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Nonanesthetics (Nonimmobilizers) and Anesthetics Display Different Microenvironment Preferences

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THE sites of action of the volatile general anesthetics remain to be determined conclusively, despite extensive research over a number of decades.[1] Nevertheless, current consensus favors membrane proteins that function as ion channels and neurotransmitter receptors as the likely targets,[2] despite limited direct evidence for this. Indirect support comes from studies showing that volatile general anesthetics alter the *activity* of a number of membrane proteins, such as voltage- and ligand-gated ion channels.[3,4]

The Meyer-Overton rule has played a central role in general anesthetic mechanisms research.[5] This relation shows that the olive oil:gas partition coefficients of anesthetic molecules correlate positively with their potency in animals. By extension then, definition of a compound's lipid solubility should allow a prediction to be made regarding anesthetic potency. Recently, a number of compounds have been described that are predicted to be potent anesthetics based on lipid-solubility criteria and their overall molecular configuration but fail to display full anesthetic effects (both immobility and amnesia) in animals.[6] These highly lipophilic compounds have been termed nonanesthetics, or nonimmobilizers, because they may have amnestic properties.[7,8]

In a previous study,[9] it was shown that four volatile general anesthetics (halothane, isoflurane, enflurane, and sevoflurane) interacted better with solvents displaying some polar characteristic (aromatic, alcohol, thiol, or sulfide), compared with the purely aliphatic solvent *n*-hexane. Herein, we report that the nonanesthetic molecules 2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobutane interact more favorably with *n*-hexane compared with the polar solvents. Data obtained with the anesthetic molecules chloroform and 1-chloro-1,2,2-trifluorocyclobutane are also included. It is clear that using isotropic solvents to model anisotropic structures, such as proteins and lipid membranes, may only provide approximate interaction energies. Nevertheless, the use of octanol as a model for biologic membranes has been successfully and extensively used in medicinal chemistry, providing a framework for detailed quantitative structure–activity relations.[10] The results of the current study suggest that nonanesthetic molecules are likely to occupy microenvironments in biologic membranes that differ from those favored by anesthetic molecules. The different microenvironment preferences of the anesthetics and the nonanesthetics are likely to account for their contrasting pharmacologic effects on intact animals.

Materials and Methods

Materials

The compounds 2,3-dichlorooctafluorobutane (F8), 1-chloro-1,2,2-trifluorocyclobutane (F3), and 1,2-dichlorohexafluorocyclobutane (F6) were purchased from PCR Incorporated (Gainesville, FL). Chloroform (99.8%), benzene (99.8%), and ethyl methyl sulfide (99%) were from Aldrich Chemical Co. (Milwaukee, WI), and *n*-hexane (99+%, capillary gas chromatography grade) was from Sigma Chemical Co. (St. Louis, MO). Methanol (99.9+%, high-pressure liquid chromatography grade) was obtained from Fisher Scientific (Fairlane, NJ).

Determination of Solvent: Gas Partition Coefficients and Transfer Free Energies

Partition coefficients and transfer free energies of the anesthetics and nonanesthetics between the gas and solvent phases were determined as described.[9]

Statistics and Linear Regression

Partition coefficients were determined between four and six times for each individual anesthetic–solvent and nonanesthetic–solvent combination. Data are expressed as mean ± SD.

Results

The partition coefficients for chloroform, 1-chloro-1,2,2-trifluorocyclobutane, 2,3-dichlorooctafluorobutane, and 1,2-dichlorohexafluorocyclobutane, between the gas phase and the four organic solvents, are given in table 1. For the two anesthetics (chloroform and 1-chloro-1,2,2-trifluorocyclobutane), partitioning was found to be more favorable into the solvents that either have aromatic character or contain an alcohol group or a sulfide group, compared with the aliphatic solvent *n*-hexane. In contrast, the two nonanesthetic molecules (2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobu-

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Table 1. Solvent:Gas Partition Coefficients for the Four Halogenated Alkanes for Four Different Solvents

Solvent	Chloroform	1-Chloro-1,2,2-trifluorocyclobutane	2,3-Dichloroocta-fluorobutane	1,2-Dichlorohexa-fluorocyclobutane
n-Hexane	448 ± 13 (n = 6)	335 ± 4 (n = 4)	221 ± 7 (n = 4)	301 ± 2 (n = 5)
Benzene	1,055 ± 31 (n = 5)	1,556 ± 19 (n = 5)	126 ± 4 (n = 4)	193 ± 2 (n = 5)
Methanol	851 ± 37 (n = 5)	907 ± 5 (n = 4)	71 ± 2 (n = 5)	105 ± 2 (n = 4)
Ethyl methyl sulfide	1,085 ± 105 (n = 5)	966 ± 19 (n = 4)	135 ± 3 (n = 4)	199 ± 2 (n = 5)

For each case, the partition coefficient is given followed by n, the number of experiments. The errors are SD.

tane) preferred to interact with *n*-hexane rather than with the somewhat polar solvents.

Compared with an aliphatic environment (*n*-hexane), the presence of an aromatic group, an alcohol group, or a sulfide group improved solvent:gas partitioning, by factors of 1.9–2.4 for chloroform and 2.7–4.6 for 1-chloro-1,2,2-trifluorocyclobutane. As shown in figure 1A, the most favorable environments for chloroform were ethyl methyl sulfide, a model for methionine, and benzene, a model for the aromatic amino acid side-chains. Figure 1B shows that 1-chloro-1,2,2-trifluorocyclobutane partitioned to the greatest extent into benzene, a model for the aromatic amino acid side-chains. Figures 2A and B show that the two nonanesthetic molecules 1,2-dichlorohexafluorocyclobutane and 2,3-dichlorooctafluorobutane partitioned most favorably into *n*-hexane, a model for the aliphatic amino acid side-chains of alanine, valine, leucine, and isoleucine.

The overall free energy of solvation of these halogenated alkanes can be divided into two components.[11] The first is related to the energy required to form a cavity in the solvent and is proportional to the volume of the solute. The second term is a favorable energy term associated with solute–solvent interactions. Molecular volumes for the four halogenated alkanes examined in this study are listed in table 2. Figures 3 and 4 show plots of the free energy of solvation *versus* the volume of the solvated molecule for the four halogenated alkanes examined in the current study, along with data for four additional anesthetic molecules (halothane, isoflurane, enflurane, and sevoflurane) taken from an earlier report.[9]

For benzene (fig. 3A) and ethyl methyl sulfide (fig. 3B), the overall free energy of solvation correlates with the molecular volume of the eight solutes. For methanol (fig. 4A), the correlation between the overall free energy of solvation and the volume of the solvated molecule is not quite as good (correlation coefficient, r = 0.8192), and this is probably related to the occurrence of favorable hydrogen bonding between the ether anesthetics (enflurane and isoflurane with molecular volumes of 133 $Å^3$ and sevoflurane with a molecular volume of 142 $Å^3$) and methanol. For *n*-hexane (fig. 4B), the correlation between the overall free energy of solvation and the volume of the solvated molecule is poor (r = 0.4406) unless the anesthetic and the nonanesthetic molecules are separated (r = 0.9298 for the six anesthetics), suggesting that the nonanesthetic molecules 2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobutane form particularly favorable interactions with *n*-hexane.

Discussion

The nonanesthetic molecules 2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobutane do not influence γ-aminobutyric acid type A $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_{2s}$ receptor currents in *Xenopus* oocytes, whereas the structurally related 1-chloro-1,2,2-trifluorocyclobutane potentiates chloride currents in the same manner as other anesthetic molecules.[12] Similarly, current con-

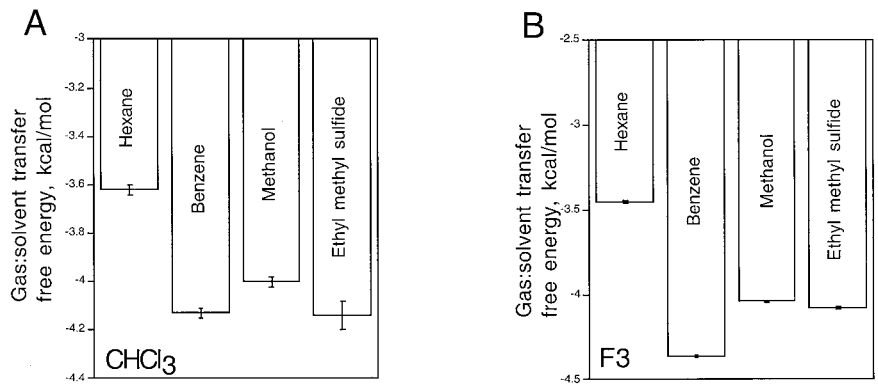

Fig. 1. Transfer free energies for (*A*) chloroform and (*B*) 1-chloro-1,2,2-trifluorocyclobutane between the gas and four solvent phases that model various microenvironments present in biologic membranes. Error bars are SD. The transfer free energies of the anesthetics between the gas and solvent phases were calculated as $\Delta G_{g\rightarrow s} = -RT\ln(C_s/C_g)$, where R is the gas constant (1.987 cal/mol K), T is the temperature in kelvins, and C_s and C_g are the solvent and gas phase concentrations of the anesthetic molecules at equilibrium.

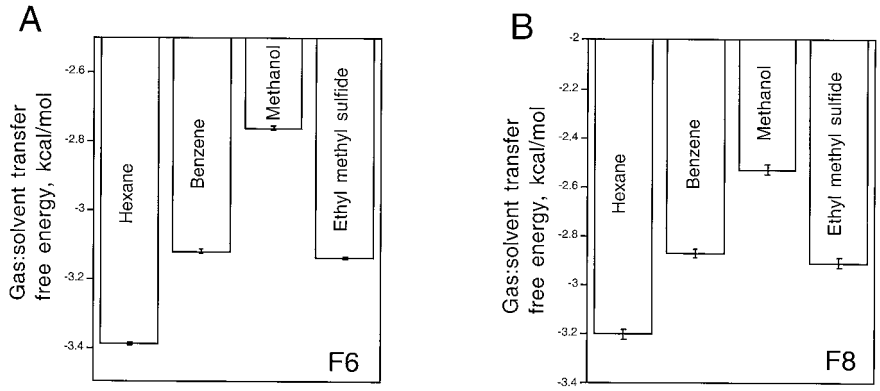

Fig. 2. Transfer free energies for (*A*) 1,2-dichlorohexafluorocyclobutane and (*B*) 2,3-dichlorooctafluorobutane between the gas and four solvent phases that model various microenvironments present in biologic membranes. Error bars are SD. The transfer free energies of the nonanesthetics were calculated as defined for the anesthetics in the legend to figure 1.

ducted through human neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes is inhibited by isoflurane and 1-chloro-1,2,2-trifluorocyclobutane but is insensitive to the nonanesthetics 2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobutane.[13] These studies suggest that ligand-gated ion channels may represent central nervous system sites contributing to the immobility component of anesthesia. In contrast, mouse skeletal muscle nicotinic acetylcholine receptor currents are inhibited by both enflurane and the nonanesthetics 2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobutane, although the kinetics of inhibition differ, indicating that the two classes of halogenated compounds may favor different conformations of the protein.[14] Binding studies using human serum albumin[14] indicate that 2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobutane bind to the same overall site as halothane and chloroform,[15–17] suggesting that this site in subdomain IIA may serve as a better model of an *in vivo* target responsible for the amnestic component of the anesthetic state.

A ^{19}F nuclear magnetic resonance study on the distribution of 1-chloro-1,2,2-trifluorocyclobutane, 2,3-dichlorooctafluorobutane, and 1,2-dichlorohexafluorocyclobutane in egg phosphatidylcholine lipid vesicles is in accordance with the current findings. The anesthetic molecule 1-chloro-1,2,2-trifluorocyclobutane was found to preferentially localize at the membrane–water interface, whereas the nonanesthetic molecules 2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobutane solubilized more deeply in the lipid core.[18] Also using ^{2}H and ^{19}F nuclear magnetic resonance in palmitoyl oleoylphosphatidylcholine membranes, 1-chloro-1,2,2-trifluorocyclobutane was shown to localize at the membrane interface, whereas the nonanesthetic 1,2-dichlorohexafluorocyclobutane was distributed evenly in the hydrocarbon region of the lipid acyl chains.[19]

Table 2. Volumes of Six Anesthetics and Two Nonanesthetics

Halogenated Alkane or Ether	Volume (Å^3)
Chloroform	102
Halothane	123
Enflurane	133
Isoflurane	133
Sevoflurane	142
1-Chloro-1,2,2-trifluorocyclobutane	123
1,2-Dichlorohexafluorocyclobutane	151
2,3-Dichlorooctafluorobutane	176

Volumes calculated using the approach outlined by Abraham and McGowan.[20]

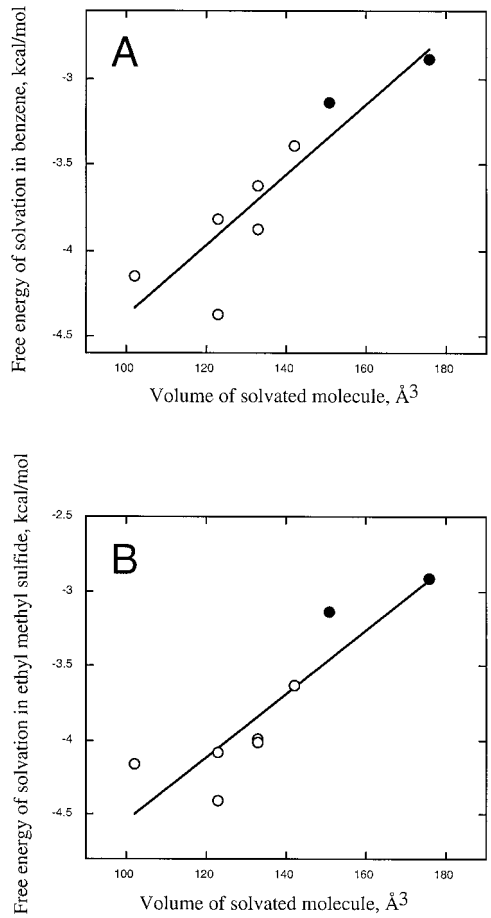

Fig. 3. Plots of the overall free energy of solvation into (*A*) benzene and (*B*) ethyl methyl sulfide for six anesthetic and two nonanesthetic molecules (from table 2) as a function of molecular volume. Linear regression least squares fits were generated using KaleidaGraph version 3.0.5 (Abelbeck Software, Reading, PA, 1994). The correlation coefficients (r) are (*A*) 0.8933 and (*B*) 0.8920. The nonanesthetic data are the filled circles.

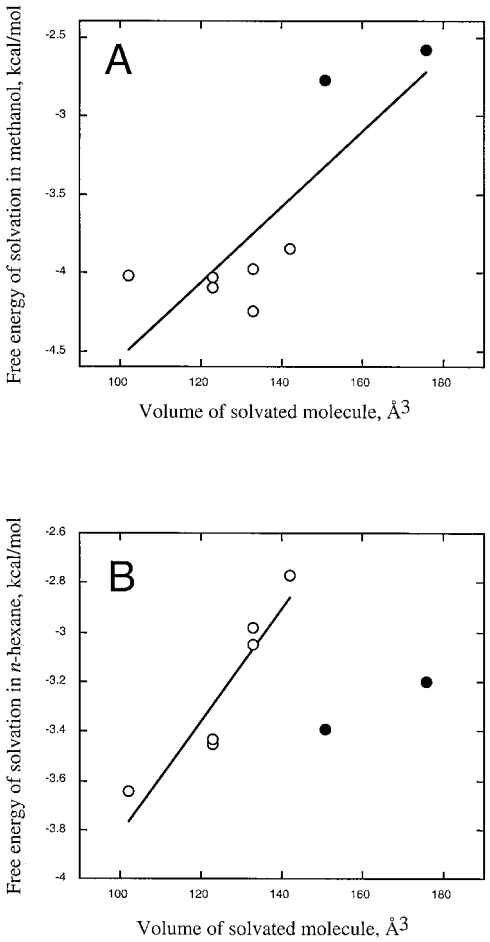

Fig. 4. Plots of the overall free energy of solvation into (*A*) methanol and (*B*) *n*-hexane for six anesthetic and two nonanesthetic molecules (from table 2) as a function of molecular volume. Linear regression least squares fits were generated using KaleidaGraph version 3.0.5 (Abelbeck Software, Reading, PA, 1994). The correlation coefficients (r) are (*A*) 0.8192, and (*B*) 0.9298 (if anesthetics and nonanesthetics are separated) *versus* 0.4406 for the entire data set (for *B* only). The nonanesthetic data are the filled circles.

The current results suggest that the nonanesthetic molecules 2,3-dichlorooctafluorobutane and 1,2-dichlorohexafluorocyclobutane interact preferentially with the purely aliphatic phase represented by *n*-hexane. This solvent serves as a model for the aliphatic amino acid side-chains of alanine, valine, leucine, and isoleucine and also for the saturated portions of the phospholipid acyl chains.[9] In contrast, the anesthetics chloroform and 1-chloro-1,2,2-trifluorocyclobutane interact more favorably with the somewhat polar solvents benzene, methanol, and ethyl methyl sulfide, which serve as models for the aromatic amino acids, serine, and methionine, respectively, in agreement with the earlier study on halothane, enflurane, isoflurane, and sevoflurane.[9] Anesthetic molecules are therefore predicted to favor several microenvironments present in proteins because of the potential for energetically more favorable interactions. In contrast, nonanesthetic molecules are predicted to prefer more apolar environments on proteins or the saturated portion of the lipid bilayer. Finally, solvation studies of this type may allow differentiation of nonanesthetic and anesthetic molecules without the necessity of animal studies.

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