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Effects of Barbiturates on the Fluidity of Phosphatidylethanolamine Model Membranes[1]

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ABSTRACT

Intramolecular excimer formation with 1,3-di(1-pyrenyl)propane (Py-3-Py) and fluorescence polarization with 1,6-diphenyl-1,3,5-hexatriene (DPH) were used to evaluate the effects of barbiturates on the bulk fluidity of the model membranes of phosphatidylethanolamine fraction of synaptosomal plasma membrane vesicles (SPMVPE) isolated from bovine cerebral cortex. In the SPMVPE, barbiturates decreased the excimer to monomer fluorescence intensity ratio (I'/I) of Py-3-Py and increased the fluorescence polarization (P), anisotropy (r), limiting anisotropy (r_∞), order parameter (S) and rotational relaxation time ($\overline{P}$) of DPH in a dose-dependent manner. The relative potencies of barbiturates to order the SPMVPE were in the order: pentobarbital > hexobarbital > amobarbital > phenobarbital. Hence, it is concluded that barbiturates have ordering effects on the SPMVPE. And the membrane-ordering potencies of barbiturates appear to be correlated with the potencies for enhancement of GABA-stimulated chloride influx and with the anesthetic effects of barbiturates.

Key Words: Bulk fluidity, Fluorescent probe technique, Membrane-ordering effects

INTRODUCTION

With increasing frequency, it is being recognized that the effect of drugs on the physical state of biological membranes is no less important than the drug-specific receptor interaction in studying the mechanism of action of drugs, especially those having anesthetic and tranquillizing actions. The physical state or fluidity of the lipid bilayer component of biological membranes has been shown to influence a number of cellular functions, including carrier-mediated transport, the properties of certain membrane-bound enzymes, binding to the insulin and opiate receptors, phagocytosis, endocytosis, depolarization-dependent exocytosis, immunologic and chemotherapeutic cytotoxicity, prostaglandin production and cell growth (Schachter, 1984; Stubbs and Smith, 1984; Spector and Yorek, 1985).

There is evidence that barbiturates penetrate into membrane lipid and alter the physical state of the lipid. Resultant changes in ion channels and membrane-bound enzymes have been hypothesized as a mechanism of action of barbiturates (Seeman, 1972; Lee, 1976). Barbiturates have been shown to decrease the temperature of the gel-to-liquid crystalline phase transition of phosphatidylcholine and phosphatidylethanolamine (PE) vesicles (Lee, 1976). Studies with electron spin resonance (ESR) probes indicate phospholipid-cholesterol vesicle fluidization by barbiturates (Lawrence and Gill, 1975;

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Miller and Pang, 1976; Pang and Miller, 1978). Harris and Schroeder (1980), using fluorescent probe techniques, demonstrated that pentobarbital and secobarbital increase the fluidity of the hydrophobic core of synaptic membranes prepared from mouse brain. In contrast, one ESR study (Rosenberg *et al.*, 1977) indicates that thiopental increases synaptic membrane surface rigidity without affecting membrane core fluidity.

In the present study, to get a better basis for studying the molecular mechanism of action of barbiturates, the effects of barbiturates on the bulk fluidity of the SPMVPE were investigated employing the fluorescence probe technique.

MATERIALS AND METHODS

The fluorescent probe, 1,6-diphenyl-1,3,5- hexatriene (DPH), was purchased from Sigma Chemical Co. (St. Louis, MO., USA). The barbiturates were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). Phosphatidylethanolamine (PE) was extracted from synaptosomal plasma membrane vesicles (SPMV) isolated from fresh bovine cerebral cortex. All other reagents were of the highest quality available. Water was double-distilled. 1,3-Di-(1-pyrenyl)propane (Py-3-Py) used in our studies was prepared by the previously reported synthesis (Bachmann and Carmack, 1941; Chandross and Demster, 1970; Huang-Minlon, 1946). Acetylpyrene obtained by a Friedel-Crafts acylation of pyrene (Bachmann and Carmack, 1941), was condensed with pyrene carboxaldehyde to produce pyrene chalcone, 1-pyrenoyl-2-pyrenylethylene (Chandross and Demster, 1970). The pyrene chalcone was converted to Py-3-Py through a sequential reduction using Pd-charcol and modified Wolf-Kishner reduction (Huang-Minlon, 1946). The prepared Py-3-Py showed the same value of melting point (Electrothermal melting point apparatus) with the reported (Zachariasse and Kühnle, 1976). And the Py-3-Py showed the same R_f value (thin layer chromatography) with the Zacharasse's Py-3-Py. Further we examined its infrared (IR) spectrum and nuclear magnetic resonance (^{1}HNMR) spectrum. ^{1}HNMR ($CDCl_3$) spectrum was recorded by using Varian EM-360 and chemical shifts were reported in values in parts per million downfield from tetramethylsilane employed as internal standard. IR spectrum was obtained by using Perkin-Elmer 710 B. Peaks at 2.36 ppm (quintet, J = 7Hz) corresponding two hydrogens and 3.42 ppm (triplet, J = 7Hz) corresponding four hydrogens in the ^{1}HNMR spectrum clearly gave support to the presence of three methylene's ($-CH_2CH_2CH_2-$) and peaks at 7.56-8.05 (multiplet) showed eighteen aromatic hydrogens in two pyrenyl rings. IR (KBr) spectrum showed peaks at 3050cm^{-1} (aromatic C-H stretching) and peaks at 2940cm^{-1} (C-H stretching).

To isolate SPMV from bovine fresh cerebral cortex, the methods previously established in our laboratory were used (Yun and Kang, 1990). Protein of SPMV was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. Electron microscopic examination showed that the membranes were in vesicular form. The purity of SPMV was also confirmed by monitoring the specific activities of Na, K-ATPase (Yun and Kang, 1990), 5′-nucleotidase (Yun and Kang, 1990) and acetylcholinesterase (Ellman *et al.*, 1961). The specific activities of Na, K-ATPase, 5′-nucleotidase and acetylcholinesterase were 5.6-fold, 3.3-fold, and 2.5-fold, respectively, enriched in the SPMV with respect to crude homogenate. Total lipids of SPMV were extracted as previously described (Kang, 1990; Yun and Kang, 1990). The individual phospholipid classes were separated by thin layer chromatography (Kang, 1990; Yun and Kang, 1990). The PE was quantitated by measuring the amounts of inorganic phosphate (Bartlett, 1959) after hydrolysis of PE at 180°C in 70% $HClO_4$ (Madeira and Antunes-Madeira, 1976). The PE model membranes (SPMVPE) were prepared and separated by the procedure of modified Melnick *et al.* (1981). The SPMVPE were suspended in phosphate buffered saline (PBS) containing NaCl 8g/l, KCl 0.2g/l, KH_2PO_4 0.2g/l, $Na_2HPO_4 \cdot 7H_2O$ 1.15g/l and N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (Hepes) 0.48g/l to PE concentration of 0.7mg/ml (Harris and Bruno, 1985). The pH was adjusted to 7.4 with NaOH and, as necessary, with HCl.

Stock solutions of Py-3-Py in absolute ethanol (10^{-4}M) and DPH in tetrahydrofuran (10^{-3}M) were made and kept in a cold dark place. The incorporation of the probes was carried out by adding aliquots of the stock solutions to the SPMVPE so that the final concentrations of Py-3-Py and DPH were less than 5×10^{-7}M and 1μg/70μg PE, respectively, as described (Almeida *et al.*, 1984; Schroeder *et al.*, 1984). Barbiturates were dissolved in a minimum volume of 0.1N-NaOH, diluted with deionized water and the pH adjusted to 9 to 10. Barbiturates, at the desired concentrations, were added directly to SPMVPE resuspended in PBS. The pH of the buffered sample was not changed significantly by addi-

tion of barbiturates. Measurements commenced usually within 1 min after addition. No effect of longer incubation times was noted.

The excitation wavelength for Py-3-Py in SPMVPE was 330nm (4nm excitation and 6nm emission slits). The excimer to monomer fluorescence intensity ratio of Py-3-Py, I'/I, was calculated from the 490nm to 378nm signal ratio. The excitation wavelength for DPH in SPMVPE was 362nm, and fluorescence emission was read at 424nm. Fluorescence lifetime was measured with an SLM-4800 using modulation frequencies of 6, 18 and 30MHz. Fluorescence lifetimes were measured with excitation polarizer set at 0 and the emission polarizers set at 55°C in order to correct for instrumentally induced anisotropy (grating correction). Fluorescence lifetimes were measured relative to a reference solution of dimethyl p-bis [2-(5-phenyloxazolyl)]benzene (dimethyl POPOP) in absolute ethanol as described previously (Lakowicz *et al.*, 1980).

All fluorescence measurements were obtained with a T-format SLM-4800 subnanosecond spectrofluorometer (SLM Instruments Inc., Champaign-Urbana, IL) and performed at 37°C. Before the fluorescence spectra were obtained, all samples were bubbled by dry nitrogen through the solution for at least 5 min in order to eliminate oxygen that might be a quencher and might denaturalize SPMVPE. Blanks (SPMVPEs not incorporated with fluorescent probes), prepared under identical conditions, served as controls for the fluorometric measurements.

RESULTS

Intramolecular excimer formation with the fluorescent probe Py-3-Py and the fluorescence polarization with DPH were used to evaluate the effects of pentobarbital, hexobarbital, amobarbital, and phenobarbital on the organization of SPMVPE. The excimer to monomer fluorescence intensity ratio of Py-3-Py, I'/I, changes with the fluidity of viscous media and has been used to monitor the fluidity of synthetic (Zachariasse *et al.*, 1980) and native membranes (Almeida *et al.*, 1984; Almeida *et al.*, 1982; Zachariasse *et al.*, 1982). DPH is the most widely used membrane probe because it is a small uncharged, rod-shaped molecule that distributes evenly throughout the hydrophobic regions of membrane lipids, and its fluorescence lifetime is ideally suited to measurement of its motion in the membrane. In order to evaluate the effects of barbiturates on the bulk fluidity of SPMVPE, it is first necessary to demonstrate that these molecules do not interact directly with DPH or Py-3-Py and thereby quench its fluorescence. Quenching of absorbance-corrected fluorescence intensity by barbiturates was not observed over the entire concentration range used for pentobarbital, hexobarbital, amobarbital, and phenobarbital in the SPMVPE. Furthermore, if direct quenching of Py-3-Py and DPH by barbiturates occurred, fluorescence lifetime would decrease. However, the fluorescence lifetimes of Py-3-Py and DPH were not changed by pentobarbital, hexobarbital, amobarbital, and phenobarbital in the SPMVPE. For example, the lifetimes of DPH in the SPMVPE were 10.21 ± 0.07, 10.17 ± 0.13, 10.14 ± 0.10, 10.03 ± 0.07, and 9.96 ± 0.05ns at 0.1, 0.5, 1.0, 5.0, and 10.0mM of pentobarbital, respectively. Similar results were obtained with hexobarbital, amobarbital, and phenobarbital. A similar pattern was observed for the fluorescence lifetime of Py-3-Py. Therefore, direct quenching of DPH and Py-3-Py fluorescence by barbiturates is ruled out.

Effects of barbiturates on the excimer to monomer fluorescence intensity ratio of Py-3-Py in the SPMVPE: *In vitro* addition of barbiturates decreased the excimer to monomer fluorescence intensity ratio of Py-3-Py in the SPMVPE (Fig. 1). Barbiturates decreased the excimer to monomer fluorescence intensity ratio of Py-3-Py, I'/I, in a dose-dependent manner. In SPMVPE, the relative potencies of barbiturates to order the lipid organization were in the order: pentobarbital > hexobarbital > amobarbital > phenobarbital. As discussed below, these results indicate that barbiturates decreased the mobility (Almeida *et al.*, 1984; Almeida *et al.*, 1982; Zachariasse *et al.*, 1982) or lateral diffusion (Schachter, 1984) of this hydrophobic probe within the SPMVPE.

Effects of barbiturates on the static and dynamic properties of DPH in the SPMVPE: The fluorescence polarization of membrane-bound DPH, P, is given by $P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$. The intensities of the emitted light in the parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) orientations are the fundamental data supplied by the instrument. The results of fluorescence polarization determination are conveniently expressed as the fluorescence anisotropy, r, where $r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$. The fluorescence anisotropy includes the static and the dynamic properties of membrane-bound DPH. The fluorescence limiting anisotropy of membrane-bound DPH, r_{∞}, where $r_{\infty} = 4/3r - 0.10$, $0.13 < r < 0.28$ (van Blitterswijk *et al.*, 1981). Alterations in polarization were converted to rotational relaxation time, $\bar{P}$, calculated from the

Perrin equation (Shinitzky and Inbar, 1976) : $(1/r-1/3) = (1/r_o-1/3)(1+3\tau/\bar{P})$. In this equation, r_o is the maximal limiting anisotropy of the probe determined under conditions where it can not rotate, is equal to 0.362 for DPH (Lakowicz *et al.*, 1979), and τ is the fluorescence lifetime of the excited state. The quantity r_∞ reflects the hindrance to full 90° rotation of a fluorophore in particular microenvironment. In natural and artificial membranes, the r_∞ values of DPH are high and largely determine r. The structural organization of the lipid environment in the bilayers limits the extent of rotation of this probe and can be converted to an order parameter (Heyn, 1979; Jähnig, 1979), S, where $S=(r_\infty/r_o)^{1/2}$. The static properties of DPH (limiting anisotropy, order) indicate that at 37°C there is still considerable restriction to motion of DPH in the SPMVPE since the limiting anisotropy

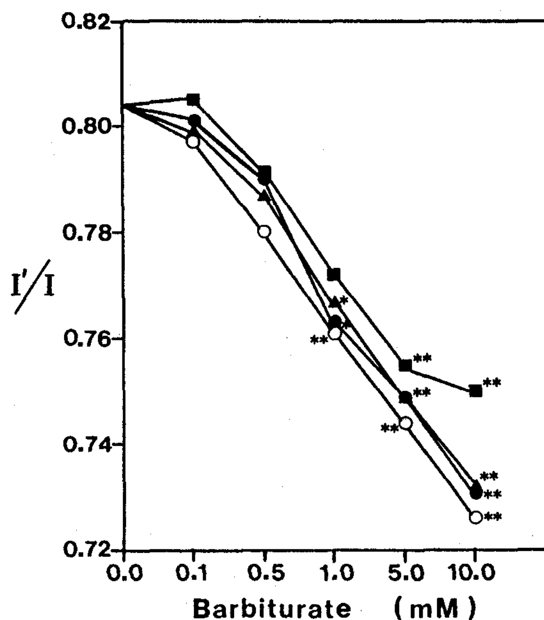

Fig. 1. Effects of barbiturates on the excimer to monomer fluorescence intensity ratio, I'/I, of 1,3-di (1-pyrenyl) propane in phosphatidylethanolamine model membranes. Fluorescence measurements were performed at 37°C. Each point represents the mean ± SEM of 4 determinations. An asterisk and double asterisk signify $p<0.05$ and $p<0.01$, respectively, according to Student's t-test. O: pentobarbital, ●: hexobarbital, ▲: amobarbital, ■: phenobarbital.

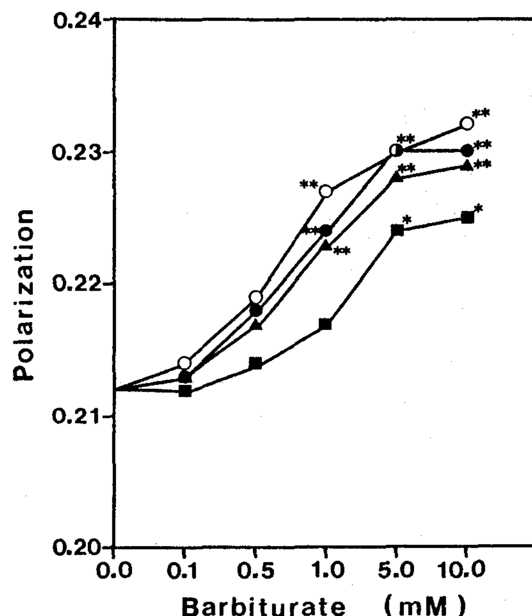

Fig. 2. Effects of barbiturates on the fluorescence polarization (P) of 1,6-diphenyl-1,3,5-hexatriene in phosphatidylethanolamine model membranes. Fluorescence measurements were performed at 37°C. Each point represents the mean ± SEM of 4 determinations. An asterisk and double asterisk signify $p<0.05$ and $p<0.01$, respectively, according to Student's t-test. O: pentobarbital, ●: hexobarbital, ▲: amobarbital, ■: phenobarbital.

Tabe 1. The excimer to monomer fluorescence intensity ratio (I'/I) of 1,3-di(1-pyrenyl)propane and fluorescence parameters of 1,6-diphenyl-1,3,5-hexatriene in phosphatidylethanolamine model membranes

I'/I	Polarization, P	Anisotropy, r	Limiting anisotropy, r_∞	Order parameter, S	Rotational relaxion time, $\bar{P}$
0.804 ± 0.009	0.212 ± 0.002	0.152 ± 0.001	0.103 ± 0.002	0.532 ± 0.004	19.24 ± 0.06

Fluorescence measurements were performed at 37°C. Values represent the mean ± SEM of 4 determinations.

and order parameter are not zero (Table 1). The dynamic property, rotational relaxation time, indicates rapid motion of DPH in the SPMVPE near 19.24 ± 0.06ns. The limiting anisotropy is primarily determined by the degree of limitation to rotation and may vary independently of rotational rate. The effects of increasing concentration of barbiturates on the fluorescence polarization (P), the anisotropy (r), the limiting anisotropy (r_∞), order parameter (S), and the rotational relaxation time ($\bar{P}$) of DPH in the SPMVPE are shown in Figs 2-6. Barbiturates increased the fluorescence polarization (P), the anisotropy (r), the limiting anisotropy (r_∞), and rotational relaxation time ($\bar{P}$) of SPMVPE-bound DPH in a dose-dependent manner. For example, the anisotropy of the SPMVPE-bound DPH was significantly increased at 1.0mM, 5.0mM, and 10.0mM pentobarbital (Fig. 3). In the SPMVPE, the relative potencies of barbiturates to order the lipid organization were in the order : pentobarbital > hexobarbital > amobarbital > phenobarbital.

In summary, barbiturates decreased the lateral and rotational diffusion of the SPMVPE.

DISCUSSION

In vitro exposure to barbiturates decreased the excimer to monomer fluorescence intensity ratio of Py-3-Py incorporated into the SPMVPE. The advantage of intramolecular over intermolecular excimer probes lies primarily in the possibility that the former offer to use the probe molecule in very small concentrations, 10^{-6}M and lower (Almeida *et al.*, 1984; Almeida *et al.*, 1982; Zachariasse *et al.*, 1980; Zachariasse *et al.*, 1982). This minimizes the perturbation of the medium under investigation and avoids the formation of probe aggregates, a phenomenon that has been encountered with pyrene (Zachariasse *et al.*, 1978). At low temperatures, or at sufficiently high viscosities, the excimer to monomer fluorescence

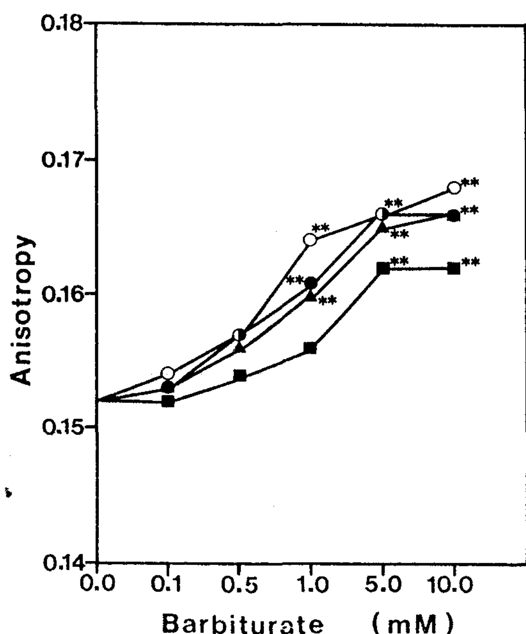

Fig. 3. Effects of barbiturates on the anisotropy (r) of 1,6-diphenyl-1,3,5-hexatriene in phosphatidylethanolamine model membranes. Fluorescence measurements were performed at 37°C. Each point represents the mean ± SEM of 4 determinations. An asterisk and double asterisk signify $p<0.05$ and $p<0.01$, respectively, according to Student's t-test. O: pentobarbital, ●: hexobarbital, ▲: amobarbital, ■: phenobarbital.

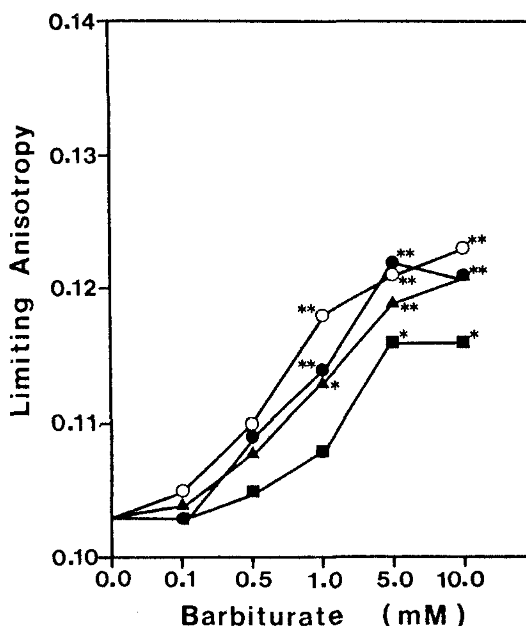

Fig. 4. Effects of barbiturates on the limiting anisotropy (r_∞) of 1,6-diphenyl-1,3,5-hexatriene in phosphatidylethanolamine model membranes. Fluorescence measurements were performed at 37°C. Each point represents the mean ± SEM of 4 determinations. An asterisk and double asterisk signify $p<0.05$ and $p<0.01$, respectively, according to Student's t-test. O: pentobarbital, ●: hexobarbital, ▲: amobarbital, ■: phenobarbital.

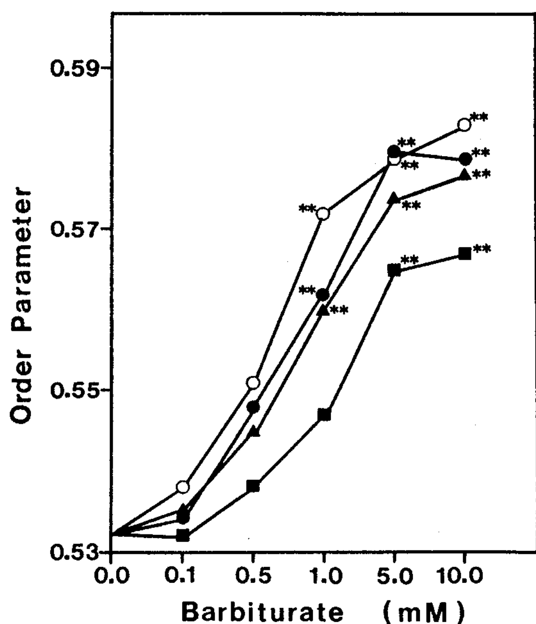

Fig. 5. Effects of barbiturates on the order parameter (S) of 1,6-diphenyl-1,3,5-hexatriene in phosphatidylethanolamine model membranes. Fluorescence measurements were performed at 37°C. Each point represents the mean ± SEM of 4 determinations. An asterisk and double asterisk signify $p<0.05$ and $p<0.01$, respectively, according to Student's t-test. O: pentobarbital, ●: hexobarbital, ▲: amobarbital, ■: phenobarbital.

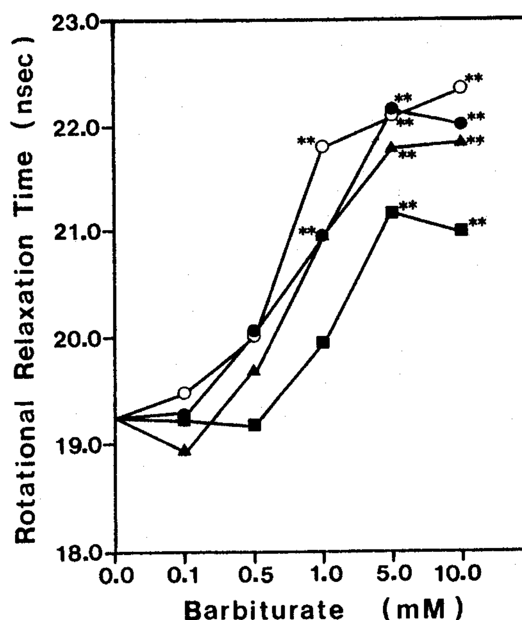

Fig. 6. Effects of barbiturates on the rotational relaxation time ($\overline{P}$) of 1,6-diphenyl-1,3,5-hexatriene in phosphatidylethanolamine model membranes. Fluorescence measurements were performed at 37°C. Each point represents the mean ± SEM of 4 determinations. An asterisk and double asterisk signify $p<0.05$ and $p<0.01$, respectively, according to Student's t-test. O: pentobarbital, ●: hexobarbital, ▲: amobarbital, ■: phenobarbital.

ratio of Py-3-Py, I'/I, is primarily determined by the rate constant of excimer formation, since the radiative rate constants and the excimer lifetime have been found to be essentially independent of temperature (Zachariasse *et al.*, 1980). Since I'/I of Py-3-Py has been shown to increase with fluidity in highly viscous media (Zachariasse *et al.*, 1978), the technique is suitable to determine the fluidity of the probe environment in media such as biomembranes (Almeida *et al.*, 1982; Zachariasse *et al.*, 1982). Therefore, the decrease in the excimer to monomer fluorescence intensity ratio of Py-3-Py, I'/I, observed in this study indicates that barbiturates order the hydrophobic regions of the SPMVPE.

Barbiturates increased the fluorescence polarization, limiting anisotropy, order paramter, and rotational relaxation time of DPH incorporated into the SPMVPE. DPH is a nonpolar molecule which distributes throughout the hydrophobic core of the SPMVPE (Shinitzky and Barenholz, 1978; Lentz *et al.*, 1978). The fluorescence polarization, the limiting anisotropy, and the order paramter of DPH indicate the property of statics of the probe (Schachter, 1984) and thus reflect the ordering of the membrane core. In relation to the modified Perrin equation, some authors suggest that the term "fluidity" be used only in relation to the dynamic parameter, $\overline{P}$, i.e., the speed of rotation of lipid molecules. Others, however, consider that r_{∞}, which reflects a structural parameter or extent of rotation, is also a useful parameter of "fluidity" (van Blitterswijk *et al.*, 1981).

The increase in the excimer to monomer fluorescence intensity ratio of Py-3-Py observed in unpublished results (our laboratory) indicates that pentobarbital, hexobarbital, amobarbital, and phenobarbial disorder or fluidize the hydrophobic regions of intact SPMV isolated from bovine brain. The barbiturates had little effects on the ratio of Py-3-Py in the unilamellar liposomes of SPMV total

lipids but the barbiturates decreased it in the unilamellar liposomes of SPMV phospholipids (unpublished data: our laboratory). And the decrease in the fluorescence polarization, the anisotropy, limiting anisotropy, order parameter, and rotational relaxation time observed in unpublished results (our laboratory) indicate that pentobarbital, hexobarbital, amobarbital, and phenobarbital have fluidizing effects on the hydrophobic regions of intact SPMV isolated from bovine brain. The barbiturates had little effects on the fluorescence polarization, the anisotropy, limiting anisotropy, order parameter, and rotational relaxation time of DPH in the unilamellar liposomes of SPMV total lipids (unpublished data: our laboratory). The barbiturates, however, increased on the fluorescence polarization, the anisotropy, the limiting anisotropy, and rotational relaxation time of DPH in the hydrocarbon core of the unilamellar liposomes of SPMV phospholipids (unpublished data; our laboratory). Harris and Schroeder (Harris and Schroeder, 1980), using fluorescent probe techniques, demonstrated that pentobarbital and secobarbital increase the fluidity of the hydrophobic core of synaptic membranes prepared from mouse brain. They are in agreement with the study of Pang and Miller (1978) of the effects of pentobarbital on the order parameter of 5-doxyl stearic acid in liposomes containing phosphatidylcholine, phosphatidic acid and cholesterol. These investigators reported that pentobarbital increases the order parameter of phosphatidylcholine-phosphatidic acid liposomes containing no cholesterol, does not affect the order parameter at 14 mol of cholesterol/100 mol of lipid and decrease the order parameter at 32 mol of cholesterol/100 mol of lipid. These findings are analogous to our observations that pentobarbital increased the fluorescence polarization, the anisotropy, the limiting anisotropy, the order parameter, and rotational relaxation time of DPH in the SPMV phospholipids and the SPMVPE.

As discussed above, whether barbiturates order or disorder a membrane appears to depend upon the initial rigidity of the membrane. The effect of proteins on the limiting anisotropy of DPH must be considered (Hoffmann *et al.*, 1981). In model system, it has been proposed not only that DPH may not report the average disorder of the hydrocarbon chains but also that its proximity to intramembrane proteins may lead to greatly hindered motion (Hoffmann *et al.*, 1981). However, tryptophan quenching experiments suggest that DPH is not associated with protein in biological membranes (Klausner *et al.*, 1980), whereas DPH has been shown to bind to hydrophobic regions of proteins in aqueous media (Mely-Goubert and Freedman, 1980). There is evidence that barbiturates also interact with proteins of gamma-aminobutyric acid (GABA)-receptor chloride ionophore complex and this may be the site of stereoselectivity for barbiturate action (Ho and Harris, 1981; Leeb-Lundberg and Olsen, 1982; Allan and Harris, 1986; Olsen, 1982). The sites at which barbiturates exert these chloride-dependent, picrotoxinin-sensitive effects on benzodiazepine receptors show a chemically specific and stereospecific profile which correlates with the pharmacological activity of barbiturates (Leeb-Lundberg and Olsen, 1982). The relative potencies of barbiturates to enhance the GABA-stimulated $^{36}Cl^-$ uptake were in the order: pentobarbital > hexobarbital > amobarbital > phenobarbital (Allan and Harris, 1986). The anesthetic potencies of the barbiturates were significantly correlated with the potencies for enhancement of GABA-stimulated $^{36}Cl^-$ uptake (Allan and Harris, 1986). This order is also in agreement with the order of the present study. Hence, it is strongly suggested that there might be a relationship between SPMVPE-ordering potencies and the potencies of GABA-stimulated $^{36}Cl^-$ uptake and these potencies are correlated with the anesthetic effects of barbiturates.

Barbiturates appear to modulate the fluidity of neuronal membranes to a certain level, which in turn facilitates chloride fluxes. There is also a possibility that specific fluidity induced by barbiturates might help the interaction of them with chloride channels. Even though direct evidence for the interaction between membrane disordering and chloride fluxes should be elucidated, these experiments strongly suggest that barbiturates act, in part, their pharmacological effects by modulating the fluidity of neuronal membranes to a specific level.

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= 국문초록 =

Barbiturates가 소의 신선한 대뇌피질 Synaptosomal Plasma Membrane Vesicles로 부터 추출하여 제제한 Phosphatidylethanolamine 인공세포막의 유동성에 미치는 영향

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Barbiturates의 분자적 약리작용기전 탐구에 기초자료를 제공하기 위하여 소의 신선한 대뇌피질 synaptosomal plasma membrane vesicles로부터 분리제제한 phosphatidylethanolamine 인공세포막 (SPMVPE)의 유동성에 미치는 barbiturates의 영향을 형광 probe법으로 검색한 결과는 다음과 같다.

1. Pentobarbital, hexobarbital, amobarbital 및 phenobarbital이 기재한 순위로 SPMVPE내 Py-3-Py의 monomer 형광세기에 대한 excimer 형광세기의 비 (I′/I)를 감소시켰다.

2. Pentobarbital, hexobarbital, amobarbital 및 phenobarbital이 기재한 순위로 SPMVPE내 DPH의 polarization, anisotropy, limiting anisotropy, order parameter 및 rotational relaxation time을 증가시켰다.

3. 따라서 위에 제시한 barbiturates가 SPMVPE의 유동성을 유의성있게 감소시킨다는 것을 확인할 수가 있었다.