

# Transbilayer Effects of n-Alkanols on the Fluidity of Model Membranes of Total Lipids Extracted from Synaptosomal Plasma Membrane Vesicles<sup>1</sup>

Il Yun<sup>2</sup> and Jung-Sook Kang<sup>3</sup>

Departments of <sup>2</sup>Dental Pharmacology and Biophysics and <sup>3</sup>Oral Biochemistry and Molecular Biology, College of Dentistry, Pusan National University, Pusan 602-739, Korea

## ABSTRACT

Selective quenching of 1,6-diphenyl-1,3,5-hexatriene (DPH) by trinitrophenyl groups was utilized to examine the transbilayer fluidity asymmetry of model membranes of total lipids (SPMVTL) extracted from synaptosomal plasma membrane vesicles (SPMV). The polarization (P), anisotropy (r), limiting anisotropy ( $r_{\infty}$ ), and order parameter (S) of DPH in the inner monolayer were 0.031, 0.025, 0.033, and 0.070, respectively, greater than calculated for the outer monolayer of SPMVTL. Selective quenching of DPH by trinitrophenyl groups was also utilized to examine the effects of n-alkanols on the individual monolayer structure of SPMVTL. n-Alkanols fluidized the hydrocarbon region of bulk SPMVTL, and the potencies of n-alkanols up to 1-nonanol increased with carbon chain length. It appears that the potencies in bilayer fluidization increase by 1 order of magnitude as the carbon chain length increases by two carbon atoms. The cut-off phenomenon was reached at 1-decanol, where further increase in hydrocarbon length resulted in a decrease in pharmacological activity. The n-alkanols had greater fluidizing effects on the outer monolayer as compared to the inner monolayer of SPMVTL, even though these selective effects tended to become weaker as carbon chain length increased. Thus, it has been proven that n-alkanols exhibit selective rather than nonselective fluidizing effects within transbilayer domains of SPMVTL.

**Key Words:** n-Alkanols, Transbilayer fluidity asymmetry, Total lipid model membranes, Fluorescent probe technique

## INTRODUCTION

Over the past decade, it has been well established that the inner and outer monolayers of the eukaryotic cell plasma membrane have different lipid composition as well as protein compo-

sition (Bick *et al.*, 1987). This compositional transbilayer asymmetry is expected to confer asymmetry of structure between the monolayers, provided that they are not coupled. Indeed, no, or only a weak, coupling of lipid motion across the bilayer has been experimentally verified (Hunt and Tipping, 1978; Flamm and Schachter, 1982; Sillerud and Barnett, 1982). The only reported exception is sphingomyelin containing long-chain fatty acids (n-tetracosanoic acid), which illustrated coupling between pure sphingomyelin monolayers (Schmidt *et al.*, 1978).

Not only is asymmetry present with respect to

<sup>1</sup>This research was supported in part by research grant 88-1013-01 from the Korea Science and Engineering Foundation.

<sup>2</sup>To whom requests for reprints should be addressed.

fluidity and lipid distribution, but certain drugs that differ in their charge properties have been found to differentially affect one monolayer or the other (Schroeder, 1988). This selective effect was dependent on the charge properties of the membrane lipids. Cationic drugs had a greater effect on the negatively charged inner monolayer, whereas anionic drugs acted on the outer monolayer. *n*-Alkanols are neutral compounds that would not be attracted to one specific monolayer on the basis of charge. However, the more fluid regions in the membrane core are more easily perturbed by ethanol than are the stiffer surface regions (Chin and Goldstein, 1981). *n*-Alkanols should have an asymmetric effect if one monolayer differed in fluidity as compared to the other monolayer.

In the present study, selective quenching of 1,6-diphenyl-1,3,5-hexatriene (DPH) by trinitrophenyl groups was utilized to examine the transbilayer fluidity asymmetry of the model membranes of total lipids (SPMVTL) extracted from synaptosomal plasma membrane vesicles (SPMV) and to examine the effects of *n*-alkanols on the individual monolayer structure of SPMVTL.

## MATERIALS AND METHODS

### Chemicals

The fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) was obtained from Molecular Probes (Junction City, OR, USA). *n*-Alkanols (methanol, ethanol, 1-propanol, 1-butanol, 1-propanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, and 1-decanol) were purchased from Fluka (Buchs, Switzerland). *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), Ficoll (70,000 M.W.), Sepharose, 2,4,6-trinitrobenzenesulfonic acid (TNBS), and bovine serum albumin (BSA) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of the highest quality available, and water was deionized.

### Membrane preparations

The SPMV were isolated from bovine cerebral cortex and characterized by the formerly reported method in our laboratory (Yun and Kang, 1990;

Yun *et al.*, 1990a). Lipids were extracted from the SPMV as described previously (Yun and Kang, 1990). The cholesterol to phospholipid molar ratio was  $0.60 \pm 0.01$ . Large unilamellar liposomes were prepared by the method described earlier (Yun and Kang, 1992a,b).

### TNBS labelling reactions

TNBS labelling reactions were performed by the method of Yun and Kang (1992a,b). The SPMVTL were gently resuspended in 0.5 mM TNBS plus buffer A or buffer A alone. Buffer A was composed of 30 mM NaCl, 120 mM NaHCO<sub>3</sub>, 11 mM glucose, and 2% BSA. The pH of reagent was adjusted to 8.5 with NaOH. CO<sub>2</sub> was bubbled through the solution, and the treatment was carried out at 4°C for 20 min. The TNBS labelling reaction was terminated by addition of 2% BSA in PBS (pH 7.4), at 4°C.

### Fluorescence measurements

The fluorescent probe DPH was dissolved in tetrahydrofuran and a volume of 0.5  $\mu$ l of tetrahydrofuran per ml of PBS was added directly to the membrane suspension at a concentration of 1  $\mu$ g/70  $\mu$ g of phospholipids described previously (Yun *et al.*, 1990b). After incorporation of the probe, the membrane suspension was placed in cuvettes. Control levels of fluorescence were then determined, an aliquot of *n*-alkanols was added directly to the cuvette, and fluorescence was again determined. The excitation wavelength for DPH was 362 nm, and fluorescence emission was read at 424 nm. All fluorescence measurements were obtained with a SPF-500C spectrofluorometer (SLM Aminco Instruments, Inc., Urbana, IL, USA) and performed at 37°C. Before the fluorescence spectra were obtained, all samples were bubbled by dry nitrogen through the solution for at least 30 min in order to eliminate oxygen. Blanks, prepared under identical conditions without DPH, served as controls for the fluorometric measurements.

The intensity of the components of the fluorescence that were parallel (*I*<sub>||</sub>) and perpendicular (*I*<sub>⊥</sub>) to the direction of the vertically polarized excitation light was determined by measuring the emitted light through polarizers oriented vertical-

ly and horizontally. The 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 is polarized in the horizontal direction. The polarization was expressed as the anisotropy [ $r = 2P / (3 - P)$ ], limiting anisotropy ( $r_{\infty}$ ), and order parameter (S). The limiting anisotropy of DPH was determined directly from the anisotropy value using the following relationship (van Blitterswijk *et al.*, 1981);

$$r_{\infty} = (4/3)r - 0.10 \quad 0.13 < r < 0.28$$

The limiting anisotropy reflects restriction to probe motion and can be converted to an order parameter,  $S = (r_{\infty}/r_0)^{1/2}$  (Kawato *et al.*, 1978) where  $r_0$ , the anisotropy in the absence of motion, is equal to 0.362 for DPH (Lakowicz *et al.*, 1979).

#### Determination of individual monolayer structure in SPMVPL: Selective quenching of DPH

The experimental determination of individual monolayer structure in SPMVTL was carried out by the method described previously (Yun and Kang, 1992a,b). The method does not simply provide a theoretically calculated or average value but is based on the assumption that the system is composed of fluorescing compartments of different accessibility to TNBS. If the fluorescence intensity, F and anisotropy, r are measured simultaneously, then

$$r = \sum F_j r_j$$

where  $F_j$  is the fraction of fluorescence intensity in compartment j. For a binary system composed of the outer and inner monolayers of the SPMVTL, this leads to

$$r = \frac{F}{F} r_i + \frac{F - F_i}{F} r_0$$

where F and  $F_i$  are fluorescence of DPH obtained for SPMVTL incubated with buffer A and buffer A plus TNBS at 4°C (nonpenetrating conditions), respectively. The value of the fluorophore concentration independent parameter anisotropies, r (anisotropy for both monolayers) and  $r_i$  (inner

monolayer anisotropy), were determined for DPH in SPMVTL incubated with buffer A and buffer A plus TNBS at 4°C (nonpenetrating conditions), respectively. The equation was then solved for  $r_0$  (outer monolayer anisotropy). Similar calculations were performed by simultaneous measurement of fluorescence intensity and either limiting anisotropy or order parameter.

## RESULTS

### Fluidity asymmetry in SPMVTL: Quenching of DPH fluorescence by trinitrophenyl groups

A wide variety of techniques in many laboratories have provided results consistent with the interpretation that the motional properties (structure) of lipids in the outer and inner monolayers of biological membranes differ (Chabanel *et al.*, 1985; Seigneuret *et al.*, 1984; Kinoshita *et al.*, 1981). Hence, in the present study, an impermeable reagent, TNBS, covalently linked to outer monolayer amino groups, was used to quench the fluorescence of DPH, a probe which distributes in both monolayers. Approximately half of the DPH fluorescence is quenched in the trinitrophenylated SPMVTL (Table 1). If the TNBS labelling was conducted under penetrating conditions (37°C), nearly 100% of the fluorescence of DPH was quenched. The values of fluorescence parameters of DPH in intact SPMVTL (both monolayers) as compared to those for TNBS-treated SPMVTL (inner monolayer) are listed in Table 2. The polarization (P), anisotropy (r), limiting anisotropy ( $r_{\infty}$ ), and order parameter (S) of DPH in the inner monolayer were 0.031, 0.025, 0.033, and 0.070, respectively, greater than calculated for the outer monolayer of SPMVTL.

### Effects of n-alkanols on transbilayer fluidity of SPMVTL

The bulk lipid fluidity change will represent an average of the affected and unaffected portions of the membrane and may underestimate the effect on specific domains. Very little attention has been given to the selective effects of n-alkanols on transbilayer membrane domains. Selective quenching of DPH by trinitrophenyl groups was also

**Table 1.** Effects of n-alkanols on transbilayer distribution of 1,6-diphenyl-1,3,5-hexatriene in the model membranes of total lipids extracted from synaptosomal plasma membrane vesicles

n-Alkanol	Concentration, mM	% Quenching of 1,6-diphenyl-1,3,5-hexatriene
None		52.5 ± 1.5
Methanol	100	53.0 ± 1.6
Methanol	2500	54.8 ± 1.6
Ethanol	25	53.4 ± 1.0
Ethanol	800	54.9 ± 2.3
1-Propanol	10	53.5 ± 1.6
1-Propanol	250	54.9 ± 1.6
1-Butanol	2.5	52.8 ± 1.1
1-Butanol	80	55.1 ± 2.1
1-Pentanol	1	53.8 ± 1.5
1-Pentanol	25	55.6 ± 1.9
1-Hexanol	0.25	54.6 ± 1.0
1-Hexanol	8	58.6 ± 2.4
1-Heptanol	0.1	53.8 ± 1.5
1-Heptanol	2.5	57.8 ± 2.3
1-Octanol	0.025	53.8 ± 1.1
1-Octanol	0.8	57.6 ± 1.4
1-Nonanol	0.01	52.7 ± 1.3
1-Nonanol	0.25	56.7 ± 2.8
1-Decanol	0.25	52.4 ± 1.4
1-Decanol	8	56.8 ± 2.5

Total lipid model membranes were treated ± 0.5 mM trinitrobenzenesulfonic acid, pH 8.5, at 4°C for 20 min. 1,6-Diphenyl-1,3,5-hexatriene was incorporated, and the fluorescence was determined in the absence and presence of n-alkanols at the concentrations given at 37°C. Values represent the mean ± SEM of 4 determinations.

utilized to examine the specific effects of n-alkanols on the fluidity of transbilayer domains of SPMVTL. In order to determine the effects of n-alkanols on individual monolayer structure, it is first necessary to demonstrate that these drugs do not interact directly with DPH and thereby quench its fluorescence. As shown in Table 1, significant changes in DPH fluorescence intensity distribution between monolayers in the presence of n-alkanols were not detected over the entire concentration range used for n-alkanols. Hence, the possibility of direct quenching of DPH fluo-

rescence by n-alkanols is ruled out.

The effects of increasing concentrations of n-alkanols on the anisotropy of DPH in SPMVTL are shown in Fig. 1. All n-alkanols fluidized the bulk lipid (Fig. 1, closed squares), and the potencies of n-alkanols up to 1-nonanol increased with the carbon chain length. It appears that the potencies in bilayer fluidization increase by 1 order of magnitude as the carbon chain increases by two carbon atoms. And the cut-off phenomenon was reached at 1-decanol, where further increases in hydrocarbon length resulted in a decrease in pharmacological activity. The n-alkanols preferentially decreased the anisotropy of DPH in the outer monolayer (Fig. 1, closed circles), and there was little effect on the inner monolayer (Fig. 1, closed triangles). This indicates that n-alkanols had greater fluidizing effects on the outer monolayer as compared to the inner monolayer, even though these selective effects tended to become weaker as the carbon chain length increased. Thus, it has been proven that n-alkanols exhibit selective rather than nonselective fluidizing effects within transbilayer domains of the SPMVTL.

In biological membranes, the anisotropy ( $r$ ) reflects mainly the range of motion rather than the rate and membrane perturbants, such as cholesterol and proteins, primarily alter the range of motion rather than rate (Kinosita *et al.*, 1981). Hence, the alterations in the anisotropy ( $r$ ) are likely to be due to changes in range of motion of the probe. Accordingly, we studied the effects of n-alkanols on the range component of DPH motion in more detail. The range of motion is generally expressed as either the limiting anisotropy ( $r_{\infty}$ ) or the order parameter ( $S$ ). The selective effects of n-alkanols on the limiting anisotropy and order parameter of DPH are shown in Table 3. The result corroborates the above point.

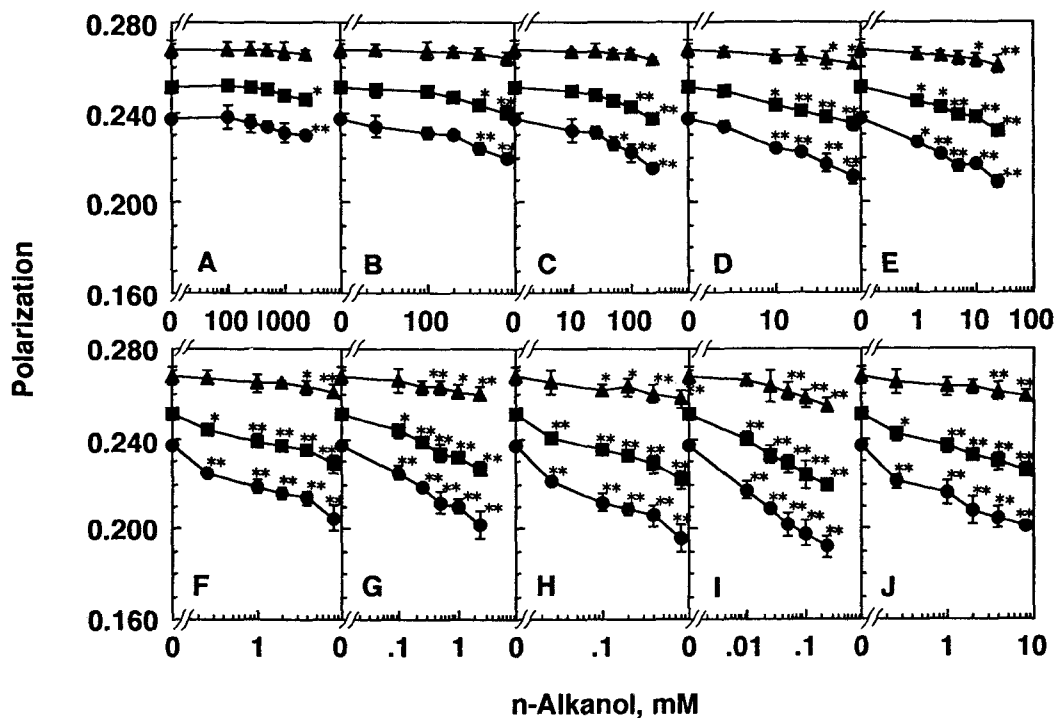
## DISCUSSION

Our data explicitly show the transbilayer fluidity asymmetry of SPMVTL (Table 2). The outer monolayer was significantly more fluid than the inner monolayer. An explanation for this fluidity gradient may result from asymmetric distribution

**Table 2.** Asymmetry of 1,6-diphenyl-1,3,5-hexatriene motion in the model membranes of total lipids extracted from synaptosomal plasma membrane vesicles

Membrane	Polarization (P)	Anisotropy (r)	Limiting Anisotropy ( $r_{\infty}$ )	Order Parameter (S)
inner+outer	$0.251 \pm 0.002$	$0.183 \pm 0.002$	$0.144 \pm 0.003$	$0.630 \pm 0.006$
inner	$0.268 \pm 0.004$	$0.196 \pm 0.003$	$0.161 \pm 0.004$	$0.667 \pm 0.008$
outer	$0.237 \pm 0.002^{**}$	$0.171 \pm 0.002^{**}$	$0.128 \pm 0.002^{**}$	$0.597 \pm 0.005^{**}$

Total lipid model membranes were treated  $\pm 0.5$  mM trinitrobenzenesulfonic acid, pH 8.5, at 4°C for 20 min. 1,6-Diphenyl-1,3,5-hexatriene was incorporated, and fluorescence measurements were performed at 37°C. Values from untreated membranes represent inner + outer monolayer; Values from 2,4,6-trinitrobenzenesulfonic acid (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  $\pm$  SEM of 4 determinations. Double asterisk signifies  $P < 0.01$  according to Student's t-test.



**Fig. 1.** n-Alkanols alter the anisotropy ( $r$ ) of 1,6-diphenyl-1,3,5-hexatriene (DPH) in outer monolayer of the model membranes of total lipids extracted from synaptosomal plasma membrane vesicles. (A) Methanol; (B) ethanol; (C) 1-propanol; (D) 1-butanol; (E) 1-pentanol; (F) 1-hexanol; (G) 1-heptanol; (H) 1-octano; (I) 1-nonanol; (J) 1-decanol. Total lipid model membranes were treated  $\pm 0.5$  mM trinitrobenzenesulfonic acid, pH 8.5, at 4°C for 20 min. 1,6-Diphenyl-1,3,5-hexatriene was incorporated, and fluorescence measurements were performed at 37°C. Untreated (inner and outer monolayers,  $\blacksquare$ ); 2,4,6-trinitrobenzenesulfonic acid (TNBS) treated (inner monolayer,  $\blacktriangle$ ); calculated for outer monolayer ( $\bullet$ ). Each point represents the mean  $\pm$  SEM of 4 determinations. An asterisk and double asterisk signify  $P < 0.05$  and  $P < 0.01$ , respectively, compared to control by Student's t-test.

Table 3. Effects of n-alkanols on limiting anisotropy ( $r_{\infty}$ ) and order parameter (S) of 1,6-diphenyl-1,3,5-hexatriene (DPH) in the model membranes of total lipids extracted from synaptosomal plasma membrane vesicles

n-Alkanol (mM)	Limiting Anisotropy ( $r_{\infty}$ )			Order Parameter (S)		
	Inner+Outer	Inner	Outer	Inner+Outer	Inner	Outer
Control	0.144±0.003	0.161±0.004	0.128±0.002	0.630±0.006	0.667±0.008	0.597±0.005
Methanol (2500)	0.138±0.001*	0.159±0.003	0.121±0.001*	0.618±0.003*	0.663±0.006	0.582±0.003*
Ethanol (400)	0.135±0.002*	0.159±0.003	0.115±0.003**	0.611±0.005*	0.663±0.006	0.567±0.007**
Ethanol (800)	0.132±0.003**	0.157±0.003	0.111±0.002**	0.604±0.008**	0.659±0.007	0.558±0.005**
1-Propanol (100)	0.134±0.002*	0.160±0.002	0.112±0.004**	0.609±0.004*	0.664±0.004	0.561±0.009**
1-Propanol (250)	0.128±0.002**	0.156±0.000	0.105±0.003**	0.595±0.006**	0.656±0.001	0.545±0.007**
1-Butanol (10)	0.135±0.002*	0.159±0.003	0.114±0.002**	0.610±0.004*	0.663±0.007	0.564±0.005**
1-Butanol (80)	0.126±0.003**	0.156±0.003*	0.102±0.004**	0.589±0.007**	0.655±0.005*	0.535±0.011**
1-Pentanol (1)	0.136±0.001*	0.159±0.003	0.117±0.002*	0.613±0.003*	0.663±0.006	0.571±0.004*
1-Pentanol (10)	0.129±0.002**	0.156±0.003*	0.107±0.001**	0.597±0.005**	0.657±0.006*	0.549±0.004**
1-Hexanol (0.25)	0.136±0.003*	0.161±0.004	0.115±0.002**	0.612±0.006*	0.666±0.007	0.567±0.005**
1-Hexanol (4)	0.126±0.001**	0.156±0.003**	0.105±0.003**	0.591±0.003**	0.656±0.006**	0.542±0.007**
1-Heptanol (0.1)	0.136±0.003*	0.159±0.005	0.117±0.003**	0.614±0.008*	0.662±0.011	0.571±0.007**
1-Heptanol (0.5)	0.126±0.004**	0.156±0.003*	0.103±0.004**	0.591±0.009**	0.655±0.006**	0.539±0.011**
1-Octanol (0.025)	0.134±0.002**	0.159±0.005	0.112±0.002**	0.607±0.005**	0.662±0.011	0.561±0.003**
1-Octanol (0.1)	0.127±0.003**	0.155±0.003*	0.103±0.004**	0.591±0.006**	0.654±0.006**	0.538±0.009**
1-Nonanol (0.01)	0.132±0.003**	0.160±0.003	0.108±0.004**	0.604±0.007**	0.664±0.006	0.551±0.010**
1-Nonanol (0.05)	0.120±0.004**	0.155±0.004**	0.092±0.006**	0.576±0.011**	0.653±0.009**	0.513±0.014**
1-Decanol (0.25)	0.134±0.003*	0.157±0.006	0.112±0.004**	0.608±0.007*	0.659±0.011	0.560±0.009**
1-Decanol (4)	0.122±0.004**	0.154±0.004**	0.096±0.005**	0.581±0.010**	0.652±0.009**	0.523±0.012**

All conditions were as described in the legend to Table 2. Values are represented as the mean ± SEM of 4 determinations. An asterisk and double asterisk signify  $P < 0.05$  and  $P < 0.01$ , respectively, compared to control by Student's t-test.

of cholesterol and phospholipids. Although the stoichiometry of phospholipid-cholesterol interactions is still controversial, recent studies on either model or native membranes show that cholesterol does not ideally mix with phospholipids. Cholesterol-rich and cholesterol-poor domains were detected in binary mixtures of cholesterol and phospholipid using electron microscopy (Copeland and McConnell, 1980), electron diffraction (Hui and Parsons, 1975), fluorescent cholesterol analogs (Smutzer and Yeagle, 1985), and differential scanning calorimetry (DSC) (Estep *et al.*, 1978). Moreover, DSC experiments on ternary mixtures indicate that cholesterol has varying affinities for phospholipids. van Dijk (1979) reported that phospholipids show a decrease in affinity for cholesterol in the following order: SP ≫ PS, phosphatidylglycerol > PC ≫ PE. The non-uniform distribution of cholesterol may partly ex-

plain that the differences in transbilayer fluidity asymmetry in SPMVTL were larger than those in SPMVPL (Yun and Kang, 1992a).

As shown in Fig. 1 and Table 3, all n-alkanols exerted specific fluidizing effects on the outer monolayer as compared to the inner monolayer of SPMVTL. In view of the point that alcohols have greater effects on fluid membranes as compared to more ordered membranes, our results are in agreement with those of previous studies (Chin and Golstein, 1981; Schroeder *et al.*, 1988; Chabanel *et al.*, 1985). However, it still remains to be established whether changes in fluidity in turn affect the functional activities of the membrane such as transport, signal transduction, drug sensitivity, and tolerance. It has been proposed that there is an optimal fluidity required for membrane function (Sanderman, 1978). This hypothesis was based on the fluidity of the bulk lipid of

the membrane. Perhaps optimal fluidity is required not for the bulk membrane but rather for specific membrane domains in relative to each other. In order for normal cell to function, optimal structural asymmetry of the monolayers in terms of fluidity and lipid distribution has been proposed (Lubin *et al.*, 1981; Schachter *et al.*, 1983; Schroeder, 1984). The physiological consequences of altered asymmetry are only now being reported. A reduction in asymmetry of membranes has been found to be associated with various disease, e. g., sickle cell disease, acanthocytosis (Lubin *et al.*, 1981; Schachter *et al.*, 1983).

However, n-alkanols' effects on membranes whether bulk or domains have been studied under the assumption that the membrane is in a bilayer form (Janoff *et al.*, 1988). In fact, increasing evidence indicates that membrane lipids can adopt a nonbilayer form. In addition, the results of investigations on the effects of higher alkanols and the corresponding alkanes on membrane luciferases indicate that the anesthetic site could be hydrophobic pockets on membrane proteins rather than the lipid part of the membrane (Franks and Lieb, 1987). Furthermore, the hypothesis that ethanol is a nonspecific drug that produces its actions via perturbation of neuronal membrane lipids is now being challenged by recent data showing that ethanol specifically and selectively affects the function of certain membrane-bound proteins (Gonzales and Hoffman, 1991; Sanna *et al.*, 1991). A recent data on the interactions of ethanol and certain receptor- and voltage-gated ion channels concluded that the receptor-gated ( $\gamma$ -aminobutyric acid and N-methyl-D-aspartate) ion channels are more sensitive to acute effects of ethanol than the voltage-gated  $\text{Ca}^{2+}$  channels (Gonzales and Hoffman, 1991).

Opinions have been divided as to whether n-alkanols interfered with membrane protein function by direct action 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 n-alkanols readily diffused. Since biological membranes are of highly complex compositions, it has not been feasible to monitor changes in the local lipid environment and to determine its effect on membrane protein functions at the same time. Still, a large, diverse collection of physiological

agonists produces the alterations in membrane fluidity as well as their specific ligand-receptor interaction (Manevich *et al.*, 1988). So, the function of membrane proteins may be modulated secondarily to changes in membrane fluidity. In addition, it cannot be ruled out that n-alkanols concurrently interact with neuronal membrane proteins and membrane lipids since the receptor-gated ion channels were found to be tightly associated with membrane lipid through covalent or noncovalent bonds. Thus, it may be premature to take sides in the controversy about whether membrane lipids or membrane proteins are the site of general anesthetic action.

In summary, it is strongly postulated that n-alkanols, in addition to their direct interaction with the ion channels, concurrently interact with membrane lipids, fluidize the membrane, and thus induce conformational changes of the ion channels which are known to be tightly associated with membrane lipids.

## REFERENCES

- Bick RJ, Van Winkle WB, Tate CA and Entman ML: *Phospholipid fatty acyl chain asymmetry in the membrane bilayer of isolated skeletal muscle sarcoplasmic reticulum*. *Biochemistry* 26: 4831-4836, 1987
- Chabanel A, Abbott RE, Chien S and Schachter D: *Effects of benzyl alcohol on erythrocyte shape, membrane hemileaflet fluidity, and membrane viscoelasticity*. *Biochim Biophys Acta* 816: 142-152, 1985
- Chin JH and Goldstein DB: *Membrane-disordering action of ethanol. Variation with membrane cholesterol content and depth of the spin label probe*. *Mol Pharmacol* 19: 425-431, 1981
- Copeland BR and McConnell HM: *The rippled structure in bilayer membranes of phosphatidylcholine and binary mixtures of phosphatidylcholine and cholesterol*. *Biochim Biophys Acta* 599: 95-109, 1980
- Estep TN, Mountcastle DB, Biltonen RL and Thompson TE: *Studies on the anomalous behaviours of aqueous dispersions of dipalmitoylphosphatidylcholine-cholesterol mixtures*. *Biochemistry* 17: 1984-1989, 1978
- Flamm M and Schachter D: *Acanthocytosis and cholesterol enrichment decrease lipid fluidity of only the outer human erythrocyte membrane leaflet*. *Nature* 298: 290-292, 1982
- Franks NP and Lieb WR: *What is the molecular nature of general anaesthetic target sites?* *TIPS* 8: 169-174,

1987

- Gonzales RA and Hoffman PL: *Receptor-gated ion channels may be selective CNS targets for ethanol. TIPS 12: 1-3, 1991*
- Hui S-W and Parsons DF: *Direct observations of domains in wet lipid bilayers. Science 190: 383-384, 1975*
- Hunt GR and Tipping LRH: *A <sup>1</sup>H-NMR study of the effects of metal ions, cholesterol and n-alkanols on phase transitions in the inner and outer monolayers of phospholipid vesicular membranes. Biochim Biophys Acta 507: 242-261, 1978*
- Janoff AS, Boni LT and Rauch J: *Phase-defined lipid domains in biological membranes: a perspective. In Advances in Membrane Fluidity Vol. 2. 1st ed. (Aloia RC, Curtain CC and Gordon LM, eds). Alan R. Liss Inc., New York, p 101-109, 1988*
- Kawato S, Kinoshita K and Ikegami A: *Effect of cholesterol on the molecular motion in the hydrocarbon region of lecithin bilayers studied by nanosecond fluorescence techniques. Biochemistry 17: 5026-5031, 1978*
- Kinoshita K, Kataoka R, Kimura Y, Gotoh O and Ikegami A: *Dynamic structure of biological membranes as probed by 1,6-diphenyl-1,3,5-hexatriene: a nanosecond fluorescence depolarization study. Biochemistry 20: 4270-4277, 1981*
- Lakowicz JR, Prendergast FG and Hogen D: *Fluorescence anisotropy measurements under oxygen quenching conditions as a method to quantify the depolarizing rotations of fluorophores. Application to diphenylhexatriene in isotropic solvents and in lipid bilayers. Biochemistry 18: 520-527, 1979*
- Lubin B, Chiu D, Bastacky J, Roelofsen B and Van Deenen LLM: *Abnormalities in membrane phospholipid organization in sickled erythrocytes. Clin Invest 67: 1643-1649, 1981*
- Manevich EM, Kõiv A, Järv J, Molotkovsky JG and Bergelson LD: *Binding of specific ligands to muscarinic receptors alters the fluidity of membrane fragments from rat brain. A fluorescence polarization study with lipid-specific probes. FEBS Letters 236: 43-46, 1988*
- Sanderman HJR: *Regulation of membrane enzymes by lipids. Biochim Biophys Acta 515: 209-237, 1978*
- Sanna E, Concas A, Serra M, Santoro G and Biggio G: *Ex vivo binding of t-[<sup>35</sup>S] butyl bicyclopentylphosphorothionate: a biochemical tool to study the pharmacology of ethanol at the  $\gamma$ -aminobutyric acid-coupled chloride channel. J Pharmacol Exp Ther 256: 922-928, 1991*
- Schachter D, Abbott RE, Cogan U and Flamm M: *Lipid fluidity of the individual hemileaflets of human erythrocyte membranes. Ann NY Acad Sci 414: 19-28, 1983*
- Schmidt CF, Barenholz Y, Huang C and Thompson TE: *Monolayer coupling in sphingomyelin bilayer systems. Nature 271: 775-777, 1978*
- Schroeder F: *Role of membrane lipid asymmetry in aging. Neurobiol Aging 5: 323-333, 1984*
- Schroeder F: *Use of fluorescence spectroscopy in the assessment of biological membrane properties. In Advances in Membrane Fluidity Vol. 2. 1st ed. (Aloia RC, Curtain CC and Gordon LM, eds). Alan R. Liss Inc., New York, p 161-192, 1988*
- Schroeder F, Morrison WJ, Gorka C and Wood WG: *Transbilayer effects of ethanol on fluidity of brain membrane leaflets. Biochim Biophys Acta 946: 85-94, 1988*
- Seigneuret M, Zachowski A, Hermann A and Devaux PF: *Asymmetric lipid fluidity in human erythrocyte membrane: new spin-label evidence. Biochemistry 23: 4271-4275, 1984*
- Sillerud LO and Barnett RE: *Lack of transbilayer coupling in phase transitions of phosphatidylcholine vesicles. Biochemistry 21: 1756-1760, 1982*
- Smutzer G and Yeagle PL: *A fluorescence anisotropy study on the phase behavior of dimyristoylphosphatidylcholine/cholesterol mixtures. Biochim Biophys Acta 814: 274-280, 1985*
- Van Blitterswijk WJ, Van Hoeven RP and van der Meer BW: *Lipid structural order parameters (reciprocal of fluidity) in biomembranes derived from steady-state fluorescence polarization measurements. Biochim Biophys Acta 644: 323-332, 1981*
- Van Dijk PWM: *Negatively charged phospholipids and their position in the cholesterol affinity sequence. Biochim Biophys Acta 555: 89-101, 1979*
- Yun I and Kang JS: *The general lipid composition and aminophospholipid asymmetry of synaptosomal plasma membrane vesicles isolated from bovine cerebral cortex. Mol Cells 1: 15-20, 1990*
- Yun I and Kang JS: *Transbilayer effects of n-alkanols on the fluidity of phospholipid model membranes. Arch Pharm Res 15: 152-161, 1992a*
- Yun I and Kang JS: *Effects of barbiturates on transbilayer fluidity domains of phospholipid model membrane monolayers. Korean J Pharmacol 28: 103-114, 1992b*
- Yun I, Kim YS, Yu SH, Chung IK, Kim IS, Baek SW, Cho GJ, Chung YZ, Kim SH and Kang JS: *Comparison of several procedures for the preparation of synaptosomal plasma membrane vesicles. Arch Pharm Res 13: 325-329, 1990a*
- Yun I, Kim HI, Hwang TH, Kim JR, Kim IS, Chung YZ, Shin YH, Jung HO and Kang JS: *Effects of barbiturates on the fluidity of phosphatidylethanolamine model membranes. Korean J Pharmacol 26: 209-217, 1990b*



=국문초록=

## n-Alkanols가 소의 대뇌피질 Synaptosomal Plasma Membrane Vesicles로부터 추출 제제한 총지질 인공세포막 이중층간에 형성된 비대칭적 유동성에 미치는 비대칭적 영향

부산대학교 치과대학 치과약리학교실 및 구강생화학교실\*

윤 일·강 정 숙\*

한국산 2년생 소의 대뇌피질로부터 synaptosomal plasma membrane vesicles(SPMV)를 분리한 후 이 SPMV로부터 추출한 총지질로서 인공세포막(SPMVTL)을 제제하였다. SPMVTL의 outer monolayer에 trinitrophenyl group을 공유결합시킴으로써 SPMVTL이중층에 분포된 형광 probe 1,6-diphenyl-1,3,5-hexatriene(DPH)중 outer monolayer에 분포된 DPH 형광만을 소광케 하였다. 형광분석기를 통하여 SPMVTL의 inner monolayer에 비하여 outer monolayer의 유동성이 크다는 것을 확인하였다. n-Alkanols는 SPMVTL지질 이중층(inner + outer monolayers)의 회전확산운동을 증가시키되 탄소수 두개가 증가될 때마다 그 효력이 약 10배 가량 증가된다는 것을 알았다. 그러나 1-decanol의 경우에는 1-nonanol에 비하여 그 효력이 낮아지는 소위 cut-off현상이 나타났다. 뿐만 아니라 n-alkanols는 SPMVTL의 inner monolayer에 비하여 outer monolayer의 회전확산운동을 주로 증가시켰다. 하지만 n-alkanols의 탄소수가 증가됨에 따라 outer monolayer에 대한 선택적인 작용이 감소된다는 것도 확인되었다.