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MODE OF ACTION OF TERTIARY AMINE LOCAL ANESTHETICS ON AXON MEMBRANE EXCITABILITY

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Summary

A study was made on the mode of action of tertiary amine local anesthetics on lobster axon excitability. A minimum concentration of extracellularly applied local anesthetics to exert narcotic action on the axon membrane, and the time to exert narcotic action after application of a given concentration of local anesthetic to the extracellular solution were determined. A theoretical relationship describing the mode of action was obtained in terms of the internal and external pH values, the external concentration of local anesthetics, their permeability through the membrane and the time to exert narcotic action. The experimental data were analyzed in comparison with the theoretical formulation for the action mode. It is concluded that the predominant mode of action of tertiary amine local anesthetics is, first, to penetrate into the axon interior in a neutral form, and then to react in a cationic form electrostatically from the intracellular phase and, it is speculated that finally, both the electrostatic and hydrophobic interactions exert narcotic action on the nerve membrane.

In addition, it was found that the permeability of the neutral form of procaine was about 3.5 times greater than that of tetracaine and almost independent of the extracellular pH.

Introduction

There have been a number of studies on how local anesthetics act on membrane excitability [1—3]. The most widely accepted role of local anesthetics in a descriptive theory centers on their interaction with membrane constituents and their related ionic environment. One of these which is based on the lipoid theory originally proposed by Overton in 1896 [4] and others [35], emphasizes the predominant role of the uncharged form of the anesthetic

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in effecting local anesthetic action [5—10]. However, because many local anesthetics are basic tertiary amine compounds, they are dissociated into cationic form or neutral form depending on the pK_a of the anesthetics and the pH of the bathing solution. Thus, another theory has been proposed concerning the interaction of the cationic form of local anesthetics with certain charged sites (polar groups of lipids or proteins) in or on the membranes [11,12]. In connection with the theories involving charge interaction, some [13,14] have suggested that the local anesthetic may bind with negatively-charged phospholipid polar-group sites on the outer membrane surface. It has been earlier reported that there is a competitive action between local anesthetics and calcium ions on the nerve cell excitability [15].

Recently, Narahashi and his associates [16—18] have shown, using the internal perfusion technique on squid axons, that local anesthetics penetrate into the axon in the neutral form and that the charged form is neuroactive at the interior membrane of the axolemma. Although there is still a possibility that the former theory (interaction of local anesthetics in lipid or hydrophobic phase) may be responsible for the narcotic action by the local anesthetics (supported by recent NMR and ESR spectroscopy studies [19—25]), we take a stand on Narahashi's theory [3], set up a theoretical relationship based on this mode of action, and have determined the most probable mode of action of local anesthetics on nerve axon.

Theory

Since most local anesthetics are secondary or tertiary amine compounds, they may exist both as uncharged molecules (B) and as positively-charged molecules (BH^+), and their relative proportions depend upon the pH of the solution and on the pK_a of the anesthetics according to the Henderson-Hasselbalch equation:

$$\mathrm{pH} = pK_a - \log([BH^+]/[B]) \tag{1}$$

Here, let us assume that only the uncharged form of local anesthetics can diffuse into the axon. This assumption is approximately valid since most biological membranes are known to be more permeable to uncharged molecules than to positively-charged molecules [26].

Then, the flux, J, of uncharged anesthetic across the axon membranes may be expressed by

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

where P is the permeability coefficient of the uncharged local anesthetic across the axon membrane, and substripts o and i refer to the extracellular and intracellular phases of the axon, respectively.

The total concentration of local anesthetic ($C_i = [B]_i + [BH^+]_i$) in the intracellular phase will approximately be given by

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

where A and V are the area and volume per unit length of the axon, respectively, t is the time elapsed after a local anesthetic is applied to the extracellular solution and r is the radius of the axon.

With Eqns. 1, 2 and 3, the total extracellular anesthetic concentration ($C_0 = [B]_0 + [BH^+]_0$) is given by

$$C_0 \cdot \frac{10^{pK_a-pH_i}}{1+10^{pK_a-pH_0}} = [B^+]_i \cdot \left\{1 + \frac{(1+10^{pK_a-pH_i})\, r}{2pt}\right\} \tag{4}$$

where $[BH^+]$ is abbreviated as $[B^+]$.

If we assume that only the cationic form of local anesthetic is neuroactive from the intracellular phase, it is expected that there may be a minimum concentration of the intracellular cationic form, $[B^+]_i^{min}$ at which a certain degree of narcotic action is exerted on the axon. We shall take as the measure of narcotic action the reduction to half of the maximum of the time derivative of the action potential (also see ref. 16). Then Eqn. 4 can be written by

$$C_0 \cdot \frac{10^{pK_a-pH_i}}{1+10^{pK_a-pH_0}} = [B^+]_i^{min} \cdot \left\{1 + \frac{(1+10^{pK_a-pH_i})\, r}{2pt}\right\} \tag{5}$$

In Eqn. 5, t is the time for the maximum value of the time derivative of the action potential to decline to half after a local anesthetic at a given concentration, C_0, is applied to the external solution of the axon. The minimum concentration, $[B^+]_i^{min}$, is determined by measuring the time, t, for a given concentration C_0, since the pH of the external solution, pK_a of the local anesthetic, and the radius, r, of the axon are available, provided that the pH of the internal solution of the axon is known.

Furthermore, if, as in the present case, $(10^{pK_a-pH_i}) \cdot r/2pt << 1$, Eqn. 5 is approximated as

$$\log C_0 \cdot \frac{10^{pK_a-pH_i}}{1+10^{pK_a-pH_0}} \cong \log[B^+]_i^{min} + \frac{r}{4.6p} \cdot \frac{10^{pK_a-pH_i}}{t} \tag{6}$$

This suggests that if the assumption of the action mode adopted here is valid, when experimental data (C_0 and t) are plotted with $\log C_0 \cdot 10^{pK_a-pH_i}/1 + 10^{pK_a-pH_0}$ as the ordinate vs. $10^{pK_a-pH_i}/t$ as the abscissa, the experimental points should show a straight line relationship. The slope of the line corresponds to $r/4.6p$ and the intercept indicates the minmum concentration $[B^+]_i^{min}$ as defined.

Methods and Procedures

Circumesophageal and thoracic segments of lobster giant axons (diameter 80—100 μm) dissected from fresh lobsters weighing about 1—1.25 lbs. were used in the experiments. Desheated axon bundles about 4 cm in length were mounted in a plexiglass chamber. The membrane potentials were measured by a standard electrophysiological technique. The extracellular potential recording electrode was a calomel electrode (a reference electrode for pH meter) and the

intracellular potential recording electrode was a glass capillary microelectrode filled with 3 M KCl. The resistance of all the microcapillary electrodes used was about 10 MΩ. The intracellular potential was fed to a high input impedance preamplifier via a silver-silver chloride wire placed in the 3 M KCl of the microelectrode. The membrane potential was measured by taking the difference in potentials between those of the external calomel and intracellular electrodes through the amplifier. A pair of platinum wires was used as the external stimulation electrodes, and square pulses were applied to the electrodes via a stimulus isolation unit. Resting membrane potentials were recorded with a chart recorder (Heath, EU20V), and the action potential and its time derivative were displayed on the storage oscilloscope screen (Tektronix Type 564).

The 'standard' extracellular solution consisted of NaCl 457 mM, KCl 8.9 mM, $CaCl_2$ 10 mM, $MgCl_2$ 24.9 mM, $MgSO_4$ 27 mM and Tris-base buffer 1 mM, pH was adjusted by HCl. Local anesthetics used were procaine-HCl and tetracaine-HCl (Mann Research Lab., over 95% pure). Local anesthetics were added in the above standard extracellular perfusing solution. The pH of the solution was carefully checked and adjusted at the beginning of each experiment.

In each experiment, the axon was first placed in the standard external solution (pH 7.9) without local anesthetics. After the application of each local anesthetic solution, the standard external solution was flushed around the axon for a sufficient time to bring back to complete recovery the resting and action potentials, including its time derivative. For each experiment, the flow rate of the external flushing solution was kept the same. The solution in the perfusion chamber could be changed completely within 10 s. The experimental temperature was room temperature, about 23°C.

Experimental results and analysis

In Fig. 1, the logarithm of the externally-applied procaine concentration, C_0, is plotted against the inverse of the time, at which the maximum of the time derivative ($dV/dt_{max} \equiv \dot{V}_{max}$) of the action potential ($\dot{V}$) is reduced to half ($\dot{V}_{max}/2 \equiv V_{max/2}$), at various pH values (6.0, 7.0 and 8.5). The experimental points obtained at external pH 6.0 and 7.0 fall on curves which are concave downward with time. The points corresponding to pH 8.5 are slightly concave upward with time.

Experimental data plots similar to those given above, obtained under the application of tetracaine at various pH values (6.0, 7.0 and 8.0) are shown in Fig. 2. The experimental points obtained at pH 8.0 fall approximately on a straight line. The pH dependency of the experimental points were similar to that of procaine. The concave downward tendency of the experimental points with time at the lower pH (6.0, 7.0) in both experiments (Figs. 1 and 2) suggests that the internal pH may have decreased toward the external pH with time. Also, the slightly concave upward tendency of the experimental points for the higher pH (8.5) indicates that the internal pH has increased toward the external pH value with time. In addition, it is clearly shown from Figs. 1 and 2, that tetracaine is more potent than procaine. Each point in the figures is the average value of at least four experiments.

Figs. 3 and 4 show the experimental results of the minimum concentrations

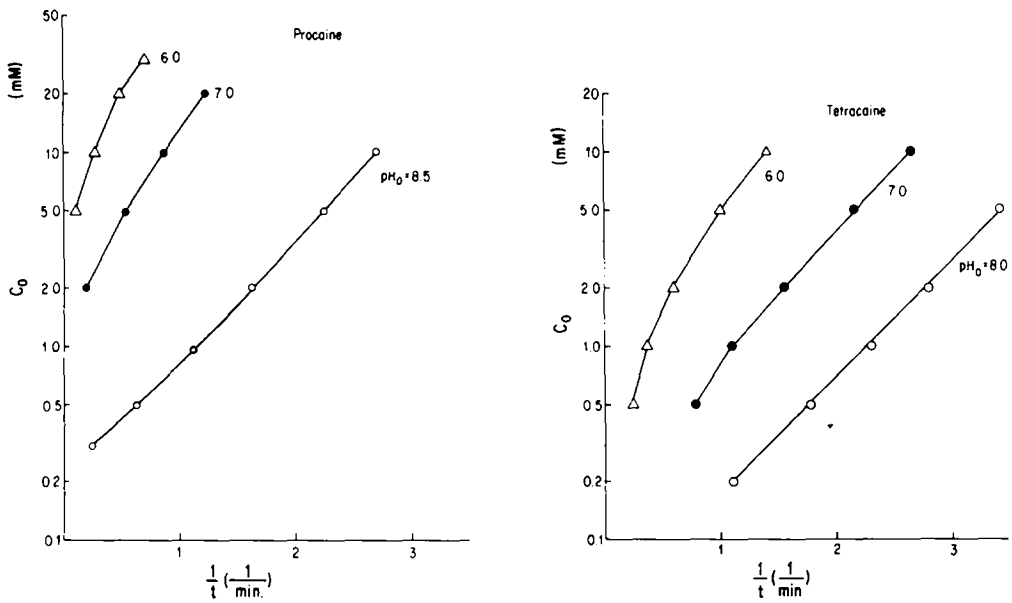

Fig. 1. The relation of a minimum concentration of procaine applied externally at various external pH and the inverse of the time in which the maximum of the time derivative of the action potential is reduced to half. △, Extracellular pH = 6.0; ●, extracellular pH = 7.0; ○, extracellular pH = 8.5.

Fig. 2. The same relation as in Fig. 1, but for the case of tetracaine. △, Extracellular pH = 6.0; ●, extracellular pH = 7.0; ○, extracellular pH = 8.0.

of local anesthetic applied externally to suppress $\dot{V}_{max}$ to half ($\dot{V}_{max/2}$) within 2 min for procaine, and 1 min for tetracaine, respectively, at different pH. The filled circles in Figs. 3 and 4 stand for the cases where pH change and the local anesthetic application were made simultaneously, and the open circles are for the cases where the local anesthetic was applied 15 min after the pH of the solution surrounding the axon had been changed.

For the former case, the logarithm of the minimum concentration of a given local anesthetic applied externally is approximately linearly-related to the external pH with a slope of about −0.5 for procaine and −0.7 for tetracaine in the pH range lower than that of the corresponding pK_a values of the local anesthetics. The lower the external pH is, the higher is the concentration of local anesthetic needed to exhibit the narcotic action. However, at pH near the pK_a value, the slope of the minimum concentration vs. pH tends to be less steep. This suggests that the anesthetic action parallels the amount of uncharged form in the extracellular solution. Similar results (for rabbit cornea) have been obtained by others [5].

On the other hand, the latter case (Figs. 3 and 4, open circles) shows that there is not as much pH dependency with respect to the minimum concentration of local anesthetics. The reason for this tendency, however, will be given in Discussion.

Following Eqn. 6, Figs. 6 and 7 show the relationship between $\log C_0 \cdot 10^{pK_a - pH_i}/1 + 10^{pK_a - pH_0}$ on the ordinate and $10^{pK_a - pH_i}/t$ on the abscissa. In

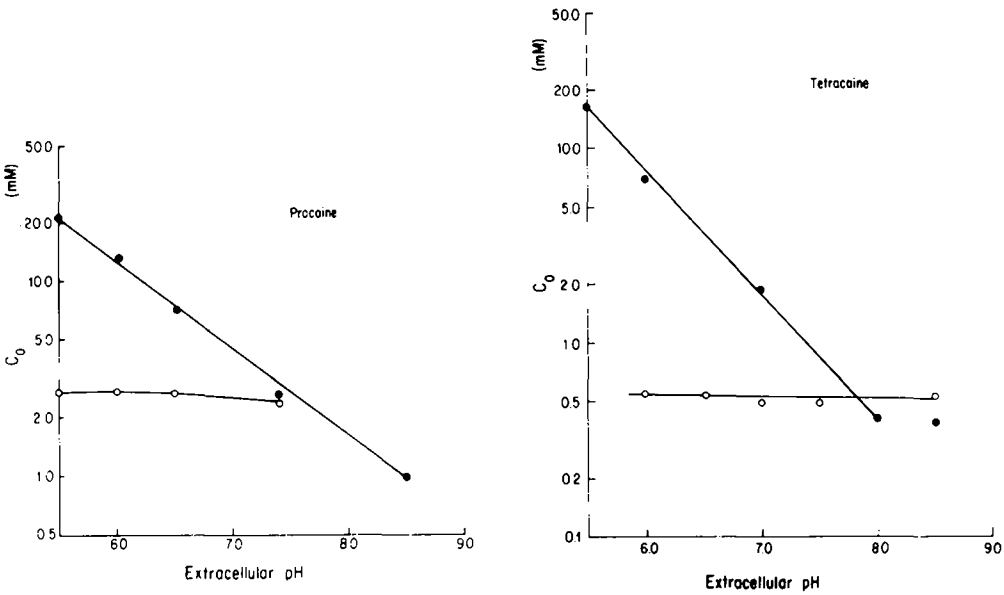

Fig. 3. The filled circles indicate the minimum concentration of procaine applied externally to suppress the maximum of the time derivative of the action potential to half within 2 min after the anesthetic is applied to the extracellular phase at various pH values. The open circles correspond to the minimum extracellular concentration of procaine to exert the same narcotic action as the above, where the procaine solution was applied 15 min after the pH of the extracellular solution was changed to the same pH as the local anesthetic solution.

Fig. 4. The filled circles indicate the minimum concentrations of tetracaine in the extracellular phase to exert the same narcotic action as the above, but within 1 min. The open circles correspond to the minimum concentration of tetracaine to exert the same narcotic action where the tetracaine solution was applied extracellularly 15 min after the pH of the extracellular solution was changed to the same pH as the local anesthetic solution.

order to obtain $\log C_0 \cdot 10^{pK_a - pH_i}/1 + 10^{pK_a - pH_0}$, we have used the experimental data shown in Figs. 1 and 2, pK_a values of 8.9 for procaine and 8.24 for tetracaine from Skou [7], and pH_i obtained with squid axons (Bicher and Ohki [27], and our unpublished data) which are shown in Fig. 5. The final reduced

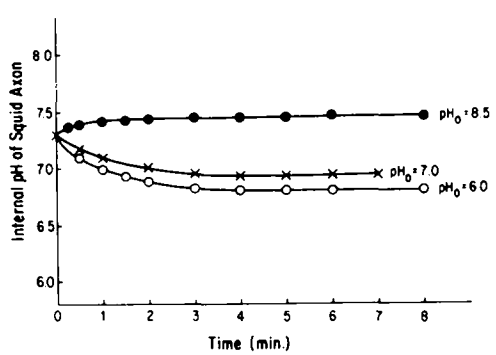

Fig. 5. Time course of internal pH of squid axons at fixed extracellular pH.

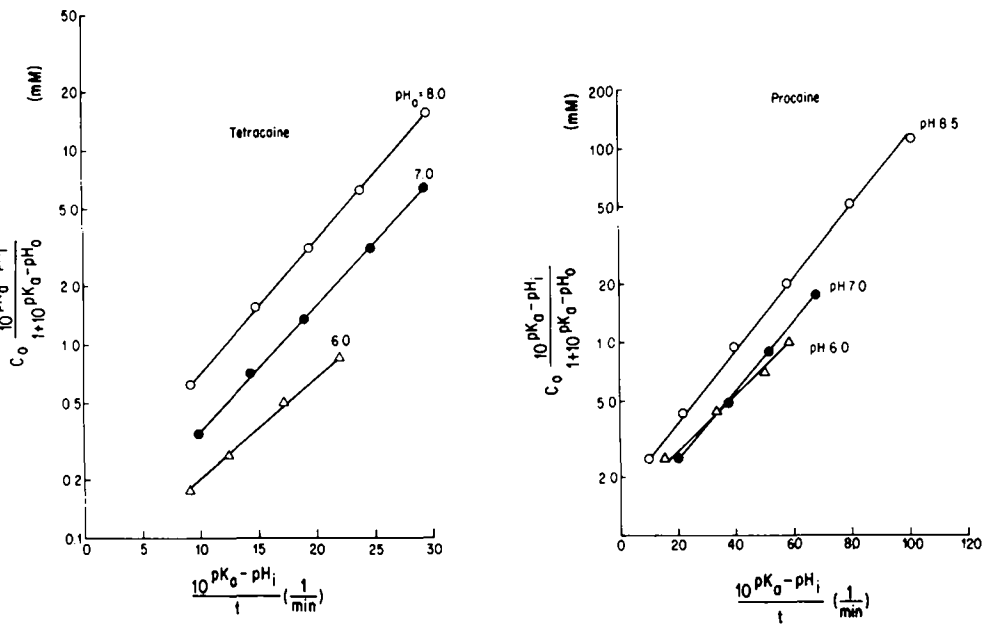

Fig. 6. The relationship between log $C_0 \cdot 10^{pK_a - pH_i}/1 + 10^{pK_a - pH_0}$ on the ordinate and $10^{pK_a - pH_i}/t$ on the abscissa for procaine at various extracellular pH values. △, Extracellular pH = 6.0; ●, extracellular pH = 7.0; ○, extracellular pH = 8.5. The pK_a value of procaine, 8.9, was used in the calculation.

Fig. 7. The similar relationship as in Fig. 6, but for tetracaine. The pK_a value of tetracaine was 8.24.

experimental points fall approximately on the straight line. It is seen from the figures that the minimum intracellular concentration $[BH^+]_i^{min}$ is about 1—2 mM for procaine, and about 0.1—0.2 mM for tetracaine. The slope of the straight line corresponds to $r/4.6p$. If we take the diameter of lobster giant axons to be about 80 μm, we can obtain the permeability for the uncharged form of the local anesthetics across the axon membrane from the slope of the line (Table I). For example, the permeabilities of procaine and tetracaine are $8.2 \cdot 10^{-4}$ cm $\cdot$ s^{-1} and $2.3 \cdot 10^{-4}$ cm $\cdot$ s^{-1}, respectively, at $pH_0 = 7.0$.

As seen in Figs. 6 and 7, and Table I, there seems to be no appreciable pH_0 dependency in permeability of the neutral form of local anesthetics. It is interesting to note that the permeability of the neutral form of procaine is greater (about 3.5 times) than that of tetracaine over the pH range tested. However, as already seen, the minimum intracellular concentration of positively-charged local anesthetic to effect narcotic action is about 10 times lower for tetracaine than for procaine (Figs. 6 and 7 and Table I).

Finally, the following experiment was done in order to further examine the assumed mode of action of local anesthetics. The experiment involved measuring the recovery time of the action potential in the presence and absence of NH_4Cl in the extracellular solution after the action potential had been completely abolished by the application of local anesthetics.

First, the axon was immersed in standard extracellular solution, pH 7.9. Then, standard solution containing 5 mM tetracaine at pH 7.0, buffered with 20 mM Tris, was applied to the axon. The action potential was thus com-

TABLE I

PERMEABILITIES OF NEUTRAL FORM OF LOCAL ANESTHETICS

(r = 40 μm)

	Permeability (cm · s^{-1})				$[BH^+]_i^{min}$ (mM)
	pH_0 = 6.0	pH_0 = 7.0	pH_0 = 8.0	pH_0 = 8.5	
Procaine	9 · 10^{-4}	8.2 · 10^{-4}		8 · 10^{-4}	1—2
Tetracaine	2.7 · 10^{-4}	2.3 · 10^{-4}	2.1 · 10^{-4}		0.1—0.2

pletely abolished within 0.8 min on the average. Immediately after the action potential had been abolished, standard solution containing 10 mM NH_4Cl, pH 7.0, with 20 mM Tris, was applied to the axon. The action potential reappeared in about 0.7 min (average) and 3 min were required for complete recovery.

On the other hand, after blocking the axon excitability with the 5 mM tetracaine solution, when the external solution was changed to standard solution (also, at pH 7.0, 20 mM Tris), but without NH_4Cl, the action potential first reappeared in 6.4 min, and more than 20 min were required to bring the action potential back to full recovery.

This distinct difference in recovery time with and without NH_4Cl indicates that, for the case where NH_4Cl was present, the internal pH of the axon was incresed by the rapid uptake of NH_3 into the axon [44,45], and consequently, the concentration of the charged form of the anesthetic inside the axon was more rapidly reduced than in the case of its absence. These experiments further substantiate the assumed mode of action of local anesthetics, as will be mentioned in the Discussion.

Discussion

In order to deduce the most probable action mode of tertiary amine local anesthetics, various possible modes of action are examined and compared with the experimental results obtained. The following actions can be considered:

(1) The local anesthetic acts in the neutral form only from the extracellular phase. The experimental results indicated with the filled circles in Figs. 3 and 4 seem to be in accordance with this assumption except that the slope of the logarithm of the minimum concentration vs. the external pH is time dependent. Ideally, the slope should be —1 for this action mode in the pH range which is lower than pK_a. The results indicated with the open circles do not agree with this action mode because the amount of uncharged form in the extracellular solution should depend on the extracellular pH. The experimental results (open circles in Figs. 3 and 4) show practially no pH dependency. Therefore, this action mode is not satisfactory.

(2) The local anesthetic acts in the charged form only from the extracellular phase. For this assumed action, the minimum effective concentration of local anesthetic should decrease with decreasing extracellular pH which is lower than the pK_a of the local anesthetics. The experimental results indicated with the

filled circles in Figs. 3 and 4 show, however, a completely opposite tendency. Therefore, this action mode cannot be adopted.

(3) Another mechanism of action in which the local anesthetic penetrates into the axon in its charged form more easily than its neutral form and acts from the intracellular axon phase. This seems to be unlikely to occur predominantly because, in most of the biological membranes the uncharged form of a permeant molecule is more permeable than its charged form. Indeed, a permeability study of a tertiary amine local anesthetic (procaine) by use of the radioisotope tracer method indicates that at higher pH, the uptake of the local anesthetic into squid axons is greater than that at lower pH [28]. This suggests that the uncharged form of local anesthetic is more permeable than the charged form to the axon membrane.

(4) The local anesthetic penetrates into the axon predominantly in an uncharged form and acts in this form from the intracellular phase. This action can be eliminated by examining the experimental results obtained at the external pH 6.0 shown in Figs. 1 and 2. By applying an extracellular solution having a lower pH (6.0) than the normal physiological internal pH (approx. 7.3), then since the internal pH of the axon follows the external pH with time [27], the amount of uncharged form in the intracellular solution should decrease as the internal pH decreases with time. Therefore, the curve should be concave upward, rather than concave downward, with time. The experimental results (pH 6.0) in Figs. 1 and 2 show concave downward curves with time.

(5) The local anesthetic penetrates as the uncharged form into the axon and acts in the charged form from the intracellular phase. For this mode of action, the experimental results obtained here are all consistent with the possible consequences arising from this assumption. The results indicated with the filled circles in Figs. 3 and 4 show that the amount of the uncharged form parallels the potency of the narcotic action with various pH, although, as pointed out earlier, the correlation between the external pH, (which is related to the amount of neutral form) and the potency, (which is related to the local anesthetic concentration C_0) has a time-dependent feature. Those indicated with the open circles in Figs. 3 and 4 suggest that because the internal pH somewhat follows the external pH with time, e.g. when the external pH is lower than the physiological intracellular pH (approx. 7.3), the amount of the charged form in the intracellular phase is increased more than is expected for the case where the internal pH would not change. Therefore, the slope of the minimum concentration vs. the external pH should become lower and lower with time. This tendency can be seen for the experimental results shown with the filled circles in Figs. 3 (slope = -0.5) and 4 (slope = -0.7). If the time which is allowed to elapse before the application of the local anesthetic is longer than 15 min, the minimum concentration of local anesthetic becomes almost independent of the extracellular pH values which are below the pK_a (open circles in Figs. 3 and 4).

The concave downard nature of the relation between C_0 and $1/t$ at lower pH (6.0) (Figs. 1 and 2) suggests that the internal pH would be lowered from the normal internal pH with time, and therefore, if the charged form is neuroactive, the amount of charged form in the axon will be increased from that expected in the case where the internal pH would not change. Therefore, less time is

needed to reach a certain minimum concentration to exert narcotic action. This should result in a concave downward curve, as the experimental results at pH 6.0 indicate in Figs. 1 and 2. The experimental results obtained at higher pH (8.5) showing a slightly concave upward curve (Figs. 1 and 2) are explained quite well by similar arguments.

From these analyses, it seems clear that the mode of action of the tertiary amine local anesthetics is the one mentioned last, (5). Although this has been suggested earlier by the experiments with perfused squid axons [16,17], there seems to be some relevance in our present work as evidence confirming their proposal by performing different experiments and a theoretical analysis of the obtained experimental results. In addition, in our work there are two new results: (1) the permeability of neutral form of local anesthetic is not pH dependent. Similar results can be obtained by analyzing the data from the direct measurements of the local anesthetic uptake into the axon interior by a radioisotope tracer assay (see Table II), although a direct measurement of neutral anesthetic (such as *n*-butanol) permeability shows a slight pH dependency [28]. (2) The second point concerns a comparison of the potency of the local anesthetics used. It is well known that tetracaine is more potent than procaine. However, if the permeabilities of the neutral form of the two species are compared in our study, the neutral form of the tetracaine molecule is less (1/3.5 times) permeable to the membrane than procaine (Table I). At the same time, the minimum concentration of the charged form of tetracaine in the axon interior to effect narcotic action is about 10 times less than that of procaine (see Figs. 6 and 7, and Table I). It seems, therefore, that in this case (procaine and tetracaine) the factors to determine the potency of the local anesthetic are not primarily due to the permeability of the neutral form, but both the amount of the neutral form at the membrane sites which relates to both the pK_a value of the local anesthetics and pH of the solution, and the interaction affinity (hydrophobic interaction [19—23], and charge interaction [19], with the membrane) of the local anesthetic with the axon membrane.

It has been reported [44,45] that the presence of NH_4Cl in the extracellular phase results in a rapid uptake of NH_3 into the axon interior, and consequently, an increase in internal pH. The present observation of the rapid recovery of axon excitability with the presence of NH_4Cl in the extracellular solution, therefore, definitely suggests that the charged form of tertiary amine local anesthetics is more potent than the neutral form.

With the results described in this paper and various other experimental evidence reported in the literature, the following action of a tertiary amine local anesthetic on the axon membrane may be suggested: first, the neutral form of local anesthetics penetrate into the axon interior, where it becomes a charged form according to its pK_a and the internal pH; secondly, the positively-charged amine-group of the local anesthetic molecule interacts strongly with negatively-charged sites of lipid [19] and protein molecules of the membrane located on the axon interior surface, which include those constituting the so-called "ionic channels" [29—32]; and finally, while the local anesthetic is anchored on the negatively-charged sites of the membrane, its lipophilic portion containing the aromatic ring interacts with hydrophobic parts [19—21] of the membrane molecules.

TABLE II

Data for extracellular pH from Dettbarn et al. [28]. Values indicate % equivalent distribution of local anesthetic uptake in the axon with respect to those in the extracellular solution.

	Extracellular pH					Permeability	Permeability ratio	
	$pH_0 = 5.7$	$pH_0 = 5.8$	$pH_0 = 7.0$	$pH_0 = 7.9$	$pH_0 = 8.6$	$pH_0 = 7.0$	$\frac{P(pH_0 = 5.7)}{P(pH_0 = 8.6)}$	$\frac{P(pH_0 = 5.8)}{P(pH_0 = 7.9)}$
Procaine		17	91	402		$1.4 \cdot 10^{-3}$ *		≈ 1.3 *
n-Butanol	44				26		1.6	

* Calculated values using Dettbarn et al. [28] data.

On the outer membrane surface, the positively-charged form of local anesthetic may not compete well (shown for squid axons [33]) with the extracellular divalent ions of high concentration which have high screening and binding affinities [34], for their negatively-charged sites [36]. Although the positively-charged form of local anesthetics may interact with the negatively-charged sites on the surface of biological membranes [14,15,37—39] as well as artificial membranes [40—42], competition between Ca^{2+} and local anesthetics depends strongly on their concentrations near the membrane surface. On the other hand, it is easily seen that the internal membrane surface may be more susceptible to the positively-charged local anesthetic because of the small amounts of free divalent cations in the intracellular phase [43]. Although the neutral form of local anesthetic alone may exert narcotic action through hydrophobic interaction with the membrane, in the case of tertiary amine local anesthetics, it appears as if the cationic form of local anesthetics is neuroactive at the inner surface of the membrane. Because the effect of the charge interaction is so strong, the former mechanism (hydrophobic interaction) of narcotic action may be masked by the charge effect. This interpretation of the interaction mode of local anesthetics can accommodate both action modes of the charged form of local anesthetics and the neutral form of local anesthetics (such as benzocaine, etc.) including *n*-butanol [28]. Although we have made several approximations in our treatment to analyze the experimental data (such as internal pH, pK_a values, linearization involved in Eqn. 6, etc.) which have to be improved in the future, we believe that our treatment is correct as an initial step in understanding the mechanism of action of local anesthetics on the membrane excitability.

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References

1 Ritchie, J.M. and Greengard, P. (1966) Annu. Rev. Pharmacol. 6, 405—430
2 Seeman, P. (1972) Pharmacol. Rev. 24, 583—653
3 Narahashi, T. and Frazier, D.T. (1971) in Neurosciences Research (Ehrenpreis, S. and Solnitzky, O.C., eds.), Vol. 4, pp. 65—99, Academic Press, New York
4 Overton, E. (1896) Z. Phys. Chem. 22, 189—209
5 Trevan, J.W. and Boock, E. (1927) Brit. J. Exptl. Pathol. 8, 307—315
6 Ferguson, J. (1939) Proc. Roy. Soc. B127, 387—404
7 Skou, J.C. (1954) Acta Pharmacol. Toxicol. 10, 297—304
8 Skou, J.C. (1954) Acta Pharmacol. Toxicol. 10, 317—324
9 Skou, J.C. (1954) Acta Pharmacol. Toxicol. 10, 281—291
10 Shanes, A.M. and Gershfeld, N.L. (1960) J. Gen. Physiol. 44, 345—363
11 Ritchie, J.M. and Greengard, P. (1961) J. Pharmacol. Exp. Ther. 133, 241—245
12 Ritchie, J.M., Ritchie, B. and Greengard, P. (1965) J. Pharmacol. Exp. Ther. 150, 160—164
13 Feinstein, M.B. (1964) J. Gen. Physiol. 48, 357—374
14 Blaustein, M.P. and Goldman, D.E. (1966) J. Gen. Physiol. 49, 1043—1063
15 Aceves, J. and Machne, X. (1963) J. Pharmacol. Exp. Ther. 140, 138—148
16 Narahashi, T., Frazier, D.T. and Yamada, M. (1970) J. Pharmacol. Exp. Ther. 171, 32—44
17 Frazier, D.T., Narahashi, T. and Yamada, M. (1970) J. Pharmacol. Exp. Ther. 171, 45—51

18 Narahashi, T. and Frazier, D.T. (1975) J. Pharmacol. Exp. Ther. 195, 506—513
19 Hauser, H., Penkett, S.A. and Chapman, D. (1969) Biochim. Biophys. Acta 183, 466—475
20 Cerbon, J. (1972) Biochim. Biophys. Acta 290, 51—57
21 Giotta, G.J., Gargiulo, R.J. and Wang, H.H. (1973) J. Membrane Biol. 13, 233—244
22 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1973) Biochim. Biophys. Acta 291, 321—327
23 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1973) Biochim. Biophys. Acta 291, 328—334
24 Boggs, J.M., Yoong, T. and Hsia, J.C. (1976) Mol. Pharmacol. 12, 127—135
25 Boggs, J.M., Roth, S.H., Yoong, T., Wong, E. and Hsia, J.C. (1976) Mol. Pharmacol. 12, 136—143
26 Davson, H. (1966) A Textbook of General Physiology, Little, Brown and Co., Boston
27 Bicher, H.I. and Ohki, S. (1972) Biochim. Biophys. Acta 255, 900—904
28 Dettbarn, W.D., Heilbronn, E., Hoskin, F.C.G. and Kitz, R. (1972) Neuropharmacology 11, 727—732
29 Chandler, W.K., Hodgkin, A.L. and Meves, H. (1965) J. Physiol. Lond. 180, 821—836
30 Rojas, E. and Atwater, I. (1968) J. Gen. Physiol. 51, 131s—145s
31 Hille, B. (1968) J. Gen. Physiol. 51, 221—236
32 Gilbert, D.L. and Ehrenstein, G. (1969) Biophys. J. 9, 447—463
33 Narahashi, T., Frazier, D.T. and Takeno, K. (1976) J. Pharmacol. Exp. Ther. 197, 426—438
34 Hauser, H. and Dawson, R.M.C. (1967) Eur. J. Biochem. 1, 61—69
35 Meyer, H.M. (1899) Arch. Exp. Path. Pharmak. 42, 109—118
36 Segal, J.R. (1968) Biophys. J. 8, 470—487
37 Kwant, W.O. and Seeman, P. (1969) Biochim. Biophys. Acta 193, 338—349
38 Madeira, V.M.C. and Carvalho, A.P. (1972) Biochim. Biophys. Acta 266, 670—683
39 Bondani, A. and Karlev, R. (1970) J. Cell Physiol. 75, 199—211
40 Hauser, H. and Dawson, R.M.C. (1968) Biochem. J. 109, 909—916
41 Ohki, S. (1970) Biochim. Biophys. Acta 219, 18—27
42 Papahadjopoulos, D. (1970) Biochim. Biophys. Acta 211, 467—477
43 Baker, R.F., Hodgkin, A.L. and Ridgway, E.B. (1971) J. Physiol. 218, 709—755
44 Thomas, R.C. (1974) J. Physiol. 238, 159—180
45 Boron, W.F. and de Weer, P. (1976) J. Gen. Physiol. 67, 91—112