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Molecular orientation of volatile anesthetics at the binding surface: ^{1}H- and ^{19}F-NMR studies of submolecular affinity

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The shift of ^{1}H- and ^{19}F-NMR peaks in the frequency domain was used to resolve the solubilization of volatile anesthetics into sodium dodecylsulfate micelles to submolecular level. Enflurane has protons at both ends of the molecule, and the solubilization parameters (partition coefficients in a broad sense) of each end were estimated by ^{1}H-NMR. The values were: 2130 for the hydrophobic end and 1980 for the hydrophilic end. The hydrophobic end of halothane is CF_3, hence ^{19}F-NMR was used: 4330 for the hydrophobic end and 2670 for the hydrophilic end. The ratios of the solubilization parameters between hydrophobic and hydrophilic ends were methoxyflurane 1.9 (Kaneshina et al. (1981) Biochim. Biophys. Acta 647, 223–226), enflurane 1.1, and halothane 1.6. The results indicate that methoxyflurane and halothane adsorb perpendicular to the membrane surface, whereas enflurane molecules stay parallel to the interface. The averaged solubilization parameters of both ends of these anesthetics were in good agreement with their conventional partition coefficients between dipalmitoylphosphatidylcholine (DPPC) membranes and water. The solubilization parameter of chloroform (^{1}H-NMR) was 1580 in agreement with the reported values of DPPC-water partition coefficient.

When anesthetics are transferred from aqueous phase to hydrophobic domain, the NMR signal peaks in the frequency domain are shifted to upfield according to the change in the magnetic environment. From the magnitude of the displacement of the signals, the solubilization parameters (often designated as partition coefficient) of the submolecular parts of anesthetics can be estimated separately.

A characteristic of volatile anesthetics, currently in clinical use, is the presence of acidic protons in their halogenated (mainly fluorinated) hydrophobic molecular structure. Because the acidic proton part is hydrophilic, these anesthetic molecules are amphiphilic and tend to stay at the macromolecule/water interfaces. The interfacial preference of volatile anesthetics for their interaction site has been demonstrated by ^{19}F-NMR chemical shift change during halothane solubilization into sodium dodecylsulfate micelles [1], and by two-dimensional nuclear Overhauser effect of proton NMR on methoxyflurane interaction with dipalmitoylphosphatidylcholine vesicle membranes [2].

When these dipolar molecules are bound to the interface, the hydrophilic and hydrophobic parts may not interact with lipid structures identically. From the difference in the solubilization parameters of each end of the rod-like methoxyflurane molecule estimated by ^{1}H-NMR, Kaneshina et al. [3] demonstrated that the hydrophobic end of the anesthetic penetrated into the hydrophobic core of surfactant micelles while the hydrophilic end did not lose contact with the aqueous phase. The present study extended the above work to halothane, enflurane and chloroform.

Anesthetic interaction with membranes is generally expressed by the term 'membrane/buffer partition coefficient'. Because partition implies distribution of a drug between two homogeneous phases, partition coefficient is improper to describe interaction of amphiphilic volatile anesthetics with strongly structured membranes or micelles, which are pseudophases. The numerical values obtained in the present study may better be designated as affinity or solubilization parameter. Nevertheless, 'partition coefficient' is a convenient term

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because of the familiarity of the traditional expression, and its liberal use is not uncommon.

Sodium dodecylsulfate (SDS) was obtained from Nakarai Chemical (Kyoto) and was twice recrystallized from acetone/water (95 : 5, v/v) mixture. Halothane (Takeda Co., Osaka, Japan), enflurane (Dainabot, Tokyo), and chloroform (Nakarai Chemical) were passed through activated aluminum oxide columns to remove water. The stabilizer contained in the halothane preparation is removed by this procedure. Heavy water (isotope purity of 99.85%, CEA, France) was used as received.

Anesthetics were added to the SDS-D_2O in a glass ampoule with a microsyringe, and the added amount was confirmed by weighing the ampoule with a chemical balance. The opening of the ampoule was flame-closed and the content was mixed by shaking in a waterbath maintained at 30.0 ± 0.5 °C for 48 h. After equilibration, the sample was transferred into a 5 mm internal diameter NMR tube and was closed air-tight by a Teflon cap.

^{19}F-NMR spectra were obtained by a Hitachi R-20B spectrometer with an A-1600A signal averaging analyzer (Hitachi Ltd., Tokyo) at 30 °C and 2 ppm sweep width. Trichlorofluoromethane (Tokyo Chemical, Tokyo) was used as the external standard. ^{1}H-NMR spectra were obtained by Varian XL-200 NMR spectrometer with a spectral width of 2600 Hz, 15 600 points in the time domain and acquisition time of 3 s. The ^{1}H chemical shifts were referenced to the proton peak of HDO in the heavy water used as the solvent.

Enflurane has two protons at both end of the rod-like molecule. In D_2O solution, the two proton peaks of enflurane ($HFClC \cdot CF_2 \cdot O \cdot CF_2H$) were observed at 6.971 and 7.427 ppm, representing the HFClC and CF_2H protons, respectively [4]. Because the bound anesthetic molecules are exchanging with the free anesthetic molecules in the aqueous phase faster than the NMR time scale, the peak position is the weighted average of these positions.

From the magnitude of displacement of proton signals, Kaneshina et al. [3] assigned solubilization parameter to each signal as follows.

$$\frac{1}{\Delta\delta} = \frac{1}{\delta_M - \delta_W}\left(1 + \frac{50 A_W}{A_M}\left(\frac{1}{C - C_0}\right)\right) \qquad (1)$$

where $\Delta\delta$ is the displacement of the peak position relative to the aqueous solution, δ_M and δ_W are the peak position in the micelle and water, respectively, A_M is the saturating amount of the anesthetic solubilized in the micelle, A_W is the anesthetic solubility in water, C is the surfactant concentration, and C_0 is the critical micelle concentration (8.0 mM at 30 °C [5]). The SDS concentration was varied between 9 and 380 mM. Because D_2O was the aqueous phase, the molarity was taken as 50.

When the reciprocal of $\Delta\delta$ was plotted against the reciprocal of micellar concentrations, a straight line was obtained. From the slope of the plot, solubilization parameter, A_M/A_W, was estimated. A_M/A_W is a ratio of anesthetic concentrations between micelles and water, and is equivalent to partition coefficient in a broad sense as discussed before. Because the distribution of anesthetic molecules is a weighted average of the residence sites at (hydrophobic and in the micelle and hydrophilic end in water) and near (entire anesthetic molecule either in the micelle or in the vicinal water) the micelle/water interface, different values of A_M/A_W are obtained for each end of anesthetic molecules.

Fig. 1 is the double-reciprocal plots between the proton chemical shifts and micellar concentrations on enflurane and chloroform at saturation in various SDS concentrations. The values for enflurane were 2130 for the hydrophobic end and 1980 for the hydrophilic end. The hydrophobic end of halothane ($CHClBr \cdot CF_3$) is CF_3, hence ^{19}F-NMR was used: 4330 for the hydrophobic end and 2670 for the hydrophilic end (not shown). Error estimates in these values were less than

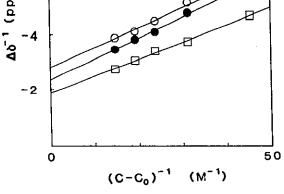

Fig. 1. The plot between the reciprocal of the displacement of the chemical shifts of proton peaks and the reciprocal of the micellar concentration. Enflurane HFClC (○) and CF_2H (●) protons, and chloroform proton (□)

TABLE I

Comparison between the present micelle-water partition coefficient of volatile anesthetics (averaged values of the solubilization parameter of each ends) with the reported values on DPPC membrane-buffer partition coefficients

	Halothane	Enflurane	Methoxyflurane	Chloroform
This study	3500	2055	2086	1580
Ref. 6	3790	–	–	962
Ref. 7	2570	1680	2350	1220
Ref. 8	4000	–	–	–

8%. Our previous data [3] on methoxyflurane showed 2736 for the hydrophobic end and 1435 for the hydrophilic end. The ratio of the partition coefficients between hydrophobic and hydrophilic ends were methoxyflurane 1.9, enflurane 1.1, and halothane 1.6. The results indicate that methoxyflurane and halothane adsorb perpendicular to the membrane surface, whereas enflurane molecules stay parallel to the interface. The averaged partition coefficients of both ends of these anesthetics were in good agreement with their conventional partition coefficients between phospholipid (dipalmitoylphosphatidylcholine, DPPC) membranes and water (Table I) in the literature [6–8]. Chloroform molecule is spherical, hence single partition coefficient was obtained. The value was 1580 and also agreed with the DPPC-water partition coefficient [7].

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