Contrasting Membrane Localization and Behavior of Halogenated Cyclobutanes that Follow or Violate the Meyer-Overton Hypothesis of General Anesthetic Potency

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ABSTRACT The membrane localization and properties of two halogenated cyclobutanes were examined using ^2H and ^{19}F NMR. The common predictors of potency indicate that these two compounds will have anesthetic activity; however, 1,2-dichlorohexafluorocyclobutane (c(CClFCClFCF$_2$CF$_2$)) is not an effective anesthetic, whereas 1-chloro-1,2,2-trifluorocyclobutane (c(CClFCF$_2$CH$_2$CH$_2$)) is an effective general anesthetic. Using ^2H NMR, the effect of these compounds on the acyl chain packing in palmitoyl (d_{31}) oleoylphosphatidylcholine membranes was examined. The addition of the anesthetic c(CClFCF$_2$CH$_2$CH$_2$) results in small increases in the segmental order near the headgroup, whereas segments deeper in the bilayer show decreases in order. These results are consistent with those obtained previously for halothane, isoflurane, and enflurane. On the addition of the nonanesthetic c(CClFCClFCF$_2$CF$_2$), the segmental order is virtually unchanged, except for a slightly changed order near the segments 10–12 of the palmitoyl chains. These results, and the ^{19}F chemical shifts, indicate that the anesthetic c(CClFCF$_2$CH$_2$CH$_2$) exhibits a preference for the membrane interface, as do the other general anesthetics, whereas the nonanesthetic c(CClFCClFCF$_2$CF$_2$) resides within the membrane hydrocarbon core. The compound c(CClFCClFCF$_2$CF$_2$) and other nonanesthetic halocarbons have lower molecular dipole moments compared to effective anesthetic halocarbons, which may account for their altered distribution within the membrane. These data strongly suggest that preferential localization of a halocarbon within the membrane interface is a predictor of anesthetic potency. Furthermore, the data indicate that the properties and forces in the membrane interface deserve consideration as mediators of anesthetic activity.

INTRODUCTION

For many years the Meyer-Overton rule has been used as a general predictor of anesthetic potency. Meyer and Overton noticed that anesthetics had no consistent structural specificity, but found that potency was correlated with hydrocarbon solubility, as measured by partitioning into olive oil (Meyer, 1899). The Meyer-Overton rule works well for a wide range of anesthetics and anesthetic potencies, and it is consistent with the anesthetic "cutoff" in long-chain alcohols (Miller, 1985). The Meyer-Overton rule suggests that the site of anesthetic activity is a hydrophobic but structurally nonspecific site, such as the membrane hydrocarbon. Indeed, mechanisms involving an anesthetic modification in the biophysical properties of the membranes have been proposed, including changes in bilayer curvature (Gruner and Shyamsunder, 1991) and modulation of membrane electrostatic properties (Qin et al., 1995). It has also been proposed that anesthetics are brought into the proximity of membrane proteins because of their partitioning into the bilayer, but that they bind directly to membrane proteins at specific sites to affect activity through allosteric mechanisms (Eyring et al., 1973; Franks et al., 1990). Not enough is known about how membrane protein function is modified by anesthetics at a molecular level to provide conclusive support for either a membrane or protein site as the primary anesthetic target.

To gain an increased understanding of the target and physical mechanisms of anesthesia, compounds have been sought that violate the conventional predictors of anesthetic potency. Recently a series of halogenated and perfluorinated compounds was examined for their ability to induce anesthesia (Koblin et al., 1994). Compounds such as perfluorinated cyclobutane (c(CF$_2$)$_4$) and c(CClFCClFCF$_2$CF$_2$) were not anesthetics at levels predicted by the Meyer-Overton hypothesis, whereas c(CClFCF$_2$CH$_2$CH$_2$) was. The most obvious difference between these compounds is that molecules such as c(CF$_2$)$_4$ are symmetrical and will therefore have a much lower molecular dipole moment than compounds such as c(CClFCF$_2$CH$_2$CH$_2$). Whereas c(CF$_2$)$_4$ and c(CClFCClFCF$_2$CF$_2$) partition into oil and lipids, they have a low water solubility and might be expected to prefer the hydrocarbon core to the interfacial region. Other compounds that lacked anesthetic activity also had low solubilities in aqueous solution. In the case of the compounds tested, the differences in the behavior of these molecules apparently were not due to differences in their pharmacokinetics (Koblin et al., 1994).

The membrane localization of small molecules such as anesthetics can readily be deduced from their effect on the segmental order along the lipid chain (Boden et al., 1991). The insertion of a small molecule into the hydrocarbon decreases the *gauche-trans* isomer ratio of segments near the binding site, which is seen as an increase in segmental

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order. At chain segments remote from the binding site, the *gauche-trans* isomer ratio increases because of the larger volume available to the chain segments. This is observed as a decrease in molecular order. The molecular order is easily observed in labeled lipids using ^2H NMR from a measurement of the residual quadrupolar splitting of a deuterated chain segment (Seelig and MacDonald, 1987), and this technique was previously used to investigate the distribution of the volatile anesthetics halothane, enflurane, and isoflurane within membranes (Baber et al., 1995). These anesthetics increase molecular order in segments near the interface and lower it in the center of the hydrocarbon, indicating that these general anesthetics exhibit a preference for the membrane interface.

In this paper the biophysical properties and membrane localization of two related polyhalogenated cyclobutanes (Fig. 1, I and II) are compared. Although they both strongly partition into membranes, the compound 1-chloro-1,2,2-trifluoro-cyclobutane (c(CClFCF$_2$CH$_2$CH$_2$) or I) is an effective anesthetic, whereas 1,2-dichloro-hexaflourocyclobutane (c(CClFCClFCF$_2$CF$_2$) or II) is not. The effects of these compounds on the dynamics of the palmitoyl chains in a palmitoyl(d_{31})oleoyl-phosphatidylcholine (POPC) dispersion are examined using ^2H NMR. The ^{19}F chemical shifts of these compounds are also examined in various environments, along with the general anesthetics halothane and isoflurane. The data indicate dramatic and clear differences in the membrane localization of the anesthetic versus nonanesthetic compound. These differences further suggest explanations for the violation of the Meyer-Overton hypothesis by these compounds and improved predictors of anesthetic action.

EXPERIMENTAL

Materials

Deuterated P(d_{31})OPC and unlabeled POPC were obtained from Avanti Polar Lipids (Birmingham, AL), and dimyristoylphosphatidylcholine (DMPC) was obtained from Sigma (St. Louis, MO). These lipids were used without further purification. The anesthetic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) was obtained from Halocarbon Laboratories (Hackensack, NJ), and isoflurane (1-chloro-2,2,2-trifluoroethydifluoromethyl ether) was obtained from Anaquest (Madison, WI). The compounds 1-chloro-1,2,2-triflouro-cyclobutane (I) and 1,2-dichlorohexafluoro-cyclobutane (II) were obtained from PCR (Gainesville, FL).

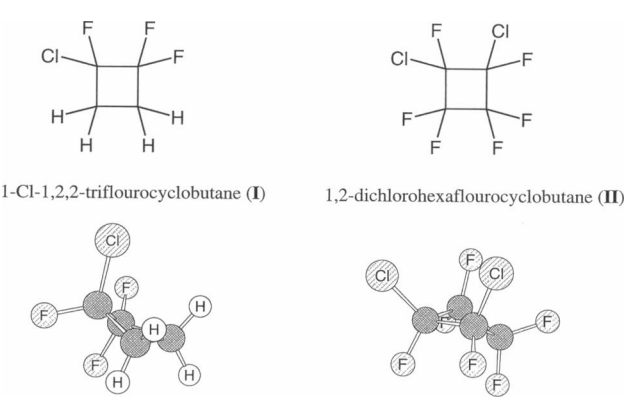

FIGURE 1 Structures of the anesthetic and nonanesthetic cyclobutanes. The partially substituted halogenated cyclobutane I is a good anesthetic, whereas the fully halogenated cyclobutane II is not (Koblin et al., 1994).

METHODS

Deuterium NMR of membranes

Labeled and unlabeled POPC were mixed in a roughly 50/50 proportion to maximize sample size and optimize the signal within the dynamic range of the NMR receiver. POPC in CH_3Cl was evaporated to dryness and then placed under vacuum overnight. The dry powdered POPC was placed into quartz sample tubes, and deionized water or buffer (100 mM NaCl, 10 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7) was injected into the sample at a level of 50% by weight. Samples were stirred with a microspatula and then gently centrifuged to alternate ends of the sample tube to promote mixing between freeze thaw cycles. The sample was taken through at least five freeze-thaw cycles to ensure homogeneity. Anesthetics and halogenated compounds were injected directly into the sample using microliter syringes, and the quantities of the compounds added were confirmed gravimetrically. These samples were sealed, allowed to equilibrate overnight, and again mixed by centrifugation before data acquisition.

The ^2H NMR spectra were measured at 55 MHz with a highly modified Nicolet NT-360 spectrometer at 35°C, using a quadrupolar echo sequence, a sweep width of 100,000 Hz, a 2.2-μs pulse length, and a 200-ms recycle delay. Each spectrum was the sum of 12,000 acquisitions. Quadrupolar splittings were determined from the highest points of the $\sigma_\perp$ peaks, and resonances were assigned by taking the innermost CD_3 segment to be represented by the smallest splitting and assigning increasing splittings to outer segments in order. Assignment of the resonances from inside to outside assumes a steady progression of higher order from the end of the acyl chain, as has been demonstrated previously (Seelig and Seelig, 1974). Relative order parameters were calculated from the ratios of the splittings for a given sample relative to a reference sample with no anesthetic present.

^{19}F NMR of anesthetics

Fluorine 19 spectra at 470 MHz were taken on a GE Omega 500 NMR spectrometer for the anesthetics halothane, isoflurane, I, and the nonanesthetic halogenated cyclobutane II at 1% v/v in the following solvent environments: H_2O, hexanes (predominately *n*-hexane), *n*-decane, and 0.1 μm diameter-extruded DMPC vesicles in H_2O (1:4 w/w). The concentration of II in H_2O was 0.3%, so that signals from suspensions of undissolved II would be eliminated. The NMR spectra were the average of 64 acquisitions, except the spectrum for II in H_2O, which is an average of 4096 acquisitions. Isoflurane was further examined in a number of solvents of varying dielectrics. A single pulse was used without proton decoupling at 35°C. Chemical shift references are relative to the center of CF_3 of external neat trifluoroethanol.

RESULTS

Compound I prefers the membrane interface, as do other volatile anesthetics

Shown in Fig. 2 is a series of ^2H NMR spectra for labeled P(d_{31})OPC in the absence and presence of the anesthetic I. The spectra in the absence of anesthetic are similar to those reported by Baber et al. (1995), with segments 2–8 showing up under a single broad resonance. Upon the addition of I, the residual quadrupolar coupling for segments 2–8 exhibits a slight increase, corresponding to an increase in segmental

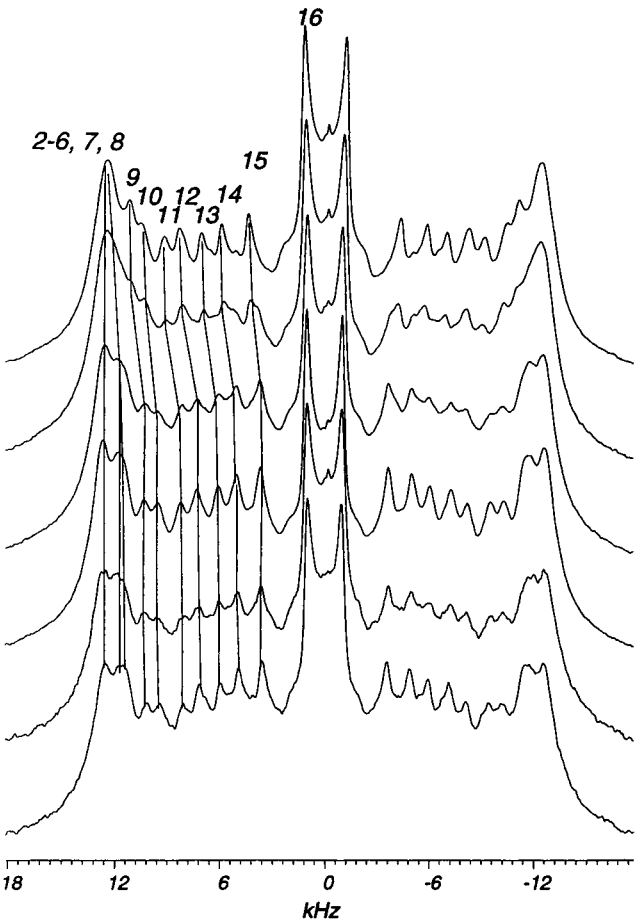

FIGURE 2 ^{2}H NMR spectra for P(d_{31})OPC in the presence of the halogenated cyclobutane I. Concentrations of I in POPC are 0, 24, 35, 44, 49, 56 mol% (*top to bottom*).

order, whereas the residual quadrupolar coupling for segments deeper in the membrane hydrocarbon decreases upon the addition of anesthetic. Fig. 3 shows the relative order parameters for this lipid upon the addition of anesthetic. Also shown for comparison is the relative order parameter profile for P(d_{31})OPC in the presence of isoflurane obtained previously (Baber et al., 1995). It can be seen that the profile for the cyclobutane anesthetic is similar to that obtained previously for isoflurane and consistent with the profiles obtained for other inhalation anesthetics.

The relative changes in molecular chain order seen in Fig. 3 indicate an increase in *gauche-trans* isomer ratio for deeper segments in the membrane, resulting in a shortening of these segments of the chain. This decrease in chain length is a result of the lateral expansion of the chains produced by binding of anesthetic near the interface. From the segmental order parameters, $S_{CD}(i)$, an estimate of the average length of any acyl chain segment can be obtained from

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

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, and m and n are the first and last segments considered, respectively (Salmon et al., 1987, Seelig and Seelig, 1974). Using a formalism presented previously (Baber et al., 1995, Seelig and Seelig, 1974, Seelig and Macdonald, 1987), we used a simple two-site model to analyze the values of S_{CD} in terms of the membrane distribution of anesthetic. The acyl chains are treated as a cylin-

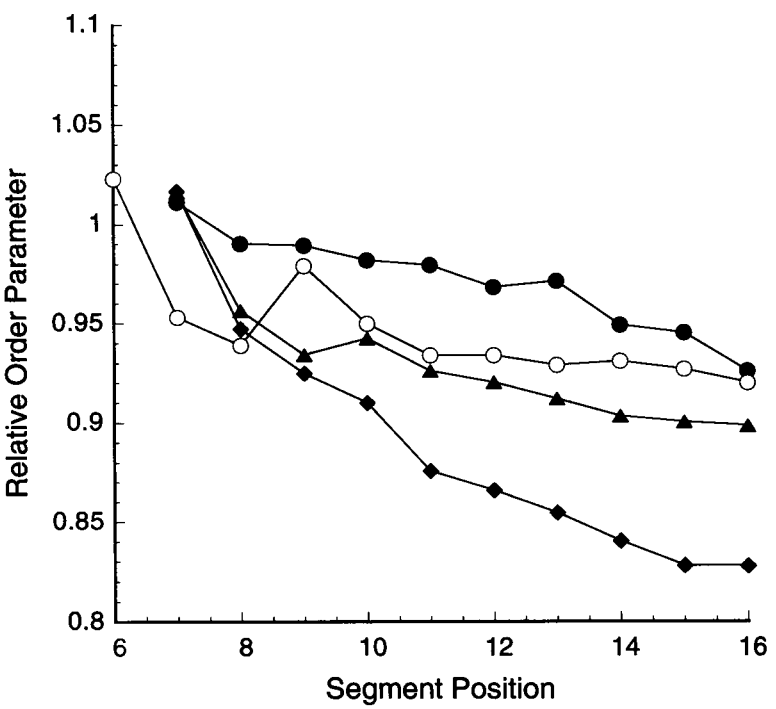

FIGURE 3 Relative order parameter (S_{CD}) for P(d_{31})OPC as a function of the segment position in the presence of I or isoflurane (the isoflurane data were obtained previously; Baber et al., 1995). Mole fractions in POPC: ●, 0.24 of I; ▲, 0.30 of I; ◆, 0.49 of I; ○, 0.24 of isoflurane. The deuterium spectra for compound I are overlapped for positions 2–7, and the value at position 7 represents the value for these segments.

der, and the average cross-sectional area $\langle A \rangle$ is estimated from

$$\langle A \rangle = \frac{V}{\langle L \rangle}, \quad (2)$$

where V is the volume of a methylene segment in the liquid crystalline state. We assume two sites for anesthetic binding, one from segments 1–7 and another from segments 8–16. In the presence of even high concentrations of I, the changes in the lengths of the hydrocarbon segments are quite small. For I at a concentration of $\chi = 0.49$, the length of the site from segments 8 to 16 decreases by 0.16 Å, whereas the length of the site from segments 1 to 7 increases by just 0.04 Å. From these data one finds an average increase in the lateral area of the palmitoyl chain of 1.0 $Å^2$, which corresponds to an average volume increase of 6.12 $Å^3$ per lipid chain. This is the volume that would be available for solute occupation in the site from segments 1 to 7. Assuming that the oleoyl chain is similarly affected and that all of the anesthetic is bound inside of the first hydrocarbon segment, one obtains a fractional occupation of $F_I = 0.53$ for the region adjacent to segments 1–7 relative to segments 8–16 (a detailed discussion of this analysis can be found elsewhere; Baber et al., 1995). This result is comparable to previous findings, where a slight preference for occupancy in the interfacial region was obtained for halogenated general anesthetics (Baber et al., 1995). However, as discussed below, this value of F_I could easily be an underestimate, and the fraction of anesthetic localized near the interface may be higher than that reported here.

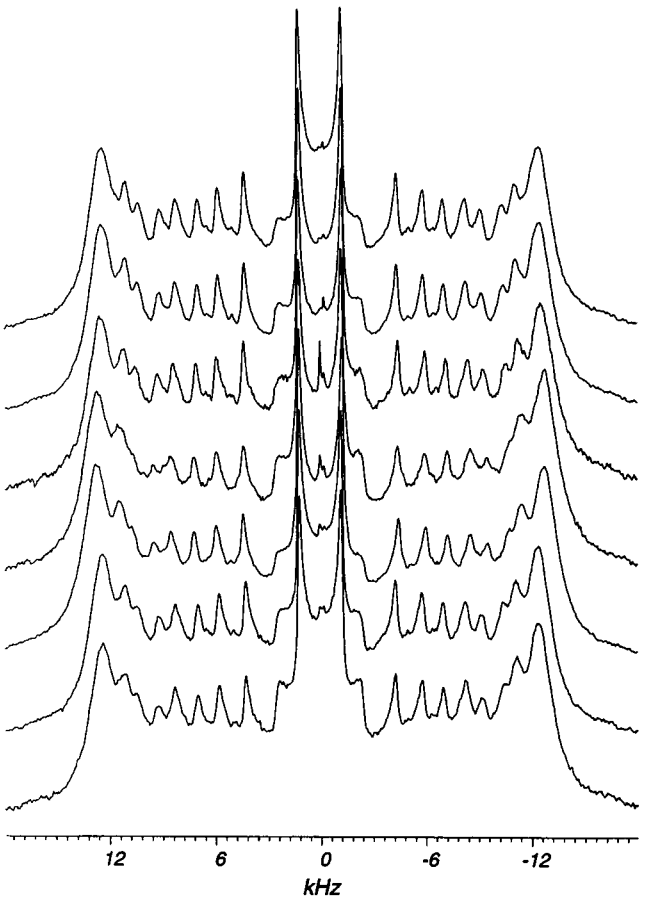

FIGURE 4 ^2H NMR spectra for $P(d_{31})OPC$ in the presence of the perhalogenated cyclobutane II. Concentrations of II in POPC are 0, 11, 22, 35, 41, 47, 55 mol% (*top to bottom*).

The nonanesthetic II localizes within the membrane hydrocarbon interior

Shown in Fig. 4 is a series of ^2H NMR spectra for labeled POPC in the presence of the nonanesthetic II, and Fig. 5 shows the relative order parameters for this compound plotted as a function of segment position. In contrast to the anesthetics previously examined, the splittings and order parameters are nearly unchanged as the concentration of II is increased. The relative order parameters show a slight increase along the chain as the concentration of this compound is increased, with the highest increase in order parameters occurring in the vicinity of C_{10} to C_{12}. This suggests that compound II is very evenly distributed in the hydrocarbon region of the lipids, with just a slight preference for localization near C_{10} to C_{12}. The acyl chains of the bilayer require relatively little change in dynamics to accommodate this substituted nonanesthetic cyclobutane.

Fluorine-19 chemical shifts indicate an interfacial location for anesthetics

The ^{19}F chemical shifts of the two halogenated cyclobutanes, along with the inhalation anesthetics isoflurane and halothane, were examined in environments of varied dielectric and compared with the chemical shifts in unilamellar lipid vesicles. The concentrations of lipid used in these samples is high enough that all of the anesthetic will be associated with the membrane phase. Under these conditions, the observed chemical shift should provide an approximate indication of the relative dielectric of the environment around the anesthetic. And because there is a gradient of the dielectric within the bilayer, this chemical shift will provide a relative indication of position. If anesthetic is exchanging in several domains (for example, the membrane interface and the hydrocarbon domain), a time average-weighted chemical shift intermediate to these environments will be indicated (provided that the exchange of the compound is fast on the NMR chemical shift time scale).

Shown in Fig. 6 is a series of ^{19}F NMR spectra taken for halothane at a frequency of 470 MHz. Halothane shows a single ^{19}F resonance at this frequency, where the chemical shift exhibits a 0.81 ppm difference between a 1% solution in H_2O and 1% in hexanes. The peak of the ^{19}F resonance in DMPC is about 0.04 ppm upfield from the aqueous resonance. These data indicate that in DMPC halothane experiences an environment that is on average closer to that of water than bulk hydrocarbon.

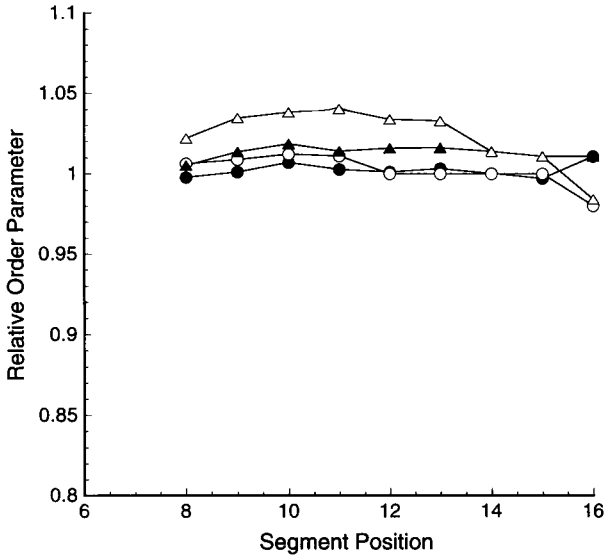

FIGURE 5 Relative order parameter (S_{CD}) for P(d_{31})OPC as a function of the segment position in the presence of II. Mole fractions in POPC: ●, 0.11; ○, 0.18 mol; ▲, 0.22; △, 0.35. The deuterium spectra for compound II are overlapped for positions 2–8, and the value at position 8 represents the value for these segments.

The ^{19}F NMR spectra for isoflurane are shown in Fig. 7. There is a singlet downfield representing the three equivalent CF_3 nuclei, and a resonance upfield from CHF_2. In water, the CHF_2 resonance is a doublet, indicating that equivalent fluorines are being split by the attached proton. In low dielectric solvents, the CHF_2 resonance appears as a quadruplet of doublets. The splitting by the proton is retained, whereas the fluorines become distinct and have different chemical shifts on the order of the scalar couplings, which are also expressed. This results in the quadruplet, which has splittings that can fit to a smooth function

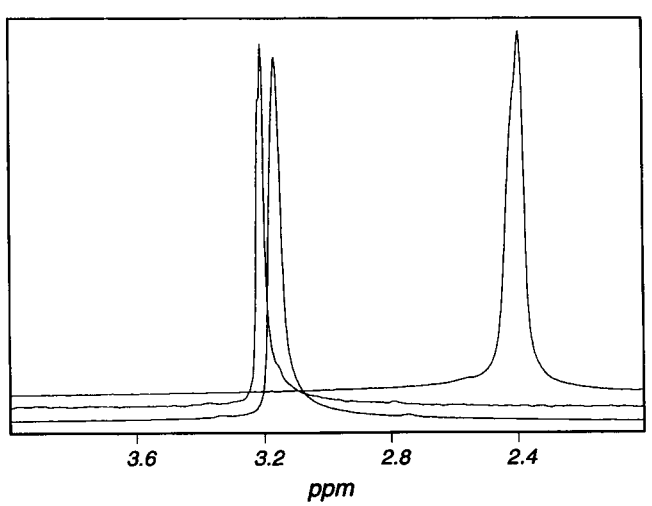

FIGURE 6 Fluorine-19 NMR spectra of halothane (1% by volume) in hexanes, H_2O, and 0.1-μm-diameter DMPC vesicles (~200 mg lipid/ml) (*top to bottom*).

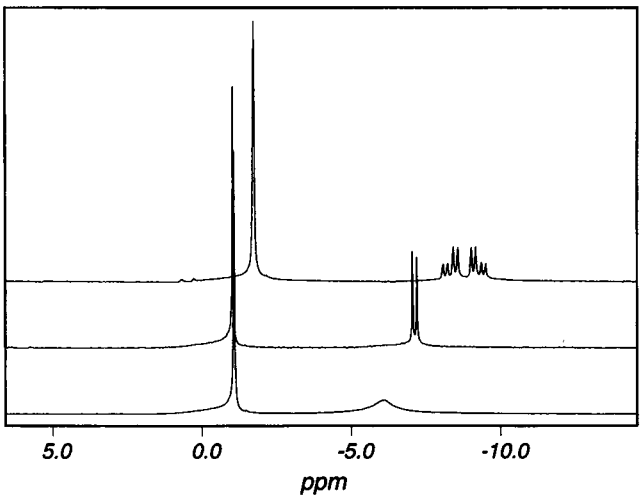

FIGURE 7 Fluorine-19 NMR spectra of isoflurane (1% by volume) in hexanes, H_2O, and 0.1-μm-diameter DMPC vesicles (~200 mg lipid/ml) (*top to bottom*).

of the dielectric constant for 1% solutions in several solvents (spectra not shown).

The CF_3 peak is shifted upfield in hexane by 0.71 ppm compared to H_2O. In the DMPC/H_2O sample at 35°C the signal is a peak between the water and *n*-hexane chemical shift positions near the aqueous signal. The multiplet from CHF_2 is also dramatically broadened and shifted downfield of both the aqueous and oil chemical shifts. The appearance of the CHF_2 ^{19}F resonance is dramatically dependent on the solvent environment, and the observed broadening is likely due to exchange between differing environments. These spectra indicate that isoflurane resides in membranes in an environment that is intermediate between that of a pure hydrocarbon and an aqueous phase with a time-averaged position in a more aqueous environment.

Shown in Fig. 8 are the ^{19}F NMR spectra of I, which shows three resonances in all solvents: a single peak from CFCl and two doublets from the CF_2, which are distinct because of the relative *cis* or *trans* position of Cl. The CF_2 resonances are each split by 193 Hz by the other fluorine. The signals in hexane are shifted upfield relative to water by 0.28 and 0.48 ppm for the two fluorines on CF_2 and 0.125 ppm for the CFCl resonance. The peak of the resonance in DMPC is nearer to the aqueous chemical shift and downfield by 0.05, 0.28, and 0.25 ppm for the two fluorines on CF_2 and CFCl, respectively, suggesting that I spends most of its time in an environment near that of water.

Fluorine 19 chemical shifts indicate an hydrocarbon location for II

As shown in Fig. 9, two singlets and four doublets are observed for nonanesthetic II in H_2O, hexane and in DMPC dispersion. Some fluorines should be equivalent; however, *cis* and *trans* isomers are present, and the number of signals is due to the two chiral centers. Within each population

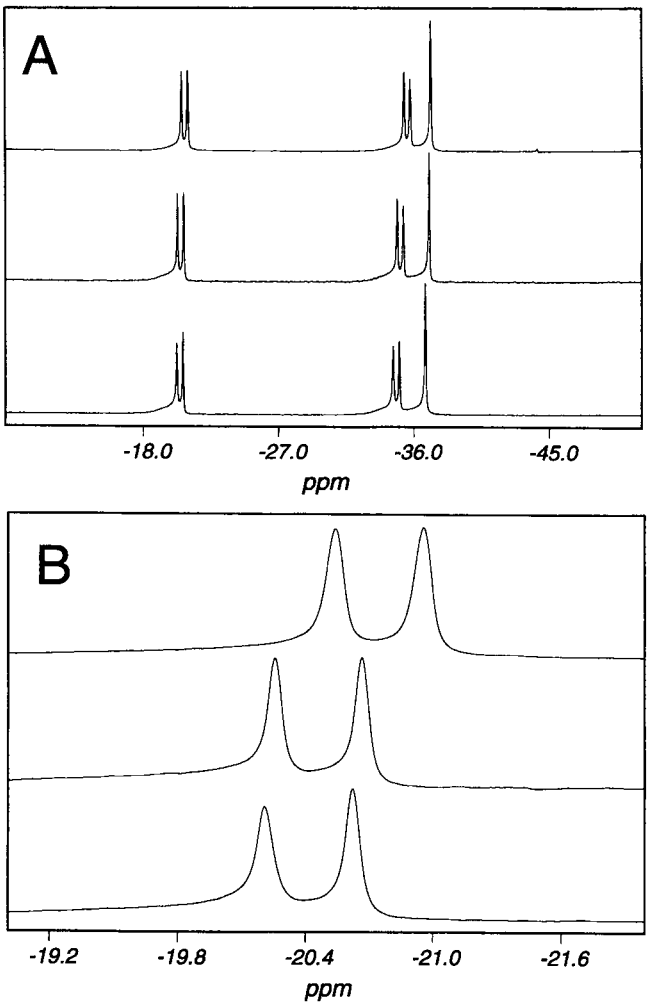

FIGURE 8 Fluorine-19 NMR spectra of I (1% by volume) in hexanes, H_2O, and 0.1-μm-diameter DMPC vesicles (~200 mg lipid/ml) (*top to bottom*).

there are three pairs of equivalent fluorines exhibiting three signals each from two populations, with the chlorines being *cis* or *trans*. There are chemical shift differences for the doublets between the water and hexane samples of 0.64–0.93 ppm upfield. The singlets show chemical shift differences of 0.24 and 0.35 ppm between aqueous and oil phases. The signals for II in DMPC vesicles are more than half of the way toward the shifts in hexane. Thus, in contrast to the anesthetics, the nonanesthetic II yields a chemical shift in DMPC dispersions that is closer to that found for the compound in oil than in water.

DISCUSSION

The partially substituted halogenated cyclobutane I is known to act as an anesthetic (Koblin et al., 1994), and it is shown here to have effects on membrane chain dynamics and packing similar to those found previously for clinically relevant inhalation anesthetics (Baber et al., 1995). The differential effects on molecular order along the length of

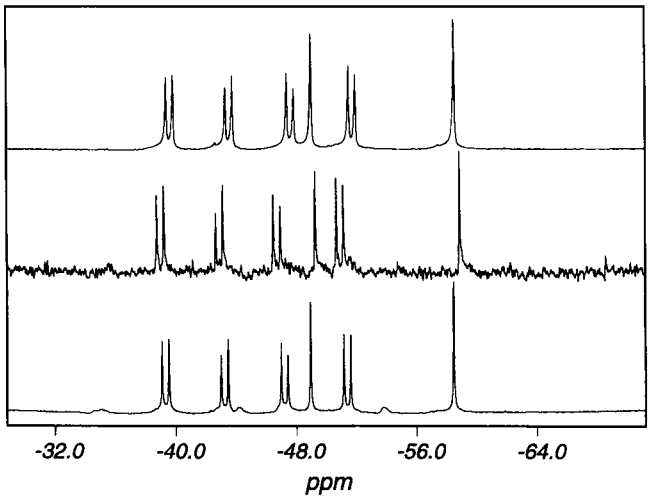

FIGURE 9 Fluorine-19 NMR spectra of 1% by volume II in hexanes, 0.3% in H_2O, and 1% in 0.1-μm-diameter DMPC vesicles (~200 mg lipid/ml) (*top to bottom*).

the lipid hydrocarbon chain indicate a preferential partitioning for all of these anesthetic compounds into the membrane interface.

As described above, I exhibits a fractional partitioning into the region adjacent to segments 1–7 of the palmitoyl chain of $F_I = 0.53$ at a minimum, indicating a preference for spending time near the interface. It is important to point out that this number is based on several simplifying assumptions that could underestimate the occupation of anesthetic in the interfacial domain. This estimate of the fractional occupation due to the volume expansion produced by the anesthetic was assumed to occur entirely in the interfacial hydrocarbon region C_1-C_7 (Baber et al., 1995). This may not be the case if the anesthetic occupies volume near the glycerol backbone or headgroup regions. If the lateral area equal to that in segments 1–7 occupied by anesthetic extends through the headgroup region, ~30 Å^3 of additional volume could be occupied by anesthetic. In this case, the fractional occupation for the region on the aqueous side of acyl segment 7 would be $F_I = 0.62$. Furthermore, it has been suggested that anesthetics displace some water in the interface (Ueda et al., 1986). If one water molecule per lipid were displaced by anesthetic, its fractional occupation in the interface region could be as high as 70%. Thus for I as well as for halothane, isoflurane, and enflurane, the fractional occupation of these anesthetics in an interfacial domain could be higher than previously estimated (Baber et al., 1995), and twice as much anesthetic may reside in the interfacial region as in the hydrocarbon core.

The ^{19}F chemical shifts of c(CClFCF$_2$CH$_2$CH$_2$), halothane, and isoflurane when bound to membranes more closely resemble that in an aqueous environment than shifts in a hydrocarbon environment, also indicating a preference of the anesthetics for the interfacial domain. The chemical shifts of ^{19}F in halothane were previously shown to correlate with a parameter related to dielectric, the Hildebrand δ, and

could be used to examine the binding of anesthetics to lipids (Koehler et al., 1977, 1980). In contrast, the nonanesthetic II is only slightly soluble in aqueous solvents, and the ^{19}F spectrum of II in DMPC dispersions closely resembles the spectrum observed in hexane. Furthermore, the uniform profile of relative order parameters (Fig. 5) suggests that this compound partitions throughout the bilayer interior with a small preference for the region near segments 10–12 of the palmitoyl chain of POPC. The fully substituted cyclobutane II actually slightly "stiffens" the interior of the bilayer because of its preferential partitioning into the hydrocarbon.

The data shown here provide a strong indication that effective anesthetics must reside preferentially in the membrane interfacial region. At the present time, the role, if any, for anesthetic localization in the interface is not understood. It has been suggested that anesthetics act by directly interacting with membrane proteins. In this case, localization in the interface, although a reflection of the properties of anesthetics, might otherwise be of no more direct consequence for anesthesia other than to increase the concentration near a binding site. On the other hand, the physical properties of the membrane interface are known to be important for membrane-protein function, and the consequences of anesthetics in this region could well account for anesthesia. For example, anesthetics appear to alter the membrane dipole potential, a feature that is likely associated with localization in the interface (Qin et al., 1995). Dipole potentials are altered by about 10 mV at clinical levels of anesthetics, a change that could modulate the activity of proteins exhibiting electrically active conformational transitions. The localization of anesthetics in the interface should also change the interfacial tension or spontaneous radius of curvature of the lipid interface (Gruner and Shyamsunder, 1991). It is well documented that changes in the spontaneous curvature of the membrane lipid can alter the activity of channels such as alamethicin (Gruner and Shyamsunder, 1991; Keller and Gruner, 1996). G-protein coupled receptors such as rhodopsin also appear to be sensitive to lipid compositions that alter lateral forces in the membrane interface (Baldwin and Hubbell, 1985; Brown, 1994; Cline and Cafiso, 1986).

The molecular dipoles of the anesthetic compounds halothane, isoflurane, and I were modeled using BIOSYM/MSI Insight II software along with a number of nonanesthetic molecules. The range of dipole moments obtained is shown in Table 1 along with a mean dipole moment. The range and magnitude of the molecular dipole moments clearly correlate with anesthetic potency previously reported (Koblin et al., 1994) and with interfacial localization. The dipole moments for the anesthetics are found to be variable over a relatively wide range, depending on the conformation of the molecule, and this variability may allow these compounds to interact favorably in environments of varying polarity. A low molecular dipole moment is common to the compounds that violate the Meyer-Overton rule tested previously (Koblin et al., 1994). As seen in Table 1, the molecular dipoles of these compounds are uniformly small, and there is much less variability due to molecular dynamics than in the anesthetic molecules. This may explain why molecules such as II partition deep into the bilayer.

TABLE 1 Molecular dipole moments (in Debye units) of anesthetics and nonanesthetics*

Compound	Minimum dipole	Maximum dipole	Mean dipole ± SD
Halothane	0.54	3.4	2.26 ± 0.36
Isoflurane	0.35	5.0	2.98 ± 1.02
CTFCB	1.66	4.3	3.05 ± 0.38
cis-DCHFCB	0.27	1.2	0.48 ± 0.20
trans-DCHFCB	0.05	1.4	0.52 ± 0.21
CCl_2F_2	0.27	0.46	0.23 ± 0.09
$CClF_2CF_3$	0.13	1.2	0.54 ± 0.22
$(CClF_2)_2$	0.04	0.71	0.32 ± 0.13
$CF_3CClFCF_3$	0.12	1.5	0.63 ± 0.25
$CF_3(CClF)_2CF_3$	0.59	1.4	0.64 ± 0.26
CBr_2F_2	0.04	1.0	0.39 ± 0.20
$CBrF_2CF_3$	0.11	1.2	0.52 ± 0.28
$CF_3CBrFCF_3$	0.08	1.1	0.54 ± 0.22

*Dipole moments were calculated using the BIOSYM/MSI Insight II software package. The first three molecules in this list are anesthetics. The minimum and maximum values for the dipole moments were obtained from conformations encountered during a molecular dynamics simulation. Simulations were run using the ESFF forcefield provided with Discover 95.0/3.00 for a single molecule in vacuum at 300K for 500 ps after 100 ps of equilibration.

The results obtained here are in agreement with the results of recent modeling calculations (Pohorille et al., 1996). In this study, molecular dynamics was used to determine the free energy profile of the anesthetic trifluoroethane and nonanesthetic perfluoroethane in passing from the bulk aqueous phase to the interior of a glycerol monooleate model membrane. It was found that the anesthetic had an energy minimum just inside the interface and a local minimum in the headgroup region. The perfluorinated nonanesthetic had a broad minimum throughout the membrane interior.

In conclusion, the results obtained here reveal a remarkably different behavior for anesthetic versus nonanesthetic halogenated cyclobutanes in membranes. The anesthetic compound chlorotrifluorocyclobutane (I) is distributed throughout the bilayer, but preferentially resides in the interfacial domain. The effects of this compound on membranes and its localization are consistent with that observed for other inhalation anesthetics. The nonanesthetic dichlorohexafluorocyclobutane (II) resides deep within the bilayer interior and does not demonstrate an affinity for the membrane interface. Thus anesthetic potency is not simply indicated by lipophilicity, as suggested by Meyer and Overton, but is indicated by the ability to partition into the interfacial region of the bilayer. For this to occur, the compounds must be hydrophobic, but have some dipolar character (either permanent or inducible).

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