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# Effect of Lidocaine · HCl on Microviscosity of Phosphatidylcholine Model Membrane

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In order to provide a basis for studying the molecular mechanism of pharmacological action of local anesthetics and to develop a fluorescence spectroscopic method which can detect the microviscosity of native and model membranes using intramolecular excimerization of 1,3-di(1-pyrenyl)propane (Py-3-Py), we examined the effect of lidocaine · HCl on the microviscosity of model membranes of phosphatidylcholine fraction extracted from synaptosomal plasma membrane vesicles (SPMVPC). The excimer to monomer fluorescence intensity ratio ($I'/I$) of Py-3-Py in liquid paraffin was a simple linear function of $T/\eta$. Based on this calibration curve, the microviscosity values of the direct probe environment in SPMVPC model membranes ranged from $234.97 \pm 48.85$ cP at 4°C to $19.21 \pm 1.11$ cP at 45°C. At 37°C, a value of $27.25 \pm 0.44$ cP was obtained. The lidocaine · HCl decreased the microviscosity of SPMVPC model membranes in a concentration-dependent manner, with a significant decrease in microviscosity value by injecting the local anesthetic even at the concentration of 0.5 mM. These results indicate that the direct environment of Py-3-Py in the SPMVPC model membranes is significantly fluidized by the lidocaine · HCl. Also, the present study explicitly shows that an interaction between local anesthetics and membrane lipids is of importance in the molecular mechanism of pharmacological action of lidocaine · HCl.

Key Words: Lidocaine · HCl, Fluorescence probe technique, 1,3-Di(1-pyrenyl)propane, Membrane microviscosity

## INTRODUCTION

The effects of local anesthetics on fluidity of model and native membranes have been extensively studied for over a century to understand the molecular mechanism of local anesthesia (Seeman, 1972; Strichartz, 1973; Lee, 1976; Neal et al, 1976; Singer, 1977; Boulanger et al, 1981; Strichartz & Richie, 1987; Sweet et al, 1987; Yun et al, 1987; Auger et al, 1988; Seeling et al, 1988; Butterworth & Strichartz, 1990; Guo et al, 1991; Smith et al, 1991). Information on local anesthetics partitioning between water/ lipid or into olive oil supported the experimental search for the site of anesthetic action in the lipid membrane. The results obtained by previous experimental studies (Sweet et al, 1987; Yun et al, 1990a; Catterall et al, 1991; Guo et al, 1991; Agnew et al, 1991; Yun et al, 1993, 1994; Shibata et al, 1995; Kang et al, 1996; Park, 1997) are difficult to examine critically because diverse lipids and different local anesthetics at varying concentration have been used. The effects and location of local anesthetics at clinical concentrations are not well defined. Comple-

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mentary experimental studies in the last twenty years or so shifted the focus from the lipids to the direct binding to the proteins (Strichartz, 1973; Strichartz & Richie, 1987; Butterworth & Strichartz, 1990; Guo et al, 1991). More recently, it was suggested that the action of local anesthetics may involve function to modulate a number of transmembrane ligand-gated ion channels (Agnew et al, 1991; Catterall et al, 1991; Guo et al, 1991). Indeed, despite an overwhelming body of experiments, the controversy as to whether the primary site(s) of action of local anesthetics is the membrane lipid or membrane proteins remains unresolved, rendering the mechanism for local anesthetic action still unknown. Most recently, renewed interest in the role of membrane lipids was ignited by a hypothesis regarding modifications in membrane structure induced by the presence of local anesthetics, which in turn may indirectly alter membrane protein function (Park, 1997; Min, 1998; Lee, 1999).

The effects of local anesthetics on motions, order and phase transitions of bulk bilayer systems of native or model membranes have received considerable attention in the past decades. This is due to the interest in biological membranes, as well as the unique information on intermolecular interactions that can be derived from the investigation of volume changes (Yun et al, 1987, 1993, 1994; Kang et al, 1996; Park, 1997; Min, 1998; Lee, 1999). It is known that the potency of anesthetic increases roughly in proportion with its lipid/water partition coefficient. This strongly suggests an amphiphilic site for anesthetic molecules (Miller et al, 1986).

There have been some studies (Seeman, 1972; Lee, 1976; Neal et al, 1976; Singer, 1977; Boulanger et al, 1981; Sweet et al, 1987; Yun et al, 1987, 1993, 1994; Auger et al, 1988; Seeling et al, 1988; Smith et al, 1991; Kang et al, 1996; Park, 1997; Min, 1998; Lee, 1999) which back up the membrane expansion theory, although without full satisfaction. The membrane expansion theory is one of the more important theories regarding the mechanism of pharmacological action of local anesthetics. Numerous methods including the fluorescent probe technique (Seeman, 1972; Lee, 1976; Neal et al, 1976; Singer, 1977; Boulanger et al, 1981; Sweet et al, 1987; Yun et al, 1987; Auger et al, 1988; Seeling et al, 1988; Smith et al, 1991) have been employed to determine the biophysical properties of native and model membranes or to study the influence of drugs on the biophysical properties of the membrane lipids. Clearly, no single experimental approach can encompass the richness and complexity inherent in the problems of membrane structure and dynamics. A number of quite different physical and chemical techniques must be applied and their results must be correlated. Most of published data about the effect of local anesthetics on the biophysical characteristics of native and model membranes has been obtained from the analysis of the influence on the phase transition temperature of model membranes (Lee, 1976; Yun et al, 1987; Racansky et al, 1988; Kaminoh et al, 1989) or on the rotational mobility of native membranes (Sweet et al, 1987). If local anesthetics cause expansion of neuronal membranes, this expansion is probably due to the increased fluidity in neuronal membrane lipid bilayer induced by local anesthetics. Our questions were what the role of local anesthetics (which is believed to have more interactions with protein than other lipids) was and to what degree the neuronal membrane lipid bilayer was expanded by local anesthetics. More specifically, our questions were how much of a decrease the local anesthetic brought to microviscosity (primarily due to lateral mobility). Attempting to answer this question, we studied the effect of lidocaine · HCl on the lateral mobility of 1,3-di(1-pyrenyl)propane (Py-3-Py) in model membranes of phosphatidylcholine fraction extracted from synaptosomal plasma membrane vesicles (SPMVPC model membranes) and now report the results.

## METHODS

### *Materials*

2-years-old Korean cattle of both sexes were killed at Daeyoung slaughterhouse (#192 Kupo-dong, Book-Goo, Pusan, Korea). The whole brain was rapidly excised, placed in ice-cold 0.32 M sucrose + 3 mM Hepes, pH 7.5, and then immediately carried to our laboratory. Lidocaine · HCl was purchased from Sigma Chemical Co. (St. Louis, MO., USA). Liquid paraffin was purchased from Shinyo Chemicals Co., LTD. (Osaka, Japan). Liquid paraffin was purified chromatographically over $Al_2O_3$ as a (1 : 1) mixture with n-hexane. The n-hexane was removed from mixture by distillation. The liquid paraffin was subsequently distilled under high vacuum. The fluorescent probe, Py-3-Py, was purchased from Molecular Probes, Inc. (Junction City, OR., USA). All other reagents

were purchased commercially and were of the highest quality available. Water was deionized.

*Preparation of SPMV*

Preparation of SPMV was performed according to the procedure of eariler studies (Yun & Kang, 1990; Yun et al, 1990b). The specific activities of Na, K-ATPase, acetylcholinesterase and 5'-nucleotidase were approximately 4-, 2.5- and 3-times, respectively, higher in the plasma membrane fraction compared to those in crude homogenates. The electron microscopic examination of the prepared SPMV showed very high purity. The vesicles, which were separated according to size, demonstrated a homogeneous distribution and no longer showed the presence of intracellular organelles or leakage.

*Preparation of model membranes*

Total lipids were extracted from the SPMV as previously described (Yun & Kang, 1990). The phosphatidylcholine (PC) was separated by a High Performance Thin Layer Chromatography (CAMAG, Switzerland). The PC was quantitated by measuring the amounts of inorganic phosphate (Bartlett, 1959) after hydrolysis of PC at 180°C in 70% $HC1O_4$ (Madeira & Antunes-Madeira, 1976). PC was composed of hexadecanoic acid (16 : 0, 40.88±1.22%), 9-hexadecanoic acid (16 : 1, 1.50±0.18%), octadecanoic acid (18 : 0, 15.02±0.64%), 9-octadecanoic acid (18 : 1, 31.31±0.65%), 5,8,11,14-eicosatetraenoic acid (20 : 4, 5.93±0.80%) and 4,7,10,13,16, 19-docosahexaenoic acid (22 : 6, 5.37±0.61%).

The characteristics of the lipid samples, such as size, lamellarity, radius of curvature, and shape, are strongly dependent on the method used to form the vesicles (Lasic, 1988). As a consequence of the preparation method, the parameters that characterize the lipid phase equilibrium in lipid mixtures are affected by the lipid sample characteristics. This is because the size of the giant unilamellar vesicles is on the same order as the size of the cells. Giant unilamellar vesicles are becoming objects of intense scrutiny in diverse areas that focus on membrane behavior (Menger & Keiper, 1998). Stock solution of PC was made in chloroform. The concentration of the PC stock solution was 0.7 mg/ml (Phosphate-buffered saline: 8 g/l NaCl, 0.2 g/l KCl, 0.2 g/l $KH_2PO_4$, 1.15 g/l $Na_2HPO_4 \cdot 7H_2O$, 0.48 g/l Hepes, pH 7.4). For the SPMVPC preparation we followed the electroformation method developed by Angelova and Dimitrov (Angelova & Dimitrov, 1986; Dimitrov & Angelova, 1987; Angelova et al, 1992). To grow the SPMVPC, a special temperature-controlled chamber, which were previously described (Bagatolli & Gratton, 1999), was used.

*Determination of microviscosity*

The kinematic viscosity of liquid paraffin has been determined with Physica-Reometer (Physica). Dynamic viscosities of liquid paraffin were calculated by multiplying kinematic viscosity by density, and densities ( $\rho$) were calculated from the following equation:

$$\rho(g/cm^3)=1.4967-2.8369\times10^{-3}\times T+9.3232\times10^{-8}\times T^2+7.7915\times10^{-9}\times T^3$$

where T is absolute temperature ($K$, $K$=°C+273.15).

The incorporation of Py-3-Py was carried out by adding aliquots of a stock solution of $5\times10^{-5}$ M in absolute ethanol to SPMVPC model membranes or liquid paraffin, so that the final probe concentration was less than $5\times10^{-7}$ M. The mixtures were vigorously vortexed for 10 seconds at room temperature, and then incubated at 4°C for 18 hours under gentle stirring. A concentrated solution of the lidocaine · HCl was prepared in phosphate-buffered saline (PBS) and added at desired concentrations to SPMVPC model membranes previously labeled with Py-3-Py. Measurements commenced usually within 1 min after addition. No effect of longer incubation time was noted. Blanks, prepared under identical conditions without Py-3-Py, served as controls for the fluorometric measurements. The mixture was stirred for 20 min at room temperature in order to reduce the concentration of ethanol that might alter the microviscosity of the SPMVPC model membranes. Also, the mixture was bubbled by dry nitrogen for 1 min with 20 min intervals in order to eliminate oxygen that might be a quencher and denaturalize the membranes. The pH of the buffered sample was not changed significantly by addition of lidocaine · HCl. The measurements were carried out with a Multi Frequency Cross-Correlation Phase and Modulation Fluorometer (ISS K2-003, USA). The excitation wavelength was 330 nm. The excimer to monomer fluorescence intensity ratio was calculated at 480 nm

versus 379 nm signal ratio (*I'/I*).

## RESULTS

In the present study, using fluorescence technique, we examined the effect of cation of lidocaine · HCl on the microviscosity of SPMVPC model membranes. In order to determine the effect of the local anesthetic on the aforementioned microviscosity, it is first necessary to demonstrate that the anesthetic does not interact directly with fluorescent probe and thereby quench its fluorescence. Siginificant changes in fluorescence intensities of the probe in the SPMVPC model membranes by the anesthetic were not detected over the entire concentration range used for lidocaine · HCl. Hence, the possibility of direct quenching of fluorescence of the probe by local anesthetic is ruled out.

Intramolecular excimer formation of Py-3-Py was studied in liquid paraffin as a function of temperature. The present methodology could be used since the fluorescence spectra of Py-3-Py are identical in the SPMVPC model membranes and liquid paraffin. The excimer to monomer fluorescence intensity ratio of Py-3-Py in the liquid paraffin, *I'/I*, obtained various temperatures has been plotted as a function of T(*K*)/ $\eta$(cP), as shown in Fig. 1. A linear relationship existed between *I'/I* and T/ $\eta$. The dynamic viscosity of liquid paraffin changed from 31.031 cP at 45°C to 710.520 cP at 4°C. When the temperature was raised from 4 to 45°C, the excimer to monomer fluorescence intensity ratio of Py-3-Py dissolved in the liquid paraffin, *I'/I*, increased from a value of 0.034 to 0.527. This ratio can shows the microviscosity of the probe environment, here, in the SPMVPC model membranes.

Fig. 2 shows the temperature dependence of the microviscosity of the direct environment of Py-3-Py in the SPMVPC model membranes. The microviscosity of the probe environment in the SPMVPC model membranes, based on the calibration curve (Fig. 1), ranged from 234.97±48.85 cP to 22.61±1.11 cP over the temperature range from 4 to 45°C. At the physiological temperature (37°C), a value of 27.25±

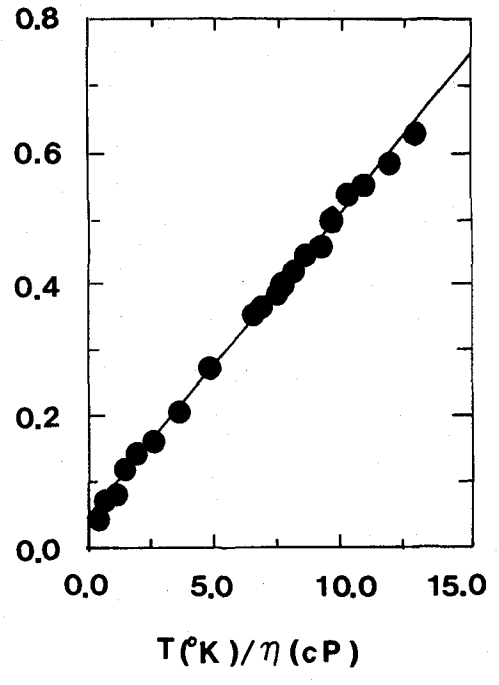


**Fig. 1.** Excimer to monomer fluorescence intensity ratio (*I'/I*) of Py-3-Py in liquid paraffin as a function of T/ $\eta$. This line served as a standard curve in determining the microviscosity. Each point represents the mean of 5 determinations.

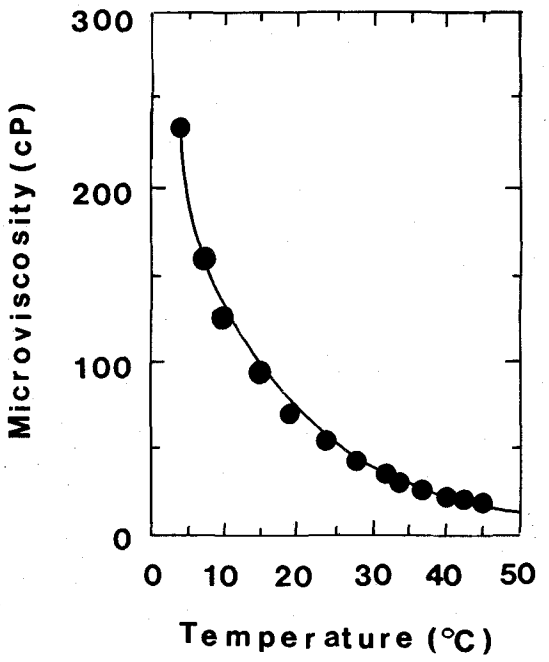


**Fig. 2.** The temperature dependence of the microviscosity of the direct environment of Py-3-Py in the model membranes of phosphatidylcholine (SPMVPC) fraction extracted from synaptosomal plasma membrane vesicles (SPMV). SPMVPC model membranes were suspended in PBS (pH 7.4) and each point represents the mean of 5 determinations.

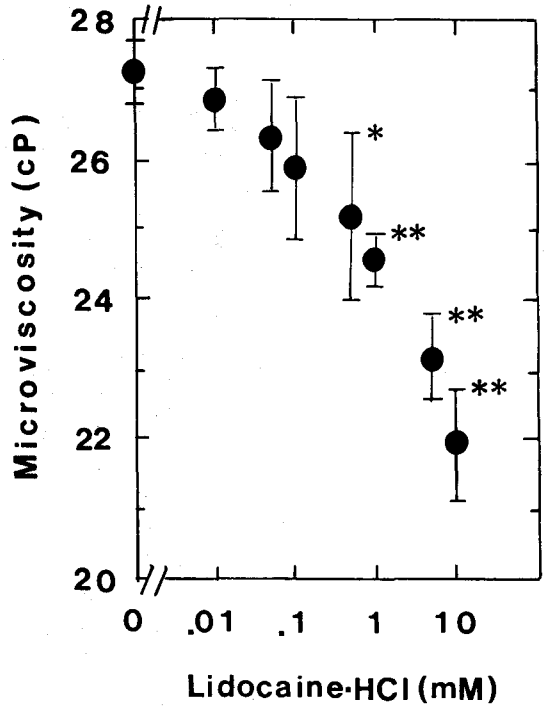


**Fig. 3.** The effect of lidocaine·HCl on the microviscosity of SPMVPC model membranes. Fluorescence measurements were performed at 37°C and each point represents the mean±SEM of 5 determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to by Students *t*-test.

0.44 cP was obtained.

The effect of lidocaine · HCl on the microviscosity of the direct environment of Py-3-Py in the SPMVPC model membranes at 37°C is shown in Fig. 3. The concentration of lidocaine · HCl in the SPMVPC model membranes was varied between 0.01 and 10 mM. The microviscosity of SPMVPC model membranes changed from 26.86±0.47 cP at 0.01 mM to 21.97±0.79 cP at 10 mM. When the concentration of lidocaine · HCl was raised from 0.01 to 10 mM, the excimer to monomer fluorescence intensity ratio of Py-3-Py in the SPMVPC model membranes, *I'/I*, increased from a value of 0.58±0.01 cP to 0.70± 0.03 cP. The lidocaine · HCl significantly decreased the microviscosity of SPMVPC model membranes in a concentration-dependent manner with a significant decrease noted even at 0.5 mM.

## DISCUSSION

Owing to the organization of native and model membrane structures, the resistance to flow at any point in the lipid is quite dependent on the direction of the applied force and on the mode of motion which contributes to the fluidity or microviscosity. Such motions include the following: lateral mobility of lipid molecules in the plane of the monolayer, with diffusion coefficients in the range of $10^{-9}$ to $10^{-7}$ cm second$^{-1}$ (Devaux et al, 1972; Trauble & Sackman, 1972), rotational mobility of a lipid molecule around an axis perpendicular to the plane of the bilayer, with reported rotational mobility frequencies in the range of $10^{7}$ to $10^{8}$ second$^{-1}$ (Hubbell & McConnell, 1969); in contrast to the relatively rapid motions of the preceding modes, transverse diffusion or "flip-flop" from one monolayer to the other is energetically less favored and relatively slow, particularly in model bilayers (Kornberg & McConnell, 1971); lateral flow of arrays of lipid molecules in bulk under pressure gradients; vertical displacements in and out of the bilayer; rotations of lipid substituents around single bonds, including trans-gauche movements, and vibratory changes in bond length within one molecule (Ladbrooke & Chapman, 1969). A precise definition of "fulidity or microviscosity" in an anisotropic bilayer could be described as a weighted sum of all of these modes of motion.

Unfortunately, no single method is now available to assess all of these motions and to provide such a weighted parameter. Current thinking, as a result, holds that the "fluidity or microviscosity" parameters of bilayers assessed by extant techniques are operational quantities which are technique-dependent and do not necessarily reflect a bulk property of the membrane lipid (Lakowicz et al, 1979).

The traditional method for studying microviscosity has been fluorescence depolarization which measures the resistance of the probe molecule to rotational and reorientational motion. Attempts have been made to use intermolecular excimer fluorescence for microviscosity measurements (Pownall & Smith, 1973). However, a difficulty occurs in the interpretation of such results in lipid bilayer, because lipid bilayers with 0, 1, 2, 3, ... probe molecules are present. The excimer emission occurs alongside the partly quenched monomer fluorescence in the lipid bilayers with double or more probes, whereas the one with single probe alone shows the unquenched monomer fluorescence. This situation complicates the determination of the excimer to monomer fluorescence intensity ratio which is used to determine the microviscosity

of a medium (Pownall & Smith, 1973), leading to an over-estimation of the monomer fluorescence intensity to be related to the intensity of excimer emission, and too low a value for the microviscosity will result. In fact, it is clear that the apparent microviscosity measured this way will decrease with decreasing probe concentration. This is probably the cause of the large discrepancy found between the microviscosities of lipid bilayers obtained via intermolecular excimer formation (Pownall & Smith, 1973) and those resulting from fluorescence depolarization measurements (Shinitzky & Henkart, 1979).

Excimers can also be formed intramolecularly in a pseudomonomolecular process, with molecules consisting of aromatic hydrocarbons (benzene, naphthalene, anthracene or pyrene) linked with an alkane chain (Chung et al, 1993a, b; Yun et al, 1993, 1994; Kang et al, 1996). These probes offer the opportunity to study a bimolecular reaction with a single molecular entity. Intramolecular excimer formation of bichromophoric systems is affected by the viscosity of the medium, and the dipyrenylalkanes [$Py(CH_2)_n$ Py] are no exception. This is caused by the activation energy for diffusion of the end-groups in the solvent and by the (viscosity-dependent) activation energy involved in the reorientation of the alkane chain necessary to allow the end-groups to reach the excimer configuration. The ratio of the intensity of the excimer fluorescence $I'$ to the intensity of the residual monomer fluorescence $I$ is in general a function of the rate constants of excimer formation, dissociation, and deactivation (Zachariasse et al, 1982).

Py-3-Py, in particular, is well suited for intramolecular excimer studies such as here in model membranes, because of its large quantum yield of excimer fluorescence (Zachariasse et al, 1980, 1982; Melnick et al, 1981; Chung et al, 1993a, b; Yun et al, 1993, 1994; Kang et al, 1996), and because of the fact that no photochemical reactions have been found to occur upon excimer formation. This methodology has an advantage over its counterpart based on intermolecular excimer formation, since very small probe concentrations can be employed ($<10^{-6}$ M) (Zachariasse et al, 1980, 1982; Chung et al, 1993a, b; Yun et al, 1993, 1994; Kang et al, 1996). The use of low probe concentrations is possible as intramolecular excimer formation is a monomolecular process, independent of concentration (Zachariasse et al, 1980, 1982; Chung et al, 1993a, b; Yun et al, 1993, 1994; Kang et al, 1996). In this manner, the formation of aggregates in viscous media such as native and model membranes is avoided (Zachariasse et al, 1980, 1982; Chung et al, 1993a, b; Yun et al, 1993, 1994; Kang et al, 1996). Furthermore, perturbations of the membrane by the probe molecule are minimized.

The relatively nonspecific nature of anesthetic action has long been recognized. Extensive studies have shown that the anesthetic potency is closely related to the partition coefficient of anesthetic (Seeman, 1972; Miller et al, 1972, 1986; Chung et al, 1993a, b; Yun et al, 1993, 1994; Kang et al, 1996). In addition, the only obvious similarity between compounds with local anesthetic action is their hydrophobicity. Protonated local anesthetics such as carbisocaine and lidocaine induced changes in modulus of elasticity. They were shown to depend on both the lipid composition of the membrane and the electrolyte pH. In addition, neutral forms of local anesthetics induced marked changes in the elasticity (Hianik et al, 1990). The authors suggested that the action of both carbisocaine and lidocaine may be nonspecific. The anesthetics such as procaine and dibucaine lower the phase transition temperature of phosphatidylcholines and phosphatidylethanolamines (Lee, 1976). Procaine, lidocaine, tetracaine, and dibucaine significantly lowered the phase transition temperature, broadened the thermogram peaks, and reduced the size of the cooperative unit in dimyristoylphophatidylcholine multilamellar liposomes (Yun et al, 1987). Tetracaine, lidocaine, cocaine, dibucaine and heptacaine derivatives have been shown to decrease the temperature of the gel-to-liquid crystalline phase transition of dipalmitoylphosphatidylcholine model membranes (Racansky et al, 1988). Cationic local anesthetics decreased the transition temperature of the anionic dimyristoylphosphatidic acid vesicles and the differences in the partition coefficients between solid-gel and liquid-crystalline membranes correlated to the nerve blocking potencies (Kaminoh et al, 1989). Furthermore, Park (1997) reported that tetracaine · HCl, bupivacaine · HCl, lidocaine · HCl, prilocaine · HCl and procaine · HCl increased the rate and range of rotational and lateral mobility of SPMV and increased annular lipid fluidity of the SPMV, and caused protein clustering in SPMV and induced interdigitation of the lipid bilayers. In this study, lidocaine · HCl significantly decreased the microviscosity of the direct environment of Py-3-Py in the SPMVPC model membranes at 37°C (Fig. 3), suggesting an increase

in the rate and range of the lateral mobility of the membrane bilayers.

Strichartz & Richie (1987) suggested that the major mechanism of sodium conductance block of local anesthetics involves their interaction with a specific binding site within the sodium channel. However, there is evidence that local anesthetics can interact electrostatically with membrane proteins as well as membrane lipids (Wang et al, 1983; Chan & Wang, 1984). Whether or not all actions of local anesthetics are mediated by a common site remains unclear (Courtney & Strichartz, 1987; Strichartz & Richie, 1987). Purified sodium channels can be dissolved in micelles of nonionic detergent stabilized with phospholipids. The unusually hydrophobic sodium channel macromolecule has an anomalously high detergent-binding capacity, due in part to more than a dozen long-chain fatty acids associated with each channel molecule (Levinson et al, 1986). Bound to the protein by covalent or noncovalent bonds, these acyl chains may anchor and orient the channel in the membrane, stabilizing the channel's three-dimensional structure. Long-chain fatty acids also may participate in binding of hydrophobic drugs such as local anesthetics (Levinson et al, 1986). Sodium channels in nerve membranes are postulated to be surrounded by lipid molecules in the gel (or crystalline) phase (Lee, 1976). Addition of local anesthetics may trigger a change in the surrounding lipids to the fluid, liquid-crystalline phase, allowing the sodium channel to close and resulting in local anesthesia (Lee, 1976).

Opinions have been divided as to whether anesthetics interfered with membrane protein function by directly binding to the proteins, or whether the main modes of action occurred indirectly through a change in the physicochemical properties of the lipid membranes into which the local anesthetics readily dissolved (Richards, 1980; Yun & Kang, 1992). Because biological membranes are of highly complex composition, it has not been feasible to monitor changes in the local lipid environment and to determine its effect on membrane protein function at the same time. It is possible to explain the multiplication effects citing the increased mobility of protein triggered by lipids, but the reverse case of protein triggering change in lipids is more likely. It is certain that local anesthetics increase the mobility of the neuronal lipid bilayer but the direct effects of local anesthetics on protein appear to have magnified such effects on the lipid. That is to say, before or during or even after the interaction of lidocaine · HCl with sodium channels, the fluidization of membrane lipids may provide an ideal microenvironment for optimum local anesthetic effects. In conclusion, the present data suggest that lidocaine · HCl, in addition to its direct interaction with sodium channels, concurrently interacts with membrane lipids, fluidizes the membrane, and thus induces conformational changes of sodium channels which are known to be tightly associated with membrane lipids. This conclusion can be drawn because we confirmed that the increase in the rotational and lateral mobility of the lipid bilayer structure of the neuronal (Park, 1997) and liposomes of total lipids (Min, 1998) and phospholipids (Lee, 1999) extracted from neuronal membranes induced by the local anesthetics was significantly less than the increasing effect of local anesthetics on the annular lipid fluidity of SPMV. Furthermore, the SPMV showed a higher sensitivity to the local anesthetics than the liposomes of total lipids and phospholipids without proteins (Park, 1997; Min, 1998; Lee, 1999).

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