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Effect of local anesthetics on the electrical characteristics of an excitable model membrane composed of dioleyl phosphate

II. Dynamic response

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The effects of local anesthetics (tetracaine, procaine and lidocaine) on self-sustained electrical oscillations were studied for a lipid membrane comprising dioleyl phosphate (DOPH). This model membrane exhibits oscillation of the membrane potential in a manner similar to that of nerve membranes, i.e., repetitive firing, in the presence of an ion-concentration gradient, on the application of d.c. electric current. Relatively weak anesthetics such as procaine and lidocaine increased the frequency of self-sustained oscillation, and finally induced aperiodic, rapid oscillation. The strong anesthetic tetracaine inhibited oscillation.

1. Introduction

Excitability is one of the most important properties among the various functions of biological membranes. The excitation of membranes involves transient depolarization or repetitive firing (i.e., oscillation) of the membrane potential. In spite of several studies aimed at the elucidation of nerve excitation, the physicochemical mechanisms remain unclear. Consequently, investigation of electrical oscillations in artificial membranes is important for the clarification of the mechanism. The most well-known system may be the Theorell oscillator constructed from a glass filter placed under an electrochemical and hydrodynamical gradient [1,2]. Other examples showing excitation are those of an oil membrane lacking proteins [3,4] and an oil/water interface [5,6].

DOPH membranes also display self-sustained oscillations of the membrane potential, as has been extensively investigated [7–11]. In the present paper, the effect of local anesthetics on the excitability of DOPH membranes was studied. In the preceding article [12], local anesthetics were shown to affect the static and steady electrical properties of the membrane potential and resistance. Whilst there are some reports [13–17] describing the effect of anesthetics on the static properties of model membranes, the present study is the first reported attempt to establish the dynamic properties with the application of anesthet-

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ics on excitable lipid membranes. The effect on the electrical oscillations exerted by the anesthetics was so drastic that regular oscillation was suppressed or changed to aperiodic, fast oscillations.

2. Materials and methods

Tetracaine, procaine hydrochloride and lidocaine free base were obtained from Sigma, the latter being used as the salt with hydrochloric acid.

Membranes were prepared according to the same method as that mentioned in ref. 12. A DOPH Millipore membrane was placed between two cells, filled with 1 and 100 mM KCl solutions. The solution of 1 mM KCl was grounded; hence, the membrane potential corresponds to the electrical potential on the 100 mM KCl side.

As regards the electrical oscillations of the membrane, the d.c. component is not important. Thus, the salt bridges were omitted and a manometer for measurement of pressure was mounted on the apparatus. An electrical oscillation was induced by imposing an electric current and a pressure difference on the membrane from the 100 mM KCl side to 1 mM KCl side.

Oscillation appeared above a threshold value of about 0.1 μA under 30 cmH_2O pressure difference. This phenomenon can be regarded as representing the appearance of a temporary ordered structure bifurcating from a thermodynamic branch, where steady ionic flow occurs [9,10]. The frequency of oscillation increased with the electric current.

Anesthetics were added to the 1 mM KCl solution, which was stirred throughout the experiments, performed at $25 \pm 2°C$.

3. Results

3.1. Effect of anesthetics on electrical oscillation of the membrane

The DOPH membrane exhibits self-sustained oscillation of the electrical potential [7–11]. The frequency was usually 0.1–0.5 Hz with an amplitude of about 100 mV under the conditions of a pressure difference of about 30 cmH_2O and an electric current of 0.05–0.5 μA imposed on the membrane from the 100 mM to 1 mM KCl side. Under fixed experimental conditions, oscillation continued stably for a few hours. The membrane can be considered as a model of the excitable nerve membrane. In biological systems, local anesthetics are known to affect the nerve membrane [18].

The effects of the anesthetics on the oscillation were complex. In some cases, the anesthetics suppressed oscillation (fig. 1), whereas at other times they disturbed the wave form and accelerated the oscillation, causing a sudden, approx. 10-fold increase in frequency (figs 2 and 3). This fast, aperiodic oscillation is referred to as a burst for

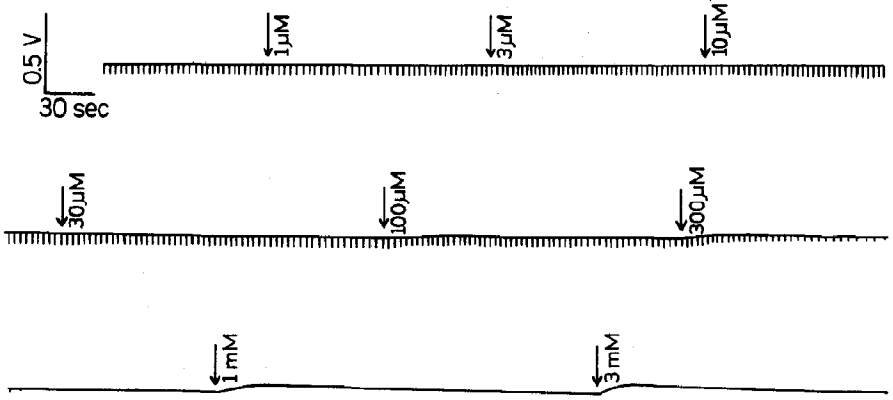

Fig. 1. Suppression of oscillations by tetracaine. Experimental conditions: 30 cmH_2O and 0.10 μA were imposed on the membrane.

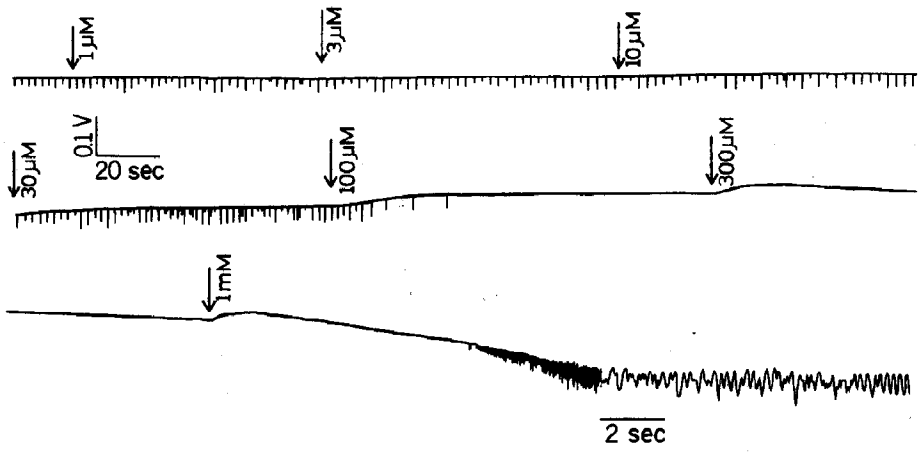

Fig. 2. Induction of burst of oscillation by lidocaine. Experimental conditions: 30 cmH_2O and 0.16 μA. In the lowest chart, the time scale has been expanded.

the sake of convenience. Experiments with procaine, lidocaine and tetracaine were performed 10, 10 and 11 times, respectively. Among these, three, four and seven treatments in the respective groups halted the oscillations temporarily or for prolonged periods. Eight, nine and four treatments in the respective groups changed the pattern of oscillation to that of bursts finally. Occasionally the burst appeared after the temporary cessation of oscillation (fig. 2); this is the reason why the total number of cessation and burst periods is larger than the number of experiments using procaine and lidocaine. Tetracaine, which is a more potent anesthetic than the other two, tended to depress oscillation, while procaine and lidocaine readily induced the burst.

The cessation of oscillation caused by tetracaine is represented in fig. 1 for the case in which a membrane displayed electrical oscillations of 0.3 Hz before treatment. The anesthetic did not affect the oscillation at concentrations between 1 and 100 μM, whereas it suppressed and then finally

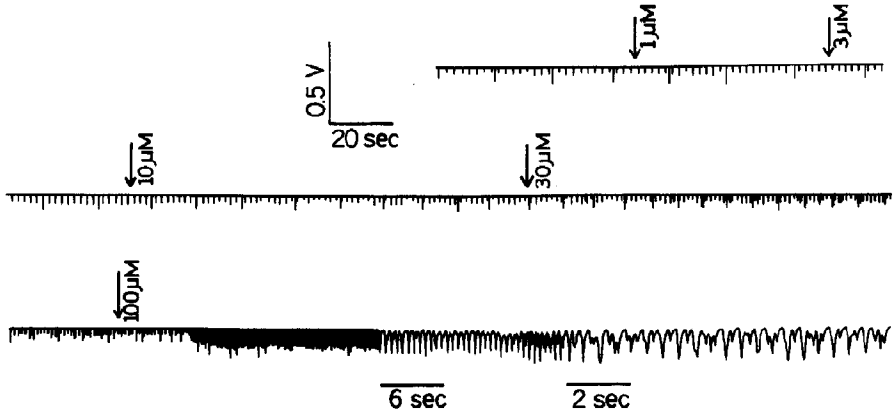

Fig. 3. Induction of burst of oscillation by procaine. Experimental conditions: 30 cmH_2O and 0.14 μA.

stopped the oscillation at 300 μM. Subsequently, the concentration of tetracaine was raised to 1, 3 and 10 mM; however, oscillation did not reappear. The spikes in the oscillation occurred in a downward direction due to the reduced membrane resistance [11] in the case where the membrane potential was polarized toward the positive side on application of a d.c. current.

Fig. 2 shows an example of the burst induced after the cessation of oscillation. When addition of lidocaine was made to a membrane oscillating at 0.4 Hz, the oscillation was halted at 100 μM anesthetic. However, at 1 mM, lidocaine decreased the baseline of the membrane potential to a certain extent, and subsequently evoked a sudden, fast oscillation, i.e., the burst. The frequency reached 5 Hz, i.e., about 10-fold greater than that of the untreated membrane.

The burst without cessation of the oscillations is shown in fig. 3, being induced by procaine treatment. The oscillation of frequency 0.4 Hz was gradually accelerated by successive additions of 1–30 μM procaine. During this process, the rate of acceleration was not as large and the increase in frequency was only 2-fold the initial frequency. Application of 100 μM procaine induced the burst, the frequency being increased suddenly and markedly.

3.2. Events underlying the changes in oscillations

In general, the baseline of the membrane potential was upwardly directed immediately after the addition of anesthetics, followed by a gradual change toward the downward direction. The upward and downward changes in the baseline were accompanied by the depression and acceleration of oscillation, respectively (fig. 4). Thus, the depression and acceleration are closely related to the change in resting level of the potential.

The downwardly directed spikes in oscillation originate from the decrease in membrane resistance [11]. Similarly, the downward change in the resting potential observed with the anesthetics can be attributed to the reduced membrane resistance. In contrast, the upward change in the resting potential occurring immediately after the addition of anesthetics is not connected with the change in membrane resistance, as demonstrated in fig. 5, where the anesthetics at 0.3 mM or above gradually decreased the resistance.

Treatment with procaine increased the frequency of oscillations, seven examples being shown in fig. 6. Whereas the effect of the anesthetic was negligible at low concentrations, a steep increase in frequency occurred near 10^{-4} M. Lidocaine induced fast, aperiodic oscillation at about 10^{-3}

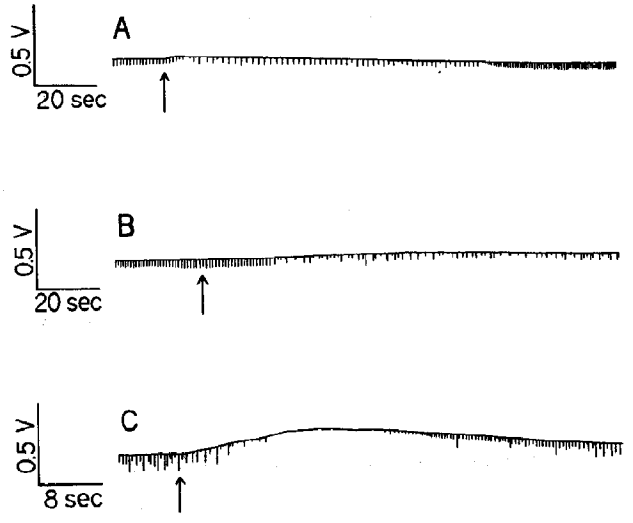

Fig. 4. Temporary suppression of oscillation after addition of anesthetics. Anesthetics were added at the time points indicated by arrows: (A) 0.3 mM, tetracaine, (B) 0.1 mM lidocaine, (C) 1 mM lidocaine.

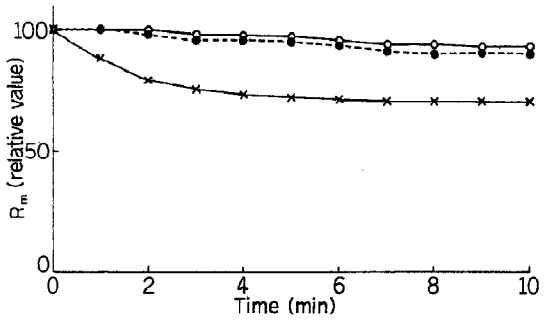

Fig. 5. Time course of change in membrane resistance for a membrane showing no oscillation. Each anesthetic was added at 0.3 mM and the membrane resistance was determined at 1-min intervals. (×——×) Tetracaine, (●- - - -●) procaine, (○——○) lidocaine.

M. Tetracaine induced the burst with a probability of 1/2, while the concentration giving rise to this phenomenon ranged from 10^{-4} to 10^{-3} M.

4. Discussion

We investigated the action of local anesthetics on the dynamic electrical properties of DOPH membranes. The self-sustained electrical oscillations in DOPH membranes occur as a result of the repetitive phase transitions of DOPH molecules coupled with diffusion of K^+ across the membrane [9–11]. The states with low and high values of the membrane resistance are associated with release and accumulation of K^+ within the membrane, respectively. These changes in membrane resistance are reflected by the alterations in the measured values of the membrane potential on application of a d.c. electric current.

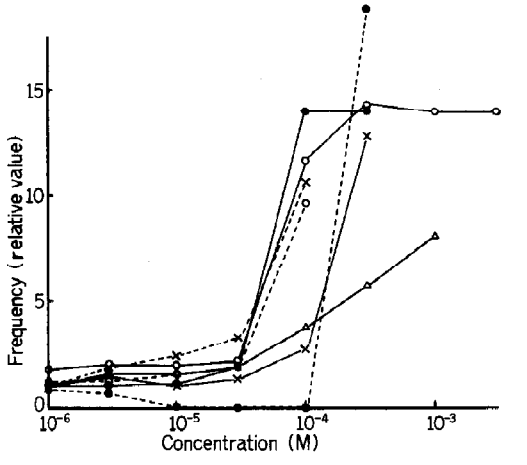

Fig. 6. Effect of procaine on the frequency of oscillation. The frequency of oscillation for untreated membranes varied over the range 0.1–0.5 Hz; hence, frequencies are shown as relative values, with the initial values being set to unity. Seven experiments are summarized.

The anesthetics suppressed oscillations of the membrane potential or resulted in their conversion to aperiodic, rapid oscillations. Strong anesthetics, such as tetracaine, tended to inhibit oscillation. The relatively less potent anesthetics, procaine and lidocaine, induced seemingly chaotic oscillations, thus being indicative of irregular and aperiodic oscillations. We shall now discuss the effects of the anesthetics with referral to fig. 2. The phenomenon of transient upward changes in membrane potential resembles that observed for membrane resistance in fig. 4 of ref. 12. However, the initial upward change in the resting potential after the addition of anesthetic is not associated with the variation in membrane resistance, as shown in fig. 5. Therefore, the initial difference can be attributed to the change in electrical potential of the surface. The decrease in magnitude of this parameter on the 1 mM KCl side causes the upward change in membrane potential. In the case of the resting potential being deflected upwards, the oscillation was suppressed as can be observed on the basis of the results for treatment at 100 μM.

The later, downwardly directed, slow change in the resting potential following the initial upward deflection was usually accompanied by bursts; this may be considered to originate from the decrease in membrane resistance, as confirmed by the data in fig. 5. The fall in membrane resistance is, therefore, related to the increase in oscillatory frequency inducing a chaotic pattern of oscillation. Evidence in support of such a mechanism is provided by the theoretical result that, on average, frequency increases with decreasing membrane resistance [10,11].

A similar phenomenon was observed for the case where bitter substances such as quinine were

applied to the DOPH membrane [19]. Here, the bitter substances penetrate into the hydrocarbon region of the lipid molecules, thereby decreasing the membrane resistance, and hence inducing aperiodic oscillations.

According to Shanes [18], anesthetics suppress the action potential without affecting the resting potential of nerve membrane. The suppression of the action potential can readily be linked with the effects of anesthetics. For electrical oscillations of the DOPH membrane, however, local anesthetics exert two contrasting effects: suppression of oscillations and induction of fast oscillations. Concerning the first effect, this model membrane is capable of reproducing the characteristic property of nerve membranes; i.e., excitability was inhibited by anesthetics. The rapid oscillations induced by anesthetics appear to be of the chaotic (irregular) type, as seen in fig. 2. Furthermore, chaotic oscillations were induced by the application of d.c. electric current on DOPH membranes between two compartments containing low, equimolar salt solutions [20]. Such types of chaotic oscillations have been reported for nerve membranes on performing stimulation by using a sinusoidal current [21]. The question as to whether chaotic oscillations appear in nerve membranes following the application of anesthetics remains to be investigated.

The situation where oscillations are influenced by chemical substances such as anesthetics is similar to that existing in electronic oscillator systems. In the latter systems, the type of oscillation occurring shows strong sensitivity to the input of a small signal to, e.g., a gate part of the transistor. We have not detected stable oscillations but have clearly observed irregular oscillations, i.e., fluctuations as shown in fig. 2 of ref. 12 on application of anesthetics on the membrane in the absence of electric current. A steady, continuous flow across the membrane may be necessary for the appearance of stable electrical oscillations. In fact, oscillations of greater regularity were observed during applications of electric current and pressure difference than in the presence of a salt-concentration gradient only [22].

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