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Electric oscillation in an excitable model membrane impregnated with lipid analogues

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A model membrane constructed from a Millipore filter, whose pores were impregnated with dioleyl phosphate, exhibited an electric self-oscillation under nonequilibrium conditions. The membrane interposed between two solutions with the same KCl concentrations showed no temporal change in membrane potential. However, the potential became oscillatory on application of an electric current to the membrane. The frequency was proportional to the magnitude of the electric current. When both KCl solutions were replaced by NaCl solutions, a similar trend was observed, although the oscillation was not as regular as in the case of KCl. A membrane placed between equimolar solutions of KCl and NaCl, on the other hand, gave rise to an oscillation even without current application. When a membrane was placed between 5 mM KCl and 100 mM KCl, it was found that NaCl added to the 5 mM KCl side had a pronounced effect on the membrane with respect to the frequency response of the oscillation. These results indicate that the dioleyl phosphate membrane discriminates Na^+ from K^+.

1. Introduction

Excitation is one of the most important properties among various functions of the biological membrane. In spite of many investigations on nerve excitation, the physicochemical mechanisms of these phenomena remain unclear. Studies on an electric self-oscillation of artificial membranes are thus important for the elucidation of the mechanisms. One simple system studied previously is the Teorell oscillator constructed from a glass filter placed under an electrochemical gradient [1]. Another example is an oil membrane containing no proteins, showing repetitive firing [2].

A dioleyl phosphate (DOPH)-Millipore membrane also belongs to the types of artificial membrane systems which have been developed for this kind of investigation [3]. This model membrane shows a self-sustained oscillation of electric potential under a salt concentration difference, an electric current and a pressure difference across the membrane [4–9]. While various kinds of oscillations related to a phase transition of DOPH have been investigated experimentally and theoretically, the usual experimental conditions above seem to be too far from equilibrium, if we take into account the biological situation. In real biological systems, Na^+ and K^+ concentration gradients only exist across the membrane in principle, whereas these gradients are maintained by an active transport of ions with the help of ATP. An electric oscillation can appear under this resulting situation of ionic conditions [10]; many nonequilibrium factors, such as a concentration gradient, electric current and pressure, have been applied in the DOPH system. Therefore, it should be rather interesting to study

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the oscillatory behavior of a model membrane under similar conditions to biological systems. Although an electric oscillation of sorbitan monooleate membranes has been studied under these conditions, i.e., a concentration difference of Na^+/K^+ [11,12], a major merit of using the DOPH membrane system lies in the fact that the basic characteristics of its phase transition have been well elucidated [3–9,13].

This paper reports that even a membrane comprising the lipid analogue DOPH alone can lead to an electric self-sustained oscillation on being placed under a nonequilibrium state of the Na^+/K^+ concentration gradient without an applied electric current. The oscillation was also found to appear under conditions of equimolar KCl or NaCl at both sides of the membrane, only if an electric current was imposed upon it. It was demonstrated that sensitivity to Na^+ is amplified by using an electric oscillation occurring under the usual conditions of a K^+ concentration gradient with the application of an electric current and pressure.

2. Materials and methods

A DOPH-Millipore membrane was prepared by the same method as reported previously [4–9]. The quantity of the lipid analogue DOPH adsorbed within the filter paper was adjusted to about 5 mg/cm^2. Each membrane was placed between two cells with two pairs of Ag/AgCl electrodes; one was used for measuring the electric potential across the membrane, the other for the purpose of supplying d.c. current. In experiments where the membrane was placed between 100 mM KCl and 100 mM NaCl solutions, the membrane potential was measured by means of the Ag/AgCl electrodes via two salt bridges placed in the solutions. The cells were also constructed such that a pressure difference could be imposed on the membrane. The electric potential was registered by means of a chart recorder (Riken Denshi F-42CP) through a high-impedance transducer of gain unity. The DOPH-Millipore membrane was pre-conditioned in 100 mM KCl solution over 12 h. The membrane with a large amount of DOPH adsorbed changed much more slowly to the state of low electric resistance and nearly zero electric potential between 100 mM NaCl and/or KCl solutions due to the phase transition taking place over 1 or 2 days. The experiment on oscillations was performed while the membrane showed high electric resistance.

3. Results and discussion

3.1. Oscillations induced by nonequilibrium conditions in the equimolar case

A DOPH-Millipore membrane usually exhibits a rhythmic electric oscillation under the three nonequilibrium factors of a salt concentration difference, electric current and pressure difference. The oscillation is attributable to repetitive processes of the phase transition of DOPH molecules between hydrophobic oil droplets and hydrophilic multi-bilayers, coupled with salt accumulation and release [3–9].

The three nonequilibrium factors mentioned above have usually been employed in the DOPH system. In the present study, the possibility of excitability of the membrane was investigated under nonequilibrium conditions similar to the biological situation: a DOPH-Millipore membrane was interposed between equimolar 100 mM NaCl and/or 100 mM KCl solutions, and the membrane potential was measured. In the case of no additional treatment such as electric current supply, the behavior of the membrane potential was observed continuously for at least 12 h. For application of current, 0.01–0.35 μA d.c. was employed; in this case, measurement was made over a few hours. This experimental time is sufficient for the observation of oscillation coupled with ionic flow under an electric voltage gradient [6].

The results are summarized in table 1: when a membrane was kept under equilibrium conditions, i.e., in identical solutions in both cells, no oscillation appeared as would reasonably be expected. The application of an electric current as an effective external force on the membrane can bring the system to a nonequilibrium state: this resulted in electric oscillation in both cases of equimolar KCl

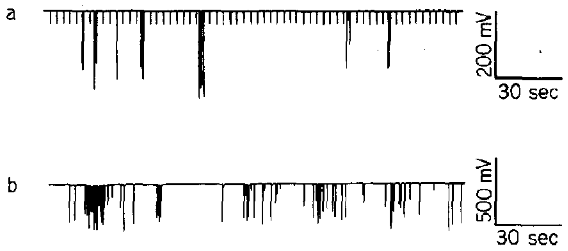


Fig. 1. Typical examples of electric oscillations under conditions of equimolar salt concentrations with an electric current. (a) KCl, 0.23 μA; (b) NaCl, 0.04 μA. Note that the oscillation is more regular for KCl than with NaCl.

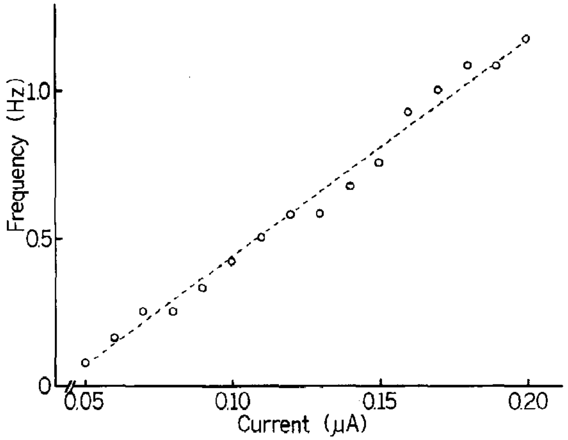


Fig. 2. Dependence of frequency on electric current for equimolar KCl solution.

and NaCl solutions. Fig. 1 shows two examples of oscillations. As can be seen, the oscillation is more regular in the presence of K^+ than Na^+. This tendency holds under the usual conditions of the presence of a concentration gradient, electric current and pressure difference (see fig. 5). The reproducibility in the wave forms was fairly good, although the value of the electric current necessary for the oscillation was not very reproducible, as occurs under the usual experimental conditions used for the DOPH system [6–8].

Fig. 2 illustrates the dependence of the oscillatory frequency on electric current in the case of equimolar KCl. It can be seen that the frequency is proportional to the applied electric current. A similar trend is observed for the DOPH membrane under a 5 mM/100 mM KCl gradient with an applied pressure [6]. Since the imposed current is d.c. and the output voltage is a.c., this membrane behaves as a kind of D-A converter of a current-controlled oscillator.

When a membrane was interposed between 100 mM NaCl and 100 mM KCl, on the other hand, an electric oscillation appeared spontaneously without an applied electric current. An oscillation induced by the NaCl/KCl gradient is shown in fig. 3. The oscillation started abruptly 20–60 min after the DOPH-Millipore membrane had been placed between the NaCl and KCl solutions. The amplitude was approx. 10 mV and the period approx. 1 min. In many cases the oscillations

Table 1

Induction of electric self-oscillation under conditions of equimolar salt

A DOPH-Millipore membrane was interposed between 100 mM NaCl and/or 100 mM KCl. (○) Occurrence of oscillation; (×) nonoccurrence. I, implies current application; I-K, current from KCl side; I-Na, current from NaCl side; N.T., current was not applied. Typical examples of oscillations are shown in the corresponding figures.

Solution in cell		Treatment	Oscillation
Left cell	Right cell		
KCl	KCl	N.T.	×
		I	○ fig. 1a
KCl	NaCl	N.T.	○ fig. 3
		I-K	○ fig. 4a
		I-Na	○ fig. 4b
NaCl	NaCl	N.T.	×
		I	○ fig. 1b

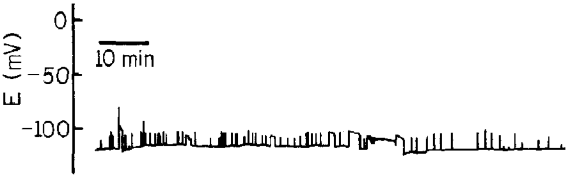


Fig. 3. Electric oscillation induced by an NaCl/KCl gradient. E, electric potential. The NaCl side was taken as a zero standard. The oscillation appeared spontaneously after the membrane had been placed between 100 mM NaCl and 100 mM KCl solutions.

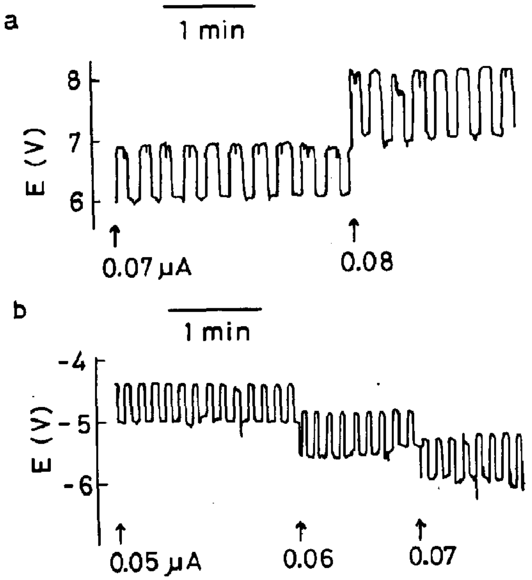


Fig. 4. Electric oscillations under an NaCl/KCl gradient with an electric current applied from the KCl side (a) and NaCl side (b). Values of the applied current are shown. The electric potential is positive (a) and negative (b), since the origin is taken on the NaCl side.

continued for 5–10 h. When the membrane was placed between 100 mM salt solutions, the resting level of the electric potential tended to approach zero owing to the phase transition of DOPH to multi-bilayers. The lifetime of oscillation is partly related to the situation where the DOPH membrane shows a stable high potential while DOPH remains in the oil droplet phase.

The KCl side was negative with respect to the NaCl side throughout measurements. The observed potential difference at this resting level should, therefore, originate mainly from the diffusion potential of K^+. The upward temporary change in electric potential, i.e., an action potential, may indicate the flow of Na^+ from the opposite side of membrane to the KCl side. The existence of several plateaus in fig. 3 may suggest participation of two steady states dominated by K^+ flux and Na^+ flux. This phenomenon showing channel-type behavior is very analogous to actual nerve excitation. In electrophysiological terms, therefore, ions participating in the resting potential and action potential are the same as those playing such a role in nerve cells [10,14].

An electric oscillation induced by an NaCl/KCl gradient was also found in another type of artificial membrane comprising sorbitan monooleate [11,12]. An oscillation was observed in a reconstituted membrane of *Nitella* protoplasmic droplet under similar conditions including Ca^{2+} [3,15]. Excitability appearing under this kind of concentration difference between Na^+ and K^+ may be interesting in examining the excitability of nerve membranes; the nonequilibrium state connected with phase transitions of membrane constituents would be important in considering excitability.

When an electric current was imposed on a membrane placed between 100 mM NaCl and 100 mM KCl solutions, an oscillation with a rectangular wave form appeared, irrespective of the direction of current application (see also table 1). Examples are given in fig. 4. It can be seen that the oscillation is fairly rhythmic and that its frequency is very high compared with that exhibited in fig. 3. This result may suggest that the electric oscillation becomes more rhythmic with the addition and elevation of a nonequilibrium factor. Since the salt concentration difference and electric current are combined with the phase transition of DOPH, the oscillation can be considered to become more regular. In the case of one nonequilibrium factor for an NaCl/KCl concentration gradient, the oscillation is not very regular, as shown in fig. 3.

3.2. Effect of Na^+ and K^+ on oscillation under a KCl concentration gradient

In this section, we study the case where a membrane is placed between aqueous solutions composed of the same salt species (KCl or NaCl) with different concentrations in the two cells. Fig. 5 shows an example of regular self-oscillation, when a DOPH-Millipore membrane was placed under a KCl or NaCl gradient with a pressure difference and electric current. The oscillations are more rhythmic than those shown in fig. 1, where only an electric current is imposed on the membrane under conditions of equimolar salt. The frequency of oscillation in one membrane usually

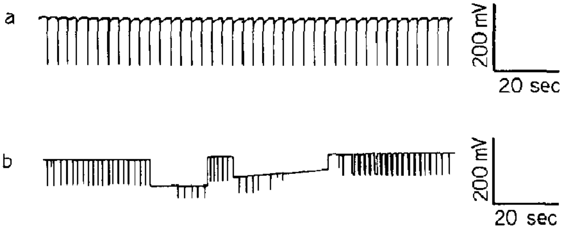


Fig. 5. An example of self-sustained oscillation. (a) 5 mM KCl/100 mM KCl; electric current 0.32 μA; pressure difference 25 cmH_2O and (b) 5 mM/100 mM NaCl; 0.20 μA; 40 cmH_2O. The low-concentration side was taken as a zero standard, and hence the electric potential was positive for the electric current usually applied.

differed from that in another even when the amounts of DOPH adsorbed were equal. This may imply that the semi-microscopic adsorbed state of DOPH molecules within pores strongly affects the oscillation, as pointed out previously [6]. In most cases, the KCl gradient induced stabler oscillations than the NaCl gradient, as can be seen from a comparison of fig. 5a and b; this is similar to the case in fig. 1a and b.

Na^+ does seem not to differ much from K^+ in the sense that two ions are monovalent cations. The effect of these ions on the phase transition of a DOPH-Millipore membrane does not differ [3,4,16]. In addition, the permeability of both ions through lipid membranes is almost the same [17,18]. As for the electric oscillation of the DOPH-Millipore membrane, however, a KCl concentration gradient induces a stabler oscillation than an NaCl gradient (fig. 5). Moreover, the nonequilibrium situation of an Na^+/K^+ concentration gradient causes an electric oscillation even without an applied electric current, as shown in fig. 3. These two results demonstrate that the effect of each ion species on the oscillation of a DOPH-Millipore membrane is different. To confirm this expectation, the membrane was interposed between 5 mM KCl and 100 mM KCl, and the change in oscillatory frequency was studied by adding NaCl or KCl to the 5 mM side. An Na^+ gradient is unsuitable for this purpose, since it does not bring about a stable oscillation. Fig. 6 shows the frequency change as a function of salt concentration. Both salts accelerated the oscillation. However, NaCl is much more effective than KCl, since NaCl increased the frequency by a very small amount of a few hundred μM. This concentration is one-tenth that of KCl. While the increase of frequency may be attributable to a reduction in charge of DOPH molecules [8], the extent may differ between Na^+ and K^+.

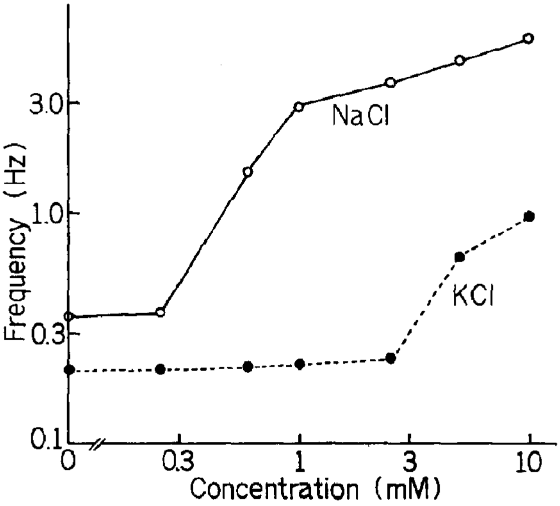


Fig. 6. Effect of NaCl and KCl on self-oscillation. A DOPH-Millipore membrane was interposed between 5 mM and 100 mM KCl. Conditions: 0.28 μA electric current and 30 cmH_2O pressure difference were applied in the KCl treatment, and 0.30 μA and 30 cmH_2O in that with NaCl. Although the initial frequencies differ from each other (0.2 and 0.33 Hz for KCl and NaCl, respectively), this difference is not essential since the tendency for NaCl to be more effective than KCl was always obtained irrespective of the difference in initial frequency.

Many biological systems discriminate Na^+ from K^+. In their static state, macromolecules such as proteins may possibly discern both ions, but lipids not. The present study, however, shows that even lipids will be able to discriminate between them in a dynamic state exhibiting an electric oscillation. The self-oscillation arises by nonlinear hydrophilic and hydrophobic coupling of ion flow with the phase transition of DOPH. It seems that NaCl and KCl interact with this nonlinear component in a somewhat different fashion. This slight difference can be enlarged by the electric oscillation. This can be considered as originating partly from a qualitative difference between structure-making and structure-breaking ions. Na^+ and K^+ belong to the classes of structure-making and structure-breaking ions, respectively [19]. It is reported that, in the presence of Na^+, a nearest-neighbor interaction between proton dissociation sites becomes important for a phosphatidylglycerol monolayer in the gel state [20,21]. A detailed theoretical calculation treating the site-site interaction occurring in polyelectrolytes [22] might be effective for dealing with this sort of problem.

It has been reported that the sign of the resting potential is inverted between membranes composed of different lipids such as, monoolein, sorbitan-monooleate and triolein [11,12]. From this viewpoint, the DOPH system shows the same behavior as that of triolein, since the K^+ side is electrically negative. The present system exhibits a stabler oscillation in KCl solution than in NaCl, as shown in fig. 5. It is therefore reasonable to suppose that the phase transition of DOPH molecules interacts successfully with the K^+ flow, although the microscopic details have not been elucidated. This may suggest greater permeability of K^+ than Na^+ when a membrane is placed between KCl and NaCl solutions. This results in a negative value for the electric potential on the KCl side.

3.3. Some types of oscillations in DOPH-Millipore membranes

The excitation phenomenon in biological membranes has attracted much attention with respect to the mechanism of reception of biological information and, more broadly, in relation to the formation of dynamic (dissipative) structures in non-equilibrium systems [3,23,24]. During the past few decades, some model membranes producing self-oscillating phenomena have been proposed. These are divided into four categories: (i) polymer membranes [1,25,26], (ii) liquid membranes [27–29], (iii) lipid-analogue membranes [2–9,11,12] and (iv) bilayer and multi-bilayer (reconstituted) membranes [3,15,30–34].

The DOPH-Millipore membrane investigated falls within the category of case iii. Fig. 7 summarizes typical oscillations appearing in this model membrane [3,5–9,15]. A long-period oscillation appears spontaneously after a membrane has been placed between a concentration gradient, e.g., 5 mM and 100 mM KCl solutions. The wave form is usually of the pulse type and its frequency ranges from several minutes to a few hours, depending on the quantity of DOPH adsorbed [5,6]. A theoretical analysis showed that the occurrence of oscillations is the result of a hard-mode instability leading to a limit cycle associated with slow ion penetration into the membrane [5]. A short-period oscillation usually appears under a salt concentration gradient with a pressure difference and electric current (see fig. 5). Its wave form shows a type of relaxation oscillation which is often observed in an electrical circuit. The frequency and wave form can respond to various chemicals such as Ca^{2+}, sucrose and bitter substances [8,9]. The occurrence of an oscillation is brought about by the elevation of a nonequilibrium factor, i.e., an increase in a bifurcation parameter such as pressure difference or electric current. This appears in a region very far from equilibrium, compared with long-period oscillations and oscillation under an Na^+/K^+ gradient.

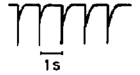
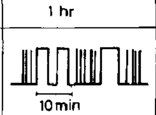
Oscillation	Waveform	Experimental condition	Comment
Long-period oscillation	 	ΔK^+	HMI due to ion penetration
Short-period oscillation	 	ΔK^+, pressure, electric current	HMI due to elevated bifurcation parameter
Burst	 	ΔK^+	Coupled oscillation (Neuron)
Oscillation (Na^+/K^+)	 	Na^+/K^+ gradient	Similarity to nerve excitation

Fig. 7. Four types of oscillations observed in a DOPH-Millipore membrane. The K^+ concentration gradient across the membrane is denoted by ΔK^+, and the hard-mode instability by HMI. The nomenclature for the long-period oscillation, short-period oscillation and burst is according to ref. 6.

A burst appears as a consequence of the nonlinear coupling between a short-period oscillation in the surface region of a filter and a long-period oscillation within a pore [6]. This mechanism is closely analogous to a real burst observed in a neuron, where the slow periodic change in membrane conductance causes a rapid oscillation [35].

The oscillation induced by an Na^+/K^+ concentration gradient occurs in a similar situation to real biological systems. This may be due to the small degree of difference between Na^+ and K^+ in interacting with DOPH molecules. This possibility already appeared in the short-period oscillation (fig. 5). It may be interesting that lipids can discriminate Na^+ from K^+ in the temporary ordered state. In real biological systems, such a dissipative structure may also act in information processing [15,36]. In this case, a dynamic ordered structure including spatio-temporal organization may play an essential role.

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