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

I. Static and steady-state response

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The local anesthetics, tetracaine, procaine and lidocaine, interacted with a negatively charged lipid membrane composed of dioleyl phosphate (DOPH), which exhibited a self-sustained oscillation of the membrane potential. The anesthetics depolarized the membrane potential when present in increasing concentrations, whereas they increased the membrane resistance at low concentrations and decreased it at high concentrations. The above results were analyzed on the basis of electrochemical theory taking into account ion flux across the membrane. The electrical characteristics are affected by both the hydrophobicity and the diffusion constant of local anesthetics within the membrane.

1. Introduction

The site of action of anesthetics is considered to be nerve cell membranes. The mode of action on the membrane is still unclear despite numerous investigations [1–11]. Long-chain alcohols with carbon number exceeding 12 increase the phase-transition temperature of the membrane, and at the same time reduce the effect of anesthetics [12]. This fact suggests a close relationship between the fluidity of membranes and the anesthetic effect.

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The purpose of the present paper is to study the effect of local anesthetics on the electrical characteristics of an excitable model membrane constructed from a porous filter and lipid analogue, dioleyl phosphate (DOPH). We have investigated the interaction of taste substances with excitable artificial membranes including the DOPH membrane [13–15]; four kinds of taste substances, namely, sour, salty, bitter and sweet, were found to affect the electrical characteristics in different fashions. The three local anesthetics, tetracaine, procaine and lidocaine, were applied on the DOPH membrane, and the effects on the static properties such as membrane potential and resistance were investigated. An electrochemical theory was proposed to explain the results obtained. It was pointed out that both the hydro-

phobicity and the diffusion constant of anesthetics affect the electrical properties of the membrane. The following paper [16] deals with the dynamic response where a self-sustained electrical oscillation generated by the DOPH membrane is affected by the anesthetics.

2. Materials and methods

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

The membranes were processed as reported previously [13,14,17]. DOPH was synthesized by hydrolysis of the reaction product of oleyl alcohol and phosphorus oxychloride. A filter (Millipore Corp.) of cellulose ester with an average pore size of 5 μm was immersed for 1 min in a solution of DOPH in benzene, then dried in air. The quantity of DOPH adsorbed within the filter was adjusted to about 3 mg/cm^2 by regulating the concentration of the DOPH-benzene solution. The DOPH Millipore membrane was conditioned in 100 mM KCl solution for over 12 h, and then immersed in 1 mM KCl solution for a few more hours.

A DOPH membrane was placed between two cells, as shown in fig. 1. One cell was filled with 100 mM KCl and the other with 1 mM KCl. Both cells had a circular opening so that each solution could come into contact with the membrane. The membrane potential was detected with Ag/AgCl electrodes via two salt bridges, and was recorded with an XY recorder (Riken Denshi F-42CP) through a high-impedance transducer with a gain of unity. The 1 mM KCl side was grounded. The membrane resistance was measured by the potential change accompanying the application of 0.01 μA d.c. electric current for 1 s.

Concentrated solutions of each anesthetic were prepared. 1 mM KCl was added to the solutions, and the pH was adjusted to a neutral value. The anesthetics were added to the 1 mM KCl side, which was stirred throughout the experiments. The concentration of each anesthetic was increased stepwise at 30-min intervals. The membrane potential was recorded continuously, and the membrane resistance was determined at the end of each treatment. The temperature was maintained at 25 ± 2° C.

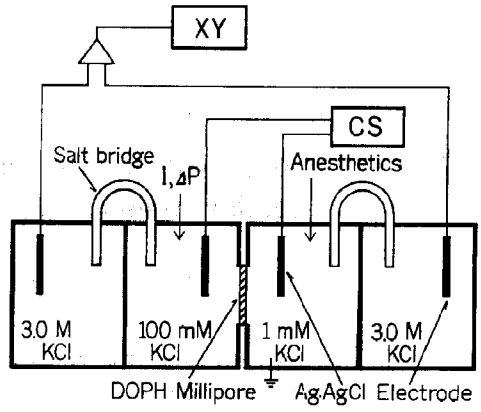

Fig. 1. Experimental apparatus. A DOPH Millipore membrane was placed between two cells containing 1 and 100 mM KCl solutions. The electrical potential was measured with Ag/AgCl electrodes via two salt bridges, and was recorded on an XY recorder (XY). The membrane resistance was evaluated from the potential change accompanying the application of a weak current with a current sweeper (CS). When d.c. current (I) and/or pressure (ΔP) were necessary to induce electric oscillation, they were applied using the current sweeper and a manometer, respectively. Anesthetics were added to the 1 mM KCl solution.

3. Results

3.1. Response of the DOPH membrane to anesthetics

Tetracaine, procaine and lidocaine were used as representative local anesthetics, the equimolar concentration of effectiveness of anesthesia being as follows: tetracaine > lidocaine > procaine [3, 10]. Fig. 2 shows the response of the membrane potential to each anesthetic. Experiments were carried out about five times for each substance. The electrical potential of the untreated membrane was about −118 mV, although the value varied between −110 and −125 mV for each membrane preparation. The negative value for the potential indicates that the membrane is permeable to cations due to the negatively charged phosphate group in the lipid DOPH.

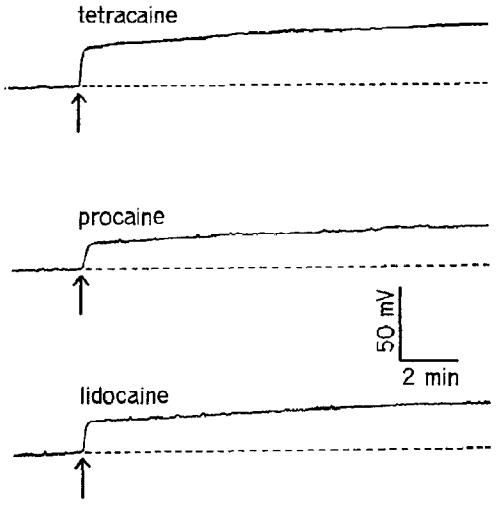

Fig. 2. Transient profiles of electrical potential. The time of addition of 0.3 mM anesthetic is shown by the arrow. Since the electrical potential was originally negative, the upward potential changes indicate depolarization.

The anesthetics increased the membrane potential, i.e., they resulted in depolarization. At concentrations between 0.1 and 1 mM, the response of the membrane potential displayed two phases; one fast, the other slow.

The rates of depolarization were about 100 and 1 mV/min, respectively. Above 1.0 mM anesthetic, the slow phase of the membrane potential response disappeared.

3.2. *Effect of anesthetics on the membrane potential*

Fig. 3 shows the potential change as a function of each anesthetic concentration. The averages and the standard deviations are shown by theoretical curves (discussed later). The anesthetic effects are presented as relative to the control. With increasing concentration of each anesthetic, the potential was shifted upwards. At higher anesthetic concentrations, the membrane potentials were shifted to positive values. Of these anesthetics, the action of tetracaine was the strongest, since its effect could be observed even at 10^{-6} M. Although procaine and lidocaine appear to be approximately equal in strength, quantitative adjustment of the experimental data by the electrochemical theory shows that lidocaine is slightly stronger than procaine.

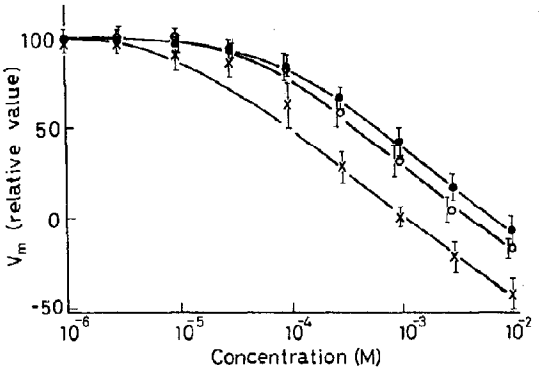

Fig. 3. Depolarization of electrical potential with anesthetics. The potential (V_m) of the untreated membrane differed slightly for each membrane, and the values were normalized to a relative value of 100. (×) Tetracaine, (●) procaine, (○) lidocaine. The standard deviations are denoted by vertical bars, and the theoretical results are shown by unbroken lines.

3.3. *Effect of anesthetics on the membrane resistance*

The change in membrane resistance caused by anesthetics is shown in fig. 4, where the averages and the standard deviations are shown together with theoretical curves. The resistance of untreated membranes was 4–6 MΩ. The effects of the anesthetics on the membrane resistance were

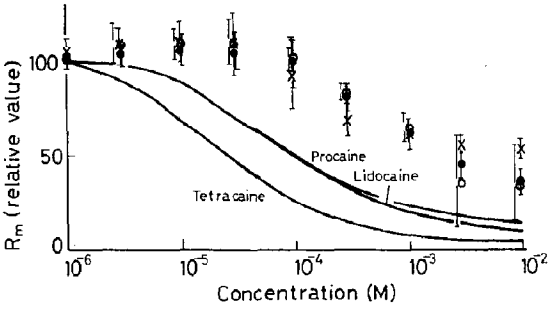

Fig. 4. Change in membrane resistance with anesthetics. The membrane resistance (R_m) was normalized in the same manner as that in fig. 3. (×) Tetracaine, (●) procaine, (○) lidocaine. The standard deviations are denoted by vertical bars with the unbroken theoretical lines.

found to be biphasic. With increasing concentration, the resistance was slightly increased up to a maximum point and further increase in the concentration decreased the resistance. This characteristic held for all the anesthetics investigated. For each anesthetic, the concentration at which the resistance began to decrease agrees approximately with that which affected the potential, as shown in fig. 3. Despite the decrease in membrane resistance, the function of the membrane as an ionic diffusional barrier is considered to be retained, since the magnitude of the resistance was as high at 2–3 MΩ even at 10^{-2} M.

4. Theory

We shall analyze the experimental results of membrane potential and resistance in figs 3 and 4. Based on constant field theory [18], we express the diffusion potential V_d within the membrane by taking the lower salt-concentration side (II) as the reference:

$$V_d = -\frac{RT}{F}\ln\Big\{\big[D_K P_K C_{Ks}(I) + D_L P_L C_{Ls}(I) + D_{Cl} P_{Cl} C_{Cls}(II)\big] \times \big[D_K P_K C_{Ks}(II) + D_L P_L C_{Ls}(II) + D_{Cl} P_{Cl} C_{Cls}(I)\big]^{-1}\Big\}, \quad (1)$$

where R, T and F denote the gas constant, absolute temperature and Faraday constant, respectively. The diffusion constants within the DOPH membrane are designated D_i where i represents K^+ (K), anesthetics (L) and Cl^- (Cl). The partition coefficient between the lipids and water is denoted by P_i. The concentrations C_{is} (I) and C_{is} (II) correspond to those of species i ($= K^+$, anesthetics and Cl^-) at the membrane surface in contact with the aqueous solutions I and II, respectively, defined by

$$C_{is}(j) = C_i(j)\exp(-z_i F V_s(j)/RT) \quad (i = K, L, Cl;\ j = I, II) \quad (2)$$

where z_i is the valency of species i, $C_i(j)$ being the bulk concentration of species i in the aqueous phase, j = I, II. The surface electrical potential, which is formed in the aqueous phase near the membrane surface, is expressed by $V_s(j)$ for each phase.

The membrane potential V_m is composed of the sum of V_d and the surface-potential difference across the membrane, $V_s(II) - V_s(I)$:

$$V_m = V_d + V_s(II) - V_s(I). \quad (3)$$

The membrane resistance R_m can be expressed as (see the appendix):

$$R_m = \frac{RTd}{F^2 D_c(I)} \cdot \frac{1 - (D_c(I)/D_c(II))\exp(-FV_a/RT)}{[1 - \exp(-FV_a/RT)](1 + V_d/V_a)}, \quad (4)$$

where V_a is the induced increment of voltage on application of d.c. electric current for measuring the membrane resistance, and d the membrane thickness. $D_c(i)$ for i = I or II is defined by

$$D_c(i) = D_K P_K C_{Ks}(i) + D_L P_L C_{Ls}(i) + D_{Cl} P_{Cl} C_{Cls}(\hat{i}), \quad (5)$$

where $\hat{i}$ signifies the other side of i (if i = I, then $\hat{i}$ = II). Since the relation between the electric current and the electrical potential across the membrane is nonlinear, the membrane resistance depends on the potential. R_m must be solved numerically from eq. 4, which includes V_a.

In the present experimental situation, $C_K(I)$ = 100 mM, $C_K(II)$ = 1 mM and $C_L(I) = 0$. Since the DOPH membrane is negatively charged, Cl^- scarcely penetrates into the membrane; then we can put $P_{Cl} = 0$. Eq. 3 with eqs 1 and 2 is reduced to

$$V_m = -\frac{RT}{F}\ln\left[\frac{C_K(I)}{C_K(II) + gC_L(II)}\right], \quad (6)$$

where the relative parameter g, which expresses the hydrophobicity multiplied by the diffusion constant, is defined by

$$g = D_L P_L / D_K P_K. \quad (7)$$

Note that the surface potentials $V_s(I)$ and $V_s(II)$ disappear in V_m and that eq. 6 does not corre-

spond to the concentrations of K^+ and anesthetics at the membrane surface but in the aqueous solution.

Similarly, the membrane resistance R_m is reduced to

$$R_m = \frac{RTd}{F^2 D_K P_K C_K(\mathrm{I})} \times \{\exp(FV_s(\mathrm{I})/RT) - [C_K(\mathrm{I})/(C_K(\mathrm{II}) + gC_L(\mathrm{II}))] \times \exp[F(V_s(\mathrm{II}) - V_a)/RT]\} \times \{[1 - \exp(-FV_a/RT)](1 + V_d/V_a)\}^{-1}. \quad (8)$$

By taking a limit of $V_a = 0$, we obtain

$$R_m = \frac{R^2T^2d}{F^3 D_K P_K C_K(\mathrm{I}) V_d}\left[\exp(FV_s(\mathrm{I})/RT) - \frac{C_K(\mathrm{I})}{C_K(\mathrm{II}) + gC_L(\mathrm{II})}\exp(FV_s(\mathrm{II})/RT)\right]. \quad (9)$$

Comparisons with the experimental data are made in figs 3 and 4 using eqs 6 and 8. The values of g are 17.3, 21.1 and 116.4 for procaine, lidocaine and tetracaine, respectively. This order is in agreement with those of the degree of hydrophobicity [19,20] and anesthetic potency [3,10]. Dependence of the surface potential on the anesthetic concentration has not been observed, and hence we assumed $V_s(j) = 0$ in R_m of eq. 8 for simplicity in order to observe the overall trend.

Although the agreement between the theoretical and experimental results is excellent for the membrane potential, it is poor in the case of the membrane resistance. In the experiment, the resistance increases once and then decreases, but a monotonic decrease is expected according to the theory. This discrepancy may be due to three factors. The first is neglecting to account for the surface potential (discussed later). The second is concerned with an unstirred layer formed near the membrane, which affects the membrane electrical characteristics [21]. The third may be the assumption that D_i and P_i for $i = \mathrm{K}$, L stay constant during the variation of the concentration of anesthetics. These quantities may be altered with the adsorption of anesthetics into the membrane, leading to changes in the states of aggregation of DOPH molecules.

5. Discussion

We investigated the action of local anesthetics on the static and steady electrical properties of the DOPH Millipore membrane. The electrical potential of the membrane under the condition of no oscillation was observed to increase in proportion to the logarithms of the concentration of anesthetics. Increase in anesthetic concentrations up to 10^{-2} M increased the potential to zero and above. For artificial lipid membranes, changes in membrane potential have been reported by Papahadjopoulos [22] and McLaughlin [10]. Nevertheless, membrane potentials do not change in nerve membranes [23]. On gustation, the electrical response of the DOPH membrane [13,14] resembles that of biological membranes [24,25]. The DOPH membrane might also reproduce the strength of the anesthetic effects for changing the membrane electrical properties in accordance with that for blocking the nerve.

The effect of the anesthetics on membrane resistance was biphasic; these substances increased it at low concentrations but lowered it at high concentrations. The biphasic action of anesthetics is not an uncommon observation. Inhalation anesthetics such as halothane increase the order parameter of synaptic membranes at low concentrations and decrease it at high concentrations [26]. Various authors have suggested that halothane fluidizes [27], rigidifies [28] or has no effect [29] on the internal motion of probes incorporated into lipid bilayers at the clinical concentration of 1 mM.

The surface electrical potentials $V_s(\mathrm{I})$ and $V_s(\mathrm{II})$ disappeared formally in the expression for the membrane potential, V_m. The same situation occurs in the taste cell [30]. This result implies that the surface potential cannot be ignored even if the membrane potential apparently obeys the equation for the diffusion potential. In fact, the mem-

brane resistance remained unchanged soon after the application of anesthetics on the DOPH membrane (see fig. 5 in ref. 16) in spite of the alteration in membrane potential shown in fig. 2. This result is in support of a large contribution of the surface potential to the membrane potential.

The explicit expression for the surface potential V_s may include the Donnan potential or sometimes the Gouy-Chapman double-layer potential according to the conditions actually existing, as shown theoretically [31]. We assumed V_s to be zero in the estimation of R_m. The surface potential is a function of the fixed charge density of the membrane, but in the present case the manner in which anesthetics affect the charge density is unknown. Qualitatively, the anesthetics may decrease the magnitude of the charge density, which is a negative quantity due to the phosphate group of the DOPH molecule. This process leads to a decrease in magnitude of V_s(II). The membrane resistance R_m, therefore, is increased as indicated by eq. 9 even when the diffusion potential V_d (< 0) is constant. This results in an improvement in the theory for R_m. It is necessary to consider an adsorption equilibrium between the membrane and anesthetics for discussing quantitatively the effect of the surface potential, as has been carried out in other systems composed of bilayers and Ca^{2+} [32–34].

The discrepancy in the values of the membrane resistance R_m between theory and observation may be due to two factors in addition to neglecting of the surface electrical potential as discussed above, i.e., the effect of the unstirred layer formed near the membrane surface and the assumption of constant D_i (and P_i) for i = K, L. The effect of the unstirred layer may require further study, although preliminary experiments showed that the membrane potential increased by several millivolts and the membrane resistance decreased by a few percent when a 1 mM solution was stirred vigorously.

If we assume that decreases in D_K and D_L occur with successive addition of anesthetics, the membrane resistance R_m in eq. 9 can increase, as observed experimentally (fig. 4). Whereas R_m changes directly in this way, the membrane potential V_m in eq. 6 is scarcely altered because the ratio $g = D_L P_L / D_K P_K$ remains constant when D_L is decreased almost in proportion to D_K. Furthermore, the decrease in D_L is not necessary at such low anesthetic concentrations as $C_L(II) \ll C_K(II)$: V_m of eq. 6 is unaltered due to $C_K(II) + gC_L(II) \simeq C_K(II)$ even if we assume the decrease to occur in D_K only. We can therefore expect a substantial improvement by considering such decreases in D_K (and in D_L) in the theory, whereas a reliable expression for the dependence of D_K on anesthetic concentration has not been derived as yet. These variations in the diffusion constants may occur due to conformational changes in DOPH assemblies on the adsorption of anesthetics on the membrane, as observed using scanning electron microscopy to study the adsorption of monosodium glutamate, where the membrane resistance was also increased [35].

At concentrations where the membrane resistance was decreased by the anesthetics, the membrane potential was changed (figs 3 and 4). This suggests that the anesthetics act on the diffusion potential of the membrane. Since the hydrophobicity of the anesthetics facilitates the penetration of anesthetic molecules into the DOPH membrane, the anesthetics can also affect the diffusion potential.

The theory gave a quantitative explanation of the observed membrane potential. As can be seen from eq. 6, the membrane potential is determined not only by the partition coefficient P_i but also by the diffusion constant D_i within the membrane. The kinetic parameter reflecting diffusion may also have an effect on the anesthetic potency, although previous works have dealt solely with the hydrophobicity [5–10].

Appendix

The electric current I_i flowing across the membrane from phase I to phase II is given by [18]

$$I_i = -\frac{D_i P_i F^2 V_{ma}}{RTd} \times \left[\frac{C_{is}(I) - C_{is}(II)\exp(-z_i F V_{ma}/RT)}{1 - \exp(-z_i F V_{ma}/RT)}\right] \quad \text{for } i = K, L, Cl, \tag{A1}$$

where z_i is the valency of species i and V_{ma} is defined by

$$V_{ma} = V_d + V_a, \qquad (A2)$$

with V_d designating the diffusion potential within the membrane without an applied d.c. electric current and V_a the increase in potential brought about by the applied current. The concentration at the membrane surface $C_{is}(j)$ is given by eq. 2. Eq. 1 is readily obtained from the condition where the total electric current I, which is the summation of I_i with respect to i, equals zero when there is no applied current. The membrane resistance R_m is given by V_a/I. By using eqs 1 and A1, we obtain eq. 4.

Let us evaluate the magnitude of membrane resistance without application of anesthetics by assuming $V_s(I) = V_s(II) = 0$ in eq. 9. The membrane thickness d is about 10^{-4} m and the diffusion constant within the membrane D_K approx. 10^{-9} cm^2/s [17]. Assuming the partition coefficient P_K to be of the order of 0.1, we estimate the numerical value of R_m in eq. 9 at 3.1 MΩ cm^2. This agrees well with the observed value.

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