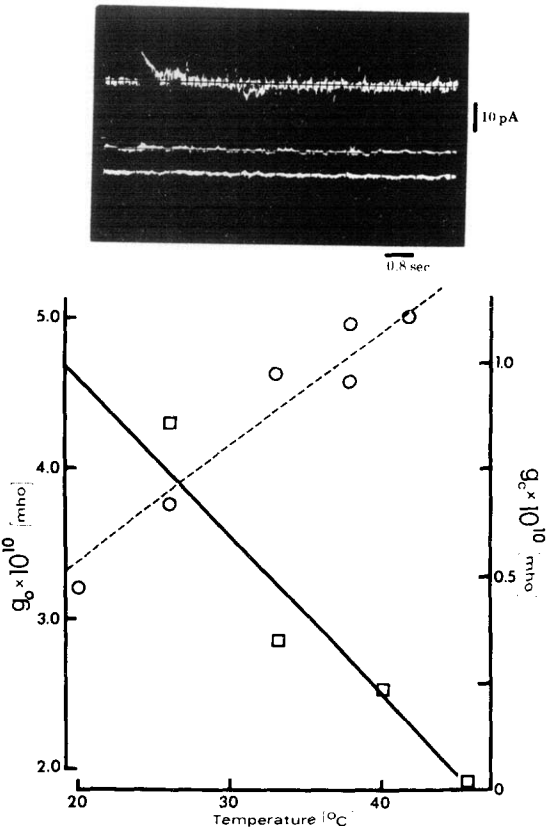

Fig. 2. Upper part: Current levels in an oxidized cholesterol membrane with one excitability inducing material channel at 33 °C. Lower trace is zero current. Intermediate trace is the current passing through the bilayer and through the closed channel, applied potential 60 mV. Upper trace is the current passing through an open channel at 60 mV applied potential. Time constant of the system 1.5 ms. Lower part: Effect of temperature on the conductance of the closed channel, g_c (□) and on the open channel conductance, g_0 (○). Broken line is the expected conductance assuming that the excitability inducing material channel is a cylindrical pore with a radius of 10 Å and filled with 0.1 M K^+. λ_0 for each temperature was taken from Harned and Owen [10].

membranes containing many channels indicate that the ratio of maximum to minimum conductance increases with increasing temperature. This reflects the decrease in conductance of the closed channel at high temperatures.

On the basis of selectivity measurements, it has been postulated that the excitability inducing material pore is a rather wide water-filled hole [3]. In addition to excitability inducing material, gramicidin [8, 11] and alamethicin [12, 13] have been shown to produce holes through black lipid films. The alamethicin pore presents a rather poor selectivity between cations and anions [13] and although the gramicidin channel excludes polyvalent cations and anions, the sequence of univalent cations conductance is similar to that for the corresponding electrolytes in aqueous solutions [11]. These findings have led to the conclusion that these channels also behave like water-filled pores.

The temperature dependence of the conductance of the open excitability inducing material channel is just that expected from the temperature dependence of the bathing electrolyte filling a pore. These measurements provide more support to the model previously postulated [3]. For a membrane thickness of 40 Å the average diameter for the excitability inducing material channel calculated from Eqn (1) is 20 Å. This means that the open configuration acts simple like a pore, and that the conductance of the closed channel represents some structure (perhaps a leakage between lipid and protein) that is lost as a consequence of increasing temperature.

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