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INTERFACIAL PREFERENCE OF ANESTHETIC ACTION UPON THE PHASE TRANSITION OF PHOSPHOLIPID BILAYERS AND PARTITION EQUILIBRIUM OF INHALATION ANESTHETICS BETWEEN MEMBRANE AND DEUTERIUM OXIDE

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The half-height linewidth ($\nu_{1/2}$) of the ^{1}H-NMR spectra of dipalmitoylphosphatidylcholine vesicles changes abruptly at the phase transition temperature. In the absence of inhalation anesthetics, proton signals from the choline head group (hydrophilic interface) and acyl-chain tails (lipid core) change at the same temperatue of 39.6°C. The present study compared the effect of four inhalation anesthetics, i.e., methoxyflurane, chloroform, halothane and enflurane, upon the ligand-induced phase transition of phosphatidylcholine vesicle membranes at 37°C. The anesthetics showed differential action upon the phase transition of the phospholipid vesicle membranes between the lipid core and the hydrophilic interface. The concentrations of anesthetics which induced the phase transition of the lipid core were about 2-fold greater than those required for the phase transition of the interfacial choline head groups. From the area under the proton signals of inhalation anesthetics in the NMR spectra, the maximum solubilities of methoxyflurane, chloroform and halothane in ^2H_2O at 37°C were determined to be $0.671 \cdot 10^{-4}$, $2.637 \cdot 10^{-4}$ and $1.398 \cdot 10^{-4}$ (expressed as mole fractions), or 3.35, 13.17 and 6.98 mmol/1 000 g ^2H_2O, respectively. The solubilities of the anesthetic vapor in ^2H_2O expressed as mole fractions according to Henry's law were $9.586 \cdot 10^{-4}$, $6.432 \cdot 10^{-4}$ and $2.311 \cdot 10^{-4}$/atm ($1.013 \cdot 10^5$ Pa) partial pressure, respectively. The presence of phospholipid vesicles in ^2H_2O increased the solubility of the inhalation anesthetics. From difference between solubility in ^2H_2O and a dipalmitoylphosphatidylcholine vesicle suspension, the partition coefficients of methoxyflurane, chloroform and halothane between the phospholipid vesicle membranes and ^2H_2O were estimated. These values, calculated from the mole fractions, were 3364, 1660 and 3850, respectively at 37°C.

Introduction

The need for accurate data on the partition coefficients of volatile anesthetics between membranes and water in order to elucidate the molecular mechanism of general anesthesia has recently been emphasized [1,2]. It has been noted [1] that the bulk of the available data on partition coefficients is mainly derived from thermodynamic arguments and is indirect.

Accordingly, two reports appeared about the direct measurement of the phospholipid membrane/water partition coefficient of an inhalation anesthetic, halothane, by the use of gas chromatography [1] and a radioactive tracer method [2].

^{1}H-NMR spectroscopy is frequently used to probe the dynamic structure of phospholipid vesicle membranes and to study its perturbation by anesthetics. In this case, the phospholipid vesicles are dispersed in ^2H_2O in order to avoid a large signal from water

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protons masking the spectra. There is an obvious need to know the membrane/^2H_2O partition coefficient of anesthetics and their solubility in ^2H_2O.

All inhalation anesthetics currently in clinical use contain hydrogen atom(s) in their structure. The intensity of proton signals of these anesthetics in solution is a linear function of the concentration of the anesthetics in the solvent. When the time elapsed between pulses is long enough to allow complete return from the activated to the ground state, the spectral intensity is expressed by the area under the proton signal in the NMR spectra. A 98% recovery is estimated when the pulse delay time is 5-times that of the spin-lattice relaxation time, T_1 [3].

By measuring the area under the proton signal and comparing it with that of known concentrations of nonvolatile molecules such as sodium acetate dissolved in ^2H_2O, the concentration of anesthetics in solution can be obtained.

When the concentration of anesthetics exceeds their solubility in ^2H_2O, a different proton peak appears upfield in the NMR spectra. By measuring the area under the first peak and comparing it with the calibration curve, the saturated concentration of the anesthetic in solution can be obtained.

The present study reports a novel method to determine the concentration of inhalation anesthetics dissolved in phospholipid suspension in ^2H_2O according to the above principle. The solubility of halothane, chloroform and methoxyflurane in ^2H_2O and the partition coefficient between dipalmitoylphosphatidylcholine vesicle membranes and ^2H_2O will be presented.

Phospholipid membranes undergo a quasi-first-order thermotropic phase transition between the solid-gel and liquid-crystalline states. It has been established that anesthetics depress the phase transition temperature of phospholipid vesicle membranes [4–14].

Shieh et al. [9], using ^{1}H-NMR, reported that inhalation anesthetics decreased the linewidth of the signal of the choline protons at lower concentrations compared to those of the methylene protons of the acyl chain.

When the half-height linewidths ($\nu_{1/2}$) of the proton signals of dipalmitoylphosphatidylcholine vesicles are plotted against temperature, a break is observed which corresponds to the phase transition temperature. In the absence of anesthetics, the phase transition is observed simultaneously in the choline and acyl-chain protons. The present study reports the dissociation of the anesthetic-induced phase transition of phospholipid membranes between the choline methyl protons and the acyl-chain methylene protons.

Materials and Methods

Synthetic dipalmitoylphosphatidylcholine (1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine) was obtained from Sigma and its purity was checked by thin-layer chromatography. It was mixed in a glass ampule with 99.8% ^2H_2O (Stohler Isotope Chemical, Waltham, MA) which was deoxygenated by bubbling with oxygen-free nitrogen gas. The opening was fused in a flame. The concentration of dipalmitoylphosphatidylcholine was 10 mM.

After preincubation at 55°C for 10 min, the ampule was placed in a cup horn of a Cell Disruptor Model W-370 (Heat Systems, Plain View, NY) and sonicated for 60 min at 1°C. The vesicles were annealed for 10 min at 55°C to repair structural defects [15]. The suspension obtained was centrifuged at 20 000 × g for 30 min in a Beckman L5-40 ultracentrifuge and the floating multilamellar vesicles were discarded [16]. The uniformity of vesicle size among preparations was checked by taking ^{1}H-NMR spectra and confirming that the half-height linewidths of the choline methyl protons were identical between batches.

The final concentration of dipalmitoylphosphatidylcholine in the vesicle suspension was determined by labelling vesicles with [^{14}C]dipalmitoylphosphatidylcholine (New England Nuclear, Boston, MA) and measuring the remaining radioactivity in the suspension with a Beckman LS-3150-T liquid scintillation counter. Under the present experimental condition, it was found that, on average, 62.5% of the radioactivity remained in the final preparation.

Proton signals were obtained by a 100 MHz JEOL FX-100 NMR spectrometer at single-pulse mode ($\pi/2$ pulse) and under deuterium internal lock. Since the area under the proton signals represents the spectral intensity when the signals are obtained in the completely relased state, an interval of 120 s was chosen for the pulse delay. The number of data points was 4096 and spectral width was 100 Hz. The signals were

Fourier transformed after 25 accumulations. The area under the proton signal was measured by planimetry.

Stabilizer-free halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) was a gift from Halocarbon Labs. (Hackensack, NJ). Chloroform was spectroscopic and chromatographic grade (MCB, Cincinati, OH). Methoxyflurane (2,2-dichloro-1,1-difluoroethylmethyl ether) (Abbott Labs, North Chicago, IL) contained 0.01% (w/w) 2,6-bis(1,1-dimethylethyl)-4-methylphenol as a stabilizer. Enflurane (2-chloro-1,1,2-trifluoroethyldifluoromethyl ether) was obtained from Ohio Medical Products (Madison, WI). Sodium acetate and tetramethylsilane were obtained from Sigma.

The anesthetics were mixed with the phospholipid vesicle suspension in a 5 mm NMR tube by a microsyringe and the tube was capped tightly to prevent escape of the volatile anesthetics. The amount added was determined by weighing the tube on an analytical balance.

In order to estimate anesthetic concentration from the proton signal area, varying concentrations of sodium acetate in ^2H_2O were used for calibration. Proton signals of the anesthetics were obtained in the fully relaxed state and the anesthetic concentration was estimated by comparing the area under the proton signal with that of sodium acetate. This method circumvents the difficulty of accurate estimation of the volatile anesthetics in solution due to evaporation during sample preparation.

The temperature of the sample was maintained at 37°C by the temperature controller of the spectrometer. The accuracy of the temperature was checked by inserting a filament thermistor into the NMR tube and by a Digitec thermometer (Dayton, OH) with 1/100 K resolution.

Results

The calibration curve for the concentration/area under the proton signal was constructed from ^1H-NMR spectra of varying concentrations of sodium acetate. The relationship between the area of the CH_3 signal and the concentration of sodium acetate was linear, and the following linear regression equation was obtained: $y = 0.0177x$ ($r = 0.999$), where y is the area of the spectrum per proton and x the sodium acetate signal and the anesthetic concentration were also linearly related and the line overlapped upon the regression line constructed with sodium acetate.

When the anesthetic concentration exceeded the solubility in ^2H_2O, a second peak appeared upfield. Further increase of the concentration of anesthetics did not increase the area under the original signal, while the area under the second signal was increased.

Apparently, the second peak represents anesthetics in the pure liquid form undissolved in ^2H_2O and in equilibrium with the solution. This was confirmed by comparing the chemical shift of the second peak to that of the pure liquid anesthetics. When expressed in ppm downfield shift from the peak of the internal reference tetramethylsilane, the values were: chloroform, 7.259; enflurane, CHF_2O-, 6.651 and $CHClFCF_2$-, 6.087; halothane, 5.751; and methoxyflurane, $CHCl_2CF_2$- 5.731, and CH_3O-, 3.663. The area under the original signal in the presence of the second signal was used as the maximum solubility of anesthetics in ^2H_2O.

The maximum solubilities of halothane, chloroform and methoxyflurane in ^2H_2O at 37°C were 6.98, 13.17 and 3.35 mmol/1 000 g ^2H_2O, or when expressed as mole fractions $1.398 \cdot 10^{-4}$, $2.637 \cdot 10^{-4}$ and $0.671 \cdot 10^{-4}$, respectively.

From the differences in solubilities between ^2H_2O and the phospholipid suspension, the maximum solubilities of halothane, chloroform and methoxyflurane in dipalmitoylphosphatidylcholine membranes were obtained. These values were 1550, 1035 and 388 mmol/1 000 g membrane, or when expressed as mole fractions 0.5383, 0.4376 and 0.2257, respectively.

The partition coefficients between dipalmitoylphosphatidylcholine vesicles and ^2H_2O calculated from the mole fractions were: halothane, 3850; chloroform, 1660; and methoxyflurane, 3364.

In the absence of anesthetics, the plot of $\nu_{1/2}$ of the choline methyl protons and acyl-chain methylene protons vs. temperature showed a sudden change at the phase transition as shown in Fig. 1. The phase transition temperature estimated from the discontinuity was 39.6°C in both groups. The phase transition temperature measured by the midpoint of the change in light scattering [10] was 41.3°C and was higher than the present results. The reason for this discrepancy is unknown.

Fig. 2 illustrates the plot of $\nu_{1/2}$ of the choline methyl protons vs. anesthetic concentration at 37°C.

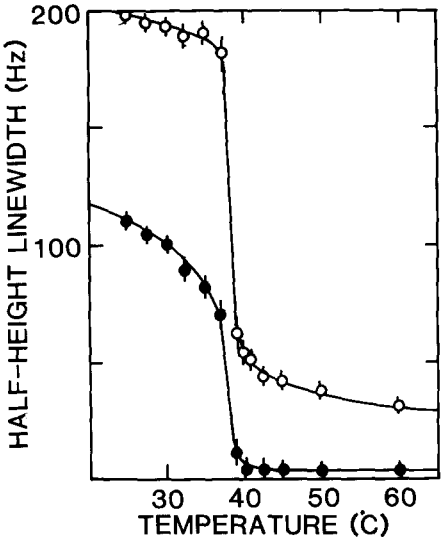

Fig. 1. Plot of temperature vs. the half-height linewidth ($\nu_{1/2}$) of signals from the choline group and acyl chain. The linewidth changes suddenly at the phase transition. Ordinate: $\nu_{1/2}$ expressed in Hz. Acyl-chain methylene protons (○), choline methyl protons (●). Vertical bars denote S.D.

The plot produced rectilinear curves consisting of two components. The anesthetic concentration at the phase transition was estimated from the intercept of the linear regression of the two components. The concentration of halothane which caused the phase transition of choline methyl protons at 37°C was 7.78 mM. The values for other anesthetics are listed in Table I.

TABLE I

THE CONCENTRATIONS (mM) OF ANESTHETICS WHICH INDUCED PHASE TRANSITION IN DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLES AT 37°C

	Choline methyl protons	Acyl-chain methylene protons
Methoxyflurane	4.74	9.32
Chloroform	6.80	17.29
Halothane	7.78	19.90
Enflurane	16.41	32.75

The anesthetic effects upon $\nu_{1/2}$ of acyl-chain methylene protons are shown in Fig. 3. Methoxyflurane and enflurane produced rectilinear plots. With halothane and chloroform, however, $\nu_{1/2}$ continued to decrease with increasing anesthetic concentrations. The anesthetic concentration at the phase transition was estimated from the plot as above. The halothane

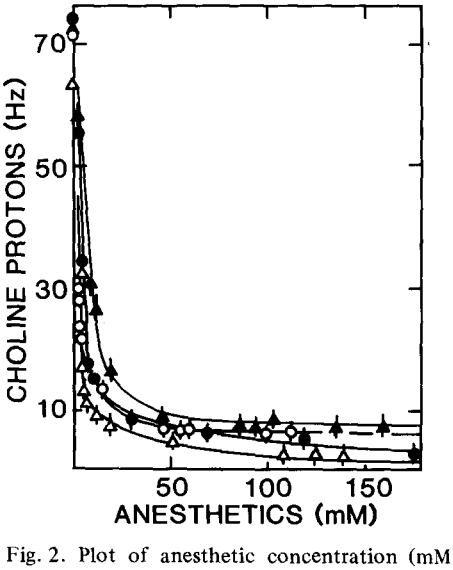

Fig. 2. Plot of anesthetic concentration (mM) vs. the linewidth (Hz) of choline methyl protons. Methoxyflurane (○), chloroform (●), halothane (△), enflurane (▲).

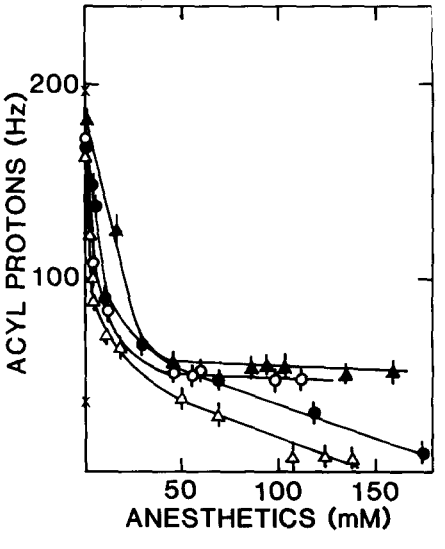

Fig. 3. Plot of anesthetic concentration (mM) vs. linewidth (Hz) of acyl-chain methylene protons. Methoxyflurane (○), chloroform (●), halothane (△), enflurane (▲). The linewidths without anesthetics are shown by X at 30°C (upper) and 50°C (lower).

concentration which caused the phase transition in the acyl-chain methylene protons at 37°C was 19.90 mM. The values for other anesthetics are listed in Table I.

Discussion

In the present study, partition coefficients were measured at 37°C, which is below the phase transition temperature, but the anesthetic concentrations were such that the membranes were in the liquid-crystalline state.

Maximum solubilities were determined at saturating concentrations of these anesthetics at 37°C in the presence of the liquid anesthetics. Therefore, membrane, ^2H_2O, liquid anesthetics and anesthetic vapor coexist in equilibrium. The vapor pressures of halothane, chloroform and methoxyflurane at 37°C represent the partial pressures of these anesthetics in equilibrium with the membrane suspension; these values are 0.605, 0.410 and 0.070 atm, respectively. From the solubilities of these anesthetics in ^2H_2O, expressed as mole fractions according to Henry's law, the above values translate into $2.311 \cdot 10^{-4}$, $6.432 \cdot 10^{-4}$ and $9.586 \cdot 10^{-4}$ mole fraction/atm vapor pressure, respectively.

The partition coefficient of halothane between dipalmitoylphosphatidylcholine and ^2H_2O was 3850 at 37°C and is in reasonable agreement with that reported by Simon et al. [2] which was 4323 for dipalmitoylphosphatidylcholine/H_2O at 40°C. The solubilities of these anesthetics in ^2H_2O may presumably be higher than those in H_2O, because Wen and Muccitelli [17] demonstrated that the solubilities of perfluorocarbon gases in ^2H_2O, expressed as mole fractions according to Henry's law, are larger than those in H_2O. The higher solubility in ^2H_2O would decrease the partition coefficients, and this may account, at least in part, for the smaller values of the partition coefficients found in the present study compared to the value in H_2O. These two figures, however, were obtained at different temperatures and are not directly comparable.

It was reported that addition of cholesterol to the phospholipid membrane decreased the partition coefficient of anesthetics [2] in a dose-dependent fashion. Therefore, the present result is not comparable with that reported by Mastrangelo et al. [1], because their preparation contained cholesterol.

The phase transition of phospholipid membranes has been measured by a number of techniques, including differential scanning calorimetry, change in turbidity of the vesicle solution, dilatometry, exclusion of probe molecules from the gel phase, ESR spectroscopy and NMR spectroscopy, (see, for instance, a review by Lee [18]). Due to the limitations of many of these methods being macroscopic, it has been assumed that the phase transition of phospholipid membranes is a single event where the tail hydrocarbon chains transform into a more disordered conformation from the more ordered conformation. The exact meaning of the so-called pretransition, which may involve the head group, is still unclear.

The advantage of using ^{1}H-NMR spectroscopy is that it is a microscopic technique and visualizes the movement of protons of each part of the phospholipid molecules.

The present results show that the thermal phase transition in the absence of anesthetics occurs simultaneously at the same temperature among choline methyl protons and acyl-chain methylene protons, while the anesthetic-induced phase transition at reduced temperature occurs differentially in the lipid core and in the choline group surface. About 2-fold greater concentrations of anesthetics were required to induce phase transition in the lipid core than in the interfacial region.

The difference between the effect of ether-type and alkane-type anesthetics upon $\nu_{1/2}$ of acyl protons indicates that the actions of ether-type anesthetics are confined to the interfacial region while those of alkane-type anesthetic proceed into the lipid core when the concentrations are increased.

The molar concentrations of anesthetics which induced the phase transition at 37°C in the lipid core and in the choline interface are plotted against the minimal alveolar concentrations [19] of these anesthetics which induce the surgical stage of anesthesia in humans (Fig. 4). In both cases, a straight line was obtained. From these plots, the linear regression equations were obtained. For the choline methyl protons: $y = 7.77x + 2.99$ ($r = 0.991$), and for the acyl-chain methylene protons: $y = 14.67x + 8.59$ ($r = 0.990$), where y is the minimal alveolar tension of anesthetics required to induce surgical stage anesthesia, expressed in atm, and y is the anesthetic con-

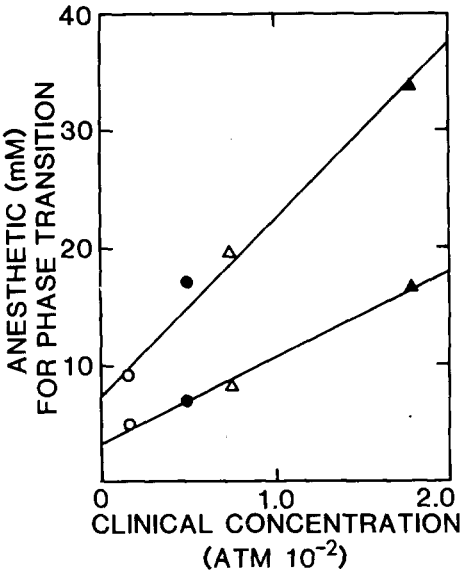

Fig. 4. Correlation between the minimal alveolar tension (atm) of anesthetics required to induce surgical stage anesthesia in humans and the concentrations (mM) required to induce phase transition in acyl-chain methylene protons (upper plot) and choline methyl protons (lower plot). Methoxyflurane (○), chloroform (●), halothane (△), enflurane (▲).

centration required to induce phase transition at 37°C. The correlation coefficients, r, are quite high in both cases.

The present results indicate that the action of anesthetics on the phase transition of phospholipid membranes is more pronounced at the interfacial region than in the lipid core. The fact that incorporation of a slight dipolar moment into the lipophilic molecules enhances the anesthetic potency [20] suggests that the action of inhalation anesthetics is directed primarily to the interfacial region.

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References

1 Mastrangelo, C.J., Trudell, J.R., Edmunds, H.N. and Cohen, E.N. (1978) Mol. Pharmacol. 14, 463–467
2 Simon, S.A., McIntosh, T.J., Bennett, P.B. and Shrivastav, B.B. (1979) Mol. Pharmacol. 16, 163–170
3 Farrar, T.C. and Becker, E.D. (1971) Pulse and Fourier Transform NMR, Introduction to Theory and Methods, Academic Press, New York
4 Trudell, J.R., Hubbell, W.L. and Cohen, E.N. (1973) Biochim. Biophys. Acta 291, 321–327
5 Hill, M.W. (1974) Biochim. Biophys. Acta 356, 117–124
6 Jain, M.K., Wu, N.Y. and Wray, L.V. (1975) Nature 255, 494–495
7 Trudell, J.R., Payan, D.G., Chin, J.H. and Cohen, E.N. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 210–213
8 Lee, A.G. (1976) Biochemistry 15, 2448–2454
9 Shieh, D.D., Ueda, I., Lin, H.C. and Eyring, H. (1976) Proc. Natl. Acad. Sci. U.S.A. 76, 3999–4002
10 Ueda, I., Tashiro, C. and Arakawa, K. (1977) Anesthesiology 46, 327–332
11 Tsong, T.Y., Greenberg, M. and Kanehisa, M.I. (1977) Biochemistry 16, 3115–3121
12 MacDonald, A.G. (1978) Biochim. Biophys. Acta 507, 26–37
13 Mountcastle, D.B., Biltonen, R.L. and Halsey, M.J. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 4906–4910
14 Kamaya, H., Ueda, I., Moore, P.S. and Eyring, H. (1979) Biochim. Biophys. Acta 550, 131–137
15 Lawaczek, R., Kainosho, M. and Chan, S.I. (1976) Biochim. Biophys. Acta 443, 313–330
16 Chan, S.I., Sheetz, M.P., Seiter, C.H.A., Feigenson, G.W., Hsu, M.C., Lou, A. and Yau, A. (1976) Ann. N.Y. Acad. Sci. 222, 499–522
17 Wen, W.Y. and Muccitelli, J.A. (1979) J. Solution Chem. 8, 225–246
18 Lee, A.G. (1977) Biochim. Biophys. Acta 472, 237–281
19 Eger, E.I., II (1974) Anesthetic Uptake and Action, Williams & Wilkins, Baltimore
20 Suckling, C.W. (1957) Br. J. Anaesth. 29, 466–472