BBA Report

BBA 71065

Sustained, coupled electrical and mechanical oscillations in a bimolecular lipid membrane in the presence of inorganic ions

HARISH C. PANT and BARNETT ROSENBERG

Biophysics Department, Michigan State University, East Lansing, Mich. 48823 (U.S.A.)

(Received December 8th, 1970)

SUMMARY

Bimolecular lipid membranes of oxidized cholesterol or phosphatidylethanolamine separating bathing solution compartments of potassium ferricyanide and potassium iodide can be set into regular, sustained electrical oscillations when the pH's of the two compartments are adjusted to 5 and 10, respectively. The period of the oscillations are in the order of 30 sec, and the amplitudes are about 30–40% of the self electromotive force, or conduction in an applied field. A mechanical oscillation of the membrane can be detected simultaneously, which has the same period as the electrical.

Bimolecular lipid membranes are a good model system for biological membranes. As such, it is of direct interest if sustained electrical oscillations can be demonstrated in such models. Teorell[1] first showed oscillatory electric current flows in thick porous membranes with fixed charges, and developed a theory based upon superposed electrochemical and hydrostatic pressure gradients which led to non-linear differential equations with oscillatory solutions. The theory was developed further by Franck[2] and Katchalsky and Oster[3], and additional experimental work on such thick membranes was reported by Monnier[4] and Shashoua[5].

Sustained oscillations in bimolecular lipid films were first reported by Mueller and Rudin[6] when proteinaceous substances (EIM and protamine sulfate) were adsorbed on the lipid bilayer. A later paper reported that a small cyclic peptide (alamethicin) produced the same type of "action potentials"[7]. The theoretical models suggested by these and other authors[8,9] invoke "gates", "channels" or "pumps", singly or in combination, to account for the electrokinetic effects.

We report here the appearance of sustained, coupled, electrical and mechanical oscillations in a bimolecular lipid membrane caused by the presence of simple inorganic ions in the bathing solution. These oscillations occur under a rather restricted set of

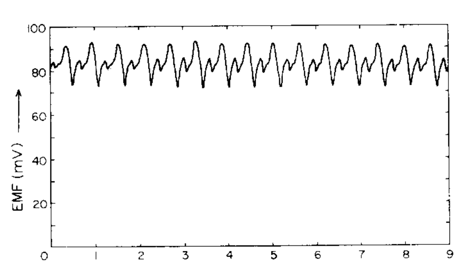
Abbreviation: EMF, electromotive force.

conditions, all of which are necessary for its occurrence.

The lipid used was oxidized cholesterol prepared in our laboratory using the method of Tien *et al.*[10]. The bilayer was formed in the aperture of a teflon cup according to standard procedures[11]. The bathing solution on both sides of the membrane was 0.1 M KCl. The electrodes on each side were identical calomel electrodes with KCl bridges. The electrodes were checked for symmetry before forming the membrane. To the inner cup, a solution of potassium ferricyanide was added to make a $1.3 \cdot 10^{-3}$ M bathing solution of the complex. This produced a small electromotive force (EMF) (approx. −8 mV) across the electrodes; the inner cup electrode was the active one, and attached to a Keithley 610BR electrometer, the outer cup electrode was connected to ground as a reference electrode. The conductance of the membrane, normally about 10^{-12} $(\Omega \cdot \text{cm})^{-1}$ is not changed by the presence of ferricyanide ions. However, a marked photo EMF and photoconductance change does occur in this system[12] and all experiments reported here were done in subdued room light.

To the outer cup, a solution of potassium iodide was added to make a $7.4 \cdot 10^{-2}$ M bathing solution in iodide. The solutions in both cups were well stirred with magnetic stirrers. The addition of the potassium iodide produces an EMF of about −120 mV, and a conductance increase by a factor of 10^3. The membrane now exhibits a large rectification effect (> 100) in the presence of an applied voltage. The forward rectifying direction is that of negative charges moving from the iodide compartment to the ferricyanide compartment.

If now the pH of the ferricyanide compartment is adjusted to about 5, and that of the iodide compartment to about 10, the membrane is set into regular electrical oscillations. Fig.1 shows the records of such oscillations for the open circuit condition (the electrometer in the voltage mode). The total EMF in this experiment was −130 mV, and the magnitude of the oscillations are about 33% of the total. The oscillations will last for periods of hours, with constant amplitude and frequency. After a few hours, the amplitude and frequency decrease and the oscillations finally disappear. A fresh membrane prepared after breaking the old one, will again show oscillations but with a somewhat different frequency and wave shape.



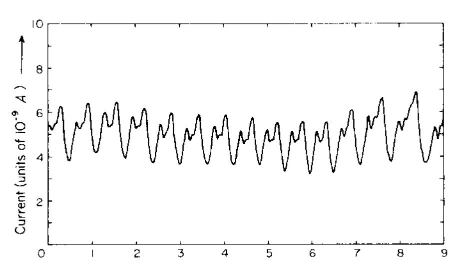


Fig.1. Electrical oscillations of the trans membrane potential in a bimolecular lipid membrane of oxidized cholesterol, in the presence of bathing solutions of ferricyanide ions on one side and iodide ions on the other at appropriate pH's.

Fig.2. Electrical oscillations of the trans membrane conductance in a bimolecular lipid membrane of oxidized cholesterol in the presence of bathing solutions of ferricyanide ions on one side and iodide ions on the other at appropriate pH's. The applied voltage is 20 mV.

The appearance of the oscillations is very sensitive to the adjustment of the pH's of two compartments. At a pH > 10 in the iodide compartment, the amplitudes of the oscillations are the same, but the frequency decreases. At low iodide concentrations ($< 10^{-4}$ M) the amplitudes of the oscillations are markedly decreased.

Fig.2 shows the oscillations in the current mode. The applied voltage is 20 mV. The bilayer is highly conductive under these conditions, the total current flow is of the order of 10^{-8} A. Increasing the applied voltage increases the amplitude of the oscillations, but the frequency is unchanged. At applied voltages $\geqslant$ 120 mV, the oscillations fade away after a few minutes.

We have observed the membrane through a binocular microscope using a focused lamp placed so that a reflection can be seen from the region where the bilayer broadens into the supporting thicker torus of lipid material. When the electrical oscillations begin, the position on the circumference where the reflected light can be seen also begins to oscillate. The period of these mechanical movements coincides with the period of the electrical oscillations. Thus these two oscillations, the electrical and mechanical, are coupled; they appear together, have the same period, and disappear together.

Our first hypothesis was that a periodic hydrostatic pressure change was causing a periodic bowing out of the membrane, thus increasing its area, and therefore the conductance, and also causing the observed mechanical oscillations. However, even slight bowing of the membrane could be observed by reflections from the bilayer surface, and we have not observed such an effect. At present we cannot ascribe the observed movement to any particular mode of mechanical oscillation.

These coupled oscillations have also been observed with bimolecular membranes composed of phosphatidylethanolamine and are thus not unique for the oxidized cholesterol membrane.

The periods and amplitudes of these oscillations are very different from those caused by EIM and cyclic peptides. However, we feel that their occurrence in the presence of simple inorganic ions reflects a simpler set of underlying mechanisms, more consonant with the theory of Teorell, which can now be applied to bimolecular lipid membranes.

This work was supported by Contract No. ONR-2587 from the Office of Naval Research.

REFERENCES

1 T. Teorell, *Exptl. Cell Res. Suppl.*, 3 (1955) 339.
2 U.F. Franck, *Ber. Bunsenges, Phys. Chem.*, 67 (1963) 657.
3 A. Katchalsky and G. Oster, in D.C. Tosteson, *The Molecular Basis of Membrane Function*, Prentice-Hall, New Jersey, 1969, p.1.
4 A.M. Monnier, *J. Gen. Physiol.*, 51 (1968) 26.
5 V.E. Shashoua, in D.C. Tosteson, *The Molecular Basis of Membrane Function*, Prentice-Hall, New Jersey, 1969, p.147.
6 P. Mueller and D.O. Rudin, *J. Theoret. Biol.*, 18 (1968) 222.
7 P. Mueller and D.O. Rudin, *Nature*, 217 (1968) 713.
8 G. Ehrenstein, H. Lecar and R. Nossal, *J. Gen. Physiol.*, 55 (1970) 119.
9 R.C. Bean, W.C. Shepherd, H. Chan and J.T. Eichner, *J. Gen.Physiol.*, 53 (1969) 741.
10 H.T. Tien, S. Carbone and E.A. Dawidowicz, *Nature*, 212 (1966) 718.
11 P. Mueller, D.O. Rudin, H.T. Tien and W.C. Wescott, *Nature*, 194 (1962) 979.
12 B. Rosenberg and H.C. Pant, *Abstr. 3rd Intern. Biophys. Congr., 1969, Cambridge, Mass.*, p.67.