Intra-axonal continuous measurement of lidocaine concentration and pH in squid giant axon

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Purpose: To measure the dynamic penetration process of lidocaine, lidocaine concentration (Ci) and pH (pHi) in squid giant axon, and to determine the times and Ci of disappearance and reappearance of action potentials (AP).

Methods: Lidocaine solutions adjusted to four different pHs (pH = 5.5, 6.8, 7.8 and 9.0) were externally administered to the axon and Ci and pHi were measured using lidocaine and pH microensors. The times and Ci when the AP just disappeared and reappeared were recorded. In addition, for comparison with Ci, the lidocaine content in the whole axon (Cw) was measured with high-performance liquid chromatography (HPLC).

Results: The Ci (charged plus uncharged) was 1.5 times greater than the uncharged form of administered lidocaine. The changes in pHi depended on the increase in Ci. The AP disappeared only after administration of high pH lidocaine solutions (pH = 7.8, 9.0) and reappeared by washing out the solution in the chamber. Nerve block occurred more rapidly at pH 9.0 than at pH 7.8, and the time after washing out the lidocaine was longer at pH 9.0 than at pH 7.8. The mean Ci and charged lidocaine concentration in the axoplasm, when the AP disappeared or reappeared, were lower at pH 9.0 than at pH 7.8 ($P < 0.05$).

Conclusion: Uncharged lidocaine penetrates the axon membrane to the axoplasm where it changes to the charged form and is concentrated in the axon membrane and axoplasm. External application of uncharged lidocaine plays a role in modulating nerve conduction.

Objectif : Mesurer le processus de pénétration dynamique de la lidocaïne, la concentration de lidocaïne (Ci) et le pH (pHi) dans un axone géant de calmar et déterminer les temps et la Ci de disparition et de réapparition des potentiels d'action (PA).

Méthode : Les solutions de lidocaïne, préparées selon quatre pH différents (pH = 5,5; 6,8; 7,8 et 9,0), ont été administrées sur la paroi externe de l'axone et la Ci et le pHi ont été mesurés en utilisant de la lidocaïne et des microdétecteurs de pH. Les temps et les Ci correspondants au moment de disparition et de réapparition du PA ont été enregistrés. De plus, pour comparer avec la Ci, le contenu de lidocaïne de l'axone complet (Cc) a été mesuré par chromatographie liquide à haute pression (CLHP).

Résultats : La Ci (ionisée plus non ionisée) a été 1,5 fois plus élevée que la forme non ionisée de lidocaïne administrée. Les changements de pHi dépendaient de l'augmentation de Ci. Le PA a disparu seulement après l'administration de solutions de lidocaïne à pH élevé (pH = 7,8; 9,0) et a réapparu quand on a rincé la solution dans la cuve. Le blocage nerveux est survenu plus rapidement sous un pH de 9,0 que sous un pH de 7,8 et le temps après le rinçage de la lidocaïne a été plus long sous un pH de 9,0 que sous un pH de 7,8. La Ci moyenne et la concentration de lidocaïne ionisée dans l'axoplasme, au moment où le PA disparaissait ou réapparaissait, ont été plus faibles sous un pH de 9,0 que sous un pH de 7,8 ($P < 0,05$).

Conclusion : La lidocaïne non ionisée pénètre la membrane axonale jusqu'à l'axoplasme où elle se transforme en lidocaïne ionisée et est concentrée dans la membrane axonale et l'axoplasme. L'application externe de lidocaïne non ionisée joue un rôle en modulant la conduction nerveuse.

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It has been suggested that local anesthetics (LAs) penetrate cell membranes and bind to the cytoplasmic opening of the sodium channel only in a charged form.¹ However, this theory is contradicted because uncharged benzocaine is a powerful LA. Many other hydrophobic molecules also inhibit nerve conduction.

When obtaining a comprehensive picture of the anesthetic action of LAs, it is important to investigate the direct outside-to-inside permeation process of LAs in the axon. Hille² developed a simplified diffusion model of LAs in the axon, and the membrane permeability of LAs was estimated. However, the concentration of LAs in the axoplasm has not been measured. Dettbarn³ and Ohki *et al.*⁴ measured the influx of LAs into the axoplasm using a radioactive isotope, but the permeation process of LAs was not clarified by this approach.

We developed lidocaine and pH microsensors consisting of coated wire electrodes.^{5,6} Since the diameter of these microsensors is only 200 μm , they are suitable for measuring the lidocaine concentration (C_i) and pH (pH_i) in the axoplasm of a single squid giant axon directly and continuously. In the present study, using these lidocaine and pH microsensors, we measured lidocaine distribution from the outside to the inside of the axon by varying extracellular pH ($\text{pH} = 5.5, 6.8, 7.8, 9.0$), as the first step in the elucidation of the mechanism of action of LAs. We also investigated the values of G_i on disappearance and reappearance of the AP. In addition, for comparison with C_i which was measured with the lidocaine microsensor, the lidocaine content in the whole axon (C_w) was measured with high-performance liquid chromatography (HPLC).

Methods

Experimental set-up

Single giant axons of squids, *Doryteuthis bleekeri* and *Sepioteuthis lessoniana*, were dissected (ID; 350–550 μm) from the first stellar nerve and kept in cold sea water until used.

Lidocaine hydrochloride (Astra Japan, Tokyo) was dissolved in artificial sea water (ASW; Na^+ ; 450 mM, K^+ ; 10 mM, Cl^- ; 580 mM, Ca^{++} ; 10 mM and Mg^{++} ; 50 mM). The solution was adjusted to four different pHs by the addition of 1 M HCl or NaOH, (i.e., pH 5.5, 6.8, 7.8 and 9.0) with a buffer system; H_2PO_4 (Kanto Kagaku Co., Tokyo) for pH 5.5, HEPES (Dojin Kagaku Co., Tokyo) for pH 6.8 and 7.8, and CAPSO (Dojin Kagaku) for pH 9.0. The final lidocaine concentration, was adjusted to 10 mM in each solution. The charged and uncharged concentrations in the 10 mM lidocaine solution at pH = 5.5, 6.8, 7.8 and 9.0 were,

FIGURE 1 (A) Schematic diagram of the experimental system. The lidocaine concentration and pH in the axoplasm of a single squid giant axon were measured continuously and directly by lidocaine and pH microsensors.

(B) Structure of the microsensors for lidocaine and pH. PVC = Polyvinyl Chloride; TTFPB = Tetrakis [3,5-bis (trifluoromethyl) phenyl] borate; DOP = Dioctyl Phthalate

10 mM charged, 0 mM uncharged; 9.4 mM charged, 0.6 mM uncharged; 6.1 mM charged, 3.9 mM uncharged; and 0.9 mM charged, 9.1 mM uncharged, respectively ($\text{pK}_a = 7.99$ (20°C); the pK_a value was estimated by the equation described by Kamaya *et al.*⁷ Ten mM lidocaine at pH 7.8 blocked nerve conduction reversibly in a preliminary study.

Figure 1A is a schematic diagram of the experimental system. A giant axon was placed horizontally in the chamber filled with ASW. The solution in the chamber was stirred continuously. After the cannulation of a micro-glass capillary filled with perfusate (400 mM KF (Kanto Kagaku), glycerin 4% (Kanto Kagaku) and 10 mM HEPES (Dojin Kagaku) from one end of the axon, the lidocaine or pH microsensor was inserted through the capillary to the centre of the axon. A ref-

FIGURE 2 Relationship between lidocaine concentration in the perfusate at pH 6.8 ($\square$), 7.2 ($\diamond$), 7.5 ($\circ$) and 7.9 (Δ) and the electrode potential relative to the Ag/AgCl electrode.

FIGURE 3 Lidocaine concentrations from the calibration curve at pH 7.2 as a function of the analytical concentration at each pH: pH 6.8 ($\square$), 7.2 ($\diamond$), 7.5 ($\circ$) and 7.9 (Δ).

reference electrode was connected to the micro-glass capillary. The electrode potential from the sensor was measured using a multi-ion monitor (MIM-1, Yamashita Giken, Tokushima, Japan), and the data were stored in a personal computer (PC- 9800, NEC). Since the axon was too small to install both lidocaine and pH sensors simultaneously, C_i and pH_i were measured in another axon of the same squid. The two portions of the axon attached to the stimulating and recording electrodes were covered by a thin sheet of glass and sealed with hydrocarbon gel (Plastibase@,

Taisho Pharmaceutical Co., Tokyo) to prevent them from drying and to isolate them from the lidocaine.

Measurement of action potential (AP)

The action potential (AP) was evoked by outer stimulation using an electric stimulator (SEN-3301, Nihon Kodens, Tokyo; 10 V, 0.5 Hz, 200 msec). The wave form was recorded from outside the axon, amplified with a bioelectric amp (Polygraph system 366 - 4124, NEC Sanei, Tokyo) and monitored with an oscilloscope (COR 5541, Kikusui Electronics Co., Yokohama, Japan) throughout the experiment to evaluate nerve conduction.

A nerve blocking effect in the area bathed in lidocaine was determined by the disappearance of the AP. Since, in a preliminary study, the time of disappearance and reappearance of the AP were the same despite internal or external recording, the AP was recorded from outside the axon in this study. The disappearance and reappearance of AP were examined under the conditions described above, and the times and C_i when these phenomena just occurred were recorded.

Microsensors and the theory of the measurement of lidocaine and pH

The lidocaine microsensor was prepared as described previously (Figure 1B).^{5,6} The composition of the sensor membrane was a polyvinyl chloride 67.9% (Wako, Tokyo) matrix incorporating lidocaine- tetrakis [3,5 - bis (trifluoromethyl) phenyl] borate 1.2% (Wako) and dioctylphthalate 30.9% (Tokyo Kasei, Tokyo) (Figure 1B). The sensor diameter was approximately 200 μ m. The response time to changes in the concentration of lidocaine was within 5-10 sec. The sensor was used as the indicator electrode, and an Ag/AgCl electrode connected to a normal saline agar bridge was used as a reference electrode.

Because the lidocaine sensor can only measure the charged form, the uncharged form was calculated from the Henderson-Hasselbalch equation. Since the lidocaine sensor is sensitive to pH, correction of the electrode potentials is necessary because pH_i changes after the administration of lidocaine solution.

If only the concentration of charged [HA] and uncharged [A] lidocaine and potassium ions [K^+] affects the electrode potential, then the electrode potential can be calculated from the following equation;

$$E = E_0 + 0.059 \log ([HA] + K_A [A] + K^{K^+} [K^+]) \quad (1) \quad (25^\circ C)$$

where K_A is a contribution constant of uncharged lidocaine for the electrode potential and K^{K^+} is the ion selectivity of K^+ by the sensor.

Since $[HA]$ and $[A]$ are calculated by using;

$$[HA] = C_t / (1 + 10^{pH-pK_a}) = C_t / (1 + P) \quad (2)$$

$$[A] = C_t 10^{pH-pK_a} / (1 + 10^{pH-pK_a}) = C_t P / (1 + P) \quad (3)$$

where C_t is the total concentration of charged plus uncharged lidocaine and P is defined by $P = 10^{pH-pK_a}$, then Eq. 1 is rewritten as follows;

$$E = E_0 + 0.059 \log [C_t \{1 / (1 + P) + K_A P / (1 + P)\} + K^{K+} [K^+]] \quad (4)$$

Lidocaine concentrations from the sensor at each pH are calculated as follows,

$$C_{t\ 7.2} = C_t \{1 / (1 + P) + K_A P / (1 + P)\} + K^{K+} [K^+] \quad (5)$$

where the calibration curve measured at pH 7.2 is defined as the standard. Thus, Eq. 5 can be written as

$$C_{t\ 7.2} = A C_t + B \quad (6)$$

$$\text{where } A = 1 / (1 + P) + K_A P / (1 + P), B = K^{K+} [K^+]$$

From Eq. 6, the lidocaine concentration from the calibration curve at pH 7.2 can be calculated as a primary equation.

In order to validate the above equation, we measured the dose-response curves at pH 6.8, 7.2, 7.5 and 7.9 (Figure 2). Figure 3 shows lidocaine concentrations from the calibration curve at pH 7.2 as a function of the analytical concentration at each pH from Figure 2. All concentrations at each pH show linear regression and intersect at 0.72 ± 0.01 mM. These results indicate that, if the pH of the solution can be measured, lidocaine concentration can be calculated from the calibration curve at pH 7.2, because the effect of uncharged lidocaine in a solution with the same pH on the electrode potential will be constant. Plotting the $\log [\text{slope}]$ of Figure 3 as a function of pH, a linear relation is obtained (Figure 4);

$$\log [\text{slope}] = 0.498 \text{ pH} - 3.588 \quad (7)$$

The lidocaine concentrations at various pH values obtained from the calibration curve at pH 7.2 are listed in Table I. Thus, lidocaine concentration can be measured by the sensor in various pH solutions containing lidocaine of > 1 mM with a high degree of accuracy ($\pm 2-10\%$).

The pH microsensor was prepared from the sensor material of a glass pH electrode (Figure 1B).⁸ Its diameter was approximately 200 μm , and the response to changes in the hydrogen ion concentration was immediate. The reference electrode was an Ag/AgCl electrode connected to a normal saline agar bridge, as

FIGURE 4 The relationship between pH and the $\log [\text{slope}]$ of the data shown in Figure 3.

with the lidocaine microsensor. The slope of the standard curve was -43 mV/pH in perfusate.

Measurement of lidocaine in the axoplasm (C_i) and pH in the axoplasm (pH_i)

After installation of the microsensor to the axon, recordings of both the electrode potential from the sensor and the AP were started. When the electrode potentials became stable, the ASW in the chamber was replaced with lidocaine solution at various pHs (pH = 5.5, 6.8, 7.8 and 9.0).

After immersion of the axon in lidocaine solution for 15 min, the lidocaine solution was removed from the chamber. The lidocaine was immediately washed out four times and the chamber was filled with 500 μl of ASW, the pH of which was the same as that of the lidocaine solution, except that in the case of pH 9.0, ASW of pH 7.8 was used in the chamber solution. The elution of lidocaine was observed for 30 min in each experiment. All measurements were done at a water temperature of 18–21°C.

Measurement of lidocaine content in the whole axon (C_w)

After lidocaine elution for 30 min, the lidocaine contents in 13 μl of the chamber solution was measured with HPLC (GGPE - II, Tosoh, Tokyo).⁹ The C_w was calculated from the quantity of eluted lidocaine divided by the axonal volume, considering the axon to be a cylinder with its entire diameter and length immersed in ASW of the chamber.

The elution was complete within 30 min. To confirm whether all lidocaine was eluted within 30 min, any remaining lidocaine was extracted from the axon

FIGURE 5 (A) Changes in lidocaine concentration in the axoplasm (Ci) after the administration of lidocaine at various pH values (pH = 5.5 (●), 6.8 (□), 7.8 (+) and 9.0 (○)). (B) Changes in pH in the axoplasm (pHi) before and after the administration of lidocaine at various pH values (pH = 5.5 (●), 6.8 (□), 7.8 (+) and 9.0 (○)). Add = administration of lidocaine solution.

by diethylether and measured with HPLC. These values at pH = 5.5, 7.8 and 9.0 were negligible.

Statistical analysis

Data are expressed as mean $\pm$ SD. Statistical analyses were performed by using analysis of variance with Bonferroni's adjustment for multiple comparison, paired t-tests, and unpaired t-tests, where appropriate (StatView® 4.5; Abacus Concepts, Inc., Berkeley, CA). A value of $P < 0.05$ was considered significant.

Results

Lidocaine concentration in the axoplasm (Ci)

Figure 5A shows the typical time course of Ci changes

FIGURE 6 Relationships between the concentration of uncharged form in the administered lidocaine, lidocaine in the axoplasm (Ci (□)) and that in the whole axon (Cw (♦)). The horizontal axis represents the concentration of the uncharged form administered, and the vertical axis represents the Ci and Cw. The Ci was in direct proportion to the concentration of the uncharged form administered, and 1.5 times higher ($Ci = 1.5x + 0.4$, $r = 1.00$). The Cw also increased in accordance with the concentration of the uncharged form administered, but did not show a simple relationship.

* $P < 0.05$ between Ci and Cw at pH = 5.5 and 6.8.

after the administration of lidocaine at various pH values in the chamber. The Ci increased rapidly after the administration of lidocaine to the outside of the membrane. After the lidocaine solution was washed out from the chamber, the Ci decreased gradually and returned almost to the control level within 30 min. The values of Ci in the uptake process at pH 5.5, 6.8, 7.8 and 9.0 just before the wash-out were 0.65 ± 0.59 , 0.89 ± 0.74 , 6.13 ± 1.01 and 13.75 ± 1.64 , respectively. The Ci was higher in higher-pH lidocaine solutions than in lower-pH solutions (Table II).

pH in the axoplasm (pHi)

Figure 5B shows typical patterns of pHi changes. The time courses at pH 7.8 or 9.0 were similar to those observed for Ci. The ASW at pH 7.8 and 9.0 did not affect the pHi. At pH 5.5 and 6.8, the pHi decreased slowly, having no connection with existence of lidocaine.

The pHi differences between the post- and pre-administration of lidocaine (ΔpHi) were -0.05 ± 0.04 , 0.14 ± 0.15 , 0.42 ± 0.09 and 0.59 ± 0.05 at pH 5.5, 6.8, 7.8 and 9.0, respectively. The ΔpHi was larger in the chamber solution rich in the uncharged form compared to those rich in the charged form ($P < 0.01$) (Table II).

TABLE I Lidocaine concentration at various pH obtained from the calibration curve at pH 7.2

Lidocaine administered (mM)	Lidocaine concentration obtained from the calibration curve at pH 7.2 (mM)		
	pH=6.8	pH=7.5	pH=7.9
15.85	15.14 ± 0.45 (n=4)	15.76 ± 0.28 (n=4)	15.14 ± 0.70 (n=4)
10.00	9.83 ± 0.18 (n=4)	10.00 ± 0.30 (n=4)	9.78 ± 0.50 (n=4)
3.16	3.07 ± 0.14 (n=4)	3.20 ± 0.20 (n=4)	3.09 ± 0.24 (n=4)
1.00	0.98 ± 0.20 (n=4)	0.98 ± 0.07 (n=4)	1.00 ± 0.12 (n=4)
0.10	0.097 ± 0.02 (n=4)	0.093 ± 0.02 (n=4)	0.11 ± 0.04 (n=4)
0.01	0.015 ± 0.02 (n=4)	0.006 ± 0.01 (n=3)	0.02 ± 0.05 (n=3)

TABLE II Lidocaine concentration in the axoplasm (Ci), in the whole axon (Cw), and pH in the axoplasm (pHi)

Lidocaine	Ci (mM)	Cw (mM)	pHi(unit)
pH=5.5	0.65 ± 0.59 (n=4)	2.35 ± 0.25 (n=4)	-0.05 ± 0.04 (n=3)
pH=6.8	0.89 ± 0.74 (n=4)	4.04 ± 0.60 (n=4)	0.14 ± 0.15 (n=3)
pH=7.8	6.13 ± 1.01 (n=6)	6.28 ± 0.85 (n=5)	0.42 ± 0.09 (n=7)*†
pH=9.0	13.75 ± 1.64 (n=4)	13.51 ± 1.62 (n=4)	0.59 ± 0.05 (n=3)*†

pH = pHi differences between post- and pre- administration of lidocaine

Values are mean ± SD.

* $P < 0.01$ vs pH = 5.5

† $P < 0.01$ vs pH = 6.8

TABLE III The mean time, lidocaine concentration (Ci), and charged lidocaine concentration in the axoplasm when the action potential (AP) just disappeared and reappeared

Lidocaine solution	AP Disappearance Time (min)	Ci (mM)	Ci-charged (mM)
pH = 7.8 (n=6)	8.45 ± 3.38	4.33 ± 1.08	3.49 ± 0.86
pH = 9.0 (n=4)	2.83 ± 1.91*	1.96 ± 0.83*	1.47 ± 0.50*
	AP Reappearance		
pH = 7.8 (n=6)	3.95 ± 1.40	5.47 ± 1.77	4.36 ± 1.35
pH = 9.0 (n=4)	20.33 ± 3.41*	2.24 ± 1.36*	1.75 ± 1.01*

Ci-charged = charged lidocaine concentration in the axoplasm

Values are mean ± SD

* $P < 0.05$ vs pH = 7.8

Lidocaine concentration in the whole axon (Cw)

The Cw in each pH solution is listed in Table II, which shows that the Cw values in the higher-pH solutions were greater than those in the lower-pH solutions. The Cw was 2.35 ± 0.25 mM at pH 5.5, while a trace of lidocaine was detected in the axoplasm.

Change in action potential (AP) and lidocaine concentration in the axoplasm

The AP disappeared after the administration of higher-pH lidocaine solutions (pH = 7.8, 9.0) and reappeared by washing out the solution in the chamber. In contrast, the AP did not disappear after the application of the lower-pH solutions (pH = 5.5, 6.8).

Table III shows the mean time, Ci, and charged lidocaine concentration in the axoplasm when the AP

just disappeared and reappeared at pH = 7.8 and 9.0. The time when the AP just disappeared was more rapid at pH 9.0 than at pH 7.8, and the time when the AP just reappeared was slower at pH 9.0 than at pH 7.8 ($P < 0.05$). The Ci and charged lidocaine concentration of the axoplasm in both situations were lower at pH 9.0 than at pH 7.8 ($P < 0.05$).

Discussion

The penetration process of lidocaine across the nerve membrane and its concentration in the axoplasm

The results of the present study demonstrated the continuous penetration of lidocaine across the nerve membrane. Lidocaine in the axoplasm (Ci) increased after the administration of lidocaine in the chamber, and was decreased by washing out the lidocaine solu-

tion (Figure 5A). Since the lidocaine microsensor was inserted into the centre of the axoplasm, a delay in the increase of C_i was observed after the addition of lidocaine. In almost all of the experiments, discontinuity of C_i was found when the lidocaine solution was washed out from the chamber. Vibration of the axon by the washing out process might have caused drifts in the electrode potentials of a lidocaine microsensor.

The C_i increased in accordance with the extracellular pH changes. By plotting the C_i and lidocaine in the whole axon (C_w) as a function of the concentration of the uncharged form, a simple equation was obtained for C_i but not for C_w (Figure 6). At pH 5.5, only 0.65 mM lidocaine was detected in the axoplasm, while 2.35 mM existed in the axonal membrane (axon without axoplasm). This means that the charged form cannot penetrate the axoplasm through the membrane but exists in the axon membrane, which includes a small amount of connective tissue. At pH > 7.8, no differences between C_i and C_w were found, which means that the uncharged form diffuses equally into both the axon membrane and the axoplasm because of its hydrophobicity. If uncharged lidocaine only penetrates through the axon membrane and does not bind with it, the C_i should be the same as that for the uncharged form administered. However, the C_i was 1.5 times greater than that for the uncharged form administered. Thus, lidocaine may be bound or concentrated in the axon membrane and axoplasm. The oil/water partition coefficient of the uncharged form of lidocaine is greater than that of the charged form. The octanol/water partition coefficient of the charged form of lidocaine is 3.07 (M·1000 ml⁻¹ solution), and that of the uncharged form is 65.5 (M·1000 ml⁻¹ solution).¹⁰ Although the axon membrane/axoplasm partition coefficient of lidocaine was not identified in the present study, lidocaine is thought to be partitioned in the axoplasm rather than in the extracellular solution, because both membrane and axoplasm have lipophilic properties. Thus, it is reasonable to suggest that lidocaine could be concentrated in the axon membrane and axoplasm.

Changes of pH in the axoplasm

After the administration of lidocaine, the pH in the axoplasm (pHi) increased for each pH solution except at pH 5.5, but each pH change in the axoplasm for the higher-pH solutions was greater than that for lower-pH solutions. Since the pHi returned to the control values after the replacement of the lidocaine solution with ASW without lidocaine, these pH changes were shown to be reversible.

Uncharged lidocaine which penetrates the axoplasm is thought to change to the charged form by

binding with hydrogen ions in the axoplasm.¹ If true, the pHi should increase in proportion to the concentration of uncharged lidocaine in the axoplasm. In the present study, the pHi did not vary in direct proportion to the uncharged form of lidocaine. Since there are many intracellular pH regulatory systems such as Na⁺-K⁺ and/or Cl⁻-HCO₃⁻ exchangers in a squid giant axon,¹¹ these might contribute in some part to the modulation of pHi changes after a lidocaine influx.

At lower pH (pH=5.5, 6.8), the pHi decreased gradually even though no lidocaine was present. Because extracellular hydrogen ions inhibit various channels in the plasma membrane,^{12,13} a large amount of hydrogen ion exceeding the physiological range might inhibit normal pH regulatory systems.

Nerve blocking action and the form of lidocaine

The AP disappeared with the addition of higher-pH lidocaine solutions (pH = 7.8, 9.0), but did not disappear after the application of lower-pH solutions (pH = 5.5, 6.8). The time to disappearance of the AP was faster at pH 9.0 than at pH 7.8, and the nerve blocking period after washing out the lidocaine was also longer at pH 9.0 than at pH 7.8. The C_i was higher in higher-pH solutions than in lower-pH solutions. These findings showed that external application of the uncharged form was responsible for the nerve blocking action, but that of the charged form was not.

The mean charged lidocaine in the axoplasm, when the AP just disappeared or reappeared, was lower at pH 9.0 than at pH 7.8. Nerve conduction in the area bathed in lidocaine was blocked by lower axoplasmic charged concentration at pH 9.0 than at pH 7.8. Other factors (i.e. the increase in uncharged form in the axon membrane etc.) may modulate nerve conduction aside from the increase in charged form in the axoplasm in high pH lidocaine solutions.

The effect of pH on local anesthetic activity in clinical situations

The effects of pH adjustment and carbonation of LAs have been reported in several clinical situations. Our present study shows that external application of uncharged lidocaine can penetrate into the axon and modulate nerve conduction. Therefore, alkalization should increase the effect of LAs, because it increases the uncharged form. The effect of carbonation of LAs was unknown in this study. Since acidification with CO₂ decreases the uncharged form, it should decrease the effect of LAs. However, CO₂ decreases nerve conduction on its own.¹⁴ Adjustment of pH and carbonation of LAs are often clinically ineffective, probably because the pH of LAs is rapidly brought to that of

the extracellular fluid, regardless of the pH of the solution with which it is injected.

The changes in acid-base status of animals and patients also might affect the nerve blocking action and toxicity of LAs. However, this effect was not identified in this study because the effect of intracellular alkalization or acidification on nerve conduction cannot be investigated by the present methods.

In summary, our results indicate that uncharged lidocaine penetrates the axon membrane into the axoplasm where it changes to the charged form, and is concentrated in the axon membrane and axoplasm. Our findings also suggest that external application of uncharged lidocaine plays a role in the modulation of nerve conduction.

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