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INTERACTION OF GENERAL ANESTHETICS WITH PHOSPHOLIPID VESICLES AND BIOLOGICAL MEMBRANES

J.M. VANDERKOOI, R. LANDESBERG, H. SELICK II and G.G. McDONALD

Department of Biochemistry and Biophysics, School of Medicine, University of Pennsylvania, Philadelphia, Pa. 19174 (U.S.A.)

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Summary

Low concentrations of general anesthetics, including halothane, ethrane, trilene, diethyl ether and chloroform are observed to shift the phase transitions of phospholipid vesicles to lower temperatures, and from these data partition coefficients for the anesthetic between lipid and water can be calculated. In contrast to the anesthetics, high concentrations of ethanol are required to shift the phase transition of lipids and glycerol causes no effect. Above the phase transition general anesthetics alter nuclear magnetic resonance spectra of phospholipid dispersions and increase the rotational and lateral diffusion rates of fluorescent probes located in the hydrocarbon core of the bilayer, indicating that they induce disorder in the structure. In red blood cell membranes and sarcoplasmic reticulum fragments, the rotational diffusion rate of 1-phenyl-6-phenylhexatriene is increased in the presence of general anesthetics. The 220 MHz nuclear magnetic resonance spectra of sarcoplasmic reticulum reveal some resolved lines from the lecithin fatty acid protons; addition of general anesthetic increases the contribution of these peaks. The data from the NMR and fluorescence techniques lead to the conclusion that general anesthetics increase the pool size of melted lipids in the bimolecular phospholipid layers of biological membranes; this would account for the ability of general anesthetics to increase passive diffusion rates of various substances in membranes.

Introduction

Involvement of general anesthetics with the lipids of membranes was suggested four decades ago to be a primal cause of narcosis [1]. Subsequently, interactions of general anesthetics with proteins [2,3] or with the cellular water [4] have been proposed as the mode of action of anesthetics. While most

Abbreviation: DHP, 1-phenyl-6-phenylhexatriene.

evidence, as is summarized in several reviews [5—9] would lead to the conclusion that anesthetics interact with both proteins and lipids, data accumulated over the past fifty years indicate that general anesthetics do have a direct effect in altering permeability of cell membranes [10,11]. Although they often inhibit mediated transport processes, they nearly always produce an increase in non-mediated diffusion [12].

It has previously been shown that small hydrophobic molecules including general anesthetics shift the melting profile of phospholipid vesicles to lower temperatures [13—17]. These results are here repeated and used to calculate partition coefficients for commonly used anesthetics. Furthermore, above the phase transition the general anesthetic alters the nuclear magnetic resonance spectrum of the phospholipid dispersion and increases the rotational and lateral diffusion rates of fluorescent hydrocarbons which have been incorporated into the phospholipid vesicles indicating a change in the packing of the phospholipid molecules.

Similarly, in red blood cell membranes and in sarcoplasmic reticulum membranes, the rotational diffusion rates of a fluorescent dye are increased in the presence of general anesthetics. That this is due to an increase in the pool size of lipids above the phase transition can be shown by observing an increase in resolved peaks in NMR spectra of sarcoplasmic reticulum membranes. These results are discussed in terms of the "membrane expanding" properties of general anesthetics (reviewed by Seeman, ref. 18).

Materials and Methods

Chemicals. 1-phenyl-6-phenylhexatriene, (DHP) obtained from Aldrich Chemical Company (Milwaukee, Wis. 53233), was recrystallized from tetrahydrofuran and water. Pyrene (Eastman Chemical Co., Rochester, N.Y.) was twice recrystallized from ethanol. The purity of the fluorescence dyes was ascertained by their exponential decay of fluorescence in ethanol. L-α-dimyristoyl phosphatidylcholine and L-α-dipalmitoyl phosphatidylcholine were supplied by Sigma Chemical Co. (St. Louis, Mo.); the lipids showed only one spot on thin-layer chromatography using chloroform/methanol/water (65 : 25 : 4 v/v) as solvent and staining with iodine. 2-chloro-1,1,2-trifluoroethyl difluoromethyl ether ("ethrane", "enflurane") was obtained from Ohio Medical Products (Madison, Wis. 53701) and trichloroethylene ("trilene") was obtained from Ayerst Laboratories, Inc. (New York, N.Y.). The blue dye used to identify the trilene brand was removed by passage through a dry silica gel column (2 cm × 0.4 cm). Thymol-free 2-bromo-2-chloro-1,1,1-trifluoroethane ("halothane") was a gift from Sue Reed (U. of P. Hospital) and from L.A. Small (ICI United States Inc. Dighton, Mass. 02715). Chloroform and anhydrous ether were purchased from Fisher Chemical Co. (Fair Lawn, N.J. 07410). Deuterium oxide was obtained from Wilmad Glass Co. (Buena, N.J. 08310). Distilled and deionized water was used throughout. All other reagents were the highest quality commercially available.

Preparation of membranes. Red blood cell "ghosts" were prepared by modification of the procedure of Dodge et al. [19]. Red blood cells were isolated from the plasma by centrifugation for 10 min at 3020 × *g* and were then

washed twice with solution I (145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$ and 5 mM Na-PO_4 buffer, pH 7.0). The cells were lysed with a 1/8 dilution of solution I and the fragmented membranes were collected as a pellet after centrifugation at 17 300 $\times g$ for 20 min. This procedure was repeated until the membrane no longer retained visible red coloration. Solution III (250 mM KCl, 10 mM $MgCl_2$, 4 mM Na-PO_4, pH 7.0) was then added to the membranes to allow them to reseal. All of the above procedures were carried out at 4°C. Sarcoplasmic reticulum membranes were purified according to Martonosi et al. [20] except that 10 mM phosphate buffer, pH 7.2 was substituted for the histidine buffer during all isolation steps. The isolated membrane was lyophillized and suspended in D_2O-phosphate buffer for the NMR studies. The protein concentration of the membrane suspensions were determined by the Lowry et al. method [21]. Fluorescence labelling of the biological membranes was accomplished by adding an aliquot of the concentrated dye solution and incubating for 1 h at 4°C before the fluorescence was measured.

Phospholipid membranes were prepared by sonicating the phosphatidylcholine in a buffered solution containing the fluorescent dye for 1—3 min with a Branson sonifier at maximum power output. Phosphatidylcholine concentration was determined gravimetrically or from total phosphate [22].

Fluorescence intensities and spectra were obtained with an Hitachi MPF-2A spectrofluorometer (Hitachi, Ltd. Tokyo, Japan). Cuvettes were stoppered with a teflon plug to avoid loss of anesthetic by evaporation. Polacoat 4B polarizers were used to polarize the exciting and emitting beams for the polarization measurements. Fluorescence polarization, p, is defined as:

$$p = \frac{I_{11} - cI_{\perp}}{I_{11} + cI_{\perp}} \tag{1}$$

where I_{11} and $I_{\perp}$ are the fluorescence intensities observed with the analyzing polarizer respectively parallel and perpendicular to the polarized excitation beam and c is a constant to correct for the inability of the instrument to detect the two polarized light beams equally. Temperature control of the sample was achieved with the use of circulating water through the cell block.

Fluorescence decay times were obtained using an Ortec photon-counting fluorescence lifetime instrument (EGG Ortec, Oak Ridge, Tenn.). For measurement of DPH fluorescence decay rates, the data from the Ortec multichannel analyzer was transferred to a PDP-10 computer by cassette magnetic tape and analyzed for exponential decay by a nonlinear regression program which deconvolutes the data to account for the finite duration of the lamp flash [23]. For measurement of pyrene fluorescence decay under conditions where pyrene excimers were not formed, deconvolution of the data was not necessary because the decay time of pyrene is much longer than the lamp duration; these data were transferred to a Hewlett-Packard desk computer and then plotted on an x-y recorder. The slope of the semi-log plot in the time range of $\approx$50 to 350 ns after the flash was used to calculate the lifetimes.

Rotational relaxation time, ρ, for DPH were calculated according to the Perrin equation [24]:

$$\left(\frac{1}{p} - \frac{1}{3}\right) = \left(\frac{1}{p_0} - \frac{1}{3}\right)\left(1 + \frac{3\tau}{\rho}\right) \tag{2}$$

where τ is the fluorescent lifetime. The polarization in the absence of rotation, p_0, was taken to be 0.46 (25). The effect of temperature on the rotational relaxation time can be expressed according to Arrhenius:

$$\rho = A \exp(\Delta E/RT) \tag{3}$$

where ΔE is the energy of activation and T is the absolute temperature.

The bimolecular rate constant, k, for a quencher of fluorescence was calculated from the Stern-Volmer equation [26]:

$$\frac{F_0}{F} = \frac{\tau_0}{\tau} = 1 + k\tau_0[Q] \tag{4}$$

where F_0 and τ_0 are the fluorescent intensities and lifetimes in the absence of quencher and F and τ are the intensities and lifetimes in the presence of quencher and $[Q]$ is the apparent quencher concentration. The apparent rate constant, k, is related to the diffusion coefficient D by:

$$k = 4\pi N' RD(1 + [R/(\pi Dt)^{1/2}]) \tag{5}$$

where R is the sum of the radii interaction of fluorescent probe and quencher. D is the sum of the diffusion coefficients of fluorescent probe and quencher and N' is Avagadro's number per mmol [27—29]. In the case of oxygen, diffusion rates are several orders of magnitude larger than the diffusion than for hydrocarbons in the membrane. In this case the time-dependence of diffusion becomes negligible [30] and the equation becomes:

$$k = 4\pi N' RD \tag{6}$$

Determination of the partition coefficients of general anesthetics in phospholipids. The relationship between the freezing point depression and the partition coefficient of a solute is discussed by Hill [31]. Assuming that the concentration, Cm, of the anesthetic in the melted phase is much greater than in the gel phase, the freezing point depression can be expressed by:

$$\Delta T = \frac{RT^2}{Q} Cm \tag{7}$$

where Q is the latent heat of transition. Taking the enthalpy to be 8.66 kcal/mol^{-1} [32], this relationship predicts that the concentration of solute in the membrane required to shift the phase transition 1°C will be 0.0442. The partition coefficient P is expressed by:

$$P = \frac{Cm}{Caq} \tag{8}$$

where Cm is the concentration in the phospholipid, expressed as mol anesthetic/mol phospholipid and Caq in the concentration in the water, expressed as mol anesthetic/mol water.

Proton nuclear magnetic resonance spectra were obtained at 220 MHz using a Varian HR-220 spectrometer operating in Fourier transform mode. The radio-frequency pulse sequences, accumulation of signal and Fourier transform operations were controlled by a Varian 620-I dedicated computer. The tem-

perature of the sample, controlled to ±0.5° C was measured with a thermister thermometer.

Results

Phase transitions of phospholipid dispersions

The fluorescence of DPH in lipid vesicles is highly polarized at temperatures below the phase transition but becomes depolarized above the transition [25,33,34]. From this data the melting profiles of phospholipid vesicles can be determined and the effect of general anesthetics on the phase transition can be evaluated. As is shown in Fig. 1, low concentrations of ethrane, trilene, chloroform and ether are shown to shift the phase transition of dimyristoyl phosphatidylcholine vesicles to lower temperature, while at near saturating aqueous concentration the transition is lowered more than 20° C and the transi-

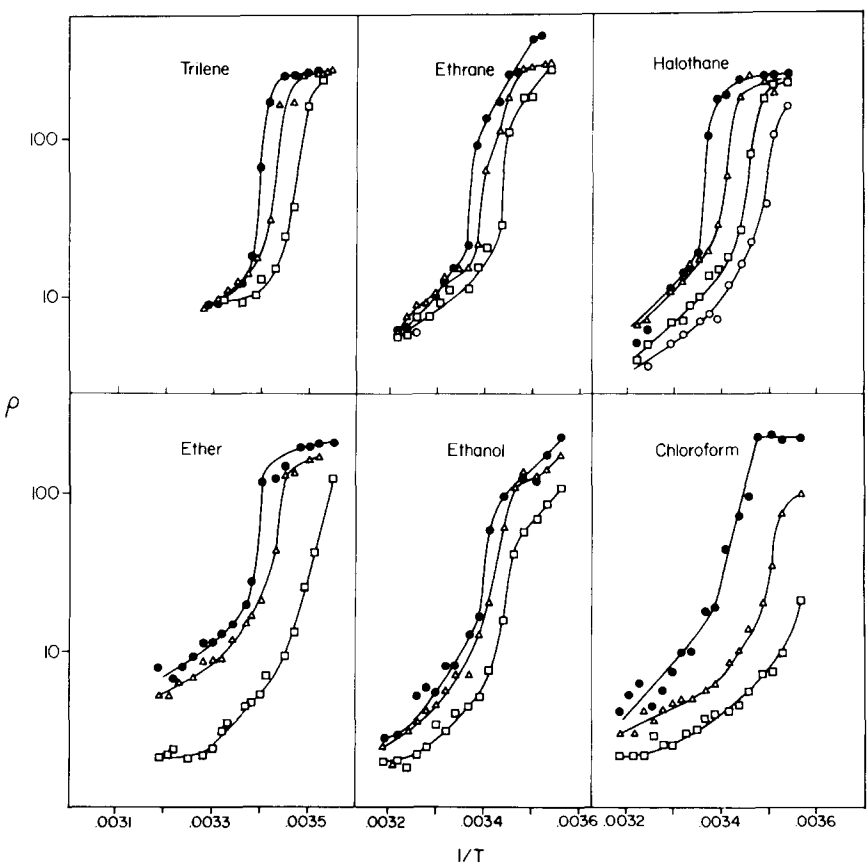

Fig. 1. Effect of anesthetics on the rotational correlation time of DPH in dimyristoyl phosphatidylcholine vesicles. Sample contained 0.1 mg dimyristoyl phosphatidylcholine/ml, 0.05 M $NH_4C_2H_3O_2$ pH 7.0 and 0.2 μM DPH. •, no additions; △, 5.4 mM ethrane, 6.7 mM trilene, 14.4 mM halothane, 1.8 M ethanol, 11.4 mM chloroform or 0.30 M ether; □, 10.7 mM ethrane, 10.1 mM trilene, 21.8 mM halothane, 3.6 M ethanol, 37.6 mM chloroform or 0.89 M ether; ○, 44.0 mM halothane. Excitation: 360 nm; emission: 428 nm.

tion is broadened. Ethanol, like the general anesthetics, produces a shift in the melting temperature; however, concentrations considerably higher than what can be obtained physiologically (i.e. 25% v/v) were required to shift the midpoint melting temperature significantly. In contrast 20 and 30% glycerol had no effect on the melting profile which can be attributed to the low partition coefficient for this molecule [35].

All experiments shown on Fig. 1 were carried out at low lipid concentrations. Increasing the dimyristoyl phosphatidylcholine concentration 30 times did not change the melting profile at a given anesthetic concentration; it can therefore be concluded that the amount of anesthetic in the lipid does not affect the concentration in the aqueous phase. In Table I, the data from Fig. 1 are presented, along with values for the partition coefficients of the anesthetics between lipid and water.

The partition coefficients for the respective anesthetic are of the same order of magnitude as those found in dipalmitoyl phosphatidylcholine [32,36]. It should be noted that the observed partition coefficient for ethanol in dimyristoyl phosphatidylcholine is about a factor of six smaller than that reported by Katz and Diamond [35]; they report a partition coefficient of 0.44 compared to our 0.07 when expressed in concentration in grams lipid/concentration in grams H_2O. We observe that the partition coefficient increases at increasing anesthetic concentration. The difference between the values may then reflect the difference in concentrations used for the experiments. Furthermore, it has not been shown whether there is a difference in anesthetic partition coeffi-

TABLE I

THE MELTING POINT TEMPERATURE (T_M) OF DIMYRISTOYL PHOSPHATIDYLCHOLINE IN THE PRESENCE OF GENERAL ANESTHETICS

Anesthetic	Aqueous concentration (M)	T_M °C	ΔT °C	Mol anesthetic/ mol phospholipid	Partion coefficient
Ethrane	0	23.2	—	—	—
	0.0054	21.2	2	0.0884	$9.02 \cdot 10^2$
	0.0107	18.1	5.1	0.225	$1.18 \cdot 10^3$
Trilene	0	21.6	—	—	—
	0.0067	20.5	1.1	0.0486	$4.05 \cdot 10^2$
	0.0101	15.0	6.6	0.292	$1.62 \cdot 10^3$
Halothane	0	23.0	—	—	—
	0.0144	20.3	2.7	0.119	$4.54 \cdot 10^2$
	0.0218	16.9	6.1	0.270	$6.75 \cdot 10^2$
	0.0440	13.5	9.5	0.420	$5.25 \cdot 10^2$
Chloroform	0	21.8	—	—	—
	0.0114	15.2	6.6	0.292	$1.39 \cdot 10^3$
	0.0376	8.6	13.2	0.583	$8.57 \cdot 10^2$
Diethyl ether	0	23.0	—	—	—
	0.2956	21.0	2	0.0884	$1.64 \cdot 10^1$
	0.8867	14.4	8.6	0.380	$2.38 \cdot 10^1$
Ethyl alcohol	0	21.1	—	—	—
	1.8	20.0	1.1	0.0486	1.47
	3.6	18.0	3.1	0.137	2.11

cients in sonicated (used by us) and unsonicated (used by Katz and Diamond) phospholipid dispersions.

Passive diffusion in phospholipid bilayers above the phase transition

As can be seen in Fig. 1, above the transition temperature there is a decrease in the rotational correlation times of DPH. For example, at 30°C, the rotational correlation time of DPH in dimyristoyl phosphatidylcholine is 11 ns with no anesthetic present but is 5.4 ns and 4.8 ns for 0.022 M and 0.044 M halothane, respectively. Phospholipid vesicles above the phase transition only approximate a two-dimensional liquid, i.e. the packing of phospholipids in the bilayer above the phase transition is still structured. The results indicate that the anesthetics disturb this structure. In order to show that the change in structure increases the passive diffusion rates of other molecules, the lateral diffusion of the hydrocarbon pyrene was measured. Since low concentrations of general anesthetics have a very small effect on the fluorescence yield and lifetime of pyrene monomer (Table II), the dimer/monomer ratio of pyrene can be used as a qualitative indication of pyrene diffusion [29]. As is shown in Fig. 2, the presence of chloroform or ether increase the dimer/monomer ratio, indicating that pyrene diffusion is increased (Fig. 2). Similar results were obtained in the presence of ethrane and trilene. Using data such as shown in Fig. 3, the diffusion coefficient for pyrene under conditions described in the legend was determined to be $2.5 \cdot 10^{-7}$ cm^2 per s in the absence of pyrene and $6.8 \cdot 10^{-7}$ cm^2 per s in the presence of ether.

The anesthetics (with the exception of halothane whose bromide quenches fluorescence) do not have a direct effect on the decay rate of pyrene fluorescence. The small quenching observed in the presence of the anesthetics may be due to the changes in the structure of the membrane which increases various

TABLE II

FLUORESCENCE LIFETIMES, γ, OF PYRENE IN DIMYRISTOYL PHOSPHATIDYLCHOLINE VESICLES

Sample was composed of 0.3 mg dimyristoyl phosphatidylcholine/ml, 0.1 μM pyrene and 50 mM $NH_4C_2H_3O_2$. Temperature, 30°C.

Anesthetic	Concentration (M)	Gas	ns
None	—	air	132
Chloroform	0.0125	air	128
	0.0413	air	126
Trilene	0.0074	air	109
	0.00112	air	94
Ethanol	1.8	air	116
	3.6	air	87
Ether	0.96	air	130
Ethrane	0.0082	air	127
	0.0164	air	118
None	—	oxygen, 1 atm	43.7
Ethrane	0.0082	oxygen	34.0
	0.0164	oxygen	28.0

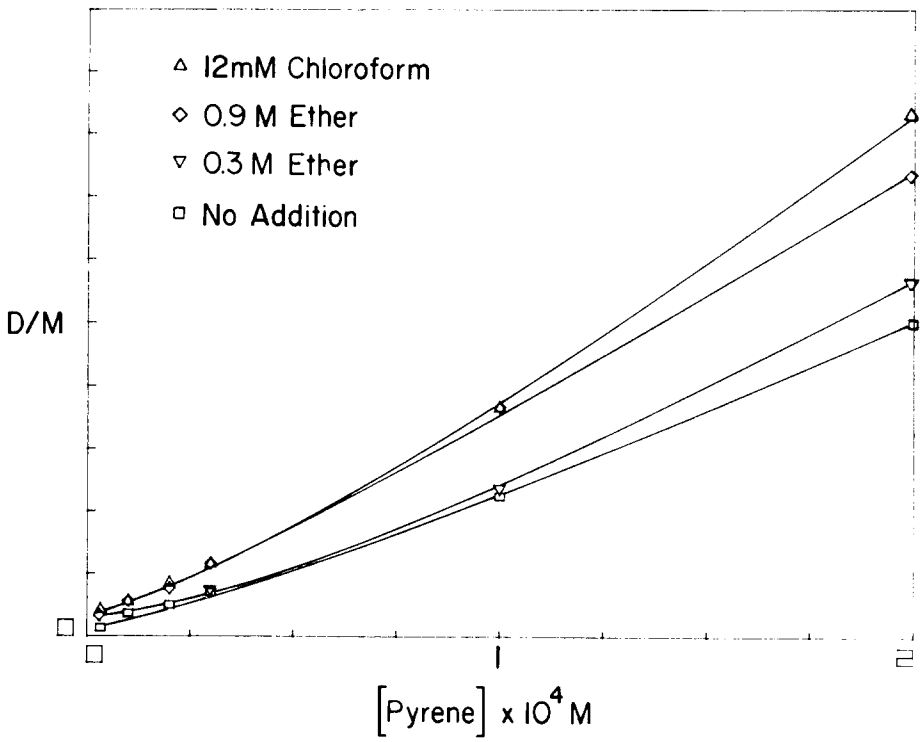

Fig. 2. Pyrene dimer formation in dimyristoyl phosphatidylcholine vesicles. The sample contained 0.3 mg dimyristoyl phosphatidylcholine/ml, 0.2 μM pyrene and 50 mM $NH_4C_2H_3O_2$, pH 7.0.

quenching processes. One of these quenching processes is caused by diffusional quenching by oxygen [30]. The fluorescence lifetime of pyrene in dimyristoyl phosphatidylcholine vesicles saturated with air was 132 ns in the absence of anesthetic, 127 and 118 ns in the presence of 0.2 and 0.33% ethrane. At saturating oxygen concentrations the lifetimes are 42, 37, and 33 ns for no additions, 0.2 and 0.33% ethrane, respectively. The shorter lifetime at 1 atm oxygen can be taken to mean that the diffusion coefficient of oxygen becomes larger in the presence of anesthetics, that partition coefficient for oxygen between the lipid and aqueous phase increases, or both.

Additional evidence that general anesthetics alter the packing of phospholipids in vesicles above the phase transition is obtained from the NMR spectra of these vesicles. In Fig. 4, spectra at 220 MHz of dimyristoyl phosphatidylcholine are presented. All of the peaks are altered; sharpening of the peaks is an indication of disorder induced by the anesthetic. The effect of the altered membrane structure by the anesthetics on the self diffusion of general anesthetics will be discussed subsequently.

Mixed lipid vesicles

Lipids of biological membranes are thought to be arranged in a bilayer in which there is lateral separation between lipids below and above the phase transition [37]. Previously it has been shown that DPH partitions into both

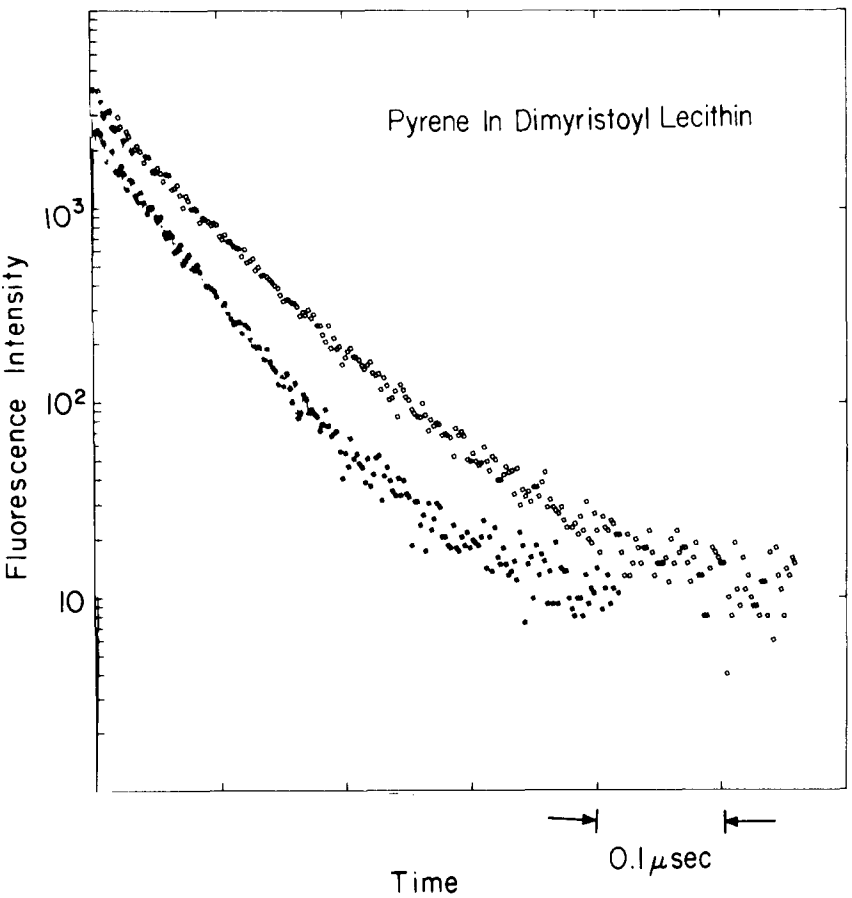

Fig. 3. Pyrene monomer fluorescence decay. Experimental points for fluorescent decay of pyrene in a sample containing 0.1 mg dimyristoyl phosphatidylcholine/ml, 0.05 M $NH_4C_2H_3O_2$, pH 7.0 and 10 μM pyrene without added anesthetic (○) or with 0.2 M ether (●). Temperature was 26°C.

phases, enabling one to construct phase diagrams [34]. The effect of a general anesthetic on DPH fluorescence polarization in vesicles composed of dipalmitoyl and dimyristoyl phosphatidylcholines is shown in Fig. 5. The temperature at which the transition is 50% complete is 34°C for the 50% dimyristoyl/dipalmitoyl phosphatidylcholine mixed vesicles without anesthetic, and 26°C in the presence of trilene. In contrast, the presence of the same amount of trilene shifted the phase transitions of the pure phosphatidylcholines about 3—5°C. The greater effect on the melting temperature of mixed phosphatidylcholines than the respective pure phosphatidylcholines is probably due to the fact that the partitioning in the melted phase is much greater than in the phase below the transition.

Effect of general anesthetics on biological membranes

The temperature dependence of DPH fluorescence polarization in the presence of red blood cell membranes and sarcoplasmic reticulum membranes is monophasic between 8 and 35°C (Fig. 6). As with the model membrane

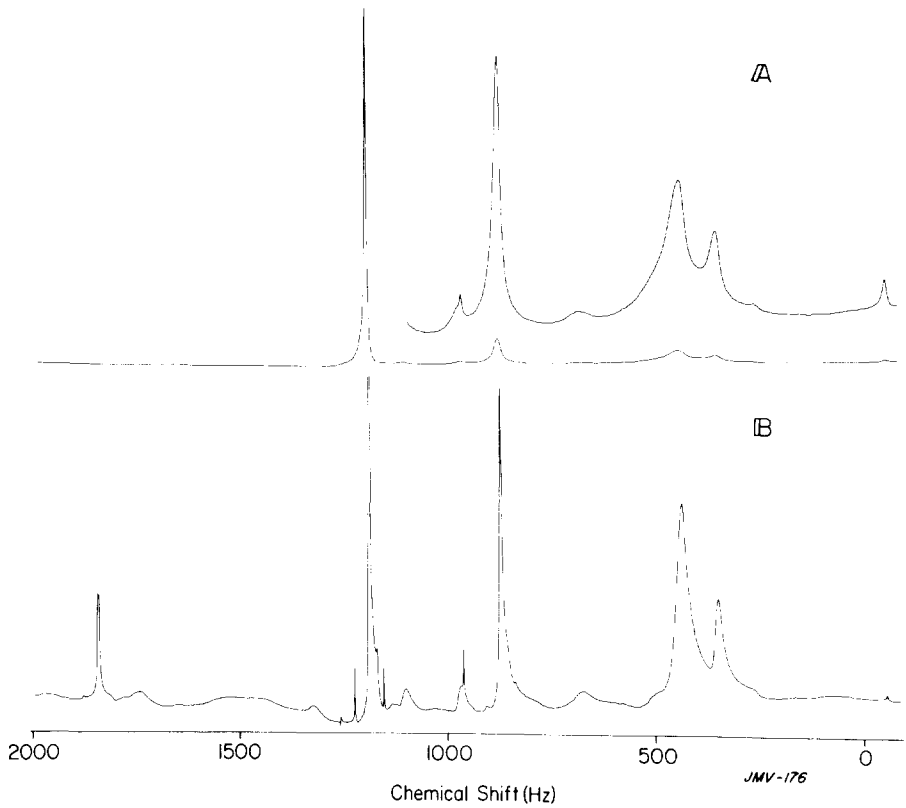

Fig. 4. 220 MHz NMR spectra of dimyristoyl phosphatidylcholine. A. 40 mg dimyristoyl phosphatidylcholine/ml in 10 mM sodium phosphate buffer at pH 7.0 and 37°C. B. Same as A but 28 mM chloroform was added. Peak at 1.25 KHz is from residual HDO.

systems, addition of general anesthetics results in a depolarization of DPH fluorescence, indicating an increase in the rotational diffusion rate of DPH. The fluorescence polarization of DPH is higher in the red blood cell membrane than in sarcoplasmic reticulum membranes, very likely reflecting the high amounts of cholesterol in the red blood cell membranes which tend to decrease the mobility of the lipids.

Since DPH partitions into lipid which is either above or below the phase transition, the depolarization induced by the general anesthetic in these biological membranes may be due to a shifting of the phase transition of the lipids such that the pool size of melted lipids increases. This idea is substantiated by NMR spectra of sarcoplasmic reticulum. As previously described [38] some of the fatty acid chains of the lipids are sufficiently mobile in sarcoplasmic reticulum so that they can be resolved by 220 MHz NMR spectroscopy. These peaks are sufficiently removed from peaks arising from the protein so that one can distinguish them from contributions due to the protein protons. Since the protons of the fatty acid chains are resolved only when the lipid is mobile; i.e. melted, conditions which increase the pool size of the melted lipid should increase the pool size of the melted lipid should increase the contribution to the

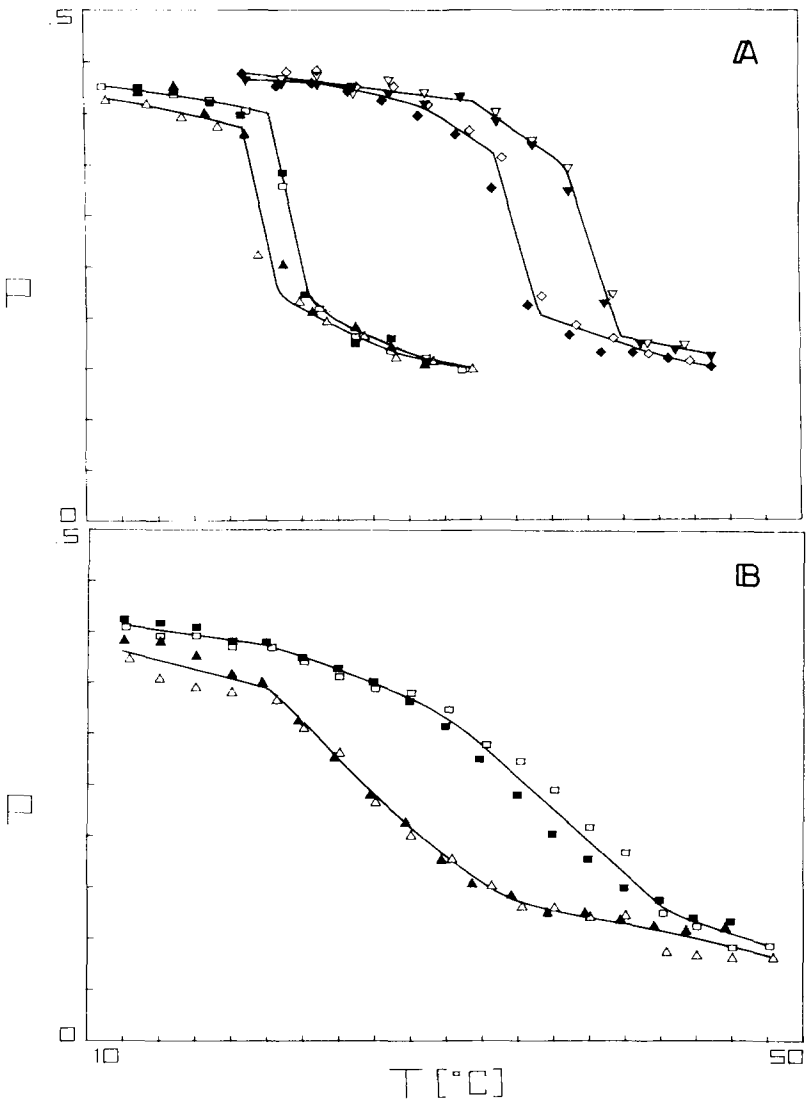

Fig. 5. Effect of trilene on DPH fluorescence polarization in mixed dipalmitoyl and dimyristoyl phosphatidylcholine. Open symbols represent measurements made with increasing temperature and closed symbols represent decreasing temperature.A. Sample contained 0.2 μM DPH, 0.05 M $NH_4C_2H_3O_2$ pH 7.0 and phosphatidylcholines as follows: □■, 0.1 mg dimyristoyl phosphatidylcholine/ml; △▲, 0.1 mg dimyristoyl phosphatidylcholine/ml and 6.7 mM trilene; △▲, 0.1 mg dipalmitoyl phosphatidylcholine/ml; ◇◆, 0.1 mg dipalmitoyl phosphatidylcholine/ml 0.1 mg dipalmitoyl phosphatidylcholine/ml and 6.7 mM trilene. B. Sample contained 0.05 mg dipalmitoyl phosphatidylcholine/ml and 0.05 mg dimyristoyl phosphatidylcholine/ml, 0.2 μM DPH, 0.05 M $NH_4C_2H_3O_2$ pH 7.0 and 6.7 mM trilene (△▲) or no additions (□■). Excitation: 360 nm, emission: 428 nm.

lipid peaks. Such is the case when halothane (Fig. 7) or chloroform (not shown) is added. It can be seen in the figure that the ratio of the fatty acid peaks to the protein peaks increases when halothane is added. Similarly, increasing the temperature results in an increase in the fatty acid peaks [38]. Taking this result,

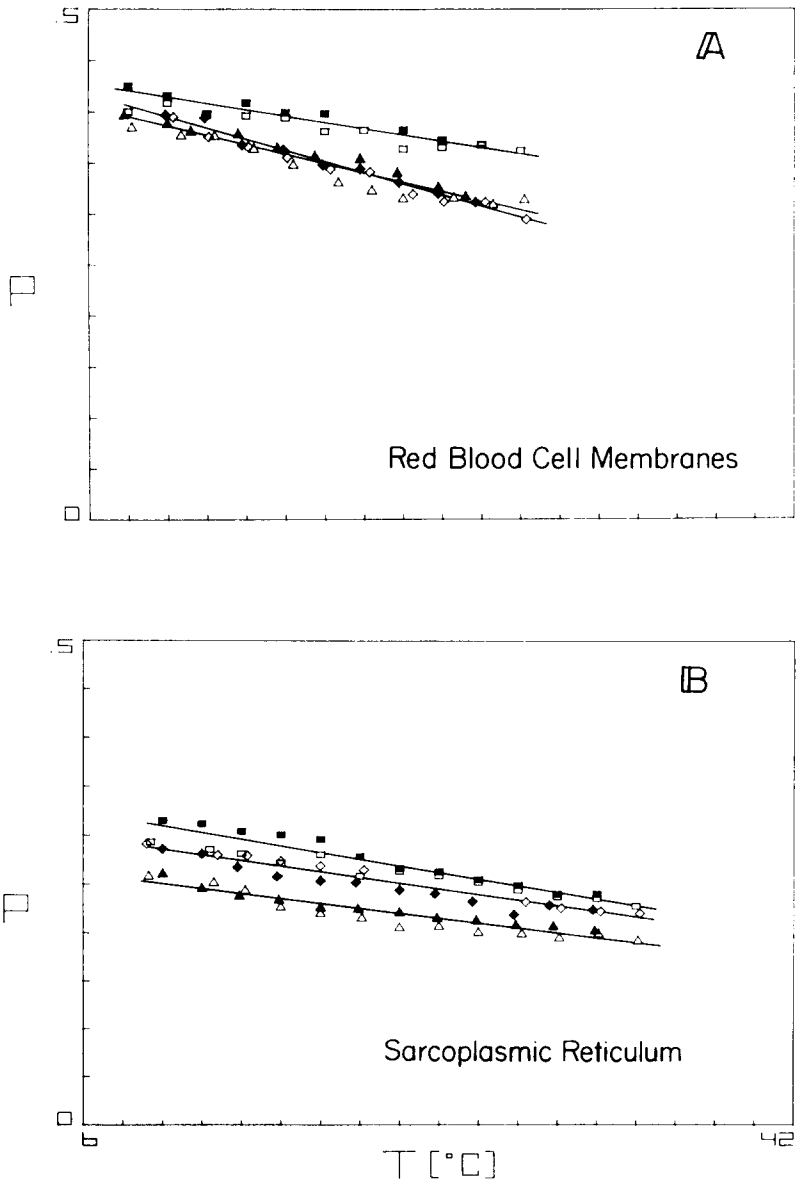

Fig. 6. Effect of general anesthetics on DPH fluorescence polarization in red blood cell membranes and sarcoplasmic reticulum membranes. A. Sample contained 0.1 mg red blood cell membrane protein/ml, 0.2 μM DPH, 250 mM KCl, 10 mM $MgCl_2$ and 4 mM $Na\text{-}PO_4$, pH 7.0. □■, no anesthetic; △▲, 37.6 mM chloroform, ◇◆, 249 mM ether. B. Sample contained 0.3 mg sarcoplasmic reticulum membrane protein/ml, 0.2 μM DPH and 10 mM $Na\text{-}PO_4$, pH 7.0. □■, no anesthetic; △▲, 6.7 mM trilene; ◇◆, 37.6 mM chloroform.

with the previous results which showed a shift in the phospholipid phase transition by the general anesthetics, the conclusion can be drawn that in a biological membrane the general anesthetic shifts the phase transition of the phospholipid thereby increasing the pool size of melted phospholipids.

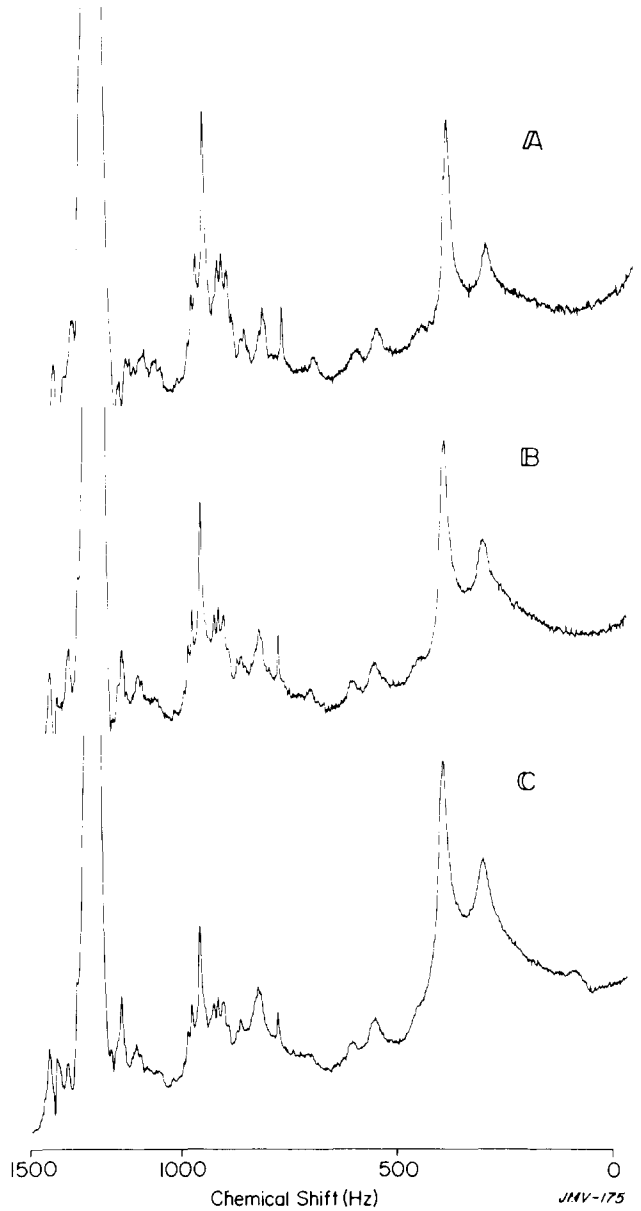

Fig. 7. 220 MHz NMR spectra of sarcoplasmic reticulum. Sarcoplasmic reticulum (40 mg dry weight/ml) were suspended in 10 mM PO_4 pH 7.0 deuterium oxide buffer. Temperature was 17°C. The two major peaks between 250 and 500 Hz are assigned to phospholipid methylene and terminal methyl protons. A. no additions; B. 38 mM halothane added; C. 76 mM halothane added.

Discussion

General anesthetics are small hydrophobic molecules which can be expected to partition into hydrophobic structures of the cell, including both cellular membranes and proteins. There are a great many possibilities where general anesthetics may act which are not excluded by this study; however, the obser-

vation that general anesthetics shift phase transitions of phospholipids to a lower temperature explains two phenomena associated with these substances. These are: (1) general anesthetics produce an increase in non-mediated diffusion in cell membranes, and (2) general anesthetics expand the surface area of membranes in an amount larger than can be accounted for by the molecule itself [18]. These properties can be accounted for by an anesthetic-induced change in the phase transition and by anesthetic-induced change in the phospholipid bilayer structure above the phase transition.

With regard to the first point, it is well known that phospholipid vesicles which are above the phase transition show increased diffusibility of a wide range of substances including fluorescent and electron paramagnetic probes [39—44], cations and anions [45,46], oxygen [30] and other substances. An increase in the size of the pool of melted phospholipids in a biological membrane could account for the increase in nonmediated diffusion across biological membranes [12]. The increase in the contribution of peaks in the NMR spectra due to the protons of the methylenes of phospholipids in sarcoplasmic reticulum provides evidence that anesthetics also shift the phase transition of lipids in biological membranes.

Lieb and Stein [47] point out that anesthetics may be considered analogous in action to plasticisers in polymer chemistry. If so, anesthetics should increase the rates of passive diffusion nondiscriminately. We find that the rotational diffusion rates of two fluorescent hydrocarbons are increased by the presence of anesthetics. Furthermore, the NMR spectra of dimyristoyl phosphatidylcholine are altered by the anesthetics, indicating that the structure is altered. In agreement with this, Metcalfe and coworkers [49] found that the proton relaxation of benzyl alcohol in erythrocyte membranes increases at high anesthetic concentration indicating that the structure is altered.

The surgical concentration of anesthetic is estimated to be 8 nmol/mg membrane protein of 25 000 sites/μ^2 [4,18]. This is approximately the concentration at which the NMR spectra of sarcoplasmic reticulum is affected. Furthermore, assuming that a biological membrane contains approximately 1 mg of lipid/1 mg protein, this is the concentration at which the shift in the phase transition can be detected with the fluorescence depolarization technique. A shift in the phase transition will change the surface area of the membrane. Below the phase transition the surface area of a phospholipid molecule is 38 Å^2, while above the transition the area expands inversely to the surface pressure. If 45 Å^2 is taken as the area above the transition it is apparent that shifting a few percent of the phospholipids will significantly increase the total membrane surface area.

Finally, it may be stated that although the data show that the anesthetics interact with phospholipids, that does not mean that anesthetics do not interact with membrane proteins. Indeed, quite the opposite conclusion can be taken. Since the interiors of most proteins are hydrophobic, the "hydrocarbon" interior of proteins and lipid bilayers can be expected to have similar physical characteristics and anesthetics can be expected to partition equally well into both. As with the lipid, the protein structure is likely to be altered, due to a weakening of the Van der Waals and hydrogen bonds leading to an expanded structure, and ultimately affecting the function of the protein.

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