Journal of Biochemistry and Molecular Biology, Vol. 33, No. 3, May 2000, pp. 221-227

JOURNAL OF
Biochemistry
and
Molecular Biology

The Distribution of Barbiturates in Model Membranes of Total Lipids and Total Phospholipids Extracted from Brain Membranes

Chang-Sik Park[†], Seong-Moon Lee[†], In-Kyo Chung[‡], Jin-Bom Kim[§], Woo-Sung Son[‖], Hye-Ock Jang[#] and Il Yun[†,*]

Departments of [†]Dental Pharmacology and Biophysics, [‡]Oral and Maxillofacial Surgery and Clinical Pharmacology,
[§]Preventive and Community Dentistry and [‖]Orthodontics, College of Dentistry and Research Institute for Oral Biotechnology,
Pusan National University, Pusan 602-739, Korea,
[#]Department of Oriental Pathology and Prescription, College of Oriental Medicine, Dong-Eui University, Pusan 614-054, Korea

Received 21 January 2000, Accepted 9 March 2000

The distribution of barbiturates in the model membranes of total lipids (SPMVTL) and total phospholipids (SPMVPL) extracted from synaptosomal plasma membrane vesicles was determined by employing a fluorescent probe technique. The two fluorescent probes 2-(9-anthroyl)stearic acid and 12-(9-anthroyl)stearic acid were utilized as probes for the surface and the hydrocarbon interior of the outer monolayer of the SPMVTL and SPMVPL, respectively. The Stern-Volmer equation of fluorescent quenching was modified to calculate the relative distribution. The analysis of preferential quenching of these probes by barbiturates indicates that pentobarbital, hexobarbital, amobarbital and phenobarbital are predominantly distributed on the surface area, while thiopental sodium has an accessibility to the hydrocarbon interior of the outer monolayer of the SPMVTL and SPMVPL. From these results, it is strongly suggested that the more effective penetration into the hydrocarbon interior of the outer monolayer of the membrane lipid bilayer could result in a higher general anesthetic activity.

Keywords: Distribution of barbiturates, Fluorescence quenching, Model membranes, Modified Stern-Volmer equation.

Introduction

It has been thought that barbiturates, as well as ethanol and volatile anesthetics, exerted their depressant effects on the central nervous system (CNS) by dissolving in the lipid membranes; thereby perturbing the function of ion channels and other proteins embedded therein (Yun *et al.*, 1994; Kang *et al.*, 1996). Kang (1990) and other investigators (Sweet *et al.*, 1987; Yun *et al.*, 1990b; Kang *et al.*, 1992; Chung *et al.*, 1992; Yun and Kang, 1992) take the position that membrane lipids are an important site of action of barbiturates, but that barbiturates have a very specific effect on different lipid areas or domains in the membrane. However, the membrane-fluidizing hypothesis of barbiturates in the CNS is now being strongly challenged by recent data showing that barbiturates specifically and selectively affect the function of the γ-aminobutyric acid-coupled chloride channel (Rall, 1991) as well as the function of sodium channel (Frenkel *et al.*, 1990). At the present time, the exact mechanism(s) of action of barbiturates in the CNS is unclear.

Important basic materials for the research of pharmacological actions of drugs are being provided by the investigation of the drug distribution region at a cellular level. This is because the region of drug distribution at a cellular level coincides with the site(s) of drug action most of the time. Indeed, despite overwhelming evidence the controversy about whether or not the primary site(s) action of barbiturates is the membrane lipids/proteins or the membrane surface head groups/phospholipid acyl chains remains unresolved. Thus, the mechanism for the barbiturates' action is still unknown.

The characteristics of the lipid samples, such as size, lamellarity, radius of curvature, and shape, are strongly dependent on the method used to form the vesicles (Lasic, 1988). As a consequence of the preparation method, the parameters that characterize the lipid phase equilibrium in lipid mixtures are affected by lipid sample characteristics. This is because the size of the large unilamellar vesicles is on the same order as the size of the cells. Large unilamellar vesicles are becoming objects of intense scrutiny in diverse areas that focus on membrane behavior (Menger and Keiper, 1998).

In detecting the site of distribution, or the binding site(s) of drugs, a radiolabelled isotope has been widely used. However, this method has several disadvantages. It not only requires expensive laboratory equipment, but also has difficulty in the

*To whom correspondence should be addressed
Tel: 82-51-240-7813; Fax: 82-51-254-0576
E-mail: iyun@hyowon.pusan.ac.kr

disposition of waste materials. Hence, it has become a serious contributing factor to environmental pollution. On the contrary, the method adopted in this research, namely fluorescence analysis, requires only a minimal experimental expense and is very simple and easy. To our knowledge, the results presented herein are unprecedented in demonstrating the region of distribution of barbiturates in model membranes by specifically employing the fluorescence probe technique. Lee and Yun (1995) reported that pentobarbital, hexobarbital, amobarbital and phenobarbital are predominantly distributed on the surface region; whereas, thiopental has an accessibility to the hydrocarbon interior of the outer monolayer of the synaptosomal plasma membrane vesicles isolated from rat brain. In order to elucidate the role of proteins, cholesterol and phospholipids in the changes in distribution of the barbiturates, we examined the penetration site(s) of barbiturates into model membranes of total lipids (SPMVTL) as well as total phospholipids (SPMVPL) extracted from rat neuronal membranes, employing two fluorescent probes 12-(9-anthroyl) stearic acid (12-AS) and N-octadecyl naphthyl-2-amine-6-sulfonic acid (ONS). The analysis of preferentially quenching these probes by barbiturates revealed the relative accessibility of these drugs into the hydrocarbon interior of the outer monolayers of SPMVTL and SPMVPL.

The purpose of this research is two-fold: (i) Provide a basis for studying the mechanism of pharmacological action of barbiturates through the investigation of the primary site of action of such drugs. (ii) Develop a fluorescence analysis in detecting the site of the distribution of drugs and various substances at the cellular level.

Materials and Methods

Materials Two-week-old Sprague-Dawley rats of either sex were used for this laboratory research. Upon their demise the whole brain was rapidly excised, placed in ice-cold 0.32 M sucrose+3 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (Hepes), pH 7.5. The fluorescent probes 12-AS and ONS were purchased from Molecular Probes, Inc. (Junction City, OR., USA). Barbiturates and Hepes were purchased from Sigma Chemical Co. (St. Louis, MO., USA). All other reagents were purchased commercially and were of the highest quality available. Water was deionized.

Preparation of SPMV Preparation of SPMV were performed according to the procedure of earlier studies (Yun and Kang, 1990; Yun *et al.*, 1990a). The specific activities of Na,K-ATPase, acetylcholinesterase and 5'-nucleotidase were approximately 4-, 2.5- and 3-fold, respectively, enriched in the plasma membrane fraction with respect to crude homogenates. The electron microscopic examination of the prepared SPMV showed a very high purity. The vesicles were separated, according to size, and demonstrated a homogeneous distribution and no longer showed the presence of intracellular organelles or leakage.

Preparation of large unilamellar vesicles Total lipids were extracted from the SPMV as described previously (Yun and Kang, 1990). The cholesterol was determined according to the Liebermann-Buchard reaction (Huang *et al.*, 1961). Phospholipids were quantitated by measuring the amounts of inorganic phosphate (Bartlett, 1959) after hydrolysis of the phospholipids at 180°C in 70% $HClO_4$ (Madeira and Antunes-Madeira, 1976). The SPMV had a high lipid to protein ratio (0.931 mg/total lipids/mg protein) and a low cholesterol to phospholipid ratio (0.62 ± 0.01), the phospholipids were composed of phosphatidylcholine (42.5%), phosphatidylethanolamine (37.5%), sphingomyelin (3.8%), phosphatidylinositol (3.2%) and lysophosphatidylcholine (1%).

Large unilamellar vesicles (SPMVTL or SPMVPL; 0.7 mg of total lipids or total phospholipids/ml, pH 7.4) were prepared by the method previously described (Angelova and Dimitrov, 1986; Dimitrov and Angelova, 1987; Angelova *et al.*, 1992; Yun and Kang, 1992). The extracted total lipids, or total phospholipids in chloroform solution, were deposited on the sides of a round-bottom flask by the removal of the organic solvent by rotary evaporation. The lipids were then redissolved in diethyl ether which had been redistilled in the presence of $NaHSO_3$ immediately prior to use. Phosphate-buffered saline (PBS: 8 g/l NaCl, 0.2 g/l KCl, 0.2 g/l KH_2PO_4, 1.15 g/l $Na_2HPO_4 \cdot 7H_2O$, 0.48 g/l Hepes, pH 7.4) was added to the solution of lipids and the organic/aqueous mixture was placed in an ultrasonic processor (Sonics & Materials, Inc., Danbury, CT, USA) under N_2 at 30°C. It was sonicated for 5 min in order to form a milky white, homogeneous emulsion. The emulsion was then transferred to a rotary evaporator and the organic solvent was removed under reduced pressure. During evaporation of the solvent, the system foamed. As the process continued, a progressively higher vacuum was needed to maintain foaming. As the majority of the solvent was removed, the material first formed a viscous gel and subsequently (within 5-10 min) it became an aqueous suspension. At this time, additional PBS was added, the preparation then foamed and was vented again several times until the foaming ceased. The procedure was finished when no foaming occurred. The preparation was then dialyzed and passed through a Sepharose 4B column.

Fluorescence measurements The SPMVTL and SPMVPL were suspended in PBS (pH 7.4) to a concentration of 0.7 mg of total lipids or total phospholipids/ml. Barbiturates were dissolved in a minimum volume of 0.1 N NaOH, diluted with deionized water and the pH adjusted to 9 to 10. The solutions were prepared immediately before use. The barbiturates, as the concentrations indicated, were added directly to membranes resuspended in PBS. The pH of the buffered sample was not changed significantly by the addition of barbiturates.

The stock solutions of 12-AS (1 mM) in tetrahydrofuran (THF) and ONS (1 mM) in dimethylsulfoxide (DMSO) were made and kept in a cold dark place. The incorporation of these probes was carried out by adding aliquots to the stock solutions of the membrane, so that the final concentrations of 12-AS and ONS were 10 and 20 μM, respectively. The mixture was stirred for 2 h at room temperature in order to reduce the concentration of THF and DMSO that might alter the permeability of model membranes. Also, the mixture was bubbled by dry nitrogen for 5

min with 20 min intervals in order to eliminate oxygen that might be a quencher and oxidize the model membranes. The fluorescence measurements were carried out with a Multi Frequency Cross-Correlation Phase and Modulation Fluorometer (ISS K2-003), equipped with a thermostated cell holder, and performed at pH 7.4. Before the fluorescence spectra were obtained, all of the samples were degassed by bubbling dry nitrogen through the solution for at least 30 min. The ONS was excited at 360 nm and the emission was read at 420 nm. The fluorescent probe 12-AS was excited at 386 nm and its emission recorded at 440 nm. Blanks (the model membrane suspensions without fluorescent probes), prepared under identical conditions, served as controls for the fluorometric measurements. All of the experiments were carried out at least five times and the averages of these values were obtained. These relative values did not differ by more than ±5% from each value measured. All of the measurements were performed at 37 ± 0.1°C.

Results

Fluorescence quenching in homogeneous solution was described in the terms of the Stern-Volmer equation:

$$F_o/F = I + kq\tau_o[Q] = I + K[Q] \quad (1)$$

In this equation, F_o and F are the fluorescence intensities in the absence and presence of the quencher, respectively. kq, is the bimolecular quenching constant. τ_o is the lifetime of the fluorophore in the absence of quencher. [Q] is the concentration of quencher, and $K = kq\tau_o$ is the Stern-Volmer quenching constant. A plot of F_o/F versus [Q] yields an intercept of one on the y axis and a slope equal to K. In the present study, the Stern-Volmer equation was modified for uneven distribution of the quencher in the SPMVTL and SPMVPL. For water-soluble barbiturates, the concentration in the aqueous phase is excessively larger than that in the lipid bilayer at pH 7.4. Consequently, $[Q]_L \cong P[Q]_T$ and equation 1 can be modified:

$$F_o/F = 1 + fKP[Q]_T \quad (2)$$

where $[Q]_L$ and $[Q]_T$ are the concentrations of the quencher in the outer monolayers of the lipid bilayer structures of SPMVTL and SPMVPL. The total concentration of the quencher in the system, respectively is: P is the partition coefficient, and f is a regional correction factor for uneven distribution of the quencher between the surface and the hydrocarbon interior region in the outer monolayers of the SPMVTL and SPMVPL. Studies have shown that the most probable position of the naphthalene sulfonate moiety of the ONS molecule is at the surface of a membrane's outer monolayer. Also, the anthroyl moiety of the 12-AS molecule is most likely located in its hydrocarbon interior region of the outer monolayers of the membrane's lipid bilayer structures (Bradley *et al.*, 1973; Lesslauer *et al.*, 1973; Koblin *et al.*, 1975; Lee and Yun, 1995). Hence, ONS or 12-AS quenching in the SPMVTL and SPMVPL gives the following:

$$F_o/F = 1 + f_s K_{ONS} P[Q]_T \quad (3)$$

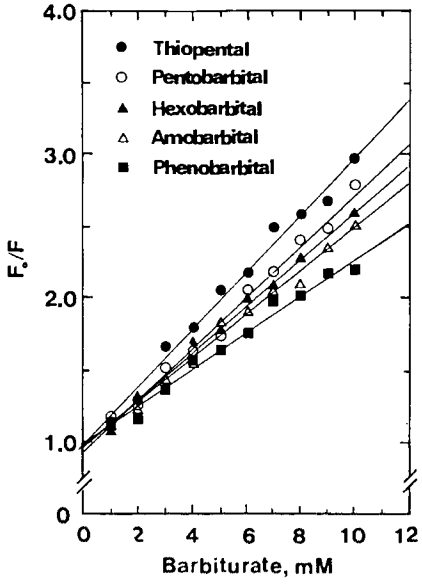

Fig. 1. Stern-Volmer plot of quenching of ONS fluorescence in model membranes of total lipid fraction (SPMVTL) by thiopental sodium, pentobarbital, hexobarbital, amobarbital and phenobarbital. Total lipids were extracted from synaptosomal plasma membrane vesicles isolated from rat whole brain. Lines were fitted by a least-squares analysis.

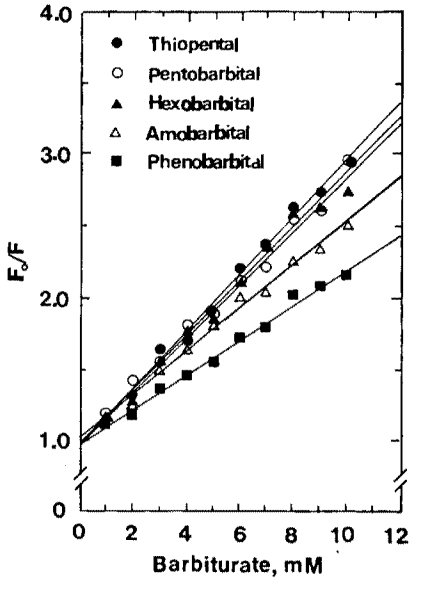

Fig. 2. Stern-Volmer plot of quenching of ONS fluorescence in model membranes of total phospholipid fraction (SPMVPL) by thiopental sodium, pentobarbital, hexobarbital, amobarbital and phenobarbital. Total phospholipids were extracted from synaptosomal plasma membrane vesicles isolated from rat whole brain. Lines were fitted by a least-squares analysis.

$$F_o/F = 1 + f_i K_{12\text{-}AS} P[Q]_T \quad (4)$$

where K_{ONS} and $K_{12\text{-}AS}$ are the Stern-Volmer constants of the fluorescence quenching of the ONS and the 12-AS by the quencher, respectively. f_s/f_i is the ratio of the regional

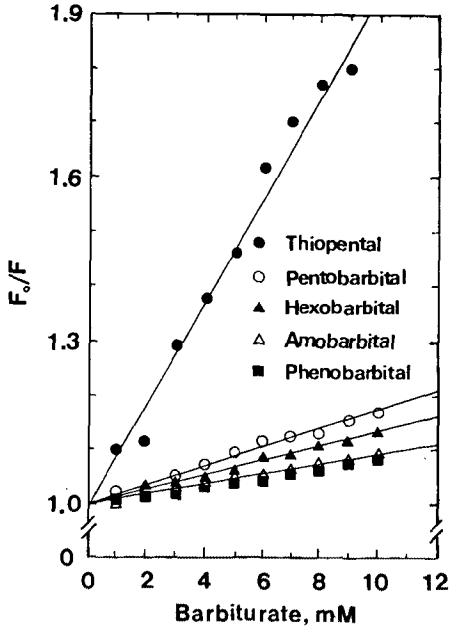

Fig. 3. Stern-Volmer plot of quenching of 12-AS fluorescence in model membranes of total lipid fraction (SPMVTL) by thiopental sodium, pentobarbital, hexobarbital, amobarbital and phenobarbital. Total lipids were extracted from synaptosomal plasma membrane vesicles isolated from rat whole brain. Lines were fitted by a least-squares analysis.

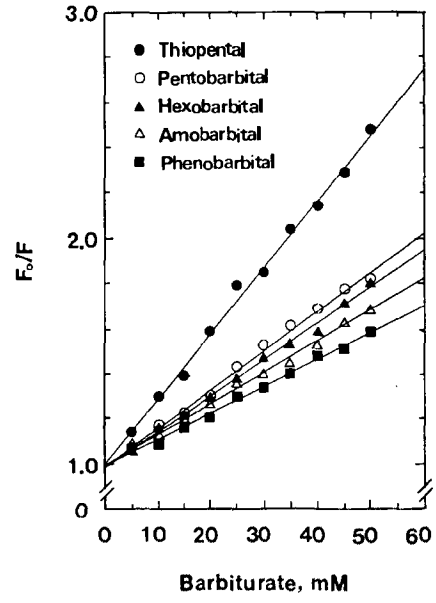

Fig. 4. Stern-Volmer plot of quenching of 12-AS fluorescence in model membranes of total phospholipid fraction (SPMVPL) by thiopental sodium, pentobarbital, hexobarbital, amobarbital and phenobarbital. Total phospholipids were extracted from synaptosomal plasma membrane vesicles isolated from rat whole brain. Lines were fitted by a least-squares analysis.

correction factors in the surface and the hydrocarbon interior region of the outer monolayers of the model membranes and becomes the concentration gradient of the quencher between these two regions. The plots of F_o/F vs. $[Q]_T$ of equation 3 are shown in Figures 1 (SPMVTL) and 2 (SPMVPL). The plots of F_o/F vs. $[Q]_T$ of equation 4 are shown in Figures 3 (SPMVTL) and 4 (SPMVPL). From these lines, f_s/f_i can be obtained:

$$f_s/f_i = \frac{S_s K_{12\text{-}AS}}{S_i K_{ONS}} \quad (5)$$

In this equation, S_S and S_i are the slopes of the plots of equations 3 and 4, respectively, and are listed in Table 1. For water-insoluble barbiturates, $[Q]_L \cong [Q]_T$, and equation 5 could be easily reached.

Stern-Volmer plots were also drawn for the quenching of ONS and 12-AS fluorescence by barbiturates in 1:1 mixture of DMSO and THF (Figs. 5 and 6). The slopes of these plots yield the K_{ONS} and $K_{12\text{-}AS}$ values and the values are listed in Table 2. Assuming K_{ONS} and $K_{12\text{-}AS}$ values in bulk solution are not much different from the values in the model membranes, these values can be substituted into equation 5. The values of f_s/f_i calculated by this method are listed in Table 3.

Discussion

The processes that result in fluorescence quenching include: excited state reactions, energy transfer, complex formation and collisional quenching. The quenching in this study, known as collisional or dynamic quenching, results from collisional encounters between fluorophore and a quencher during the lifetime of the excited state (Koblin *et al.*, 1975; Lee and Yun, 1995; Bagatolli and Gratton, 1999; Koubi *et al.*, 1999). Therefore, the extent of fluorescence quenching depends upon the effective concentration of the barbiturates surrounding the fluorophore.

The reasons for calculating the ratio (f_s/f_i) of the concentrations of barbiturates in the surface region to those in the hydrocarbon interior of the outer monolayers of the model membranes by the modified Stern-Volmer equation (equation 5) were: (i) The Stern-Volmer equation cannot be applied to

Table 1. S_s and S_i values in model membranes of total lipid fraction (SPMVTL) and total phospholipid fraction (SPMVPL) extracted from synaptosomal plasma membrane vesicles isolated from rat brain

Barbiturates	SPMVTL[a]		SPMVPL[b]	
	$S_s(M^{-1})$	$S_i(M^{-1})$	$S_s(M^{-1})$	$S_i(M^{-1})$
Thiopental sodium	199.6	95.1	201.3	86.1
Pentobarbital	178.9	17.3	187.2	14.9
Hexobarbital	161.4	13.7	187.0	12.4
Amobarbital	151.2	9.2	153.2	7.6
Phenobarbital	128.1	8.2	123.5	6.0

[a]Values are taken from Figures 1 and 3.
[b]Values are taken from Figures 2 and 4.

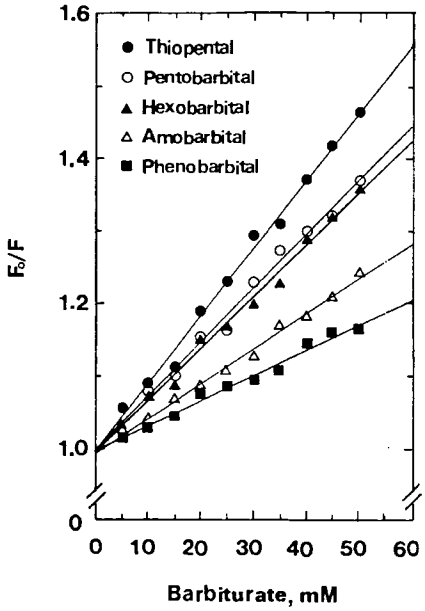

Fig. 5. Stern-Volmer plot of quenching of ONS fluorescence in dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) mixture (1 : 1) by thiopental sodium, pentobarbital, hexobarbital, amobarbital and phenobarbital. Lines were fitted by a least-squares analysis.

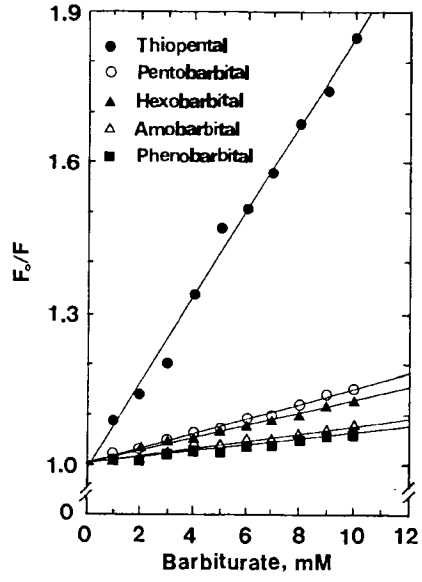

Fig. 6. Stern-Volmer plot of quenching of 12-AS fluorescence in dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) mixture (1:1) by thiopental sodium, pentobarbital, hexobarbital, amobarbital and phenobarbital. Lines were fitted by a least-squares analysis.

heterogeneous conditions. (ii) There is a good possibility that THF and DMSO cannot be completely removed from the suspension of the SPMVTL and SPMVPL in spite of our best efforts. In addition, ONS, 12-AS and barbiturates were still present in the solutions of THF, DMSO and water.

The analysis of the preferential quenching of ONS and 12-AS fluorescence by barbiturates revealed that the penetration of barbiturates into the hydrocarbon interior increased with their lipid solubility. This indicates that pentobarbital, hexobarbital, amobarbital and phenobarbital are predominantly distributed on the surface region of the outer monolayers, while the thiopental sodium is accessible to the hydrocarbon interior of the monolayers of the SPMVTL and SPMVPL. Lee and Yun (1995) also reported that the major distribution region of barbiturates in the SPMV isolated from rat brain was the surface region of the outer monolayer. The results of this study, together with our previous report (Lee and Yun, 1995), demonstrated the interesting phenomenon that barbiturates have a strong tendency to distribute more to the surface region of the outer monolayers of the SPMV, SPMVTL and SPMVPL than to the hydrocarbon interior of the membranes outer monolayers. This may be due to the electrostatic attraction between the surface of the outer monolayer of the membrane, which is positively charged, and the barbiturates, which are negatively charged in aqueous media.

The relative penetrabilities of individual barbiturates into the hydrocarbon interior were identical to a previous study (Lee and Yun, 1995). The degrees of distribution of the barbiturates into the hydrocarbon interior of the monolayer occurred in the following order: thiopental sodium> pentobarbital>hexobarbital>amobarbital>phenobarbital. This order was identical when the barbiturates were clinically used (general anesthetic efficacy). However, the important point is the different degree of penetrability of barbiturates into SPMV, SPMVTL and SPMVPL in terms of the amount of penetration of the drugs into the hydrocarbon interior of the outer monolayers of the membranes. The penetrability of barbiturates in SPMV, SPMVTL and SPMVPL was in the following the order: SPMV>SPMVTL>SPMVPL. According to previous studies (Kang, 1990; Yun *et al.*, 1990b; Yun and Kang, 1992), the relative fluidity of SPMV, SPMVTL and SPMVPL, in relation to the range and the rate of the lateral and the rotational mobility, was in the order of SPMVPL, SPMVTL, SPMV. Thus, it seems likely that there might be a correlation between intrinsic membrane fluidity and the penetrability of the barbiturates into the hydrocarbon interior of the outer monolayers of native and model membranes. The results of this study and a previous study (Lee and Yun, 1995) strongly suggest that the penetrabilities of the barbiturates into the hydrocarbon interior of the outer monolayers are inversely proportional to the intrinsic fluidity of native and model membranes. It is highly probable that the membrane fluidity and the penetrability of drugs to the membrane are directly proportional. In this respect, barbiturates seem to be an exception and the reasons for this can be delineated as follows. The penetrability of barbiturates into the SPMV may be amplified by the presence of proteins (the lipid-protein interactions) which are found to be tightly associated with lipids through covalent or noncovalent bonds. The difference

Table 2. Stern-Volmer constant of quenching of ONS and 12-AS fluorescence by barbiturates in dimethylsulfoxide and tetrahydrofuran mixutre (1 : 1)

Barbiturates	Stern-Volmer Constant (M^{-1})[a]	
	K_{ONS}	$K_{12\text{-}AS}$
Thiopental sodium	9.4	29.6
Pentobarbital	7.5	17.4
Hexobarbital	7.3	16.1
Amobarbital	4.9	13.8
Phenobarbital	3.5	12.0

[a]Values are taken from Figures 5 and 6.

Table 3. Ratio (f_s/f_i) of the concentration of barbiturates in the surface regions to the concentration in the hydrocarbon interiors of the outer monolayers of model membranes of total lipid fraction (SPMVTL) and total phospholipid fraction (SPMVPL) extracted from synaptosomal plasma membrane vesicles isolated from rat brain

Barbiturates	Ratio[a]	
	SPMVTL	SPMVPL
Thiopental sodium	6.6	7.4
Pentobarbital	24.0	29.1
Hexobarbital	26.0	33.1
Amobarbital	46.3	56.8
Phenobarbital	53.6	70.6

[a]The ratio values were obtained from equation 5 where the values of S_s, S_i, K_{ONS} and $K_{12\text{-}AS}$ are those as shown in Tables 1 and 2.

between SPMVTL and SPMVPL in the penetrabilities of barbiturates cannot be fully explained. However, such a phenomenon can be explained by the following mechanisms. Cholesterol is one of the major components in biological membranes, yet the role of cholesterol in membranes is unclear. In native and model membranes, there is evidence that cholesterol is inhomogeneously distributed and creates regions of differing fluidity (Gordon and Mobley, 1985). Although native and model 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). Thus, the penetrability of barbiturates into a hydrocarbon interior may be amplified by cholesterol (cholesterol-gel phase interactions) which is concentrated in the gel-phase regions of SPMV and SPMVTL.

Previous studies showed that barbiturates (thiopental, pentobarbital, hexobarbital, amobarbital and phenobarbital) increased the rate and range of the lateral (Kang, 1990) and rotational (Chung *et al.*, 1992) mobility of the bulk neuronal membrane lipid bilayer (SPMV). In contrast, the other investigators demonstrated that barbiturates decreased the rate and range of the lateral and rotational mobility of phospholipid model membranes (Kang *et al.*, 1992; Yun and Kang, 1992; Yun *et al.*, 1990a). Sweet *et al.* (1987) reported that pentobarbital had a greater increase on the range and rate of the rotational mobility of the outer monolayer than on the inner monolayer of the LM fibroblast membranes. More recently, Kang (1990) demonstrated that thiopental, pentobarbital, hexobarbital, amobarbital and phenobarbital preferentially increased the rotational diffusion of the outer monolayer of SPMV. Additionally, Cho (2000) reported that thiopental, pentobarbital, hexobarbital, amobarbital and phenobarbital preferentially increased the lateral mobility of the neuronal membrane's outer monolayers and all of the barbiturates increased annular lipid fluidity. It was reported in that study that the barbiturates caused protein clustering in SPMV and induced interdigitation of the lipid bilayers. In terms of the magnitude of the increasing effect on the lateral (Cho, 2000) and rotational (Kang, 1990) mobility of neuronal outer monolayers by barbiturates (thiopental, pentobarbital, hexobarbital, amobarbital and phenobarbital), thiopental showed the strongest effect, followed by pentobarbital, hexobarbital, amobarbital and phenobarbital in descending order. The relative penetrabilities of individual barbiturates into the hydrocarbon interior of the outer monolayer of the native (Lee and Yun, 1995) and model membranes (in this study) coincide with the capabilities of the drugs disordering effects on the neuronal membrane's outer monolayer (Kang, 1990; Cho, 2000). It is suggested that the more effective penetration into the hydrocarbon interior of the outer monolayers of the neuronal membranes could result in a higher perturbation of the hydrophobic core of SPMV and possibly a greater general anesthetic activity.

Acknowledgment This paper was supported in part by a grant from the Research Institute for Oral Biotechnology, Pusan National University (1998).

References

Angelova, M. J. and Dimitrov, D. S. (1986) Liposome electroformation. *Faraday Discuss. Chem. Soc.* **81**, 303-311.

Angelova, M. J., Soléau, S., Meléard, Ph., Faucon, J. F. and Bothorel, P. (1992) Preparation of giant vesicles by external AC fields. Kinetics and application. *Prog. Colloid Polym. Sci.* **89**, 127-131.

Bagatolli, L. A. and Gratton, E. (1999) Two photon fluorescence microscopy of coexisting lipid domains in giant unilamellar vesicles of binary phospholipid mixtures. *Biophys. J.* **78**, 290-305.

Bartlett, G. R. (1959) Phosphorous assay in column chromatography. *J. Biol. Chem.* **234**, 466-468.

Bradley, R. A., Martin, W. G. and Schneider, H. (1973) Dynamic behavior of fluorescent probes in lipid bilayer model membranes. *Biochemistry* **12**, 268-275.

Cho, E. S. (2000) Barbiturates alter structural parameters of synaptosomal plasma membrane vesicles isolated from bovine cerebral cortex. Ph. D. Thesis. Pusan National University.

Chung, Y. Z., Shin, Y. H., Choi, C. H., Park, H. S., Koh, Y. S. and Yun, I. (1992) Effects of barbiturates on the rotational relaxation time of 1,6-diphenyl-1,3,5-hexatriene in native and model membranes. *Arch. Pharm. Res.* **15**, 298-303.

Dimitrov, D. S. and Angelova, M. J. (1987) Lipid swelling and liposome formation on solid surfaces in external electric fields. *Prog. Colloid Polym. Sci.* **73**, 48-56.

Frenkel, C., Duch, D. S. and Urban, B. W. (1990) Molecular actions of pentobarbital isomers on sodium channels from human brain cortex. *Anesthesiology* **72**, 640-649.

Gordon, L. M. and Mobley, P. W. (1985) in Membrane Fluidity in Biology, Aloia, R. C. and Boggs, J. (eds.), Vol. 4, pp. 1-49, Academic Press, New York.

Hitzemann, R. J., Harris, R. A. and Loh, H. H. (1985) in Phospholipids and Cellular Regulation, Kuo, J. F. (ed.) Vol. 1, pp. 97-130, CRC Press, Boca Raton.

Huang, T. C., Chen, C. P., Wefler, V. and Raftery, A. (1961) A stable reagent for the Liebermann-Burchard reaction. *Anal. Chem.* **33**, 1405-1407.

Kang, J. S. (1990) Effects of barbiturates on fluidity of native and model membranes. Ph. D. Thesis. Pusan National University.

Kang, J. S. Chung, Y. Z., Cho, G. J., Byun, W. T. and Yun, I (1992) Membrane-ordering effects of barbiturates on pure phospholipid model membranes. *Arch. Pharm. Res.* **15**, 196-203.

Kang, J. S., Choi, C. M. and Yun, I. (1996) Effects of ethanol on the lateral and rotational mobility of plasma membrane vesicles isolated from cultured mouse myeloma cell line Sp2/0-Ag14. *Biochim. Biophys. Acta* **1281**, 157-163.

Koblin, D. D., Page, W. D. and Wang, H. H. (1975) The penetration of local anesthetics into the red blood cell membrane as studied by fluorescent quenching. *Arch. Biochem. Biophys.* **171**, 176-182.

Koubi, L., Tarek, M., Klein, M. L. and Scharf, D. (1999) Distribution of halothane in a dipalmitoylphosphatidylcholine bilayer from molecular dynamics calculations. *Biophys. J.* **78**, 800-811.

Lasic, D. D. (1988) The mechanism of vesicle formation. *Biochem. J.* **256**, 1-11.

Lee, A. G. (1976) Interaction between phospholipids and barbiturates. *Biochim. Biophys. Acta* **455**, 102-108.

Lee, B. W. and Yun, I. (1995) The region of distribution of barbiturates in synaptosomal plasma membrane vesicles isolated from rat brain as studies by fluorescence quenching. *Korean J. Pharmacol.* **31**, 271-278.

Lesslauer, W., Chain, J. E. and Blasie, J. K. (1973) X-ray diffraction studies of lecithin bimolecular leaflets with incorporated fluorescent probes. *Proc. Natl. Acad. Sci. USA* **69**, 1499-1503.

Madeira, V. M. C. and Antunes-Madeira, M. C. (1976) Lipid composition of biomembranes: a complete analysis of sarcoplasmic reticulum phospholipids. *Cienc. Biol. (Coimbra)* **2**, 265-291.

Menger, F. M. and Keiper, J. S. (1998) Chemistry and physics of giant vesicles as biomembrane models. *Curr. Opin. Chem. Biol.* **2**, 726-732.

Rall, T. W. (1991) in Goodman and Gilman's the Pharmacological Basis of Therapeutics, Gilman, A. G., Rail, T. W., Nies, A. S. and Taylor, P. (eds.) Vol. 1, pp. 358-360, Pergamon Press, Tokyo.

Sweet, W. D., Wood, W. G. and Schroeder, F. (1987) Charged anesthetics selectively alter plasma membrane order. *Biochemistry* **26**, 2827-2835.

Yun, I. and Kang, J. S. (1990) The general lipid composition and aminophospholipid asymmetry of synaptosomal plasma membrane vesicles isolated from bovine cerebral cortex. *Mol. Cells* **1**, 15-20.

Yun, I. and Kang, J. S. (1992) Effects of barbiturates on transbilayer fluidity domains of phospholipid model membrane monolayers. *Korean J. Pharmacol.* **28**, 103-114.

Yun, I., Kim, Y. S., Yu, S. H., Chung, I. K, Kim, I. S., Baik, S. W., Cho, G. J., Chung, Y. Z., Kim, S. H. and Kang, J. S. (1990a) Comparison of several procedures for the preparation of synaptosomal plasma membrane vesicles. *Arch. Pharm. Res.* **13**, 325-329.

Yun, I., Kim, H. I., Hwang, T. H., Kim, J. R., Kim, I. S., Chung, Y. Z., Shin, Y. H., Jung, H. O. and Kang, J. S. (1990b) Effects of barbiturates on the fluidity of phosphatidylethanolamine model membranes. *Korean J. Pharmacol.* **26**, 209-217.

Yun, I., Lee, S. -H. and Kang, J. S. (1994) The effect of ethanol on lateral and rotational mobility of plasma membrane vesicles isolated from cultured Mar 18.5 Hybridoma cells. *J. Membrane Biol.* **138**, 221-227.