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Nuclear magnetic resonance studies of the interaction of general anesthetics with 1,2-dihexadecyl-*sn*-glycero-3-phosphorylcholine bilayer

(liposome/sonication/choline/halothane/methoxyflurane)

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ABSTRACT **Sonicated 1,2-dihexadecyl-*sn*-glycero-3-phosphorylcholine forms liposomes. Studies by Fourier transform proton magnetic resonance of the interaction of these bilayers with some general anesthetics, i.e., chloroform, halothane, methoxyflurane, and enflurane, show that the addition of a general anesthetic to the liposomes and raising the temperature have a similar effect in causing the fluidization of the bilayer. General anesthetics act on the hydrophilic site (choline group) in clinical concentrations and then diffuse into the hydrophobic region with the addition of larger amount of anesthetics. There is evidence that the lecithin choline groups are involved in the interaction with protein and that the general anesthetics change the conformation of some polypeptides and proteins. We conclude that the general anesthetics, by increasing the motion of positively charged choline groups and negatively charged groups in protein, weaken the Coulomb-type interaction and cause the lipoprotein conformational changes.**

Under ultrasonic irradiation, 1,2-dihexadecyl-*sn*-glycero-3-phosphorylcholine (DHDPC) forms microvesicular liposomes in D_2O. These liposomes have been widely used as a model of a cell membrane.

We have studied the interaction of some general anesthetics, i.e., chloroform, halothane, methoxyflurane, and enflurane, with synthetic DHDPC-D_2O liposomes by Fourier transform proton magnetic resonance (PMR). Our previous work (1) showed that chloroform and halothane interacted with DHDPC-D_2O liposomes (about 1.5–2% wt/wt) that had not been sonicated or that had been sonicated in an ice-water bath (sonication temperature, 7°). The choline group was fluidized first, and the motional narrowing of the fatty acid (hexadecanoic acid) methyl and methylene groups was observed after addition of larger quantities of anesthetics at ambient temperature (28–30°). The result was compared with the temperature effect on the liposomes (2, 3). Chloroform and halothane acted on the liposomes and caused a phase transition (from crystalline to liquid-crystalline phase). The phase transition temperature occurred at 42° for the unsonicated liposomes and around 40° (in a range) for the sonicated liposomes (2, 3). The PMR intensity and linewidth below the phase transition temperature depend on the vesicle size.

The DHDPC-D_2O liposomes, whether or not they have been sonicated in an ice-water bath, show only the choline peak at 29° with the addition of methoxyflurane (>0.15 M). The linewidth of the unsonicated sample was about 30 Hz; that of the sonicated sample was about 15 Hz. The fatty acid methyl and methylene groups are only appreciably observable (linewidth 25–40 Hz) when the probe temperature is set at 37°. The interaction of enflurane at 31° with the liposome sonicated at 7° shows no choline or fatty acid methyl or methylene peaks. However, all of these anesthetics fluidize liposomes sonicated above the phase transition temperature at a probe temperature of 37°. In each case the hydrophilic choline methyl proton resonance was observed first.

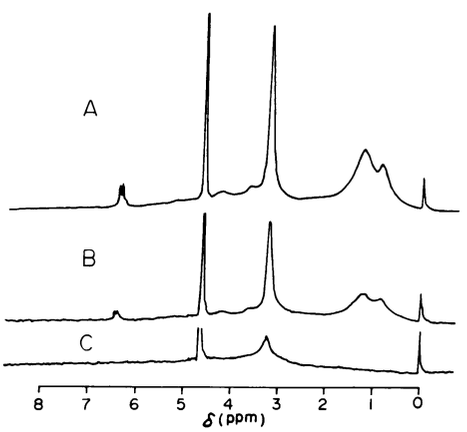

FIG. 1. High-resolution PMR 100 MHz Fourier transform spectra (100 transients) of DHDPC-D_2O (1.98% wt/wt) sonicated in the air for 10 min (final temperature 42°). (A) Halothane, 62.8 mM; (B) halothane, 31.5 mM; (C) without halothane. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate showed a peak at δ 0 ppm (parts per million).

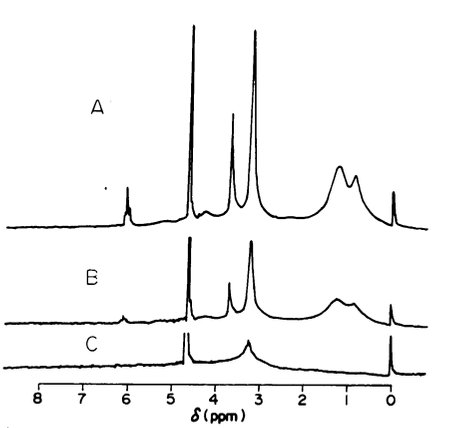

FIG. 2. High-resolution PMR 100 MHz Fourier transform spectra (100 transients) of DHDPC-D_2O (1.98% wt/wt) sonicated in the air for 10 min (final temperature 42°). (A) Methoxyflurane, 28.6 mM; (B) methoxyflurane, 14.3 mM; (C) without methoxyflurane. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate showed a peak at δ 0 ppm.

Abbreviations: DHDPC, 1,2-dihexadecyl-*sn*-glycero-3-phosphorylcholine; ppm, parts per million; PMR, proton magnetic resonance; NMR, nuclear magnetic resonance.

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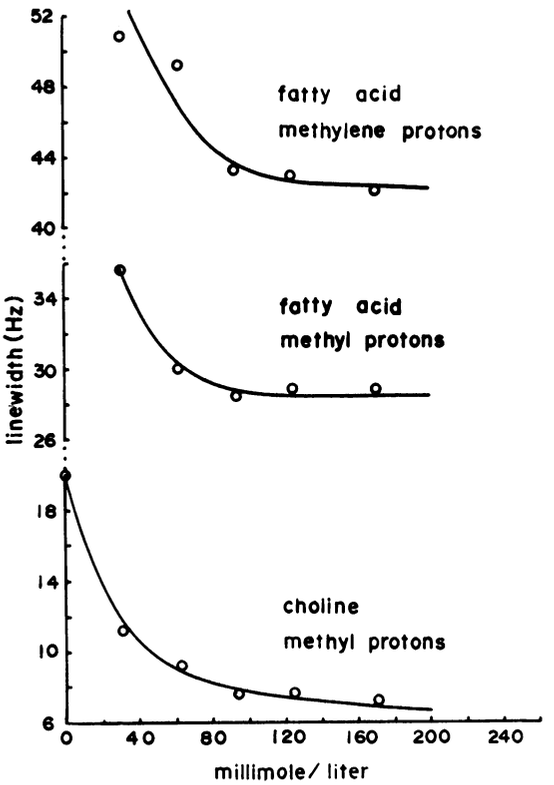

FIG. 3. The choline methyl, fatty acid methyl, and methylene PMR linewidth in Hz against concentration of halothane in the 5-mm NMR sample tube.

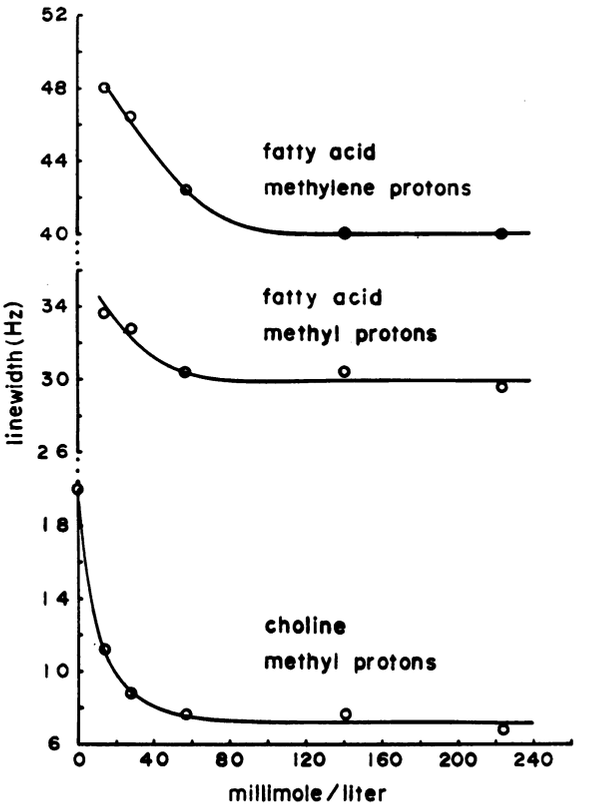

FIG. 4. The choline methyl, fatty acid methyl, and methylene PMR linewidth in Hz against concentration of methoxyflurane in the 5-mm NMR sample tube.

The effects of ultrasonic irradiation on the lecithin-water dispersion were reported by Huang (4), Hauser (5), and Finer *et al.* (6). The electron microscopic studies of Sheetz and Chan (7) on the DHDPC-D_2O sonicated above the phase transition temperature showed that the vesicle diameter may vary from 150 Å to 1400 Å. A vesicle with diameter of about 250 Å gives a choline proton peak of linewidth approximately 10 Hz and about 100% of the expected intensity (6, 7). The reason for the appearance of narrow linewidths in sonicated lecithin-water dispersion was attributed to changes in vesicle tumbling rate (rotational Brownian motion) by some authors (6, 8). Chan *et al.* (2) and Horwitz *et al.* (9) concluded that vesicle tumbling alone cannot explain the linewidth observed. Seiter and Chan (10) analyzed the PMR linewidth of both the methyl group and methylene chain proton using Anderson's stochastic theory and showed that the local motion is faster and unrestricted in sonicated lecithins formed into small sized vesicles. The internal molecular packing is, therefore, looser and more fluid for these smaller vesicles.

EXPERIMENTAL

DHDPC (Calbiochem) was used without further purification. The purity was checked by thin-layer chromatography.

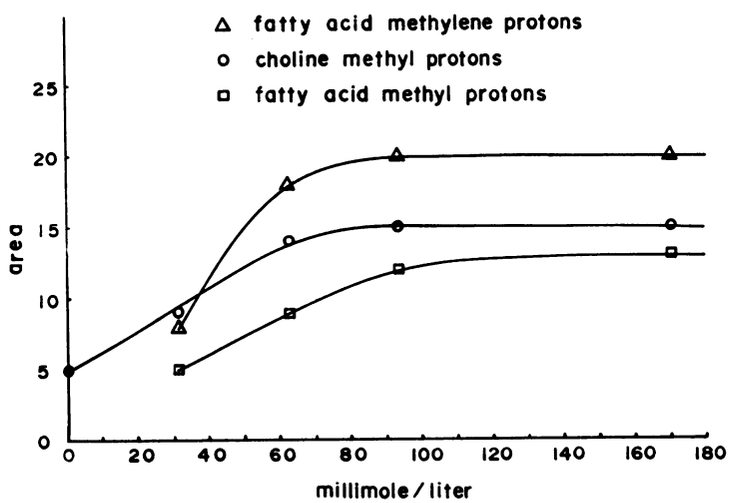

FIG. 5. The choline methyl, fatty acid methyl, and methylene PMR relative intensity (arbitary units) against concentration of halothane.

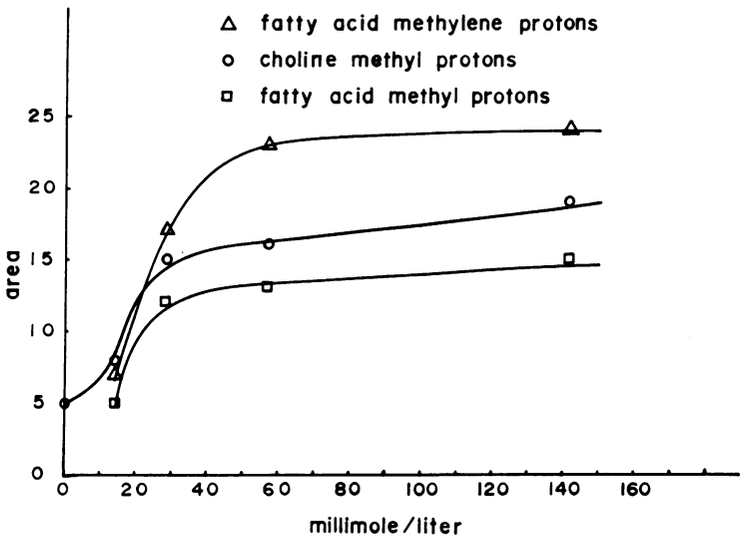

FIG. 6. The choline methyl, fatty acid methyl, and methylene PMR relative intensity (arbitrary units) against concentration of methoxyflurane.

$CHCl_3$–CH_3OH–H_2O (65:25:4 vol/vol/vol), and by a Perkin-Elmer model 141 polarimeter. The specific rotation, $[\alpha]_D^{22}$ = +6.9 (4.2 M chloroform). Deuterium oxide (D_2O, 98.8%) was purchased from Stohler Isotope Chemicals. Chloroform was Matheson, Coleman and Bell spectroquality reagent. Methoxyflurane (no inhibitor was added) was a gift from Abbott Laboratory; halothane (stabilized with 0.01% thymol) was from Ayerst Laboratories Inc.; and enflurane (without chemical stabilizers) was from Ohio Medical Products.

Sonicated liposomes were made in a glass test tube (length 10 cm and internal diameter 1.3 cm) with an ultrasonic cell disrupter (Kontes). The tip of the 4.5-inch probe was immersed to about the middle point of a 5-ml sample and the power supply was tuned to full strength for 15 min. The sonicated sample gave a single spot by thin-layer chromatography. The sonication temperature was adjusted by using an ice-water jacket or by blowing dry nitrogen on the top of the dispersion. All nuclear magnetic resonance (NMR) spectra were taken on a Varian XL-100-15 NMR spectrometer interfaced to a Varian 620/f computer operated in the pulsed Fourier transformed mode. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate was used as an internal reference (about 0.5% wt/wt) for proton NMR chemical shift measurement.

RESULTS AND DISCUSSION

A DHDPC-D_2O dispersion sonicated at or above the phase transition temperature shows a choline PMR peak with linewidths ranging from 12 to 40 Hz at 37°. The fatty acid methyl and methylene PMR peaks appear after general anesthetics are added. The linewidths of these PMR spectra decrease and the peak intensities increase when the amounts of anesthetic are increased (Figs. 1–6). The linewidths decrease sharply when less than 10 mM anesthetics is present. The peak assignments and their chemical shifts are listed in Table 1. The chemical shifts of water (HDO), choline methyl, and fatty acid methyl and methylene peaks do not change. The proton chemical shifts of the anesthetics in carbon tetrachloride (CCl_4) and in deuterium oxide (D_2O) are listed in Table 2. The proton peak of chloroform, halothane, and the ethoxy group in methoxyflurane is shifted to higher magnetic fields with the addition of more anesthetic (Fig. 7). The methoxy proton peak of methoxyflurane also shifted to a higher field, but the chemical shift change is about three to four times smaller than for the ethoxy proton shift.

The addition of general anesthetics to the liposomes and raising the temperature have a similar effect in producing sharper and larger proton peaks. This is the result of a faster proton motion and a more complete averaging of the proton magnetic dipole–dipole interactions. There is evidence that the lecithin choline groups are involved in interaction with protein (11–13). Our preliminary study of the interaction of general anesthetics with poly(L-lysine) and some other crystalline proteins with circular dichroism showed that general anesthetics induce conformational change in these macromolecules. We conclude that the general anesthetics, by increasing the motion of positively charged choline groups and negatively charged groups in protein, weaken the Coulomb-type interaction and cause the lipoprotein conformational changes. The general

Table 1. Chemical shifts δ (ppm) of water (HDO), choline methyl, fatty acid methyl, and methylene proton peaks of DHDPC-D_2O dispersion at 37°

HDO	4.60 (±0.02)
Choline methyl	3.23 (±0.02)
Fatty acid methyl	0.88 (±0.03)
Fatty acid methylene	1.25 (±0.03)

Table 2. Chemical shifts δ (ppm) of protons of chloroform ($CHCl_3$), halothane ($CHBrClCF_3$), methoxyflurane ($CHCl_2CF_2OCH_3$), and enflurane ($CHClFCF_2OCHF_2$) in carbon tetrachloride (CCl_4) and in deuterium oxide (D_2O)

	CCl_4	D_2O
Chloroform	7.23	7.68
Halothane	5.77	6.45
Methoxyflurane		
$CHCl_2CF_2$—	5.62	6.25
—OCH_3	3.65	3.72
Enflurane		
$CHClFCF_2$—	6.12	6.67
—$OCHF_2$	6.69	7.12

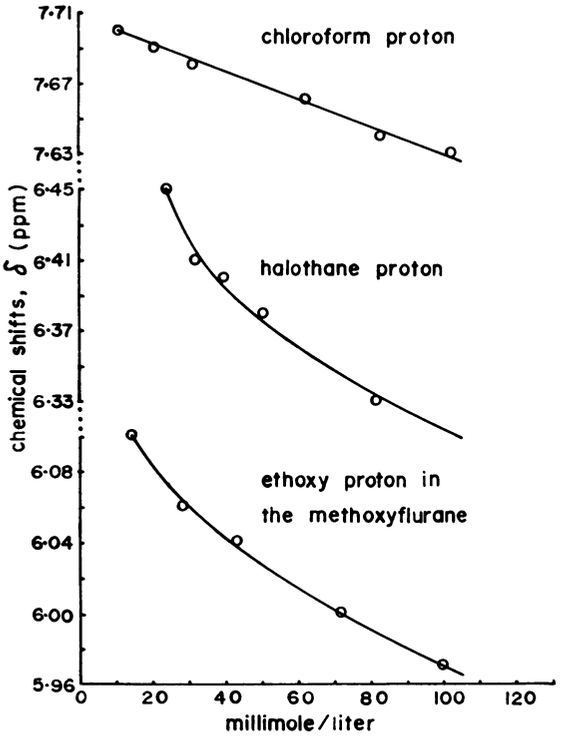

FIG. 7. The proton chemical shifts δ (ppm) of chloroform, halothane, and methoxyflurane against concentration of these anesthetics in the DHDPC-D_2O dispersion (1.5% wt/wt) sonicated above the phase transition temperature of DHDPC.

trend of our chemical shift data, from lower field (higher frequency) to higher field (lower frequency) shifts, shows that the general anesthetics start to interact in the hydrophilic region and then diffuse into the hydrophobic area of the bilayer with the addition of larger amount of anesthetics. The observed chemical shifts are the average of the chemical shifts of general anesthetics in deuterated water and in the bilayer.

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