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Molecular mechanism of inhibition of firefly luminescence by local anesthetics

(molecular theory of anesthesia/lipoprotein conformational change/electrostriction)

ISSAKU UEDA*, HIROSHI KAMAYA†, AND HENRY EYRING§

* Department of Anesthesia, University of Kansas Medical Center, Kansas City, Kans. 66103; † Veterans Administration Hospital, Salt Lake City, Utah 84113; and § Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

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ABSTRACT **The kinetics of the action of local anesthetics upon firefly luciferin and luciferase systems is presented. Clinical concentrations of local anesthetics inhibited this ATP-induced luminescence in a dose-dependent manner. From the effects of temperature and pH upon the inhibitory action of the local anesthetics, it is concluded that hydrophobic ligand–enzyme interaction is the predominant cause of the inhibition, but hydrophilic interaction also contributes to the inhibition to a lesser degree. A molecular theory of anesthesia is outlined which postulates that release of electrostricted water molecules from the hydrophilic parts of the enzyme due to the protein conformational changes induced by anesthetics is the cause of the decreased luminescence. A similar mechanism is expected to occur at the cell membrane, which probably dehydrates the sodium channel and suppresses the conductance of this ion across the membrane. These events lead to a volume expansion of the total system, and the system becomes reactive to a pressure which reverses the anesthesia by shifting the equilibrium to the nonanesthetized original volume. The pressure antagonism of anesthesia can be explained by this overall volume expansion and not by a mere swelling of the cell membrane.**

All clinically used local anesthetics contain a hydrophobic benzene ring on one end and a hydrophilic tertiary amine on the other. These compounds are solubilized by protonating the tertiary amine to form an onium ion in acid media. The ratio between unprotonated (B) and protonated (BH^+) forms is determined by the pK_a of the compound and the pH of the medium.

$$pH - pK_a = \log B/BH^+ \quad [1]$$

Because these anesthetics have pK_a values between 7.5 and 9, protonated and unprotonated forms coexist in a physiological pH. It has been a matter of wide discussion as to which is the biologically active form.

The binding of the protonated form to the cell membrane suggests electrostatic or hydrogen bond interaction to a negatively charged area, which may well constitute a specific receptor. Conversely, the binding of the unprotonated form suggests interaction with a hydrophobic region of the cell membrane. The predominant force for the hydrophobic interaction may be clusters of the water clathrate of higher energy formed around the hydrophobic molecule and the van der Waals force.

Working with bacterial luminescence and its inhibitors, Johnson, Eyring, and coworkers (1, 2) have classified ligand interaction with protein macromolecules in two types, Type I and Type II. Type I represents electrostatic or hydrogen-bonded receptor binding which does not induce protein conformational change. Type II represents hydrophobic interaction with conformational change of macromolecules. These two types are distinguishable by observing the reaction rate at varying temperatures according to the following equations (1, 2).

$$\ln\Gamma = s\ln X - \Delta H_2/RT + \Delta S_2/R \quad [2]$$

$$\ln[\Gamma(1 + 1/K_1)] = r\ln U - \Delta H_3/RT + \Delta S_3/R \quad [3]$$

$$\Gamma \equiv I_1/I_2 - 1$$

The notation has the following significance: I_1 = reaction rate without inhibitor; I_2 = reaction rate with inhibitor; K_1 = equilibrium constant for thermal inactivation; X, U = Type I and Type II inhibitor, respectively; s, r = number of X and U molecules binding to the enzyme, respectively. The remaining notations have the usual thermodynamic meaning, and subscripts 2 and 3 represent Type I and Type II inhibitions, respectively.

Ueda and Kamaya (3) have previously shown that inhalational general anesthetics inhibited the flash intensity of the soluble firefly luminescent system. The firefly tails contain a light-emitting substance, luciferin, and an enzyme, luciferase. Luciferin and luciferase can be extracted cell-free from the tail homogenate and emit light upon addition of ATP in the presence of molecular oxygen. The initial flash intensity and the quantum yield are proportional to the amount of added ATP, but the energy for the photon liberation is supplied by the oxidation of luciferin.

The kinetics of inhibition by general anesthetics was analyzed using reaction rate theory and was found to conform to Type II. We concluded that general anesthetics combined with the luciferase in the hydrophobic region and induced conformational change of the water-soluble protein. It has been postulated by Eyring *et al.* (4) that this conformational change is accompanied by a volume expansion mainly due to a release of structured water molecules attached to the hydrophilic sites of the enzyme.

The present study deals with the effects of the aromatic amine local anesthetics upon the firefly luminescent system. The inhibition was found to be a hybrid of Type I and Type II. Along with the thermodynamic data, the effect of pH is presented.

METHOD

Firefly luciferin and luciferase were extracted from dried firefly tails (Calbiochem) in arsenate buffer, as described (3). The final concentration was 2.5 mg of firefly tail (dry weight) per 1 ml of 0.1 M sodium arsenate buffer pH 7.5 with 0.04 M $MgSO_4$. The extract was prepared fresh daily and kept in ice during the experiment.

An aliquot of 1.5 ml of the homogenate was mixed with 0.2 ml of appropriate concentrations of local anesthetics.

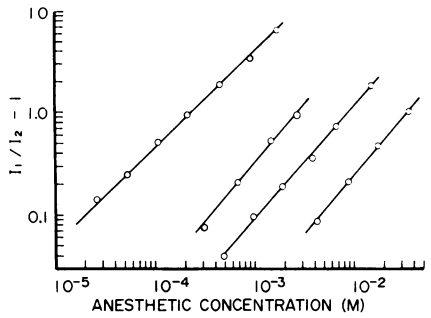

FIG. 1. Linearized dose-response curve. The lines are from left to right: tetracaine, dibucaine, procaine, and lidocaine. The slopes represent the number of local anesthetic molecules combining with the enzymes. A one-to-one ratio is observed.

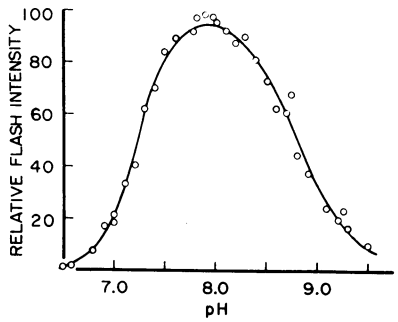

FIG. 2. The effect of pH on the flash intensity. The observed values are fitted to Eq. 4. $\Gamma = K_H(H^+) + K_{OH}(OH^-) = 10^{18.01}(H^+)^{2.49} + 10^{7.82}(OH^-)^{1.51}$. The solid curve is constructed from the equation.

The pH of local anesthetics was adjusted by the addition of NaOH and measured with a glass electrode and a Corning pH meter.

The mixture in a standard 1.0-cm light path spectrophotometry cell was placed in a thermostated cuvette compartment of a Hitachi Perkin-Elmer 139 spectrophotometer. The luminescence was induced by injecting 0.3 ml of disodium ATP (Sigma) in appropriate concentrations. The light output was measured with a 1P21 photomultiplier and was recorded on a strip chart recorder.

The local anesthetics were the gifts of the following companies: tetracaine and procaine from Sterling-Winthrop Res. Inst., dibucaine from Ciba Pharmaceut. Co., and lidocaine from Astra Pharmaceut. Prod., Inc.

RESULT

1. Dose-response relationship

All compounds inhibited the flash intensity dose-dependently. The experiment was undertaken at pH 7.5 and 20.5°. The dose-response curves were linearized by taking the logarithm of the ratio between the inhibited fraction and the uninhibited fraction of the luminescence plotted against the logarithm of the drug concentrations (Fig. 1). The mean effective doses were estimated by interpolation and were found to be: tetracaine 2.2×10^{-4} M, dibucaine 2.8×10^{-3} M, procaine 8.4×10^{-3} M, and lidocaine 3.5×10^{-2} M. The slopes of these lines represent the number of ligand molecules binding per one molecule of the luciferase, as seen in Eqs. 2 and 3. A one-to-one relationship was found with all anesthetics.

2. Effect of pH

Fig. 2 shows the relationship between pH and the flash intensity. The flash intensity shows pH optima at 7.8. This observation is explained as inhibition due to combination with either hydrogen or hydroxyl ions (see ref. 2 for detail).

The hydrogen ion (H^+) and hydroxyl ion (OH^-) bind to the native luciferase (E_n) and the thermally inactivated luciferase (E_d) indiscriminately. The numbers of (H^+) and (OH^-) that bind to the luciferase are expressed by m and n, respectively. The association constants for (H^+) and (OH^-) are represented by K_H and K_{OH}, respectively. Other notations are as follows: E_o = total luciferase = $E_n + E_d$; L = luciferin; b = proportionality constant; k' = reaction rate constant; K_1 = equilibrium constant for thermal inactivation; I_1 = light intensity at neutral pH; I_2 = light intensity under inhibition by H^+ or OH^-.

$$m(H^+) + E_n \overset{K_H}{\rightleftharpoons} E_n(H^+)_m \qquad m(H^+) + E_d \overset{K_H}{\rightleftharpoons} E_d(H^+)_m$$

$$K_H = E_n(H^+)_m/(E_n)(H^+)^m \qquad K_H = E_d(H^+)_m/(E_d)(H^+)^m$$

$$E_n(H^+)_m = K_H(E_n)(H^+)^m$$

$$E_d(H^+)_m = K_H(E_d)(H^+)^m = K_HK_1(E_n)(H^+)^m$$

The interaction with OH^- is treated similarly.

$$E_o = E_n + E_d + (E_n)(H^+)^m + (E_n)(OH^-)^n + (E_d)(H^+)^m + (E_d)(OH^-)^n$$

$$E_n = [E_o]/[1 + K_1 + K_H(H^+)^m + K_{OH}(OH^-)^n + K_1K_H(H^+)^m + K_1K_{OH}(OH^-)^n]$$

$$I_1 = bk'(E_n)(L)(ATP) = [bk'(E_o)(L)(ATP)]/[1 + K_1]$$

$$I_2 = [bk'(E_o)(L)(ATP)]/[1 + K_1 + K_H(H^+)^m + K_{OH}(OH^-)^n + K_1K_H(H^+)^m + K_1K_{OH}(OH^-)^n]$$

$$(I_1/I_2 - 1) \equiv \Gamma = K_H(H^+)^m + K_{OH}(OH^-)^n \quad [4]$$

The data in Fig. 3 are fitted to the above equation; the following numerical values were obtained.

$$(I_1/I_2 - 1) = 10^{18.01}(H^+)^{2.49} + 10^{7.82}(OH^-)^{1.51}$$

This numerical value is used to construct a curve (Fig. 2).

3. Effect of pH on the tetracaine inhibition

Fig. 3 shows the effect of pH on the inhibition of the flash intensity by tetracaine. Increased inhibition was seen in the higher pH range at each concentration of tetracaine.

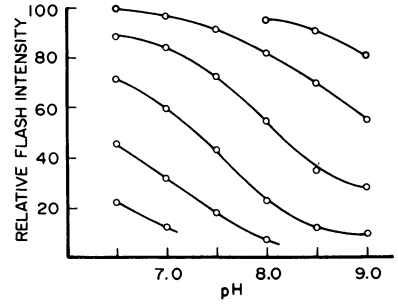

FIG. 3. The effect of pH on the tetracaine inhibition. The concentrations of tetracaine are from top to bottom: 6.49×10^{-6}, 2.60×10^{-5}, 1.04×10^{-4}, 4.16×10^{-4}, 1.66×10^{-3}, and 6.65×10^{-3} M.

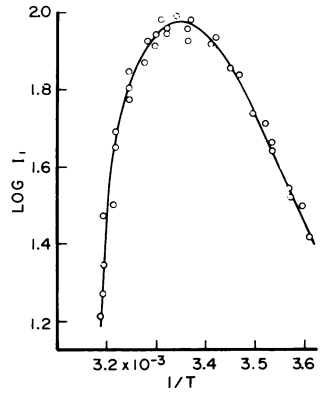

FIG. 4. Temperature effect. From the low temperature range $\Delta H^{\ddagger}$ was estimated to be 12 kcal/mol (50 kJ/mol), and from the high temperature range ΔH_1 was estimated to be 80 kcal/mol (336 kJ/mol).

The concentrations of tetracaine that inhibited flash intensity 50% at various pH values were estimated by interpolation and were found to be: 1.25×10^{-3} M at pH 6.5, 6.30×10^{-4} at pH 7.0, 2.24×10^{-4} M at pH 7.5, 1.15×10^{-4} M at pH 8.0, 5.50×10^{-5} M at pH 8.5, and 3.35×10^{-5} M at pH 9.0. When these values were converted to the concentrations of unprotonated species, the following figures were obtained: 1.24×10^{-5} M at pH 6.5, 1.93×10^{-5} M at pH 7.0, 2.04×10^{-5} M at pH 7.5, 2.77×10^{-5} M at pH 8.0, 2.75×10^{-5} M at pH 8.5, and 2.55×10^{-5} M at pH 9.0.

The 37-fold difference between uncorrected concentrations of tetracaine to achieve 50% depression of the flash intensity at pH 6.5 and at pH 9.0 reduces down to a mere 2-fold when the total concentrations were converted to the concentrations of unprotonated species.

4. Effect of temperature

An Arrhenius plot of the flash intensity against temperature is shown in Fig. 4. The constants for the activation process $\Delta H^{\ddagger}$ and the inactivation process ΔH_1 were estimated from the slopes of the lower and higher temperature range, respectively, (8) and were found to be 12.67 kcal/mol (1 cal = 4.184 *J*) and 79.02 kcal/mol, respectively. From the values of $\Delta H^{\ddagger}$ and ΔH_1, ΔS_1 was computed to be 260 e.u.

5. Effect of temperature on tetracaine inhibition

The effect of temperature on the tetracaine inhibition is shown in Fig. 5 by plotting $\ln\Gamma$ and $\ln[\Gamma(1 + 1/K_1)]$ against

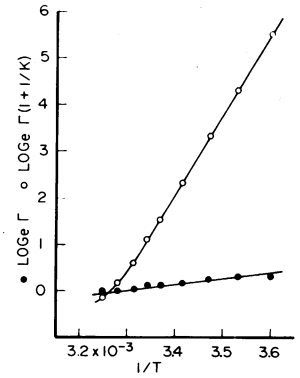

FIG. 5. Temperature effect of the inhibition of the flash intensity by tetracaine. Filled circles are the plot between log Γ and 1/T, representing Type I inhibition. Open circles are the plot between log $[\Gamma (1 + 1/K_1)]$ and 1/T, representing Type II inhibition.

1/T. Both plots were found to be linear. From the slope of $\ln[\Gamma(1 + 1/K_1)]$, ΔH_3 and ΔS_3 were estimated to be 81.05 kcal/mol and 250 e.u., respectively.

DISCUSSION

In 1954, Skou (5, 6) demonstrated that the nerve-blocking activity of local anesthetics of aromatic amine type depends upon the concentration of the unprotonated form of the compound. He also reported that the adsorption of local anesthetics to the surface monomolecular films of lipids extracted from the bovine brain correlated well with the clinical potency. From the adsorption data, he calculated that local anesthesia ensues when the number of molecules adsorbed to the nerve cell membrane exceeds 7.5×10^{13} molecules per square cm. The postulate is in accord with the Overton-Meyer theory of narcosis, which states that narcosis is produced by the solvation of a certain amount of a drug into the lipid part of the membrane.

The idea that the unprotonated form is the active species was challenged by Ritchie and Greengard in 1961 (7). They demonstrated with desheathed rabbit vagus nerve and dibucaine that the change of pH of the bathing fluid from 7.2 to 9.6 increased the size of the action potential and the change from 9.6 to 7.2 decreased it. They concluded that the local anesthetic penetrated the cell membrane in the unprotonated form and interacted with the membrane in the protonated form.

Narahashi and coworkers (8, 9), using internally perfused squid giant axon, found that the blocking potency of aromatic amine local anesthetics applied externally at a constant external pH was decreased by an increase of the internal pH. Quaternary derivatives of lidocaine, which carry a positive charge at all pH ranges used, were found to block the rate of rise of action potential more effectively from the inside than from the outside of the cell.

These observations support Ritchie's proposal that an aromatic amine local anesthetic penetrates the cell membrane in its unprotonated form and binds to the cell membrane from the cytoplasmic side after protonation. The low intracellular pH would facilitate the protonation of the penetrated drugs.

However, the transition model of Bianchi and Strobel (10), which is similar to the one used by Ritchie and Greengard (7), indicates that both protonated and unprotonated molecules are active. They used procaine or lidocaine to block the action potential of the desheathed frog sciatic nerve. After the block reached a quasi-steady state, the pH was perturbed by exchanging the bathing fluid. The change from pH 9.2 to 7.2 was associated with a dramatic potentiation of the inhibition, rapidly returning to the previous level. At the steady state, however, the inhibition was lower at pH 7.2 than at pH 9.2. They also demonstrated with [^{14}C]lidocaine and [^{14}C]procaine that the uptake of the compounds to the desheathed toad sciatic nerve is larger at pH 9.2 that at pH 7.2. They hypothesized that the protonation of the local anesthetics within the cell membrane increased the blocking action and that then the molecules dissociated from the membrane by the protonation. They concluded that both the protonated and unprotonated forms were active and worked synergistically.

The finding that the local anesthetics dissociate from the membranes at lower pH is supported by the work of Nishimura *et al.* (11) on the binding of the local [^{14}C]anesthetics with crystalline bovine serum albumin. Their data showed that the binding of the radioactive compounds was de-

creased by the decrease of pH. A sharp decrease of binding was observed with each anesthetic at a pH value close to the pK_a of the compound. Because the isoelectric point of bovine serum albumin is pH 4.9, the protein molecule is negatively charged under the conditions of the study. The predominant form of the local anesthetics that bind to this protein should be unprotonated in spite of the presence of the surface negative charge of the protein.

The observation that both protonated and unprotonated forms are active is supported by a nuclear magnetic resonance spectroscopy study by Cérbon (12). He reported that the hydrophobic end of tetracaine allowed this drug to interact with the lipid film in the absence of a net negative charge on the membrane. He also reported that the local anesthetic molecules without such a hydrophobic tail interact first electrostatically and then hydrophobically with charged films. The strength of the hydrophobic interaction apparently paralleled the clinical potency. This report is in conflict with the previous nuclear magnetic resonance result reported by Hauser *et al.* (13), who found that only negatively charged phospholipids interact with the local anesthetics.

With electron spin resonance spectroscopy, Butler and coworkers (14, 15) reported that aromatic amine local anesthetics affected the lipid bilayers, inducing disorder. They used nitroxide-labeled cholestane as a spin probe in the lipids extracted from beef brain and found that procaine, tetracaine, and butacaine increased the anisotropy of the spin label. This increase of the disorder of the molecular arrangement was enhanced by the increase of pH. Alcohols, on the other hand, showed a similar disordering effect, but the action was not influenced by the pH. Thus, unprotonated molecules increased the disorder of the lipid membrane. Butler (15), however, reported that in a lipid bilayer that contains a supranormal amount of cholesterol, molecules responded to the local anesthetics with ordering of the membrane at low pH (pH 4.5). Both excess ordering and disordering of the membrane may induce anesthesia, but the significance of the lipid model membrane with excess cholesterol is not clear at present.

Our present study shows that the inhibitory action of aromatic amine local anesthetics upon firefly luminescence was increased at an alkaline pH (Fig. 3), which indicates that the unprotonated form is more active than the protonated species. The concentrations of tetracaine that inhibited the flash intensity 50% was 1.25×10^{-3} M at pH 6.5 and 3.35×10^{-5} M at pH 9.0. The inhibitory action of tetracaine was 37-fold higher at the alkaline range. However, when the total concentrations of tetracaine were converted to the concentrations of unprotonated species, the mean effective dose was 1.24×10^{-5} M at pH 6.5 and 2.55×10^{-5} M at pH 9.0. The difference is now reduced to 2-fold, and the inhibitory action was lower in the alkaline range. This indicates that the major active species is unprotonated, and the fact that the concentrations of the unprotonated species to depress the flash intensity 50% are lower at the lower pH range suggests that the coexisting protonated species is also active to a lesser degree. The inhibition was found to be a hybrid of Type I and Type II (Fig. 5). The large increases of entropy (250 e.u.) and enthalpy (81 kcal/mol) in the Type II plot indicate that a conformational change occurred to unfold or dilate the protein macromolecule, which constitutes the Type II part of the interaction. This large disordering effect is in concert with the increase of spectral anisotropy of the electron spin resonance studies cited above (14, 15).

The dilatation of the cell membrane by the local anesthetics is reported by Seeman *et al.* (see review, ref. 16) in washed human erythrocytes. According to their calculation, the erythrocyte membrane increases its surface area about 2–3% at the anesthetic concentration that blocks nerve conduction. Seeman further estimated that the dilatation is in excess of the partial molar volume occupied by the local anesthetics adsorbed to the cell membrane by a factor of about 10.

The helix-coil transition of the α-helical protein only accounts for a few entropy units. Thus, the magnitude of ΔS (250 e.u.) induced by local anesthetics is far too large to be explained solely by the random coiling of the protein. Eyring *et al.* (4) explained the increase of ΔS of the luciferase by general anesthetics by the release of structured water from the hydrophilic part of the protein.

Before discussion of the dilatation of the cell membrane and luciferase by local anesthetics, it would be informative to discuss the dilatational effects of general anesthetics because the latter has been studied more extensively.

Dilatation of the red cell membrane by general anesthetics at the surgical stage of anesthesia is reported to be about 0.3% by Seeman and Roth (17). Again, the magnitude of the dilatation was larger by a factor of 10 than the partial molar volume occupied by the adsorbed general anesthetic molecules. It is interesting to note that the adsorption of the general anesthetics to dry elastomers like latex in the air dilates the volume exactly by the size of the partial molar volume of the adsorbed general anesthetics (18). The excess dilatation is seen only when the system contains water.

Proteins in water are like oil covered by an ionized film. The hydrophobic parts are folded inside, exposing the hydrophilic sites outside which attract water molecules to the ionized parts. These water molecules assume a crystalline lattice structure by forming hydrogen bonds. The ionic force exerted on the water dipole is large and acts to reduce its volume.

Eyring *et al.* (4) suggest that the water molecules attached to the ionized sites probably occupy a volume approximately that of Ice III, the density of which is about 10% higher than that of bulk water. The polymorphism of ice structure under pressure is well known. Ordinarily Ice III is formed under a pressure of about 2 kbar. The hydrophilic sites exert electrostatic force on the water dipoles of a magnitude that might well form Ice III. The volume of the system decreases in forming this structured water. This shrinkage is known as electrostriction.

General anesthetics interact with the hydrophobic interior, increase fluidity, and unfold the protein into a new conformation. As a result, surface positive and negative charges can neutralize each other, and the structured water is released. The system expands, and consequently ΔS increases. With the neutralization of the surface charges, the hydrophobic interior would be exposed by the unfolding. The exposure of the hydrophobic parts induces hydrophobic hydration by the formation of the clathrate around the hydrophobic sites. The clathrate structure ordinarily has a larger volume than the bulk water and contributes further to the expansion of the system by the anesthetics.

Because the volume of the system is larger under anesthesia, hydrostatic pressure antagonizes general anesthesia. This pressure reversal of anesthesia was initially demonstrated by Johnson, Eyring, and Williams (1) in bacterial luminescence. It was this theory that led Johnson and Flagler (19, 20) to demonstrate the pressure reversal of ethanol anesthesia in tadpoles. Subsequently the pressure reversal was confirmed

in newts and mice anesthetized by modern anesthetic agents (21, 22). The pressure reversal is characteristic of Type II inhibition.

The Type II part of local anesthetic action demonstrated by the present study indicates hydrophobic interaction of local anesthetics, causing an unfolding of the luciferase. This unfolding or conformational change leading to volume dilatation by local anesthetics is supported by the observation of the pressure reversal of the procaine-inhibited light output in luminous bacteria (23). The pressure reversal of the local anesthetic activity of ethanol upon squid giant axon was reported by Spyropoulos (24).

Since pressure reversal by anesthesia measures the volume change of the whole system due to each individual molecule of a cluster of molecules changing from the nonanesthetized to the anesthetized state, there would be no pressure reversal if anesthetic molecules migrated from outside the membrane into it without changing the overall volume of the membrane and its surroundings. A small homogeneous swelling of the membrane has nothing to do with pressure reversal of anesthesia. Pressure reversal can only arise from a cluster of molecules changing cooperatively between two states, with the simultaneous disappearance of about five ion pairs and the consequent release of the water bound by electrostriction expanding 75 cm^3/mole of such clusters. It is difficult to imagine anything but some conformational change of a protein that would cause the simultaneous disappearance of five ion pairs.

Our study (to be reported) demonstrates that the action of the alcohol anesthetic was independent of the pH and was in addition to the action of tetracaine. The additive effects of the aromatic amine local anesthetics and the alcohols were also reported in the nerve preparation (25). These facts indicate a unified mode of action between these two types of anesthetics, consistent with a hydrophobic interaction.

Presumably, the local anesthetics insert their hydrophobic centers into the hydrophobic interior of the cell membrane and the hydrophilic amine stays at the phase boundary and interacts with the negatively charged surface. The electrostatic interaction between the excess negative charge of the membrane and the positive end of the local anesthetics would represent the Type I part of the interaction because it is largely independent of the thermal conformational change.

The extent of the positive charge of the tertiary amine would influence the neutralization of the surface negative charge. Protonation of the local anesthetics at lower pH should facilitate the release of the structured water molecules from the negatively charged sites and enhances anesthesia. However, the protonation of the tertiary amine decreases the binding of the local anesthetics to the membrane. This sequence is well demonstrated by Bianchi and Strobel (10) in their pH jump experiment as described earlier. A sudden protonation of the local anesthetics after their binding to the nerve cell enhanced the blocking activity during a transitory phase and later decreased the blocking activity as the steady state was attained.

The Type I interaction may also occur with general anesthetics, although the kinetics of the inhibition of firefly luciferase showed almost pure Type II inhibition (3). It has been known that dipolar molecules like halothane or chloroform are a stronger anesthetic than apolar hydrocarbons like ethylene or cyclopropane. The polarity of the general anesthetics might well assist in the neutralization of the surface negative charges by donating protons.

We propose that the perturbation of the hydrophobic domain is the initial event of local anesthesia. The structured water bound to the hydrophilic sites of luciferase is released by the conformational change and the neutralization of the surface charges. The release of the structured water is reflected in the increased entropy and volume. The protonation of the tertiary amine assists the anesthesia by neutralizing the excess surface negative charges. These events should also occur at the surface of the nerve cell membrane. The breaking of the hydrogen bonds between the cell membrane hydrophilic parts and the structured water molecules dries out the interfacial passage ways and prevents the penetration of sodium ions into the cell membrane interior; thus, the depolarization is antagonized.

These arguments may not apply to the biotoxins like tetrodotoxin, suxitoxin, etc. It has been proposed that these molecules bind specifically to the sodium channel from outside of the nerve cell membrane.

Finally, the discrepancy between the relative potency of the local anesthetics upon the inhibition of firefly luminescence and the nerve blocking activity is to be expected if there is a difference in the structure of the hydrophobic core and the surface charge characteristics of the two systems.

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