^2H and ^{31}P NMR Study of the Interaction of General Anesthetics with Phosphatidylcholine Membranes†

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ABSTRACT: The effect of the general anesthetics α-chloralose and chloral hydrate as well as the nonanesthetic β-chloralose on the order of phosphatidylcholine and phosphatidylcholine–cholesterol liposomes has been examined by ^2H nuclear magnetic resonance. Chloral hydrate interacts with the hydrophilic head-group region, causing a change in the torsion angle of the C_α–C_β bond. The membrane interior is also disordered by the presence of this agent. α-Chloralose, on the other hand, disorders only the central position of the membrane. β-Chloralose produces little significant change in bilayer order.

The mechanisms of induction of general anesthesia are probably quite diverse. Also, they are not well understood especially on a molecular level, although most theories involve changes in the lipid bilayers of nerve cell membranes (Franks & Lieb, 1978). Possible formation of ion channels (Lee, 1976), interaction with membrane protein (La Bella, 1981), phase changes or separations (Cullis et al., 1980; Koehler et al., 1980), and disruption of lipid (Pang et al., 1980) are among the phenomena which have been postulated to be involved in the overall process (Seeman, 1972). In addition, the anesthetic molecules themselves range from atomic xenon to complex organic heterocycles (Kaufman, 1977). Therefore, it is quite possible that the mechanism of general anesthesia as induced by inhalational anesthetics may well be different from that produced by intravenous agents. In other words, the pathway may depend on the molecular size and/or shape and the number and distribution of the functional groups present in these central nervous system depressants.

Clinical concentrations of general anesthetics are very often measured in terms of the concentration in the bloodstream which must be maintained for the continuation of anesthesia. The anesthetic agent continuously partitions out of the bloodstream and into biological membranes where it causes some type of reversible perturbation of neuronal behavior (Roth, 1979). Seeman (1972) has noted that the concentrations of general anesthetics in the membrane required to produce general anesthesia are typically one-tenth to one-twentieth that required for local anesthesia and that, in general, the concentration of anesthetic *in the membrane* required to produce general anesthesia is on the order of 0.003 mol/kg of dry membrane. Taking into account that approximately 50% of the membrane is protein, as well as the presence of sterols and carbohydrates, it is readily seen that the minimum concentration of drug required *in the membrane* to produce general anesthesia is approximately 0.01 mol of anesthetic/mol of phospholipid. Furthermore, it has been reported (Seeman, 1972) that saturation of the membrane binding sites for general anesthetics occurs at a concentration of 0.07 mol of anesthetic present in 1 kg of dry membrane, or approximately 0.20 mol of drug present in 1 mol of membrane phospholipid. It must be emphasized that these concentrations are concentrations of anesthetic in the membrane only and do not take into account that quantity which is present in the aqueous phase. Therefore, in this investigation of chloral-type general anesthetics, we have used concentrations of anesthetic *in the sample* ranging from 1% to 50% of the total number of moles of anesthetic plus phospholipid. Obviously, the concentration of anesthetic in the membrane will be lowered by an amount which depends on the partition coefficient of the particular agent. For example, chloral hydrate has a partition coefficient of close to unity (Barker, 1975; Pang et al., 1980; Leo et al., 1971), and thus, the anesthetic present in the sample will be largely present in the aqueous phase.

In order to examine the effect of general anesthetics on the ordering of lipid membrane components, we have used deuterium NMR of saturated and unsaturated ^2H-labeled phospholipid "guests" present in a fluid liquid-crystalline matrix of egg yolk phosphatidylcholine (PC).[1] Deuterium labeling itself introduces a negligible perturbation, and thus, the labeled species will accurately report the order parameters of both saturated and unsaturated membrane phospholipids in the L_α phase (vide infra). The anesthetics chosen were chloral hydrate (2,2,2-trichloro-1,1-ethanediol) and its glucose derivative α-chloralose [1,2-*O*-(2,2,2-trichloroethylidene)-α-D-glucofuranose]. The nonanesthetic isomer β-chloralose was also studied.

Very few investigations have been carried out, of the interactions of general anesthetics, especially of the "chloral type", on membrane systems at a molecular level. Extensive work has been done with several "caine"-type local anesthetics (Guerin et al., 1980; Singer & Jain, 1980; Boulanger et al., 1980, 1981; Westman et al., 1982; Kelusky & Smith, 1983, 1984). Considerably less is known about molecular level general anesthetic–membrane interactions. In addition, a large portion of these studies has employed electron spin resonance spectroscopy of nitroxide-labeled fatty acids, sterols, and occasionally phospholipids (Paterson et al., 1972; Pang et al., 1980; Gordon et al., 1980; Ogiso et al., 1981; Gaffney et al., 1983). Although it has long been argued that the bulky nitroxide groups themselves perturb membrane organization, it has more recently been demonstrated that certain fatty acid

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[1] Abbreviations: NMR, nuclear magnetic resonance; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine; PC, phosphatidylcholine; CSA, chemical shift anisotropy; CHOL, cholesterol.

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nitroxide spin probes do not even qualitatively correctly describe such phenomena as the membrane ordering effect of cholesterol incorporation into liquid-crystalline bilayer membranes or transitions between lamellar and hexagonal phases (Taylor & Smith, 1980, 1981a,b.) Therefore, the effects of the presence of chloral-type general anesthetics on phospholipids have been studied by using nuclear magnetic resonance. Not only was the hydrophobic interior of the bilayer membrane probed but also the hydrophilic head group was deuterium labeled α and β to the phosphate group (DPPC-α,β-d_4) as well as at the trimethylammonium group (DPPC-γ-d_9). ^{2}H NMR of the head-group complements that obtained from ^{31}P NMR in that the quadrupole splittings of DPPC-α,β-d_4 are sensitive to changes in head-group conformation (Akutsu & Seelig, 1981).

Materials and Methods

DPPC-d_{62}, DPPC-*2,2,2′,2′*-d_4, DOPC-*9,9′,10,10′*-d_4, and DPPC-γ-d_9 were synthesized as previously described (Forrest et al., 1984). DPPE-α,β-d_4 was prepared as reported by Taylor & Smith (1981a) and exhaustively methylated to form DPPC-α,β-d_4. Egg yolk PC was extracted according to the method of Singeton et al., (1965) and purified by chromatography on alumina and silica gel. Chloralose was codissolved with lipid in chloroform, followed by exhaustive solvent removal. The samples were dispersed in excess deuterium-depeleted water by vortex mixing and numerous freeze–thaw cycles. Chloral hydrate was added in the aqueous phase. The lipid concentration was kept constant at 450 mM, and the concentration of added anesthetic is expressed (as a percentage) as the number of moles of anesthetic divided by the number of moles of anesthetic plus phospholipid. The deuterium-labeled DPPC's constituted less than 10% of the total lipid present. At this concentration at 20 °C, the lipids are completely miscible, and all phospholipids are in the liquid-crystalline phase (Davis et al., 1980). This fact was also confirmed by subsequent NMR spectra (vide infra).

NMR spectra were recorded on a Nicolet 360 NB spectrometer operating at approximately 55.4 MHz for deuterium and 146.2 MHz for phosphorus. The normal probe temperature was 20 °C. ^{2}H spectra were recorded either by using a Nicolet variable-frequency probe head employing a single pulse technique with 90° pulses of approximately 40-μs duration or, in the latter stages of this work, by using a fixed-frequency probe head (Probe Systems Inc.) employing a quadrupole echo technique (Davis et al., 1976; Siminovitch et al., 1980) with 90° pulses of approximately 7-μs duration and a delay of 60–80 μs between the 90° pulses. Recycle times were on the order of 200–1000 ms. ^{31}P spectra were recorded with broad-band proton decoupling using a two-level decoupling sequence with 60° pulses of 15-μs duration.

Results

Hydrophilic Head-Group Region. The proton-decoupled ^{31}P NMR spectrum of egg PC in the liquid-crystalline L_α phase consists of an asymmetric line shape dominated by the residual chemical shift anisotropy (~46.2 ppm) of the phosphate group. Increases in this CSA are associated with decreased mobility of this moiety while a decrease in this parameter indicates additional motional freedom, assuming that the head-group conformation remains unchanged. However, it has been shown that if conformational changes do occur which alter the orientation of the axis of motional averaging with respect to the principal axis frame of the chemical shielding tensor, then large changes in the apparent ordering of the phosphate group could occur (Thayer & Kohler, 1981). For this reason, confirmative experiments were performed using ^{2}H NMR of the head-group deuterium-labeled phospholipids DPPC-α,β-d_4 and DPPC-γ-d_9 (Taylor & Smith, 1981a). The effect on the ^{31}P CSA of the addition of chloral hydrate to egg PC liposomes is shown in Figure 1. A small gradual decrease to 45.8 ppm at 18 mol % chloral hydrate is observed, followed by a sharper decrease to 44.4 and 41.8 ppm at 33 and 50 mol %, respectively. α- and β-chloralose provoked little change in the ^{31}P CSA at concentrations up to 20 and 25 mol %.

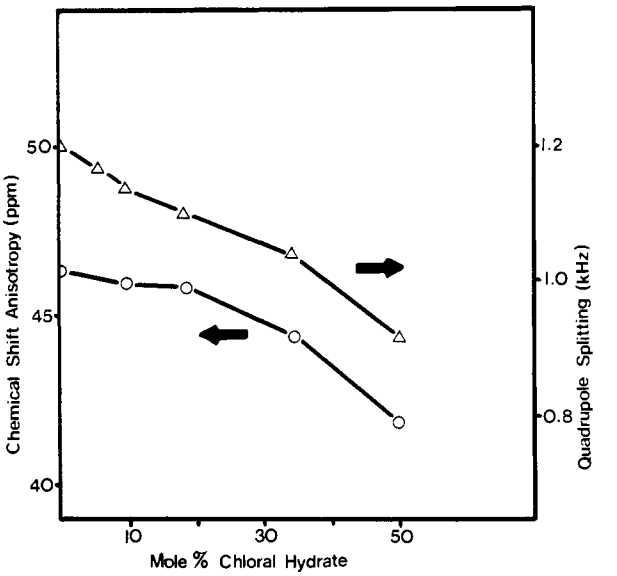

FIGURE 1: ^{31}P chemical shift anisotropy of the phosphate group of PC (O) and ^{2}H quadrupole splittings of DPPC-γ-d_9 (Δ) as a function of chloral hydrate addition.

Also shown in Figure 1 is the variation in quadrupole splitting of DPPC-γ-d_9. In the absence of anesthetic, a small splitting of approximately 1200 Hz is observed which decreases in a roughly linear fashion with increasing chloral hydrate concentration, reaching a value of 916 Hz at 50 mol % addition, i.e., a decrease of approximately 25%. Addition of 25 mol % α- or β-chloralose produced lesser decreases to 1130 and 1120 Hz, respectively.

The hydrophilic region was also probed by using DPPC-α,β-d_4 which is deuterium labeled at the two methylene groups α and β to the phosphate group. In the pure PC system, the resonances from these two positions are partially overlapped, with an average quadrupole splitting of approximately 6.7 kHz. The spectrum remains relatively unchanged upon addition of up to 25 mol % α- or β-chloralose. However, the situation is substantially different when chloral hydrate is introduced into the membrane system. Figure 2 shows that the order parameters of the α- and β-methylenes diverge with increasing anesthetic concentration with the α-methylene showing an increasing quadrupole splitting and the β-methylene a decreasing one. Assignments were made on the basis of the relative intensities and the different percentage of deuterium incorporation at the two positions, and also on the basis of chemical shift. Figure 3 shows the ^{2}H NMR spectrum of this molecule at 50 mol % chloral hydrate addition. Two main quadrupole splittings of 3.1 and 7.6 kHz are resolved. Also, there is some indication that the peaks with the largest of these splittings may be split into a doublet, implying a very slight nonequivalence of the two deuterons adjacent to the phosphate.

Saturated Acyl Chains. The palmitate chains of DPPC were both selectively labeled at carbon 2, next to the ester linkage, and massively deuterium substituted at all positions.

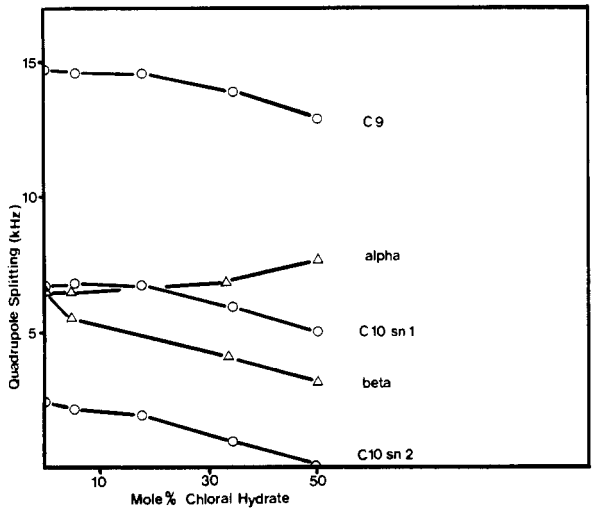

FIGURE 2: ^2H NMR quadrupole splittings of the head-group methylenes of DPPC-α,β-d_4 (△) and positions 9 and 10 of DOPC-*9,9',10,10'*-d_4 (○) as a function of chloral hydrate addition.

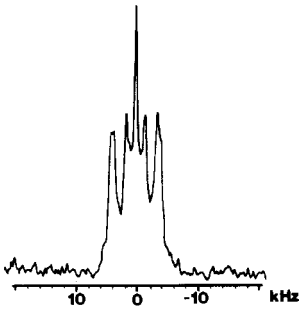

FIGURE 3: ^2H NMR spectrum of DPPC-α,β-d_4 in the presence of 50 mol % chloral hydrate.

Assignments of the three major quadrupole splittings of the C-2 deuterons of the *sn*-1 and *sn*-2 chains have been made previously (Seelig & Seelig, 1974; Haberkorn et al., 1977; Engel & Cowburn, 1981). The incorporation of up to 20 mol % β-chloralose had no effect on the quadrupole splittings of the two C-2 deuterons on the bent *sn*-2 chain, their values remaining constant at 12.3 and 18.6 kHz. However, the incorporation of a like ratio of α-chloralose produced an increase of about 15% in both of these splittings to 13.5 and 19.8 kHz. Similar increases were found for the incorporation of chloral hydrate. Quadrupole splittings of 15.8 and 20.5 kHz were found with 50 mol % addition of this agent, i.e., an increase of up to 25% in the order parameter of the C–D bond axes of the *sn*-2 chain. While the *sn*-2 chain reports higher order at the C-2 position in the presence of this anesthetic, the quadrupole splitting of both equivalent deuterons at carbon 2 of the *sn*-1 chain *decreases* by 10% from approximately 30 to 26.8 kHz.

Turning to the membrane interior, the addition of up to 25 mol % of β-chloralose had a negligible effect on the quadrupole splittings of either the terminal methyl groups in the center of the bilayer or the methylene groups, $(CD_2)_n$, which give rise to the characteristic plateau region of uniform order of the upper positions of the hydrocarbon chains. Results for the incorporation of α-chloralose and chloral hydrate are given in Table I. It is clear that the major perturbation of α-chloralose is felt near the center of the membrane. The quadrupole splittings of the terminal methyl groups of DPPC decrease by 21% from 2870 to 2267 Hz, while positions 15 and 14 decrease by 23% and 22%, respectively. The main methylene envelope, however, which includes roughly positions

Table I: Quadrupole Splittings (kHz) of DPPC-d_{62} in the Presence of α-Chloralose and Chloral Hydrate at 20 °C

	(A) α-Chloralose conen of anesthetic (mol %)				
position	0	1	5	17	25
16	2.87	2.66	2.67	2.32	2.28
15	11.10	10.16	10.16	8.91	8.59
14	12.90	12.03	11.94	10.63	10.00
$(CD_2)_n$	30.30	29.56	29.57	28.80	27.87
	(B) Chloral Hydrate conen of anesthetic (mol %)				
position	0	5	17	33	50
16	2.87	2.78	2.70	2.43	1.91
15	11.10	11.25	10.00	9.22	7.50
14	12.90	12.25	11.88	10.63	8.44
$(CD_2)_n$	30.30	29.73	29.06	27.82	24.77

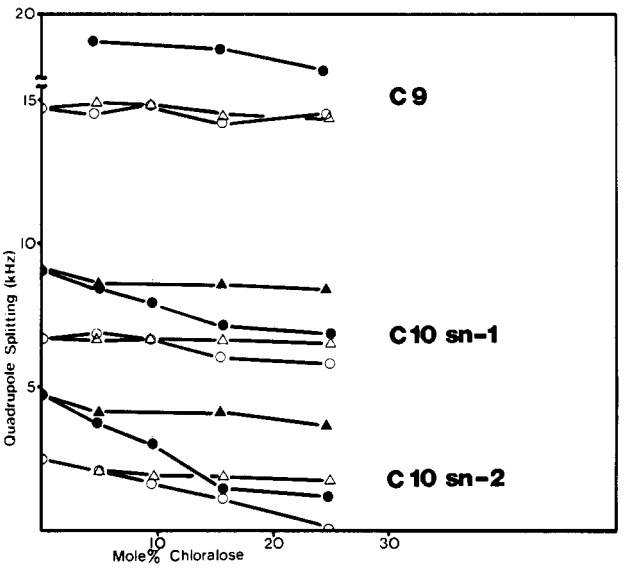

FIGURE 4: ^2H NMR quadrupole splittings of positions 9 and 10 of DOPC-*9,9',10,10'*-d_4 in the presence of α-chloralose (○) and β-chloralose (△). The solid symbols (●, ▲) indicate that 25 mol % of the lipid component of the membrane is cholesterol.

3–9, decreases only by 8% from 30.3 to 27.9 kHz. The addition of like amounts of chloral hydrate also causes slightly less large decreases in the order of positions 14–16 or the CD_2 envelope. An equimolar PC–chloral hydrate system results in lowering of the observed quadrupole splittings by over 30% for the terminal end of the chain and by only an average of 18% for the overlapping methylenes higher in the chain.

Unsaturated Acyl Chains. In the absence of any anesthetic, the spectrum of DOPC-d_4 in egg PC consists of three quadrupole splittings of 14.7, 6.7, and 2.4 kHz. These have been assigned to C-9 of both acyl chains, C-10 of the *sn*-1 chain, and C-10 of the *sn*-2 chain, respectively (Seelig et al., 1981; Tamm & Seelig, 1983). Figure 4 shows the effect of α- and β-chloralose on the double-bond region. Position 9 is little affected by either anesthetic up to 25 mol %. Position 10 of the *sn*-1 chain is not perturbed by β-chloralose, but a small decrease is observed for α-chloralose. The order at position 10 of the *sn*-2 chain is decreased marginally by small amounts of either α- or β-chloralose, but as the concentration of these two agents is increased, no further effect is manifested by β-chloralose, while the original 2.4-kHz quadrupole splitting collapses to a singlet as more α-chloralose is added. Figure 2 shows the results for the DOPC-d_4 probe in the presence of chloral hydrate. Lower concentrations up to 20 mol % show slight changes in order while addition of up to 50 mol % results

in larger decreases in the quadrupole splittings for positions 9 and 10 of both chains, with the resonance for the 10-position of the *sn*-2 chain once again collapsing to a singlet.

The effect of α- and β-chloralose on a membrane containing 25 mol % cholesterol was also investigated. As shown in Figure 4, the presence of cholesterol increases the order of the double-bond region. Anesthetics in this mixed membrane system mimic their effect in the absence of cholesterol with the result that the order profiles are shifted to higher values in the cholesterol-containing membrane.

Discussion

The deuterium NMR order parameter, S_{CD}, is proportional to $\Delta\nu_Q$, the separation of the two most intense peaks of the quadrupolar powder pattern, according to

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{CD} \quad (1)$$

where e^2qQ/h is the static quadrupole coupling constant, which for a C–D bond of a methylene group is approximately 170 kHz. Assuming axially symmetric motion of the labeled moieties, S_{CD} may be broken down into (Seelig, 1977; Seelig & Waespe-Sarcevic, 1978; Dufourc et al., 1983)

$$S_{CD} = S_\alpha S_\gamma \quad (2)$$

where S_α, which is often referred to as the molecular order parameter, S_{mol}, reflects the angular fluctuations of the motion of the deuterium-labeled subunit of the phospholipid molecule with respect to the main axis of motion which is usually the bilayer normal. S_γ, in this notation, monitors the time-average orientation of a given C–D bond of the subunit with respect to the axis of subunit motion. The order parameter S_{CD} and thus the quadrupole splittings themselves are a measure of the flexibility of the labeled segment in that they give a time average of the angular excursions of the C–D bond vector. One major type of increased motional freedom is associated with gauche–trans isomerization of the hydrocarbon chains which produce kinks and jogs in the hydrophobic region, leading to decreased bilayer thickness (Seelig & Seelig, 1974). It should also be mentioned that although the ^{2}H NMR order parameters and quadrupole splittings measure motional freedom, they do not measure changes in rates of motion.

It must be reemphasized at this point that the phospholipids of the membrane system in the present study are in the liquid-crystalline L_α state; i.e., even at 20 °C, DPPC-d_{62} and the other saturated deuterium-labeled phospholipids are not in the gel phase because they are present in small quantities in an egg PC matrix which has a gel–liquid-crystalline phase transition temperature of well below 0 °C. In addition to calorimetric evidence (Davis et al., 1980), this fact is confirmed by the ^{2}H NMR spectra themselves. In the gel state, the NMR spectrum of DPPC-d_{62}, for example, consists of a very broad nonaxially symmetric resonance due to the chain methylenes whose total width is approximately 125 kHz (Pauls et al., 1983). Superimposed on this is a quadrupole splitting of approximately 12 kHz which is due to the terminal methyl groups of the hydrocarbon chains. In the liquid-crystalline L_α state, however, an axially symmetric spectrum consisting of a number of resolved quadrupole splittings is observed. The maximum quadrupole splitting for the acyl chain methylenes is on the order of 30 kHz, while that for the terminal methyl groups is approximately 3 kHz. The reduction in $\Delta\nu_Q$ in going through the phase transition is due mainly to the onset of rapid rotation about the molecular long axis, as well as trans–gauche isomerizations of the fatty acyl chains. In the absence of added anesthetic, we have verified by ^{2}H NMR that all of the deuterium-labeled lipids used in this study are in the fluid liquid-crystalline phase (Forrest et al., 1984; Forrest & Rodham, 1985). Thus, by incorporation of various labeled phospholipids, the order parameters of both saturated (DPPC) and unsaturated (DOPC) phospholipids may be successively determined in a fluid membrane at the same temperature. While excitable membranes possess a certain degree of unsaturation, they are not totally unsaturated. The method of doping a fluid membrane with different types of phospholipids allows for a fuller description of the ordering of the different phospholipids which are present in natural membranes.

Neither the phosphate group nor the two intervening methylene groups between it and the trimethylammonium group is affected by the anesthetic α-chloralose or its nonanesthetic isomer. The much more water-soluble anesthetic chloral hydrate, however, has been shown to decrease the CSA of the phosphate group and also to decrease the order parameter of the trimethylammonium group of PC, indicating increased motional freedom within the hydrophilic region. However, more detailed information is available from the divergence of the quadrupole splittings of the methylenes of the choline group. The α-deuterons give rise to a slightly increased order parameter while that of the deuterons β to the phosphate decreases sharply. This response to the presence of chloral hydrate is opposite to that caused by ions such as Na^+, Ca^{2+}, or La^{3+} but in the same direction as the introduction to the membrane of chloroform, cholesterol, or dimyristoylphosphatidylglycerol (Gally et al., 1975; Akutsu & Seelig, 1981; Sixl et al., 1984; Sixl & Watts, 1982). It is also interesting to note that the effect of chloral hydrate on the hydrophilic region of the membrane is exactly the opposite of that found for the addition of the protonated form of the local anesthetic tetracaine (Boulanger et al., 1981); i.e., this local anesthetic when present in an anesthetic:PC ratio of up to 1:1 causes a decrease in the quadrupole splitting of the methylene α to the phosphorus atom and increases the quadrupole splittings of the β-methylene and γ-methyl groups as well as causing an increase in the ^{31}P CSA. The effect caused by chloral hydrate in the present study is the same as that which can be caused by a temperature elevation of roughly 40 °C (Gally et al., 1975). Previous investigations have shown that the divergence of these quadrupole splittings, with the β-position decreasing, can be explained in terms of the conformation of the C_α–C_β bond. The time-average torsion angle changes to such that the C_α–C_β bond moves toward a gauche conformation, and as a consequence, the trimethylammonium group moves closer to the phosphate. Therefore, it is probable that in this case, the water-soluble chloral hydrate is present in the interface and disrupts the normal intermolecular electrostatic interaction of the zwitterion groups of neighboring PC molecules. The much less water-soluble chloralose does not interact strongly with the head-group region. In this regard, it should be noted that the octanol–buffer partition coefficient of α-chloralose is 40, while for chloral hydrate it is close to unity (Barker, 1975; Pang et al., 1980; Leo et al., 1971).

As has been shown, both α-chloralose and chloral hydrate influence the ordering of the membrane interior, with the larger effect *per molecule added to the system* being manifested by α-chloralose. However, due to the differences in partition coefficients, it is apparent that while the bulk of the chloralose partitions into the membrane, a large proportion of the chloral hydrate is associated with the aqueous matrix. Therefore, on the basis of *per molecule associated with the membrane*, the intrinsic perturbation of chloral hydrate is the

greater. Since increases in order were found for the C-2 *sn*-2 deuterons in the presence of both chloral hydrate and α-chloralose, it is concluded that both these agents can penetrate to this level in the membrane with chloralose being intercalated among the hydrocarbon chains. The region below this depth then undergoes freer motion because of the mismatch of the lengths of the anesthetic molecule and the lipid chains.

Since the C–D bond axes at the 10-position of the *sn*-1 and *sn*-2 chains of DOPC are close to the magic angle with respect to the bilayer normal, they give rise to lower quadrupole splittings than the same position of saturated chains. In other words, the small quadrupole splittings of DOPC-*9,9',10,10'-d*$_4$ in the liquid-crystalline phase are primarily due to the geometry of the cis double bonds. Because of their orientation with respect to the director, their quadrupole splittings are quite sensitive to small alterations in motional freedom. In fact, the only significant effect of β-chloralose was seen at low concentrations at the C-10 position of the *sn*-2 chain of DOPC. As for α-chloralose and chloral hydrate, the decrease in the order parameter at this position is continuous with both agents collapsing the quadrupole splitting to a singlet when roughly 25 mol % of the anesthetic is associated with the membrane. The complete collapse of the ^{2}H quadrupole splitting by itself does not imply zero order or isotropic motion. The intrisic order may be relatively high since the direction of the axis of motional averaging is not accurately known (Seelig & Waespe-Sarcevic, 1978; Dufourc et al., 1983). However, the continuous decrease to a null quadrupole splitting upon increasing addition of α-chloralose and chloral hydrate indicates progressive perturbation of the membrane interior through decreases in S_α or S_γ or both.

Conclusions

From the foregoing, it may be concluded that chloral hydrate and α- and β-chloralose interact in a different fashion with phosphatidylcholine membranes. The perturbation by β-chloralose is very slight and is felt only at highly sensitive fragments in the membrane interior. α-Chloralose intercalates into the upper part of the hydrophobic region and disorders the hydrophobic region below its position by creating a relatively freer volume for movement of the acyl chains. The much more water-soluble chloral hydrate exerts its effect mainly because of a perturbation of the head-group region. The disruption of electrostatic interactions will result in a larger surface area per lipid molecule, and as a direct consequence, disordering of the membrane interior results. The change in the C_α–C_β torsion angle found for the incorporation of chloral hydrate is equal to that which can be produced thermally by a temperature increase of 40 °C but opposite to that caused by the local anesthetic tetracaine (Boulanger et al., 1981).

On the whole, it appears that general anesthetics do not all disorder the same regions of PC membranes. In a qualitative sense, α-chloralose is similar in its effect to chlorpromazine (Forrest et al., 1984). The site of action of chloral hydrate is the head-group region. While this points to the importance of the relative hydrophilicity of the anesthetic as being of great importance, the matter does not rest there. The water-insoluble anesthetic trichloroethylene does not perturb the ordering of the lamellar phase of PC, but instead induces a change to a hexagonal H_{II} phase (Forrest & Rodham, 1985). It is then probable that if membrane lipid is the site of anesthetic action, there exist a variety of specific mechanisms which depend upon the individual anesthetic agent involved.

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Registry No. α-Chloralose, 15879-93-3; chloral hydrate, 302-17-0.

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^{19}F NMR Investigation of Molecular Motion and Packing in Sonicated Phospholipid Vesicles†

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ABSTRACT: Dimyristoylphosphatidylcholine (DMPC) labeled with a $C^{19}F_2$ group in the 4-, 8-, or 12-position of the 2-acyl chain has been investigated in sonicated unilamellar vesicles (SUV) by fluorine-19 nuclear magnetic resonance (NMR) at 282.4 MHz from 26 to 42 °C. The ^{19}F NMR spectra exhibit two overlapping resonances with different line widths. Spin–lattice relaxation time measurements have been performed in both the laboratory frame (T_1) and the rotating frame ($T_{1\rho}$) in order to investigate the packing and dynamics of phospholipids in lipid bilayers. Quantitative line-shape and relaxation analyses are possible by using the experimental chemical shift anisotropy ($\Delta\nu_{CSA}$) and the internuclear F–F vector order parameter (S_{FF}) values obtained from the ^{19}F powder spectra of multilamellar liposomes. The following conclusions can be made: (i) The ^{19}F chemical shift difference between the inside and outside leaflets of SUV can be used to monitor the lateral packing of the phospholipid in the two SUV monolayers. The hydrocarbon chains in the outer layer are found to be more tightly packed than those of the inner one, and the differences between them become smaller near the chain terminals. (ii) The effective correlation time [$(1–4) \times 10^{-7}$ s] obtained from either the motional narrowing of the line widths or off-resonance $T_{1\rho}$ measurements is shorter than that estimated from the Stokes–Einstein diffusion model (10^{-6} s), on the basis of a hydrodynamic radius of 110 Å for SUV. (iii) $T_{1\rho}$, but not T_1, is found to be different for the inside and outside resonances of the SUV, indicating that slower motions (10^{-7} s) are responsible for the different line widths of the two resonances. (iv) The Arrhenius activation energies determined from T_1 values for the 4- and 8-^{19}F-labeled positions (5 kJ/mol) are lower than those obtained for the 12-position (10 kJ/mol). ^1H T_1 measurements on both the ^{19}F-labeled and unlabeled sonicated DMPC vesicles indicate that there is no perturbation of the fatty acyl chain packing by the CF_2 group. (v) The curvature in the SUV perturbs the fatty acyl chain packing mainly in the outside leaflet of the bilayer.

Fluorine-19 has recently received attention as an attractive nuclear magnetic resonance (NMR)[1] probe for membrane studies [for a recent review, see Ho et al. (1985)]. To investigate lipid fatty acyl chain order and dynamics in both model and biological membranes, myristoyl([$^{19}F_2$]difluoromyristoyl)phosphatidylcholine ([$^{19}F_2$]DMPC) has been used as a sensitive probe. By applying various NMR techniques such as the Carr–Purcell–Meiboom–Gill multiple-pulse sequence (Post et al., 1984) and line-shape analysis (Engelsberg et al., 1982; Dowd et al., 1984) to both oriented multilayers and random multilamellar liposomes of [$^{19}F_2$]DMPCs, this laboratory has determined the order parameter, S_{FF}, for the F–F internuclear vector from the Pake doublet splitting. In addition, it has become clear that, at high magnetic fields (7

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[1] Abbreviations: NMR, nuclear magnetic resonance; DMPC, dimyristoylphosphatidylcholine; [$^{19}F_2$]DMPC, myristoyl([$^{19}F_2$]difluoromyristoyl)phosphatidylcholine; 2-[4,4-$^{19}F_2$]DMPC, 1-myristoyl-2-(4,4-[$^{19}F_2$]difluoromyristoyl)-*sn*-glycero-3-phosphocholine; 2-[8,8-$^{19}F_2$]-DMPC, 1-myristoyl-2-(8,8-[$^{19}F_2$]difluoromyristoyl)-*sn*-glycero-3-phosphocholine; 2-[12,12-$^{19}F_2$]DMPC, 1-myristoyl-2-(12,12-[$^{19}F_2$]difluoromyristoyl)-*sn*-glycero-3-phosphocholine; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; TFA, trifluoroacetic acid; S_{FF}, the internuclear fluorine–fluorine vector order parameter; CSA, chemical shift anisotropy; SUV, sonicated unilamellar vesicle(s); T_1, spin–lattice relaxation time in the laboratory frame; $T_{1\rho}$, spin–lattice relaxation time in the rotating frame; T_2, spin–spin relaxation time; T_2^*, apparent spin–spin relaxation time; M_L, steady-state value of the magnetization component aligned with the main magnetic field; M_{eff}, steady-state value of the magnetization component aligned with the effective magnetic field in the presence of the off-resonance field.

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