The Penetration of n-Alkanols into Model Membranes of Phospholipids Extracted from Brain Membranes

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= 국문초록 =

n-Alkanols가 인지질들로서 제제한 인공세포막에서의 침투정도

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소의 신선한 대뇌피질로부터 synaptosomal plasma membrane vesicles(SPMV)를 분리한 후 이 SPMV로부터 추출한 모든 인지질들로서 제제한 인공세포막(SPMVPL)에서의 n-alkanols 침투 정도를 형광 probe를 이용한 형광 소광법을 통하여 검색하였다. n-alkanols는 SPMVPL 외부 단층(outer monolayer)의 표면에 주로 분포하되 그 탄소수에 비례하여 소수성 부위에 분포되는 양이 증가되는 경향을 나타내었다. methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol 및 1-decanol은 SPMVPL 외부 단층의 표면(친수성 부위)에 분포되는 것이 소수성 부위에 분포되는 것에 비하여 각각 432.4, 208.9, 125.6, 88.2, 19.3, 7.9, 2.6, 1.0, 0.42, 1.36배가 되었다. 1-decanol은 C_{10}인데도 불구하고 C_8인 1-octanol에 비하여 적은 양이 소수성 부위에 침투 분포된다는 것이 확인되었다. n-alkanols의 침투에 대하여 저자등이 이미 보고한 바 있는 SPMV 및 SPMVTL (cholesterol+phospholipids)의 경우보다도 본 연구에서의 SPMVPL의 경우가 현저하게 많은 양이 소수성 부위로 침투 분포된다는 것도 확인되었다.

Key Words: n-Alkanols, Penetration site, Phospholipid model membranes, Fluorescence probe technique, Modified Stern-Volmer equation

INTRODUCTION

It has been generally accepted that n-alkanols are members of membrane perturbing agents that fluidize native and model membranes[1]. Changes in membrane fluidity are known to be linked to alterations in physiological processes of the cell membrane like carrier-mediated transport, activities of membrane bound enzymes, receptor binding, phagocytosis, endocytosis, depolarization dependent exocytosis, cytotoxicity, and cell growth[2]. Membrane fluidity appears to change during development, aging, and drug therapy of cultured cells[3~5]. 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+}, 5'nucleo-

tidase, acetylcholinesterase, and adenylate cyclase; the mitochondrial electron transport chain; and ion channels such as those for Caion[6].

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[7]. Still, a large, diverse collection of physiological agonists produces the alterations in membrane fluidity as well as their specific ligand-receptor interaction[8].

At the present time, the exact mechanism(s) of action for ethanol and related alkanols in the central nervous system is unclear. The present study on synthetic lipid systems is included to extent that it contributes to an understanding of the behavior of naturally occurring lipid species in the native membranes. The investigation of the primary site of action of n-alkanols can provide a basis for studying the mechanism of action of these drugs. We investigated the penetration of n-alkanols into model membranes of phospholipids(SPMVPL) extracted from synaptosomal plasma membrane vesicles(SPMV) employing two fluorescent probes N-octadecyl naphthyl-2-amine-6-sulfonic acid(ONS) and 12-(9-anthroyl) stearic acid(AS). The analysis of preferential quenching of these probes by n-alkanols revealed the relative accessibility of these drugs to the hydrocarbon region of the outer monolayers of SPMVPL lipid bilayer structures.

METHODS

1) Materials

The fluorescent probe, 12-(9-anthroyl) stearic acid(AS), was purchased from Sigma Chemical Co.(St. Louis, MO, USA). The other fluorescent probe, N-octadecyl naphthyl-2-amine-6-sulfonic acid(ONS) was synthesized by Dr. Yun(Department of Dental Pharmacology and biophysics, College of Dentistry, Pusan National University) and was kindly donated to us. 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. Water was double-distilled.

2) Membrane preparations

The SPMV were isolated from bovine cerebral cortex and characterized by the formerly reported method in our laboratory[9]. Lipids were extracted from the SPMV as described previously[10]. The cholesterol to phospholipid molar ratio was 0.60 ± 0.01. The individual phospholipid classes were separated by thin layer chromatography and quantitated by measuring the amounts of inorganic phosphate after hydrolysis of the phospholipids at 180℃ in 70% $HClO_4$[10]. Phospholipids were composed of phosphatidylcholine(PC, 43%), phosphatidylethanolamine (PE, 36%), phosphatidylserine(PS, 13%), sphingomyeline(SP, 4%), phosphatidylinositol(PI, 3%), and lysophosphatidylcholine(LPC, 1%). In the present study, gangliosides might have been lost during the isolation of the phospholipids and neurtal lipids. Large unilamellar liposomes (SPMVPL; 0.7 mg of phospholipids/ml, pH 7.4) were prepared by the method described earlier[11].

3) Fluorescence measurements

These measurements were identical to procedures of our previous studies[12,13].

RESULTS AND DISCUSSION

Fluorescence quenching in homogeneous solu-

tion has been described in terms of the Stern-Volmer equation:

$$F_o/F = 1 + k_q t_o[Q] = 1 + K[Q] \quad \cdots\cdots \text{equation 1}$$

In quencher, respectively, k_q is the bimolecular quenching constant, t_o is the lifetime of the fluorophore in the absence of quencher, [Q] is the concentration of quencher, and $K = k_q t_o$ 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 SPMVPL. For water-soluble n-alkanols, the concentration in the aqueous phase is excessively larger than that in the lipid bilayer at pH 7.4 Consequently, $[Q]_L = P[Q]_T$ and equation 1 can be modified:

$$F_o/F = 1 + fKP[Q]_T \quad \cdots\cdots \text{equation 2}$$

In this equation $[Q]_L$ and $[Q]_T$ are the concentrations of the quencher in the outer monolayers of the SPMVPL lipid bilayer structures and the total concentration of the quencher in the system, respectively, P is the partition coefficient, and f is a regional correction factor for uneven distribution of the quencher between the surface area and the interior area in the outer monolayers of the SPMVPL lipid bilayer structures. Studies have shown that the most probable position of the naphthalene sulfonate moiety of the ONS molecule is at the surface of membranes' outer monolayer, and the anthroyl moiety of the AS molecule is most likely located in the hydrocarbon region of the outer monolayers of the membrane lipid bilayer structures[1-4]. Hence, ONS or AS quenching in the SPMVPL gives the following:

$$F_{so}/F = 1 + f_s K_{ONS} P[Q]_T \quad \cdots\cdots \text{equation 3}$$

$$F_o/F = 1 + f_i K_{AS} P[Q]_T \quad \cdots\cdots \text{equation 4}$$

In these equations K_{ONS} and K_{AS} are the Stern-Volmer constants of the fluorescence quenching of ONS and AS by the quencher, respectively. f_s/f_i is the ratio of the regional correction factors in the surface area and the interior area of the outer monolayers of the SPMVPL lipid bilayer structures, and becomes the concentration gradient of the quencher between these two areas.

The plots of F_o/F vs. $[Q]_T$ of equations 3 is shown in Fig. 1. and Fig. 2. And the plots of F_o/F vs. $[Q]_T$ of equations 4 is shown Fig. 3, 4, and Fig. 5. From this line, f_s/f_i can be obtained:

$$f_s/f_i = \frac{S_s K_{AS}}{S_i K_{ONS}} \quad \cdots\cdots \text{equation 5}$$

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 n-alkanols, $[Q]_L = [Q]_T$ and equation 5 could be easily reached.

Our previous study, the Stern-Volmer plots were drawn for quenching of ONS and AS fluorescence by n-alknols in 1: 1 mixture of DMSO

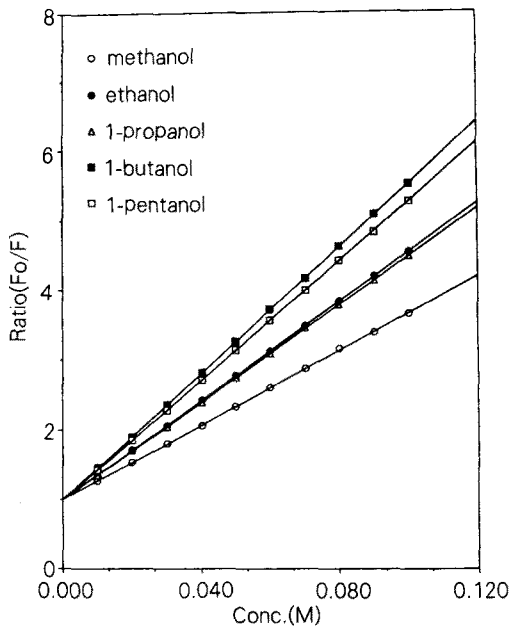

Fig. 1. Stern-Volmer plot of quenching of ONS fluorescence in SPMVPL by methanol, ethanol, 1-propanol, 1-butanol and 1-pentanol. Lines were fitted by a least-squares analysis.

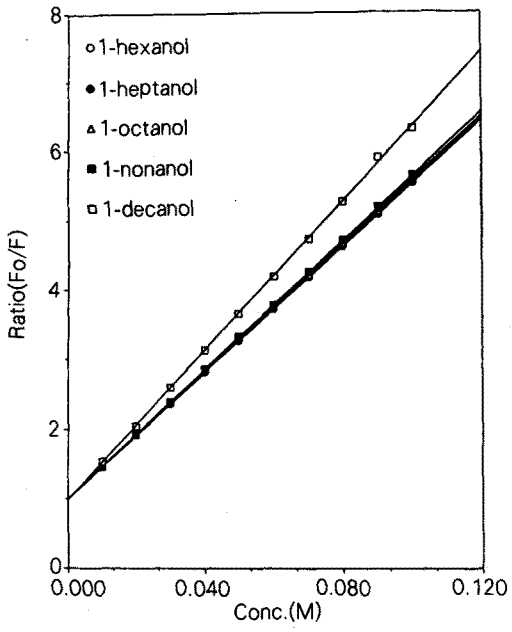

Fig. 2. Stern-Volmer plot of quenching of ONS fluorescence in SPMVPL by 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol and 1-decanol. Lines were fitted by a least-squares analysis.

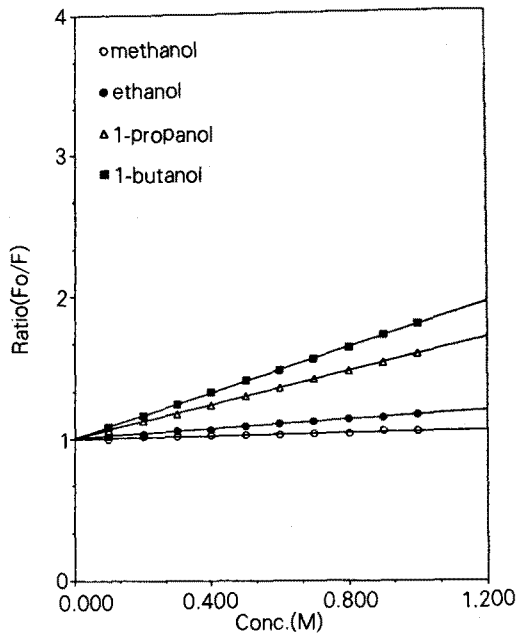

Fig. 3. Stern-Volmer plot of quenching of AS fluorescence in SPMVPL by methanol, ethanol, 1-propanol, and 1-butanol. Lines were fitted by a least-squares analysis.

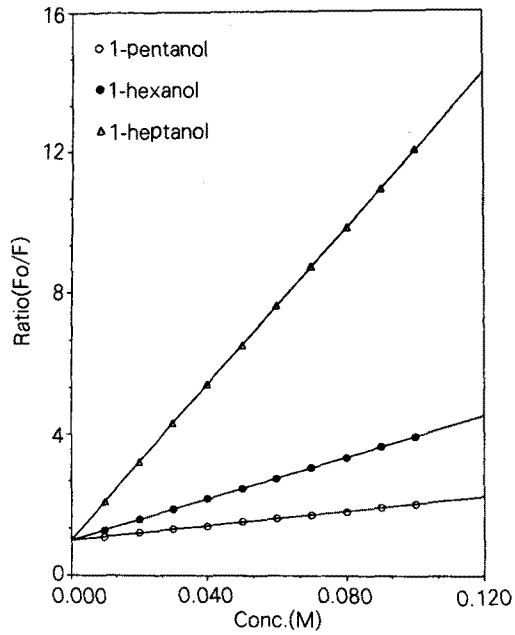

Fig. 4. Stern-Volmer plot of quenching of AS fluorescence in SPMVPL by 1-pentanol, 1-hexanol and 1-heptanol. Lines were fitted by a least-squares analysis.

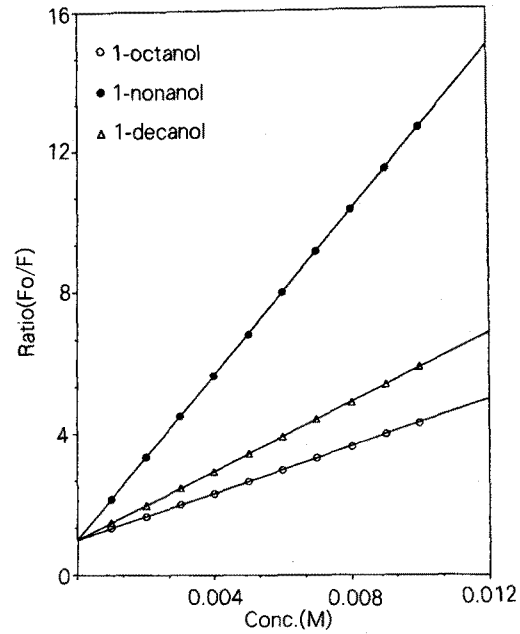

Fig. 5. Stern-Volmer plot of quenching of AS fluorescence in SPMVPL by 1-octanol, 1-nonanol and 1-decanol. Lines were fitted by a least-squares analysis.

Table 1. Stern-Volmer Constant of Quenching of ONS and AS Fluorescence by n-alkanols in SPMVPL Liposomes

n-alkanols	Stern-Volmer constant(1/M)[a]	
	ONS(S_s)	AS(S_i)
methanol	26.60	0.04
ethanol	35.40	0.16
1-propanol	34.80	0.58
1-butanol	45.20	0.79
1-pentanol	42.60	10.30
1-hexanol	45.60	29.20
1-heptanol	45.30	110.40
1-octanol	45.90	328.10
1-nonanol	46.40	1160.90
1-decanol	53.20	483.60

[a]; Values are taken from Fig. 1, 2, 3, 4 and 5.

Table 2. Stern-Volmer Constant of Quenching of ONS and AS Fluorescence by n-alkanols in Dimethylsulfoxide and Tetrahydrofuran Mixture(1 : 1)

n-alkanols	Stern-Volmer constant(1/M)[a]	
	K_{ONS}	K_{AS}
methanol	0.20	0.13
ethanol	0.38	0.36
1-propanol	0.52	0.76
1-butanol	0.68	1.50
1-pentanol	0.82	3.83
1-hexanol	1.02	5.18
1-heptanol	1.26	8.03
1-octanol	2.00	14.28
1-nonanol	2.92	28.07
1-decanol	5.14	63.33

Data were adapted from our previous study(reference number, 12).

and THF[12]. The slopes of these plots yield the K_{ONS} and K_{AS} values, and the values are listed in Table 2. Assuming K_{ONS} and K_{AS} values in bulk solution are not much different from the values in the SPMVPL, these values can be substituted into equation 5. The values of f_s/f_i calculated by this method are listed in Table 3.

Table 3. Ratio of the Concentration of n-alkanols in the Surface Area to the Concentration in the Hydrocarbon Interior of the Lipid Bilayer of SPMVPL Liposomes(f_s/f_i)

n-alkanols	Ratio[a]
Methanol	432.4
Ethanol	208.9
1-propanol	125.6
1-butanol	88.2
1-pentanol	19.3
1-hexanol	7.90
1-heptanol	2.60
1-octanol	1.00
1-nonanol	0.42
1-decanol	1.36

[a]; The ratio values were obtained from equation(5) where the values of K_{ONS}, K_{AS}, S_s and S_i were those as shown in tables 1 and 2.

Collisional quenching in this study is due to the interaction with fluorescent probe and hydroxyl group of n-alkanols. The extent of fluorescence quenching depends upon the effective concentration of the n-alkanols surrounding the fluorophore. In accordance to the analysis of preferential quenching of ONS and AS fluorescence by n-alkanols the penetration of the drugs to hydrocarbon interior increased directly proportionlal to their number of carbons(except 1-decanol). The analysis for uneven distribution of n-alkanols in the surface and hydrocarbon region of the outer monolayers that methanol is predominantly distributed on the surface area, while 1-nonanol has a greater accessibility to the hydrocarbon interior of the monolayer of the SPMVPL lipid bilayer structures. The cut-off phenomenon was reached at 1-decanol, where further increase in hydrocarbon length resulted in a decrease in the amount of penetration(Table 3). This indicates that the penetra-

tion in the hydrocarbon interior of the outer monolayers of the SPMPL lipid bilayer structures of the n-alkanols increases with an increase lipid solubility(except 1-decanol). We reported that the major distribution area of n-alkanols in the SPMV and SPMVTL was the surface of the outer monolayers of the membrane lipid bilayer structures[12,13]. Our study extended from the above experiments demonstrated a very interesting phenomenon in the SPMV, SPMVTL and SPMVPL. This result is in agreement with those of our studies, indicating n-alkanols have a larger distribution on the surface area of the outer monolayers of the SPMV, SPMVTL and SPMVPL than on the hydrocarbon region of the membranes' monolayers. The important point is the different abilities of n-alkanols among SPM, SPMVTL and SPMVPL in terms of the amount of penetration of the anesthetics into the hydrocarbon region of the outer monolayers of the membrane bilayers. According to previous studies[15~17], the relative fluidity of SPMV, SPMVTL and SPMVPL in relation to range and rate of lateral and rotational mobility were in the order of SPMVPL, SPMVTL. SPMV. There is an excellent correlation between intrinsic membrane fluidity and penetrability of the n-alkanols into SPMV, SPMVTL and SPMVPL. The results of this study strongly suggest that penetrability of the n-alkanols to hydrocarbon region of outer monolayer increase directly proportionate to intrinsic fluidity of native and model membranes. Comparing the penetrability of n-alkanols in SPMV, SPMVTL and SPMVPL, SPMVPL was the most permeable. Then was SPMVTL, SPMV being the least. The reasons of the different penetrability of the n-alkanols can be analyzed as follows: (i) the penetrability of n-alkanols into the SPMV may be inhibited by the presence of proteins(the lipid-protein interactions) which are found to be tightly associated with lipids through covalent or noncovalent bonds, (ii) gangliosides may have a significant role in the penetrability of the n-alkanols, and (iii) the differences between SPMVTL and SPMVPL in the penetrabilities of n-alkanols cannot be fully explained.

However, such phenomenon can be explained by following mechanisms. Cholesterol is one of the major components in biological membranes, yet the role of cholesterol in membranes is not clear enough. In native and model membranes, there is evidence that cholesterol is inhomogeneously distributed and creates regions of differing fluidity[18]. 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[19]. Thus, the penetrability of n-alkanols may be inhibited by the cholesterol(cholesterol-gel phase interactions) which are concentrated in the gel-phase regions. To the best of our knowledge, the results presented herein are the first to demonstrate the penetration site of n-alkanols into SPMVPL.

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