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PERMEABILITY OF AXON MEMBRANES TO LOCAL ANESTHETICS

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Summary

The permeability of the neutral form of tertiary amine local anesthetics across squid axon membranes was studied by utilizing three different experimental methods: (1) narcotic action of axon excitability was measured by monitoring the time derivative of action potential and the results were analyzed in terms of a diffusion reaction equation of local anesthetics to obtain their permeabilities; (2) the influx of local anesthetic into the axon was measured by use of the radioisotope tracer technique; and (3) the desorption rates of the neutral form of local anesthetics from lipid monolayers were measured and the desorption rate was correlated with permeability.

The relative permeabilities obtained for procaine, lidocaine and tetracaine by the above three methods were comparable. The order of relative permeabilities was procaine > lidocaine > tetracaine, and had an inverse correlation with the partition coefficients of anesthetics at oil/water phases. Some discussion concerning the concept of permeability is made when the partition coefficient of a permeant molecule is high.

Introduction

In the previous study [1] we have examined the mode of action of tertiary amine local anesthetics on axon membranes, and confirmed that the mode of action proposed first by Narahashi et al. [2] is the most probable one among others; a local anesthetic molecule penetrates through the membrane into the axon interior and acts in a charged form [3] on the axon membrane from the intracellular phase.

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Our analysis was based on a diffusion reaction equation of local anesthetics through the membrane system. Through these analyses, we were also able to estimate the permeability coefficients of uncharged forms of local anesthetics across the membrane.

The uptake of local anesthetics into squid axons with variation of extracellular pH was measured by use of the isotope tracer technique, and we concluded [4] that the neutral form of local anesthetics is indeed more permeable than the charged form of local anesthetics through the axon membranes. Similar observations were made with sciatic nerves of frog and toad by Strobel and Bianchi [5] and Akerman [6]. However, the authors of the latter two works did not isolate the anesthetic uptake in the axon interior from those of the compartments other than the axoplasm.

Collander and Barlund [17], and others, studied the permeability of cell membranes to various non-electrolytes and related their permeability to their partition between oil and water phases. However, the partition coefficients for the molecules used by these authors were relatively small (<1) compared with those of the neutral forms of local anesthetics. Recently, the permeability of axon membranes to local anesthetics has been discussed and an attempt for their estimation has been made [8] with the use of data from model membrane studies [9,10].

Our previous results on the permeabilities of local anesthetics [1] showed rather different results from what was anticipated, in terms of their partitioning into oil and water phases.

In order to investigate this further, we re-examined the diffusion reaction equation of local anesthetics and obtained more exact solutions. Experimental results obtained for squid axons by similar electrophysiological methods as used in the previous experiment [1] were analyzed according to the theory developed below. Also, in order to compare the results obtained using the electrophysiological method, the direct measurements of local anesthetic molecules transported across the axon membrane by use of the isotope tracer technique was performed. In addition, in order to investigate the permeability further, the rate of desorption of local anesthetic from lipid monolayers was measured and the permeability of the neutral form of local anesthetic was inferred and discussed with relation to the desorption rate of local anesthetic molecules from the lipid monolayers. These types of studies have not been previously reported and may provide important information regarding anesthetic action processes on nerve membrane excitability. Finally, the results of our studies on the permeability of local anesthetics and other related studies are discussed.

Theory

The distribution of charged and uncharged molecules of tertiary amine local anesthetics in an aqueous solution is approximately expressed by the Henderson-Hasselbach equation as follows:

$$\mathrm{pH} = \mathrm{p}K_a - \log([\mathrm{BH}^+]/[\mathrm{B}]) \qquad (1)$$

where $\mathrm{p}K_a$ is the dissociation constant of the local anesthetics, and $[\mathrm{BH}^+]$

and [B] are the concentrations of positively charged and uncharged local anesthetic molecules, respectively.

Let us consider the flux of the neutral form of local anesthetics entering into the axon when a given concentration of local anesthetics is applied to the extracellular phase. By assuming that only the uncharged form of local anesthetics is able to penetrate through the membrane, which is a reasonable approximation [4], the flux, J, of the uncharged anesthetic molecules across the axon membrane may be expressed by

$$J = P([B]_o - [B]_i) \tag{2}$$

where P is the permeability coefficient of the uncharged local anesthetic molecule through the membrane, and the subscripts, o and i, refer to the extracellular and intracellular phases, respectively.

The time change of the total concentration of the local anesthetic ($C_i = [BH^+]_i + [B]_i$) in the intracellular phase, is

$$\frac{\partial C_i}{\partial t} = J\frac{A}{V} = J \cdot \frac{2}{r} \tag{3}$$

where A and V are the area and volume per unit length of the axon, respectively, and r is the radius of the axon. Then, the total concentration (C_i) of local anesthetic in the intracellular phase at time t after the extracellular application of a given concentration (C_o) of the local anesthetic, in the case where the extracellular and intracellular pH remain constant, will be given by integrating Eqn. 3 with respect to time:

$$C_i = C_i^+ \frac{\beta}{\beta - 1} = C_o \cdot \frac{\beta}{\alpha} \cdot \left(1 - \exp\left(-\frac{2Pt}{r\beta}\right)\right) \tag{4}$$

where $\alpha = 1 + 10^{pK_a - pH_o}$, $\beta = 1 + 10^{pK_a - pH_i}$, and pH_o and pH_i are the pH values at the extracellular and intracellular phases, respectively, and $C_i^+ \equiv [BH^+]_i$.

Eqn. 4 is the expression for the intracellular concentration of local anesthetic (either C_i or C_i^+) at time t after the extracellular application of a given concentration C_o of local anesthetic.

If we choose 't' as the time to exhibit a defined anesthetic action (any degree of anesthetic action can be defined as one chooses), C_i^+ should be the minimum concentration $C_i^{+,min}$ of the positively charged form of local anesthetic to exert a 'defined anesthetic' action in the intracellular phase. One of the ways to obtain the minimum intracellular concentration of the charged form of local anesthetic $C_i^{+,min}$ is the use of Eqn. 4. By knowing the minimum concentration C_o^{min} ($t = \infty$) of local anesthetic applied extracellularly to exert the defined narcotic action at infinite time, the minimum intracellular concentration of the charged form is obtained:

$$C_i^{+,min} = \frac{\beta - 1}{\alpha} C_o^{min}(t = \infty) \tag{5}$$

Since α, β and r (radius of the axon) are measurable quantities and $C_i^{+,min}$ can be determined as described above, we should be able to obtain the permeability, P, of the uncharged form of local anesthetics through the membrane from the

relation (Eqn. 4) between a concentration of local anesthetic applied extracellularly and the time to exert a 'defined' narcotic action.

This type of analysis for the permeability of local anesthetic can be done by measuring the narcotic action of local anesthetics on axon membrane excitability by use of a standard electrophysiological technique [1].

Another way to measure the permeability, is as follows: If the concentration of local anesthetics in the axon interior phase C_i is measured with respect to time after the application of a given extracellular concentration of local anesthetic, C_o, the permeability of the neutral form of anesthetics through the axon membrane will be obtained from Eqn. 4, provided that α, β and r are known. The internal concentration of the local anesthetic can be measured by the radioisotope tracer technique where radioactively labeled anesthetics are used.

Another way to relate the permeability of local anesthetics through membranes may be done by measuring the desorption rate of local anesthetics from a lipid monolayer to an aqueous subphase. Let us consider that at the beginning of the experiment there are anesthetic molecules of the neutral form only in the monolayer phase but not in the subphase solution. We then measure the desorbed anesthetic molecules from the monolayer into the subphase solution where the subphase solution has much higher pH than the pK_a value of local anesthetics, so that most anesthetic molecules desorbed into the subphase solution should be in the neutral form. Also, we suppose that the volume of the subphase solution is much larger (over 10^6) than that of the monolayer phase, so that at the equilibrium state, a very little amount of local anesthetic would stay in the monolayer phase, which would not exhibit any appreciable effect of anesthetic molecule on the physical properties of the lipid monolayers.

In this situation the change in concentration of local anesthetic in the lipid monolayer with respect to time, after spreading a monolayer at the air/water interface would be expressed by:

$$\frac{dC}{dt} = -\frac{dJ_d}{dx} \tag{6}$$

where $J_d \cong P_d C$, J_d is the flux of local anesthetic molecules desorbed from a monolayer. P_d is a constant which may relate to the permeability of local anesthetic molecules. Then, Eqn. 6 is rewritten as:

$$\frac{dC}{dt} = -P_d \frac{dC}{dx} \equiv P_d \frac{C}{\Delta x} \tag{7}$$

where Δx is related to the thickness of a diffusion layer just below the monolayer, which is a constant. This equation corresponds to that describing the steady-state desorption process, where $P_d/\Delta x$ corresponds to the factor $B_2 \exp(-w/kT)$ given in the literature [11]. Here, B_2 is a constant depending on the hydrodynamics of the diffusion process, and w is the energy of desorption of the molecule from the monolayer. This steady-state desorption kinetics would represent well that of the molecular desorption process occurring from the axon membranes. Because the steady-state desorption process of local anesthetics from a monolayer membrane occurs after a water stagnant layer has been established, while in the axon membrane system the stagnant layer is always established. Then, Eqn. 7 can be integrated easily with respect

to time, and gives

$$C(t) = C_o \cdot [\exp(-P_d t/\Delta x)] \tag{8}$$

where C_o is the concentration of local anesthetics in the monolayer when the monolayer is first spread. Although C_o is a rather ill-defined quantity, it can be considered a constant as long as the spreading conditions to form monolayers are kept the same for all experiments.

If we suppose that all local anesthetic molecules (tetracaine, procaine and lidocaine) occupy the same area in the lipid monolayer, the amount of local anesthetic desorbed from the monolayer would reflect an increase in the area, A, per lipid molecule when the total area of monolayer is kept constant. Then the change in area per lipid molecule would be proportional to the change in the concentration of local anesthetic in the monolayer.

$$A(t) - A(0) \equiv \Delta A(t) \propto C(0) - C(t) \tag{9}$$

Thus,

$$\Delta A(t) \propto C(0) - C(t) = C_o \cdot \left(1 - \exp\left(-\frac{P_d}{\Delta x} t\right)\right). \tag{10}$$

This equation shows the time-dependence of the change in area per lipid molecule in terms of C_o and P_d. Consequently, by performing this type of measurement for each anesthetic, the relative degree of adsorption of local anesthetics can be obtained from Eqn. 10.

Materials and Methods

Electrophysiological studies

The axons used in the experiments were from the squid, *Loligo pealii*, available at Marine Biological Laboratories, Woods Hole, MA. A single giant axon about 400 ± 25 μm in diameter was isolated and cleaned by removing the surrounding connective tissues under a dissecting microscope. The length of axons between the two cut ends was about 5—6 cm. The isolated axon was tied with thread at each end and immersed in an extracellular solution in a lucite cell. The extracellular solution was continuously flushed around the axon. The cell arrangement was such that a complete change of solution could be carried out within 20 s.

Membrane potentials were measured by the intracellular microelectrode technique. The membrane potential and its time derivative were recorded on an oscilloscope screen (Tektronix type 564) and on a chart recorder. The details of the method have been described in the previous paper [1]. The 'standard' extracellular solution consisted of 457 mM NaCl; 8.9 mM KCl; 10 mM $CaCl_2$; and 24.9 mM $MgCl_2$; 37 mM $MgSO_4$ and 1 mM Tris base, and the pH of the solution was adjusted by HCl to 8.0.

Local anesthetics used were procaine-HCl and tetracaine-HCl, both obtained from Mann Research Lab., and lidocaine, which was given by Dr. Takmann, Astra Pharmaceutical Co. They were added in the above standard extracellular solution. The pH of the solution was again adjusted with HCl or NaOH to 8.0 at the beginning of each experiment.

In each experiment, the axon was first placed in the 'standard' extracellular solution (pH 8.0) without local anesthetics. Resting and action potentials were checked. Then a standard extracellular solution containing a given concentration of a local anesthetic was flushed around the axon. The time to exert a 'narcotic action', which we defined as the reduction to two-thirds of the maximum of the time derivative of the action potential, was recorded. After the application of each local anesthetic solution, the extracellular medium was switched back to the standard solution without local anesthetics for a sufficient time to bring back to complete recovery the resting and action potentials, including their time derivatives. For each experiment, the flow rate of the external flushing solution was kept the same. The experiments were done at 20°C.

Permeability study by radioisotope tracer technique

The axons used were the same as described above. The axon was carefully dissected in order not to cut short the branches from the axon. The length of the axon dissected was about 5.5 cm. The isolated axon was tied with thread at each end, and then immersed in a standard extracellular solution containing a given concentration (approx. 0.5 μCi/ml) of radioactively labeled local anesthetics. The specific activities of radioactive anesthetics in these solutions were 18 μCi/mg for procaine and 21 μCi/mg for lidocaine. After a certain time of immersion, the axon was taken out and rinsed well in the standard extracellular solution of pH 6.0 for about 15 s. Then, the axon was taken from the rinsing solutions and placed on filter paper to remove as much adsorbed extracellular solution from the axon as possible. The axon was then placed on a rubber platform and one end of the axon was cut so that the total length became about 4.0—4.5 cm. Then the cut end was placed on a thin parafilm and the axoplasm was squeezed out from the axon onto the thin parafilm with a micro-rubber roller.

The radioactivity of each axoplasm extruded was counted using a liquid scintillation counter. The ratio of concentration of local anesthetic in the intracellular phase to that in the extracellular phase was determined by taking the ratio of radioactivity of the axoplasm to that of an equal volume of the standard extracellular solution containing radioactively labeled local anesthetic.

Radioactively labeled local anesthetics used were ^{14}C-labeled procaine-HCl and also ^{14}C-labeled lidocaine-HCl purchased from New England Nuclear. A liquid scintillation counter (Beckman, LS 9000) available at Marine Biological Lab., Woods Hole, MA, was used for counting the β-ray activity.

Electrical activity of axons was checked by observing the action potential by means of extracellular stimulus and recording techniques before and after immersing the axons in local anesthetic solutions. Only the axons showing the activity before and after immersing in the experimental solution containing local anesthetics were used in the experiments. The local anesthetic concentration used (approx. 0.5 μCi/ml) should not affect the excitability of axons appreciably. All the experiments were done at room temperature of 20°C.

Desorption study of local anesthetics from lipid monolayers

Egg lecithin, purchased from Avanti Biochemical Co., was used to form a

monolayer at the air/water interface. Local anesthetics used were procaine base and tetracaine base, both purchased from Sigma Chemical Co. and lidocaine base (Astra Pharmaceutical Co.). They were all USP grade. Monolayer spreading solutions contained 1 mM phosphatidylcholine and 3 mM local anesthetic in *n*-hexane/chloroform (19 : 1) solution. Chloroform was needed to dissolve the local anesthetics completely. Hexane and chloroform were 'Instra-Analyzed' grade from Baker Chemical Co.

Instead of measuring the change in area per molecule of monolayers, the surface tension was measured. The monolayer was formed on an aqueous surface of 61 cm^2. The aqueous solution was 0.5 M NaCl/1 mM Tris-HCl, pH 10.5, which may be considered analogous to natural sea water in ionic strength. NaCl used was purchased from Fisher Chemical Co., and was roasted at 400—500°C for 1 h. Water was triple-distilled water including the process of alkaline $KMnO_4$ distillation. The monolayer-forming solution was spread so that the area per lipid molecule corresponds to 90 $Å^2$. Surface tension was measured by an electronic balance (Beckman) with the use of a microscope cover slip glass (18 × 18 × 0.2 mm) as a Wilhelmy plate.

The output of the electromicrobalance was monitored by a strip chart recorder (Omniscribe, Houston Inst.). The experiments were performed at 24 ± 2°C.

Results and Discussion

Fig. 1 shows the relationship between a given concentration of extracellularly applied local anesthetic, C_o, and time, t, to exert the 'narcotic action' for the case of procaine. The ordinate stands for the quantity of $C_o \cdot 10^{pK_a - pH_i}/(1 + 10^{pK_a - pH_o})$, and the absissa stands for $(1 + 10^{pK_a - pH_i})/t$. The pK_a for procaine is 8.9, the extracellular pH (pH_o) = 8.0, and the intracellular pH (pH_i) = 7.3. The internal pH of 7.3 was measured experimentally in the similar manner reported previously [12] when the extracellular pH was 8.0. There was no appreciable change in internal pH from the application of local anesthetic under this condition. Since r (axon radius) and $C_i^{+,min}$ (the minimum intracellular concentration of positively charged local anesthetics) are obtainable quantities, the permeability P of the neutral form of local anesthetic can be obtained by searching of the best fitting value for P to the experimental points shown in Fig. 1. In Figs. 2 and 3, the same relationships as in Fig. 1 are shown for the cases of lidocaine and tetracaine. In these cases, pK_a for lidocaine and tetracaine are 7.9 and 8.4, respectively.

The minimum concentration of local anesthetic applied externally to exert the defined 'narcotic action' at $t = \infty$ was 0.3 ± 0.1 mM for procaine. With this value and Eqn. 4, $C_i^{+,min}$ is calculated to be 1.3 ± 0.5 mM. Taking an average diameter of 400 μm for axons used, and with Eqn. 4, the permeability through the axon membranes of the uncharged procaine molecule is estimated to be about $5.3 \cdot 10^{-4}$ cm/s (Table II). For lidocaine and tetracaine, the minimum extracellular concentrations at an infinite time were 0.3 ± 0.07 mM and 0.035 ± 0.01 mM, respectively. Thus, the minimum intracellular concentrations of the positively charged form of local anesthetics are estimated to be 0.66 ± 0.3 mM and 0.12 ± 0.06 mM, respectively (given in Table I). The per-

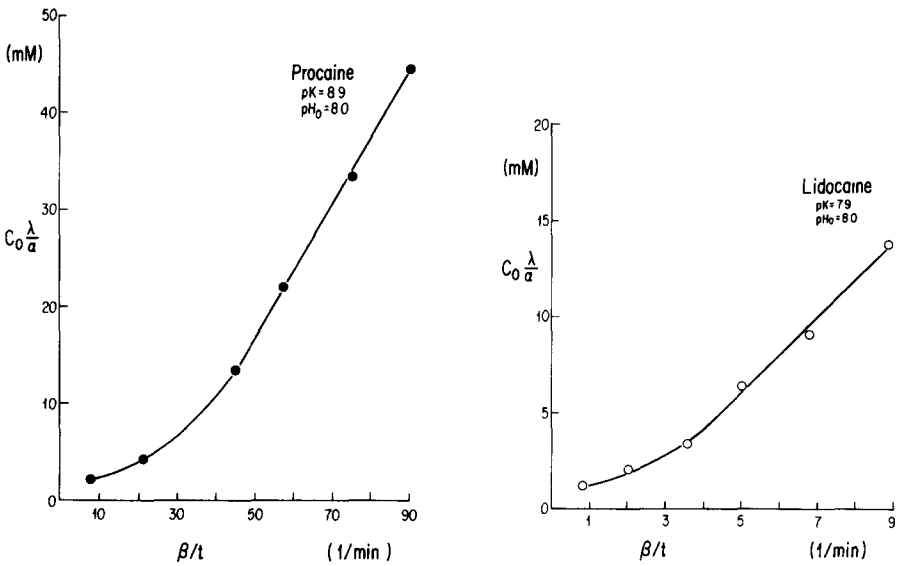


Fig. 1. The relationship between $C_o\lambda/\alpha$ and β/t for procaine at extracellular $pH_o = 8.0$. $\alpha = 1 + 10^{pK_a - pH_o}$; $\beta = 1 + 10^{pK_a - pH_i}$; $\lambda = 10^{pK_a - pH_i}$; $pK_a = 8.9$.

Fig. 2. A similar relationship to that shown in Fig. 1, but for lidocaine. $pH_o = 8.0$; $pK_a = 7.9$.

TABLE I

Observed minimum concentrations of local anesthetics in the extracellular phase to exert a 'defined narcotic action' at infinite time C_o^{min} $(t = \infty)$ and the calculated minimum concentrations $C_i^{+,min}$ of the charged forms of local anesthetic in the same narcotic action on the neme axon. $\alpha = 1 + 10^{pK_a - pH_o}$, $\beta = 1 + 10^{pK_a - pH_i}$. Values are given in mM and represent the mean ± S.E.

	C_o^{min} $(t = \infty)$	$C_i^{+,min} = ((\beta - 1)/\alpha) \cdot C_o^{min}$ $(t = \infty)$
Procaine	0.3 ± 0.1	1.3 ± 0.5
Lidocaine	0.3 ± 0.07	0.66 ± 0.3
Tetracaine	0.035 ± 0.01	0.12 ± 0.06

TABLE II

PERMEABILITY OF NEUTRAL FORM OF LOCAL ANESTHETICS

Values are given in cm/s and represent the mean ± S.E. ($\times 10^4$).

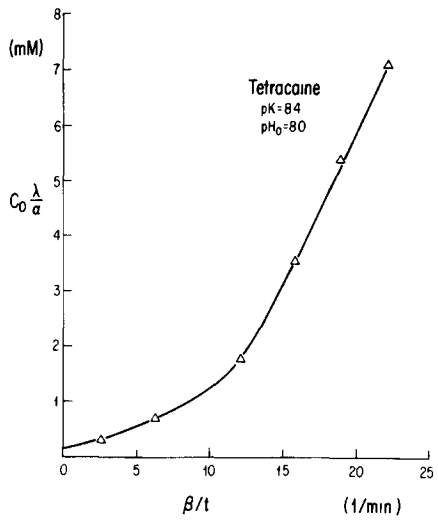
	Electrophysiological technique	Isotope tracer technique
Procaine	5.3 ± 0.5	4.1 ± 1.2
Lidocaine	1.1 ± 0.2	0.76 ± 0.3
Tetracaine	0.52 ± 0.1	

TABLE III

RELATIVE PERMEABILITY OF NEUTRAL FORM OF LOCAL ANESTHETIC

Values represent mean ± S.E.

	Electrophysiological technique	Isotope tracer technique	Desorption	Partition coefficient at oil/water phases
Procaine	1	1	1	45 [15]
Lidocaine	0.21 ± 0.5	0.18 ± 0.6	0.33 ± 0.6	225 [15]
Tetracaine	0.10 ± 0.3		0.19 ± 0.4	273 [16]



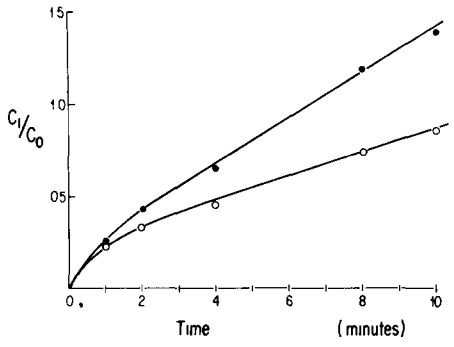


Fig. 3. A similar relationship to that shown in Fig. 1, but for tetracaine. $pH_o = 8.0$; $pK_a = 8.4$.

Fig. 4. The ratio of intracellular concentration of local anesthetic to extracellular concentration at various times after the axon was exposed to the local anesthetic solution (0.5 μCi/ml) at pH 8.0. ●, procaine; ○, lidocaine.

meabilities obtained for lidocaine and tetracaine are $1.1 \cdot 10^{-4}$ cm/s and $0.52 \cdot 10^{-4}$ cm/s, respectively (Table II). The relation of the extracellular concentration of lidocaine and the time to exert the narcotic action at $pH_o = 8.0$, is similar in magnitude to those in the case of procaine. Tetracaine shows stronger potency within a short time. However, for the permeability of the neutral form, those of lidocaine and tetracaine are more similar to each other than that of procaine. Each experimental point in Figs. 1, 2 and 3 is an arithmetic average over at least four successful experiments. The order and relative magnitudes of permeabilities of uncharged local anesthetics are the same as those reported previously [1].

The experimental results obtained from the isotope tracer studies are shown in Fig. 4. The ratios of the total intracellular concentration of local anesthetics to that of the extracellular local anesthetics are plotted against the time during which the axon was immersed in the local anesthetic solution, for the cases of procaine and lidocaine.

The relationship between the concentration ratios (C_i/C_o) and time should tend to saturate at the limiting values after a long time. The permeabilities of these anesthetics are calculated to be $4.1 \cdot 10^{-4}$ cm/s and $0.76 \cdot 10^{-4}$ cm/s, respectively, which are comparable to those obtained by the electrophysiological techniques shown in Table II.

It was found [4] that the ratio of the internal distribution of procaine due to the uptake of procaine into axons with respect to the external distribution was about 400% at pH 7.9, which was measured at 10 min exposure of axons to the procaine solution. This value is rather large compared with the present experimental results (150%). According to Eqn. 4, the ratio of the internal concentration of local anesthetic at maximum uptake with respect to

the extracellular local anesthetic concentration should be β/α, which is 4.5 (450%) for procaine. The value even at the extracellular pH 7.9 obtained by Dettbarn et al. [4] is close to the maximum uptake value. However, our experiments show that the anesthetic uptake is not saturated after 10 min exposure to the anesthetic solution. The difference may come from different experimental techniques used to measure the volume of extruded axoplasm from axons. However, it should be mentioned that the pH dependence of uptake of procaine which we have obtained is similar to those obtained by the earlier workers [4].

A typical experimental result of the change in surface tension with respect to time is shown in Fig. 5,I. In Fig. 5,I, curve A corresponds to the case of a control experiment in which a phosphatidylcholine monolayer does not contain local anesthetic; curve B refers to the case of a phosphatidylcholine monolayer containing procaine and curve C the case of the phosphatidylcholine-lidocaine monolayer and D the case of the phosphatidylcholine-tetracaine monolayer. There are clear differences among B, C and D with respect to the time to reach a stationary state (or an equilibrium state). For B, C and D, they are 1.5 ± 0.2 min, 3.5 ± 0.4 min, 8 ± 0.5 min, respectively. By subtracting the control experimental value A from B, C and D, we can obtain the time-dependent behavior of surface tension changes due to desorption of local anesthetics, which is shown also in Fig. 5,II. Although surface tension γ of the monolayer versus area per lipid molecule is not quite a linear relation, we may consider that they are linearly related within a small change in area per molecule. Then,

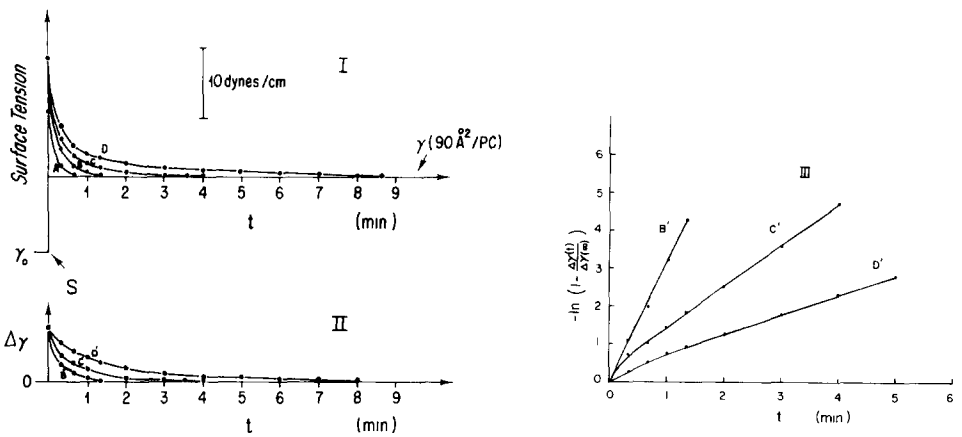


Fig. 5. I, Time behavior of surface tension of monolayers. The arrow S indicated the point where a single drop as an aliquot (approx. 10 μl) of the monolayer spreading solution was spread on 0.5 M NaCl buffer solution. The lipids were spread so as to form the monolayers having area per lipid molecule of 90 Å^2. (A) phosphatidylcholine (PC) monolayer without anesthetics, (B) PC (1 mM) + procaine (3 mM) monolayer, (C) PC (1 mM) + lidocaine (3 mM) monolayer, (D) PC (1 mM) + tetracaine (3 mM) monolayer. γ_0, surface tension of 0.5 NaCl (72 dyn); γ, surface tension of the PC monolayer of 90 Å^2/molecule on 0.5 M NaCl (61 dyn). II, Time behavior of difference in surface tension between those of PC monolayers with and without anesthetics. (B'): B — A — procaine; C': C — A — lidocaine; D': D — A — tetracaine. III, The desorption kinetics of local anesthetics from lipid monolayer. The quantities related to the change in surface tension of lipid monolayers due to the desorption of local anesthetics from the monolayers ($-\ln(1 - \Delta\gamma(t)/\Delta\gamma(\infty))$) are plotted against time t, where $\Delta\gamma(t) \equiv \gamma(t) - \gamma(0)$, $\Delta\gamma(\infty) \equiv \gamma(\infty) - \gamma(0)$.

the change in surface tension is proportional to the change in concentration of local anesthetic in the monolayer.

$$\gamma(t) - \gamma(0) \equiv \Delta\gamma(t) \propto C_o\left(1 - \exp\left(-\frac{P_d t}{\Delta x}\right)\right),\ \Delta\gamma(t) = \Delta\gamma(\infty)\left(1 - \exp\left(-\frac{P_d t}{\Delta x}\right)\right) \tag{11}$$

where $\Delta\gamma(\infty) \equiv \gamma(\infty) - \gamma(0)$.

Eqn. 11 reads

$$\frac{P_d t}{\Delta x} = -\ln\left(1 - \frac{\Delta\gamma(t)}{\Delta\gamma(\infty)}\right). \tag{12}$$

Therefore, by plotting the quantities of $\ln(1 - \Delta\gamma(t)/\Delta\gamma(\infty))$ in Eqn. 12 with respect to time t for each local anesthetic case (Fig. 5,III), the relative 'permeabilities' P_d for local anesthetics can be obtained from the slope of the curve at the steady-state desorption process. The analysis of the experimental results gives the ratios of permeabilities of procaine to lidocaine and tetracaine 0.33 and 0.19, respectively. These relative permeabilities are comparable to those obtained from the electrophysiological technique as well as the isotope tracer technique (see Table III). The monolayer studies on the correlation between local anesthetic uptake to the monolayer and its partition coefficient at the oil/water phase were first done by Skou [13].

Although the above described experiments are not a complete set of experiments (e.g., missing tetracaine experiment in the isotope tracer study, etc.), it seems clear that the order of permeabilities of the three local anesthetics used through squid axon membranes is: procaine > lidocaine > tetracaine.

The permeability results estimated from local anesthetic uptake by Strobel and Bianchi [5] are not comparable with our results because they have collected a total uptake of local anesthetics not only into the axon interior, but also to the axon membrane as well as the extracellular surrounding tissues. The order of permeabilities of anesthetics obtained here is not the one expected from the commonly held concept used to relate permeability of a membrane to a substance, and its partition between oil and water phases. The factors relating non-electrolyte permeabilities are considered as follows, according to Danielli [14]: (a) species characteristic, (b) lipid solubility, (c) molecular volume, (d) specific chemical groups, (e) position of the groups on the molecule. When the partition of a substance between lipid and water is not very large (say, less than 0.1), the usual concept concerning permeability should hold. This relation indicates a positive correlation between permeability through membranes and its partition between the membrane/water phases (Collander and Barlund [7], Danielli [14], Stein [15]). However, when a partition coefficient is much greater than 1, as in the neutral form of local anesthetics (e.g., partition coefficients of the neutral forms of procaine and lidocaine at oleyl alcohol/water, 45 and 225, respectively, [16] and that of tetracaine at cod liver oil/water, 273 [17]) the above correlation does not necessarily hold [18], because in some cases other factors responsible for permeation of substances across the membrane may become more predominant. For example, when the interaction of specific molecular groups in a permeant substance with the membrane is very strong compared with the aqueous phase,

the desorption of the substance from the membrane to an aqueous solution may become a rate-determining step for diffusion of the substance across the membrane. Since the neutral form of local anesthetics containing benzene rings and hydrocarbon chains may be associated strongly with the membrane, they could be considered even as a component of the membrane rather than a permeant molecule. The desorption studies for such systems showed that the desorption rate of a membrane component from membranes depends primarily upon the cohesive energy of such a component in the membrane phase [11,19]. Because of the existence of the $NH\text{-}C_4H_9$ molecular group in the tetracaine molecule, it may have stronger hydrophobic interaction with the membrane phase than a procaine molecule which has the $-NH_2$ group as the molecular group corresponding to the one in tetracaine. Therefore, the desorption rate of tetracaine is slower than that of procaine. In lidocaine, two CH_3 groups associated with the benzene ring could make the molecule more hydrophobic in nature than procaine.

The order of desorption rates of neutral forms of such local anesthetics is correlated inversely to that of the partitioning of the neutral forms between oil and water phases. The order of permeability seems to have a inverse correlation to the partitioning of the neutral form between the oil and water phases (Table III).

Recent magnetic resonance spectroscopic studies [20,21] demonstrated different degrees of hydrophobic interaction for various local anesthetics in membranes. Cerbon [20] observed that the relative order of strength of the hydrophobic interaction of tertiary amine local anesthetics (dibucaine, tetracaine, procaine) agreed well with the potency and length of duration of the anesthetic effect. Giotta et al. [21] studied the residence time of intracaine derivatives with ethoxy, butoxy and hexyloxy in lobster axon membranes by ESR techniques, and showed that the residence time of those compounds in the membrane was the same order as that of partition into the hydrocarbon region, indicating the inverse correlation between desorption and partition of such local anesthetics at oil/water phases. These experiments also support the present proposed concept concerning the permeability of uncharged local anesthetics.

The surface charges on the axon membrane could affect our treatment described in the theoretical section to the certain extent. The surface charges on the outer membrane surface will alter the concentration of the charged form of local anesthetic but not the neutral form, which are determined for a given external bulk concentration of anesthetic and a given external bulk pH. Therefore, the external surface charge does not affect the present treatment. On the other hand, the significant surface charges on the inner membrane surface do alter the surface concentration of the charged form of local anesthetic, and consequently this would modify the present theory. However, since the nature of surface charge is not well understood and its charge density is considered to be relatively small in magnitude [22], at the present time we do not take into account the effect of the inner surface charge on the mode of action of local anesthetics.

The present study is still at the preliminary stage, however, we believe that this type of study may add new information concerning the process of action of local anesthetics with excitable membranes.

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