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OSCILLATION PHENOMENA IN BLACK LIPID MEMBRANES INDUCED BY A SINGLE ALAMETHICIN PORE

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SUMMARY

In this paper we show how alamethicin (a small cyclic peptide of molecular weight 1691) can produce voltage oscillations in black lipid membranes and how a nonactin-alamethicin oscillator can be constructed. Alamethicin alone induces oscillations only with an applied bias current, but with nonactin and appropriate salt solutions oscillations occur with no bias current. Both kinds of oscillations can be quantitatively understood in terms of the known properties of alamethicin and nonactin and both depend on the statistical nature of the formation of pores in the membrane by alamethicin.

INTRODUCTION

One way in which biological membranes differ from model membranes is in the number and variety of transport systems which each contains. In model membranes generally only one system is studied, while in biological membranes many systems operate simultaneously and in parallel. The degree to which these systems interact with one another is a matter of some concern. In this paper we consider two transport systems each of which has been extensively studied alone.

Alamethicin, a macrocyclic polypeptide antibiotic [1], forms ion-conducting pores in black lipid membranes [2–4]. The probability of pore formation increases as the potential applied to the alamethicin-containing compartment is made more positive. A single alamethicin pore exhibits several discrete conductance values which are not integral multiples of any elementary conductance. Alamethicin is slightly cation selective in a many channel system, but the intercationic selectivity is small [3, 5].

Excitability phenomena induced by alamethicin which are similar to those observed with biological nerve membranes have been reported by Mueller and Rudin [5, 6]. These phenomena are seen in lipid membranes modified by alamethicin and the basic polypeptide protamine and are quite different in nature from those reported in this paper. Another pore-forming protein called excitability-inducing material [6] exhibits a voltage dependence in an inverse manner to alamethicin. Pore formation probability of excitability-inducing material decreases with increasing voltage [7]. Consequently with this protein the oscillation phenomena described below would not be observed.

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Neutral macrocyclic antibiotics such as nonactin (M_r 750) and valinomycin act as cation carriers in lipid membranes [8–11]. In contrast to alamethicin these carriers distinguish strongly between Na^+ and K^+ [12].

In this paper we will consider two methods by which alamethicin may induce voltage oscillations in black lipid films. Both result from the statistical nature of the switching process of a single pore. In the first case, the membrane current is clamped at a suitable value, and in the second, nonactin, a potassium-selective antibiotic, is used to provide a resting potential which enables oscillations to take place with no bias current. The fundamental point of this research is that it is possible to understand the observed oscillations completely and quantitatively in terms of the already known properties of the two antibiotics and the black lipid film. We thus demonstrate that these two transport systems are combined in parallel and act separately and independently.

Qualitative Analysis

First, we will analyze the case of constant bias current. For simplicity we will assume, even though more than one pore may open and these pores may fluctuate among discrete conductance states, that the conductance increases in a single step to the value λ_p. Fig. 1 shows schematically the voltage-current characteristics of a system

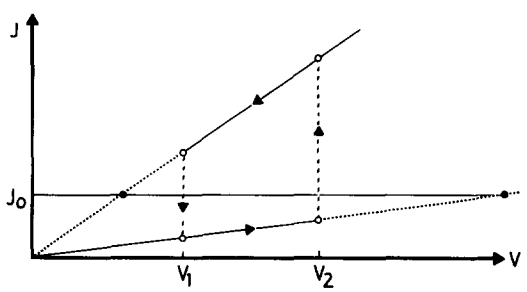

Fig. 1. Voltage-current characteristic of a two-state pore system showing increasing pore formation probability with increasing voltage.

with two conductance states. The low conductance state, λ_m, arises from the conductance of the bare membrane and the high conductance state, λ_p, arises from the opening of a single pore. If the membrane is initially at zero voltage and a current J_0 is then imposed by an external circuit, the system will be in the pore-off state and the imposed current will have two components: an ohmic current flowing through the membrane and a capacitive charging current. The differential equation for the membrane voltage V thus becomes

$$C\frac{dV}{dt} + \lambda_m V = J_0 \tag{1}$$

C: membrane capacitance
λ_m: membrane pore-off state conductance
J_0: external current

The solution for this equation is

$$V = \frac{J_0}{\lambda_m}(1 - e^{-t/t_m}) \tag{2}$$

where $t_m = C/\lambda_m$ is the membrane pore-off state relaxation time.
According to Eqn (2) the voltage would then increase to a final J_0/λ_m. Let V_2 be the voltage above which pore formation is nearly certain and V_1 be the voltage below which pore formation probability approaches zero. In Fig. 1, J_0/λ_m is assumed to be greater than V_2, the voltage at which pore formation is almost certain to occur. Thus at V_2 the pore will open and the membrane conductance will become λ_p. The differential equation for the membrane voltage in the pore-on state now becomes

$$C\frac{dV}{dt} + \lambda_p V = J_0 \tag{3}$$

λ_p: membrane pore-on state conductance
with the solution

$$V = \frac{J_0}{\lambda_p} + \left(V_2 - \frac{J_0}{\lambda_p}\right) \cdot e^{-t/tp}, \tag{4}$$

where $t_p = C/\lambda_p$ is the pore-on state relaxation time. The membrane voltage will decrease with the time constant t_p toward $V = J_0/\lambda_p$. It is easy to see that the voltage will only reach V_1, the voltage at which the pore is almost certain to turn off and the equation for the voltage will become

$$V = \frac{J_0}{\lambda_m} - \left(\frac{J_0}{\lambda_m} - V_1\right) e^{-t/t_m} \tag{5}$$

The voltage now begins to increase toward V_2 and the cycle will continue to repeat.

This analysis has not taken into account the fact that pore formation is statistical and that V_1 and V_2 are not fixed quantities. In fact, a pore may turn on or off at any voltage at any time. This makes it necessary to consider the mean life-time for the pore-off state $\bar{\tau}_m$ and for the pore-on state $\bar{\tau}_p$, respectively. $\bar{\tau}_m$ and $\bar{\tau}_p$ are strongly voltage dependent and are functions of the rates of pore formation and decay measured under voltage-clamp conditions [3].

The analysis above assumed that the pore remains open or closed long enough for the voltage to swing for enough to change the pore's conductance state. For oscillations to occur, we must, therefore, have

$$\bar{\tau}_m > t_m \quad \text{and} \quad \bar{\tau}_p > t_p. \tag{6}$$

In the case of constant external current where

$$\bar{\tau}_m \ll t_m \quad \text{and} \quad \bar{\tau}_p \ll t_p \tag{7}$$

membrane current fluctuations would occur at nearly constant voltage.

A further assumption of the analysis is that the relaxation times of single pore

formation and single pore decay are much shorter than the mean lifetimes in the different pore states. From voltage-clamp experiments we estimate these switching times to be less than 1 ms [4].

To introduce the statistical nature of pore formation in another time-dependent equation, we consider the following reaction process:

pore-off state pore-on state

$$R \underset{k_L(V)}{\overset{k_R(V)}{\rightleftharpoons}} L$$

The kinetic treatment of a many pore system yields

$$\frac{dn_L}{dt} = k_R(V)n_R - k_L(V)n_L \tag{8}$$

n_L = number of pores in the on-state
n_R = number of pores in the off-state
$k_R(V)$ = rate constant of pore formation
$k_L(V)$ = rate constant of pore decay

We introduce the probabilities for a pore to be in the on-state

$$P_L = \frac{n_L}{n_L + n_R}$$

and in the off-state

$$P_R = 1 - P_L = \frac{n_R}{n_L + n_R}$$

From Eqn (8) :

$$\frac{dP_L}{dt} = k_R(V) - [k_L(V) + k_R(V)] \cdot P_L \tag{9}$$

Eqn (9) applies to a system with many channels. In a single-pore system the pore can exist only in the on or off state. Thus we introduce a random function $\delta(P_L) = f(t, V)$ which equals 0 for the off-state and 1 for the on-state. The probability that $\delta = 1$ during statistical fluctuation with time is P_L, the probability that $\delta = 0$ is P_R.

We are now able to rewrite Eqns (1) and (3) with $\lambda_p = \lambda_m + \Lambda_p$. Λ_p is the single channel conductance.

$$C \cdot \frac{dV}{dt} + \lambda_m \cdot V + \Lambda_p \cdot \delta(P_L) \cdot V = J_0 \tag{10}$$

Eqns (9) and (10) give a complete description of the problem discussed. As shown above, an interval with no stable state for corresponding external currents J_0, i.e. a region of instability [13, 14] exists.

Oscillations can be induced without bias current if, for instance, the sodium/potassium selectivity of another antibiotic (nonactin) is used to impose a 'resting potential' on the membrane. In this case the resting potential is chosen to be greater

than V_2. An essential assumption is that the transport mechanisms of alamethicin and nonactin do not interfere with each other [15]. The membrane potential V is now determined by the equation

$$C\frac{dV}{dt} + \lambda_m V + \lambda_{non}(V - V_{non}) + \Lambda_p \delta(P_L) \cdot (V - V_{ala}) = 0 \tag{11}$$

with $J_0 = 0$ and

λ_{non}: nonactin carrier conductance

V_{non}: selectivity potential for nonactin carrier ion transport in the salt gradient

V_{ala}: selectivity potential for alamethicin pore ion transport in the salt gradient.

If we choose the experimental conditions such that $\lambda_{non} \gg \lambda_m$, V_{non} is given in a Na^+/K^+ salt gradient close to the Nernst Potential for K^+, whereas V_{ala} only results in a small potential value [3, 5]. If we rewrite Eqn (11) neglecting λ_m and V_{ala}, we obtain

$$C\frac{dV}{dt} + \lambda_{non} V + \Lambda_p \cdot \delta(P_L) \cdot V = \lambda_{non} V_{non} \tag{12}$$

which is of the same form as Eqn. (10), now with

$$t_{non} = \frac{C}{\lambda_{non}}; \qquad t_p = \frac{C}{\lambda_{non} + \Lambda_p}.$$

Consequently the system works in the same way as described above. The voltage increases toward V_{non} until $V_2 < V_{non}$ is reached and the pore opens. The voltage decreases toward

$$V_p = \frac{\lambda_{non} V_{non}}{\lambda_{non} + \Lambda_p}$$

until $V_1 > V_p$ is reached, where the pore is turned off. With a salt concentration gradient and no external bias current we obtain oscillations in just the same way as without a gradient but with an external bias current.

MATERIALS AND METHODS

Black lipid membranes were formed from bovine brain phosphatidylserine (Koch-Light Ltd) which was repurified by column chromatography and from dierucoyllecithin (di-(22 : 1)-lecithin) synthesized by K. Janko [16]. Both lipids were dissolved in *n*-decane.

Alamethicin was purchased from the Microbiological Research Establishment, Porton Down, Salisbury. Our experiments were made with the pure R_F 30 fraction of the natural alamethicin mixture.

Nonactin samples were gifts from Ciba A.G., Basel and from Squibb Inc., New Brunswick, N. J.

The measuring assembly has been described elsewhere [4]. The voltage-clamp design was changed to a current-clamp circuit by introducing the membrane resistance in the amplifier feedback loop.

Voltage will be designated positive if the more positive potential is applied

to the side of alamethicin addition. Current direction is defined as positive for cation transfer from the alamethicin-containing compartment to the opposite one.

RESULTS

Fig. 2 shows oscillations induced by alamethicin with a bias current of 1 pA. The bare membrane conductance was $\lambda_m = 8 \cdot 10^{-12}\Omega^{-1}$ for a membrane area of

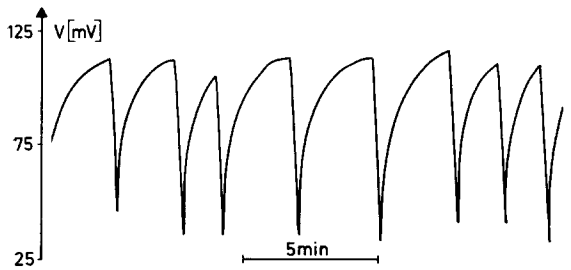

Fig. 2. Oscillations of the alamethicin pore system. Membrane solution: 5 mg phosphatidylserine + 1 mg cholesterol in 1 ml decane; salt solution: 0.1 M NaCl, unbuffered; antibiotic concentration: 10^{-6} g/ml alamethicin (R_F 30) to one compartment; temperature: 10 °C; external current: 1 pA.

about 10^{-3}cm^2. We estimated the membrane capacitance to be $C = 0.4$ nF. The relaxation time in the pore-off state calculated from Fig. 2 is $t_m = 48$ s, in good agreement with the value of 50 s estimated from the membrane conductance and capacitance. The relaxation time obtained from Fig. 2 for the pore-on state is in the order of $t_p = 10$ s. This corresponds to a conductance of $\lambda_p = 4 \cdot 10^{-11}\Omega^{-1}$, which yields a value of about $\Lambda_p = 3.2 \cdot 10^{-11}\Omega^{-1}$ for the pore conductance. This is nearly the value of the most probable conductance state of a single pore measured with voltage-clamp experiments under conditions equivalent to these of Fig. 2 (G. Boheim, unpublished results). The lifetimes of the pore-off state and pore-on state are $\bar{\tau}_m = 120$ s and $\bar{\tau}_p = 21$ s, respectively, yielding a mean period of $T = \bar{\tau}_m + \bar{\tau}_p = 141$ s.

Fig. 3 shows oscillations induced at zero current using nonactin. The chloride concentration was held constant on both sides so that only a Na^+, K^+ concentration gradient was present. Nonactin was added to both compartments but alamethicin only to that of higher Na^+ concentration. This side would be positive if the resting potential was determined by the K^+ gradient. The experimental conditions are such that the steady state zero current potential (resting potential) in the presence of nonactin alone yields about $V_{non} = 91.5$ mV compared to a Nernst potential for K^+ of $V_K = 99$ mV (for a gradient of factor 50). The nonactin-induced conductance was measured and found to be about $1.2 \cdot 10^{-9}\Omega^{-1}$. From Fig. 3 we obtain a relaxation time $t_{non} = 0.3$ s. This agrees with the value estimated using $t_{non} = C/\lambda_{non}$ with $C = 0.4$ nF as above.

The resting potential in the pore-on state estimated from Fig. 3 is about $V_p = 60$ mV. Variations in the value of V_p result from alamethicin pore fluctuations between different states before the pore is turned off.

We can calculate the value of the alamethicin conductance in the pore-on state

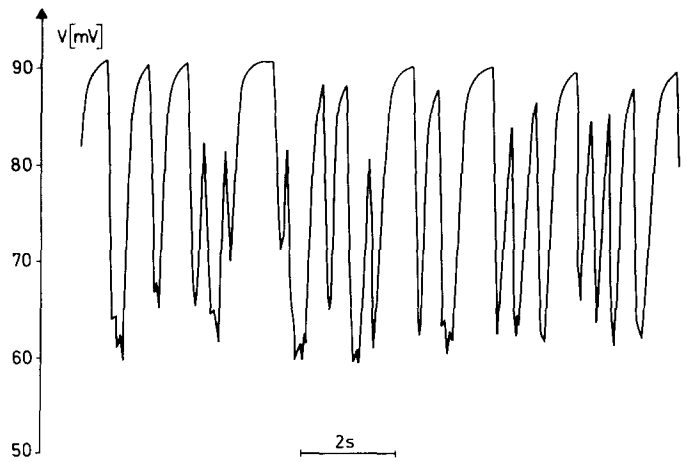

Fig. 3. Oscillations of the nonactin carrier-alamethicin pore system. Membrane solution: 10 mg di-(22 : 1)-lecithin in 1 ml decane; salt solution: 0.5 M NaCl+0.01 M KCl/0.01 M NaCl+0.5 M KCl, unbuffered; antibiotic concentration: 10^{-7} g/ml nonactin to both compartments, $5 \cdot 10^{-7}$ g/ml alamethicin (R_F 30) to the compartment of higher Na^+ concentration; temperature: 20 °C; external current: 0 pA.

using the relation (from Eqn (11))

$$V_p = \frac{\Lambda_p V_{ala} + \lambda_{non} V_{non}}{\lambda_{non} + \Lambda_p} \tag{13}$$

Using the value of $V_{ala} = 20$ mV (estimated from the data of Eisenberg et al. [3]), we calculate $\Lambda_p = 9.5 \cdot 10^{-10} \Omega^{-1}$. This is about the conductance of the most probable state of a single pore [4] under the conditions of Fig. 3. The estimated relaxation time $t_p = C/(\lambda_{non} + \Lambda_p) = 0.19$ s is in approximate agreement with the experimental value, which is difficult to determine from Fig. 3.

The mean lifetime in the pore-off state is $\bar{\tau}_{non} = 0.39$ s and in the pore-on state $\bar{\tau}_p = 0.24$ s yielding a mean period of $T = 0.63$ s.

DISCUSSION

The results demonstrate that these kind of oscillations can be predicted from an understanding of the properties of the individual components. Fig. 2 shows that the statistical formation and disappearance of alamethicin pores results in quite regular oscillations. Because in Fig. 3 the mean lifetimes of the pore-off state and pore-on-state are fairly close to the relaxation times of these membrane states, there are more pronounced statistical fluctuations in the oscillation period and amplitude.

The mean lifetimes in Figs 2 and 3 differ by two orders of magnitude. This is primarily due to the difference in membrane lipid composition [4] and to a minor extent to the difference in temperature. In addition, the voltage range and amplitude of the oscillations and to some extent the period can be varied by changing the concentrations of the antibiotics.

We have thus constructed a system from components with well studied properties and have demonstrated that the system's properties arise naturally from the properties of the components alone. We point out that oscillations like those we have observed characterized by first-order relaxation processes are similar to (but not identical with) phenomena observed in excitable membranes. We note that our oscillations are induced in thin lipid films by molecules whose total molecular weight is less than 2000 and that such molecules as these synthesized by micro-organisms might have been the precursors of more complex components performing similar functions in specialized membranes.

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