Pergamon

Biochemical Pharmacology, Vol. 47, No. 8, pp. 1461–1464, 1994.

0006-2952/94 $6.00 + 0.00

0006-2952(93)E0095-O

Interaction of trichloroethylene with phosphatidylcholine and its localization in the phosphatidylcholine vesicles: a ^{1}H-NMR study*

(*Received* 8 *June* 1993; *accepted* 30 *November* 1993)

Abstract—The interaction of trichloroethylene, an inhalation general anesthetic, with egg yolk phosphatidylcholine (EYPC) and its localization in small unilamellar vesicles (SUV) has been studied by Nuclear Magnetic Resonance. These studies demonstrate that inhalation anesthetic can interact nonspecifically with lipid molecules. Furthermore, it suggests that the action of inhalation anesthetics is directed primarily to the interfacial region of the lipid membrane.

Key words: anesthetic; temperature; nuclear Overhauser enhancement; interface; nonspecific interactions; T_1 measurements

Membrane lipids have been proposed to play a key role in the action of anesthetics [1, 2]. Studies on model membrane systems have shown that inhalation anesthetics dilate, fluidize and disorder phospholipid membranes [3, 4] and a direct interaction between anesthetic molecules and lipid core is thought to occur. In very few cases has the specific interaction of the interfacial region of the lipid molecules with the anesthetics been reported [5–7]. Studies on the membrane distribution of anesthetics and their interaction with the lipid molecules at higher level of resolution may help to better our understanding of the site and sensitivity of anesthetic action.

In the present paper interaction of trichloroethylene with EYPC† and the localization of trichloroethylene in EYPC SUV with increasing temperature has been studied by ^{1}H nOe difference spectroscopic method and proton T_1 measurements.

Materials and Methods

Trichloroethylene was purchased from Aldrich Chemical Co. and was distilled before use. EYPC (Milwakee, WI, U.S.A.) was isolated and purified according to the published procedure [8]. Small unilamellar vesicles of egg PC (10 mM) were prepared by the method described earlier [9]. The concentration of EYPC in SUV was determined by estimation of total phosphorus using the method of Ames and Dubin [10]. For NMR experiments trichloroethylene was added and incubated at the desired temperature for 30 min. before recording the spectra.

NMR measurements. Proton magnetic resonance measurements were carried out on a Bruker WM-400 FT NMR spectrometer. The spectrometer was equipped with 5 mm ^{1}H/^{13}C dual probehead and ASPECT 2000 computer and temperature control unit.

Typical experiment conditions for the nOe difference experiments were as follows: pulse width 60°; irradiation time 150 msec; acquisition time 1.64 sec, relaxation time 2 sec, i.e. a total relaxation time of 3.64 sec; the decoupler power was chosen so that only 90% of the signal was saturated. A number of 256 transients were acquired in each experiment at 4 K data space. For each condition different off resonance spectra at about 400 Hz downfield with respect to trichloroethylene proton signal, were recorded. The FID's were line broadened by 3 Hz prior to substraction.

^{1}H-T_1 measurements. The T_1 experiments at different temperatures were carried out using the inversion recovery technique and a relaxation time of 20 sec. The T_1 calculations were performed using standard software programme incorporated in the Bruker-WM 400 spectrometer.

Results and Discussion

Based on the high olive oil/water partition coefficient, high fat solubility of trichloroethylene [11, 12] and NMR studies on trichloroethylene induced bilayer to hexagonal phase transition of EYPC membranes [13], it has been hypothesized that trichloroethylene molecules interact predominantly with the hydrocarbon interior of phospholipid bilayers.

In order to analyse this hypothesis studies on interaction and location of trichloroethylene in EYPC membranes was carried out using ^{1}H NMR nOe difference spectroscopic method. The dipole–dipole relaxation, responsible for nOe depends on spatial proximity and relative motions of the interacting sets of nuclei [14], thus providing information on interaction and location of the molecules. Figure 1 shows the 1D-NMR spectrum of an EYPC-trichloroethylene mixture. The peak at δ 6 ppm corresponds to the only hydrogen atom present in the trichloroethylene molecule. The effect of increasing temperature on trichloroethylene : EYPC (2.3 : 1 mol/mol) system studied by nOe difference method is illustrated in Fig. 2. On irradiation of the proton signal of trichloroethylene at 23°, no nOe between trichloroethylene and lipid protons was observed. However, trichloroethylene showed positive nOe of 18.4% with the HOD signal, suggesting that the bulk of trichloroethylene molecules was primarily distributed in the water medium. The, positive nOe indicates that ($w_o\tau_c \ll 1$), i.e. the correlation time of trichloroethylene proton is too short implying a direct but nonspecific interaction between interacting nuclei (water and trichloroethylene in this case). The reason for not observing any nOe between trichloroethylene and lipid molecules under these conditions is due to the fact that the lipid and the anesthetic molecules are in rapid conformational exchange (10^{-6} sec). The time scale of NMR detection is 10^4 sec^{-1}, hence any species which averages more rapidly than this time scale contributes

* Communication number 5041 of Central Drug Research Institute

† Abbreviations: EYPC, egg yolk phosphatidylcholine; nOe nuclear Overhauser enhancement; SUV, small unilamellar vesicles; NMR, Nuclear Magnetic Resonance.

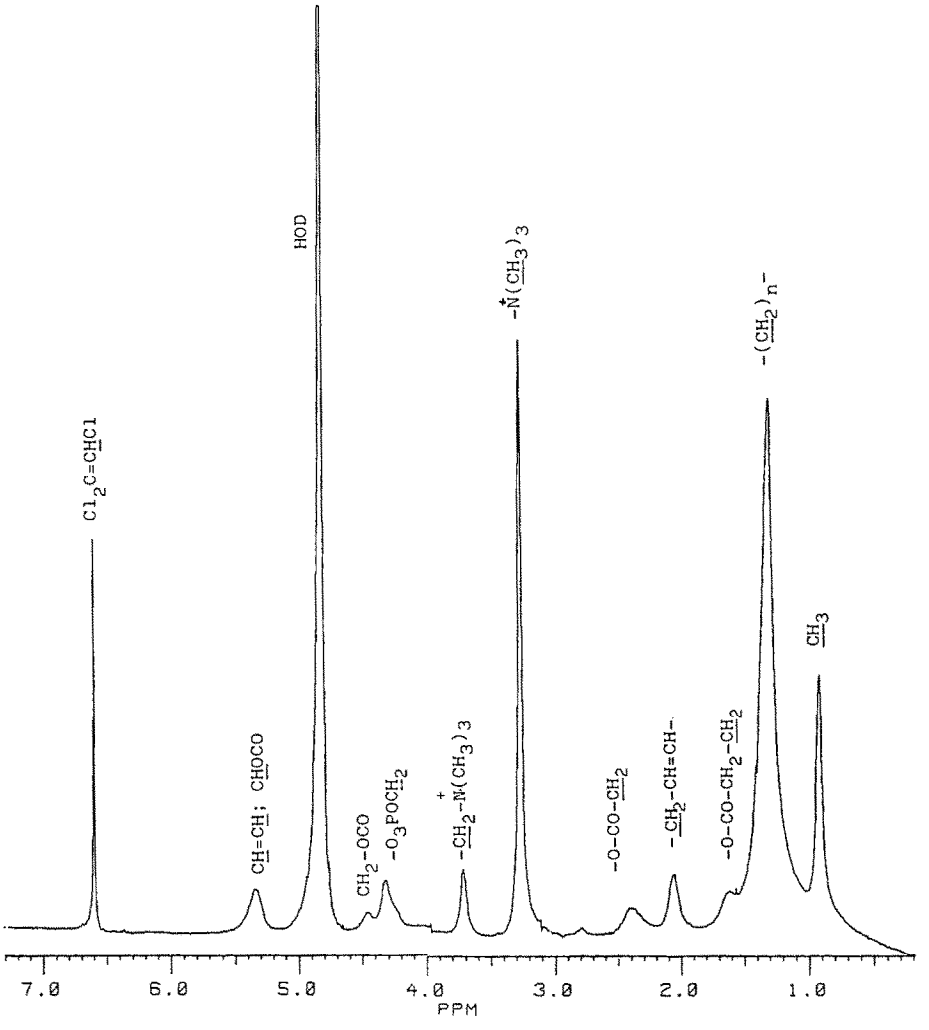

Fig. 1. A 400 MHz ^{1}H-NMR spectra of egg phosphatidylcholine vesicle membrane in the presence of trichloroethylene (trichloroethylene: EYPC 2.3:1 mol/mol) at 23°. The peak assignment is based on the basis of COSY experiment.

to the average property observed [15]. Furthermore, by NMR average ensemble information is obtained hence, for observing any effect in NMR a significant number of anesthetic or lipid binding sites must be populated. This is also the reason why higher concentrations of anesthetic are used for NMR studies [15].

At 35°, trichloroethylene proton showed positive nOes with the lipid proton signals corresponding to the interfacial region of the lipid bilayer [—O—CO—CH_2 (2.4%), —O—CO—CH_2—CH_2 (2.9%), CH_2—O—CO— (1.8%) and —$POCH_2$ (3%)] and with the HOD signal, suggesting that trichloroethylene molecules were primarily located at or close to the interface region of the lipid bilayer and in the water medium. The results further demonstrate that trichloroethylene molecules interact nonspecifically with the EYPC molecules at the interface region of the lipid membrane. This observation supports the earlier suggestion of interfacial preference of inhalation anesthetics of membrane lipids [5–7].

At high temperature (55°) positive nOes between trichloroethylene proton and all the lipid protons except the choline protons was observed, suggesting that under these conditions the trichloroethylene molecules were dispersed throughout the system.

In order to confirm further these observations ^{1}H–T_1 studies of the trichloroethylene proton under similar conditions were carried out. The T_1 values of trichloroethylene in presence of EYPC SUV at different temperatures are given in Table 1. Two relaxation mechanisms are possible for the trichloroethylene proton in the SUVs; these are proton–proton dipolar relaxation and scalar relaxation with ^{35}Cl. For a molecule of size of trichloroethylene scalar relaxations are inefficient [16], hence the only relaxation mechanism that is possible is the dipolar relaxation.

The T_1 value of trichloroethylene proton increases with increasing temperature indicating a faster correlation time [16]. This observation suggests that the trichloroethylene

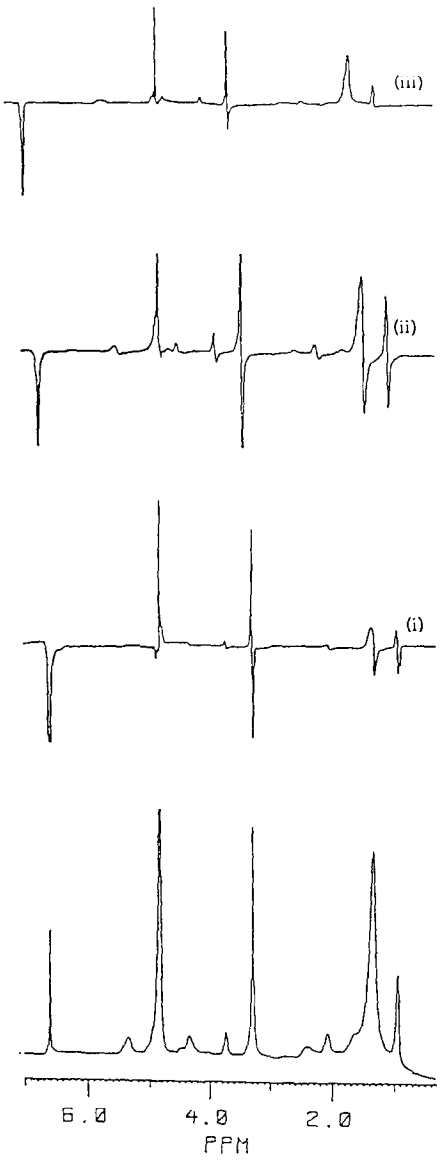

Fig. 2. A 400 MHz NMR 1D nOe difference spectra and off resonance spectrum of egg phosphatidylcholine in D_2O in the presence of trichloroethylene (trichloroethylene: EYPC 2.3:1 mol/mol) with increasing temperature (i) 23°, (ii) 35° and (iii) 55°. The off resonance spectra of (i) is plotted at the bottom for resonance assignment. For each experiment off resonance spectra under identical conditions were recorded. The experimental details are given in Materials and Methods.

Table 1. T_1 values of trichloroethylene proton in the presence of EYPC SUVs (trichloroethylene: EYPC 2.3:1 mol/mol) at different temperatures

Temperature	T_1 (sec)
23°	3.37 ± 0.02
35°	4.28 ± 0.04
55°	5.08 ± 0.02

molecules do not bind specifically to the lipid molecules, because had there been specific binding then the T_1 values of trichloroethylene proton should have shown an initial decrease in the dependence of T_1 with temperature [16]. Hence these observations further support the nOe observation that no specific binding occurs between the trichloroethylene molecules and the lipid molecules in the bilayer.

The results presented in this paper suggest that the action of inhalation anesthetic on lipid membrane is more pronounced at the interfacial region as compared to the hydrocarbon core of the lipid bilayer. Furthermore, it demonstrates that specific interactions between anesthetic and lipid molecules are not a primary requirement for anesthetic action on lipid membrane.

Division of Membrane Biology† and Medicinal Chemistry Division (RSIC)‡
Central Drug Research Institute
Lucknow-226 001
India

VINOD BHAKUNI*†
RAJA ROY‡

REFERENCES

1. Firestone LL and Kirtz RJ, Anesthetics and lipids: some molecular perspectives. *Semin Anesth* **5**: 286–300, 1986.
2. Janoff AS and Miller KW, A critical assessment of the lipid theories of general anesthetics. In: *Biological Membranes* (Ed. Chapman D), Vol. 4, pp. 417–476. Academic Press, New York, 1982.
3. Taraschi TF, Lee Y-C, Janes W and Aubin E, Anesthetic potency and conformational stability in membranes. *Ann NY Acad Sci* **625**: 698–706, 1991.
4. Ueda I and Kamaya H, Molecular mechanisms of anesthesia. *Anesth Analag* **63**: 929–945, 1984.
5. Yokono S, Shieh D and Ueda I, Interfacial preference of anesthetic action upon the phase transition of phospholipid bilayers and partition equilibrium of inhalation anesthetics between membrane and deuterium oxide. *Biochim Biophys Acta* **645**: 237–242, 1981.
6. Yokono S, Ogli K, Miura S and Ueda I, 400 MHz two-dimensional nuclear overhauser spectroscopy on anesthetic interaction with lipid bilayer. *Biochim Biophys Acta* **982**: 300–302, 1989.
7. Yokono S, Ogli K, Tsukamoto I, Yokono A and Miura S, Localization of methoxyflurane molecules in the phospholipid vesicles. *Ann NY Acad Sci* **625**: 751–754, 1991.
8. Singleton WS, Gray MS, Brown ML and White JL, Cromatographically homogeneous lecithin from egg phospholipids. *J Am Oil Chem Soc* **42**: 53–56, 1965.
9. Bhakuni V and Gupta CM, Carbamyl phosphatidylcholine/cholesterol interactions in unilamellar vesicles. *Biochim Biophys Acta* **982**: 216–222, 1989.
10. Ames BN and Dubin DT, The role of polyamine in the nutralization of bacteriophage deoxyribonucleic acid. *J Biol Chem* **235**: 769–775, 1960.
11. Sato A and Nakajima T, A structure–activity relationship of some chlorinated hydrocarbons. *Arch Environ Health* **34**: 69–75, 1979.
12. Waters EM, Gerstner HB and Huff JC, Trichloroethylene. I. An overview. *J Toxicol Environ Health* **2**: 671–707, 1977.
13. Forrest BJ and Rodham DK, An anesthetic-induced

* Corresponding author. FAX (91)-0522-243405

phosphatidylcholine hexagonal phase. *Biochim Biophys Acta* **814**: 281–288, 1985.
14. Sanders JKM and Hunter BK, Connections through space. In: *Modern NMR Spectroscopy* (Eds. Sanders JKM and Hunter BK), pp. 163–207. Oxford Press, Oxford, 1987.
15. Smith ICP, Auger M and Jarrell HS, Molecular details of anesthetic–lipid interaction. *Ann NY Acad Sci* **625**: 668–684, 1991.
16. Shaw D, Relaxations. In: *Fourier Transform NMR Spectroscopy* (Ed. Shaw D), pp. 297–344. Elsevier Scientific Press, New York, 1976.