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Effects of n-Alkanols on the Lateral Diffusion of Total Phospholipid Fraction Extracted from Brain Membranes

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We investigated the effects of *n*-alkanols on the range and rate of the lateral diffusion of 1,3-di(1-pyrenyl)propane in the model membranes of total phospholipid fraction extracted from synaptosomal plasma membrane vesicles. n-Alkanols increased the range and rate of the lateral diffusion of 1,3-di(1-pyrenyl)propane in the bulk model membrane structures (inner+outer monolayers) and the potencies of *n*-alkanols up to 1-nonanol increased 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 the lateral diffusion. However, significant changes in the I'/I value were not observed by methanol (from 100 to 2500 mM), ethanol (from 25 to 800 mM), and 1-propanol (from 10 to 250 mM) over entire concentration.

Key words: *n*-Alkanols, Fluorescence probe technique, Lateral mobility, Liposomes

INTRODUCTION

Many different physicochemical techniques have been used to provide evidence that anesthetic agents and similar drugs have a biophysical action on cell membranes that can often be described as a disordering or fluidizing effect. It is now generally accepted that two of *n*-alkanols' most significant sites of action are the cell membranes and enzymes found in the brain. More than twenty years ago, it was pointed out that small organic molecules such as *n*-alkanols can readily dissolve in the lipid bilayer of cell membranes. Since then, studies have confirmed that *n*-alkanols reduce the viscosity or increase the fluidity of the membrane of many types of cells and even of artificial systems such as liposomes. Fluidization is thus a general response to *n*-alkanols of virtually all biologic membranes and one that is likely to contribute to the great diversity of the drug's effects. The fluidizing effect of n-alkanols has been related to changes in specific membrane functions, including neurotransmitter receptors for dopamine, norepinephrine, glutamate, and opioids; enzymes such as Na^+, K^+-ATPase, Ca^{2+}-ATPase, 5'-nucleotidase, acetylcholinesterase, and adenylate cyclase; the mitochondrial electron transport chain; and ion channels such as those for Ca^{2+} (Lee and Smith, 1986).

Most of published data about the effects of *n*-alkanols on the biophysical characteristics of native and model membranes have been obtained from the analysis of the influence on the phase transition temperature of model membranes (Jain and Wu, 1977) or on the rotational diffusion of native and model membranes (Harris and Schroeder, 1981; Lyon *et al.*, 1981; Harris and Bruno, 1985; Perlman and Goldstein, 1984; Harris *et al.*, 1984; Yun and Kang, 1992a,b). We have a few experimental data on the effects of *n*-alkanols on the lateral diffusion in native and model membranes. We reported the effects of *n*-alkanols on the lateral diffusion of synaptosomal plasma membrane vesicles (SPMV) and model membranes of total lipid (SPMVTL, cholesterol+phospholipids) fraction extracted from SPMV by fluorescence probe technique (Chung *et al.*, 1993a,b). However, it is reasonable to expect that *n*-alkanols will alter the lateral diffusion of total phospholipid model membranes and that membrane function will be affected. In this paper, we studied the effects of *n*-alkanols on the range and rate of the lateral diffusion of 1,3-di(1-pyrenyl)propane (Py-3-Py) in model membranes of total phospholipid fraction (SPMVPL) extracted from SPMV.

MATERIALS AND METHODS

Materials

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The fluorescent probe, Py-3-Py, was prepared by the previously reported synthesis (Yun *et al.*, 1990a). n-Alkanols were purchased from Fluka (Buchs, Switzerland). N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes), Ficoll (70,000 M.W.), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of the highest quality available, and water was deionized.

Membrane preparation

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.*, 1990 b). Lipids were extracted from the SPMV as described previously (Yun and Kang, 1990). The individual phospholipid classes were separated by thin layer chromatography (Yun and Kang, 1990) and quantitated by measuring the amounts of inorganic phosphate (Bartlet, 1959) after hydrolysis of the phospholipids at 180℃ in 70% $HClO_4$ (Madeira and Antunes-Madeira, 1976). Phospholipids were composed of phosphatidylcholine (PC, 43%), phosphatidylethanolamine (PE, 36%), phosphatidylserine (PS, 13%), sphingomyeline (SP, 4%) phosphatidylinositol (PI, 3%), and lysophosphatidylcholine (LPC, 1%). Large unilamellar liposomes (SPMVPL) were prepared by the method described earlier (Yun and Kang, 1992b,c).

Fluorescence measurements

The incorporation of Py-3-Py was carried out by adding aliquots of stock solution of 5×10^{-5} M in absolute ethanol to SPMVPL (0.7 mg of total phospholipids/ml, pH 7.4), so that the final concentration was less than 5×10^{-7} M. The mixtures were initially vigorously vortexed for 10 s at room temperture and then incubated at 4℃
for 18 h under gentle stirring. 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 measurements were carried out with a SPF-500C spectrofluorometer (SLM Instruments Inc., Champaign-Urbana, IL, USA) and performed at 37℃ (pH 7.4). The excitation wavelength was 330 nm. The excimer to monomer fluorescence intensity ratio was calculated from the 480 nm to 379 nm signal ratio (I'/I). 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 Py-3-Py, served as controls from the fluorometric measurements.

RESULTS AND DISCUSSION

The excimer fluorescence of pyrene itself has been to assess membrane fluidity (Vanderkooi and Callis, 1974; Yun *et al.*, 1988). In such studies, however, the concentration of pyrene in the membrane lipid must be determined, since intermolecular excimer formation is highly dependent on the concentration. This requirement can be avoided by using an intramolecular excimer-forming compound such as Py-3-Py introduced by Hirayama (1965) and Zachariasse (1978). Provided that the probe concentration in the membrane is sufficiently low so as to preclude intermolecular excimer formation, estimation of the excimer to monomer fluorescence intensity ratio (I'/I) provides a convenient index of the range and rate of lateral diffusion (Schachter, 1984).

Recently our laboratory has shown that SPMV and SPMVTL differ markedly with respect to the I'/I ratio value. The I'/I values the intact SPMV (Chung *et al.*, 1993a) and SPMVTL (Chung *et al.*, 1993b) were 0.412 $\pm$ 0.005 and 0.506$\pm$ 0.007, respectively. The differences between intact SPMV and SPMVTL are due to selective protein removal, indicating the protein component of the SPMV has a large influence on the range and rate of the lateral diffusion. In this investigation, the I'/I ratio value in the SPMVPL is 0.709$\pm$ 0.007 (37° C, pH 7.4). The differences between the I'/I ratio values of the direct environment of Py-3-Py in the SPMVTL and SPMVPL are due to selective cholesterol removal, indicating the cholesterol component of the SPMVTL an important role on the lateral mobility or diffusibility of membranes.

We also reported that *n*-alkanols significantly increased the lateral diffusion of SPMV (Chung *et al.*, 1993a) and SPMVTL (Chung *et al.*, 1993b), and SPMV were more sensitive to disordering by *n*-alkanols than were total lipids (SPMVTL) extracted from them. It is suggested that the membrane disordering effects of *n*-alkanols are amplified by the presence of proteins which are found to be tightly associated with membrane lipids through covalent or noncovalent bonds. In this study, the significant increases in the I'/I values of Py-3-Py in the SPMVPL by 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, and 1-decanol were observed at 40 (I'/I value: 0.725$\pm$ 0.007), 5 (I'/I value: 0.724$\pm$ 0.005), 1 (I'/I value: 0.721$\pm$ 0.009), 0.25 (I'/I value: 0.721$\pm$ 0.006), 0.025 (I'/I value: 0.717 $\pm$ 0.006), 0.01 (I'/I value: 0.717$\pm$ 0.006), and 1.0 mM (I'/I value: 0.725$\pm$ 0.005), respectively. In a concentration-dependent manner, *n*-alkanols (except methanol, ethanol, and 1-propanol) significantly increased the ratio (I'/I) of Py-3-Py in the SPMVPL and the potencies of *n*-alkanols up to 1-nonanol increased by 1 order of magnitude as the carbon chain length increases by

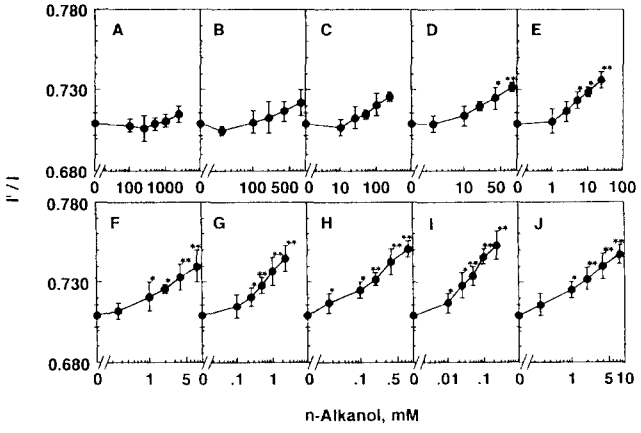

Fig. 1. Effects of *n*-alkanols on the excimer to monomer fluorescence intensity ratio, I'/I, of 1,3-di(1-pyrenyl)propane incorporated into model membranes of total lipid fraction extracted from synaptosomal plasma membrane vesicles. (A) Methanol; (B) ethanol; (C) 1-propanol; (D) 1-butanol; (E) 1-pentanol; (F) 1-hexanol; (G) 1-heptanol; (H) 1-octanol; (I) 1-nonanol; (J) 1-decanol. Fluorescence measurements were performed at 37°C (pH 7.4). Each point represents the mean SEM of 5 determinations. An asterisk and double asterisk signify $P<0.05$ and $P<0.01$, respectively, according to Student's t-test.

two carbon atoms (Fig. 1), suggesting an increase in the range and rate of the lateral mobility of the SPMVPL bilayers. The cut-off phenomenon was reached at 1-decanol (Fig. 1), where further increase in hydrocarbon length resulted in a decrease in the lateral mobility. However, significant changes in the I'/I value were not observed by methanol (from 100 to 2500 mM), ethanol (from 25 to 800 mM), and 1-propanol (from 10 to 250 mM) over entire concentration. This result is in agreement with those of our previous study (Chung *et al.*, 1993a,b), but the important point is the different potencies of *n*-alkanols among SPMV, SPMVTL, and SPMVPL, in terms of minimal *n*-alkanols' concentrations for the significant increase in the I'/I ratio values. It is suggested that the membrane disordering effects of *n*-alkanols are decreased by the absence of cholesterol. Such phenomenon can be explained by the role of cholesterol affecting the fluidity of the native and model membranes by the following mechanisms. Although biological membranes are usually in the liquid-crystalline state, it seems that they have some gel-phase regions which are interposed sparsely (Hitzemann *et al.*, 1985). Cholesterol is one of the major components in biological membranes, yet the role of cholesterol in membranes is not clear enough. Recent investigations by model membranes, however, have elucidated the effects of cholesterol may be summarized into two kinds of phenomena. One is the broadening of the phase transition, as shown by thermal analysis, and permeability measurements (Hitzemann *et al.*, 1985). Another is the dual effect on the fluidity of membranes, that is, the rigidizing effect in the liquid-crystalline phase and the fluidizing effect in the gel-phase (Hitzemann *et al.*, 1985). In summary, cholesterol acts to buffer the fluidity of model phospholipids (Oldfield and Chapman, 1972), and above the thermal transition temperature of the lipid, cholesterol increases ordering; conversely, cholesterol incorporation below the lipid melting point serve to fluidize the mixture (Shinitzky, 1986).

The old concept that proteins carry the specific functions and lipids merely provide the proper fluidity does not hold any longer. At present, most investigators agree to this view that both the lipids and the proteins influence each other's dynamic and each of them can bear specific functions. In fact, a large, diverse collection of physiological agonists produces the alterations in membrane fluidity as well as their specific ligand-receptor interaction (Sweet and Schrodeder, 1988).

n-Alkanols with lipid solubility will enter hydrophobic regions of both proteins and lipids in the core of the membrane (Goldstein, 1984). Although it is too early to explain exactly how the hydrophobic interactions affects functions, it is reasonable to predict that disruption of protein function will result from disorder in any of the hydrophobic regions (Goldstein, 1984). The results of investigations on the effects of higher alkanols and the correspondingalkanes 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 membrane-fluidizing hypothesis of ethanol action in the central nervous system is now being strongly challenged by recent data showing that ethanol specifically and selectively affects the function of the γ-aminobutyric acid-coupled chloride channel (Gonzales and Hoffman, 1991; Sanna *et al.*, 1991). 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. Conversely, there is also a possibility that *n*-alkanols may have a direct effect on certain receptors, receptor-gated ion channels, or membrane-bound enzymes, and then on membrane lipids. It may well be that *n*-alkanols concurrently interact with membrane lipids and membrane-bound proteins. At present, it may be premature to take sides in the controversy about whether membrane lipids or membrane proteins are the site of *n*-alknaols action.

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