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The Effect of Dibucaine·HCl on the Physical Properties of Neuronal Membranes

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Fluorescent probe techniques were used to evaluate the effect of dibucaine·HCl on the physical properties (transbilayer asymmetric lateral mobility, annular lipid fluidity and protein distribution) of synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex. An experimental procedure was used based on selective quenching of 1,3-di(1-pyrenyl)propane (Py-3-Py) by trinitrophenyl groups, and radiationless energy transfer from the tryptophans of membrane proteins to Py-3-Py. Dibucaine·HCl increased the bulk lateral mobility, and annular lipid fluidity in SPMV lipid bilayers, and had a greater fluidizing effect on the inner monolayer than the outer monolayer. The magnitude of increasing effect on annular lipid fluidity in SPMV lipid bilayer induced by dibucaine·HCl was significantly far greater than magnitude of increasing effect of the drug on the lateral mobility of bulk SPMV lipid bilayer. It also caused membrane proteins to cluster. These effects of dibucaine·HCl on neuronal membranes may be responsible for some, though not all, of the local anesthetic actions of dibucaine·HCl.

key words: Annular lipid fluidity, Dibucaine·HCl, Membrane protein clustering, Neuronal membranes, Transbilayer lateral mobility

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 [1,2] and the other proposes the general perturbation of the bulk membrane structure by anesthetics and its consequences on channel function [3-6]. There is a large amount of evidence in support of the specific receptor hypothesis [7]. General membrane perturbation may also contribute to an explanation of anesthetic actions [7].

The physical state of membrane lipids has been shown to influence such membrane enzymes as Na^+,K^+-ATPase [8], hormone-responsive adenylate cyclase [9], and membrane transport processes such as glucose and amino acid uptake [10, 11]. Membrane lipids also play an important role in membrane permeability to sodium, calcium, and potassium [12].

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 [13-16]. Yun et al. [14] 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. [17] 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. Yun et al. [18] reported that local anesthetics increased the rotational mobility of SPMV hydrocarbon interior, but the anesthetics decreased rotational mobility of SPMV surface region (membrane interface).

As mentioned above, there have been some sporadic studies which back up, although not fully satisfactorily, the membrane expansion theory. The membrane expansion theory is one of the more important theories regarding the mechanism of pharmacological action of local anesthetics.

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 interaction with protein than other lipids) was and to what degree the neuronal membrane lipid bilayer was expanded by local anesthetics.

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More specifically, our questions were; first, how much of an increase do local anesthetics bring to lateral mobility of the neuronal membrane lipid bilayer; second, whether such increasing effects were shown evenly on both lipid monolayers or differently between inner and outer monolayers; third, it the degree of increase is different between the inner and outer monolayers, then which monolayer has been mostly affected; fourth, whether annular lipid fluidity of the neuronal membrane lipid bilayer is increased or decreased by local anesthetics, and whether the degree of such increase or decrease is approximately the same as or much greater than the degree of changes in lateral mobility.

We are here to present the results of our study on how we solved the aforementioned questions by employing fluorescence technique, including the fluorescence quenching technique which Prof. Yun first developed specifically for the study to measure the rate and range of asymmetrical lateral mobility between inner and outer monolayers of the lipid bilayer.

MATERIALS AND METHODS

Materials

The fluorescent probe, 1,3-di(1-pyrenyl)propane (Py-3-Py) was purchased from Molecular Probes, Inc. (Junction City, OR, USA). Dibucaine·HCl, *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (Hepes) and bovine serum albumin (BSA) were purchased from Sigma Chemical (St. Louis, MO, USA). 2,4,6-Trinitrobenzenesulfonic acid (TNBS) was obtained from Fluka (Switzerland). All other reagents were purchased commercially and were of the highest quality available. Water was deionized.

Preparation of synaptosomes and TNBS labeling

Synaptosomes were prepared as described previously [15, 19]. To determine the fluorescence parameters of probe molecules in each of the membrane monolayers, TNBS labeling reactions were performed as described [15,16,20,21] with a few modifications. The synaptosomal pellet was gently resuspended in 50 ml of 4 mM TNBS in buffer A for 80 min in buffer A alone. Buffer A consisted of 30 mM NaCl, 120 mM $NaHCO_3$, 11 mM glucose and 1% BSA. Reagent pH was adjusted to 8.5 with NaOH. To assure complete exposure of all synaptosomal outer monolayers to TNBS, the pellet was passed slowly through an Eberbach tissue grinder (3 up and down strokes). Unless otherwise specified, treatment was carried out at 4°C. The TNBS labeling reaction was terminated by adding an equal volume of 1% BSA in phosphate buffered saline (PBS; 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).

Membrane isolation

SPMV were isolated from synaptosomes by the method previously reported [15,19]. Their protein concentration was determined by the method of Lowry et al. [22] with BSA as a standard.

Fluorescence measurements

All fluorescence measurements were made with a Multi Frequency Cross-Correlation Phase and Modulation Fluorometer (Model; ISS K2-003). Cuvette temperature was maintained at 37.0 ± 0.1°C in a circulating water bath (pH 7.4). Bandpass slits were 10 nm on excitation and 5 nm on emission. Blanks, prepared under identical conditions without fluorescent probes, served as controls.

Py-3-Py was incorporated by adding aliquots of a stock solution of 5×10^{-5} M in absolute ethanol to the SPMV, such that the final probe concentration was less than 5×10^{-7} M [16,21]. Mixtures were initially vigorously vortexed for 10 s at room temperature and then incubated at 4°C for 18 h with gentle stirring [16,21].

After probe incorporation the membrane suspension was placed in cuvettes, and control fluorescence was determined. Concentrated solutions of dibucaine·HCl were prepared in 10 mM Tris-HCl (pH 7.4) and added to the labeled membrane suspension (or untreated SPMV suspension) to give the desired concentration of dibucaine·HCl (in this case, for 30 min incubation).

Excitation wavelengths were 286 nm for tryptophan and 330 nm for Py-3-Py. Emission wavelengths were 335 nm for tryptophan, 379 nm for Py-3-Py monomer and 480 nm for Py-3-Py excimer. For Py-3-Py excimer emission, a GG-455 cut-off filter was used. The excimer to monomer fluorescence intensity ratio, I'/I, was calculated from the 480 nm to 379 nm signal ratio.

Effect of dibucaine·HCl on the structure of the individual monolayers of SPMV: selective quenching of Py-3-Py

The experimental determination of transbilayer asymmetric lateral mobility is based on a method previously established for the SPMV [23-25]. This method is based on the assumption that the system is composed of fluorescing compartments that are differentially accessed by TNBS. The excimer to monomer fluorescence intensity ratios, I'/I, of Py-3-Py in bulk (inner plus outer), and in the inner and outer monolayers were calculated by the following equations:

$$(I'/I)_t = I'_t / I_t \quad \text{Eq. 1}$$

$$(I'/I)_i = I'_i / I_i \quad \text{Eq. 2}$$

$$(I'/I)_o = (I'_t - I'_i) / (I_t - I_i) \quad \text{Eq. 3}$$

where $(I'/I)_t$, $(I'/I)_i$ and $(I'/I)_o$ are the excimer to monomer fluorescence intensity ratios of Py-3-Py (I'/I) in bulk, and in the inner and outer monolayers, respectively. The values of I'_t (excimer fluorescence intensity for inner plus outer monolayers) and I'_i (for the inner monolayer) were determined for Py-3-Py from SPMV incubated with buffer A and buffer A plus TNBS at 4°C (pH 8.5) (non-penetrating conditions), respectively.

Determination of annular lipid fluidity in SPMV

The experimental determination of the annular lipid fluidity

in SPMV is based on a method previously established for the synaptic plasma membrane [26,27] and SPMV [23-25]. Incorporated Py-3-Py in the SPMV was excited by radiationless energy transfer (RET) from tryptophan (excitation at 286 nm) and the excimer to monomer fluorescence intensity ratio (*I'/I*) of Py-3-Py was calculated from the ratio 480 nm to 379 nm signal. Taking into account that the Förster radius (the RET-limiting distance) for the tryptophan-Py-3-Py donor-acceptor pair is 3 nm [28], only Py-3-Py located in annular lipids (close to proteins) was excited, and the fluidity of annular lipids was considered proportional to *I'/I* [16,20,21,23-27].

Determination of protein clustering in the SPMV lipid bilayer

The experimental determination of the protein clustering in the SPMV lipid bilayer is based on a method previously established for the synaptic plasma membrane [26,27] and SPMV [23-25]. The fluorescence intensity of endogenous tryptophan in SPMV was determined. Following this measurement, the Py-3-Py probe was incorporated at 10^{-6} M (1 µl of 10^{-3} M in ethanol), and after 10 min, tryptophan emission fluorescence intensity was measured again. The efficiency of RET from tryptophan to Py-3-Py was calculated as:

$$RET = (I_d - I_{da}) / I_d \quad \text{Eq. 4}$$

where I_d and I_{da} represent the fluorescence intensity of donor (in this case, endogenous tryptophan) in the absence and presence of acceptor (in this case, Py-3-Py), respectively. The wavelengths of excitation and emission of tryptophan were 286 and 335 nm, respectively.

RESULTS

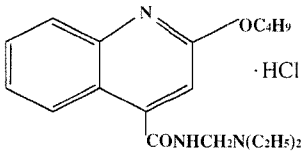
The purity of SPMV

We assessed the purity of SPMV by enzymatic and morphological criteria. The specific activities of Na^+,K^+-ATPase, acetylcholinesterase and 5'-nucleotidase were enriched about 4-, 2.5- and 3-fold, respectively, in the plasma membrane fraction with respect to crude homogenates. The transmission electron microscopic examination of the SPMV indicated very high purity. The vesicles, which were separated according to size, were homogeneous in size and there was no sign of other intracellular organelles or leakage.

Effects of dibucaine·HCl on the rate and range of lateral mobility in bulk bilayer SPMV

The *I'/I* value in intact SPMV (dibucaine·HCl-untreated) was 0.412 ± 0.007 (at 37°C, pH 7.4). Incubation with dibucaine·HCl increased the range and rate of lateral mobility of bulk (inner + outer monolayer) SPMV at concentrations as low as 0.1 mM (n = 5, $P < 0.05$), as demonstrated in Fig. 2.

The *I'/I* value of Py-3-Py in bulk SPMV incubated with 2 mM dibucaine·HCl was 0.467 ± 0.009** (n = 5, $P < 0.01$), and the change in *I'/I* value before and after adding 2 mM dibucaine·HCl was 0.055. The *I'/I* values of Py-3-Py in the bilayer were 0.412 ± 0.007 (n = 5) and 0.356 ± 0.006 (n = 5) at 37 and 25°C (pH 7.4), respectively. Hence the effect of 2 mM dibucaine·HCl was equivalent to that produced by a temperature increase of approximate 11.8°C.



Dibucaine·HCl

Figure 1. Chemical structure of dibucaine·HCl

Effects of dibucaine·HCl on the range and rate of transbilayer asymmetric lateral mobility of SPMV monolayers

The effect of increasing concentrations of dibucaine·HCl on the *I'/I* values in the individual SPMV monolayers is shown in Fig. 2. Dibucaine·HCl increased the rate and range of lateral mobility of the inner monolayer to a significant extent (0.406 ± 0.009, $P < 0.05$, n = 5) at 0.1 mM dibucaine·HCl (Fig. 2). Dibucaine·HCl caused a greater increase in the fluidity of the inner monolayer (Fig. 2, filled triangles) than of the outer monolayer (Fig. 2, filled circles). Since the changes in *I'/I* values derived primarily from the effect on the inner monolayer, we studied the selective effects of dibucaine·HCl on the rate and range of lateral mobility of the probe. To the best of our knowledge, the results presented here are the first to demonstrate that the Sheetz-Singer hypothesis [29] is valid in neuronal membranes.

Effects of dibucaine·HCl on annular lipid fluidity in the SPMV lipid bilayer

I'/I values showed that the annular lipid fluidity in SPMV (intact membrane) was 0.245 ± 0.006 (37°C, pH 7.4), and that this increased in response to concentrations of 0.1 mM dibucaine·HCl and above (Fig. 3). The *I'/I* value of Py-3-Py in the SPMV for annular lipid fluidity by 2.0 mM dibucaine · HCl was 0.305 ± 0.018 ($P < 0.01$, n = 5). The variation in the *I'/I* value of Py-3-Py in the SPMV before and after adding 2.0 mM dibucaine·HCl was 0.060. The *I'/I* values of Py-3-Py in the bilayer are 0.245 ± 0.006 (n = 5) and 0.199 ± 0.002 (n = 5) at 37 and 25°C, respectively. Hence the effect of 2.0 mM dibucaine · HCl was equivalent to that produced by a temperature increase of approximate 15.6°C. The important finding is that those increasing effects were far greater in annular lipid fluidity than on the lateral mobility.

Effects of dibucaine·HCl on protein distribution in SPMV

We evaluated protein distribution by RET from tryptophan to Py-3-Py. The RET value of untreated SPMV was 0.295 ± 0.003 (37°C, pH 7.4) and it was lowered by concentrations of

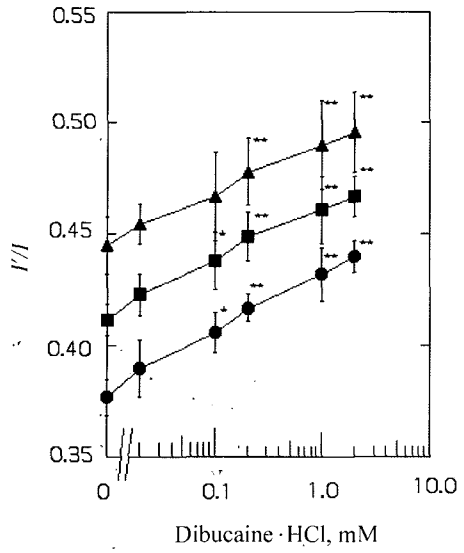


Figure 2. The effect of dibucaine·HCl on excimer to monomer fluorescence intensity ratio (*I'/I*) of Py-3-Py in SPMV. The excitation wavelength of Py-3-Py was 330 nm and the *I'/I* values were calculated from the 480 nm to 379 nm signal ratio. SPMV was treated ± 4 mM TNBS, pH 8.5, at 4°C for 80 min. Py-3-Py was incorporated into SPMV and fluorescence measurements were performed at 37°C (pH 7.4). Untreated (inner and outer monolayers, ■); TNBS treated (inner monolayer, ●); calculated for outer monolayer (▲) by eq. 3 as described in Materials and Methods. Each point represents the mean ± SEM of 5 determinations. An asterisk and double asterisks signify $P < 0.05$ and $P < 0.01$, respectively, compared to control by Student's *t*-test.

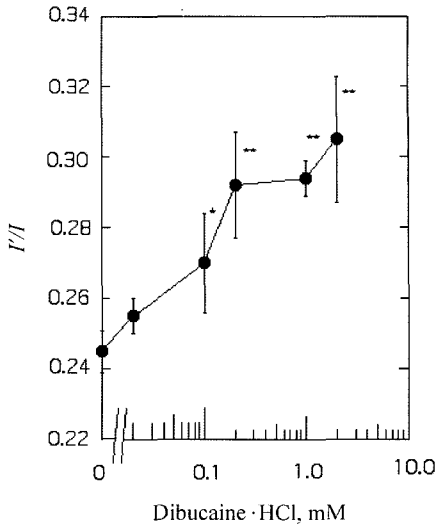


Figure 3. The effect of dibucaine·HCl on annular lipid fluidity in SPMV. Py-3-Py was excited through RET from tryptophan (excitation wave-length, 286 nm) and the excimer to monomer fluorescence intensity ratio (*I'/I*) was calculated from the 480 nm to 379 nm signal ratio. Fluorescence measurements were performed at 37°C (pH 7.4). Each point represents the mean ± SEM of 5 determinations. An asterisk and double asterisks signify $P < 0.05$ and $P < 0.01$, respectively, compared to control by Student's *t*-test.

dibucaine·HCl of 0.1 mM or more (Fig. 4). Protein clustering is probably caused by interaction between phospholipids, especially annular lipids, whose movement is increased by dibucaine·HCl and proteins around them.

DISCUSSION

We used Py-3-Py, a pyrene derivative that has been used to quantify lateral mobility within native and model membranes [16,20,21,30,31], to determine the rate and range of lateral mobility in the SPMV. With this probe one monitors emission of both the monomer (*I*) and the excimer (*I'*) components in such a way that a ratio can be derived and used as a measure of lateral mobility [16,20,21,30,31]. As the probe mobility increases emission from the excimer predominates, since formation of the intramolecular excimer is dependent upon lateral movement of its two components. Therefore, an increase in the *I'/I* ratio indicates increased lateral mobility of the probe within the membranes. The excimer fluorescence technique using Py-3-Py has the advantage over its counterpart based on intermolecular excimerization that very low probe concentrations can be used ($<10^{-7}$ M) and perturbation of the SPMV by the probe molecule is minimized.

The covalently linked trinitrophenyl group has a broad absorbance range with a maximum near 420 nm. This peak has a large overlap with the fluorescence emission of Py-3-Py. This overlap is responsible in part for the high transfer (quenching) efficiency of the probe. Approximately half of the Py-3-Py fluorescence was quenched in the trinitrophenylated SPMV. When TNBS labeling was conducted under penetrating conditions (37°C), nearly 95% of the fluorescence of the Py-3-Py was quenched. Values of the excimer to monomer fluorescence intensity ratio (*I'/I*) of Py-3-Py in intact SPMV (both monolayers) and in TNBS-treated SPMV (inner monolayer) are listed in Table 1. The *I'/I* of Py-3-Py in the outer monolayer was 0.033, greater than that calculated for the inner monolayer. This is in agreement with the results of previous studies [20,32-37] but inconsistent with two studies

Table 1. Structural parameters of intact synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex

Membrane	*I'/I* [a)]	Annular lipid fluidity	Protein clustering
Inner + Outer	0.412 ± 0.007	0.245 ± 0.006	0.295 ± 0.003
Inner	0.377 ± 0.008	-	-
Outer	0.445 ± 0.013	-	-

[a)]Synaptosomes were treated ± 4 mM 2,4,6-trinitrobenzenesulfonic acid (TNBS), pH 8.5, at 4°C for 80 min (*I'/I*), and the plasma membrane vesicles were isolated. 1,3-di(1-pyrenyl)propane was incorporated, and fluorescence measurements were performed at 37°C (pH 7.4). Values from TNBS-treated membranes represent the inner monolayer; values for the outer monolayer were calculated as described in Materials and Methods. Values are represented as the mean ± SEM of five determinations.

[38,39]. Plasma membranes consist of two monolayers that are asymmetric in lipid distribution, electrical charge, fluidity, protein distribution and function, and do not appear to be coupled [40]. It had been widely known that different lipids could affect the physical properties of the membrane. Membrane cholesterol is one of the major lipids of plasma membranes and is asymmetrically distributed in the outer and inner monolayers of membranes [32,37,41,42]. Interest in rigidifying effect on membrane above the phase transition temperature of the membrane lipid [40]. In erythrocytes, differences in fluidity between the two monolayes have not been consistently observed. Some studies have reported that the outer monolayer was less fluid [38,39], whereas other studies have found that the outer monolayer was more fluid compared with the inner monolayer [33,34]. The finding that the SPMV inner monolayer was less fluid than the outer monolayer was consistent with data showing that the SPM inner monolayer contains approximately 7-times as much cholesterol compared with the outer monolayer [37]. Thus, a possible explanation for the asymmetric lateral mobility between outer and inner monolayers of SPMV in this study is that the amount of cholesterol may differ in the outer and inner monolayers.

Although many researchers have reported that the inner and outer monolayers of native and model membranes differ in fluidity, all previous studies of asymmetric bilayer fluidity have examined the rotational range but not on the rate and range of lateral mobility. In this study, using the selective quenching of Py-3-Py fluorescence by trinitrophenyl groups, we examined transbilayer asymmetric lateral mobility.

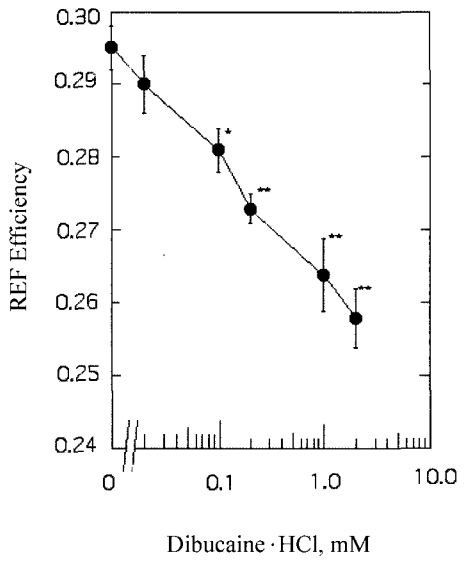


Figure 4. The effect of dibucaine·HCl on protein clustering in SPMV. Efficiency of RET from tryptophan to Py-3-Py was taken as a measure of protein clustering and calculated by eq. 4. Fluorescence measurements were performed at 37°C (pH 7.4). Each point represents the mean ± SEM of 5 determinations. An asterisk and double asterisks signify $P < 0.05$ and $P < 0.01$, respectively, compared to control by Student's *t*-test.

The TNBS labeling reaction must be carefully monitored in order to ensure that the reagent does not penetrate into the synaptosomes and label both sides of the plasma membrane. For this purpose, three control procedures are routinely used. First, as an "internal control", mitochondria and microsomes are isolated from the synaptosomes from which the trinitrophenylated plasma membranes are isolated. If any significant degree of penetration of TNBS into the synaptosome occurs, these intracellular organelles also become trinitrophenylated. Only 1.8 ± 0.2% and 2.1 ± 0.4% of microsomal and mitochondrial phosphatidylethanolamine were trinitrophenylated by our treatment. In contrast, when the TNBS treatment is performed under penetrating conditions (37°C), 60-80% of the phosphatidylethanolamine in microsomes or mitochondria is trinitrophenylated [15]. Second, approximately half of the Py-3-Py fluorescence was quenched in the trinitrophenylated SPMV. Third, the trinitrophenylation of the synaptosomes may alter membrane enzyme activities. Unlike the results obtained under penetrating conditions (37°C), the activity of neither Na^+,K^+-ATPase nor 5'-nucleotidase was significantly altered by the TNBS reaction under non-penetrating conditions [15].

It is important to note that the term "membrane fluidity" is often misused. It arose from a combination of spectroscopic studies, the realization that membranes can be regarded as two-dimensional fluids, and the desire to obtain a simple single physical parameter that would describe their properties. The difficulty with the membrane fluidity concept is that any physical parameter chosen will be a function of the spectroscopic method employed, specifically its particular time window, and the properties of the probe (shape, charge, location etc) [43]. The membrane fluidity concept also depends on the assumption that the hydrophobic region of cell membranes is structurally and dynamically homogeneous, an assumption that is now under serious challenge. Thus while it may be true to say that the bulk or average spectroscopic properties of cell membranes may not be useful in building a hypothesis for the pharmacological action(s) of drug(s), local properties pertaining to domains or the immediate environment of a membrane protein may be very relevant.

As already pointed out, membrane bilayer mobility is one of the important factors controlling membrane microviscosity or fluidity. Membrane bilayer mobility includes lateral mobility, rotational mobility and flip-flop and it is well known that the most important of these is lateral mobility. We are pleased to have been able to develop and describe, for the first time, a fluorescence quenching technique that can measure membrane transbilayer lateral mobility. We therefore believe that this study will make a contribution to the study of drug-membrane interactions.

The clear mechanism of action of the drug on the increasing effect of annular lipid fluidity in the SPMV is unknown. However, the mechanism through which dibucaine·HCl increases

the annular lipid fluidity in the SPMV lipid bilayer can be assumed as follows.

Annular lipids are known to surround proteins with or without being physically associated with them. Dibucaine·HCl may alter the stereo structure or dynamics of these proteins by combining with the lipids, especially with the annular lipids, increasing their mobility and indirectly affecting the dynamic behavior of the proteins. Because biological membranes are of highly complex composition, it has not been feasible to monitor changes in the local lipid environment and at the same time to determine their effect on membrane protein function. Nevertheless is likely that the observed effects are not only due to the influence of dibucaine·HCl on lipids, but are magnified by the interactions between lipids and proteins.

This is the first demonstration that dibucaine·HCl has a differential effect on the transbilayer lateral mobility of the inner and outer monolayers of neuronal membranes. It seems that neuronal membranes, specifically inner monolayer, are much more sensitive to the fluidizing effects of dibucaine·HCl, and this finding can be extended to the transbilayer asymmetric fluidity of neuronal membranes.

From the results of our study, it is without a doubt that dibucaine·HCl increased lateral mobility of the neuronal membrane lipid bilayer. Dibucaine·HCl which increase lateral mobility of the neuronal membrane lipid bilayer mostly increased the mobility of inner monolayer. These effects are not solely due to the influence of dibucaine·HCl on lipids, but they are magnified by the interaction between lipids and proteins. This conclusion can be drawn because we confirmed that the magnitude of increasing effect on annular lipid fluidity in SPMV lipid bilayer induced by dibucaine·HCl was significantly far greater than the magnitude of increasing effect of the drug on the lateral mobility of bulk SPMV lipid bilayer.

Opinions have been divided 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. 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 the local anesthetics 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 local anesthetics, in addition to its direct interaction with sodium channels, concurrently interact with membrane lipids, affecting fluidity of the neuronal membrane.

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