Korean J Physiol Pharmacol
Vol 7: 119–124, June, 2003

Effects of Local Anesthetics on Rotational Mobility of *n*-(9-Anthroyloxy)stearic Acid in Neuronal Membranes

Hye-Ock Jang, Chang Lee, Min-Gak Choi, Sang-Hun Shin[1], In-Kyo Chung[1], and Il Yun

Department of Dental Pharmacology and Biophysics and [1]Oral and Maxillofacial Surgery and Clinical Pharmacology, College of Dentistry and Research Institute for Oral Biotechnology, Pusan National University, Busan 602–739, Korea

To elucidate the molecular mechanism of pharmacological action of local anesthetics, we studied membrane actions of tetracaine, bupivacaine, lidocaine, prilocaine and procaine. Fluorescence polarization of *n*-(9-anthroyloxy)stearic acid (*n*-AS) was used to examine the effects of these local anesthetics on differential rotational mobility of different positions of the number of synaptosomal plasma membrane vesicle (SPMV) phospholipid carbon atoms. The four membrane components differed with respect to 3, 6, 9 and 16-(9-anthroyloxy)stearic acid (3-AS, 6-AS, 9-AS and 16-AP) probes, indicating that differences in the membrane fluidity might be present. Degrees of the rotational mobility of 3-AS, 6-AS, 9-AS and 16-AP were different depending on depth of hydrocarbon interior. In a dose-dependent manner, tetracaine, bupivacaine, lidocaine, prilocaine and procaine decreased anisotropy of 3-AS, 6-AS, 9-AS and 16-AP in the hydrocarbon interior of the SPMV. These results indicate that local anesthetics have significant disordering effects on hydrocarbon interior of the SPMV, thus affecting the transport of Na^+ and K^+ in nerve membranes and leading to anesthetic action.

Key Words: Fluorescent probe technique, Rotational mobility, Hydrocarbon interior, Local anesthetics, Neuronal membranes, AS probes

INTRODUCTION

The molecular mechanism of pharmacological action of local anesthetics has long been a subject of great interest. Two general theories have been proposed to explain the action of local anesthetics on sodium channel: one considers a direct binding of local anesthetic molecules to specific receptors on sodium channels (Strichartz, 1973; Butterworth & Strichartz, 1990) and the other proposes the general perturbation of the bulk membrane structure by local anesthetics and its consequences on channel function (Seeman, 1972; Lee, 1976; Singer, 1977; Smith et al, 1991). There is a large amount of evidence in support of the specific receptor hypothesis (Strichartz & Richie, 1987). General membrane perturbation may also contribute to an explanation of anesthetic actions (Strichartz & Richie, 1987).

The physical state of membrane lipids has been shown to influence such membrane enzymes as NaK-ATPase (Chong et al, 1985), hormone-responsive adenylate cyclase (Dipple & Houslay, 1978), and membrane transport processes such as glucose and amino acid uptake (Balcar et al, 1980; Carricre & Grimellec, 1986). Membrane lipids also play an important role in membrane permeability to sodium, calcium, and potassium (Green et al, 1980).

Effects of local anesthetics on motion, order and phase transitions of bulk bilayer systems of native or model membranes have received considerable attention in 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. It is known that the potency of an anesthetic increases roughly in proportion with its lipid/water partition coefficient, strongly suggesting an amphiphilic site for anesthetic molecules (Miller et al, 1986; Yun et al, 1987, 1994; Kang et al, 1996). Yun et al. (1987) reported that local anesthetics decreased microviscosity of synaptosomal plasma membrane vesicles isolated from the bovine cerebral cortex (SPMV). In addition, differential scanning thermograms of dimyristoylphosphatidylcholine multilamellar liposomes showed that local anesthetics significantly lowered the phase transition temperature, broadened the thermogram peaks, and reduced the size of the cooperative unit. Sweet et al. (1987) reported that prilocaine · HCl preferentially reduced the limiting anisotropy of 1,6-diphenyl-1,3,5-hexatriene in the inner monolayer of LM fibroblast plasma membrane. However, it was also true that local anesthetics' had a fluidizing effect on the outer monolayer of membrane, although the effect was smaller than that of the inner monolayer. Furthermore, recent fluorescence measurements showed that the highest affinity site for dibucaine in sarcoplasmic reticulum vesicles

Corresponding to: Il Yun, Department of Dental Pharmacology and Biophysics, College of Dentistry and Research Institute for Oral Biotechnology, Pusan National University, Busan 602-739, Korea. (Tel) 051-240-7813, (Fax) 051-254-0576, (E-mail) iyun@pusan.ac.kr

ABBREVIATIONS: SPMV, synaptosomal plasma membrane vesicles isolated from the bovine cerebral cortex; 3-AS, 6-AS, 9-AS, and 16-AP, 3, 6, 9, and 16-(9-anthroyloxy)stearic acid; BSA, bovine serum albumin.

(SRV) is a lipid site near the membrane surface (Louro et al, 1994). Yun et al. (2002) reported that tetracaine, bupivacaine, lidocaine, prilocaine and procaine decreased anisotropy of 12-(9-anthroyloxy)stearic acid (12-AS) in the hydrocarbon interior of the SPMV, but the local anesthetics increased anisotropy of 2-(9-anthroyloxy)stearic acid (2-AS) in the membrane interface. Most of the accumulated results for the analysis of local anesthetics' effect on the cell membrane fluidity used a single molecular probe for estimations of bulk membrane fluidity, thus obtaining information about one region (or average). However, the membrane fluidity may vary at different positions.

Previous studies have shown that the fluorophores of anthroyloxy derivatives locate at a graded series of levels from the surface to the center of the lipid bilayer structure (or a series of anthroyloxy fatty acids indicates that the depth of the group is almost linearly related to the number of carbon atoms between it and carboxyl group) (Tilley et al, 1979; Villalaín & Prieto, 1991; Abrams et al, 1992; Abrams & London, 1993; Mason, 1994). The fluorophores of anthroyloxy derivatives can also be used to differentiate whether the bilayer has a fluidity gradient across it, as the anthroyloxy group can be positioned at different positions of the stearic acid moiety (Ehringer et al, 1991; Han & Gross, 1992; Martinez-Azorin et al, 1992; Tricerri et al, 1994). These probes have been suggested to measure primarily the dynamic component of membrane fluidity (Schachter et al, 1982; Vincent et al, 1982; Schachter, 1984; Brasitus & Dudeja, 1985; Brasitus et al, 1986; Molitoris & Hoilien, 1987).

The aim of this research was to provide a basis for studying the molecular mechanism of pharmacological action of local anesthetics, through investigation of the effects of local anesthetics on differential rotational mobility among the number of carbon atoms in the neuronal membrane phospholipids which differ in fluidity. Employing fluorescence polarization of 16-(9-anthroyloxy) stearic acid (16-AP), 9-(9-anthroyloxy)stearic acid (9-AS), 6-(9-anthroyloxy)stearic acid (6-AS) and 3-(9-anthroyloxy) stearic acid (3-AS), we investigated the effect of tetracaine · HCl bupivacaine · HCl, lidocaine · HCl, prilocaine · HCl and procaine · HCl on rotational mobility of SPMV isolated from bovine cerebral cortex.

METHODS

Materials

The fluorescent anthroyloxy stearate probes, 16-AP, 9-AS, 6-AS and 3-AS were purchased from Molecular Probes, Inc. (Junction City, OR, USA). Tetracaine · HCl, bupivacaine · HCl, lidocaine · HCl, prilocaine · HCl, procaine · HCl, *N*-2-hydroxyethyl-piperazine-*N*'-2-ethanesulfonic acid (Hepes) and bovine serum albumin (BSA) were purchased from Sigma Chemical (St. Louis, MO, USA). All other reagents were obtained from various commercial sources and were of the highest quality available. Water was deionized.

SPMV preparation

The SPMV were prepared according to the procedure described earlier (Yun & Kang, 1990; Yun et al, 1990). The specific activities of Na,K-ATPase, acetylcholinesterase and 5'-nucleotidase in the plasma membrane fraction were approximately 4-, 2.5- and 3-times higher than 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 homogeneous distribution and showed no presence of intracellular organelles or leakage. The protein concentration was determined by the method of Lowry et al. (1951), using BSA as a standard.

Fluorescence measurements

The fluorescence measurements were taken using a modified method of earlier study (Molitoris & Hoilien, 1987). The SPMV were suspended in phosphate-buffered saline (PBS; 8 g/l NaCl, 0.2 g/l KCl, 0.2g/l KH_2PO_4, 1.15g/l $Na_2HPO_47 \cdot H_2O$, 0.48 g/l Hepes, pH 7.4) to 50 μg of protein/ ml concentration. Stock solutions of the individual probes in methanol (2×10^{-5} M) were prepared and kept in a cold dark place. Aliquots were added to the solutions of the neuronal membranes, so that the final concentrations of the individual probes were 4×10^{-8} M incorporated. The mixture was stirred for 20 min at room temperature in order to reduce the concentration of methanol that might alter the rotational mobility of the SPMV. Also, the mixture was bubbled by dry nitrogen for 1 min with 20 min intervals in order to eliminate oxygen that might act as a quencher. Concentrated solutions of local anesthetics were prepared in PBS and added to the labeled membrane suspension to give the desired concentration of the local anesthetics. The pH of the buffered sample was not changed significantly by addition of local anesthetics.

Fluorescence measurements were carried out with a Multi Frequency Cross-Correlation Phase and Modulation Fluorometer (ISS K2-003), equipped with a thermostated cell holder and performed at pH 7.4 (37 ± 0.1°C). The fluorescent probes, 16-AP, 9-AS, 6-AS and 3-AS, were excited at 360 nm (4 nm slit width) and emissions were recorded at 445 nm (8 nm slit width) through a sharp cut-off filter (Schott KV418). Corrections for light scattering (membrane suspensions without fluorescent probes) and for fluorescence in the ambient medium (quantified by pelleting the membranes after each estimation) were routinely made, and the combined corrections were less than 9% of the total fluorescence intensity observed for anthroyloxystearate-loaded suspensions. The intensity of the components of the fluorescence which were parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) to the direction of the vertically polarized excitation light was determined by measuring the emitted light through polarizers which were oriented vertically and horizontally. Polarization (P) was obtained from intensity measurements using $P = (I_{\parallel} - GI_{\perp})/(I_{\parallel} + GI_{\perp})$, where G is a grating correction factor for the monochromator's transmission efficiency for vertically and horizontally polarized light. This value is given by the ratio of the fluorescence intensities of the vertical to horizontal components, when the exciting light was polarized in the horizontal direction. The polarization was expressed as anisotropy [$\gamma=2P/(3-P)$] of the probes.

RESULTS

In the present study, using fluorescence probe technique, we examined the effects of cations of tetracaine, bupivacaine, lidocaine, prilocaine and procaine on the differential rota-

tional mobility among the number of carbon atoms of SPMV phospholipids. In order to determine effects of local anesthetics on the aforementioned rotational mobility, it was first necessary to demonstrate that the drugs did not interact directly with fluorescent probes, thereby quenching its fluorescence. Quenching of absorbance-corrected fluorescence intensity by the local anesthetics is not observed at all the concentration levels where tetracaine, bupivacaine, lidocaine, prilocaine and procaine were tested. Furthermore, if direct quenching of the probes by the local anesthetics occurred, fluorescence lifetime would decrease. However, the fluorescence lifetime of each individual probe was not changed by the local anesthetics in the SPMV. For example, the lifetime of 16-AP in the SPMV was 11.2±0.2, 11.3±0.1, 11.8 ±0.3, 11.4±0.1 and 11.5±0.2 ns at 0.01, 0.1, 0.2, 1 and 2 mM tetracaine · HCl, respectively. Therefore, direct quenching of probe fluorescence by the local anesthetics used was ruled out in the present experiments.

The anisotropy (r) values of 16-AP, 9-AS, 6-AS and 3-AS for hydrocarbon interior of intact SPMV were 0.092± 0.002 (n=5), 0.108±0.001 (n=5), 0.115±0.002 (n=5) and 0.123±0.001 (n=5) at 37°C (pH 7.4), respectively (Table 1). The rotational mobility's degrees of SPMV phospholipids differed, depending on the number of carbon atoms in the descending order of the 16-AP, 9-AS, 6-AS and 3-AS.

Disordering effects of local anesthetics on the rotational mobility of the hydrocarbon interior

It is found that the disordering effects of an local anesthetic on the hydrocarbon interior of neuronal membrane increases roughly in proportion with its depth of site of action (the more effective penetration into hydrocarbon interior could result in higher disordering effects on the neuronal membrane). The effects of increasing concentrations of the local anesthetics on the anisotropy (r) of the 16-AP in the hydrocarbon interior (the sixteen of carbon atom including near region of the membrane phospholipids) of SPMV are shown in Fig. 1. The local anesthetics decreased the anisotropy (r) of the 16-AP in the SPMV (increased rotational mobility) in a concentration-dependent manner. The significant decreases in the anisotropy (r) values by the tetracaine, bupivacaine, lidocaine, prilocaine and procaine were observed even at such low concentrations as 0.01, 0.1, 0.1, 0.1 and 0.5 mM, respectively (Fig. 1). The significant decreases in the anisotropy (r) values of the 9-AS by the tetracaine, bupivacaine, lidocaine, prilocaine and procaine were observed at 0.1, 0.1, 1.0, 1.0 and 5.0 mM, respectively (Fig. 2). The effects of increasing concentrations of the local anesthetics on the anisotropy (r) of the 6-AS in hydrocarbon interior of SPMV are shown in Fig. 3. The local anesthetics decreased the anisotropy (r) of the 6-AS in a concentration-

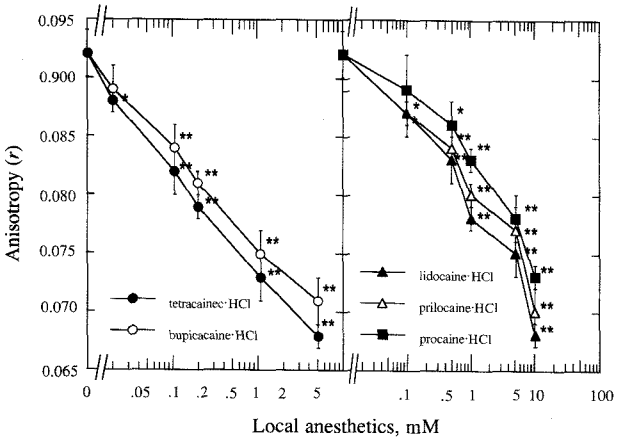

Fig. 1. Effects of local anesthetics on the anisotropy (r) of 16-(9-anthroyloxy)palmitic acid (16-AP) in synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex. Fluorescence measurements were performed at 37°C (pH 7.4). Each point represents mean±SEM of five determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to control according to Student's t-test.

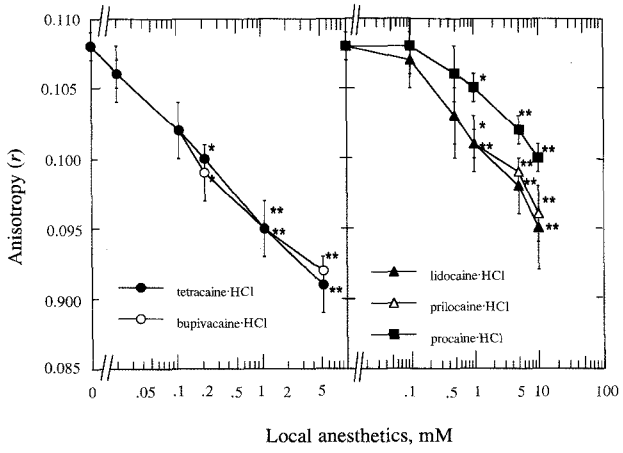

Fig. 2. Effects of local anesthetics on the anisotropy (r) of 9-(9-anthroyloxy)stearic acid (9-AS) in synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex. Fluorescence measurements were performed at 37°C (pH 7.4). Each point represents mean±SEM of five determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to control according to Student's t-test.

Table 1. Fluorescence parameters of 16-(9-anthroyloxy)palmitic acid (16-AP), 9-(9-anthroyloxy)stearic acid (9-AS), 6-(9-anthroyloxy)stearic acid (6-AS) and 3-(9-anthroyloxy)stearic acid (3-AS) in synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex

Membrane	Parameter	16-AP	9-AS	6-AS	3-AS
SPMV	Anisotropy	0.094±0.002	0.108±0.001	0.115±0.002	0.123±0.001

Fluorescence measurements were performed at 37°C (pH 7.4). Values represent mean±SEM of five determinations.

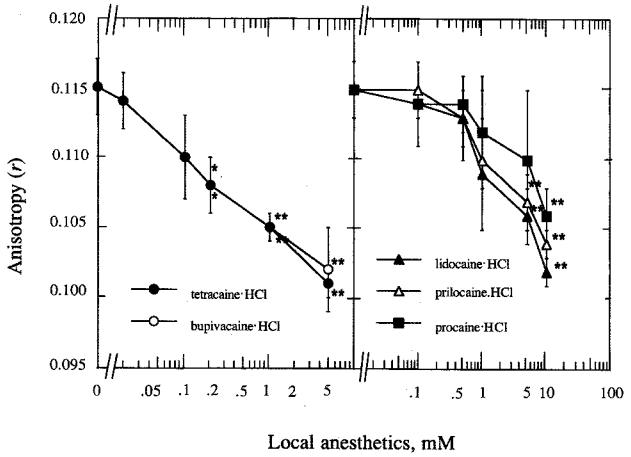

Fig. 3. Effects of local anesthetics on the anisotropy (r) of 6-(9-anthroyloxy)stearic acid (6-AS) in synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex. Fluorescence measurements were performed at 37°C (pH 7.4). Each point represents mean±SEM of five determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to control according to Student's t-test.

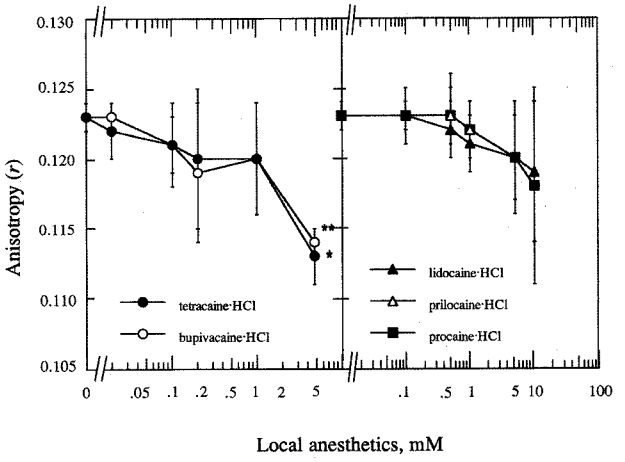

Fig. 4. Effects of local anesthetics on the anistropy (γ) of 3-(9-anthroyloxy)stearic acid (3-AS) in synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex. Fluorescence measurements were performed at 37°C (pH 7.4). Each point represents mean±SEM of five determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, compared to control according to Student's t-test.

dependent manner. The significant decreases in the anisotropy (r) values by the tetracaine, bupivacaine, lidocaine, prilocaine and procaine were observed to be significant at 0.2, 0.2, 5.0, 5.0 and 10.0 mM, respectively (Fig. 3). The local anesthetics decreased the anisotropy (r) of the 3-AS in a dose-dependent manner and the decreases in anisotropy (r) values by the tetracaine and bupivacaine were considerable at higher concentrations of 2.0 and 2.0 mM, respectively (Fig. 4). However, the significant decreases or increases in the 3-AS anisotropy values by lidocaine, prilocaine and procaine were not observed at all concentrations used (0.1, 0.5, 1.0, 5.0 and 10.0 mM).

The difference in the anisotropy (r) value of the 16-AP found in hydrocarbon interior of SPMV before and after adding 2 mM tetracaine · HCl was 0.024. This can be illustrated by comparing effects of temperature on this parameter. The anisotropy (r) of the 16-AP in hydrocarbon interior of SPMV at 37°C (pH 7.4) and 25°C (pH 7.4) was 0.092±0.002 (n=5) and 0.119±0.003 (n=5), respectively. Thus, the difference in the anisotropy (r) value of 16-AP in hydrocarbon interior of SPMV before and after adding 2 mM tetracaine was 0.024 which was as large as that produced by the raise of approximate 10.7°C temperature.

The difference in the anisotropy (r) value of the 9-AS found in hydrocarbon interior of SPMV before and after adding 2 mM tetracaine · HCl was 0.017. Variation in the anisotropy (r) value was also noticed by the change in temperature as mentioned above. The anisotropy (r) of the 9-AS in hydrocarbon interior of SPMV at 37°C (pH 7.4) and 25°C (pH 7.4) was 0.108±0.001 (n=5) and 0.138±0.002 (n =5), respectively. Thus, the difference in the anisotropy (r) value of 9-AS in hydrocarbon interior of SPMV before and after adding 2 mM tetracaine was 0.017 which was as large as that produced by the raise of approximate 6.8°C temperature.

The difference in the anisotropy (r) value of the 6-AS found in hydrocarbon interior of SPMV before and after adding 2 mM tetracaine · HCl was 0.013. This can be illustrated by comparing effects of temperature on this parameter. The anisotropy (r) of the 6-AS in hydrocarbon interior of SPMV at 37°C (pH 7.4) and 25°C (pH 7.4) was 0.115±0.002 (n=5) and 0.147±0.003 (n=5), respectively. Thus, the difference in the anisotropy (r) value of 6-AS in hydrocarbon interior of SPMV before and after adding 2 mM tetracaine was 0.013 which was produced by the raise of approximate 4.9°C temperature.

The difference in the anisotropy (r) value of the 3-AS found in hydrocarbon interior of SPMV before and after adding 2 mM tetracaine · HCl was 0.010. This can be illustrated by comparing effects of temperature on this parameter. The anisotropy (r) of the 3-AS in hydrocarbon interior of SPMV at 37°C (pH 7.4) and 25°C (pH 7.4) was 0.123±0.001 (n=5) and 0.158±0.003 (n=5), respectively. Thus, the difference in the anisotropy (r) value of 3-AS in hydrocarbon interior of SPMV before and after adding 2 mM tetracaine was 0.010 which was produced by the raise of approximate 3.4°C temperature.

DISCUSSION

The potency of local anesthetics is generally defined as the dose necessary to achieve a specified end point. Clinically, the potency is usually the total mass (or moles) of drug required to relieve or prevent pain, produce tactile numbness, or inhibit sympathetic or motor activity. By comparison, tetracaine is approximately 10 times more potent as procaine, lidocaine has approximately 2 times the potency of procaine, prilocaine is somewhat less potent than lidocaine, and bupivacaine is approximate 4 times more potent than lidocaine (Mitchell, 1982; Yagiela, 1986). Tetracaine is an ester of *p*-aminobenzoic acid in which a butyl chain replaces one of the hydrogens on the *p*-amino group. Bupivacaine is a homologue of mepivacaine which is highly lipid soluble by replacement of the *N*-methyl group with a butyl chain. Lidocaine is an aminoethylamide derivative

of xylidine, prilocaine is a secondary amino derivative of toluidine, and procaine is the ester of *p*-aminobenzoic acid and diethylaminoethanol. The physicochemical behavior of local anesthetics is a consistent and somewhat predictable function of their structure. Local anesthetic molecules with larger alkyl groups on both the tertiary amine nitrogen and the aromatic moiety show greater hydrophobicity.

In a dose-dependent manner, the local anesthetics used in the present study lowered the anisotropy (r) values of 16-AP, 9-AS, 6-AS and 3-AS in hydrocarbon interior of SPMV. The disordering effects of the local anesthetics on the lipid bilayer occurred in the order of tetracaine (ester type) $\geq$ bupivacaine (amide type) > lidocaine (amide type) $\geq$ prilocaine (amide type) > procaine (ester type), which are in accordance with the clinical potency of the local anesthetics. The local anesthetics, tetracaine and bupivacaine, showed the highest potency while lidocaine, prilocaine and procaine showed lower potencies. Using EPR, de Paula and Schreier (1995) reported that the ester-type anesthetics have greater active fluidizing effects than the amide-type ones. Our data are not in agreement with these findings. Differences between the two studies might have been due in part to differences in the type of probe molecules used and to the differences between multilamellar liposomes and neuronal membranes in the EPR and fluorescence study. In addition, due to the lower sensitivity of EPR, relatively high probe concentrations (2% of total lipid, in moles) are required that could perturb the membrane and thus alter drug effects. The concentration of 5-doxylstearic acid and it's methyl ester, the probes used in the measurement through EPR, was 80 times higher than that of the AS probes, the fluorescence probes used in this study.

The mechanism of pharmacological action(s) of local anesthetics on disordering effects on the neuronal membranes is not well understood. The interaction of the local anesthetics with the hydrocarbon interior will rearrange the intermolecular hydrogen-bonded network among phospholipid molecules and/or protein molecules that are associated with the liberation of hydrated water molecules on the neuronal membranes (shibata et al, 1995). The interaction will also change the orientation of the P-N dipole of phospholipid molecules (Scherer & Seelings, 1989). These changes should cause disordering of the hydrocarbon interior, thus affecting the transport of Na^+ and K^+ in nerve membranes and leading to anesthetic action.

The sensitivities to increasing the rotational mobility of the hydrocarbon interior by the local anesthetics differed depending on number of carbon atoms, including near region of the membrane phospholipids, in the descending order of the carbon number 16, 9, 6 and 3. The results of this study, undoubtedly led us to conclude that the local anesthetic agents increase the rotational mobility of the hydrocarbon interior of the membrane. Water plays a fundamental role in cell membrane structure in that it drives the formation of the lipid bilayer, with a polar surface facing the aqueous environment and a hydrophobic interior containing the fatty acyl chains and transmembrane proteins. In general, the structure and dynamics of proteins are also governed to a large extent by interactions with water (Teeter, 1991). Water penetrates into lipid bilayers at least as far as the glycerol backbone and also deeper between fatty acyl chain packing defects. Water at the protein-lipid interface is an additional factor involved in influencing the lipid bilayer structure. The introduction of small peptides, consisting of three amino acids, can cause a shift of water deeper into the bilyaer, indicating increased hydration (Jacobs & White, 1989). Altered hydration may have marked effects on membrane protein/lipid functioning, possibly due to the formation of hydrogen bonds between the interchain water and protein amino acid side chains facing/lipid acyl chains facing into the hydrophobic interior of the membrane. It is possible that the proteins organize the lipid in a way that makes them more susceptible to the anesthetics.

There have been divided opinions as to whether local 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 diffused. Because biological membranes are of highly complex composition, it has not been possible to monitor changes in the local lipid environment and to determine its effect on the membrane protein function at the same time. It is possible to explain the multiplication effects by citing the increased mobility of protein triggered by lipids, however 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. In conclusion, the present data suggest that local anesthetics, in addition to its direct interaction with sodium channels, concurrently interact with membrane lipids, thereby affecting fluidity of the neuronal membrane.

REFERENCES

Abrams FS, Chattopadhyay A, London E. Determination of the location of fluorescent probes attached to fatty acids using parallax analysis of fluorescence quenching: effect of carboxyl ionization state and environment on depth. *Biochemistry* 31: 532–5327, 1992

Abrams FS, London E. Extension of the parallax analysis of membrane penetration depth to the polar region of model membranes: use of fluorescence quenching by a spin-label attached to the phospholipid polar headgroup. *Biochemistry* 32: 10826–10831, 1993

Balcar VJ, Borg J, Robert J, Mandel P. Uptake of L-glutamate and taurine in neuroblastoma cells with altered fatty acid composition of membrane phospholipids. *J Neurochem* 34: 1678–1681, 1980

Brasitus TA, Dudeja PK. Correction of abnormal lipid fluidity and composition of rat ileal microvillus membranes in chronic streptozotocin-induced diabetes by insulin therapy. *J Biol Chem* 260: 12405–12409, 1985

Brasitus TA, Dudeja PK, Eby B, Lau K. Correction by 1-25-dihydroxycholecalciferol of the abnormal fluidity and lipid composition of enterocyte brush border membranes in vitamin D-deprived rats. *J Biol Chem* 261: 16404–16409, 1986

Butterworth JF, Strichartz GR. Molecualr mechanisms of local anesthesia: a review. *Anesthesiology* 72: 711–734, 1990

Carricre B, le Grimellec C. Effects of benzyl alcohol on enzyme activities and D-glucose transport in kidney brush border membranes. *Biochim Biophys Acta* 857: 131–138, 1986

Chong PIG, Fortes PAG, Jameson DM. Mechanism of inhibition of NaK-ATPase by hydrostatic pressure studies with fluorescence probes. *J Biol Chem* 260: 14484–14490, 1985

Dipple I, Houslay MD. The activity of glucagon-stimulated adenylate cyclase from rat liver plasma membranes is modulated by the fluidity of its lipid component. *Biochem J* 174: 179–190, 1978

Ehringer WD, Belcher D, Wassall SR, Stillwell W. A comparison

of α-linolenic acid (18 : 3Ω3) and γ-linolenic acid (18 : 3Ω6) in phosphatidylcholine bilayers. *Chem Phys Lipids* 57: 87−96, 1991

Green DE, Fry M, Blondin GA. Phospholipids as the molecular instruments of ion and solute transport in biological membranes. *Proc Natl Acad Sci USA* 77: 257−261, 1980

Han X, Gross RW. Nonmonotonic alterations in the fluorescence anisotropy of polar head group labeled fluorophores during the lamellar to hexagonal phase transition of phospholipids. *Biophys J* 63: 309−316, 1992

Jacobs RE, White SH. The nature of the hydrophobic binding of small peptides at the bilayer interface. Implications for the insertion of transbilayer helices. *Biochemistry* 28: 3421−3437, 1989

Kang JS, Choi CM, Yun I. Effects of ethanol on the lateral and rotational mobility of plasma membrane vesicles isolated from cultured mouse myeloma cell line Sp2/0-Ag14. *Biochim Biophys Acta* 1281: 157−163, 1996

Lee AG. Model for action of local anesthetics. *Nature* 262: 545−548, 1976

Louro SRW, Tabak M, Nascimento OR. Depth profiling of dibucaine in sarcoplasmic reticulum vesicles by fluorescence quenching. *Biochim Biophys Acta* 1189: 243−246, 1994

Lowry OH, Rosebrough NR, Farr AL, Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265−275, 1951

Martinez-Azorin F, Teruel JA, Fernandez-Belda F, Gomez-Fernandez JC. Effect of diethylstilbestrol and related compounds on the Ca2+-transporting ATPase of sarcoplasmic reticulum. *J Biol Chem* 267: 11923−11929, 1992

Mason JT. Properties of phosphatidylcholine bilayers as revealed by mixed-acyl phospholipid fluorescent probes containing *n*-(9-anthroyloxy) fatty acids. *Biochim Biophys Acta* 1194: 99−108, 1994

Miller KW, Braswell LM, Firestone LL, Dodson BA, Forman SA. Mechanism of action of local anesthetics. In: Roth H ed, *Molecular and Cellular Mechanisms of Anesthetics*. Plenum, New York, pp 157−171, 1986

Mitchell EW. Local anesthetics. In: Allyn A, Hoos A, McKearn J, Najarian D ed, *Accepted Dental Therapeutics*, A The Council on Dental Therapeutics of the American Dental Association, Chicago, pp 145−165, 1982

Molitoris BA, Hoilien C. Static and dynamic components of renal cortical brush border and basolateral membrane fluidity: role of cholesterol. *J Membr Biol* 99: 165−172, 1987

de Paula E, Schreier S. Use of a nobel method for determination of partition coefficients to compare the effects of local anesthetics on membrane structure. *Biochim Biophys Acta* 1240: 25−33, 1995

Schachter D. Fluidity and function of hepatocyte plasma membranes. *Hepatology* 4: 140−151, 1984

Schachter D, Cogan U, Abbott RE. Asymmetry of lipid dynamics in human erythrocyte membranes studied with permeant fluorophores. *Biochemistry* 21: 2146−2150, 1982

Scherer PG, Seeling J. Electric charge effects on phospholipid headgroups. Phosphatidylcholine in mixtures with cationic and anionic amphiphiles. *Biochemistry* 28: 7720−7728, 1989

Seeman P. The membrane actions of anesthetics and tranquilizers. *Pharmacol Rev* 24: 585−655, 1972

Shibata A, Ikawa K, Terada H. Site of action of the local anesthetic tetracaine in a phosphatidylcholine bilayer with incorporated cardiolipin. *Biophys J* 69: 470−477, 1995

Singer MA. Interaction of dibucaine and propanol with phospholipid bilayer membranes-effect of alterations in fatty acyl composition. *Biochem Pharmacol* 26: 51−57, 1977

Smith ICP, Auger M, Jarrell HC. Molecular details on anesthetic-lipid interaction. *Ann NY Acad Sci* 625: 668−684, 1991

Strichartz GR. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. *J Gen Physiol* 62: 37−57, 1973

Strichartz GR, Richie JM. The action of local anesthetics on ion channels of excitable tissues. In: Strichartz GR ed, *Local anesthetics*. Springer-Verlag, New York, pp 21−52, 1987

Sweet WD, Wood WG, Schroeder F. Charged anesthetics selectively alter plasma membrane order. *Biochemistry* 26: 2827−2835, 1987

Teeter MM. Water-protein interaction: theory and experiment. *Annu Rev Biophys Biophys Chem* 20: 577−600, 1991

Tilley L, Thulborn KR, Sawyer WH. An assessment of the fluidity gradient of the lipid bilayer as determined by a set of *n*-(9-anthroyloxy) fatty acids (n=2,6,9,12,16). *J Biol Chem* 254: 2592−2594, 1979

Tricerri MA, Garda HA, Brenner RR. Lipid chain order and dynamics at different bilayer depths in liposomes of several phosphatidylcholines studied by differential polarized phase fluorescence. *Chem Phys Lipids* 71: 61−72, 1994

Villalaín J, Prieto M. Location and interaction of *N*-(9-anthroyloxy)-stearic acid probes incorporated in phosphatidylcholine vesicles. *Chem Phys Lipids* 59: 9−16, 1991

Vincent M, de Furesta B, Gallay J, Alfsen A. Fluorescence anisotropy decays of *n*-(9-anthroyloxy) fatty acids in dipalmitoyl phosphatidylcholine vesicles. Localization of the effects of cholesterol addition. *Biochem Biophys Res Commun* 107: 914-921, 1982

Yagiela JA. Local anesthetics. In: Neidle EA, Yagiela JA ed, *Pharmacology and therapeutics for dentistry*, The C. V. Mosby Company, Philadelphia, pp 230−248, 1986

Yun I, Cho ES, Jang HO, Kim UK, Choi CH, Chung IK, Kim IS, Wood WG. Amphiphilic effects of local anesthetics on rotational mobility in neuronal and model membranes. *Biochim Biophys Acta* 1564: 123−132, 2002

Yun I, Han SK, Baek SW, Kim NH, Kang JS, Chung IK, Lee EJ. Effects of local anesthetics on the fluidity of synaptosomal plasma membrane vesicles isolated from bovine brain. *Korean J Pharmacol* 24: 43−52, 1987

Yun I, Kang JS. The general lipid composition and amino-phospholipid asymmetry of synaptosomal plasma membrane vesicles isolated from bovine cerebral cortex. *Mol Cells* 1: 15−20, 1990

Yun I, Kim YS, Yu SH, Chung IK, Kim IS, Baik SW, Cho GJ, Chung YZ, Kim SH, Kang JS. Comparison of several procedures for the preparation of synaptosomal plasma membrane vesicles. *Arch Pharm Res* 13: 325−329 1990

Yun I, Lee SH, Kang JS. The effect of ethanol on lateral and rotational mobility of plasma membrane vesicles isolated from cultured Mar 18.5 Hybridoma cells. *J Membr Biol* 138: 221−227, 1994