Membrane Structural Perturbations Caused by Anesthetics and Nonimmobilizers: A Molecular Dynamics Investigation

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ABSTRACT The structural perturbations of the fully hydrated dimyristoyl-phosphatidylcholine bilayer induced by the presence of hexafluoroethane C_{2F6}, a "nonimmobilizer," have been examined by molecular dynamics simulations and compared with the effects produced by halothane $CF_3CHBrCl$, an "anesthetic," on a similar bilayer (DPPC) (Koubi et al., *Biophys. J.* 2000.78:800). We find that the overall structure of the lipid bilayer and the zwitterionic head-group dipole orientation undergo only a slight modification compared with the pure lipid bilayer, with virtually no change in the potential across the interface. This is in contrast to the anesthetic case in which the presence of the molecule led to a large perturbation of the electrostatic potential across to the membrane interface. Similarly, the analysis of the structural and dynamical properties of the lipid core are unchanged in the presence of the nonimmobilizer although there is a substantial increase in the microscopic viscosity for the system containing the anesthetic. These contrasting perturbations of the lipid membrane caused by those quite similarly sized molecules may explain the difference in their physiological effects as anesthetics and nonimmobilizers, respectively.

INTRODUCTION

To understand the mechanisms of general anesthesia, membrane lipids and their interaction with inhaled volatile anesthetics have been extensively studied (Koehler et al., 1980; Franks et al., 1982, 1994; Miller, 1985; Trudell, 1991). Although anesthetics seem to act by modulating the activity of membrane proteins, the exact site and mechanism of general anesthetic action, which include transient analgesia, amnesia, and immobility in response to a noxious stimulus, are largely unknown (Franks et al., 1984; Koblin et al., 1994). There is good reason to suspect that anesthetics may act through lipid bilayers. The Meyer-Overton rule (Meyer, 1899, 1901, 1937; Overton, 1901; Miller and Smith, 1973; Curatola et al., 1991), stating that the potency of general anesthetics correlates strongly with their solubility in olive oil, has long been considered the basis of the lipid theory of narcosis. Recent studies, however, (Koblin et al., 1994; Taheri et al., 1993; Kandel et al., 1996; Fang et al., 1997) have shown that many compounds, strikingly similar to potent general anesthetics, and predicted by the Meyer-Overton rule to be good anesthetics, are devoid of anesthetics effects. Such compounds produce amnesia but do not suppress movement. The study of anesthetics and structurally resembling nonimmobilizer pairs permits a closer examination of the molecular effects on membrane shared by anesthetic but not by nonimmobilizer (Minima et al., 1997; Xu et al., 1998; Forman et al., 1998; Tang et al., 1999a,b). A detailed description of the membrane structure in the presence of anesthetics and nonimmobilizers may therefore shed new light on the molecular and cellular mechanism of anesthesia.

In our previous molecular dynamics simulation of the halothane anesthetic molecule ($CF_3CHBrCl$), in the dipalmitoylphosphatidylcholine (DPPC) hydrated lipid bilayer (Tu et al., 1998; Koubi et al., 2000), we determined the distribution of the anesthetic in the bilayer. The halothane molecules preferentially segregate in the upper part of the lipid chains with a maximum probability near the C_5 methyl groups (Baber et al., 1995; Eckenhoff, 1996; North and Cafiso, 1997). The presence of anesthetics induces a lateral expansion and a slight contraction in the bilayer thickness, as well as a decrease of the alkyl chains order parameters for the tail region of the lipid. The anesthetic location in the upper region of the acyl chain induced a modification of the head-group phosphate-nitrogen (P-N) dipole orientation. The new head-group orientation implies modifications in the electric properties of the membrane.

More recently, we have studied the nonimmobilizer pair of halothane, C_2F_6, in a fully hydrated dimyristoyl-phosphatidylcholine (DMPC) lipid bilayer (L. Koubi, M. Tarek, S. Bandyopadhyay, M. L. Klein, and D. Scharf, manuscript in preparation). Preliminary analysis shows that nonimmobilizers have a different location in the lipid membrane compared with anesthetics. They are evenly distributed along the hydrocarbon chains of the lipids with a small preference for the bilayer center. In this paper, we focus on the perturbations induced by the presence of the C_2F_6 on the lipid structure. The results of the molecular diagnostics (MD) simulation of a fully hydrated DMPC lipid bilayer, containing a mole fraction of 25% of the nonimmobilizer, C_2F_6, will be compared with the previous investigation of

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halothane at mole fractions of 6.5 and 50% (Tu et al., 1998, Koubi et al., 2000) in the DPPC lipid. The two lipids are characterized by a very small structural difference in the length of the acyl chains (14 carbon atoms instead of 16). Both simulations were performed in the lipid liquid crystalline phase (L_α). This phase is exhibited above $T = 42°C$ and $T = 24°C$ for DPPC and DMPC, respectively. The most accurate experimental data on the biorelevant hydrated L_α phase of DMPC have been obtained at $T = 30°C$ (Petrache et al., 1998). The temperature of the simulation was therefore set to $T = 30°C$ for DMPC, which is more relevant to clinical conditions and allows a comparison with experimental results. The DPPC/halothane system was simulated at $T = 50°C$. At this temperature the membrane is in the physiological phase of biological membranes, although technically, this temperature is too high to mimic clinical conditions.

COMPUTATIONAL METHODS

Because the computational protocol of the DMPC/C_2F_6 simulation has already been presented in our previous paper (Koubi et al., manuscript in preparation), we summarize here briefly the system characteristics. Sixty-four DMPC molecules, 1645 water molecules (full hydration), and 16 C_2F_6 molecules were arranged to form a bilayer/water/nonimmobilizer system. Three-dimensional periodic boundary conditions are applied to generate a multilamellar system. The lipid/nonimmobilizer ratio of 4:1, which correspond to a mole fraction of 25%, is higher than clinically relevant concentrations. These are normally expressed as the mininum alveolar concentration required to render 50% of the subjects anesthetized and is estimated at approximately a mole ratio of 0.025 (a mole fraction of 2.5%). A previous MD study, performed at near-clinical anesthetic concentrations (Tu et al., 1998), showed that to obtain a reliable probability distribution of the perturbative solute in the model membrane, a higher concentration needs to be used. Moreover, the use of a high concentration of C_2F_6 is required to compare with the key experimental results (North et al., 1997).

In this study, the 16 nonimmobilizer molecules are initially incorporated uniformly in the bilayer. To start the simulation, the C_2F_6 molecules were treated as point-masses with zero charges and van der Waals (VDW) parameters. Then, short, consecutive MD runs (20 ps each) were performed to "grow" the molecules progressively by extending their intramolecular bonds. The intra- and intermolecular interactions were simultaneously rescaled from zero to one (in 20 steps). This procedure permits the incorporation of the molecules without having to create free volume (Tu et al., 1998). The system was then equilibrated at constant volume and constant temperature (30°C) for 200 ps. This was followed by $\simeq$2 ns constant-pressure (1 atm) and constant temperature (30°C) (NPT) simulations. For the analysis, averaged quantities were evaluated over the last 1.2 ns of the NPT runs.

To carry out the MD simulation described herein we used the recently developed MD package (PINY MD) (Tuckerman et al., 2000). We used the CHARMM (*c*hemistry at *Har*vard *m*olecular *m*echanics) (Sclenkrich et al., 1996) force field and parameters for the fully atomistic representation of DMPC molecules, and the TIP3P parameters for water (Jorgensen et al., 1983). Force-field parameters for C_2F_6 were fitted based on our previous work on halothane (Scharf and Laasonen, 1996). Details about the DPPC/halothane simulation are given in Koubi et al. (2000).

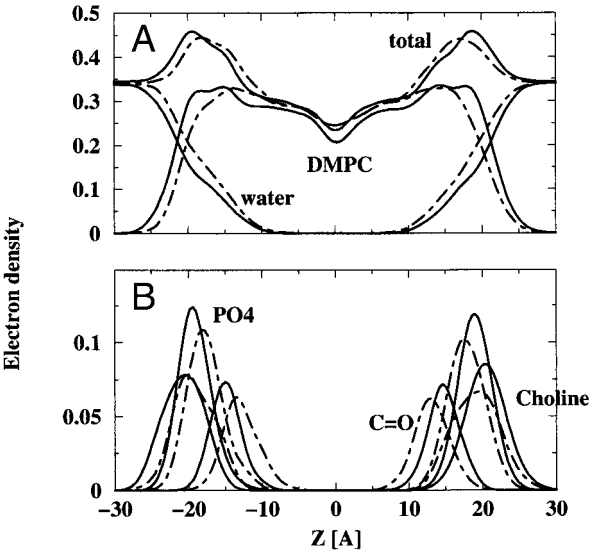

FIGURE 1 EDPs along the bilayer normal Z averaged over the last 1.2 ns of the NPT trajectory. Densities in both monolayers were averaged and displayed symmetrically. (*a*) The overall profile, the water, and the DMPC contributions. (*b*) The PO4, choline, and carbonyl carbon contributions. The dot-dashed lines represent results for the neat DMPC lipid, the solid lines the results for the DMPC/16C_2F_6 system. $Z = 0$ is the bilayer center.

RESULTS AND DISCUSSION

To monitor the changes in the overall membrane structure induced by the presence of the nonimmobilizers C_2F_6, we have computed the electron density profiles (EDPs) of the DMPC lipid and the water region from the MD trajectory. A Gaussian distribution of electrons was placed on each atomic center with a variance equal to the VDW radius, for each configuration, and the EDP was averaged over configurations. The resulting profiles are displayed in Fig. 1 *a* in comparison with the neat lipid profiles. In the presence of the nonimmobilizer, the total EDPs are shifted toward the water interface, and there is a decrease in the methyl trough region of the hydrocarbon chains. In fact, the simultaneous shift in the DMPC and water profiles indicates an overall expansion of the lipid core in the direction perpendicular to the bilayer attributable to the partial segregation of the nonimmobilizer in the middle of the bilayer (methyl trough) (Koubi et al., manuscript in preparation).

In Fig. 1 *b*, we have broken down the EDP into contributions from the phosphate, the choline, and the carbonyl carbons groups. The distance between the phosphate groups is 38.6 Å for the system containing the nonimmobilizer compared with 36 Å for the pure lipid. The increase of 7% is consistent with the enlargement of the *d*-spacing from 62.0 Å for the pure lipid to 66.7 Å (Koubi et al., manuscript in preparation). The choline density peaks at $\sim\pm$ 20.3 Å for both the pure lipid and the DMPC/C_2F_6, respectively, although the profile is sharper for the latter. Finally, the C = O density has a similar shape in presence and absence of

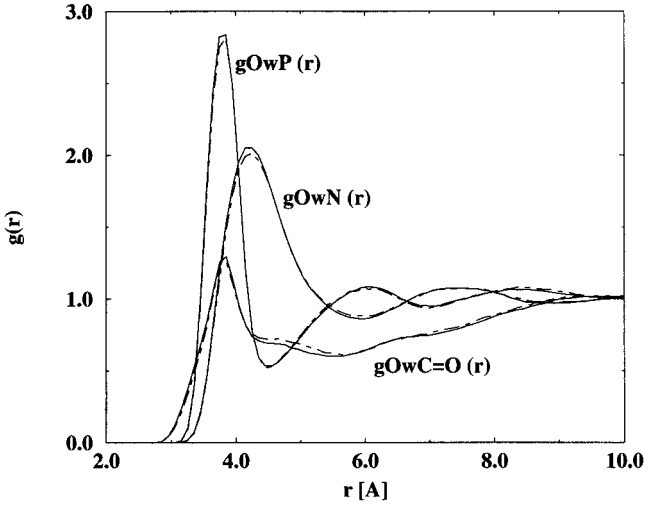

FIGURE 2 Radial distributions functions of the oxygen atoms of the water molecules around the choline, the phosphorus, and the glycerol ester carbonyl carbon atoms of the lipid head-groups. Comparison with the pure lipid (*dot-dashed lines*).

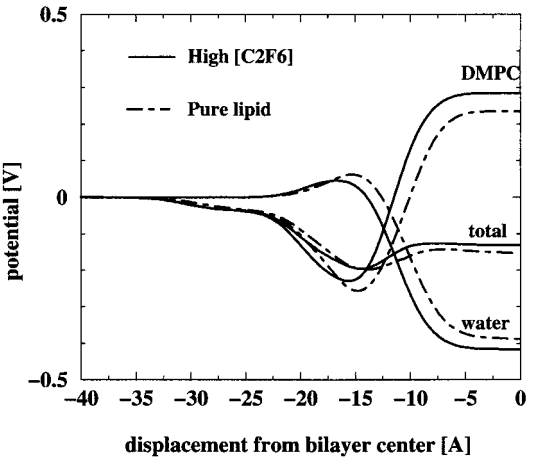

FIGURE 3 Total electrostatic potential difference relative to the bilayer center computed from the simulation and the individuals contributions from the water and the lipid molecules. The dot-dashed lines are for the pure lipid, and the solid lines for the system with perturbative molecules.

nonimmobilizers and the peaks are slightly shifted toward the water interface.

The changes in the head-group structure can further be monitored through the average distances of various carbons of the glycerol from the bilayer center. Following the nomenclature of Buldt et al. (1979), i.e., $C_{GC3}H_2$-O-P-O-$C_\alpha H_2$-C_β·H_2-N($C_\gamma H_3$)$_3$, the distances for the choline methyl, C_γ, the choline methylenes, C_α and C_β, and the glycerol methylene C_{GC3} carbons are 20.4 ± 3.5 Å, 19.3 ± 2.6 Å, 18.6 ± 2.7 Å, and 16.3 ± 2.6 Å for the pure lipid and 20.7 ± 3.8 Å, 20.1 ± 2.9 Å, 19.6 ± 2.5 Å, and 17.3 ± 2.5 Å for the DMPC/C_2F_6 system. Thus, the presence of nonimmobilizers causes the choline group to move slightly toward the exterior of the lipid, whereas the other carbons of the glycerol backbone shifted much more toward the water interface. This is in contrast to the DPPC bilayer system containing a high concentration of anesthetics, where the distances from the bilayer center did substantially decrease, compared with the values obtained for the neat lipid.

To characterize the water environment of the head-group, it is useful to consider the radial distribution function, $g_{XY}(r)$, which is proportional to the probability of finding atom Y at a distance r from atom X. We have evaluated $g(r)$ of the oxygen atoms of the water molecules around the choline, the phosphate, and the carbonyl carbon groups (Fig. 2), and by integration up to the first hydration shell of water molecules, estimated the corresponding hydration numbers. We found that, on average, in the presence of nonimmobilizers 17.1, 5.9, and 2.0, water molecules hydrate the choline, the phosphate, and the carbonyl carbon groups, respectively. As observed from the $g(r)$ plots (Fig. 2), there is essentially no difference in the hydration between the pure lipid and the lipid containing the nonimmobilizer. In contrast, in the DPPC/halothane system, we found that the choline head-group is experiencing a different water environment in the presence of anesthetics (Koubi et al., 2000). Specifically, the water coordination number amounts to 15.3 compared with 17.3 for the pure lipid phase, whereas no change was observed for the phosphate and carbonyl carbon groups.

For the DMPC/C_2F_6 system, the head-group orientation has been characterized through the probability distribution of the angle between P-N dipole vector and the normal to the bilayer (Koubi et al., manuscript in preparation). The results show clearly that the C_2F_6 molecules have no effect on the head-group orientation. To the contrary, in the MD study of the halothane molecules (Koubi et al., 2000), it was found that halothanes induce important modification of the phosphate-choline (P-N) orientation. The population of dipoles oriented toward the interior of the bilayer was drastically increased upon addition of the anesthetic.

The P-N dipole orientation observed in the DPPC lipid in presence of anesthetic molecules led to an important modification of the potential across the membrane (lipid/water interface) and, consequently, of the electrostatic properties of the bilayer. For the present system, we have plotted the potential difference across the interface and the individual contributions from the DMPC and the water molecules (Fig. 3) for the neat lipid and for the system with C_2F_6. The total potential display a slight decrease though the bilayer/water interface to a value in the water of −150 mV for the neat DMPC and −130 mV for the DMPC/C_2F_6 system. In comparison, the total potential for the DPPC/halothane system has changed from ≃−500 mV for the pure lipid to ≃−900 mV for the bilayer containing the halothane. This underlines clearly a difference between the nonimmobilizer and the anesthetic effects on the lipid electrostatic properties. Be-

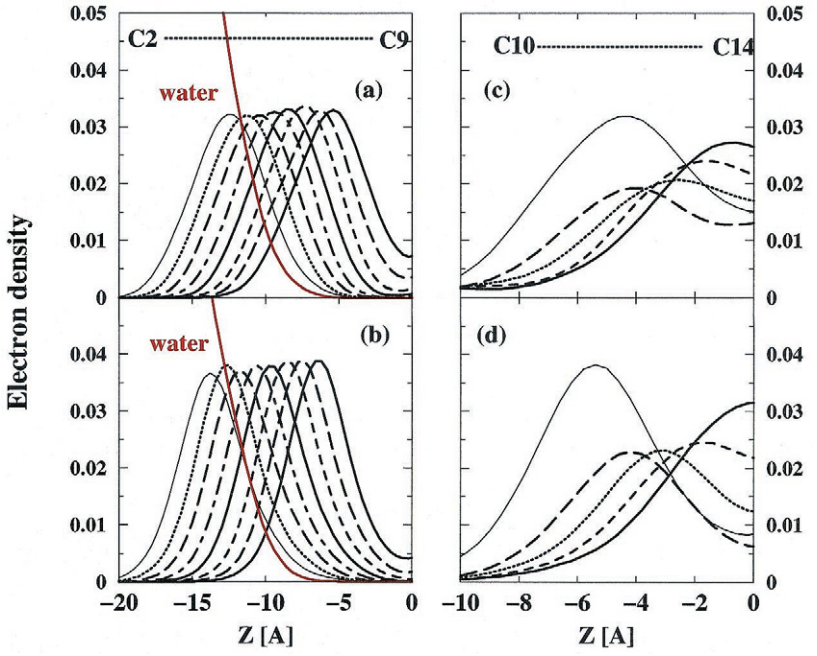

FIGURE 4 EDPs along the bilayer normal Z for different carbon atoms of the lipid chains. (*a*) and (*c*) The pure DMPC lipid. (*b*) and (*d*) DMPC/16C_2F_6 system. $Z = 0$ represents the bilayer center.

cause of the different distribution in the membrane, the latter induces a drastic change in the electrostatic potential, whereas the former hardly perturbs the lipid water interface.

It is important to address here the differences in potential observed between the neat DPPC and DMPC systems. The potential V is estimated from the MD simulation as a double integral of molecular charges density distributions neglecting the explicit electronic polarization as suggested by Tobias et al. (1997). This implies that it is dependent on the force-field parameters used (note here that the force field used in the DMPC and the DPPC simulations are different). Second, a more accurate estimate can be obtained only if the trajectory is long enough to permit a complete sampling of the head-group motions, which are known to be in the range of tens of nanoseconds time-scales (Pastor and Feller, 1996; Tobias et al., 1997). Finally, the temperatures for the two systems were set to $T = 30°C$ and $T = 50°C$, respectively, to allow both lipids to be in their liquid crystalline phase. A combination of there effects may explain the difference in the electrostatic potential estimated from the MD trajectories in each system. However, it is clear that the changes observed in the lipid in the presence of the anesthetic are not reproduced in the lipid in the presence of the nonimmobilizer. Our results suggest that the changes are large enough in the case of the anesthetic molecules (net change of $\simeq 300$ mV) to be measurable, and are almost unchanged in presence of nonimmobilizers.

From this analysis of the DMPC/C_2F_6 system, it seems that the structural and the electrical modifications occurring in the head-group region induced by the nonimmobilizer are very small. We now examine the effects on the hydrocarbon chain region. In Fig. 4, we plotted the average EDP along z, the direction normal to the bilayer surface, for different carbon atoms of the hydrocarbon chains. Here, Cn is the nth carbon in a hydrocarbon chain in which the numbering begins at the carbonyl carbon (Buldt et al., 1979), and the profiles are averaged over the two layers. Overall, the EDPs in the presence of C_2F_6 display a sharper distribution compared with the neat lipid and are increasingly shifted toward the water interface as we go from the lipid head-group to the terminal methyl group. The overall shift is attributable to the aggregation of C_2F_6 in the middle of the bilayer, hence pushing the two leaflets apart, whereas the decrease in the profile's width indicates a stiffness in the lipid core. Indeed, the percentage of gauche defects obtained for the last nine bonds amounts to 23 (C_3-C_4), 16 (C_4-C_5), 20 (C_5-C_6), 18 (C_6-C_7), 19 (C_7-C_8), 20 (C_8-C_9), 21 (C_9-C_{10}), 21 (C_{10}-C_{11}), and 27 (C_{11}-C_{12}) compared with 24, 19, 20, 22, 19, 21, 22, 24, and 28% for the pure lipid. The data show that there are slightly more gauche rotamers in the system without nonimmobilizer. The effects of the nonimmobilizer on the lipid core structure are different from those caused upon addition of anesthetics, as is indicated by the changes taking place in the lipid hydrocarbon chain order parameters. Our previous study has revealed that anesthetics have a tendency to decrease the order parameters, whereas the nonimmobilizers seem to increase the chain order parameters.

These contrasting effects on the lipid core stiffness are likely to influence the lipid dynamics, and therefore affect their permeability to other molecules. Indeed, on time-

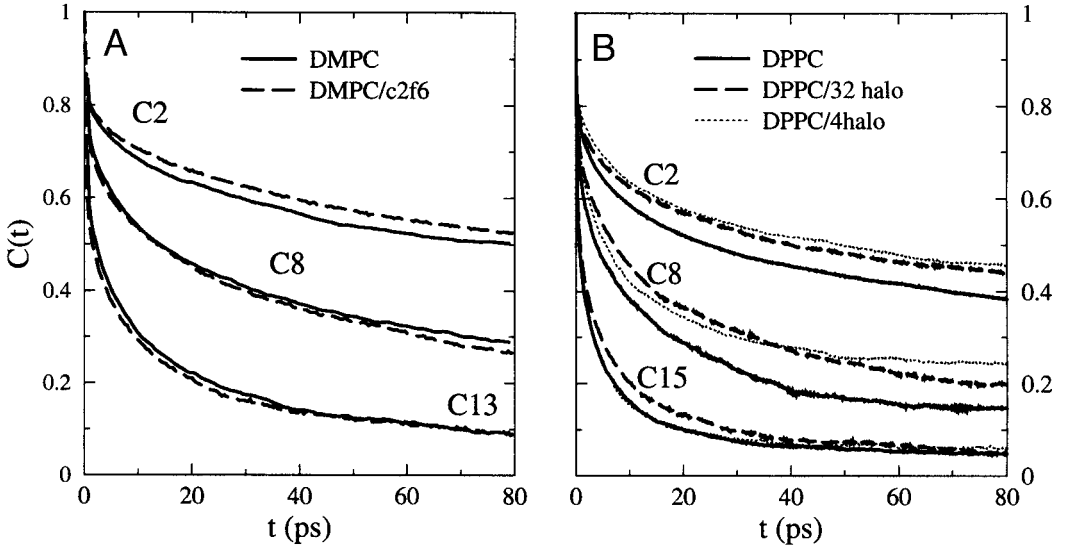

FIGURE 5 Orientational time correlation functions, $C(t)$, for selected C-H vectors of the lipid acyl chains. (*a*) DMPC with (*long dashed line*) and without (*solid line*) C_2F_6 (*b*) DPPC with a low (*dotted line*) and a high (*long-dashed line*) concentration of halothane and without (*solid line*). The numbering of the carbons begins at the carbonyl group of the acyl ester linkage.

scales ranging from the picosecond to nanoseconds, lipid molecules in bilayers exhibit a variety of whole-molecule and internal motions (amplitudes of up to 10 Å) that are important in the lateral and transbilayer transport of small molecules. These motions include single molecule protrusions, lateral "rattling in a cage," and complicated rearrangements of the acyl chains. To describe the internal dynamics of the hydrocarbon chains, or to discuss the fluidity of the bilayer interior, the time correlation functions, $C(t) = 1/2\langle 3[\mathbf{u}(t)\cdot\mathbf{u}(0)]^2 - 1\rangle$, is commonly used ($u$ is a unit vector along methylene C-H bonds). These functions describe the reorientational relaxation of the acyl chains. They generally consist of a fast (100 ps) and a slow (1 ns) component (Venable et al., 1993). In Fig. 5 *a*, we display $C(t)$ for the DMPC molecules with and without C_2F_6 molecules. This figure indicates that the short time-scale reorientation is not affected by the presence of nonimmobilizers. Fig. 5 *b* reports the results for DPPC molecules in presence of both a low and a high concentration of anesthetic halothane. We note an increase of the reorientational relaxation times along the chains, especially at the top of the chain C_2 and in the middle of the chains C_8, whereas few modifications are observed at the end of the chains C_{15}. In summary, the C-H reorientational motion is slowed in the presence of halothane whereas the nonimmobilizer C_2F_6 has no effect on it.

The effect of nonimmobilizers on the lateral rattling and the protrusion motions of the lipid molecules on the 100 ps time-scale is illustrated by the time dependence of the in-plane and out-of-plane center-of-mass mean square displacements plotted in Fig. 6 *a*. It is clear that the in- and out-of-plane motion are not affected by the presence of the C_2F_6 molecules. Results for the anesthetic reported in Fig. 6 *b* show that the in-plane motion is markedly reduced in the presence of anesthetics. The out-of-plane motion is unchanged in presence of a low concentration of halothane and decreases at high concentration.

CONCLUSIONS

We have performed an MD simulation of DMPC in the liquid crystalline phase containing a 25% mole fraction of the nonimmobilizer C_2F_6. The results have been extensively compared with the previous MD study of DPPC containing its anesthetic pair halothane ($CF_3CHBrCl$). The DMPC lipid in the presence of C_2F_6 exhibits almost no structural changes compared with those of the pure lipid. The main perturbation of the overall membrane structure is manifested by an expansion of the lipid core region in the direction perpendicular to the bilayer (increase of the bilayer thickness) because of a preferred segregation of the nonimmobilizer in the methyl tough region. This seems to have little or no effect either on the structure of lipid molecules or on their dynamics.

We have quantified the structural changes induced by the presence of the nonimmobilizer on the head-group region and the lipid core, as well as the changes in the transport properties of the membrane. First, the head-group/water interface does not suffer any changes as manifested by the nonperturbed phosphatidylcholine/water structure. We find that the distribution of P-N dipole orientations with respect to the water interface does not change, and therefore, the electrostatic potential and the head-group hydration remain

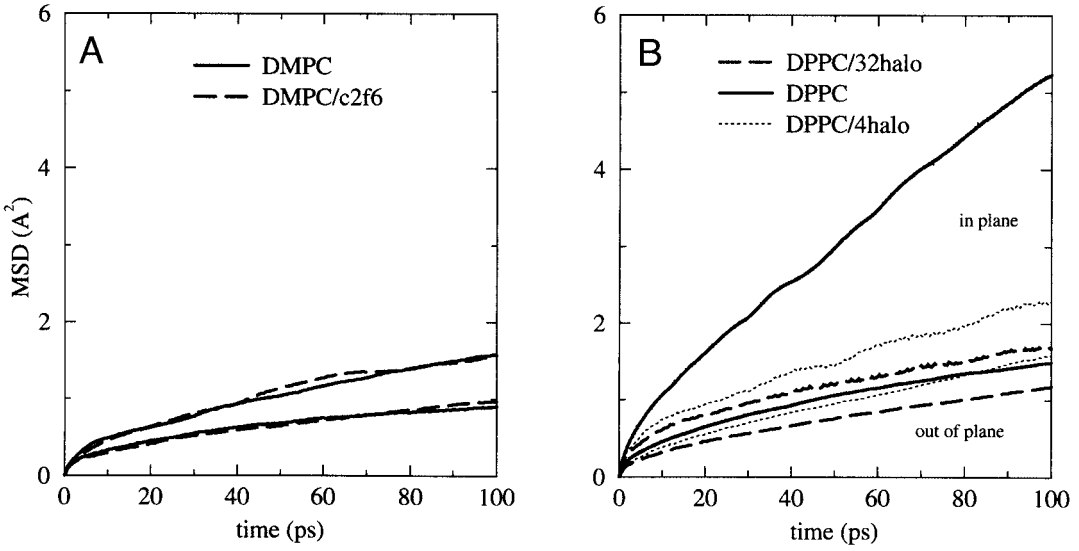

FIGURE 6 Comparison of the center-of-mass mean square displacements of lipids in the bilayer plane computed from simulations of a pure lipid and lipid containing perturbating molecules. (*a*) DMPC with (*long dashed line*) and without C_2F_6. (*b*) DPPC with a low (*dotted line*) and a high (*long-dashed line*) concentrations of halothane and without (*solid line*).

identical to those of the neat lipid system. In comparison, the anesthetic molecules were shown to have a preference for the head-group region, leading to rather an increase of the surface area per lipid. The partitioning of the anesthetic induces large structural modifications of the lipid bilayer, among which an increased hydration of the choline moiety, and more importantly, a large modification of the P-N dipole average orientation, and, therefore, of the electrostatic potential across the lipid/water interface.

The different partitioning of nonimmobilizers versus anesthetics also has different effects on the lipid hydrocarbon structure. In the presence of C_2F_6 (Koubi et al., manuscript in preparation), one hardly detects a stiffness of the lipid core, an increase of the order parameters, and a decrease of the number of gauche rotamers population. In contract, the addition of halothane was shown to decrease substantially the order parameters of the lipid chains and increase the population of gauche defects.

The effects of nonimmobilizers versus anesthetics on the lipid dynamics on the 100 ps time-scale are also shown to be different. As expected from the structural characterization, the presence of the nonimmobilizer seems to have no effect on the fluidity of the membrane. In contrast, the anesthetic increases the microscopic "viscosity," as manifested by the decrease in reorientational relaxation times of the acyl chains and the slowing down of the in-plane lipid motion.

The differences between C_2F_6 and halothane distributions can be attributed to the enhanced hydrophobicity of C_2F_6. The nonimmobilizer, being more hydrophobic and lacking a net dipole moment, preferentially migrates to the hydrophobic region of the bilayer, along the acyl chains. The affinity of C_2F_6 to the lipid acyl chain region is likely a reflection of the predominance of VDW interactions in determining its location in the membrane. Halothane, on the contrary, exhibits lesser hydrophobicity and, like most of the inhaled volatile anesthetics, has a small dipole moment. Therefore, it resides preferentially in regions of the lipid bilayer that are partially accessible to water. This is reflected in the appropriate $g(r)$. The tendency of halothane to aggregate and exhibit a non-uniform distribution across the membrane interior reflects the delicate balancing of electrostatic and VDW interactions, in which the former interactions are stronger with polar components, such as water molecules.

This study does not address the question of whether or not anesthetics and nonimmobilizers interact differently with membrane proteins. This precludes us from advancing a hypothesis with regard to the site of action. However, the high partition coefficient of these substances suggests that lipids play a significant role in anesthesia and analgesia, either by mediating or as being the site where action is rendered. Our analysis shows that as far as the lipid structure and dynamics are concerned, the effects of these molecules are rather different. In turn, their presence in the membrane is likely to affect differently the properties of membrane proteins.

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