Distribution of General Anesthetics in Phospholipid Bilayers Determined Using ^2H NMR and $^1H-^1H$ NOE Spectroscopy†

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ABSTRACT: The effect of the general anesthetics halothane, enflurane, and isoflurane on hydrocarbon chain packing in palmitoyl(d_{31})oleoylphosphatidylcholine membranes in the liquid crystalline phase was investigated using ^2H NMR. Upon the addition of the anesthetics, the first five methylene units near the interface generally show a very small increase in segmental order, while segments deeper within the bilayer show a small decrease in segmental order. From the ^2H NMR results, the chain length for the perdeuterated palmitoyl chain in the absence of anesthetic was found to be 12.35 Å. Upon the addition of halothane, enflurane, or isoflurane, the acyl chain undergoes slight contractions of 0.11, 0.20, or 0.16 Å, respectively, at 50 mol % anesthetic. A simple model was used to estimate the relative amounts of anesthetic located near the interface and deeper in the bilayer hydrocarbon region, and only a slight preference for an interfacial location was observed. Intermolecular $^1H-^1H$ nuclear Overhauser effects (NOEs) were measured between phospholipid and halothane protons. These NOEs are consistent with the intramembrane location of the anesthetics suggested by the ^2H NMR data. In addition, the NOE data indicate that anesthetics prefer the interfacial and hydrocarbon regions of the membrane and are not found in high concentrations in the phospholipid headgroup.

General anesthetics almost certainly act by modulating the activity of membrane proteins; however, whether the primary site of action resides in the lipid bilayer or in the protein is an open question (Koblin, 1994). There is good reason to suspect that anesthetics may act though lipid bilayers. Anesthetics are highly lipid soluble and there is a well-known correlation between anesthetic potency and lipid solubility (Janoff & Miller, 1982). Nonetheless, it has been difficult to identify membrane mechanisms of anesthetic action due to the dynamic, liquid-like nature of membranes and the difficulty in separating general bilayer perturbations from more specific interactions between anesthetics and membrane components. Investigations on membranes have largely centered on the effects of anesthetics on lipid dynamics, and it is clear that anesthetics have relatively small effects on the bulk dynamics of membranes at clinical anesthetic levels (Miller, 1985). Thus, changes in the rates and amplitudes of lipid motion, at least as measured in the bulk lipid phase, are unlikely to produce changes at clinical anesthetic levels that could affect membrane protein activity.

There are a number of ways in which anesthetics might modulate lipid bilayer properties and affect membrane protein function that have not generally been considered. For example, if anesthetics localize at the membrane interface, they would be expected to modulate membrane dipole potentials. Membrane dipole potentials are internal electrostatic potentials in membranes that are on the order of 300 mV, and the very large field from these potentials is present across the membrane interface. These are potentials that could easily modulate the activity of electrically active conformational transitions in membrane proteins (Cafiso, 1991). Indeed there is evidence indicating that dipole potentials are modulated by 10−20 mV at clinical anesthetic levels, voltage changes that are comparable to the voltages required to activate the voltage-gated sodium channel of nerve (Qin et al., 1995). Another possible membrane-mediated mechanism of anesthesia involves modification of the lateral tension profile along the bilayer normal or balance of forces between the headgroup and hydrocarbon domains. In some cases membrane protein function requires a mixture of two lipid types; those which tend to stabilize bilayers (such as phosphatidylcholines, PC)[1] and those which tend to destabilize bilayer phases (such as unsaturated phosphatidylethanolamines, PE) (Cullis et al., 1985). As these mixtures are varied the energy required to bend or deform a monolayer changes, and this bilayer property is referred to as the "spontaneous curvature" (Gruner, 1989). Changes in the spontaneous curvature might modulate membrane protein activity by modulating the energetics of conformational transitions that involve volume expansion in the membrane interface. Indeed, anesthetics have been shown to affect membrane spontaneous curvature and membrane phase preferences (Shyamsunder & Gruner, 1991; Taraschi et al., 1991).

Clearly information on the distribution of anesthetics along the bilayer normal would be useful in evaluating mechanisms such as the electrostatic or lipid strain models described

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[1] Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; POPC, palmitoyloleoyl-PC; DPPC, dipalmitoyl-PC; DMPC, dimyristoyl-PC; POPE, palmitoyloleoyl-PE; DPPE, dipalmitoyl-PE; egg PC, egg yolk PC; FTIR, Fourier transform infrared; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; halothane, 2-bromo-2-chloro-1,1,1-trifluoroethane; enflurane, 2-chloro-1,1,2-trifluorethyldifluoromethyl ether; isoflurane, 1-chloro-2,2,2-trifluoroethyldifluoromethyl ether; MOPS, 4-morpholinepropanesulfonate; MAC, minimum alveolar concentration.

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above. Unfortunately, information of this type is rather limited and has focused primarily on local anesthetics. For example, local anesthetics such as dibucane, tetracaine, and procaine have been studied using FTIR, deuterium NMR, ^{14}N NMR, and fluorescence (Browning & Akutsu, 1982; Kelusky & Smith, 1983; Sweet et al., 1987; Auger et al., 1988). Not surprisingly, such studies indicate that these charged anesthetics reside and have effects primarily at the membrane interface. Both ^{2}H and ^{31}P NMR have been used to measure the effects of chloralose and chloral hydrate on the segmental order of lipid bilayers and interactions with the headgroup have been demonstrated (Forrest & Mattai, 1985). Relatively little information is available on the distribution of volatile anesthetics in lipid bilayers. Early work on halothane using ^{19}F NMR and spin labels provided an indication that anesthetics were widely distributed in lipid bilayers (Trudell & Hubbell, 1976). However, the wide distribution for nitroxides on an acyl chain could make positional information difficult to interpret (Ellena et al., 1988), and more recent work suggests that general anesthetics reside primarily at the membrane interface (Kaneshina et al., 1981; Craig et al., 1987).

In this report, we present data on the effects of the volatile general anesthetics halothane, isoflurane, and enflurane on the acyl chain segmental order parameters of POPC bilayers. These order parameters are used to calculate the effect of the anesthetics on average acyl chain length and cross-sectional area (Seelig & Seelig, 1974; Salmon et al., 1987; Boden et al., 1991) and to make an estimate the intramembrane distribution of anesthetics. In addition, $^{1}H-^{1}H$ nuclear Overhauser effects between halothane and the phospholipid were measured and used along with the ^{2}H NMR data to evaluate the intramembrane distribution of the anesthetic. The results indicate that these anesthetics are widely distributed in the hydrocarbon domain of the membrane with only a slight preference for the lipid interface. In contrast to the hydrocarbon domain, these anesthetics appear to be largely absent from the headgroup region.

MATERIALS AND METHODS

Perdeuterated palmitoyl(d_{31})oleoylphosphatidylcholine (POPC-d_{31}) in chloroform was obtained from Avanti Polar Lipids (Birmingham, AL) and used without further purification. Volatile anesthetics halothane, enflurane, and isoflurane were the generous gift of Dr. Carl Lynch in the Department of Anesthesia at the University of Virginia. To prepare the membrane dispersions, chloroform was removed from 20–30 mg of the POPC-d_{31} sample by vacuum desiccation for at least 24 h. The dried lipid was then hydrated by adding an equal amount of a buffer (by weight) containing 100 mM NaCl and 10 mM morpholinopropanesulfonic acid (MOPS), pH 7.1. The resulting dispersion was then freeze-thawed at least six times. This sample was transferred to a quartz tube, anesthetic was then injected directly into the sample, and the sample tube was sealed with a Teflon cap. The sample was mixed by centrifuging the sample tube in alternate directions at low speed, and the sample was allowed to equilibrate for at least 24 h before recording spectra. After recording the NMR spectra, the sample tube was uncapped, quickly placed into a vial containing 3 mL of a chloroform/methanol 72/28 mixture, and capped with a Teflon coated septum. Anesthetic levels in these samples were determined with a Hewlett Packard 5890 series II gas chromatograph using a 30 m HP-1 capillary column having a 0.53 mm i.d. and a 2.65 μ-film thickness following a procedure similar to one described previously (Kusmierz et al., 1989). Typically, the anesthetic concentrations in these samples were found to be approximately 15% lower than expected on the basis of the original amount of anesthetic injected into the sample. Lipid concentrations following the experiment were determined using a Fiske–Subbarrow phosphate assay similar to one described previously (Bartlett, 1959).

NMR Spectroscopy. Deuterium NMR spectra were acquired using a modified Nicolet NT-360 spectrometer equipped with a wide-line ^{2}H solids probe (Cryomagnet Systems, Indianapolis, IN) and Doty 500A RF amplifier (Doty Scientific, Columbia, SC). A quadrupole echo pulse sequence was used to acquire the spectra (Davis et al., 1976), where 90° pulses were 3 μs in duration and the delay between experiments was 200 ms. The spectra were the result of at least 40 000 acquisitions. The time domain spectra were Fourier transformed and depaked (Sternin et al., 1983). The depaked peaks were fit with Gaussian line shapes using Felix 2.30 (Biosym Technologies, San Diego, CA). Buildup rates for nuclear Overhauser effects were measured using a one-dimensional truncated NOE experiment similar to that described previously (Wagner & Wüthrich, 1979) on a General Electric Omega 500 NMR spectrometer. In this experiment individual lipid resonances were selectively irradiated using an rf field strength of between 23 and 27 Hz, and the intermolecular NOE produced in the anesthetic proton resonance was measured. The time dependent NOE, $\eta_i(t)$, was calculated as a function of the duration of this selective rf field, according to

$$\eta_i(t) = [I_i(t) - I_i(0)]/I_i(0) \quad (1)$$

where $I_i(t)$ is the intensity of the observed anesthetic spin following irradiation for a time t, and $I_i(0)$ is the intensity of the spin at equilibrium in the absence of irradiation.

RESULTS

Shown in Figure 1A is the ^{2}H NMR spectrum for a dispersion of POPC with a fully perdeuterated palmitic acid chain, and in Figure 1B is the depaked spectrum showing the segment assignments for the depaked doublets (Lafleur et al., 1989). In the absence of anesthetic, nine resonances are clearly resolvable, where the resonance with the largest residual quadrupolar splitting corresponds to carbons 2–8 toward the polar end of the hydrocarbon chain. Shown in Figure 1C is a simulation of the depaked spectrum in Figure 1B. Integration of this depaked spectrum is consistent with the assignments given in Figure 1.

The addition of volatile anesthetics produces small, but easily distinguishable changes in the deuterium NMR spectrum. The depaked spectra for POPC in the absence and presence of 30 mol % isoflurane are shown in Figure 2, panels A and B, respectively. Instead of nine resolvable peaks, the deuterium NMR spectrum of POPC in the presence of isoflurane shows 11 peaks, where the outermost pair in the depaked spectrum corresponds to carbons 2–6. The residual quadrupolar splitting for the outermost pair increases in the presence of anesthetic, while splittings for peaks due to segments further down the acyl chains decrease. Thus, the spectrum in Figure 2B indicates that segments near the polar interface experience an increase in segmental order,

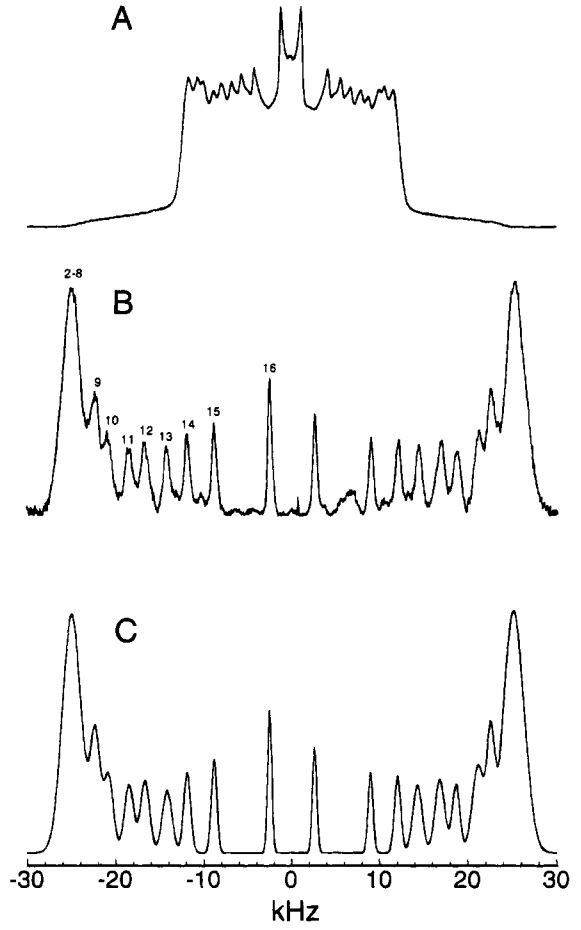

FIGURE 1: (A) Deuterium NMR spectrum of a fully hydrated multilamellar dispersion of perdeuterated POPC-d_{31} at 25 °C obtained using a modified NT-360 and wide-line ^{2}H probe. (B) Depaked spectrum produced as described in the text. This spectrum is similar to that reported previously (Lafleur et al., 1989) and shows nine resolvable doublets, where the outermost doublet results from deuteriums on the first seven methylene segments removed from the lipid carbonyl. (C) Fit to the depaked spectrum produced using the simulated annealing fitting routine in FELIX (see text).

while segments further down the hydrocarbon chain experience a decrease in segmental order with anesthetic addition.

The order parameters were calculated from fits to the depaked spectra as described above, and Figure 3 shows plots of the relative carbon−deuterium bond order parameter for POPC upon the addition of the volatile anesthetics halothane, enflurane, and isoflurane. For each of these anesthetics an increase in the segment ordering for carbons 2−6 is observed in all cases (except 13 mol % isoflurane), while a decrease in segment order is always observed for residues further down the acyl chain. The simplest interpretation of these data is that these anesthetics preferentially partition to an area near the upper portion of the acyl chain in POPC bilayers. Thus, the increase in ordering near the chain carbonyls reflects an increase in *trans* conformers created by the presence of the anesthetic, and the decrease in order down the acyl chain reflects an increase in gauche conformers promoted by the additional surface area at the membrane interface due to anesthetic.

Shown in Figure 4 are the time-dependent NOEs measured for the halothane proton resonance at 6.55 ppm when the indicated lipid resonances are irradiated with a selective rf pulse. NOE build-up rates are determined primarily by the

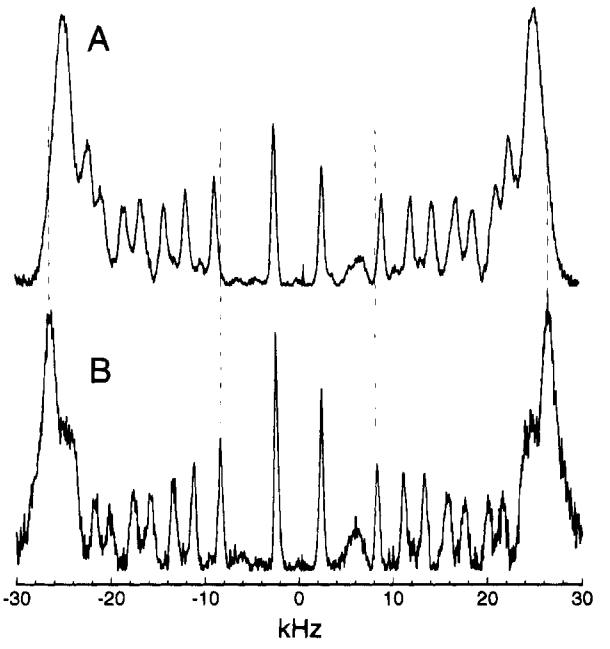

FIGURE 2: Comparison of the depaked spectra for POPC-d_{31} obtained in the absence (A) and presence (B) of 36 mol % isoflurane. Two methylene segments becomes separated from the outermost doublet resulting in 11 resonances in the presence of anesthetic. As is seen with the aid of the dashed vertical lines, the splitting of the outermost segment increases with anesthetic while the splitting of segments further down the acyl chain decrease.

proximity of the irradiated and detected protons. The largest NOEs are observed when the first and second methylene segments from the carbonyl are irradiated, and the smallest NOEs are observed for resonances in the lipid headgroup. As described previously, some of the intensity seen for the segment two positions from the carbonyl is produced by partial irradiation of the main methylene resonance in the spectrum (Ellena et al., 1987). Comparison of the build-up rates indicates that the most likely membrane binding location for halothane is near the membrane−solution interface.

A closer look at the ^{2}H and NOE data suggests that while the most probable location for the anesthetics is near the membrane−solution interface, they also spend a considerable amount of time at more central bilayer positions. The order parameters obtained from deuterium NMR can be used to determine the average length of all or part of the acyl chain projected along the bilayer normal. The average length of an acyl chain segment can be obtained using

$$\langle L \rangle = l\left[\left(\frac{n-m+1}{2}\right) - \sum_{i=m}^{n-1} S_{CD}(i) - 3S_{CD}(n)\right] \quad (2)$$

where $\langle L \rangle$ is the chain length projected along the bilayer normal, $l = 1.25$ Å is the projected length of a trans segment, m and n are the first and last segments considered, respectively, and $S_{CD}(i)$ are the segmental order parameters (Seelig & Seelig, 1974; Salmon et al., 1987). It should be noted that when the last (i.e., highest numbered) segment is not the methyl group, the final term in eq 3 is omitted and the summation runs from m to n. Shown in Figure 5A is the average palmitic acid chain length from segment 1 to 16 as a function of the concentration of halothane, enflurane, and isoflurane. Even high levels of these anesthetics result in very small reductions in acyl chain length. Shown in Figure

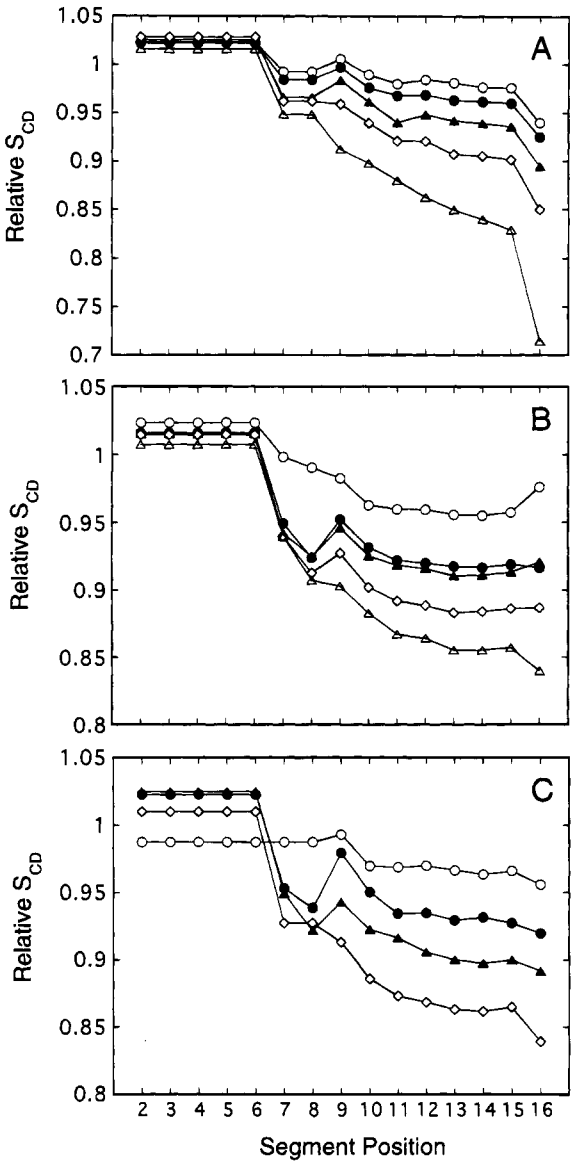

FIGURE 3: Plots of the relative order parameter (the ratio of the order parameter in the presence versus the absence of anesthetic) for (A) halothane at 0.18 (○), 0.28 (●), 0.38 (▲), 0.52 (◆), and 0.97 (△) mol %, (B) enflurane at 0.18 (○), 0.28 (●), 0.31 (▲), 0.40 and 0.52 (◆) mol %, and (C) isoflurane at 0.13 (○), 0.24 (●), 0.36 (▲), and 0.51 (◆) mol %. The relative order parameter increases slightly for segments 1−6 in the presence of anesthetic but decreases for segments toward the center of the lipid bilayer.

5 panels B and C are the lengths of segments 6−16 and 1−6 determined as a function of the anesthetic mole fractions. Segments 6−16 are shortened by 0.11, 0.20, and 0.19 Å in the presence of 50 mol % halothane, enflurane, and isoflurane, respectively, and there is virtually no change in the length of the segments 1−6. The lateral area expansion of segments 6−16 due to the presence of anesthetic can be calculated by treating the acyl chains as a cylinder and using the following expression:

$$\langle A \rangle = \frac{V}{\langle L \rangle} \tag{3}$$

where $\langle A \rangle$ and $\langle L \rangle$ are the average cross-sectional area and length, and V is the volume. Taking 27.6 Å^3, as the volume of a liquid crystalline methylene segment (Nagle & Wiener, 1988), area expansions of 1.06, 1.95, and 1.83 Å^2 are

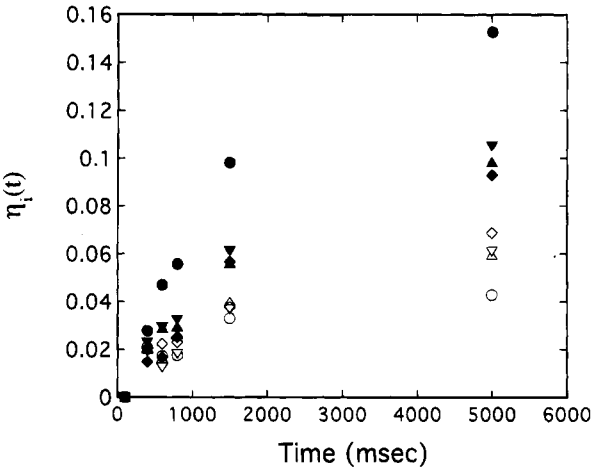

FIGURE 4: Time-dependent NOEs measured for the proton resonance in halothane dissolved in sonicated vesicles when the following lipid resonances are saturated: -CH_2-O-P-O-C-C-N (○), P-CH_2-C-N (△), HC=CH (▽), N(CH_3)$_3$ (◇), CH_2COO- (▲), CH2-C= (▼). CH2-C-COO- (●), -CH3 (◆). The vesicles were formed from POPC in 100 mM NaCl/10 mM phosphate buffer at pH 7.0 in D_2O. The POPC was at a final concentration of about 100 mM, and halothane was present at a membrane concentration of approximately 12 mol %. Sonicated vesicles were prepared following a procedure described previously (Cafiso & Hubbell, 1978).

obtained for membranes containing 50 mol % halothane, enflurane, and isoflurane, if one assumes equivalent area expansions for the palmitoyl and oleoyl chains. For positions 1−6 this area change is very small relative to that for positions 6−16, and the area is nearly concentration independent.

If we assume that the anesthetic binds adjacent to methylene segments 1−6 and that this region of the membrane experiences the same area change as the segment 6−16 region, then the apparent molar volume of the anesthetic in the membrane ($\bar{V}_{ap}$) can be calculated. These volumes are 4.7, 8.6, and 8.1 Å^3 per halothane, enflurane, and isoflurane molecule. These apparent volumes are approximately 4% of the true partial molar volumes (Miller, 1985). Thus, the presence of the anesthetics causes the length of segments 6−16 to decrease slightly and the lateral area occupied by these segments to increase relative to bilayers without anesthetics. However, the length reduction and resultant area increase of segments 6−16 is a small fraction of that expected if the anesthetics were located exclusively in the bilayer region occupied by segments 1−6. This suggests that localization of the anesthetics in the bilayer regions occupied by segments 6−16 is only slightly lower than in regions occupied by segments 1−6.

In order to estimate the relative amounts of time that the anesthetic spends near the membrane−solution interface compared to the bilayer interior, we employed a two-site model for the binding of anesthetic. When bound to site I, the anesthetic is located adjacent to positions 1−6, and when bound at site II, the anesthetic is at a deeper location. The average populations of each site were calculated by assuming that if the anesthetic only occupied site I, the average area of segments 7−16 would expand to fill the free volume created by the addition of anesthetic. If the anesthetic had an equal probability of occupying sites I or II, then no change in the chain conformation would be necessary to accommodate the anesthetic. Another way of stating the latter condition is that if sites I and II are equally occupied, the

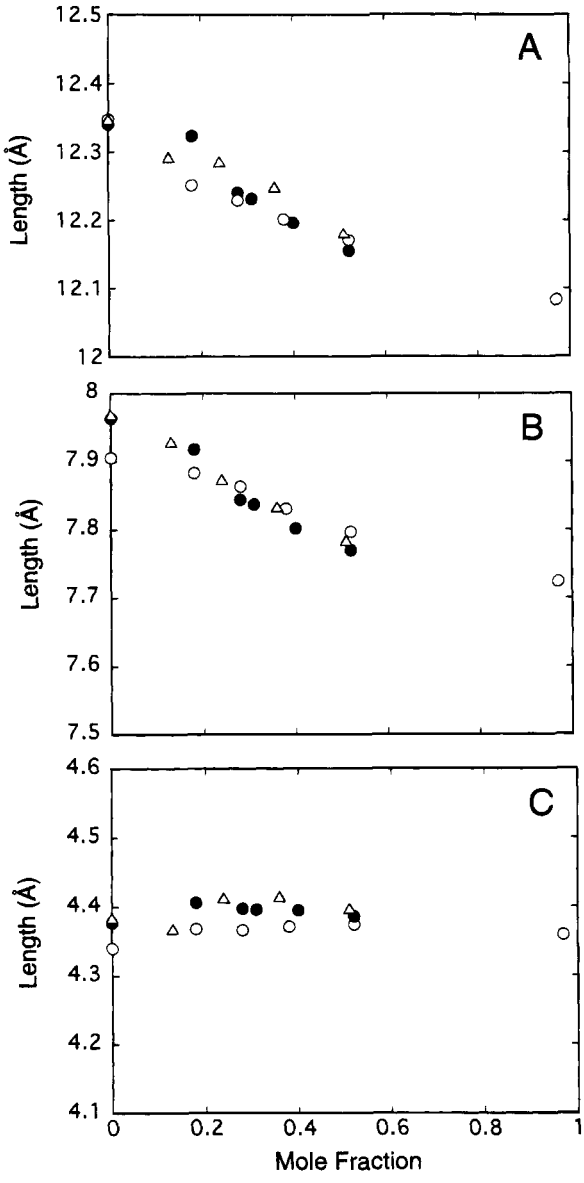

FIGURE 5: Chain length dependence of the palmitic acid acyl chain of POPC-d_{31} as a function of the mole fraction of halothane (○), enflurane (●), or isoflurane (△) added to the lipid mixture. In panel A is shown the length of the entire acyl chain encompassing positions 1−16. In panels B and C are shown the lengths of segments 6−16 and 1−6, respectively. These lengths were calculated as described in the text.

anesthetic is uniformly distributed between the membrane solution−hydrocarbon interface and the center of the bilayer. The fractional occupation of site I can be estimated (from the data at 0.5 mole fraction) from

$$F_I = \frac{\bar{V}_{ap}}{\bar{V}_{an}}(0.5) + 0.5 \quad (4)$$

where F_I is the fractional occupation of site I, $\bar{V}_{ap}$ is the anesthetic apparent molar volume as calculated above, and $\bar{V}_{an}$ is the true anesthetic partial molar volume. Consider the limiting cases where the segment 7−16 volume redistribution is equal to (a) 0 or (b) the partial molar volume of the anesthetic ($\bar{V}_{an}$). The fractional occupation of site I would be 0.5 and 1.0 for cases a and b, respectively. The anesthetic partial molar volumes ($\bar{V}_{an}$) are 170, 202, and 206 Å^3 (Firestone et al., 1986) for halothane, enflurane, and isoflurane, respectively. When one uses eq 4 and the $\bar{V}_{an}$ and V_{ap} values listed above, one obtains a value of 0.51 for the fractional occupation of site I for halothane and 0.52 for enflurane and isoflurane. Although this binding model is clearly an approximation, it demonstrates that the changes in acyl chain conformation caused by the addition of anesthetic are consistent with an almost uniform distribution of anesthetic between the interfacial region and interior with only a slight preference for the interface.

A comparison of the palmitoyl chain and anesthetic lengths and volumes supports the plausibility of the binding model. The acyl chain volume is 442 Å^3; thus, two anesthetic volumes are similar to the acyl chain volume. Since the long dimension of the anesthetics is between 5.8 and 8.0 Å depending on conformation, the length of two anesthetic molecules placed end to end is similar to the length of the POPC palmitoyl chain (12.35 Å). The above binding model has no requirement for a lock and key type of fit between anesthetic and sites I and II. It is only necessary that the anesthetics have equivalent access to regions comprising each site. We estimate that site I is in the segment 1−6 region of the membrane on the basis of anesthetic effects on segmental order parameters. Accordingly, the segment 1−6 length was used in the anesthetic apparent volume calculations above. The site I length may extend beyond segments 1−6, particularly in the interfacial direction (i.e., toward the glycerol and headgroup region of the phospholipid). This uncertainty is due to the fact that no conformational information was obtained in this area of the phospholipid. We repeated our calculation of fractional site I occupation with the assumption that site I is twice as long as segment 1−6; in this case, the fractional site I occupation was 0.53 or 0.54 rather than the original values, 0.51 or 0.52. Thus, uncertainties in the site I and II definitions have little effect on the fractional site occupation calculation. The conclusion that anesthetics must have access to both interfacial and more central regions of the bilayer (with only a slight preference for the interface) is reached directly from the observation that acyl chain ordering and consequently acyl chain average length is changed very little by the presence of these anesthetics.

The NOE data are difficult to interpret quantitatively in terms of anesthetic location because the NOE magnitudes are determined by both internuclear distances and dynamics (Ellena et al., 1987) and an accurate description of the dynamics is complex (Bloom et al., 1991). However, the NOE results are qualitatively consistent with preferential partitioning of the anesthetics to the membrane-solution interface because the largest NOEs are observed when protons in that region are irradiated. The NOEs observed when protons residing close to the center of the bilayer (-CH_3) are irradiated are only slightly smaller than those due to irradiation of the -CH2-COO- protons, which are close to the membrane−solution interface; this is consistent with the nearly equal probability of the anesthetic occupying locations near the membrane interface and the bilayer center. The relatively small NOEs observed when headgroup protons are irradiated indicates that the probability of anesthetic binding to the headgroup is considerably lower than binding to the membrane hydrocarbon region.

It should be noted that many of the experiments described here employ anesthetic concentrations that greatly exceed those used clinically. Clinically relevant membrane concentrations for the anesthetics used here should be on the order of a few mol %. Nonetheless, the effects seen here extrapolate to much lower concentrations, and there is no indication that binding sites for these anesthetics are being saturated. For example, the effects shown in Figure 5 on chain length are linear and extrapolate to lower concentrations that are clinically relevant. The NOE experiments in particular can be carried out at much lower concentrations, close to those used clinically. These NOE measurements lead to the same conclusions as those obtained from ^{2}H NMR experiments that are carried out at much higher concentrations.[2]

DISCUSSION

The effects of halothane and other general anesthetics on phospholipid bilayers have been examined in numerous studies [for review, see Koblin (1994)]. A consensus membrane binding location for general anesthetics does not exist and appears to depend on the composition and structure of the bilayer and the structure of the anesthetic. Conclusions about the binding location for halothane in phosphatidylcholine membranes have varied. Trudell and Hubbell (1976) used ^{19}F NMR to probe the environment of halothane bound to egg phosphatidylcholine bilayers containing spin labels at different distances from the bilayer surface. They also used manganese ions to evaluate the halothane accessibility to the aqueous phase of the bilayer solution. They found that halothane rapidly exchanged between aqueous and bilayer phases and had approximately equal access to the bilayer interfacial and hydrocarbon regions. Similar results were obtained for halothane (Koehler et al., 1977) for a 1:1 bovine phosphatidylcholine/phosphatidylserine mixture. However, when Raman spectroscopy was used to examine the effect of halothane on dipalmitoylphosphatidylcholine bilayers, the ability of ideal solution theory to describe the transition temperature depression due to halothane was taken as evidence that halothane is excluded from the hydrocarbon region of the bilayer (Craig et al., 1987). An NMR chemical shift analysis of anesthetic interactions with micelles concluded that methoxyflurane interacted only with the interfacial region of the micelles (Kaneshina et al., 1981). Our results are not consistent with these later studies but are consistent with earlier work which indicates that general anesthetics reside in the hydrocarbon and interfacial regions of the membrane. We find a slight preference for the interface and no evidence for significant localization of the anesthetic in the headgroup region. Our work is also consistent with X-ray diffraction experiments on DMPC bilayers which indicate that approximately 50 mol % halothane increases bilayer disorder and has no significant effect on bilayer width (Franks & Lieb, 1979).

Phospholipid aggregates can adopt several types of structures (i.e., multilamellar dispersions, small unilamellar vesicles, hexagonal and cubic phases), and anesthetics can affect phase and structure interconversion (Hornby & Cullis, 1981; Forrest & Rodham, 1985; Gaillard et al., 1991; Taraschi et al., 1991). Anesthetics can stabilize or destabilize bilayers relative to other aggregate structures depending on the anesthetic structure and bilayer composition [i.e., Hornby and Cullis (1981)]. Gaillard et al. (1991) found that when more than 50 mol % halothane is added to DPPC or DMPC, small vesicle formation occurs or changes to isotropic and hexagonal phases are promoted, respectively. Addition of 32 mol % halothane promoted hexagonal phase formation in POPE bilayers and halothane was found to promote hexagonal phase formation in bilayers containing DPPE, cardiolipin, egg PC, or the total extracts of rat liver mitochondrial or microsomal membranes (Taraschi et al., 1991). Trichloroethylene (66 mol %) caused a bilayer to hexagonal phase transition at 20 °C in egg PC bilayers (Forrest & Rodham, 1985). We found no evidence of isotropic or hexagonal phase formation in our spectra; however, we might expect to see this at higher anesthetic levels and/or temperatures.

It has been proposed that an important mechanism of general anesthesia is the anesthetic-induced alteration of the bilayer lateral tension profile which gives rise to the membrane spontaneous curvature (Shyamsunder & Gruner, 1991). The membrane or bilayer spontaneous curvature is related to the amount of strain energy in a bilayer and is predictive of a bilayer's tendency to undergo a structural or phase change. Multilamellar phospholipid dispersions and cell membranes are composed of relatively flat bilayers. Flat bilayers can convert to more highly curved structures including small vesicles and hexagonal phases if the bilayer has an appropriate spontaneous curvature; small vesicles and hexagonal phases have opposite spontaneous curvatures. The intramembrane location of anesthetics should be important in determining the bilayer spontaneous curvature. Our observation that halothane, enflurane, and isoflurane are rather evenly distributed throughout the bilayer interior suggests that anesthetics should not change the lateral tension profile within the hydrocarbon. However, the absence of anesthetic from the membrane headgroup region might create significant strain differences between headgroup and hydrocarbon domains. Our data do not allow us to determine if the anesthetic resides at the bilayer center between the leaflets; however, the work of Franks and Lieb (1991) on a similar system suggests that this does not occur. Clearly, a more complete understanding of anesthetic effects on bilayer to nonbilayer transitions could be enhanced by employing the techniques used in this study to obtain more detailed information on the distribution of these anesthetics in different phases and in the phase transition region.

In summary, the deuterium NMR and NOE data indicate that the anesthetics studied here reside in the hydrocarbon region of the membrane with a slight preference for the membrane−solution interface. In addition, anesthetics are not found in high concentration within the charged headgroup region.

[2] Clinically relevant membrane concentrations of the anesthetics used here can be estimated from the anesthetic MAC pressures and PC/bilayer gas partition coefficients given previously (Firestone et al., 1986). These estimates must be viewed as approximate since the partition coefficients are temperature dependent and since the anesthetic may have a different partition coefficient to the biologically relevant target membrane than the PC bilayers used here.

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