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Transbilayer Effects of n-Alkanols on the Fluidity of Model Membranes of Total Lipids Extracted from Synaptosomal Plasma Membrane Vesicles[1]

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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_∞), 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 estabished that the inner and outer monolayers of the eukaryotic cell plasma membarane have different lipid composition as well as protein composition (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

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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 ± 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 $NaHCO_3$, 11 mM glucose, and 2% BSA. The pH of reagent was adjusted to 8.5 with NaOH. CO_2 was bubbled through the solution, and the treatment was carried out at 4℃ for 20 min. The TNBS labelling reaction was terminated by addition of 2% BSA in PBS (pH 7.4), at 4℃.

Fluorescence measurements

The fluorescent probe DPH was dissolved in tetrahydrofuran and a volume of 0.5 μl of tetrahydrofuran per ml of PBS was added directly to the membrane suspension at a concentration of 1 μg/70 μg 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℃. 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 ($I_{\parallel}$) and perpendicular ($I_{\perp}$) 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_∞), 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, $S = (r_\infty/r_o)^{1/2}$ (Kawato *et al.*, 1978) where r_∞, 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

$$r = \Sigma F_j r_j$$

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

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

where F and F_i are fluorescence of DPH obtained for SPMVTL incubated with buffer A and buffer A plus TNBS at 4℃ (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℃ (nonpenetrating conditions), respectively. The equation was then solved for r_o (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; Kinosita *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℃), 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_∞), 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℃ 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℃. 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 fluorescence 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_∞) 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 asymmetic 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_∞)	Order Parameter (S)
inner+outer	0.251 ± 0.002	0.183 ± 0.002	0.144 ± 0.003	0.630 ± 0.006
inner	0.268 ± 0.004	0.196 ± 0.003	0.161 ± 0.004	0.667 ± 0.008
outer	0.237 ± 0.002**	0.171 ± 0.002**	0.128 ± 0.002**	0.597 ± 0.005**

Total lipid model membranes were treated ± 0.5 mM trinitrobenzenesulfonic acid, pH 8.5, at 4℃ for 20 min. 1,6-Diphenyl-1,3,5-hexatriene was incorporated, and fluorescence measurements were performed at 37℃. 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 ± SEM of 4 determinations. Double asterisk signifies $P < 0.01$ according to Student's t-test.

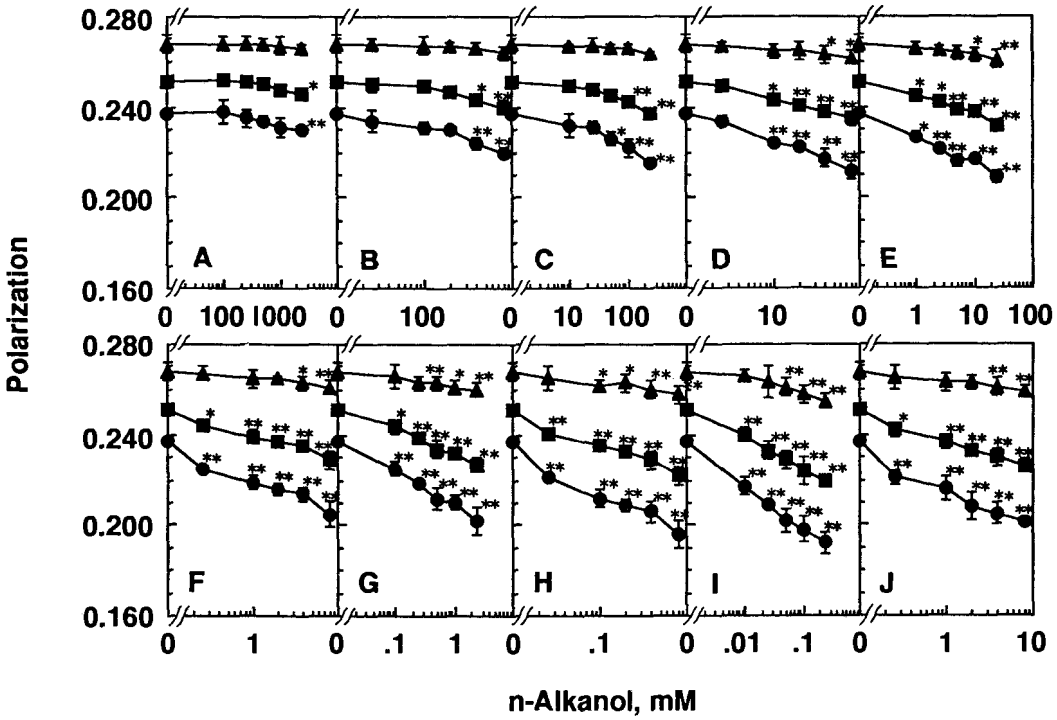

Fig. 1. n-Alkanols alter the anisotrpy (r) of 1,6-diphenyl-1,3,5-hexatriene (DPH) in outer monolayer of the model membranes of total lipids extracted from synaptosomal plasma membrane vesicles. (A) Methanol; (B) ethanol; (C) 1-propanol; (D) 1-butanol; (E) 1-pentanol; (F) 1-hexanol; (G) 1-heptanol; (H) 1-octano; (I) 1-nonanol; (J)1-decanol. Total lipid model membranes were treated ± 0.5 mM trinitrobenzenesulfonic acid, pH 8.5, at 4℃ 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, ■); 2,4,6-trinitrobenzenesulfonic acid (TNBS) treated (inner monolayer, ▲); calculated for outer monolayer (●). Each point represents 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.

Table 3. Effects of n-alkanols on limiting anisotropy (r_∞) 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_∞)			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). Morecover, DSC experiments on ternary mixtures indicate that cholesterol has varying affinities for phospholipids. van Dijck (1979) reported that phospholipids show a decrease in affinity for cholesterol in the following order: SP ≫ PS, phosphatidylglycerol > PC ≫ PE. The nonuniform distribution of cholesterol may partly explain 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 estabilished 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 (γ-aminobutyric acid and N-methyl-D-aspartate) ion channels are more sensitive to acute effects of ethanol than the voltage-gated 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.

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=국문초록=

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

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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 DPH형광만을 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.