〔ORIGINAL〕

DETERMINATION BY ^{1}H-NMR OF BINDING SITES OF LOCAL ANESTHETICS ON LIPID BILAYER MEMBRANE MODEL

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Abstract

This is a study of the binding mode of local anesthtics to the phospholipid bilayer membrane model by proton nuclear magnetic resonance spectroscopy(^{1}H-NMR). There was evidence of ionic interaction between the polar external hydrophilic part of the membrane and positively charged nitrogen atom of N-ethyl substituent of lidocaine molecule. Lidocaine ester derivatives, synthesized for prologed duration of action, indicated interaction with the membrane not only by positively charged nitrogen atoms but also by an electrostatic effect of the ester carbonyl oxygen atom. This may be related to the prolonged duration of lidocaine ester derivative action.

The results indicate that a single molecule of local anesthetic does not close a single sodium channel. Many local anesthetic molecules bind to the phospholipid bilayer surounding sodium channels, and may bring some change in sodium channel protein comformation, and lead to the obstruction of the sodium ion passage.

key wards : Local anesthetics, Bindind sites, Lipid bylayer, NMR Spectroscopy

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Introduction

Various hypotheses have been proposed to account for the mechanism by which local anesthetics exert their effects[1-5], however, none of the theories provide a full explanation for the mechanism. Hille's hypothesis[4] for the account of action mechanism of local anesthetics to sodium channel is now considered to be the most appropriate theory. This hypothesis thus assumes that local anesthetic bind to the recepter at the inside of a sodium channel. Lidocaine molecule has an overall length of 12 angstrom, so that it may be difficult for this molecule to easily penetrate into sodium channel with diameters of only 3×5 angstrom in resting state.

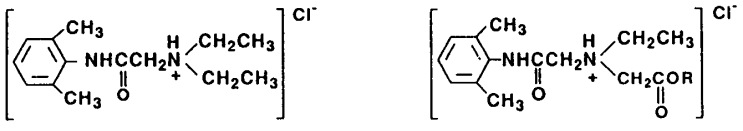
It is also difficult to postulate the existance of specific recepters for local anesthetics. Some substances are believed to produce pharmacological effects by direct binding to a specific recepter, these include tetrodotoxin, acetylcholine morphine, and are pharmacologically active at extremely low concentrations. The lethal dose of tetrodotoxin has demonstrated that sodium ion channel binding takes place with only a single molecule[6], is 0.01 mg/kg. In contrast, the maximum dose for the lidocaine administration is 7~10 mg/kg, nearly 1,000 times higher than tetrodotoxin. This underscores the unlikelines of the recepter theory for local anesthetics which cannot exhibit their pharmacological effect unless they are present at such high concentration.

Neural cell membrane consist of phospholipid bilayer and the ion channel consisting of protein, and therfore, blokage of sodium ion channel could be caused by binding of local anesthetic onto surrounding phospholipid bilayer.

The cell membrane has been accepted as the binding site of local anesthetics.

Fenstein[7] indicated that local anesthetics are capable of reacting stoichiometrically with phospholipid of the membrane. From NMR spectroscopy studies, it appears that local anesthetics were bound primarily at the membrane surface[8,9]. However, the binding site, was not clearly established.

This study examines binding site and mode of action of lidocaine and lidocaine ester derivatives(Fig.1), synthesized for the purpose of prolonged duration[10] on lipid bilayer membrane model.



Lidocaine-HCl

Lidocaine ester derivatives

R = CH_3 methyl ester
C_2H_5 ethyl ester
C_4H_9 butyl ester

Fig 1. Structure of lidocaine and lidocaine ester(methyl, ethyl, butyl)derivatives.[10]

Methods

1) Preparation of phospholipid bilayer(lecithin vesicles).

Lecithin vesicles were made by evaporating the lecithin solution(100㎎ in 1ml of n-hexane) to dryness, taking up residue in 2ml of D_2O(99.96%),and sonicating the resulting coarse dispersion with an ultrasonic desintegrator (W-220F,Haet Systems -Ultrasonic Inc) for 30㎜ in ice-cooled vessel under nitrogen atmosphere.

2) measurement of ^{1}H-NMR spectra.

0.11M of lidocaine hydrochloride or lidocaine ester derivatives were added to licithin vesicles solution. The ^{1}H-NMR spectra were recorded on a JOEL FX-900 or GX-270 spectrometer. DDS(sodium 2,3-dimethyl-2-sils-pentane-5-sulfonate) was used as the internal standard.

Results

The ^{1}H-NMR spectrum of lecithin vesicle in D_2O is illustrated in Fig 2, with a sharp choline methyl peak and broad methyl and methylene peaks of fatty acid chaines. On addition of Eu^{3+}(Paramagnetic ion such as Eu^{3+} and Mn^{2+} have been effectively employed in the NMR approach to structural problems in the field of interaction between lecithin vesicle and drugs) to the vesicle solution, internal(inward facing) and external(outward facing) choline methyl peaks were separated as shown in Fig 2 a.

This phenomenon suggests that Eu^{3+} effectively interacted with the polar part of lecithin vesicles and induced an upfield shift of the signal arising from the outward facing choline methyl,so that the choline methyl signal split into two peaks signifying outward facing(external) and inward facing(internal) component.

When lidocaine was added stepwise to this solution, the external choline methyl peak shifted toward the internal choline methyl peak(Fig 3 d). This behavior indicated that lidocaine diffused to the vesicle surfaces and interacted with the polar part of lecithin, displacing Eu^{3+} ions from the vesicle surfaces. These results showed that the prepared lecithin vesicles could be used as an biological membrane model.

Next, interaction between lidocaine and lecithin vesicles was measured by the ^{1}H -NMR spectrum. Peak assignment of lidocaine was performed by chemical shift(Fig 4). When lidocaine(15㎎, 0.11M) was added to the vesicle solution (pH 6.9) the peak from the vesicle exhibited no change, but two peaks(c and d) of lidocaine showed broadening (0.9Hz→2.2Hz, 0.8Hz→2.1Hz). Similarly in the case of lidocaine methyl ester derivatives, this broadening was observed in peak c, d and e [Fig 5. 0.9Hz→2.2Hz(c), 0.8Hz→2.5Hz (d), 0.8Hz→0.9Hz(e)]. Further, in the series of lidocaine ester derivatives, this tendency of broadening of peak e was strongthened with the size of alkyl substitution(Fig 6). Broadening of peak c resulted in no change. This also indicated that lidocaine ester

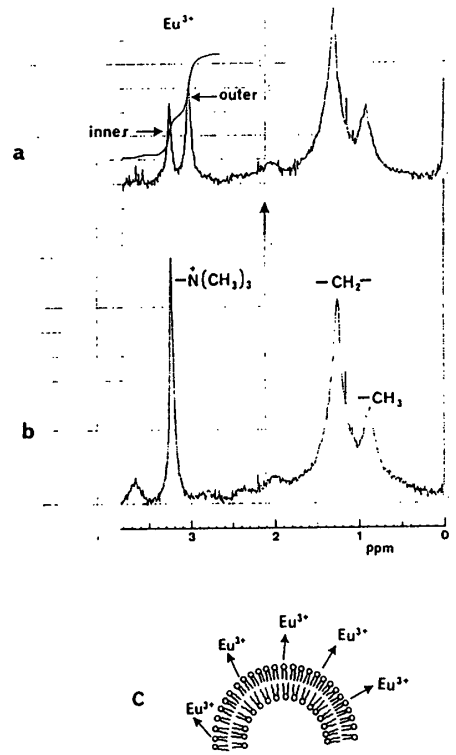


Fig 2. Effect of lidocaine-HCl on the ^{1}H-NMR signal of choline methyl of lecithin vesicle solution on containing Eu^{3+}. a) after addition of Eu^{3+} to lecithin vesicle solution. b) lecithin vesicle alone. c) effect of Eu^{3+} on vesicle. Eu^{3+} interacts only with outer choline methyl of the vesicle.

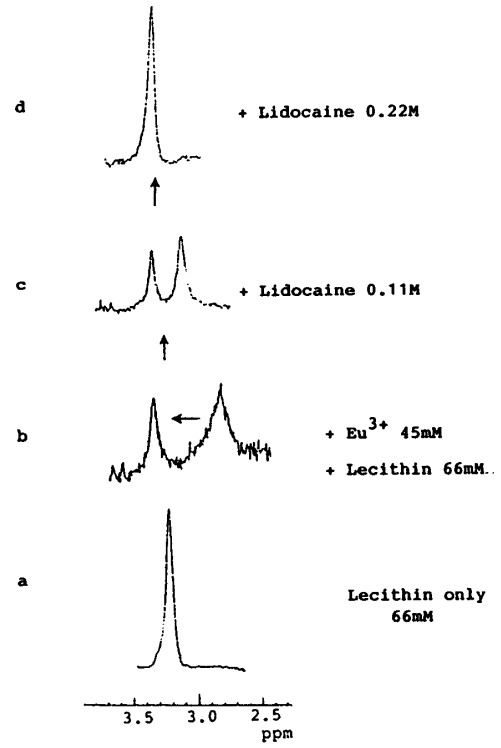


Fig 3. Revaersal of the Eu^{3+} -induced splitting of the ^{1}H-NMR signal of choline emthyl of lecithin in D_2O, as related to increasing concentration of lidocaine-HCl. Lidocaine diffused to the vesicle surfaces and interacted with the polar part of lecithin displacing the Eu^{3+} ions from the vesicle surface.

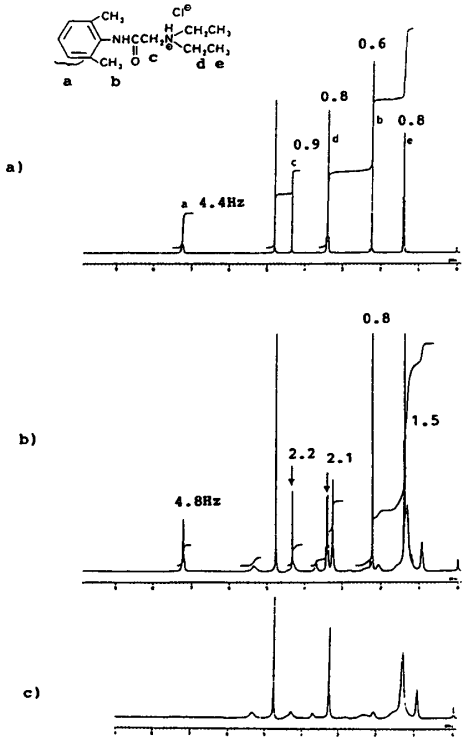


Fig 4. ^{1}H-NMR spectra of lidocaine-HCl. Values indicate half width(Hz) of each signals. a) in D_2O. b) in licithin vesicle solution. c) lecithin vesicle solution alone.

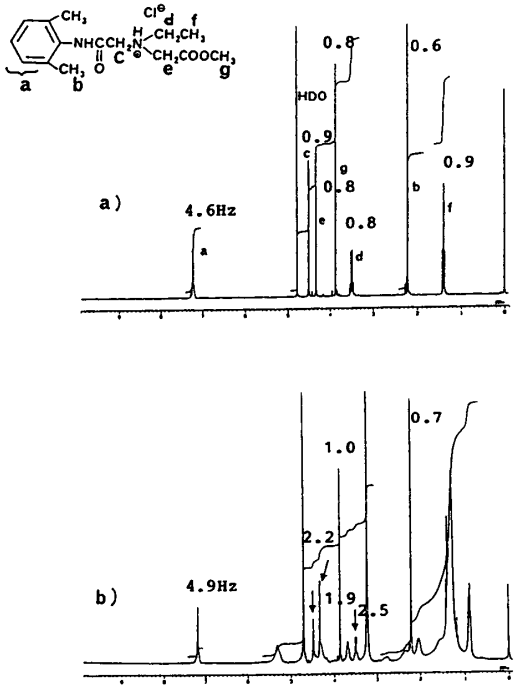


Fig 5. ^{1}H-NMR spectra of lidocaine methyl ester derivative. a) in D_2O. b) in lecithin vesicle solution.

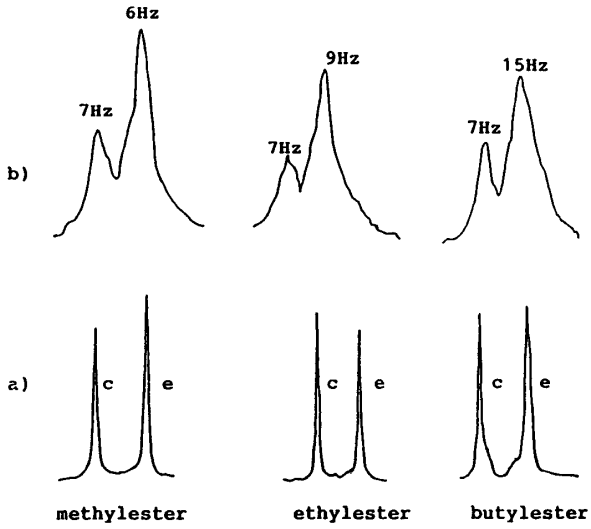


Fig 6. ^{1}N-NMR spectra of lidocaine ester derivatives (only peak of c and e. expanded) a) in D_2O. b) in lecithin vesicle solution.

derivatives, synthesized for prolonged duration of action, interact with lecithin vesicle both by positively charged nitrogen atom and by the electrostatic effect of ester carbonyl oxygen atom.

Discussion

Local anesthetics have been considered to exhibit their effect by sodium channel closure, as a result of direct binding to the recepter[11,12]. This assumption is justified since just benzene ring alone of local anesthetics is large enough (5～8 angstrom unit) to close sodium ion channels (3×5 angstrom[13]).

Hille's hypothesis[4], concerning the manner in which local anesthetics achieve sodium ion channel closure, is now considered the most approriate theory. It assumes the exsistance of two types of gates in the sodium ion channel, one the activated gate (m -gate), and the other the inactivated gate (h-gate). During the resting state of the neural cell, the sodium ion channel has a diameter of approximately 3×5 angstrom, a size that does not permit easy passsage of sodium ions. To enable sodium uptake and depolarization, it is necessary that the activated gate should be open to facilitate sodium ion passage. When a local anesthetic bind to the specific receper from the inside of sodium channel, the non-activated gate closed, and sodium ions cannot penetrate the channel (Fig 7).

We have studied the binding mode between local anesthetics and phospholipid bilayer, in an artificial membrane model, by proton nuclear magnetic spectroscopy (^{1}H-NMR). Generally, signal broadening (decreasing in height and enlargement of half width) of the drugs indicated the incorporation of the drug into the membrane model, and the mobility of the drug molecule was restricted by its penetration into the vesicle membrane. In the case of lidocaine, this broadening was observed only in the part adjacent to (peak c and d) nitrogen atoms. There was evidence of ion interaction between the polar external

hydrophilic part of the membrane model and the positively charged nitrogen atom of the N-ethyl portion of lidocaine molecule. It is therefore, likely that local anesthetics -membrane binding take place at the positively charged nitrogen atom of lidocaine and the oxygen atom of external hydrophilic part of the membrane(Fig 8).

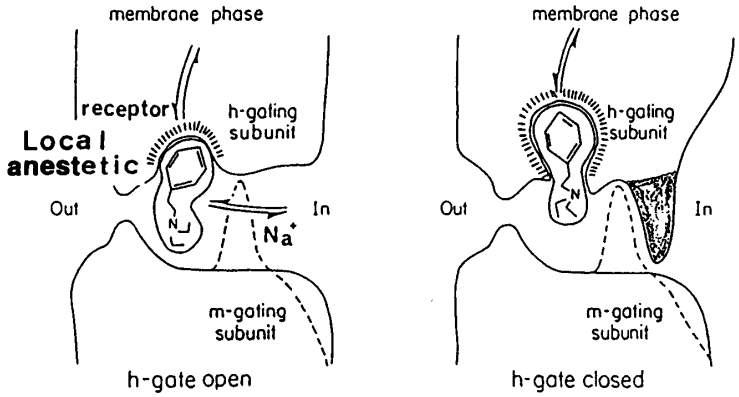


Fig 7. Hille's hypothesis of the manner in which local anesthetics achieve sodium ion channel closure[4].

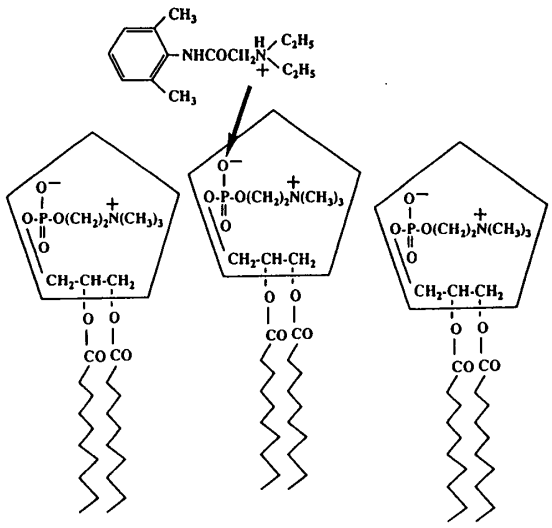


Fig 8. Binding site of lidocaine molecule and lecithin membrane.
Electrostatic interaxtion between lidocaine and lecithin vesicles may take place at the nitrogen atom of lidocaine and oxygen atom of extrnal hydrophilic part of the membrane.

The lidocaine ester derivatives, synthesized for prolonged duration of action, also resulted in strong broadening of ajacent parts (peak e) of ester carbonyl. This phenomenon indicated the interaction with membrane not only by positively charged nitrogen atoms but also by an electrostatic effect of ester carbonyl oxygen atoms(Fig 9). This may be related to the prolonged duration of lidocaine ester derivatives.

The results suggest the possibility that local anesthetics do not act by direct blockage of sodium ion channels. The neural cell membrane consists of phospholipid bilayer and ion channel. Therefore, blockage of sodium ion channels could occured by binding local anesthetics on the surounding phospholipid bilayer. A large number of local anesthetic

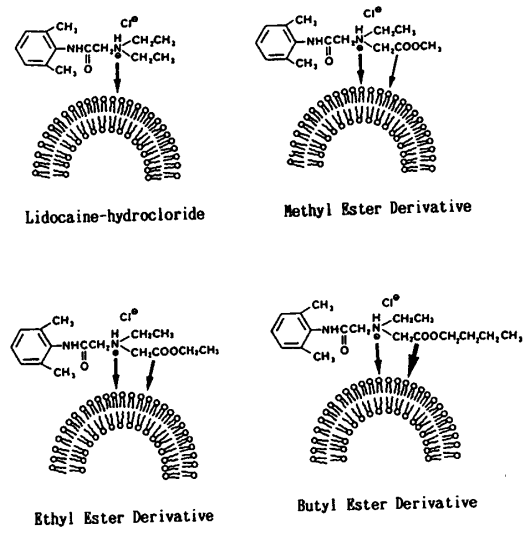


Fig 9. Binding mode for lidocaine and lidocaine ester derivatives (methyl, ethyl, butyl) to the lecithin membrane. Lidocaine ester derivatives interact with the membrane both by positively charged nitrogen atom and the ester carbonyl oxgen atom of the membrane. The width of arrows indicate the strength of binding.

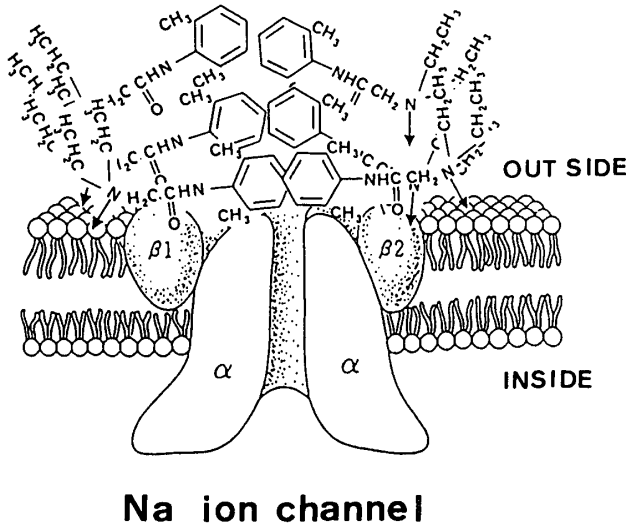


Fig 10. Kokubu's hypothesis of the manner of sodium ion channel closure with local anesthetics. A single molecule of local anesthetic does not close a channel. Large numbers of local anesthetics bind to the phospholipid bilayer surounding the channel, and may cause changes to the channel protein conformation, this leads to the resulting obstruction of the sodium ion passage.

molecurles bind to the phospholipid bilayer surounding the channel, and may cause some change in channel protein conformation, and lead to the resulting obstruction of the sodium ion passage (Fig 10)[14]. Based on this assumption, a large number of the local anesthetics would be required to block a single sodium channel. This new theory of the manner in which local anesthetics achieve sodium channel closure, is consistent with observation that local anesthetics do not manifest their effects unless administrated in large dosage.

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和文抄録

局所麻酔薬の作用機序についてはさまざまな仮説があるが，どの仮説も十分な説明をしていない。そこで我々は核磁気共鳴装置（NMR）を用いて，局所麻酔薬と神経膜モデルであるリン脂質二重膜との結合状態を検索した。この結果，局所麻酔薬分子中にある窒素原子とリン脂質二重膜の外側にある親水性部分の酸素原子とが静電結合をおこすことが分かった。また我々が作用持続時間の延長を目的に合成した，リドカインのエステル誘導体では窒素原子との結合に加えて，エステルカルボニル部分の酸素原子でも膜と結合することもわかった。さらに，この結合力の強さは局所麻酔薬の作用持続時間に影響することもわかった。これらの事実は一分子の局所麻酔薬が一つのNaイオンチャネルを閉鎖して局所麻酔作用を発現するのではなく，リン脂質二重膜と結合した多くの局所麻酔薬がNaイオンチャネルを構成するタンパク質の流動性を変化させ，結果的にNaイオンの通過を阻害して，局所麻酔作用を発現する可能性を示唆している。