J Anesth (1997) 11:121–125

Journal of
Anesthesia

Correlation between the anesthetic potency of local anesthetics and their binding ability to a model membrane

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Abstract: The interaction between various local anesthetics and the phospholipid membrane was examined by ^{1}H-NMR (nuclear magnetic resonance) spectroscopy. By examining the chemical shift value in order to measure the extent of proximity of various local anesthetics to the membrane, it was determined that tetracaine (10.7 Hz) was closest to the membrane, followed in descending order of proximity by dibucaine (8.8 Hz), bupivacaine (4.4 Hz), propitocaine (4.4 Hz), and lidocaine (3.5 Hz). Procaine and cocaine did not affect the chemical shift value. In addition, we studied the interaction of local anesthetics with the membrane by examining the broadening of the half-width, and determined that tetracaine (12.2 Hz) bound closest to the membrane, followed in descending order of proximity by dibucaine (11.0 Hz), bupivacaine (9.6 Hz), propitocaine (9.0 Hz), lidocaine (8.8 Hz), procaine (8.0 Hz) and cocaine (7.9 Hz). In the present study, the binding ability of local anesthetics to the phospholipid membrane was found to be directly in parallel with the potency and toxicity of the anesthetic.

Key words: Local anesthetics, NMR, Biomembrane, Binding ability, Site of action

Introduction

The site of local anesthetic action is well known to be the sodium channel. However, several questions have been raised in response to Hille's theory that specific receptors exist for local anesthetics [1]. For example, the effective concentration of drugs for which expression of the pharmacological effect depends on specific receptors, such as morphine, acetylcholine, and tetrodotoxin, is on the order of nanomoles, but that of local anesthetics is on the order of millimoles [2]. This difference is about 1 million-fold. It is difficult to accept that specific receptors exist for chemicals that are effective only at such high concentrations.

Also, according to Hille, local anesthetics pass through the biomembrane, and enter sodium channels inside [1]. However, there is no scientific evidence demonstrating that local anesthetics pass through the biomembrane. The results of a study we conducted using NMR showed that when local anesthetics were left for 1 month at 37°C in an environment in which the pH was raised to 10, thereby increasing the amount of basic local anesthetic 1000 times, the anesthetic did not pass through the membrane and instead remained on the surface [3].

Researchers do not dispute that the sodium channel is the site of local anesthetic action. However, local anesthetics can inhibit the influx of the sodium ion without entering the channel. For example, local anesthetics can inhibit the passage of the sodium ion by binding to the biomembrane that surrounds the sodium channel, thereby altering the conformation of channel proteins and expressing their pharmacological effect [4].

Previously, we synthesized new ester derivatives of lidocaine with a longer duration of action than that of lidocaine, and the interaction between the derivatives and phospholipid membrane was examined by NMR spectroscopy [5]. The duration of the ester derivatives was found to be three times longer than that of lidocaine [6], because the derivatives bound to the membrane at two location [4,5]. Therefore, in the present study, NMR was utilized to measure the binding ability of each local anesthetic with the phospholipid membrane in order to compare the pharmacological characteristics (potency and toxicity) of the anesthetics.

Materials and methods

The interaction between 110 mM of local anesthetics hydrochloride (lidocaine, propitocaine, bupivacaine,

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Received for publication on March 1, 1996; accepted on August 5, 1996

dibucaine, tetracaine, procaine, and cocaine, pH 6.2–6.8) and the phospholipid model membrane (66 mM of egg yolk lecithin) was examined by ^{1}H-NMR. Lecithin dispersions were obtained by evaporating lecithin solution (L-α-phosphatidyl-choline type III-E from egg yolk, 100 mg·ml^{-1} in hexane) to dryness, dissolving the residue in D_2O, and subjecting the resulting coarse dispersion to an ultrasonic disintegrator (W-220, Heat System-Ultrasonic, Inc., New York, USA) for 1 h in an ice-cold vessel under a nitrogen atmosphere. ^{1}N-NMR spectra were measured in a 5-mm tube at 27°C using a JNM-EX-400 spectrometer (JEOL, Tokyo, Japan). Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as the internal standard (0.0 ppm).

Based on the results of previous studies [3–5], it was thought that the binding between local anesthetics and the membrane occurs in the oxygen atom on the phosphate adjacent to the choline methyl, which is the hydrophilic region on the external surface of the membrane. Therefore, a hydrogen atom that exists most proximally to the oxygen atom, the choline methyl signal, was used for ^{1}H-NMR to measure the chemical shift and the broadening of half-width (peak width at half maximum height) of hydrogen atoms signal.

Results

The ^{1}H-NMR spectrum of the phospholipid membrane in D_2O is shown in Fig. 1A. The most prominent signals, identfied as choline methyl protons, methyl protons, and methylene protons, showed a chemical shift at 3.26 ppm (1302.4 Hz), 0.85 ppm (340.3 Hz), and 1.31 ppm (523.8 Hz) from DSS, respectively. When lidocaine hydrochloride (110 mM) was added to the solution, the chemical shift of the methylene and methyl signals showed no change, but the choline methyl signal shifted 3.5 Hz to upfield (1302.4 Hz → 1298.9 Hz, Fig. 1B) from the internal reference (DSS). This provides evidence of the interaction between anesthetics and lecithin vesicles on the membrane surface. A similar upfield shift was observed after the addition of propitocaine (4.4 Hz), bupivacaine (4.4 Hz), dibucaine (8.8 Hz), and tetracaine (10.7 Hz), whereas no chemical shift was observed after the addition of procaine and cocaine. These results are attributable to the change in electrical environment of the polar part of the membrane surface resulting from the approach of the incorporated anesthetics. Furthermore, the degree of the chemical shift probably depends on the extent of proximity between the phospholipid membrane and anesthetics (Fig. 2).

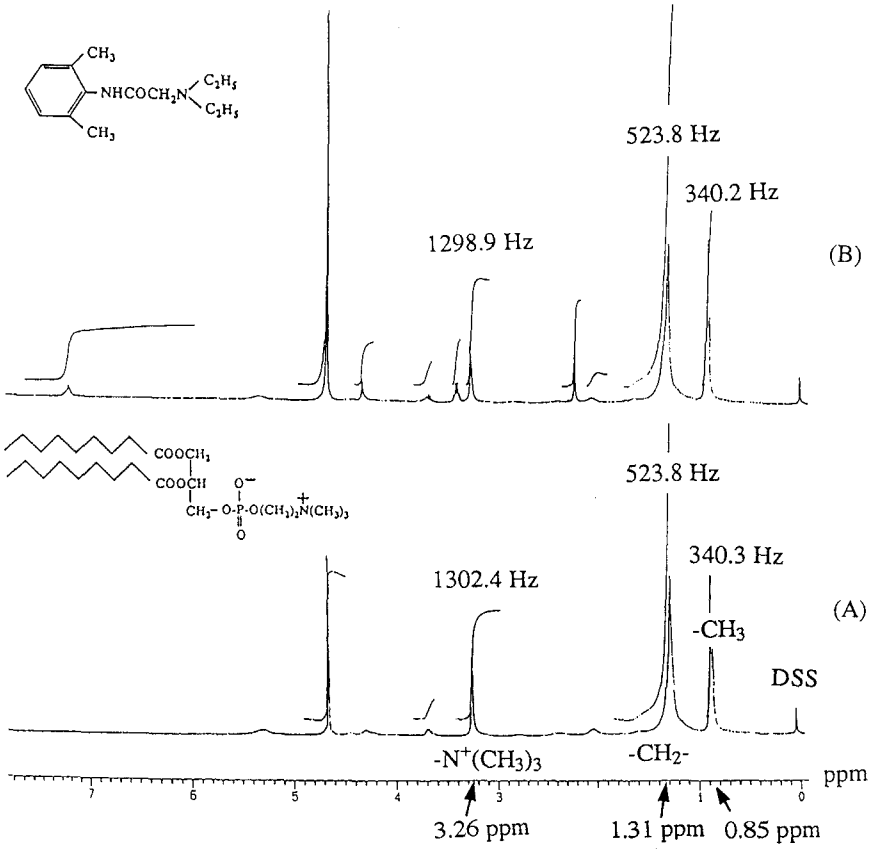

Fig. 1. A Nuclear magnetic resonance (NMR) spectrum of phospholipid membrane (66 mM). **B** NMR spectrum of phospholipid membrane with lidocaine (110 mM). *DSS*, sodium 2,2-dimethyl-2-silapentane-5-sulfonate

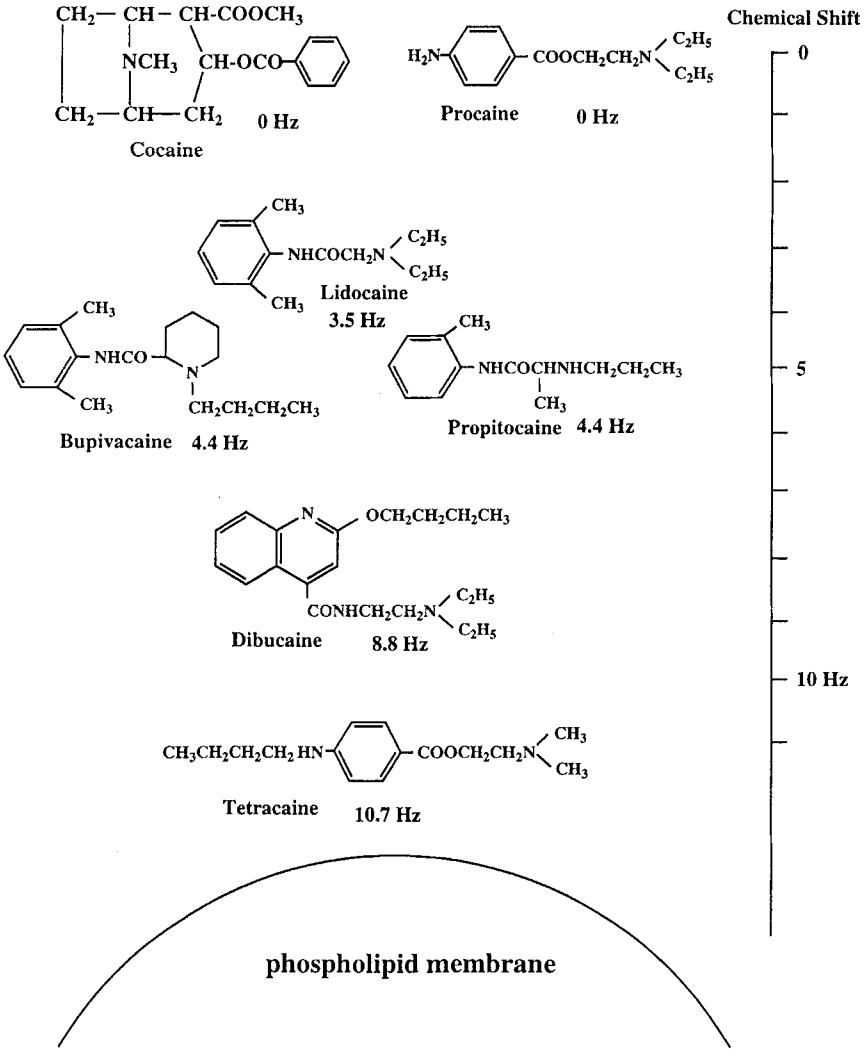

Fig. 2. Proximity between phospholipid membrane and anesthetics estimated by chemical shifts of the choline methyl signal

When lidocaine was added to the phospholipid membrane solution, the half-width of the choline methyl peak changed from 8.0 Hz to 8.8 Hz (Fig. 3), whereas other signals from the membrane were not affected. Similar broadenings were observed in the peaks of propitocaine (8.0 Hz → 9.0 Hz), bupivacine (8.0 Hz → 9.6 Hz), dibucaine (8.0 Hz → 11.0 Hz), and tetracaine (8.0 Hz → 12.2 Hz). On the other hand, the addition of procaine and cocaine did not affect the half-width of the choline methyl signal. These results suggest that broadening of the half-width increases with increasing proximity between the phospholipid membrane and anesthetics (Fig. 4).

Discussion

Previously, we synthesized ester derivatives of lidocaine and concluded that the durations of action were longer than that of lidocaine because the derivatives possess two binding sites to the membrane [4–6]. In other words, the nitrogen atom on the *N*-ethyl group of lidocaine has a positive charge and forms an electrostatic interaction with the negatively charged oxygen atom on the surface of the phospholipid membrane. In the ester derivatives of lidocaine, as well as in the nitrogen atom, an oxygen atom in the carbonyl of ester moiety also binds with the membrane, thereby extending the duration of action. Furthermore, the nitrogen atom in almost all local anesthetics has been shown to be positively charged, forming an anionic interaction with the negatively charged oxygen atom in the phosphate moiety on the external membrane surface [4,5].

The results of the present study show that the interaction between the local anesthetics and the model membrane was strong enough to affect not only electron flexibility based on the results of the broadening of half-width of hydrogen atoms on the membrane, but also electron density (results of the chemical shift) of hydrogen atoms. In addition, the strength of this interaction

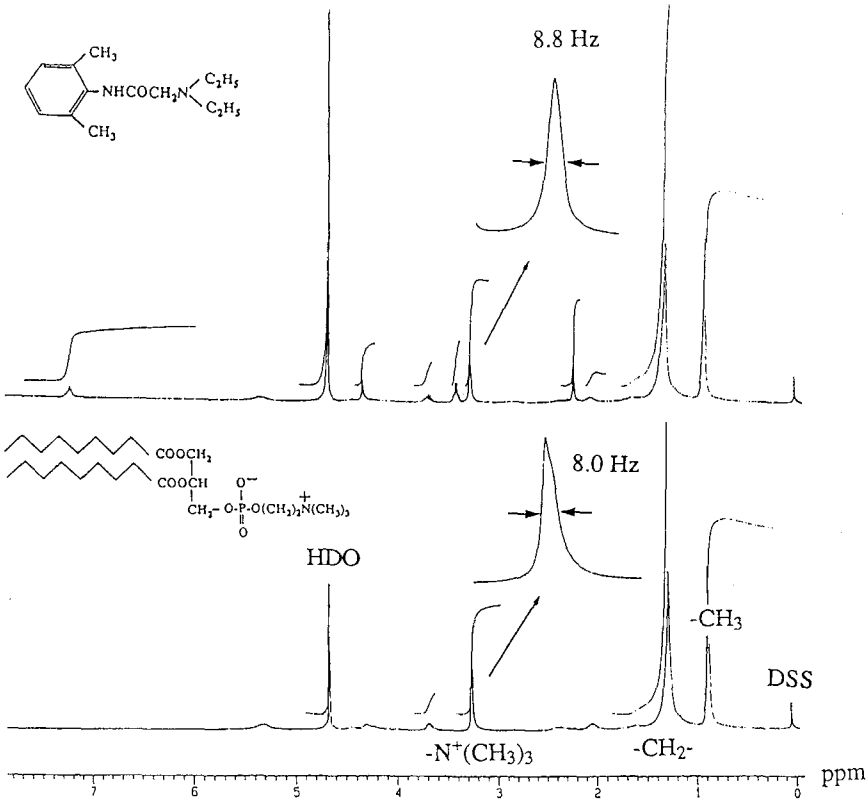

Fig. 3. Broadening of the choline methyl signal by addition of lidocaine (110 mM)

was directly in parallel with the potency of the anesthetic, i.e., the CNS toxicity of the local anesthetic. Especially, the membrane-binding ability of tetracaine and dibucaine, both of which possess two nitrogen atoms, was stronger than that of anesthetics possessing only one nitrogen atom.

On the other hand, when the positively charged nitrogen atom is surrounded by bulky carbon chains, such as in cocaine, the chemical shift and broadening of half-width of hydrogen atoms in the membrane were virtually unaffected. This indicates that cocaine did not bind with the membrane in vitro. In order for cocaine to express its anesthetic effect, its C-C bond is assumed to cleave and form a pyrrolidine ring in vivo, and the positively charged nitrogen atom must be located on the surface of the molecule.

The results of the present study also demonstrate that chemical shift values and broadening of the choline methyl signal are closely related, and that the change in the choline methyl signal in the membranes is a useful indicator of proximity between the membrane and anesthetics, independent of molecular structure.

Büchi and Perica [7] postulated three types of drug–receptor binding: van der Waal's forces, dipole-dipole interaction, and electrostatic force. The electrostatic force of the nitrogen atom is clearly the most potent of these three forces. However, we believe that the bond is formed not with a receptor, but with the oxygen atom in the phosphate moiety on the surface of the phospholipid membrane.

The role of the phospholipid membrane in the action mechanism of local anesthetics has previously been investigated using NMR. Watts and Poile [8] and Westman et al. [9] examined the interaction between tetracaine and the phospholipid membrane using NMR, but could not identify the binding site clearly. One possible explanation is that the binding ability of tetracaine increases as it proceeds further into the phospholipid membrane, thus making an assessment more difficult, as was shown in the present study.

The site of local anesthetic action is widely accepted to be the sodium channel. However, local anesthetics can inhibit the passage of the sodium ion without entering the channel and binding to the receptor. Feinstein [10] hypothesized that local anesthetics reduce the ion permeability of the membrane by binding the nitrogen atoms in local anesthetics to oxygen atoms on the surface of the phospholipid membrane, thereby altering the phase boundary potential of the biomembrane. In addition, local anesthetics might also express their pharmacological effect by binding to the biomembrane that surrounds the sodium channel and inhibiting passage of sodium ions, thereby altering the conformation of channel proteins [4,11]. Nevertheless, in this present study,

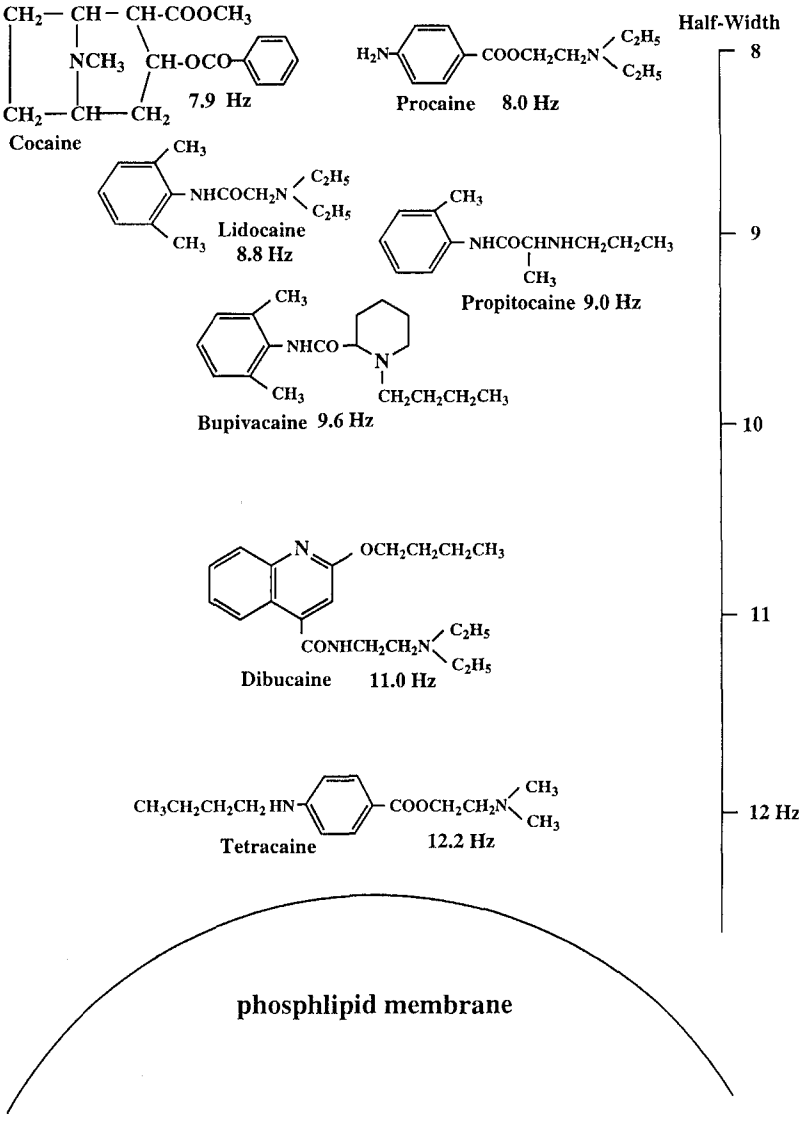

Fig. 4. Proximity between phospholipid membrane and anesthetics estimated by broadening of the choline methyl signal

the binding ability of local anesthetics to the phospholipid membrane was found to be directly proportional to anesthetic potency, provided that the local anesthetics are in the cation form (pH 6.2–6.8).

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