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Atypical Langmuir adsorption of inhalation anesthetics on phospholipid monolayer at various compressional states: difference between alkane-type and ether-type anesthetics

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Adsorption of chloroform, halothane, enflurane and diethyl ether on the air/water interface was compared with adsorption on the dipalmitoylphosphatidylcholine monolayer, spread on the air/water interface, at four compressional states; 88.5, 77.0, 66.5 and 50.5 Å^2 surface area per phosphatidylcholine molecule. Anesthetics were administered from the gas phase. The affinities of these agents to the phosphatidylcholine monolayer varied according to the state of the monolayer. Chloroform and halothane showed a stronger affinity to the highly compressed phosphatidylcholine monolayer (50.5 Å^2) than to the expanded monolayer (88.5 Å^2) or to the air/water interface without the monolayer. Diethyl ether behaved in reverse; a stronger affinity to the expanded monolayer was exhibited than to the compressed monolayer. Enflurane showed the highest affinity to the intermediately compressed monolayer (77.0 Å^2). The adsorption isotherm of anesthetics to the monolayer was characterized by atypical Langmuir-type, in which available number of binding sites changed when anesthetics were adsorbed. The mode of adsorption onto the monolayer was dissimilar to adsorption onto air/water interface, where adsorption followed the Gibbs surface excess. A theory is presented to explain the above differences. The adsorbed anestheic molecules do not stick to phosphatidylcholine molecules but penetrate into the monolayer lattice and occupy the phosphatidylcholine sites at the interface. Quantitative agreement between the theory and the experimental data was excellent. For the monolayer at 50.5 Å^2 compression, the changes in the transfer free energy accompanying the anesthetic adsorption from the gas phase to the monolayer were in the order of chloroform > halothane > enflurane > diethyl ether, in agreement with the clinical potencies.

Introduction

Lipid monolayers, spread at the air/water interface, have been used successfully as a model for cell membranes. In contrast to many reports concerning monolayer penetration from the aqueous phase by water-soluble drugs, reports on penetration from the gas phase by inhalation anesthetics are relatively few. Dean et al. [1] reported that nitrous oxide, diethyl ether and chloroform increased the surface pressure of lipid monolayers, such as stearic acid, palmitic acid and sterol. Because the saturated vapors of diethyl ether and chloroform were used, the anesthetic partial pressures exceeded clinical concentrations, and analy-

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sis of the obtained data to correlate to anesthesia mechanisms was not attempted.

Clements and Wilson [2] used monolayers of stearic acid, cholesterol and phosphatidylcholine, and analyzed actions of inhalation anesthetics upon the surface tension. They reported that the affinities of anesthetics to the lipid monolayer correlated with their clinical potencies. They concluded that equal numbers of anesthetic molecules bound to the monolayer at the same depth of anesthesia. Ueda et al. [3] reported that inhalation anesthetics (chloroform, methoxyflurane, halothane, enflurane and fluroxene) expanded the surface area of a dipalmitoylphosphatidylcholine monolayer dose-dependently, and the magnitude of expansion showed a constant value of about 0.5% at clinical partial pressures; equal magnitude of expansion at equal depth of anesthesia.

Shibata et al. [4] analyzed anesthetic adsorption on the air/water interface and reported that the entropy change differed for the adsorption of ether-type anesthetics (diethyl ether and enflurane) and the alkane type (chloroform and halothane). Chloroform and halothane were estimated to lose one degree of freedom of the rotation by adsorption, whereas enflurane and diethyl ether lose two and three degrees, respectively. When these losses were taken into account, the molar affinity potentials of the anesthetics to the interface inversely correlated to the anesthetic potency with a correlation coefficient of 0.999.

The present report analyzed the anesthetic interaction with a dipalmitoylphosphatidylcholine monolayer, spread at the air/water interface, in comparison with their adsorption on vacant water surface. To investigate the effect of the state of the phosphatidylcholine monolayer upon anesthetic adsorption, the study was performed at four different compressional states of the phosphatidylcholine monolayer.

Method

Synthetic dipalmitoylphosphatidylcholine (1,2-dihexadecanoyl-*sn*-glycero-3-phosphorylcholine) was obtained from Sigma, and was homogeneous by thin-layer chromatography. Chloroform and diethyl ether were obtained from Spectrum Chemical (Redondo Beach, CA) and J.T. Baker (Phillipsburg, NJ), respectively. Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) and enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) were gifts from Ayerst Labs (New York) and Ohio Chemical Products (Madison, WI).

Water was purified by distillation followed by passage through two mixed-bed ion-exchange columns, an activated charcoal column and an ultrafilter in a Millipore system (Bedford, MA). The specific resistance of the obtained water was maintained above 16 Mohm · cm, and the absence of surface-active impurities was checked by dynamic surface tension measurement as previously described [4].

The surface tension was measured by Langmuir's hanging-plate method with a glass-plate and a high sensitivity force transducer (Shinkoh Co., Kanagawa, Japan) in an Acoma surface tensiometer (Tokyo, Japan). The Teflon-coated stainless-steel trough ($250 \times 50 \times 10$ mm) was equipped with a Teflon compression bar driven by a servomotor. The position of the bar was measured by a follow-up linear potentiometer. The trough temperature was controlled by circulating water from a constant temperature water-bath. All measurements were performed at 25.0 ± 0.2°C. The surface tensiometer was enclosed in a plexiglass chamber of about 2.5 l internal volume.

The anesthetics were vaporized in the copper-kettle of an anesthesia machine and diluted with nitrogen gas. The partial pressures of the anesthetics were estimated from the kettle temperature and the flows of nitrogen gas, and were confirmed by a Shimadzu gas-chromatograph (Columbia, MD). The anesthetic vapor was administered into the surface-tension chamber at a flow-rate of about 4 l/min from one side and discarded into an anesthetic-scavenging system from the other side.

Dipalmitoylphosphatidylcholine was dissolved in a hexane/ethanol mixture (9 : 1, v/v), and was spread onto the air/water interface by a microsyringe. The initial spreading area was typically 62.58 cm^2 and the volume of the spreading solution was 0.2 ml.

Results and Discussion

Surface pressure-area (π–A) curve for the dipalmitoylphosphatidylcholine monolayer is a typi-

cal expanded-type and undergoes two-dimensional condensation at about 10.5 $mN \cdot m^{-1}$ (see, for instance, Phillips and Chapman [5]). The monolayer at various compressional states represents different degrees of molecular freedom (or order), resulting from the intermolecular forces in the monolayer and between the monolayer and subphase.

Adsorption of inhalation anesthetics on the phosphatidylcholine monolayer was studied at four compressional states; the initial surface pressure was 4.5, 8.5, 11.5 and 17.5 $mN \cdot m^{-1}$ (the area occupied by a phosphatidylcholine molecule was 88.5, 77.0, 66.5 and 50.5 $Å^2$, respectively). The values of initial surface parameters, i.e., surface pressure, area per phosphatidylcholine molecule, and compressibility, are shown in Table I. The value of the monolayer compressibility shows a maximum at the surface pressure of 11.5 $mN \cdot m^{-1}$, corresponding to fluctuations at the transition region of the monolayer.

Fig. 1 shows the relationship between the surface pressure and the anesthetic vapor pressure (π–P) for chloroform (A), halothane (B), enflurane (C) and diethyl ether (D) in the presence and absence of the phosphatidylcholine monolayer at the air/water interface. The standard deviation of the surface-pressure data was small. The largest deviation was found with diethyl ether adsorption onto the most compressed monolayer, and was 0.11 $mN \cdot m^{-1}$. In most cases, the standard deviations were within 0.02 $mN \cdot m^{-1}$. For this reason, the error bars were omitted.

The increment of surface pressure caused by adsorption of the alkane-type anesthetics, chloroform and halothane, was larger in highly compressed monolayers. Their adsorption on the monolayer-free air/water interface was less than on the monolayer. The effect of the ether-type anesthetics, enflurane and diethyl ether, on the surface pressure differed from the effect of the alkane-type anesthetics, chloroform and halothane. The increment of surface pressure by diethyl ether decreased according to the increase of the initial surface pressure.

When the inverse of the surface pressure, $1/\pi$, was plotted against the inverse of the anesthetic partial pressure, $1/P$, all π–P data were transformed into linear lines, with negative intercepts at $1/\pi$ axis (Fig. 2A–D). If the intercept had a positive value, the adsorption is characterized by the well-known Langmuir-type, i.e., the monolayer has certain saturable adsorption sites for anesthetic molecules. The present result of negative intercepts for all four anesthetics implies that the mode of anesthetic adsorption of phosphatidylcholine monolayer differs from the typical Langmuir adsorption isotherm. As will be shown later in the theory, the negative intercept is interpreted to indicate that the adsorbed anesthetic molecules do not bind onto the monolayer but penetrate into the phosphatidylcholine lattice and occupy the lattice sites.

In contrast, linearity of the data on the adsorption onto the water surface in the double-reciprocal plots was inferior compared to the adsorption onto the phosphatidylcholine monolayer. The intercept on the $1/\pi$ axis was positive for chloroform and halothane and negative for enflurane and diethyl ether. Apparently, the mode of anesthetic adsorption onto the air/water interface is different from that onto the phosphatidylcholine monolayer.

We propose the following model to explain the excellent linearity of the $1/\pi$ versus $1/P$ plots of anesthetic adsorption on the phosphatidylcholine monolayer, and the poor linearity for their adsorption on the air/water interface. First, we consider the case of the anesthetic adsorption on the phosphatidylcholine monolayer. Although the phosphatidylcholine molecules in a monolayer are not localized in the fixed lattice points and are mobile, we assume the number, N_s of the lattice sites in

TABLE I

MEAN VALUES OF SURFACE PARAMETERS OF MONOLAYER IN THE ABSENCE OF INHALATION ANESTHETICS

π_i, initial surface pressure; A_0, area per phosphatidylcholine molecule; δ, compressibility.

π_i ($mN \cdot N^{-1}$)	A_0 ($Å^2$/molecule)	$1/A \cdot 10^{-14}$ (molecule/cm^2)	δ (10^5 N^{-1})
4.5	88.5	1.13	0.0350
8.5	77.0	1.30	0.0382
11.5	66.5	1.50	0.0851
17.5	50.5	1.98	0.0264

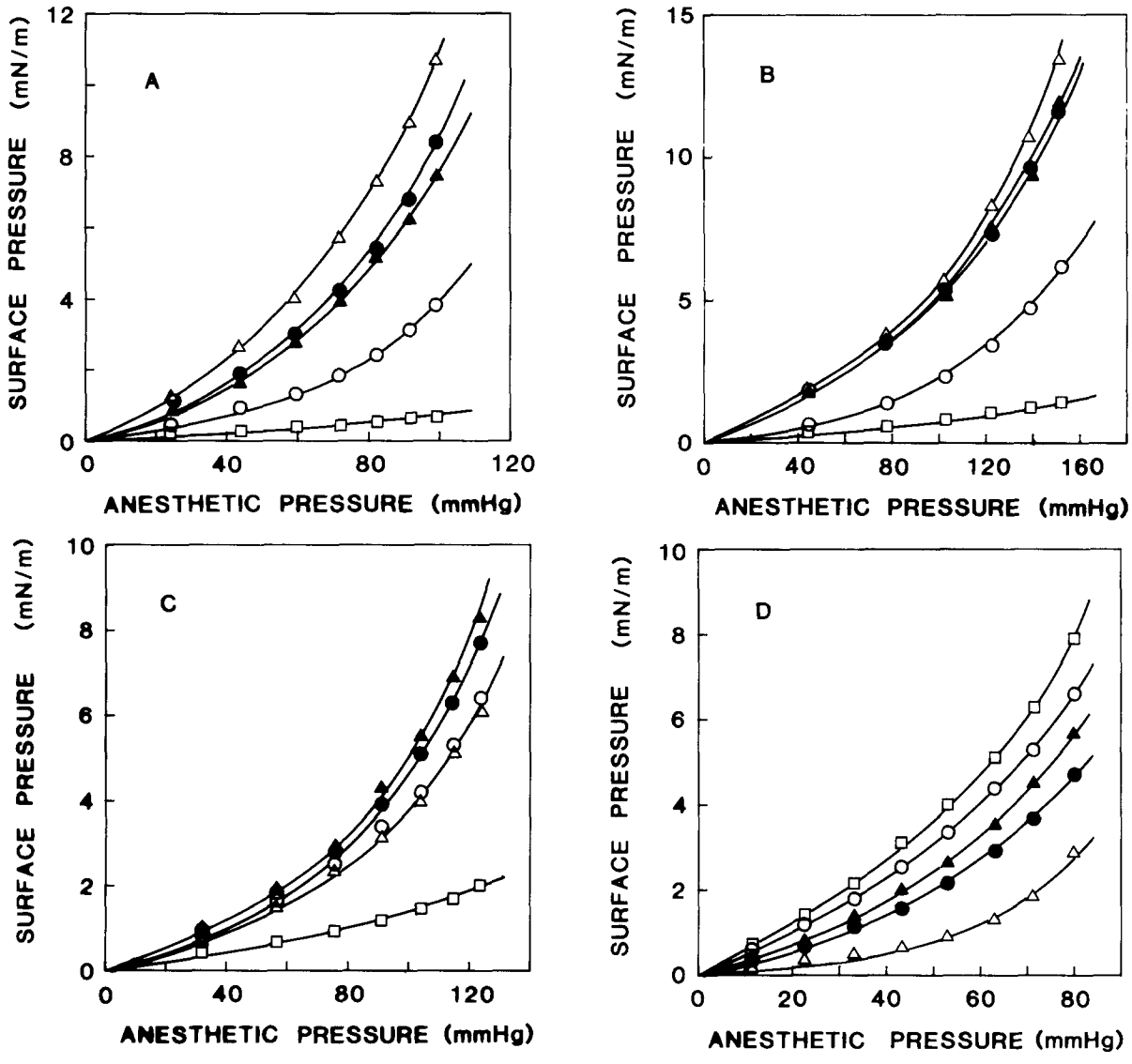

Fig. 1. Surface pressure versus anesthetic pressure ($\pi - P$) curves for adsorption of inhalation anesthetics. Chloroform (A), halothane (B), enflurane (C) and diethyl ether (D) on phosphatidylcholine monolayer and on water surface. Initial monolayer surface pressure: (○) 4.5 mN·m^{-1}, (▲)8.5 mN·m^{-1}, (●) 11.5 mN·m^{-1}, (△) 17.5 mN·m^{-1}, and (□) without monolayer. Errors were within the size of the symbol.

which phosphatidylcholine molecules reside. Let a number, n, of the total anesthetic molecules, n_A, be adsorbed on the monolayer, and the remaining $(n_A - n)$ molecules be in the gas phase in contact with the monolayer.

We postulate that the total lattice points of phosphatidylcholine and anesthetics become $(N_s + n)$ instead of N_s. If one lets this number be N_s, the adsorption becomes of the typical Langmuir-type. Physically, the present model means that the adsorbed anesthetics occupy their own sites, and have no previously specified number of adsorption sites in the monolayer. This is consistent with the fact that all four monolayer states for anesthetic adsorption are in the mobile form and not in the rigid form. According to the above assumption,

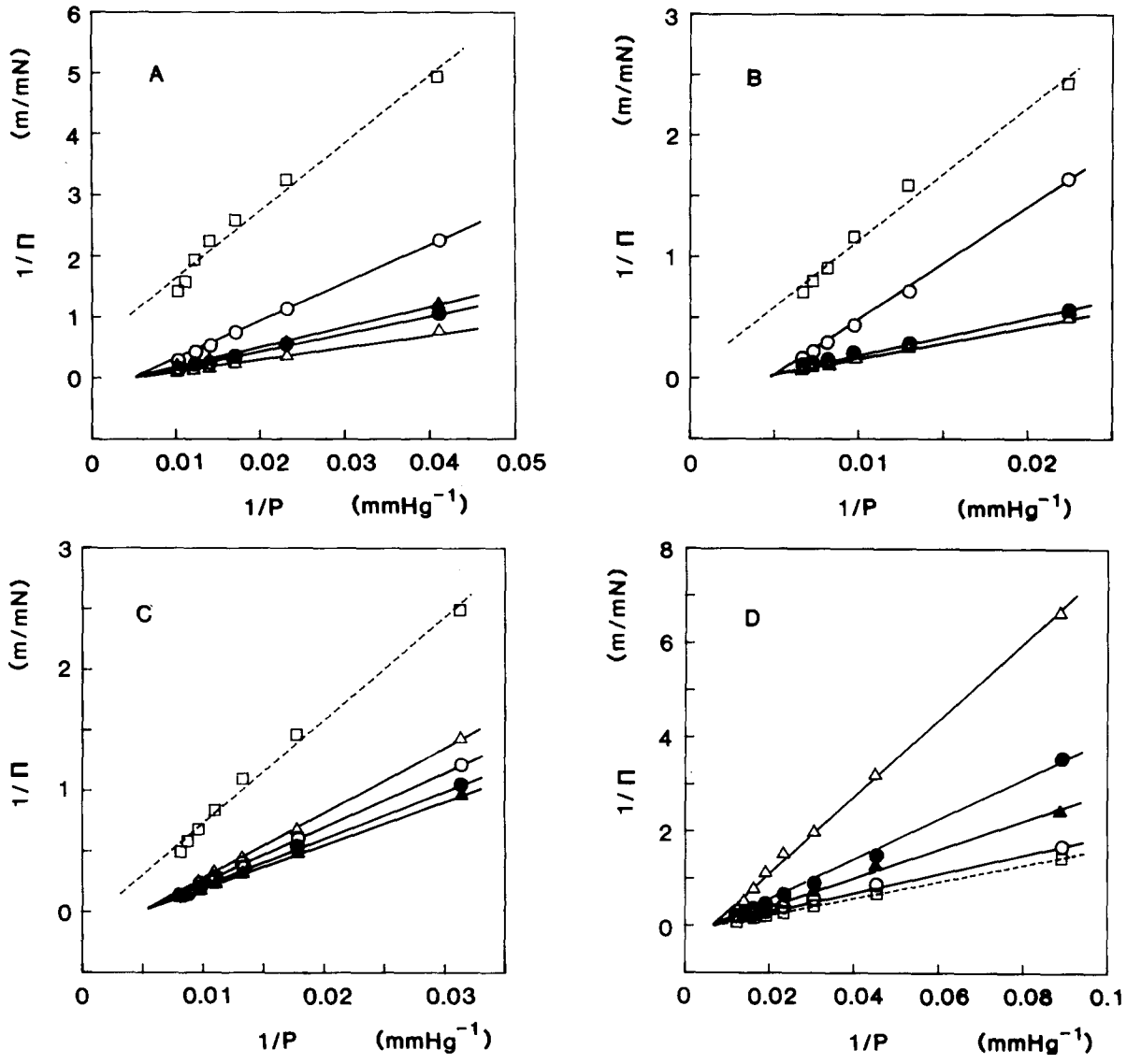

Fig. 2. Double-reciprocal ($1/\pi$ vs. $1/P$) plots for adsorption of inhalation anesthetics on phosphatidylcholine monolayer and on water surface. Chloroform (A), halothane (B), enflurane (C) and diethyl ether (D). Symbols for initial monolayer surface pressure are the same as in Fig. 2.

the partition function, Z, of the total system is written:

$$Z = \binom{N_s + n}{n} (pf)_a^n \frac{1}{(n_A - n)!} (pf)_b^{n_A - n} \exp(-\gamma_{PC} A/kT) \quad (1)$$

$$(pf)_a = \lambda_A^{-3} \Delta l \frac{A}{N_s} \exp(\epsilon_a/kT) \quad (2)$$

$$(pf)_b = \lambda_A^{-3} \Delta V_g \quad (3)$$

where $(pf)_a$ and $(pf)_b$ are the molecular partition functions of the adsorbed anesthetic and of the dissolved anesthetic in the subphase, respectively. The factor $\binom{N_s + n}{n}$ in Eqn. 1 is the number of combinations of distributing n in the $(N_s + n)$ lattice sites. λ_A is the De Brogrie wavelength of

the anesthetic molecule and is given by:

$$\lambda_A^{-3} = (2\pi mkT)^{3/2}/h^3 \quad (4)$$

where m, k, T, and h are the mass of an anesthetic molecule, the Boltzmann constant, absolute temperature, and the Planck constant, respectively. In Eqns. 2 and 3, V_g is the volume of the gas phase, Δl is the translational free length of an anesthetic molecule in the perpendicular direction at the monolayer, and ϵ_A is the affinity free energy of an anesthetic molecule from the gas phase to the monolayer. In Eqn. 1, γ_{PC} and A are the surface tension of the phosphatidylcholine monolayer and the surface area of the monolayer, respectively.

The free energy, F, of the total system is defined by:

$$\begin{aligned} F &= -kT \ln Z \\ &= \gamma_A A - kT((N_s+n)\ln(N_s+n) - n\ln n - N_s \ln N_s) - n\epsilon_A \\ &\quad - nkT \ln\left(e\lambda_A^{-3}\Delta l \frac{A}{N_s}\right) \\ &\quad -(n_A - n)kT \ln\left(e\lambda_A^{-3}\frac{V_g}{(n_A-n)}\right) \end{aligned} \quad (5)$$

The adsorbed number n is determined so as to minimize F:

$$\begin{aligned} \frac{\partial F}{\partial n} &= kT \ln\left(\frac{n}{n_s+n}\right) - kT \ln\left(\frac{A\Delta l}{nN_s}\right) - \epsilon_A \\ &\quad + kT \ln\left(\frac{V_g}{n_A - n}\right) = 0 \end{aligned} \quad (6)$$

and the surface tension, γ, of the system is:

$$\gamma = \frac{\partial F}{\partial A} = \gamma_{PC} - nkT/A \quad (7)$$

By definition, the surface pressure, π, is:

$$\pi = \gamma_{PC} - \gamma = nkT/A \quad (8)$$

Because anesthetics in the gas phase may be regarded as an ideal gas, the anesthetic concentration $(n_A - n)/V_g$ is replaced by the partial pressure, P, by the following equation:

$$PV_g = (n_A - n)kT \quad (9)$$

Then, Eqn. 6 is rewritten by the use of Eqn. 9 in the form:

$$n/(N_s + n) = \Delta l A P/(N_s kT)\exp(\epsilon_A/kT) \quad (10)$$

Eqn. 10 is further rewritten by the use of Eqn. 8:

$$\frac{1}{\pi} = -\frac{1}{\pi_0} + \left(\frac{1}{\Delta l}\exp(-\epsilon_A/kT)\right)\frac{1}{P} \quad (11)$$

where:

$$\pi_0 = N_s kT/A \quad (12)$$

By assuming $\Delta l = 1$ Å [4,6], ϵ_A of the respective membranes is calculated from the slope of Figs. 2A–D. The result is listed in Table II.

In the case of adsorption on the air/water interface, the free energy change, ϵ_0, of the anesthetic from the gas phase to the air/water interface is estimated by the formula:

$$\pi_0 = kT \ln[\pi/(P\Delta l)] \quad (13)$$

in the low concentration limit of the anesthetics. The estimated values of ϵ_0 are also listed in Table II. In this estimation, Δl in Eqn. 13 is taken to be identical to that of Eqn. 11. The difference, $\epsilon_A - \epsilon_0$ of the affinity is also listed in Table II. In reality, Δl in Eqns. 11 and 13 may be different. This will affect the value of $\epsilon_A - \epsilon_0$. For instance, a 50% change in Δl causes a 1.00 kJ · mol^{-1} change in the value of $\epsilon_A - \epsilon_0$. Nevertheless, the stronger affinity of chloroform and halothane to the lipid monolayer than to the water surface is evident.

Fig. 3 shows the relationship between the transfer free energy of anesthetic and the initial surface pressure of the phosphatidylcholine monolayer. For the less compressed monolayer (4.5 mN · m^{-1}), the transfer free energies of the ether-type anesthetics are larger than those of the alkane-type anesthetics. The difference becomes considerably smaller when the monolayer is further compressed. The hydrophobic portions of phosphatidylcholine molecules in the expanded film are in random, rather than regular, orientation. Only the polar groups are constrained to be in contact with the aqueous phase [7]. The surface area of the expanded monolayer region is regarded to be composed of a mixture of phosphatidylcholine and water molecules. When the monolayer is com-

TABLE II

ANESTHETIC ADSORPTION FROM GAS PHASE TO PHOSPHATIDYLCHOLINE MONOLAYER

Surface pressure parameter (π_0) defined in Eqn. 12, affinity free energy (ϵ_a), and the difference in affinity of anesthetics to phosphatidylcholine monolayer and water surface ($\epsilon_a - \epsilon_0$). Affinity free energy of anesthetics to vacant water surface, ϵ_0 is also shown. A_0, initial area per phosphatidylcholine molecule.

	A_0 (Å^2)	π_0 (mN·m^{-1})	ϵ_A (kJ·mol^{-1})	$\epsilon_A - \epsilon_0$ (kJ·mol^{-1})	ϵ_0 (kJ·mol^{-1})
Chloroform	88.5	2.675	17.5	2.11	15.4
	77.0	4.278	19.0	3.58	
	66.5	5.620	19.4	3.99	
	50.5	7.553	20.2	4.74	
Halothane	88.5	2.097	16.6	0.70	15.9
	77.0	9.768	19.5	3.62	
	66.5	9.140	19.4	3.56	
	50.5	9.563	19.6	3.73	
Enflurane	88.5	4.758	18.4	1.49	16.9
	77.0	5.892	18.9	2.03	
	66.5	5.430	18.7	1.84	
	50.5	3.513	17.9	1.06	
Diethyl ether	88.5	10.96	20.4	−0.65	21.0
	77.0	5.028	19.4	−1.62	
	66.5	2.538	18.5	−2.56	
	50.5	1.824	16.9	−4.10	

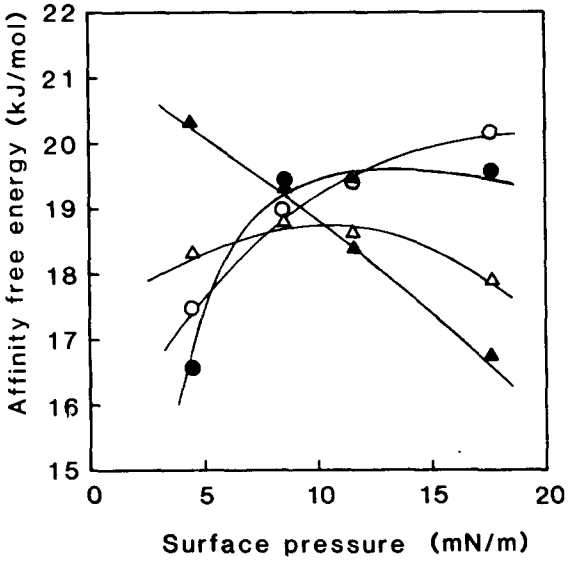

Fig. 3. Relationship between affinity free energy of anesthetic adsorption, ϵ_A and initial phosphatidylcholine surface pressure, π_i, for chloroform (○), halothane (●), enflurane (△) and diethyl ether (▲)

pressed into a condensed state, the surface water molecules are squeezed out into the bulk aqueous phase, and the hydrocarbon-hydrocarbon attractive forces between phosphatidylcholine molecules are enhanced. The affinity of the ether-type anesthetics to the water surface is much stronger than that of the alkane-type anesthetics (Table II). Therefore, the results obtained with the less compressed monolayer would be caused by the strong affinity of the ether-type anesthetics to the water surface.

It is of interest that there is little difference in ϵ_A values between monolayers compressed to 8.5 and 11.5 mN · m^{-1}, despite the fact that the film compressibility at 11.5 mN · m^{-1} surface pressure is much larger than at 8.5 mN · m^{-1}. The anesthetic adsorption to the intermediately compressed monolayers appears to be mainly dependent on the number of phosphatidylcholine molecules per unit area at the air/water interface and not on the plasticity (or fluidity in a broad sense) of the membrane. In the monolayer at 50.5 Å^2 compression, the transfer free energies of anesthetics are in

the order of chloroform > halothane > enflurane > diethyl ether, in agreement with the order of their clinical potencies.

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