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Stopped-flow study of anesthetic effect on water-transport kinetics through phospholipid membranes. Interfacial versus lipid core ligands

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We have compared ligand effects between polar and apolar anesthetic molecules upon water transport across phospholipid membranes by kinetic analysis of the osmotic swelling rate, using a stopped-flow technique. Chloroform and 1-hexanol were used as interfacial ligands, and carbon tetrachloride and *n*-hexane were used as their counterparts, representing lipid core action. Because anesthetics transform the solid-gel membrane into a liquid-crystalline state, and because phospholipid membranes display an anomaly in permeability at the phase transition, dimyristoylphosphatidylcholine vesicles were studied at temperatures above the main phase transition to avoid this anomaly. All these molecules increased the osmotic swelling rate. However, a significant difference was observed in the activation energy, ΔE_p, between polar and apolar molecules; ΔE_p was almost unaltered by the addition of polar molecules (chloroform and 1-hexanol), whereas it was decreased by apolar molecules (carbon tetrachloride and *n*-hexane). The obtained results were analyzed in terms of the dissolution-diffusion mechanism for water permeation across the lipid membrane. It is suggested that polar molecules affect water permeability by altering the partition of water between the membrane interior and water phase, and apolar molecules affect it by altering both the partition and the diffusion of water within the membrane interior.

Introduction

Anesthetics affect the physical properties of membranes, such as the phase transition between the solid-gel and liquid-crystalline states. It is generally agreed that membrane perturbation caused by anesthetic molecules is directly or indirectly related to the state of anesthesia. A number of studies have been reported about interactions between anesthetics and model membranes to elucidate molecular mechanisms of anesthesia.

Permeability to small molecules is one of the important properties of membranes, and several studies have been reported about the effect of anesthetics on permeability in model membrane systems [1,2]. In the present communication, we report the effect of anesthetics on water permeability of phospholipid vesicle membranes by measuring the vesicle swelling rate induced by osmotic shock. We used dimyristoylphosphatidylcholine membranes at above the phase-transition temperature because the permeability anomaly associated with phase transition is amply emphasized [3–5] and anesthetic binding to the membrane transforms the solid-gel membrane to a liquid-crystalline state.

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Abbreviation: DMPC, dimyristoylphosphatidylcholine

The osmotic swelling or shrinking technique has been frequently applied to the study of water transport across phospholipid vesicle membranes [6–14] (see also a review in Ref. 15). Because lipid bilayers are poorly permeable to solutes such as inorganic electrolytes, sucrose and other polar molecules, and are relatively permeable to water, phospholipid vesicles undergo swelling or shrinking when the osmolarity of the external solution is changed. It has been empirically established that the vesicle volume change associated with swelling or shrinking is directly proportional to the change in the reciprocal of absorbance at appropriate wavelengths [6]. The spectrophotometric method can be used to monitor the time-course of vesicle volume change after a sudden osmotic shock using a stopped-flow apparatus.

In general, it is difficult to determine the absolute value of the water permeability coefficient from the osmotic shock experiment in phospholipid vesicle systems, because of several unknown factors involved. Nevertheless, this technique is useful for comparative purposes, i.e., to study the difference in permeability between the absence and presence of anesthetics.

It was of interest to compare two types of anesthetics which have similar molecular structure but different polarity. We have reported [16–20] that polar anesthetic molecules accumulate at the membrane/water interface at clinical concentrations. On the other hand, it has been established [21] that apolar molecules, such as alkanes, penetrate into the hydrophobic core. Chloroform and 1-hexanol are used to represent interfacial action, and carbon tetrachloride and *n*-hexane are used to represent lipid core action. It will be shown that there is a significant difference between these two classes of ligands in activation parameters for water permeation across the membrane.

Materials and Methods

Synthetic dimyristoylphosphatidylcholine (DMPC) was obtained from Sigma. Chloroform (MCB), carbon tetrachloride (Fisher), 1-hexanol (Sigma), *n*-hexane (Fluka) and sodium chloride (Mallinckrodt) were all reagent grade and used without further purification. Water was triply distilled, once from alkaline potassium permanganate solution.

DMPC vesicle suspension in NaCl solution was prepared by sonication in a cup-horn of a Branson Sonifier Model 185 (Danbury, CN) above the phase-transition temperature. After sonication, vesicle suspension was maintained at 4°C to fuse into homogeneous size according to the method reported by Wong et al. [22]. The concentration of DMPC was 0.4 mM. Anesthetics were added to the vesicle suspension by a microsyringe which was calibrated by the weight of delivered water measured by a Perkin-Elmer electronic ultramicrobalance.

A Durrum Model D-110 stopped-flow spectrophotometer was used for kinetic meaurements. DMPC vesicle suspension in NaCl solution was mixed with hypotonic solution with a mixing ratio of 1 : 1. The swelling process of the vesicle after mixing was followed by the absorbance change at 450 nm. The output signal was stored in a Nicolet Model 3091 digital oscilloscope with a time resolution of 1 μs, and recorded on a Hewlett-Packard X-Y recorder. The temperature of the sample solution was maintained at 30.0 ± 0.1°C.

Fig. 1. shows a typical tracing of absorbance change in the time domain. At the initial stage after mixing, absorbance instability was observed. A similar response has been reported by others

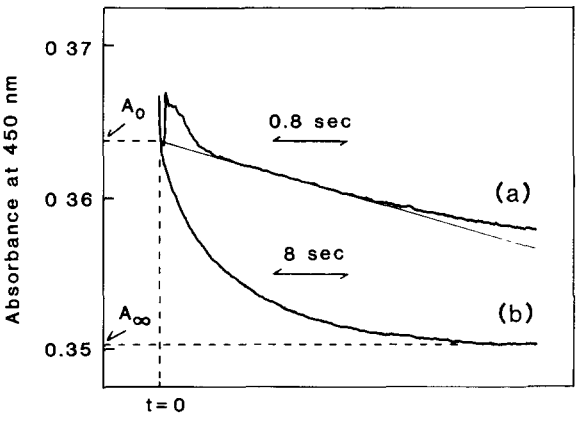

Fig. 1 Typical time-course of absorbance change at 450 nm due to osmotic swelling for DMPC vesicle suspension DMPC in 0 05 M NaCl was mixed with water at 30 0°C DMPC concentration after mixing was 0.21 mM Curve a was recorded by expanding the time-scale of curve b

[9,11], but the origin of this instability is not known.

The rate was analyzed in terms of the relative initial change (after the instability) in the reciprocal of absorbance with time, i.e.,

$$v_0 = \frac{(d(1/A)/dt)_0}{1/A_0} = -\frac{(dA/dt)_0}{A_0} \quad (1)$$

where A is the absorbance and subscript 0 indicates $t = 0$. From the slope of the initial linear portion of curve (a) in Fig. 1, $(dA/dt)_0$ was obtained. A_0 was estimated by extrapolating the same line to $t = 0$. The experiment was repeated at least four runs. All data points were stored in an Apple IIe microcomputer interfaced with a PDP 11/23 minicomputer, and linear and polynomial curve fittings were performed according to the least-squares method.

Results and Discussion

If the phospholipid membrane is impermeable to the solute, NaCl, the rate of volume increase resulting from water influx, dV/dt, is expressed as [23]:

$$\frac{dV}{dt} = P_w SRT\Delta c \quad (2)$$

where P_w is the permeability coefficient for water, S is the membrane area, R is the gas constant, T is the absolute temperature, and Δc is the concentration difference in the impermeable solutes between the inside and outside of the vesicle. Since v_0 is proportional to the initial rate of volume change, the following relation holds [12]:

$$v_0 = k\left(\frac{dV}{dt}\right)_0 = kP_w SRT\Delta c \quad (3)$$

where k is a proportionality constant. The relation expressed in Eqn. 3 has been used to analyze the osmotic behavior. The existence of linearity between v_0 and Δc has been taken as an indication that the membrane is impermeable to the solute [12].

In many osmotic shock experiments [9,10,12–14], a small amount of phosphatidic acid or other charged lipids was added to pure phosphatidylcholines to enhance osmotic response. In these systems, linearity between v_0 and Δc has been reported. We favored, however, a simpler system for analysis, and pure DMPC vesicles were used.

Fig. 2 shows the variation of v_0 as a function of Δc_{NaCl} at different temperatures above and below the phase transition temperature. (Although Δc in Eqn. 3 is the difference in ionic concentration, the abscissa of Fig. 2 is expressed as the concentration difference of NaCl; $\Delta c = 2\Delta c_{NaCl}$). As shown in Fig. 2, the linear relationship holds between v_0 and Δc_{NaCl} for pure DMPC vesicles. Hence, it may be safely assumed that DMPC vesicle membranes are practically impermeable to NaCl and k, P_w, and S in Eqn. 3 can be regarded to be dependent only on temperature in the present experimental condition.

The absolute values of v_0 at 30.0°C were smaller than the results of osmotic shrinking reported by Blok et al. [12], who used DMPC vesicle membranes doped with 4 mol% egg phosphatidic acid, and glucose as the impermeable solute. The difference may be caused in part by the presence of the charged lipid. All subsequent experiments were performed at fixed Δc_{NaCl} of 0.025 M, i.e., DMPC vesicle suspension in 0.05 M NaCl was mixed with water.

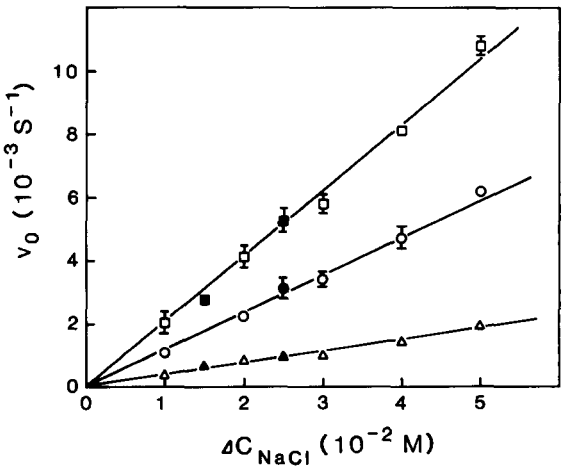

Fig. 2. Plot of the initial swelling rate for DMPC vesicle, v_0, against the NaCl concentration difference, Δc_{NaCl}, at various temperatures. 30°C, □; 24°C, ○, 18°C, △. DMPC in 0.1 M NaCl (open symbols) or in 0.05 M NaCl (filled symbols) was mixed with water or NaCl solution of lower NaCl concentration to give the Δc_{NaCl} value shown on the abscissa. Vertical bars indicate the standard deviation estimated from four repeated runs.

Effect of anesthetics on v_0

To avoid osmotic differences due to anesthetics between vesicle suspension and diluting fluid, an equal amount of anesthetics was added to the vesicle suspension and diluting fluid, except for *n*-hexane, because its water solubility is negligible. In this case, DMPC suspension was mixed with water saturated with *n*-hexane.

Fig. 3a shows the plot of v_0 against anesthetic concentration at 30.0°C. In all cases, v_0 increased almost linearly with anesthetic concentrations. To compare the effectiveness of each anesthetic, the bulk anesthetic concentration was converted to the mole fraction of anesthetics in the phospholipid membrane, using the following reported values of the partition coefficient [24]: $CHCl_3 = 1060$, 1-hexanol = 960 and $CCl_4 = 6910$. For *n*-hexane, it was assumed that all *n*-hexane molecules are incorporated into the lipid phase because of the low solubility in water. The result is shown in Fig. 3b where the anesthetic concentration is expressed by x_A^l, where superscript l indicates the lipid phase. The amount of anesthetic molecules incorporated into the membrane may vary with temperature because partition coefficients depend on temperature. Generally, however, the enthalpy change associated with the transfer of such molecules as used here have a small positive value, typically less than 1 kcal · mol^{-1}. Simon et al. [25] found ΔH^0 for [^{14}C]halothane binding to the dipalmitoylphosphatidylcholine vesicle membranes from water to be 0.0 ± 0.2 kcal · mol^{-1}. Assuming the enthalpy change of 1 kcal · mol^{-1}, it is estimated that the partition coefficient varies about 8% over the temperature range 25–40°C, and this leads to the variation of the mole fraction of anesthetics in the lipid phase by a similar extent. However, the results variation of v_0 due to the change in the partition coefficient is less than 2% (see Fig. 3b). Hence, the contribution of the variation of anesthetic content in the membrane to the change in v_0 may safely be neglected.

It is seen in Fig. 3b that polar 1-hexanol is much more effective for increasing v_0 than apolar *n*-hexane. Also, polar $CHCl_3$ is more effective than apolar CCl_4, although the difference is not as distinct as in the case of the hexanol-hexane pair. If we assume that the parameters k, the optical coefficient relating the absorbance change to the

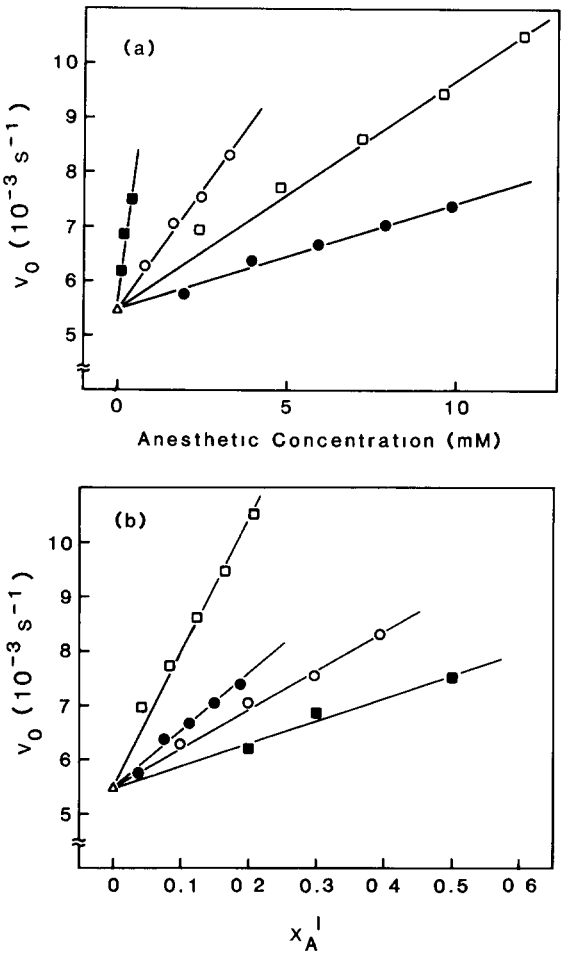

Fig. 3 Plot of the initial swelling rate for DMPC vesicle, v_0, against (a) anesthetic concentration and (b) mole fraction of anesthetics in the lipid phase, x_A^l, at 30.0°C $CHCl_3$, ●; CCl_4, ○, 1-hexanol, □, and *n*-hexane, ■ DMPC vesicle suspension in 0.05 M NaCl with anesthetics was mixed with water containing an equal amount of anesthetics Error bars are omitted for clarity, because the scatter was small and in many cases within the size of the symbol.

volume change, and S, the vesicle surface area, are constant and independent of the presence of small amounts of anesthetics (because anesthetics are mixed with vesicle suspension and diluting fluid before the osmotic shock), then v_0 can be regarded to be directly proportional to P_w at fixed Δc_{NaCl} and T (Eqn. 3). Although anesthetics increase membrane area, S is constant in the present study, because the anesthetic concentration is identical before and after the osmotic shock. Thus, the

results in Fig. 3b indicate that the water permeability of the DMPC vesicle membrane is increased by incorporation of anesthetic molecules into the membrane, and the extent of the increase is larger for polar molecules than for apolar molecules.

It is generally accepted that water permeability through phospholipid membranes is controlled mainly by two factors: partition of water molecules between the bulk water phase and membrane phase, and the diffusion rate of water molecules through the membrane [15]. A more detailed analysis about how anesthetics alter the water permeability may be obtained by examining the temperarure dependence of the swelling rate.

Temperature dependence of v_0

Swelling rates of DMPC with and without anesthetics were measured as a function of temperature above the phase transition. Again, we assume that both k and S are nearly independent of small amounts of additives or temperature. Then the temperature dependence of v_0/T may be caused by the change in P_w. Assuming that the permeability coefficient has the following form:

$$P_w = P_w^\circ \exp(-\Delta E_p/RT) \tag{4}$$

where ΔE_p is the activation energy for water permeation and P_w° is a constant, we obtain from Eqn. 3:

$$v_0/T = CP_w^\circ \exp(-\Delta E_p/RT) \tag{5}$$

where C is a constant at fixed Δc_{NaCl} ($C = kSR\Delta c$). According to Eqn. 5, ΔE_p can be obtained from the plot of $\log(v_0/T)$ vs. $1/T$.

In Fig. 4, the logarithms of v_0/T are plotted against $1/T$ for chloroform (a) and carbon tetrachloride (b). Data for 1-hexanol and *n*-hexane are omitted for brevity, but they are essentially simillar to those of chloroform and carbon tetrachloride. For pure DMPC systems, the deviation from linearity is seen at low temperature, which coincides with the phase transition between the gel and liquid-crystalline states. In the presence of anesthetics, the linearity holds for the whole temperature range examined. This is caused by anesthetic-induced depression of the transition temperature; the DMPC vesicles are in a liquid-crystalline state at the measured temperature range. The values of ΔE_p were estimated from the slopes of the straight lines in Fig. 4 and are shown in Table I.

The value of activation energy for water permeation of a pure DMPC vesicle membrane has not been reported to our knowledge. Values for other phosphatidylcholine vesicles, obtained from osmotic shock experiments at above the transition temperature, scatter from 6.6 ± 0.4 to 14.1 ± 2.6 kcal · mol^{-1} [8,12,26]. The present value for the pure DMPC vesicle is close to the lower limit of the reported values.

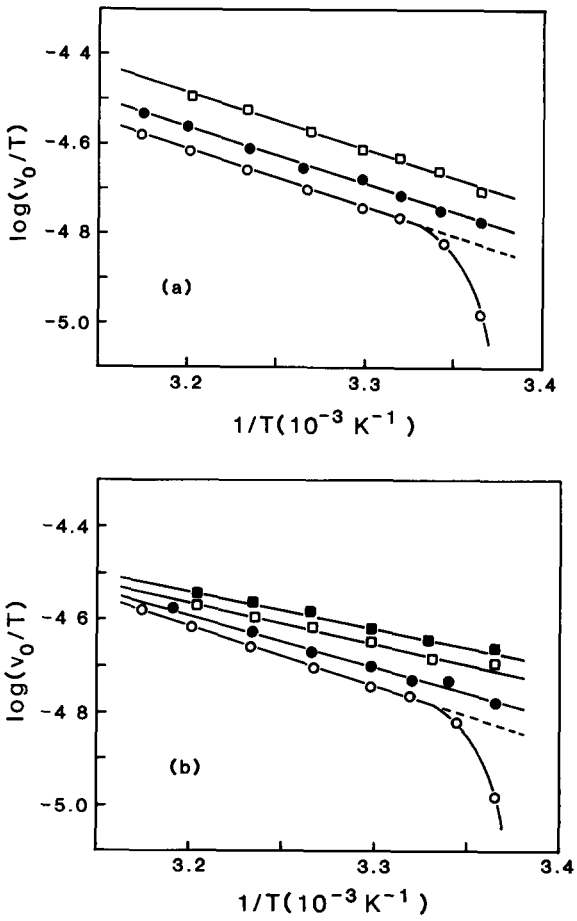

Fig. 4 Plot of $\log(v_0/T)$ against $1/T$. (a) DMPC-$CHCl_3$ system. $CHCl_3$ concentrations are 0, ○; 3.95 mM, ●; and 7.90 mM, □ (b) DMPC-CCl_4 system. CCl_4 concentrations are 0, ○; 0.83 mM, ●; 1.65 mM, □; and 2.47 mM, ■. The standard deviations of all data points were within the size of the symbol

An interesting finding in the present study is the demonstration of the distinct difference in activation energies between polar and apolar ligands. As shown in Fig. 4 and Table I, ΔE_p remained almost unchanged with the addition of chloroform and 1-hexanol, whereas ΔE_p decreased dose-dependently with the addition of carbon tetrachloride and *n*-hexane. This results indicates that the mechanism of modification of the water permeation across the vesicle membrane is different between the two groups.

Water permeation across the lipid membrane is generally interpreted in terms of the dissolution-diffusion mechanism, where the membrane core is assumed to be a homogeneous layer in which water dissolves and moves by diffusion [8,15,26]. According to this model, P_w is expressed by the following equation [27]:

$$\frac{1}{P_w} = \frac{\Delta x}{KD_m} + \frac{2l}{D_{sm}} \tag{6}$$

where K is the partition coefficient of water, D_m is the diffusion coefficient of water within the hydrocarbon core of the membrane, Δx is the core thickness, l is the width of the interfacial region containing the polar headgroup of the lipid, and D_{sm} is the diffusion coefficient across the interfacial region from the bulk aqueous phase to the membrane.

The first and second terms on the right-hand-side of Eqn. 6 represent the contributions from the hydrocarbon core and the interfacial region of the membrane, respectively. There are two possibilities for the rate-determining step for the water permeation, i.e., diffusion in the lipid core and diffusion across the interfacial region. If both of these diffusion processes are rate-limiting, then the Arrhenius plot in Fig. 4 will be in a nonlinear form.

Although there is no conclusive evidence that explicity shows which is the rate-determining step, the fact that the water permeability of black lipid membranes formed from the lipids with the same headgroups can be altered by varying the lipid chain composition [28,29] suggests that the hydrocarbon core may be the primary site that determines water permeability.

If we assume that the rate-determining step for water permeation is diffusion within the membrane core, the second term in the right-hand-side of Eqn. 6 can be neglected. Then,

$$P_r w = KD_m/\Delta x \tag{7}$$

According to the absolute reaction rate theory [30], a diffusional flow is treated as a series of successive jumps from one equilibrium position to another, and the diffusion coefficient is given by:

$$D_m = (\lambda^2 kT/h)\exp(\Delta S^{\ddagger}/R)\exp(-\Delta H^{\ddagger}/RT) \tag{8}$$

where $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are the activation enthalpy and the activation entropy for the diffusion process, respectively; λ is the distance between successive equilibrium positions along the path of diffusion; k is the Boltzmann constant, and h is Plank's constant. Also, from the thermodynamic relation

$$-RT\ln K = \Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ}$$

K is expressed as:

$$K = \exp(\Delta S^{\circ}/R)\exp(-\Delta H^{\circ}/RT) \tag{9}$$

where ΔH° and ΔS° are the standard enthalpy change and the standard entropy change, respec-

TABLE I

ARRHENIUS ACTIVATION ENERGY (ΔE_p, kcal mol^{-1}) FOR WATER PERMEATION ACROSS DMPC VESICLE MEMBRANES

Anesthetics	Concn. (mM)	Mole fraction x_A^l	Activation energy ΔE_p (kcal mol^{-1})
None	–	–	5 89 ± 0 10
$CHCl_3$	3 95	0 075	5 83 ± 0 15
	7.90	0.15	5 82 ± 0 23
CCl_4	0 82	0 099	5 12 ± 0 23
	1 65	0 20	4 03 ± 0 23
	2.47	0 30	3 51 ± 0 19
1-Hexanol	2.40	0.041	5 90 ± 0 12
	4 80	0 083	5.97 ± 0 10
	9 60	0 165	6 03 ± 0 34
n – Hexane	0.099	0 20	4 70 ± 0 32
	0 176	0.30	4 25 ± 0 33
	0.412	0.40	3.27 ± 0 26

tively, accompanied by the transfer of water molecules from the water phase into the hydrocarbon core. From Eqns. 7–9, we obtain:

$$P_w = (\lambda^2 kT/\Delta x h)\exp[(S^{\ddagger} + \Delta S^{\circ})/R] \times \exp[-(\Delta H^{\ddagger} + \Delta H^{\circ})/RT] \quad (10)$$

Comparing Eqn. 4, and according to the definition of Arrhenius activation energy [30],

$$\Delta E_p = \Delta H^{\ddagger} + \Delta H^{\circ} + RT \quad (11)$$

$$P_w^{\circ} = (\lambda^2 kT/\Delta x h)\exp[(\Delta S^{\ddagger} + \Delta S^{\circ})/R] \quad (12)$$

Thus, ΔE_p obtained from the slopes of the straight lines in Fig. 4 corresponds to the sum of the activation enthalpy for diffusion of water in the membrane core and the standard enthalpy change for partition of water. Also, information on the entropy change can be obtained from the intercept of the straight line in Fig. 4.

Although the absolute value of $\Delta S^{\ddagger} + \Delta S^{\circ}$ cannot be estimated because of the presence of unknown constant C in Eqn. 5, one can estimate the change in $\Delta S^{\ddagger} + \Delta S^{\circ}$, i.e., $\Delta(\Delta S^{\ddagger} + \Delta S^{\circ})$, by taking the difference in the intercepts between DMPC systems with and without additives, under the assumption C, λ and Δx are independent of the presence of a small amount of additives. In Fig. 5, obtained $\Delta H^{\ddagger} + \Delta H^{\circ}$ and $\Delta\ (\Delta S^{\ddagger} + \Delta S^{\circ})$ are plotted as a function of the mole fraction of anesthetics in the lipid membranes.

For chloroform and 1-hexanol, $\Delta H^{\ddagger} + \Delta H^{\circ}$ remained almost constant in the presence of additives. It may be reasonably concluded that neither $\Delta H^{\ddagger}$ nor ΔH° are altered by the presence of additives, because the possibility is remote that each of them changes without changing the sum. The activation enthalpy of solute (water) diffusion in a solvent (membrane core) is closely related to the viscosity of the solvent [30]. The present result that $\Delta H^{\ddagger}$ is unaffected by the presence of polar anesthetics indicates that these molecules do not significantly perturb the hydrocarbon core of the membrane, at least in the concentration range employed in the present study. It has now been recognized that the anesthetic effect on the order of the lipid tail is modest [31].

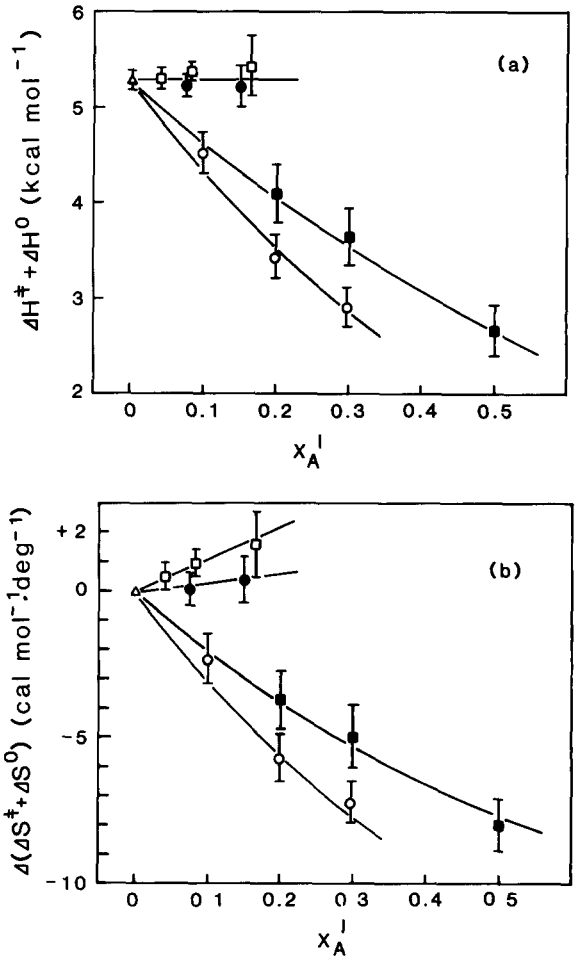

Fig. 5 Plot of (a) $\Delta H^{\ddagger} + \Delta H^{\circ}$ and (b) $\Delta(\Delta S^{\ddagger} + \Delta S^{\circ})$ against the mole fraction of anesthetic molecules in the lipid phase, x_A^l. Pure DMPC, △, $CHCl_3$, ●, CCl_4, ○; 1-hexanol, □, and *n*-hexane, ■. Vertical bars indicate the standard error estimated from the least-squares analysis.

On the other hand, $\Delta(\Delta S^{\ddagger} + \Delta S^{\circ})$ for chloroform and 1-hexanol were slightly increased. Because the membrane core is little affected by the addition of these molecules, $\Delta\Delta S^{\ddagger}$ should be close to zero. Then, the increase in $\Delta S^{\ddagger} + \Delta S^{\circ}$ is attributable to the increase in ΔS° induced by the incorporation of these molecules. This means that the entropy of water molecules dissolved in the hydrocarbon core is increased by the presence of chloroform or 1-hexanol, because the entropy of water in the bulk phase may be regarded to be unchanged. Presumably, relaxation of the membrane structure by low anesthetic concentrations

may be enough to increase the free space for water molecules in the lipid core, but may not induce *trans-gauche* rotation of the lipid tail, because the membrane is already in the liquid-crystalline state. It is concluded that polar molecules, chloroform and 1-hexanol, increase the water permeability of the DMPC vesicle membrane, primarily by increasing the partition coefficient of water through increased $\Delta S°$ rather than increasing diffusion of water molecules across the membrane core.

Carbon tetrachloride and *n*-hexane showed quite different results from chloroform and 1-hexanol. Both $\Delta H^{\ddagger} + \Delta H°$ and $\Delta(\Delta S^{\ddagger} + \Delta S°)$ decreased significantly with the increase in the mole fraction of these molecules in the membrane. Unfortunately, $\Delta H^{\ddagger}$ and $\Delta H°$ or $\Delta\Delta S^{\ddagger}$ and $\Delta\Delta S°$ cannot be estimated separately; therefore, detailed analysis is difficult. However, some qualitative conclusions can be drawn.

It may be reasonably assumed that carbon tetrachloride and *n*-hexane perturb the membrane core because they reside in the bilayer center [21]. It is likely that the presence of these molecules disorder the phospholipid hydrocarbon chains and lower $\Delta H^{\ddagger}$. On the other hand, the activation entropy for the diffusion process is generally small (close to zero) regardless of the property of the medium, except in associated liquids [30]. Hence, $\Delta\Delta S^{\ddagger}$ is considered to be small and the decrease in $\Delta S^{\ddagger} + \Delta S°$ may be mostly attributable to the decrease in $\Delta S°$. This indicates that the entropy of water in the hydrocarbon core is reduced by the presence of these additives. If the decrease in entropy of water results from some interaction between water and carbon tetrachloride or *n*-hexane in the membrane, $\Delta H°$ may also be decreased by the presence of these additives.

It is suggested that apolar molecules, carbon tetrachloride and *n*-hexane, affect water permeability through DMPC vesicle membrances by altering both the diffusion coefficient of water within the membrane core and the partition coefficient of water. These changes in D_m and K may be mainly due to perturbation of the membrane core induced by the presence of these molecules.

Effect of anesthetics on the relative volume change of DMPC vesicles

From the linear relationship between the change in the vesicle volume and the change in the reciprocal of the absorbance, one can estimate the relative volume change of DMPC vesicles, which is a measure for the extent of swelling, according to the following equation:

$$\frac{\Delta V}{V_0} = \frac{1/A_\infty - 1/A_0}{1/A_0} = \frac{\Delta A}{A_\infty} \tag{13}$$

where $\Delta A = A_0 - A_\infty$ is the absorbance at equilibrium (see curve b in Fig. 1). $\Delta V/V_0$, and hence $\Delta A/A_\infty$, may be used as measure for the 'strength' of the lipid membrane.

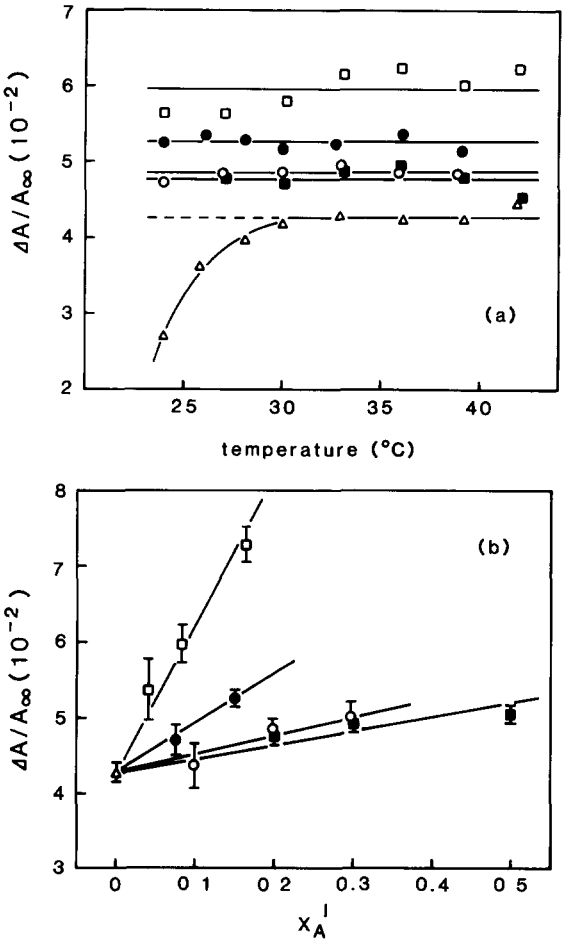

Fig 6 Plot of the relative absorbance change, $\Delta A/A_\infty$, against (a) temperature and (b) mole fraction of anesthetic molecules in the lipid phase. Pure DMPC, △, $CHCl_3$, ●, CCl_4, ○, 1-hexanol, □, and *n*-hexane, ■. Concentrations of anesthetics in (a) are $CHCl_3$ = 7.90 mM, CCl_4 = 1 65 mM, 1-hexanol = 4.8 mM, and *n*-hexane = 0 099 mM

Because the osmotic swelling experiments were performed at fixed ΔC, it may be inferred that the stronger the association forces between membrane lipids, the smaller the relative volume change of the vesicle.

Fig. 6a shows the plots of $\Delta A/A_\infty$ as a function of temperature. Notice that $\Delta A/A_\infty$ is almost constant at the measured temperature range. With pure DMPC vesicles, however, the plots deviate from linearity at the low temperature range, due to the gel-to-liquid-crystalline phase transition of the membrane. A similar finding has been reported with phosphatidylcholine vesicles containing phosphatidic acid [12].

The values of $\Delta A/A_\infty$, averaged over the temperature, are plotted against the mole fraction of anesthetics in the lipid phase in Fig. 6b. In all cases, $\Delta A/A_\infty$ increases with increasing x_A^l, and this indicates that the strength of the vesicle membrane is weakened by the presence of anesthetics in the membrane. Again, a significant difference is seen in the extent of the increase of $\Delta A/A_\infty$ between polar and apolar groups. The increment in $\Delta A/A_\infty$ is much larger for polar molecules than for apolar molecules. The molecules incorporated into the interfacial region and affecting water/membrane association are more effective in weakening the strength of the membrane than those incorporated into the membrane core. The present result shows the importance of the interfacial property for membrane stability and supports our view that polar anesthetic agents are membrane destabilizers as well as nonpolar anesthetics.

Acknowledgements

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References

1 Johnson, S M. and Bangham, A D (1969) Biochim Biophys Acta 193, 92–104
2 Hochster, H.S. and Prestegard, J H (1977) J. Membrane Biol 35, 303–307
3 Papahadjopoulos, D., Jacobson, K., Nir, S and Isac, T (1973) Biochim. Biophys Acta 311, 330–348
4 Blok, M.C , Van der Neut-Kok, E.C M., Van Deenen, L L M and De Gier, J (1975) Biochim Biophys Acta 406, 187–196
5 Tsong, T Y., Greenberg, M. and Kanehisa, M I (1977) Biochemistry 16, 3115–3121
6 Bangham, A.D , De Gier, J. and Greville, G.D (1967) Chem Phys Lipids 1, 224–246
7 De Gier, J , Mandersloot, J.G and Van Deenen, L L.M. (1968) Biochim Biophys Acta 150, 666–675
8 Reeves, J P and Dowben, R.M and (1970) J Membrane Biol 3, 123–141
9 Bittman, R and Blau, L (1972) Biochemistry 11, 4831–4839
10 Jain, M D , Toussaint, D G and Cordes, E H. (1973) J Membrane Biol 14, 1–16
11 Schullery, S.E and Garzaniti, J P. (1973) Chem Phys Lipids 12, 75–79
12 Block, M.C , Van Deenen, L.L.M and De Gier, J (1976) Biochim Biophys. Acta 433, 1–12
13 Block, M C , Van Deenen, L L M and De Gier, J (1977) Biochim Biophys. Acta 464, 509–518
14 Boehler, B A., De Gier, J and Van Deenen, L L.M (1978) Biochim Biophys Acta 512, 480–488
15 Fettiplace, R and Haydon, D.A (1980) Physiol Rev. 60, 510–550
16 Yokono, S , Shieh, D D and Ueda, I (1981) Biochim Biophys Acta 645, 237–242
17 Kaneshina, S , Lin, H C. and Ueda, I (1981) Biochim. Biophys. Acta 647, 223–226
18 Suezaki, Y , Kaneshina, S. and Ueda, I (1983) J Colloid Interface Sci. 93, 225–234
19 Yoshida, T , Mori, T Ueda, I (1983) J Colloid Interface Sci 96, 39–47
20 Yoshida, T , Kamaya, H and Ueda, I (1983) J Colloid Interface Sci. 96, 48–54
21 McIntosh, T J , Simon, S.A and MacDonald, R.C. (1980) Biochim Biophys Acta 597, 445–463
22 Wong, M , Anthony, F.H., Tillack, T W. And Thompson, T.E (1982) Biochemistry 21, 4126–4132
23 Kedem, O and Katchalsky, A (1958) Biochim Biophys. Acta 27, 229–246
24 Kamaya, H., Kaneshina, S and Ueda, I. (1981) Biochim Biophys Acta 646, 135–142
25 Simon, S.A , McIntosh, T J , Bennett, P B and Shrivastav, B.B (1979) Mol Pharmacol 16, 163–170
26 Cohen, B E (1975) J Membrane Biol 20, 205–234
27 Zwolinski, B.J , Eyring, H and Reese, C E (1949) J Phys. Colloid Chem 53, 1426–1453
28 Graziani, Y and Livne, A (1972) J. Membrane Biol. 7, 275–284
29 Fettiplace, R. (1978) Biochim Biophys Acta 513, 1–10
30 Glasstone, S , Laidler, K J and Eyring H. (1941) The Theory of Rate Process, chapter 9, McGraw Hill, New York
31 Trudell, J R (1980) in Molecular Mechanisms of Anesthesia, Progress in Anesthesiology (Fink, B R , ed), Vol 2, pp 261–270, Raven Press, New York