Biochimica et Biophysica Acta, 982 (1989) 300–302
Elsevier

BBAMEM 70611

BBA Report

400 MHz two-dimensional nuclear Overhauser spectroscopy on anesthetic interaction with lipid bilayer

Satoshi Yokono [1,*], Kenji Ogli [1], Shigetoshi Miura [2] and Issaku Ueda [3]

[1] *Department of Anesthesiology and Emergency Medicine,* [2] *Department of Biochemistry, Kagawa Medical School, Ikenobe, Mikicho, Kitagun, Kagawa 761-07 (Japan) and* [3] *Anesthesia Service, Veterans Administration Medical Center, Salt Lake City, UT (U.S.A.)*

(Received 9 December 1988)

Key words: Anesthesia theory; Vesicle membrane; Interface; Two-dimensional nuclear Overhauser effect; Nuclear Overhauser difference spectroscopy; Hydrophyl–hydrophobe balance

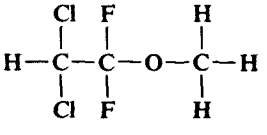
Interaction between a volatile anesthetic, methoxyflurane, and dipalmitoylphosphatidylcholine (DPPC) vesicle membrane was analyzed by nuclear Overhauser effect (NOE) difference spectroscopy and two-dimensional nuclear Overhauser spectroscopy (NOESY). The NOE difference spectra were obtained by selectively irradiating methoxy protons (hydrophobic end) of the anesthetic: a negative nuclear Overhauser effect of −2.94% was observed with the choline methyl protons of DPPC. The NOESY spectra revealed a cross-peak between the anesthetic methoxy protons and the choline methyl protons. A dipole–dipole interaction exists between the hydrophobic end of the anesthetic and the hydrophilic head group of DPPC. No other cross-peaks were observed. The anesthetic orients itself at the membrane/water interface by interacting with the hydrophilic surface of the DPPC membrane, leaving the hydrophilic end of the anesthetic molecule in the aqueous phase. The preferred residence site of dipolar volatile anesthetics is the membrane/water interface.

Anesthetics depress the temperature of order-disorder phase transition of lipid membranes (see, for instance, reviews [1–3]). Because the transition between the solid-gel and liquid-crystal phases is the property expressed by the hydrocarbon-chain conformation of phospholipid molecules, direct interaction between anesthetic molecules and lipid core is often assumed.

Yokono et al. [4] demonstrated, however, by ^{1}H-NMR that anesthetic-induced isothermal phase transition occurred in two stages. At lower anesthetic concentrations, protons at the hydrophilic head mobilized. The acyl proton mobility increased when the anesthetic concentration was increased. In contrast, thermal phase transition was characterized by a simultaneous increase in mobility of head and lipid tail protons. From the chemical shift spectra of ^{1}H-NMR, Kaneshina et al. [5] demonstrated that methoxyflurane solvate at the micellar surface, where hydrophilic end of the anesthetic molecule did not lose contact with the aqueous phase.

The nuclear Overhauser effect (NOE), when combined with difference spectroscopy or two-dimensional approach, provides a powerful method for structure determinations [6]. The dipole cross-relaxation, responsible for the nuclear Overhauser effect, depends on spatial proximity and relative motions of the interacting sets of nuclei. The two-dimensional nuclear Overhauser spectroscopy furnishes direct information on the existence of the nuclear Overhauser effect between two nuclei.

Methoxyflurane has the following structure.



Its protons are conveniently located at both ends of the rod-like molecules. The single proton (S-proton) at the left side represents the hydrophilic end of the molecule and the three methoxy protons (M-proton) at the right side represent the hydrophobic end of the molecule.

To determine the site of interaction between volatile anesthetics and lipid membranes, this study used ^{1}H-NMR nuclear Overhauser differences spectroscopy and two-dimensional nuclear Overhauser spectroscopy (NOESY).

Single-shelled vesicles were prepared by dispersing 10 mM DPPC (Sigma) in ^2H_2O (99.92% pure, CEA, France) by sonication with a Heatsystems Ultrasonics

* To whom requests for reprints should be addressed.

Correspondence: I. Ueda, Anesthesia Service 112A, VA Medical Center, Salt Lake City, UT 84148, U.S.A.

W-385A (Farmingdale, NY) at 50 °C. The obtained liposome was centrifuged at 1000 × *g* for 15 min to remove titanium particles dispersed from the sonicator tip. Methoxyflurane (a gift from Dynabot, Osaka, Japan) 2.0 μl was added to 1.0 ml of the liposome, and tetramethylsilane (TMS) was the internal reference. The mole ratio between the anesthetic and DPPC was 1.7. The NMR tube was flame-closed after addition of the anesthetic.

^{1}H-NMR nuclear Overhauser difference spectra and two-dimensional nuclear Overhauser spectra were obtained by a JEOL GX400 NMR Spectrometer (Tokyo) at 37 °C. The pulse sequence in two-dimensional nuclear Overhauser spectroscopy was $[90°\text{-}t_1\text{-}90°\text{-}\tau_m\text{-}90°\text{-}$acquisition $(t_2)]_n$, where the mixing time (τ_m) was 150 ms. For two-dimensional nuclear Overhauser spectra of DPPC/methoxyflurane mixture, 16 free induction decays were acquired for each of 256 increments in t_1. The 256 × 1024 data-point matrix was zero filled and Fourier transformed to a 1024 × 1024 data-point 2D spectrum. A sine-bell squared weighing function was used in both t_1 and t_2 domains.

The nuclear Overhauser difference spectra were obtained by irradiating the anesthetic's methoxy protons (3.67 ppm) during the pulse delay of 2 s and subtracting the off-resonance irradiated spectra. The nuclear Overhauser effects were analyzed on the choline methyl protons and the lipid tail methylene and methyl protons of DPPC. The data-points of each spectrum were Fourier transformed after 16 accumulations at 4096.

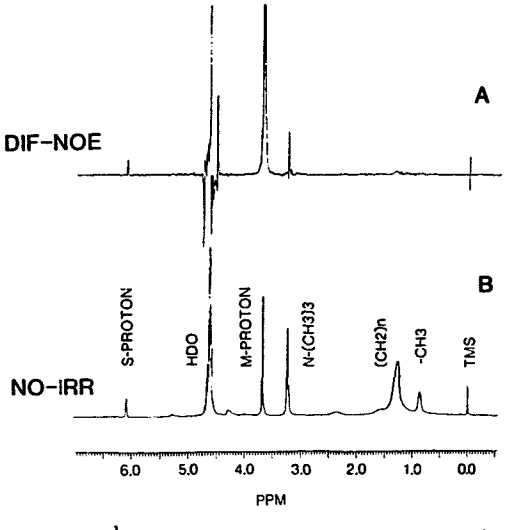


Fig. 1. 400 MHz ^{1}H-NMR spectra of dipalmitoylphosphatidylcholine vesicle membrane in the presence of methoxyflurane with tetramethylsilane as the reference. The peak assignment is based on the previous study [4]. S-proton and M-proton indicate, respectively, the single proton of the hydrophilic end and the methoxy protons of the hydrophobic end of the methoxyflurane molecule. A: One-dimensional NOE difference spectrum. B: Conventional chemical shift spectrum.

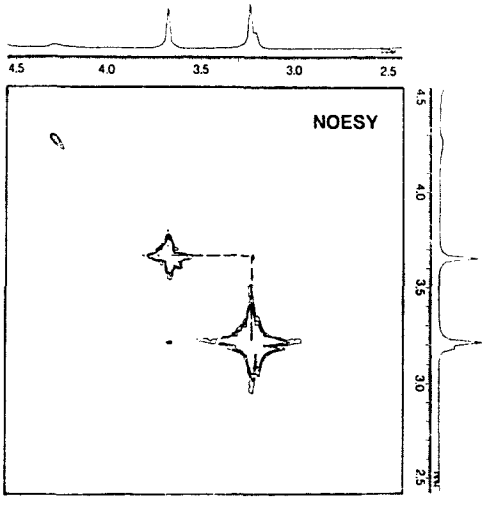


Fig. 2. Two-dimensional nuclear Overhauser effect (NOESY) spectrum. Mixing time 150 ms. The dashed lines indicate the cross-peak between the choline methyl protons of DPPC and hydrophobic methoxy protons of methoxyflurane.

The conventional chemical-shift spectrum of DPPC-methoxyflurane mixture is shown in Fig. 1 with the peak assignments. Because of the presence of methoxyflurane, the membrane was in the liquid-crystal phase [4]. Fig. 1 also shows the nuclear Overhauser difference spectrum of the methoxy protons of methoxyflurane in the lipid/anesthetic mixture. The choline methyl protons showed negative nuclear Overhauser effect of −2.94%.

The two-dimensional nuclear Overhauser spectrum is shown in Fig. 2. A clear cross-peak is observed between the choline methyl protons and the methoxy protons of the anesthetic. No other cross-peaks were found between the anesthetic protons and DPPC methylene or methyl protons.

The excellent correlation established between the anesthetic potency and oil/water partition coefficient and the anesthetic effects on the depression of the main phase-transition temperature of phospholipid membranes tend to give the impression that anesthetics interact directly with the lipid tails of phospholipid bilayers. The often overlooked fact is that the oil/water partition coefficient correlates to the anesthetic potency only when dipolar molecules, such as olive oil and 1-octanol, are used for the oil phase [7]. The correlation deteriorates when apolar solvents, such as alkanes, are used for the oil phase. The present results demonstrate that the main interaction site of the clinically used volatile anesthetics is the membrane/water interface rather than lipid core.

The dipole cross-relaxation depends on the spatial

arrangement and the relative motions of the interacting set of nuclei, and leads to the nuclear Overhauser effect [6]. The present result that the cross-peak was found only between the hydrophobic methoxy protons of methoxyflurane and the choline methyl protons of DPPC in the two-dimensional nuclear Overhauser spectroscopy is a direct proof of interfacial action of volatile anesthetics. Because no cross-peaks were observed between the hydrophilic single proton of methoxyflurane and any of the hydrophilic or hydrophobic protons of DPPC, the hydrophilic end of the anesthetics stays in the aqueous phase without interacting with the membrane molecules.

The negative nuclear Overhauser effect of −2.94%, observed between the anesthetic methoxy protons and the choline methyl protons, indicates $\omega_0\tau_c \gg 1$, i.e., the correlation time of the methoxy proton is long. A dipole–dipole interaction apparently exists between the hydrophobic end of the anesthetic and the hydrophilic head of DPPC. The motion of the methoxy protons of the anesthetic is slow, in the order of the vesicle tumbling.

The wedge-like structure of methoxyflurane, the methoxy-proton side being the sharp end, suggests easiness of the hydrophobic end in penetrating into the membrane core. The present result agrees perfectly with the ^{1}H-NMR data by Kaneshina et al. [5] in surfactant micelles where the anesthetic–micelle interaction was interfacial.

We are not implying, however, that dipolar anesthetics do not solvate into the membrane core, the dielectric constant of which was reported to be 1.9 [8]. On the contrary, we have shown that volatile anesthetics are freely miscible with decane (dielectric constant 1.9) [9,10]. The interfacial location of anesthetic molecules means that the probability of finding anesthetic molecules is highest at the interface.

This study was supported by Grant-in-Aid for Scientific Research No. 61771132 from the Ministry of Education, Japan, and by the Medical Research Service of the Veterans Administration and NIH grants GM25716 and GM27670 U.S.A.

References

1 Trudell, J.R. (1980) in Molecular Mechanisms of Anesthesia. Progress in Anesthesiology, Vol. 2 (Fink, B.R., ed.), pp. 261–270, Raven Press, New York.
2 Ueda, I. and Kamaya, H. (1984) Anesth. Analg. 63, 929–945.
3 Miller, K.W. and Roth, S.H. (1986) in Molecular and Cellular Mechanisms of Anesthetics (Roth, S.H. and Miller, K.W., eds.), pp. 261–266, Plenum, New York.
4 Yokono, S., Shieh, D.D. and Ueda, I. (1981) Biochim. Biophys. Acta 645, 237–242.
5 Kaneshina, S., Lin, H.C. and Ueda, I. (1981) Biochim. Biophys. Acta 647, 223–226.
6 Sanders, J.K.M. and Hunter, B.K. (1987) in Modern NMR Spectroscopy (Sanders, J.K.M. and Hunter, B.K., eds.), pp. 163–207, Oxford.
7 Hansch, C. (1971) in Drug Design (Ariens, E.J., ed.), pp. 271-342, Academic Press, New York.
8 Simon, S.A., McIntosh, T.J., Bennett, P.B. and Shrivastav, B.B. (1979) Mol. Pharmacol. 16, 163–170.
9 Mori, T., Matubayasi, N. and Ueda, I. (1984) Mol. Pharmacol. 25, 123–130.
10 Yoshida, T., Takahashi, K., Kamaya, H. and Ueda, I. (1988) J. Colloid Interface Sci., in the press.