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〔ORIGINAL〕

All local anesthetics bind to the biomembrane by its nitrogen atom in the molecule

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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. In order to measure the extent of proximity of various local anesthetics to the membrane, We examinined the chemical shift value and broadening of half-width of choline methyl signal of the membrane. Electrostatic interaction was seen between positively charged nitrogen atom of local anesthetics and negatively charged oxygen atom of phospholipid membrane. It was also determined that binding ability to the membrane of tetracaine and dibucaine, both of which possess two positively charged nitrogen atoms, was stronger than that of anesthetics such as lidocaine, procaine and bupivacaine, possessing only one positively charged nitrogen atom. On the other hand, when the positively charged nitrogen atom is surrounded by bulky carbon chains, such as cocaine, the chemical shift and broadening of half-width of choline methyl signal in the membrane were virtually unaffected. The binding ability of local anesthetics to the phospholipid membrane was found to be directly proportional to the anesthetic potency, suggesting that positively charged nitrogen atom of local anesthetics plays an important role in the action mechanism of local anesthetics.

Key words : Local anesthetics, Binding site, NMR-spectroscopy, Phospholipid membrane

INTRODUCTION

According to Hille's hypothesis[1], external applied local anesthetics must cross the nerve membrane, and

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enter sodium channels to reach their specific binding site to bring local anesthetic effect by promotion of sodium ion channel inactivation. However, there is no scientific evidence demonstrating that local anesthetics cross the nerve membrane. Previous our study using ^{1}H-NMR spectroscopy only indicated the electrostatic interaction of local anesthetics at the membrane surface[2], and further our study showed that local anesthetics were left for 1month at 37℃ or 2months at 22℃ in an environment in which the pH was raised from 6.5 to 10, increasing the amount of basic local anesthetics 1,000 times, they did not cross the phospholipid membrane but instead remained on the membrane surface[3].

Furthermore, we synthesized two new uncharged(neutral) lidocaine analogues in which the nitrogen atom possess almost no positive charge, and showed that their local anesthetic effect were very poor[4]. In the contrary, local anesthetics in which possess multiple positively charged nitrogen atoms showed increased local anesthetic effect[5].

We also showed that when the negative charge of oxygen atom at the membrane surface was reduced by the administration of Eu^{3+} and Na^+ ion, local anesthetic effects were reduced[6].

Therefore, local anesthetics might express their pharmacological effect by binding to the membrane surface at the positively charged nitrogen atom. The present study using ^{1}H-NMR spectroscopy was design to examine whether all local anesthetics bind to the biomembrane by its nitrogen atom in the molecule.

MATERIALS AND METHODS

The interaction between 110mM of local anesthetics(lidocaine, procaine, propitocaine, bupivacaine, dibucaine, tetracaine, and cocaine, pH6.2-6.8) and phospholipid model membrane (66mM of egg yolk lecithin) were examined by ^{1}H-NMR spectroscopy. Lecithin dispersion were obtained by evaporating lecithin (L-α-phosphatidyl-choline type Ⅲ-E from egg yolk, 100mg/ml 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 hour in an ice-cold vessel under a nitrogen atmosphere. ^{1}H -NMR spectra were measured in a 5-mm tube at 27℃ using a JNM-EX-400 spectrometer (JEOL, Tokyo, Japan). Sodium 2,2-dimethyl-2-silapentane-5-sulfpnate (DDS) was used as the internal standard (0.0ppm).

Based on the results of previous studies [2.3.4] it was thought that the binding between local anesthetics and the phospholipid 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, the choline methyl signal, was used for ^{1}H-NMR spectroscopy to measure the chemical shift and broadening of half-width (peak width at half maximum height) of hydrogen atoms signal.

RESULTS

In the ^{1}H-NMR spectrum of the phospholipid membrane, the most prominent signals, identified as choline methyl protons, methylene protons, and methyl protons, showed a chemical shift at 3.26 ppm (1302.4 Hz), 1.31 ppm (523.8 Hz), and 0.85 ppm (340.3 Hz) from internal reference (DSS), respectively. When lidocaine hydrochloride (110 mM) was added to the solution, the chemical shift of the choline methyl signal shifted 3.5 Hz to upfield (1302.4 Hz → 1298.9 Hz), but the chemical shift of the methylene

and methyl signals showed no change. This provides evidence of the interaction between anesthetics and lecithin vesicles occured on the membrane surface. A similar upfield shift was observed after addition of propitocaine (4.4 Hz), bupivacaine (4.4 H), dibcaine (8.8 Hz), and tetracaine (10.7 Hz), whereas no change of chemical shift was observed after addition of procaine and cocaine(Fig.1). In addition, the change of chemical shift of the choline methyl signal was accompanied by broadening of half-width of the choline methyl signal. Upon addition of lidocaine to the phospholipid membrane solution, the half-width of the choline methyl signal broadened from 8.0 Hz to 8.8 Hz(0.8Hz), whereas other signals of the membrane were not affected. Similar broadenings were observed in the case of propitocaine (8.0 Hz → 9.0 Hz, 1.0 Hz), bupivacaine (8.0 Hz → 9.6 Hz, 1.6Hz), dubucaine (8.0 Hz → 11.0 Hz, 3.0Hz), and tetracaine (8.0 Hz → 12.2 Hz, 4.2Hz). On the other hand, the addition of procaine and cocaine did not affect the half-width of the choline methyl signal(Fig.2). These results suggest that broadening of the half-width increases with increasing proximity between the oxygen atom of phospholipid membrane and nitrogen atom of anesthetics (Fig.3). In order to see the relation of these two values (chemical shift and half-width), values of chemical shift of choline methyl signal were plotted against half-width values of choline methyl signal in Fig. 4. As a result, the change of two values showed a good correlation (R^2=0.9622). It was also determined that binding ability to the membrane of tetracaine and dibucaine, both of which possess two nitrogen atoms, was stronger than that of anesthetics, possessing only one nitrogen atom.

DISCUSSION

According to Hille's specific receptor theory[1], external applied local anesthetics must penetrate the nerve membrane, and bind to the specific receptor from inside of nerve cell to bring local anesthetic effect, however, there is no scientific evidence demonstrating that local anesthetics cross the nerve membrane. Previous our study using ^{1}H-NMR spectroscopy and model membrane indicated that the electrostatic interaction occurred between local anesthetics and model membrane at the membrane surface[2,7], and further our study showed that local anesthetics were left for 1month at 37℃ or 2months at 22℃, they did not penetrate the phospholipid membrane but instead remained on the membrane surface[3].

We previously synthesized lidocaine ester derivatives with prolonged duration and examined their binding mode with phospholipid model membrane. The results showed that lidocaine ester derivatives indicated two interaction sites with the membrane surface not only positive charged nitrogen atom but also by electrostatic effect of ester carbonyl group[7]. Furthermore, we synthesized uncharged (neutral) lidocaine analogues in which nitrogen atom posseses almost no positive charge, and showed that their local anesthetic effect were very poor[4]. In the contrary, local anesthetics in which possess multiple positively charged nitrogen atoms showed increased local anesthetic effect[5]. We also showed that when the negative charge of oxygen atom at the membrane surface was reduced by the administration of Eu^{3+} and Na^+ ion, local anesthetic effects were reduced[6].

Present our study indicated that all local anesthetics bind to the biomembrane by its nitrogen atom in the molecule. The possibility exist that local anesthetics might express its pharmacological effect by binding to the nerve membrane.

Local anesthetics might express the anesthetic effect even if the local anesthetics molecule could not penetrate the membrane. It is generally accepted that, when the hydrophilic membrane surface is negatively charged, clusters of bound water form, the density becomes 15% heavier than that of bulk water and the volume is reduced(electrostriction). It could be assumed that local anesthetics might bring sodium channel protein conformation change by binding to the nerve membrane.

Ueda[8] hypothesized electrostriction theory that account for the mechanism of general anesthetics, when the diethylether bound to albumin, negative charge of membrane surface was reduced, and brought the volume expansion of the nerve membrane by melting of electrostricted water molecule released from the protein surface. This volume expansion suppress sodium ion channel and inhibit sodium ion passage.

The same theory is applied also to local anesthetics. When the positively charged nitrogen atoms of local anesthetics bind to the membrane and neutralize the negative charge of the membrane surface, electrostricted water is released into the bulk water, and the volume of the membrane surface expands. This expansion may cause a conformation change in the sodium channel protein, then inhibit sodium ion passage. This could be the action mechanism of local anesthetics.

In this present study, electrostatic interaction between positively charged nitrogen atom of local anesthetics and negatively charged oxygen atom of the membrane surface was seen except cocaine, however, cocaine has strong local anesthetic effect. When the positively charged nitrogen atom is surrounded by bulky carbon chains, such as is observed in cocaine, might 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.

There are many discussions whether anesthetic effect is specific or not. Some one postulated receptor theory, and another ones supported non-specific membrane lipid theory. Nevertheless, researcher do not dispute that minimum effective concentration of anesthetics is on order of mM whatever local anesthetics or general anesthetics. It is difficult to accept that specific receptor exist, because anesthetics are only effective in such a high concentration.

As mention above, all local anesthetics have the electrostatic interaction with the membrane by its positively charged nitrogen atom. It could be also appreciate that local anesthetics are effective only in such a high concentration provided the conformation change of sodium ion channel protein which was brought by many local anesthetics molecules binding to membrane surrounding the sodium ion channel, thereby inhibit the passage of sodium ions.

These results suggest that interaction with positively charged nitrogen atom of local anesthetics and negatively charged oxygen atom of the membrane play the very important role in the mechanism of local anesthetics.

CONCULUSION

In this present study, using ^{1}H-NMR spectroscopy , electrostatic interaction between positively charged nitrogen atom of local anesthetics and negatively charged oxygen atom of the membrane surface was seen. These interaction might be an action mechanism of local anesthetics.

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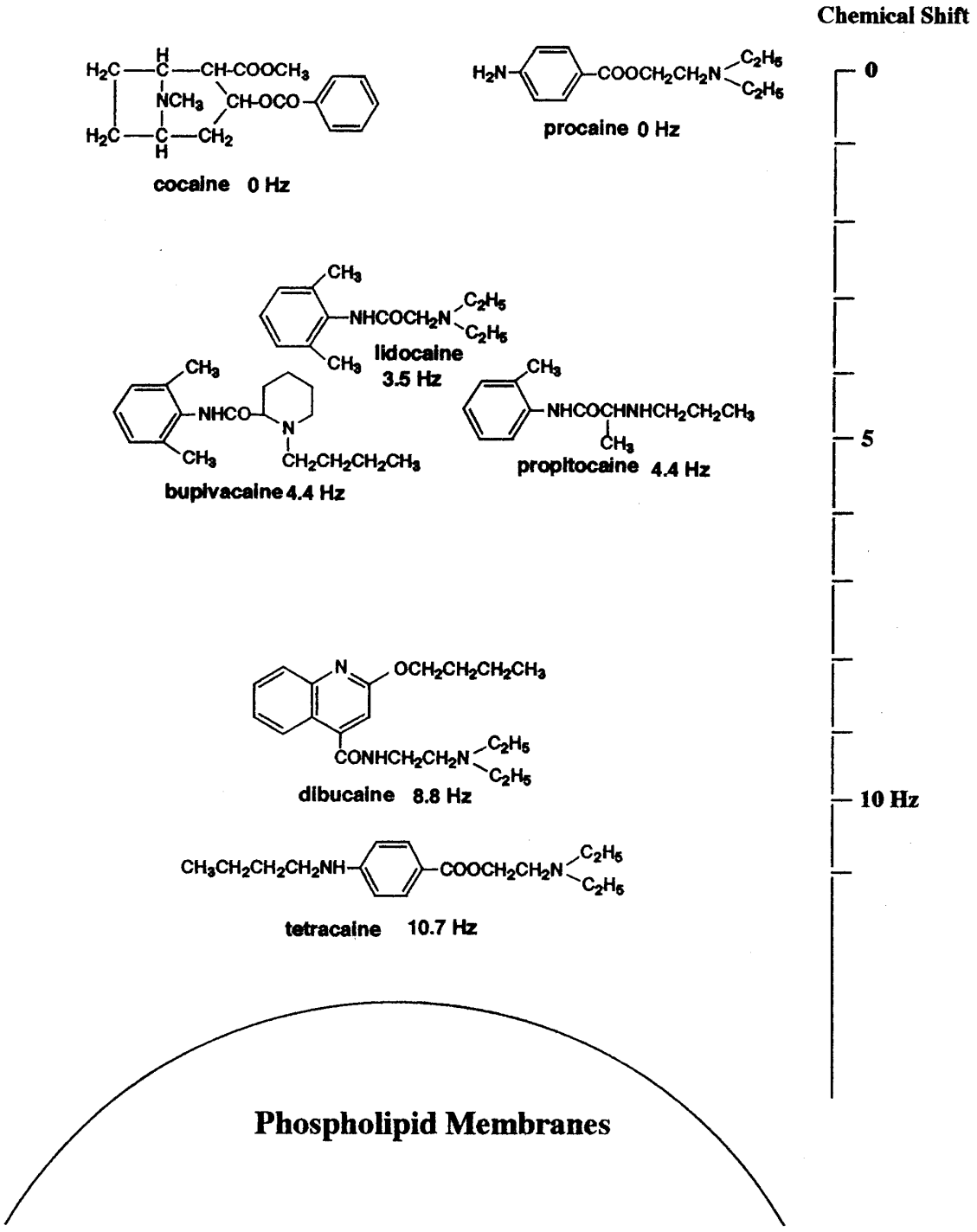

Fig.1 Proximity between phosposlipid membrane and local anesthetics estimated by chemical shifts of choline methyl signal of phospholipid membrane. Lidocaine(3.5Hz),bupivacaine(4.4Hz), propitocaine(4.4Hz), dibucaine(8.8Hz) and tetracaine(10.7Hz).

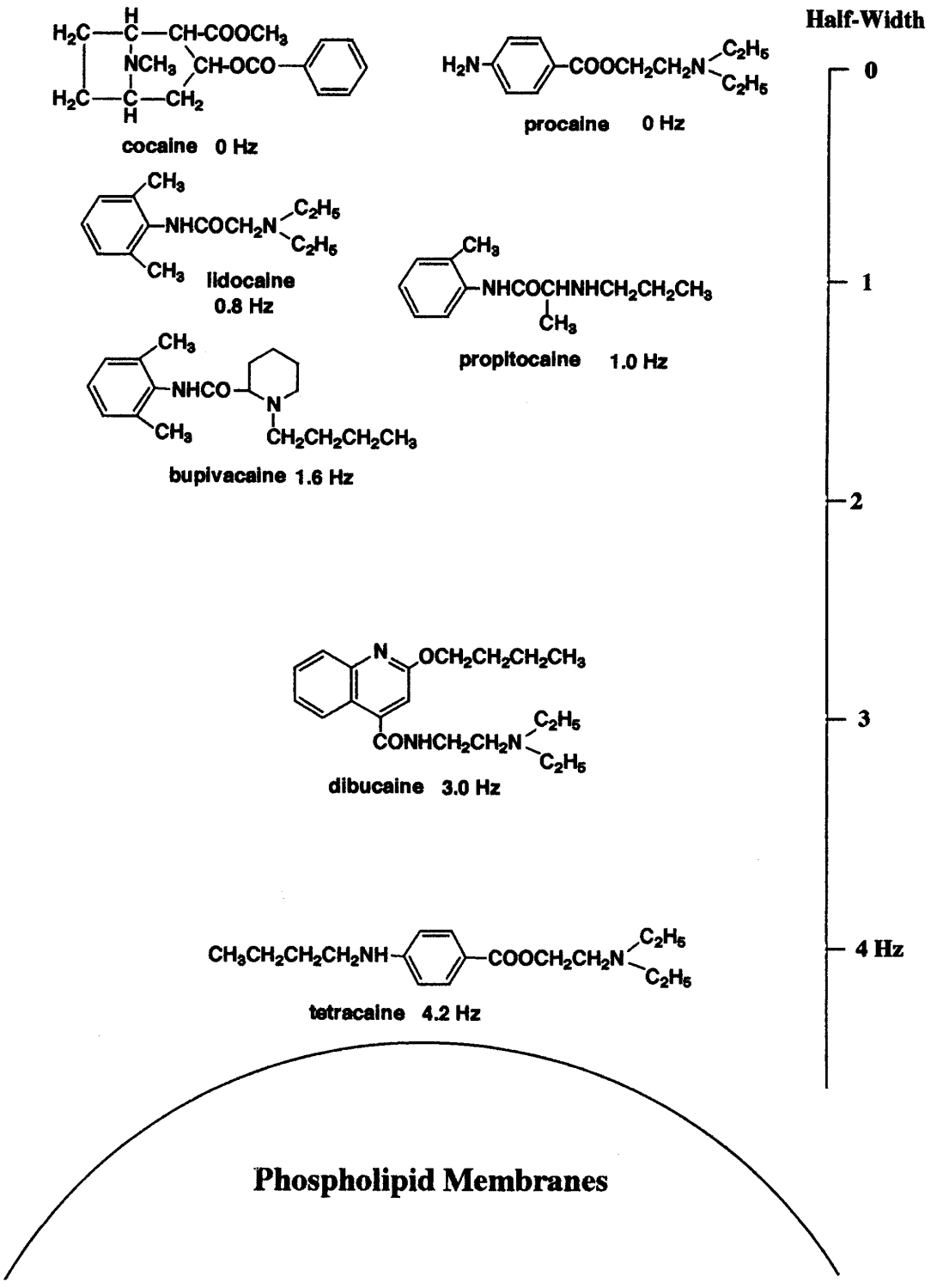

Fig.2 Proximity between phospholipid membrane and local anesthetics estimated by broadening of choline methyl signal. Lidocaine(0.8Hz), propitocaine(1.0Hz), bupivacaine(1.6Hz), dibucaine(3.0Hz) and tetracaine(4.2Hz).

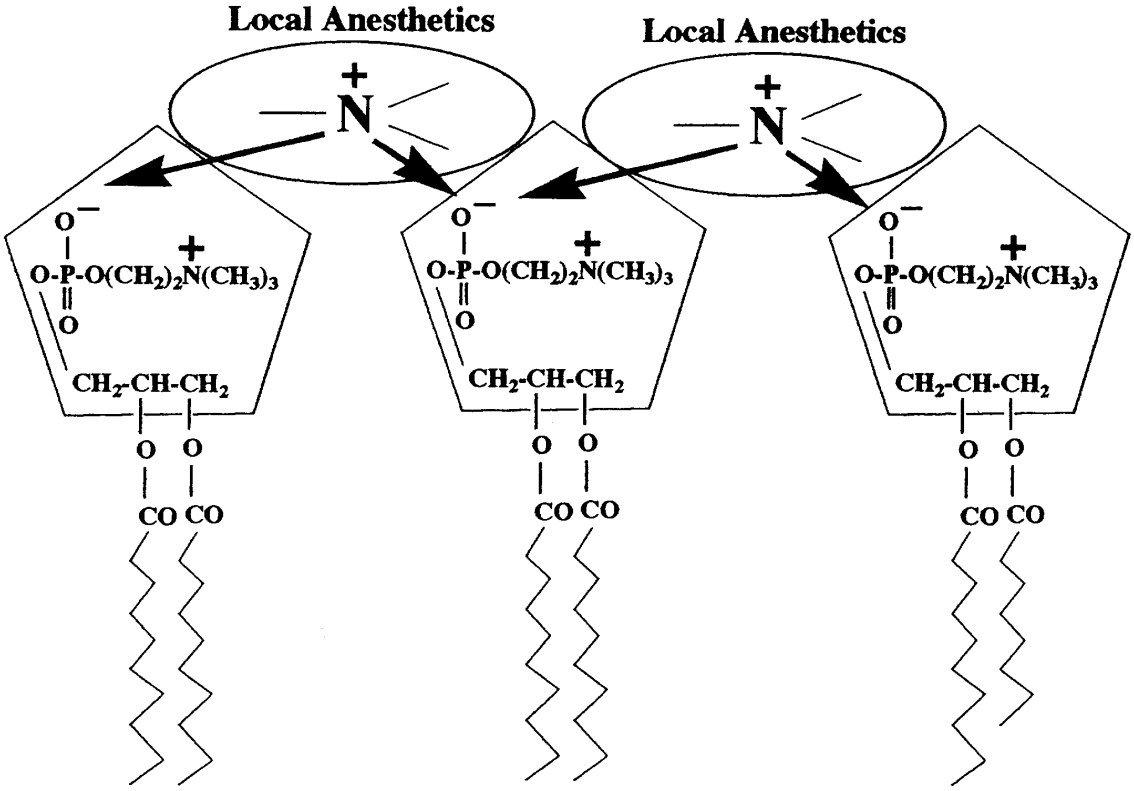

Fig. 3 Electrostatic interaction between positively charged nitrogen atom of local anesthetics and negatively charged hydrogen atom of phospholipid membrane was seen.

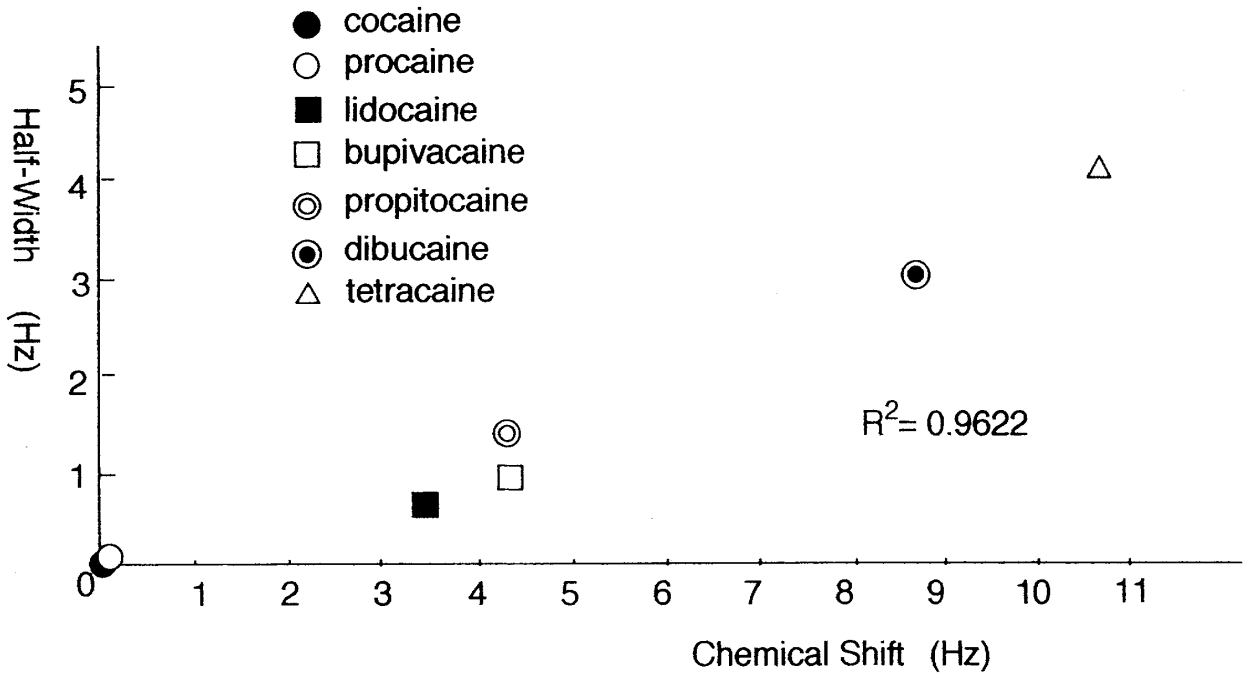

Fig.4 There was a close correlation between chemical shift and half-width (R^2=0.9622).