

MD simulations of C₂F₆ effects on gramicidin A: Implication of mechanisms of general anesthesia

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ABSTRACT

It was recently postulated that the effects of general anesthetics on protein global dynamics might underlie a unitary molecular mechanism of general anesthesia (Tang & Xu, Proc Natl Acad Sci USA, 99:16035-40, 2002). To verify that the specific dynamics effects caused by general anesthetics were not shared by non-anesthetic molecules, two parallel 8-ns all-atom molecular dynamics simulations were performed on a gramicidin (gA) channel in a fully hydrated dimyristoylphosphatidylcholine (DMPC) membrane in the presence and absence of hexafluoroethane (HFE), which structurally resembles the potent anesthetic molecule halothane but produces no anesthesia. Similar to halothane, HFE had no measurable effects on the gA channel structure. In contrast to halothane, HFE produced no significant changes in the gA channel dynamics. The difference between halothane and HFE on channel dynamics can be attributed to their distinctly different distributions within the lipid bilayer and consequently to the different interactions of the anesthetic and the non-anesthetic molecules with the channel-anchoring tryptophan residues. The study further supports the notion that anesthetic-induced changes in protein global dynamics may play an important role in mediating anesthetic actions on proteins.

INTRODUCTION

One of the greatest challenges to the scientific community in the past 150 years is to understand the action of a class of seemingly non-specific drugs on the central nervous system (CNS) to produce the physiological state of mind referred to as “general anesthesia.” The conceptual difficulties have been the lack of a functional definition of “general anesthesia” at the molecular level. A significant amount of efforts has been devoted in recent years to the identification of anesthetic targets in the CNS. Departing from the classical view of lipid perturbation theory, contemporary experiments involving mutagenesis, photo-affinity labeling, and electrophysiology have focused on several anesthetic-sensitive neuronal ion channels (Campagna *et al.*, 2003; Franks and Lieb, 1994) or even anesthetic-sensitive mutation sites in these channels (Forman *et al.*, 1995; Jenkins *et al.*, 2001; Mascia *et al.*, 2000; Mihic *et al.*, 1997; Pratt *et al.*, 2000). The lack of high-resolution structures of these ion channels, however, has impeded the progress in finding out where the anesthetic interaction sites are, and more importantly, how the interactions alter the biological functions of these channels.

High-resolution structures of these ion channel proteins, once resolved, will definitely provide important structural basis for the interpretation of their responses to general anesthetics. Given the low affinity (K_d in the sub-mM to mM range) of most general anesthetics at their putative binding sites, however, it has been questioned whether the structure-function paradigm alone is sufficient to explain the molecular mechanisms of general anesthesia (Tang and Xu, 2002). There is a growing realization that protein dynamics plays vital roles in protein functions. Because protein molecules are dynamic in nature, the conventional static view of protein structures can only provide a limited, and often incomplete, understanding of protein functions. For example, the function of myoglobin was viewed for a long time as storage of dioxygen at the heme iron based on the static myoglobin structure (Kendrew *et al.*, 1958; Perutz, 1979). Not

until recently after dynamical aspects of myoglobin were well characterized did its role other than O₂ storage start to emerge. Without integrating the dynamic properties of myoglobin, one could not even clearly pinpoint the pathway for dioxygen to enter the protein because the pathway is not apparent in the static structure (Case and Karplus, 1979; Perutz and Mathews, 1966). Systematic measurements on myoglobin dynamics have confirmed the existence of its conformational substates and identified the importance of conformational substates to the protein functions (Bourgeois *et al.*, 2003; Frauenfelder *et al.*, 2003; Srajer *et al.*, 2001). If dynamics plays such an important role in a monomeric protein like myoglobin, dynamical motion must be essential to the functions of the multi-subunit neuronal ion channels. In fact, different functional states of neuronal ion channels, including the open, close, and slow and fast desensitized states, have already been identified experimentally (Auerbach and Akk, 1998; Karlin, 2002; Neubig *et al.*, 1982). These functionally distinct states are likely resulted from shifting the equilibration of different conformational substates.

Can general anesthetics modulate different conformational substates or shift the dynamic population of the substates to exert their actions on neuronal ion channels? An unambiguous experimental proof with neuronal ion channels remains a challenge, but the results from our previous molecular dynamics (MD) simulations of the effects of halothane, a potent volatile anesthetic, on gramicidin A (gA) channel have encouraged the thinking along this line (Tang and Xu, 2002). Although the anesthetic effect on the structure of the gA channel is minimal, which is consistent with our earlier experimental findings (Tang *et al.*, 2000a; Tang *et al.*, 1999a; Tang *et al.*, 2002), the presence of halothane profoundly affects the channel dynamics, as evidenced by the changes in the root mean square (rms) fluctuation and the autocorrelation function of the gA backbone in the lipid core in the presence of halothane, even though halothane preferentially targets the anchoring residues at the channel-lipid-water interface. Our earlier simulation results

discounted the viewpoint that overrates the importance of structural fitting between anesthetic molecules and yet-unidentified hydrophobic protein pockets. Instead, the results suggest at least two important possibilities: (1) direct anesthetic interactions with some of key residues of ion channel proteins, such as tryptophans in gramicidin A, can modulate the dynamics of residues that are remote from anesthetic interaction sites; and (2) protein global dynamics might be crucial for anesthetic action. We hypothesize that drugs such as general anesthetics and alcohols with low affinity binding to proteins can still change protein function specifically by modulating protein global dynamics on various timescale. Whereas multiple conformational substates coexist dynamically for all proteins, the presence of general anesthetics at certain crucial locations within or around the protein can shift the equilibrium among different substates. When anesthetic modulations of the global dynamics of a given protein create “conformation resonance” where one of the equilibrating conformers becomes the dominating conformation, then the function carried out by the protein can potentially be changed. The enhancement of the protein dynamical motion having the characteristic time matching the timescale for the protein function will lead to anesthetic-induced potentiation, whereas matching other motion time constants will lead to anesthetic-induced inhibition or desensitization.

In the present study, we test the aforementioned hypothesis using a negative control by replacing anesthetic halothane (CF_3CHClBr) with non-anesthetic hexafluoroethane (HFE), C_2F_6 , in the previously studied simulation system (Tang and Xu, 2002). Two parallel ~ 8 ns MD simulations were performed to investigate if this non-anesthetic molecule can produce same effects as halothane on the gramicidin A channel. The comparison of the effects of structurally similar anesthetic-nonanesthetic pairs on the same ion channel will elucidate the critical properties that are relevant to the underlying mechanisms of the action of general anesthetics.

This study confirmed that the profound changes in gramicidin backbone dynamics occurred only with anesthetic halothane and not with nonanesthetic HFE.

METHODS

Simulation Systems. Detailed procedures for the preparation of simulation systems have been reported previously (Tang and Xu, 2002). Two parallel systems, in the absence and presence of HFE, were prepared using the NAMD2 (Kale *et al.*, 1999) and X-PLOR (Brünger, 1992) programs. Each system had a gA channel (1MAG (Ketchum *et al.*, 1997)) in the pre-equilibrated dimyristoylphosphatidylcholine (DMPC) membrane, consisting of 182 DMPC lipid molecules fully hydrated with 5538 water molecules (Zubrzycki *et al.*, 2000). CHARMM22 force field (Brooks *et al.*, 1983; MacKerell *et al.*, 1998) and TIP3P water model (Jorgensen *et al.*, 1983) were used in the study. The geometry of HFE molecules were optimized at the B3LYP/6-311+G(2d,p) level, and the nonbonded interaction parameters of HFE were optimized to be compatible with the CHARMM force field (Liu *et al.*, 2004). For the system with HFE, ten HFE molecules were placed at the same initial locations as for halothane in the previous study (Tang and Xu, 2002). Additional energy minimization was performed on the system after introducing HFE. The heavy atoms of the gA channel backbone were restrained with a harmonic force during the initial steps of energy minimization to ensure a stable channel structure. The force constant of the restraint was initially set at 999 kcal/mol/Å² and was reduced in a stepwise fashion to 0 kcal/mol/Å². Both energy-minimized systems, in the absence and presence of HFE, went through equilibration under NVT (constant number of atoms, volume, and temperature) and NPT (constant number of atoms, pressure, and temperature) conditions for 40 ps and 250 ps, respectively, with a harmonic constraint of 0.5 kcal/mol/Å² on gA backbone heavy atoms.

MD Simulations. After system preparations, two NPT simulations were carried out for 8 ns each in parallel in the presence and absence of 10 HFE molecules on the T3E parallel supercomputer at the Pittsburgh Supercomputing Center using the NAMD2 program. The Nosé-Hoover method with Langevin dynamics and Langevin piston pressure were applied to control the temperature at 305 K (Hoover, 1985; Nose, 1984) and pressure at 1 bar (Feller *et al.*, 1995; Martyna *et al.*, 1994), respectively. The periodic boundary condition was imposed on a flexible cell of initial dimension $80 \times 80 \times 60 \text{ \AA}^3$ with water wrapping. The time step was 1 fs for the first 3-ns simulations and was extended to 2 fs for the rest of simulations. The energies and trajectories were stored every 0.5 and 1 ps, respectively. The cutoff distance for the van der Waals interaction was 12 \AA with the pair list distance extended to 13 \AA . The pair list for nonbonded interactions were updated every 20 time steps. The long-rang full electrostatic interactions were evaluated every four time steps using the particle mesh Ewald (PME) method with a PME tolerance of 10^{-6} and PME interpolation order of 4 (Darden *et al.*, 1993). The SHAKE routine was used to restrain all bonds between hydrogen and its parent atom to a tolerance of 10^{-5} \AA (Van Gunsteren and Berendsen, 1977) in all simulations.

Data Analysis. Data analysis followed the same procedures as reported previously (Tang and Xu, 2002) using the scripts developed within the VMD software environment (Humphrey *et al.*, 1996) on a local Linux computer. Autocorrelation functions were calculated to evaluate the channel's internal motions. This was done by first superimposing trajectory frames onto an optimal common frame of reference to remove any translational motions and then evaluating the following term over a sliding time window:

$$C_i(t) = \langle P_2 [\boldsymbol{\mu}(t') \cdot \boldsymbol{\mu}(t' + t)] \rangle \quad [1]$$

Where $\boldsymbol{\mu}(t') \cdot \boldsymbol{\mu}(t' + t)$ is the projection of a unit vector pointing along a given backbone vector at time t' onto the same vector at later time $t' + t$, $P_2(x)$ is the second Legendre polynomial, and the brackets denote a time average over the trajectory.

Lipid order parameter, $|S_{CD}|$, was calculated from MD trajectories using the following equations (Huang *et al.*, 1994):

$$S_j = \langle (3 \cos^2 \beta - 1) / 2 \rangle \quad [2]$$

and

$$|S_{CD}| = 0.5 S_j \quad [3]$$

where j stands for the j^{th} carbon counted from the head group, β is the angle between the membrane normal and the vector joining C_{j-1} and C_{j+1} , and bracket denotes averaging over time and all molecules. $|S_{CD}|$ typically ranges from 0 to 0.5, representing an isotropically disordered state to highly ordered all-trans conformation of alkyl chains. Twenty-seven DMPC lipids within the 5-Å proximity to the gA channel were used for $|S_{CD}|$ calculations.

RESULTS AND DISCUSSIONS

HFE distribution in the gA-membrane system

In order to compare directly with the halothane results, HFE molecules were purposely placed at the same initial locations as the halothane molecules in our previous simulation system (Tang and Xu, 2002). This initial placement is consistent with our prior experimental knowledge (Tang *et al.*, 1999a) of halothane distribution in the membrane but somewhat artificial for HFE, thus allowing for the specificity of anesthetic and non-anesthetic interaction with channel protein to be examined. Fig. 1 shows the movement trajectories of HFE molecules in the 8-ns NPT simulation. The trajectories were generated by connecting positions of the center of mass of HFE molecules at each saved time point (every ps). The density of the trajectory lines reflected how frequently a HFE molecule sampled a particular region. As shown in Fig. 1A, all ten HFE molecules had considerable movement over the 8-ns simulation, as reflected by the large regions encompassed by each motion trajectory. There is a clear tendency for HFE to finally locate in the lipid alkyl-tail region regardless of their initial positions, which were highlighted in Fig. 1A using HFE molecules in CPK presentations. A closer examination of the trajectories showed that only some of the HFE molecules (#4, #5, #7, #8, and #10) had large displacement along the membrane normal direction. Most of these molecules were initially placed near the lipid-water interface. Instead of moving around at the interface or moving into the bulk water as their halothane counterparts did in the previous simulations (Tang and Xu, 2002), these HFE molecules moved toward inner lipid bilayer and showed their preference for the hydrophobic environment. The difference in water solubility between halothane and HFE is also evident: HFE #9 and #10 in Fig. 1A moved into and remained within lipid interior during the entire 8-ns simulations, whereas previously (Tang and Xu, 2002) halothane molecules placed at the

corresponding initial positions moved into lipid-water interface and the bulk water in the 2.2-ns simulations. Thus, unlike anesthetic halothane, none of the nonanesthetic HFE showed appreciable amount of time in water during the 8-ns simulation. The preference of HFE molecules for the lipid tail region was revealed more clearly in Fig. 1B, where the displacement of HFE molecules along the z-axis (parallel to the membrane normal) was determined. At least four out of ten HFE molecules (#4, #5, #8, and #10) were either close to or at the lipid-water interface at the beginning of the simulation, but all of them moved away from the interface and into the lipid interior at the end of the simulation. The maximum z-coordinate difference of the ten HFE molecules was reduced from ~ 30 Å to less than ~ 20 Å over the simulation. No single HFE molecule moved out of the two lines of the averaged z-coordinates of phosphorus where the lipid-water interfaces are defined. This HFE distribution in the membrane is in sharp contrast to that of the anesthetic analogue, which showed a strong preference for the lipid-water interfacial region of the membrane.

The differences between halothane and HFE molecules observed in our simulation systems are consistent with the finding from previous experimental and simulation studies. Both NMR measurements (North and Cafiso, 1997; Tang *et al.*, 1997) and MD simulations (Koubi *et al.*, 2001; Koubi *et al.*, 2002; Tu *et al.*, 1998) indicated that anesthetic and non-anesthetic molecules had different preferential localization and properties in pure lipid membrane. The former preferred amphiphilic lipid-water interfacial regions whereas the latter resided primarily within the membrane hydrocarbon core. Their difference in effective concentrations at different submolecular sites might account for their ability to target functionally crucial domains in channel proteins and ultimately for their ability to produce general anesthesia. By its hydrophobic nature, HFE has much less probability than halothane to interact with the anchoring residues of gA channel near the lipid-water interface. Such interaction in the presence of

halothane, observed by NMR (Tang *et al.*, 2000a) and MD simulations (Tang and Xu, 2002), have shown strong effects on the hydrogen bonding between tryptophan side chains and lipids, and consequently on the dynamics behavior of the gA channel.

HFE effects on gA channel structure

The structural stability of gA channel in the presence and absence of HFE was evaluated by RMSD and presented in Fig. 2. The presence of non-anesthetic HFE had little impact on the secondary and tertiary structures of the channel. The same conclusion was drawn in the previous study of a gA channel in DMPC in the presence of anesthetic halothane (Tang and Xu, 2002). These simulation results are consistent with our earlier finding from NMR experiments (Tang *et al.*, 2002; Tang *et al.*, 1999b) that anesthetics and their non-anesthetic analogues, in general, have no significant effects on gA channel structures.

HFE effects on dynamics behavior of gA channel

It has been found previously that halothane caused profound changes in gA channel dynamics (Tang and Xu, 2002). Moreover, anesthetic effects on dynamics varied with the characteristic times of the dynamical motion. In contrast, the presence of HFE had virtually no influence on the dynamics behavior of the gA channel. Fig. 3 compares the root mean square fluctuation (RMSF) of the gA backbone C_α in the presence and absence of HFE. To correct the baseline of RMSF by removing the effects of any possible accumulative translational movement of the system, the center of mass of the entire system at all saved points was fitted to a common reference point. As expected, the residues near the entrance of the channel have greater fluctuation than the residues near the center of the channel, forming a U-shaped RMSF profile along the channel. Unlike the system with halothane in which halothane seems to equalize the fluctuation along the channel (Tang and Xu, 2002), this U-shaped profile is well preserved after

the addition of HFE to the system, suggesting that HFE does not impose any sizeable effects on the gA channel dynamics.

The autocorrelation function, $C_i(t)$, of the channel backbone N-H vectors gives a more detailed description of the time dependence of the channel dynamics. For display clarity, autocorrelation functions for residues 1-8 and 9-15 were pooled and averaged separately and shown in Fig. 4. Grouping the inner and outer residues allows for possible difference of HFE effects on the anchoring and central part of the channel to be analyzed separately. In both the control and HFE simulations, $C_i(t)$ drops to about 0.9 within picoseconds for all residues, due to the ultrafast subpicosecond libration motion of the NH vectors. Inner channel residues (1-8) remain at relatively high asymptotic values of ~ 0.88 , while overall $C_i(t)$ values for outer residues (9-15) are just slightly lower (~ 0.82), indicating that the NH vectors of both inner and outer residues are well ordered on a pico-second time scale. The variations of $C_i(t)$ between the control and HFE simulations are so trivial that they must be regarded as random fluctuations. Overall, the autocorrelation function data suggested again that HFE exert no significant effects on the dynamics of the channel.

Significance of these results is at least twofold. First, it confirms that the previously observed changes in the gA channel dynamics in the presence of halothane (Tang and Xu, 2002) is indeed caused by the anesthetic interactions with the channel and not fortuitous events. Artificially replacing the anesthetics with nonanesthetics at the same initial positions cannot reproduce the anesthetic effects on protein global dynamics, suggesting that the interactions are anesthetic specific. Second, the ability of halothane and possibly other anesthetics to affect the channel dynamics and the inability of nonanesthetic HFE to do the same may represent the most fundamental difference between anesthetics and nonanesthetics in exerting their distinct actions on ion channels.

An important remaining question is why anesthetic can affect gA channel dynamics but structurally similar non-anesthetic cannot. The tryptophan indole amide hydrogen atoms of gA have been found to form stable hydrogen bonds with the phosphate oxygen in the lipid head region or the fatty acid oxygen near the glycerol bridge, depending on the depths of the indoles in the membrane (Tang and Xu, 2002). These hydrogen bonds could be disrupted frequently in the presence of nearby halothane molecules due to the replacement of the channel-membrane hydrogen bonding with hydrogen bonding between the indole amide hydrogen and the fluorine in halothane. Considering there are four tryptophans (W9, W11, W13, and W15) at each end of a gA channel to anchor the channel dimer, it is conceivable that this type of disruption between the anchoring residues and membrane head groups could affect the entire channel motion (Tang and Xu, 2002) and consequentially the channel stability and conductance (Hu *et al.*, 1993; Ketchum *et al.*, 1997) in the membranes. In contrast, non-anesthetic HFE has little effects on disrupting the association of anchoring tryptophan residues with the membrane surface. The lack of HFE interaction with membrane interfacial tryptophan residues is clearly a direct consequence of the preferred HFE partitioning in the hydrophobic lipid core of the membrane.

HFE Effects on lipids

Lipids play important roles in the stability and function of ion channels. Modification of lipid properties may potentially affect channel behavior. Previous MD simulations (Koubi *et al.*, 2002) on the pure lipid systems showed that the HFE molecules were almost evenly distributed along the lipid hydrocarbon chains with only a slight preference for the bilayer center, which is consistent with the observations in the current study (see Figure 1). The presence of HFE in pure lipid imposed little changes on the electrostatic potential across the membrane interface and on the structural and dynamical properties of the lipid core (Koubi *et al.*, 2001), whereas the presence of halothane could cause profound changes on these properties. Contrary to a decrease

of the membrane thickness and an increase of the average area per lipid induced by the presence of anesthetic halothane (Koubi *et al.*, 2000; Tu *et al.*, 1998), opposite changes were observed previously in pure lipid systems in the presence of HFE. Comparing to the control system in the present study, the system involved with HFE has greater values on membrane thickness and smaller values on the averaged area per lipid (data not shown). The observation is consistent with the findings in the pure lipid system (Koubi *et al.*, 2001), but the extent of changes is smaller in the current study, presumably because of lower HFE concentration in our system.

The dynamical properties of the lipids in the vicinity of the gA channel are crucial to the channel functions and are evaluated using the order parameters of the lipid alkyl chain. As depicted in Fig. 5, the order parameters are gradually reduced from the head group to the alkyl-tail in both sn-1 and sn-2 chains. The magnitudes of order parameters for the lipids in the immediate vicinity of the gA channel (i.e., the interfacial boundary lipids) are higher in the head group and lower in the tail region than the corresponding values of bulk lipids from earlier experimental and simulation studies (Boden *et al.*, 1991; Douliez *et al.*, 1995; Moore *et al.*, 2001). The increased order near the head group can be attributed to the four tryptophan residues at each side of membrane-water interface. The bulky tryptophan side chains served as anchors to stabilize the channel in the membrane, and in doing so, they might at same time produce similar effects to what cholesterol do to rigidify the head group of the lipid. Previous NMR experiment (Rice and Oldfield, 1979) indicated the same possibilities. Because the effect of HFE on the lipid dynamics is essentially non-existent, the variations in the boundary lipid order parameters by HFE were smaller than error bars, suggesting that the presence of about 5% mole fraction of HFE in lipid bilayer has essentially no impact on the lipid acyl chain conformations. A similar result was also obtained from MD simulations (Koubi *et al.*, 2002) of DMPC membrane with

mole fraction of HFE up to 25%, indicating the extreme insensitivity of the lipid acyl chain conformations to HFE.

Using a well-defined thermodynamic argument on the basis of membrane lateral pressure, Cantor investigated the lipid-mediated anesthetic effects on ion channels (Cantor, 1997) and predicted that incorporation of amphiphilic and other interfacially active solutes into the bilayer would selectively increase the lateral pressure near the aqueous interfaces, thereby shifting the conformational equilibrium of a multi-domain channel protein to favor closed state. He also predicted that perfluorocarbons having low accessibility to the interface would not be able to increase the membrane lateral pressure at the interface, but rather cause a gradual change in lateral pressure profile. Our simulation results are consistent with this prediction and show that no HFE effects can be found on the ordering (or stiffness) of the lipid head and tail groups.

Finally, it should be noted that the total simulation time in the present study is almost 4 times longer than our previous simulation with halothane. For the properties that can be assessed on the basis of a few nanosecond simulations, 3-ns and 8-ns seem to make no significant difference. Indeed, the same conclusions can be drawn based only on the first 3-ns of simulation in the current study. Availability of more computing power than merely 2 years ago allowed us to confirm this assertion by extending the current simulation to 8 ns. Neither 2.2 ns simulation nor 8 ns simulation is long enough to study some other interesting properties (e.g., anesthetic effects on ion permeation) that are not attempted in the current study.

CONCLUSIONS

Similar to anesthetic halothane (Tang *et al.*, 2000b), non-anesthetic HFE molecules disturbed neither secondary nor tertiary structure of gA channel in the duration of 8-ns simulations. Combining the information obtained in this study with the knowledge acquired

before (Bhattacharya *et al.*, 2000; Tang *et al.*, 2000a; Tang *et al.*, 1999b), it can be concluded that low affinity agents, such as volatile anesthetics, are likely to exert their action on proteins without strong perturbation to the protein structures.

The distribution of HFE along membrane normal at the end of 8-ns simulation clearly shows higher occurrence of HFE toward the tail region of lipids. In contrast, most of halothane molecules moved to the lipid-water interface after 2-ns simulations (Tang and Xu, 2002). Experimentally, we have compared the distributions of anesthetic-nonanesthetic pairs within the membrane (Tang *et al.*, 1997), their interactions with gA channels (Tang *et al.*, 2000a; Tang *et al.*, 1999a), and their effects on the channel structure (Tang *et al.*, 2002; Tang *et al.*, 1999b) and on channel function as measured by the unidirectional Na⁺ permeation rates (Tang *et al.*, 1999a). The same tendency in membrane distribution and in different interactions with the anchoring residues found in this study as in the previous experiments suggests the high likelihood that halothane and HFE will affect the channel function differently. The lack of HFE at the lipid-water interface precludes HFE from interacting with the channel-anchoring tryptophans or disrupting the hydrogen bonds between tryptophans and the surrounding lipids. Consequently, the channel dynamics was not affected by the presence of HFE. The immediate implication of the finding is that the dynamics changes of gA channel in our previous study in the presence of halothane (Tang and Xu, 2002) are due specifically to the anesthetic interaction with the anchoring residues at the membrane-water interface. Although both halothane and HFE have significant distribution in the lipid tail region near the channel segments deeply embedded in the lipid core, the local non-specific perturbation is not sufficient to account for the dynamics changes in the middle segments of the gA channel seen in the presence of halothane but not seen in the presence of HFE. Thus, the discriminating property that differentiates the anesthetic effects from the nonanesthetic effects in our previous and the current simulations is the ability to

modulate the global, as oppose to local, dynamics of the channel proteins. This realization may prove to be crucial for a better understanding of the action of a wide variety of low-affinity drugs on proteins.

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REFERENCES

- Auerbach A, Akk G. 1998. Desensitization of mouse nicotinic acetylcholine receptor channels. A two-gate mechanism. *J Gen Physiol* 112: 181-197.
- Bhattacharya AA, Curry S, Franks NP. 2000. Binding of the general anesthetics propofol and halothane to human serum albumin. High resolution crystal structures. *J. Biol. Chem.* 275: 38731-38738.
- Boden N, Jones SA, Sixl F. 1991. On the use of deuterium nuclear magnetic resonance as a probe of chain packing in lipid bilayers. *Biochemistry* 30: 2146-2155.
- Bourgeois D, Vallone B, Schotte F, Arcovito A, Miele AE, Sciara G, Wulff M, Anfinrud P, Brunori M. 2003. Complex landscape of protein structural dynamics unveiled by nanosecond Laue crystallography. *Proc. Natl. Acad. Sci. U. S. A.* 100: 8704-8709.
- Brooks BR, Brucoleri RE, Olafson BD, States DJ, Swaminathan S, Karplus M. 1983. CHARMM: A program for macromolecular energy, minimization and dynamics calculations. *J. Comput. Chem.* 4: 187-217.
- Brünger AT. 1992. X-PLOR, Version 3.1 : a system for X-ray crystallography and NMR. New Haven: Yale University Press.
- Campagna JA, Miller KW, Forman SA. 2003. Mechanisms of actions of inhaled anesthetics. *N. Engl. J. Med.* 348: 2110-2124.
- Cantor RS. 1997. The lateral pressure profile in membranes: a physical mechanism of general anesthesia. *Biochemistry* 36: 2339-2344.
- Case DA, Karplus M. 1979. Dynamics of ligand binding to heme proteins. *J Mol Biol* 132: 343-368.
- Darden T, York D, Pedersen L. 1993. Particle Mesh Ewald - an N.Log(N) Method for Ewald Sums in Large Systems. *J. Chem. Phys.* 98: 10089-10092.

- Douliez JP, Leonard A, Dufourc EJ. 1995. Restatement of order parameters in biomembranes: calculation of C-C bond order parameters from C-D quadrupolar splittings. *Biophys. J.* 68: 1727-1739.
- Feller SE, Zhang YH, Pastor RW, Brooks BR. 1995. Constant-Pressure Molecular-Dynamics Simulation - the Langevin Piston Method. *J. Chem. Phys.* 103: 4613-4621.
- Forman SA, Miller KW, Yellen G. 1995. A discrete site for general anesthetics on a postsynaptic receptor. *Mol. Pharmacol.* 48: 574-581.
- Franks NP, Lieb WR. 1994. Molecular and Cellular Mechanisms of General-Anesthesia. *Nature* 367: 607-614.
- Frauenfelder H, McMahon BH, Fenimore PW. 2003. Myoglobin: the hydrogen atom of biology and a paradigm of complexity. *Proc Natl Acad Sci U S A* 100: 8615-8617.
- Hoover WG. 1985. Canonical dynamics: Equilibrium phase-space distributions. *Phys. Rev. A* 31: 1695.
- Hu W, Lee KC, Cross TA. 1993. Tryptophans in membrane proteins: indole ring orientations and functional implications in the gramicidin channel. *Biochemistry* 32: 7035-7047.
- Huang P, Perez JJ, Loew GH. 1994. Molecular dynamics simulations of phospholipid bilayers. *J. Biomol. Struct. Dyn.* 11: 927-956.
- Humphrey W, Dalke A, Schulten K. 1996. VMD: Visual molecular dynamics. *J. Mol. Graphics.* 14: 33-38.
- Jenkins A, Greenblatt EP, Faulkner HJ, Bertaccini E, Light A, Lin A, Andreasen A, Viner A, Trudell JR, Harrison NL. 2001. Evidence for a common binding cavity for three general anesthetics within the GABAA receptor. *J. Neurosci.* 21: RC136.
- Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 79: 926-935.

- Kale L, Skeel R, Bhandarkar M, Brunner R, Gursoy A, Krawetz N, Phillips J, Shinozaki A, Varadarajan K, Schulten K. 1999. NAMD2: Greater scalability for parallel molecular dynamics. *J. Comput. Phys.* 151: 283-312.
- Karlin A. 2002. Emerging structure of the nicotinic acetylcholine receptors. *Nat Rev Neurosci* 3: 102-114.
- Kendrew JC, Bodo G, Dintzis HM, Parrish RG, Wyckoff H, Phillips DC. 1958. A three-dimensional model of the myoglobin molecule obtained by x-ray analysis. *Nature* 181: 662-666.
- Ketchum R, Roux B, Cross T. 1997. High-resolution polypeptide structure in a lamellar phase lipid environment from solid state NMR derived orientational constraints. *Structure* 5: 1655-1669.
- Koubi L, Tarek M, Bandyopadhyay S, Klein ML, Scharf D. 2001. Membrane structural perturbations caused by anesthetics and nonimmobilizers: a molecular dynamics investigation. *Biophys. J.* 81: 3339-3345.
- Koubi L, Tarek M, Bandyopadhyay S, Klein ML, Scharf D. 2002. Effects of the nonimmobilizer hexafluoroethane on the model membrane dimyristoylphosphatidylcholine. *Anesthesiology* 97: 848-855.
- Koubi L, Tarek M, Klein ML, Scharf D. 2000. Distribution of halothane in a dipalmitoylphosphatidylcholine bilayer from molecular dynamics calculations. *Biophys. J.* 78: 800-811.
- Liu ZW, Xu Y, Saladino AC, Wymore T, Tang P. 2004. Parametrization of 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane) and hexafluoroethane for nonbonded interactions. *J. Phys. Chem. A* 108: 781-786.

- MacKerell AD, Bashford D, Bellott M, Dunbrack RL, Evanseck JD, Field MJ, Fischer S, Gao J, Guo H, Ha S, Joseph-McCarthy D, Kuchnir L, Kuczera K, Lau FTK, Mattos C, Michnick S, Ngo T, Nguyen DT, Prodhom B, Reiher WE, Roux B, Schlenkrich M, Smith JC, Stote R, Straub J, Watanabe M, Wiorkiewicz-Kuczera J, Yin D, Karplus M. 1998. All-atom empirical potential for molecular modeling and dynamics studies of proteins. *J. Phys. Chem. B.* 102: 3586-3616.
- Martyna GJ, Tobias DJ, Klein ML. 1994. Constant-Pressure Molecular-Dynamics Algorithms. *J. Chem. Phys.* 101: 4177-4189.
- Mascia MP, Trudell JR, Harris RA. 2000. Specific binding sites for alcohols and anesthetics on ligand-gated ion channels. *Proc. Natl. Acad. Sci. U.S.A.* 97: 9305-9310.
- Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, Harris RA, Harrison NL. 1997. Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature* 389: 385-389.
- Moore PB, Lopez CF, Klein ML. 2001. Dynamical properties of a hydrated lipid bilayer from a multianosecond molecular dynamics simulation. *Biophys. J.* 81: 2484-2494.
- Neubig RR, Boyd ND, Cohen JB. 1982. Conformations of Torpedo acetylcholine receptor associated with ion transport and desensitization. *Biochemistry* 21: 3460-3467.
- North C, Cafiso DS. 1997. Contrasting membrane localization and behavior of halogenated cyclobutanes that follow or violate the Meyer-Overton hypothesis of general anesthetic potency. *Biophys. J.* 72: 1754-1761.
- Nose S. 1984. A unified formulation of the constant-temperature molecular-dynamics methods. *J. Chem. Phys.* 81: 511-519.
- Perutz MF. 1979. Regulation of oxygen affinity of hemoglobin: influence of structure of the globin on the heme iron. *Annu. Rev. Biochem.* 48: 327-386.

- Perutz MF, Mathews FS. 1966. An x-ray study of azide methaemoglobin. *J Mol Biol* 21: 199-202.
- Pratt MB, Husain SS, Miller KW, Cohen JB. 2000. Identification of sites of incorporation in the nicotinic acetylcholine receptor of a photoactivatable general anesthetic. *J. Biol. Chem.* 275: 29441-29451.
- Rice D, Oldfield E. 1979. Deuterium nuclear magnetic resonance studies of the interaction between dimyristoylphosphatidylcholine and gramicidin A'. *Biochemistry* 18: 3272-3279.
- Srajer V, Ren Z, Teng TY, Schmidt M, Ursby T, Bourgeois D, Pradervand C, Schildkamp W, Wulff M, Moffat K. 2001. Protein conformational relaxation and ligand migration in myoglobin: a nanosecond to millisecond molecular movie from time-resolved Laue X-ray diffraction. *Biochemistry* 40: 13802-13815.
- Tang P, Eckenhoff RG, Xu Y. 2000a. General anesthetic binding to gramicidin A: The structural requirements. *Biophys. J.* 78: 1804-1809.
- Tang P, Hu J, Liachenko S, Xu Y. 1999a. Distinctly different interactions of anesthetic and nonimmobilizer with transmembrane channel peptides. *Biophys. J.* 77: 739-746.
- Tang P, Mandal PK, Zegarra M. 2002. Effects of volatile anesthetic on channel structure of gramicidin a. *Biophys. J.* 83: 1413-1420.
- Tang P, Simplaceanu V, Xu Y. 1999b. Structural consequences of anesthetic and nonimmobilizer interaction with gramicidin A channels. *Biophys. J.* 76: 2346-2350.
- Tang P, Xu Y. 2002. Large-scale molecular dynamics simulations of general anesthetic effects on the ion channel in the fully hydrated membrane: the implication of molecular mechanisms of general anesthesia. *Proc. Natl. Acad. Sci.U.S.A.* 99: 16035-16040.
- Tang P, Yan B, Xu Y. 1997. Different distribution of fluorinated anesthetics and nonanesthetics in model membrane: a ¹⁹F NMR study. *Biophys. J.* 72: 1676-1682.

- Tang YZ, Chen WZ, Wang CX. 2000b. Molecular dynamics simulations of the gramicidin A-dimyristoylphosphatidylcholine system with an ion in the channel pore region. *Eur. Biophys. J.* 29: 523-534.
- Tu K, Tarek M, Klein ML, Scharf D. 1998. Effects of anesthetics on the structure of a phospholipid bilayer: molecular dynamics investigation of halothane in the hydrated liquid crystal phase of dipalmitoylphosphatidylcholine. *Biophys. J.* 75: 2123-2134.
- Van Gunsteren WF, Berendsen HJC. 1977. Algorithms for macromolecular dynamics and constraint dynamics. *Mol. Phys.* 34: 1311-1327.
- Zubrzycki IZ, Xu Y, Madrid M, Tang P. 2000. Molecular dynamics simulations of a fully hydrated dimyristoylphosphatidylcholine membrane in liquid-crystalline phase. *J. Chem. Phys.* 112: 3437-3441.

FIGURE LEGENDS

Fig. 1. (A) HFE trajectories over the 8-ns all-atom simulations in a fully hydrated membrane patch consisting of one gramicidin A channel (cyan ribbon along with anchoring tryptophan side chains), 182 DMPC lipids (gold spheres marking the phosphorus atoms in the lipid head group), and 5538 water molecules (light purple line segments). The initial positions of HFE were marked by HFE in CPK representation. Notice the preference of HFE for the alkyl tail region of the lipids. (B) The positions of HFE along the membrane normal (z -axis) are plotted over the course of the simulations. Averaged positions of lipid phosphorus atoms of each leaflet at each time point were also shown to mark the lipid-water interface.

Fig. 2. HFE has minimal effects on the structure of gramicidin A channel, as revealed by the small and nearly identical root-mean-square deviations (RMSD) of the backbone C_{α} atoms in the presence and the absence of HFE.

Fig. 3. Comparison of root-mean-square fluctuations (RMSF) for the C_{α} carbons along the gramicidin A channel in the presence and the absence of HFE. Possible accumulative drift of the entire membrane system was removed by fitting the center of mass of all simulation frames to a common point of reference. The similar RMSF profile in the two simulations suggests that HFE has little effects on the channel global dynamics on the nanosecond time scale.

Fig. 4. Comparison of the autocorrelation functions of the backbone N–H bond orientation in the presence and the absence of HFE. For display clarity, the autocorrelation functions are pooled and averaged separately for inner residues (residues 1-8, a, without HFE; and b, with HFE) and outer anchoring tryptophan residues along with the spacer leucine residues (residues 9-15, c, without HFE; and d, with HFE).

Fig. 5. The order parameters of lipid alkyl chain sn1 (A) and sn2 (B) averaged over 27 lipid molecules in the immediate vicinity of the gramicidin A channel in the absence (○) and presence (●) of HFE.

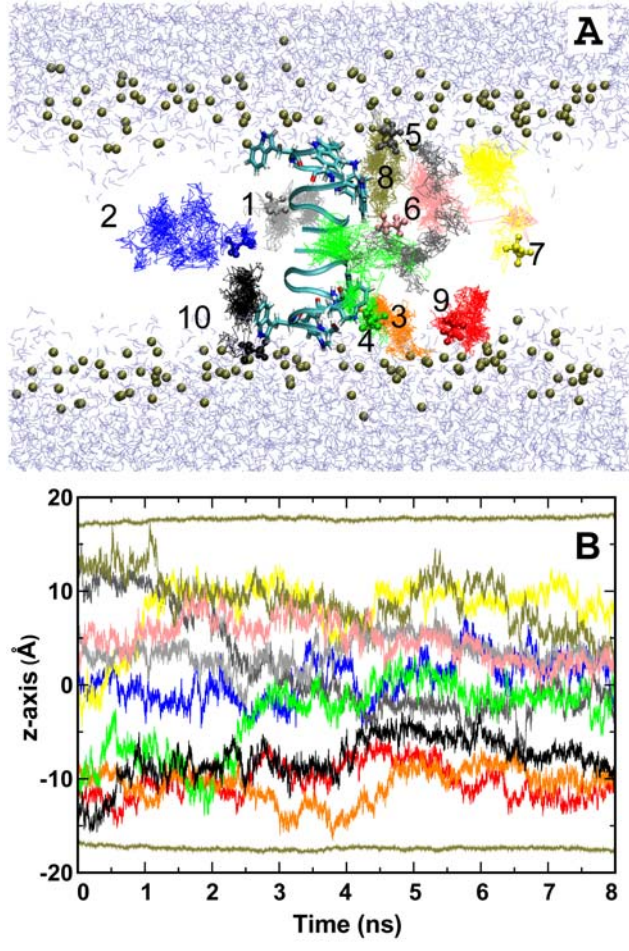


Figure 1

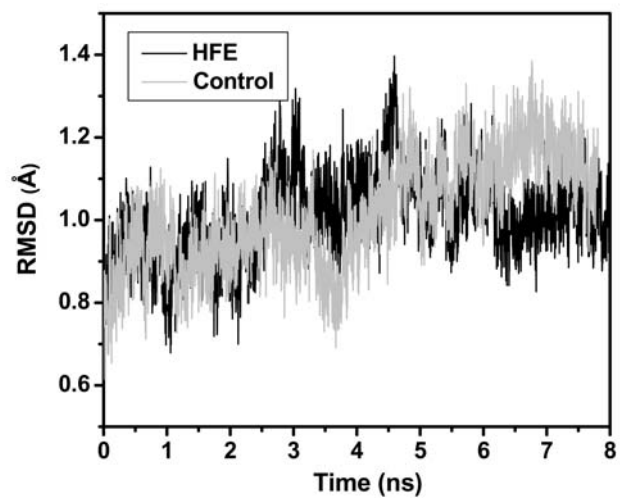


Figure 2

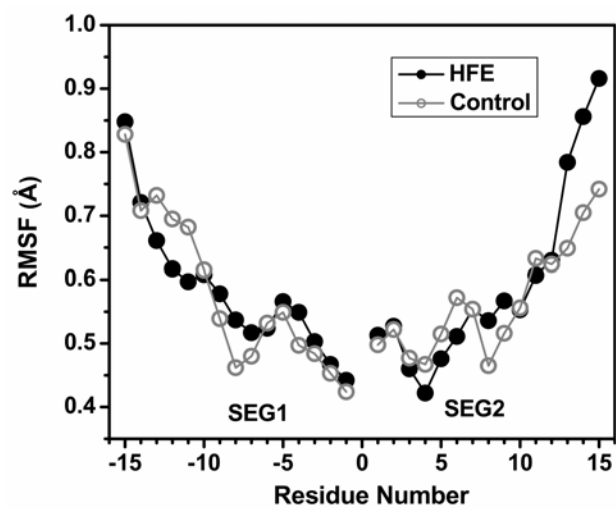


Figure 3

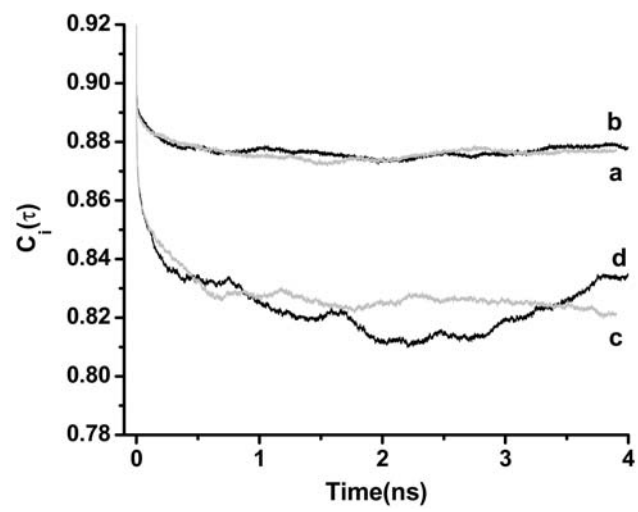


Figure 4

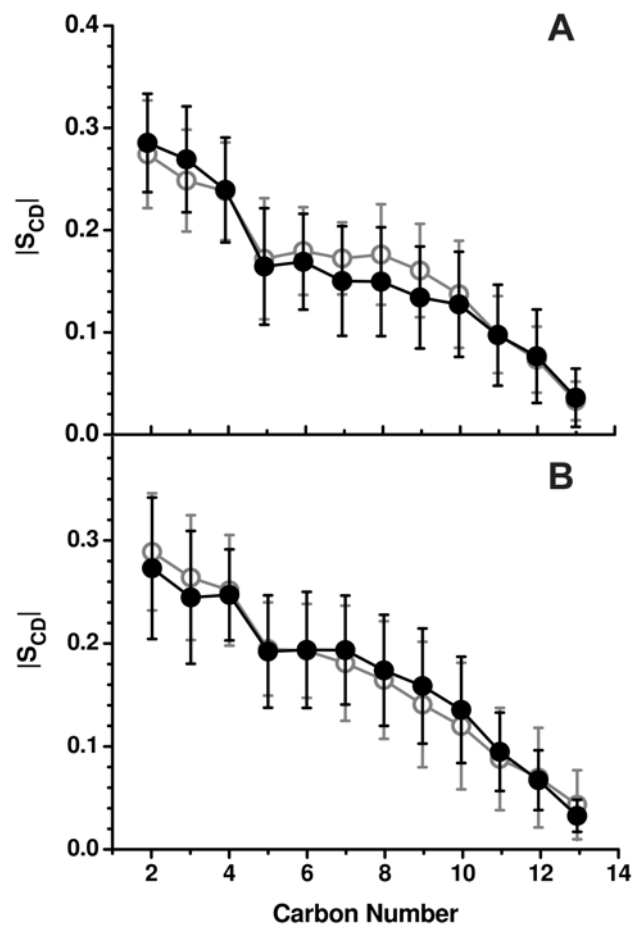


Figure 5