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Effect of Clinical Concentrations of Halothane on Phospholipid-Cholesterol Membrane Fluidity

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SUMMARY

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Wide variation exists among estimates of the concentration of the inhalation anesthetic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) in the phospholipid-cholesterol bilayer membrane of a nerve cell exposed to clinical anesthetic concentrations. Attempts to adapt octanol/water partition coefficients to phospholipid-cholesterol bilayer systems, as well as different estimates of the concentration of anesthetic that occur in the lipid region of a nerve cell exposed to a clinical concentration of halothane vapor, have resulted in conflicting conclusions regarding the effects of this anesthetic on nerve membranes. Therefore we have exposed phosphatidylcholine-cholesterol bilayer vesicles to a typical clinical concentration of 1.3% volume of halothane vapor per volume of nitrogen and measured the resultant concentration of halothane in the phospholipid bilayer by gas chromatography. We obtained a value of 30 ± 3 mmoles of halothane per mole of phospholipid-cholesterol for the 1.3% exposure. On the basis of thermodynamic principles of equal chemical potential, we suggest that this concentration obtains in membrane bilayers of the same composition in humans undergoing anesthesia with 1.3% halothane. Electron paramagnetic resonance studies of phospholipid-cholesterol bilayers exposed to concentrations of 1.3% or 3.1% volume of halothane vapor per volume of nitrogen indicate that the internal fluidity of these bilayers is increased following exposure to clinical anesthetic concentrations.

INTRODUCTION

There is conflict among the several reports that describe the effect of clinical concentrations of inhalation anesthetics on phospholipid bilayer membrane model systems. The discrepancy is not only a quantitative one, and various authors have suggested that at a clinical concentration halothane fluidizes (1–3), rigidifies (4, 5), or has no effect (6) on the internal motion of the bilayer system that they studied.

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Miller and Pang (2) have recently measured membrane effects of high and low concentrations of anesthetics on phospholipid bilayers of varying cholesterol content. They demonstrated that the anesthetic structure, the anesthetic concentration, and the phospholipid to cholesterol ratio determine whether the internal fluidity of a phospholipid bilayer is increased or decreased by a particular anesthetic. The wide variations in structure of phospholipid molecules and content of cholesterol in the systems studied by various authors partially explain the conflicting conclusions re-

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garding the effect of clinical anesthetic concentrations on membrane fluidity.

We suggest that another major cause of the discrepancies in the reported results relates to the wide variation in estimates of concentration of an inhalation anesthetic that results in a lipid bilayer of a nerve exposed to clinical anesthetic concentrations, as well as to the techniques used to obtain these concentrations in model systems. These concentrations have frequently been expressed as aqueous concentrations of anesthetic bathing the lipid region or as concentrations per liter of suspension. For example, the following halothane-water-lipid mixtures have been considered to represent the anesthetic concentration reached during clinical anesthesia: Rosenberg *et al.* (4) employed aqueous concentrations of 0.32 mM halothane; Boggs *et al.* (6) used a suspension concentration of 0.4 mM halothane; Vanderkooi *et al.* (1) used 8 mmoles of halothane per kilogram of lipid; Shieh *et al.* (7) studied a 30–170 mM suspension concentration range; Miller and Pang (2) used an 11 mM suspension concentration; while Trudell *et al.* (3) used 49 mmoles of halothane per mole of lipid.

Because of the high lipid/water partition coefficient of halothane, when this anesthetic is added to a vesicle suspension the concentration in the aqueous phase is depleted as the anesthetic partitions into the lipid bilayer. Therefore the use of aqueous concentrations that are appropriate for bathing a nerve results in subanesthetic concentrations of halothane in the bilayer region of a suspension. With a given total suspension concentration, the anesthetic concentration in the bilayer region is dependent on the percentage of lipid in the suspension, as well as on the water/lipid partition coefficient of the anesthetic for that particular preparation of lipid suspension. The type of phospholipid head group, the length and unsaturation of the fatty acid chains, the curvature of vesicles created by sonication, the addition of spectroscopic probe molecules, the percentage of cholesterol in the preparation, and the presence of buffer salts in the aqueous phase may each have large effects on the partition coefficients. Nevertheless, certain workers have used partition coefficients extrapolated from those determined in an octanol–water system as a stated basis for considering that they have studied bilayer systems containing a clinical concentration of halothane.

Because the effects of inhalation anesthetics on membranes are important to our understanding of the mechanism of anesthesia (8, 9), we have measured by gas chromatography the concentration of halothane in the lipid region of a phosphatidylcholine-cholesterol bilayer suspension equilibrated with 1.3% or 3.1% (v/v) concentration of halothane delivered from a clinical anesthetic vaporizer. A 1.3% (v/v) concentration of halothane is typically used to maintain anesthesia in humans. The over-all approach used is based on the concept of equal chemical potential for anesthetics as described by Ferguson (10). When various components of the body (fat, muscle, blood, brain) are at equilibrium with a 1.3% concentration of halothane, each cellular region contains an equal activity of halothane and attains a concentration of halothane determined solely by its composition. Thus a given nerve membrane will have the same concentration of halothane whether it is in a human whose lungs are equilibrated with 1.3% halothane or in a chamber filled with 1.3% halothane vapor, because equal activity must exist in all systems. When a phospholipid-cholesterol bilayer model nerve membrane is equilibrated with 1.3% (v/v) halothane, it likewise reaches an anesthetic concentration determined by its individual composition. The present technique eliminates errors in establishing lipid anesthetic concentrations that have arisen previously from the use of inappropriate partition coefficients or concentrations of anesthetic measured in protein-rich animal membranes.

METHODS

Chromatographically pure phosphatidylcholine was prepared from egg yolks by the method of Singleton *et al.* (11). Phosphatidylcholine (219 mg), cholesterol (57.4 mg), and a phosphatidylcholine spin label Iβ (7, 8) (2 mg) were prepared as previously described (3) and dissolved in petroleum ether. The mixture was evaporated to dryness and kept under vacuum for 16 hr.

Water (2.5 ml) was added to produce a suspension, which was sonicated at 23° for 5 min at 20 W, using a Branson model W140 Sonifier to produce vesicles. A 1.3% or 3.1% volume of halothane vapor per volume nitrogen mixture was delivered by a Fluotec Mark II halothane vaporizer with a 2 liter/min total flow, which was previously calibrated for each concentration with a Cavitron halothane analyzer as well as by gas chromatography. Mixtures of halothane in nitrogen were bubbled very slowly through a fine hypodermic needle into either the phospholipid suspensions or distilled water in a covered 10-ml vial at 20° for 2 hr, until equilibrium had been achieved as established by gas chromatography. Lack of dehydration was established by weighing the sample before and after halothane equilibration. EPR spectra were recorded at 20.2° ± 0.1° on equilibrated phospholipid samples, using a Varian E-104 A EPR spectrometer. EPR spectra were measured using a PDP 8-e computer for online data acquisition and smoothing, as well as for calculation of the order parameter S_n, corrected for changes in the nitrogen isotropic hyperfine splitting constant resulting from anesthetic-induced changes in membrane polarity (3, 12). The S_n values reported are averages of five duplicate measurements with a standard error of ±0.002. Samples of the identical phospholipid suspension, halothane vapor, or water were analyzed by gas chromatography, using a Porapak QS column at 196°, to establish lipid/water and gas/water partition coefficients for halothane. Differences between the amount of halothane in the total suspension and that in water equilibrated with the same vapor phase yielded the concentration of halothane in the lipid phase. In a separate experiment, the lipid suspension was centrifuged and halothane concentration in the separated water phase was determined. Loss of halothane during centrifugation was minimized by using sealed capillary tubes. Monitoring of the halothane concentration by gas chromatography provided no indication of significant loss during handling.

RESULTS AND DISCUSSION

The order parameter S_n was determined for the lipid suspension after treatment with 0%, 1.3%, or 3.1% halothane in the gas phase. In addition, reversibility of anesthetic action on bilayer internal fluidity was determined after extensive flushing with N_2 gas to remove the anesthetic. The data displayed in Fig. 1 show that a linear dose-response relationship exists between the two anesthetic concentrations studied and the lipid fluidity of the phosphatidylcholine-cholesterol vesicles. The effects of the anesthetic on S_n were reversible on flushing with nitrogen until gas chromatographic measurement indicated the absence of halothane. It is likely that the return to a value higher than control was caused by slight dehydration following extensive flushing. Slowly bubbling pure nitrogen for 2 hr through a fresh suspension had no measurable effect on the order parameter of the vesicles. The EPR data in Fig. 1 demonstrate that the fluidity of a bilayer of this composition is significantly increased after equilibration with 1.3% halothane, a partial pressure used to maintain clinical anesthesia.

Equilibrating distilled water with 1.3% halothane yielded a concentration of halothane of 0.91 mM at 20°, while the super-

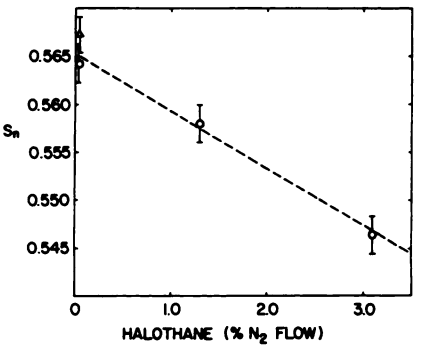

FIG. 1. *Changes in order parameter S_n as a function of added concentrations of halothane*

○, averages of five measurements after bubbling a lipid suspension with the indicated halothane-nitrogen gas mixture; △, S_n after the suspension equilibrated with 3.1% (v/v) halothane had been flushed thoroughly with N_2. The higher S_n value of the suspension after thorough flushing (△) may have been the result of some dehydration of the sample during the final flushing. - - -, best linear least-squares fit of the data. The dose response ratio at 1.3 and 3.1% halothane are within the experimental error of the concentration and S_n measurements.

natant after centrifugation of the vesicles equilibrated with 1.3% halothane exhibited a halothane concentration of 1.1 mM. This larger value may have been due to the presence of residual lipid in the centrifuged aqueous phase; however, both values agree well with the value of 0.96 mM halothane predicted for water equilibrated with 1.3% halothane, using a reported (13, 14) gas/water partition coefficient of 0.57 (grams of halothane per milliliter of gas/grams of halothane per milliliter of water) at 20°. Direct determination of this partition coefficient by gas chromatography yielded a value of 0.52 at 20°.

The lipid phase of the solution equilibrated with 1.3% halothane contained 30 ± 3 mmoles of halothane per mole of lipid, or 46 mmoles of halothane per kilogram of lipid or 9 g of halothane per kilogram of lipid. We assumed a molecular weight of 655 for the lipid mixture, based on an average molecular weight of 800 for egg phosphatidylcholine and 386 for cholesterol. This measured concentration of halothane is in agreement with the Meyer-Overton rule (15, 16) and with that concentration suggested earlier as capable of producing both anesthesia (17) and an increase in bilayer internal fluidity (3). Moreover, gas chromatographic measurement of the halothane content in the water and vapor phases equilibrated with 1.3% halothane vapor yielded partition coefficients of 1875 (moles of halothane per mole of lipid/moles of halothane per mole of water) or 50 (grams of halothane per milliliter of lipid/grams of halothane per milliliter of water) and 642 (moles of halothane per mole of gas/moles of halothane per mole of water) or 385 (grams of halothane per milliliter of gas/grams of halothane per milliliter of water). For the 3.1% halothane-equilibrated suspension the halothane content was 60 ± 5 mmoles/mole of lipid or 18 g/kg of lipid. Our molar partition coefficient of 1875 for the egg phosphatidylcholine-cholesterol-water system equilibrated with 1.3% halothane vapor may be compared with the 525 moles of halothane per mole of dimyristoylphosphatidylcholine/moles of halothane per mole of water partition coefficient of Vanderkooi *et al.* (1) and the 3790 moles of halothane per mole of dipalmitoylphosphatidylcholine/moles of halothane per mole of water partition coefficient of Hill (18). It is to be expected that these values will differ from the above as well as from each other, since they were determined in highly cooperative pure lipid systems that contained no cholesterol, and the values are based on thermodynamic arguments rather than actual measurement of halothane concentrations.

It is important to note that other authors (5, 19–21) have estimated a halothane membrane/water partition coefficient equal to 13 (grams of halothane per milliliter of lipid/grams of halothane per milliliter of water) by applying the empirical rule that the membrane/buffer partition coefficient is equal to one-fifth the octanol/water value (19) and using 64.5 for the octanol/water partition coefficient, as listed in a recent review (20). The latter value was attributed to an earlier article by Hansch and co-workers (22); however, recent studies by Hansch *et al.* have yielded an octanol/water partition coefficient of 200 (23, 24). One-fifth of this later value is 40, which more closely compares with our measured value of 50 (grams of halothane per milliliter of lipid/grams of halothane per milliliter of water).

Seeman (20) utilized the partition coefficient of 64.5, together with concentration data derived from his erythrocyte expansion studies and an empirical rule that drug concentrations that produce general anesthesia are approximately one-tenth the nerve-blocking concentration, to predict a clinical anesthetic concentration of 6.5 mmoles of halothane per kilogram of dry membrane. This calculated value has subsequently been widely used in estimating anesthetic concentrations for model membrane studies. In contrast, our measured value is 30 mmoles/mole of phosphatidylcholine-cholesterol (assumed mol wt 655), or 46 mmoles/kg of dry lipid.

We suggest that the conflicting data in the literature result in part from different assumptions of anesthetic concentrations, differences in accuracy of ΔS_n determination, and different lipid compositions of model systems. The present study provides gas chromatographically measured gas/water and lipid/water partition coefficients,

as well as halothane concentrations, in phosphatidylcholine-cholesterol vesicles at two clinically applicable partial pressures. At these partial pressures the internal fluidity of our particular lipid bilayer was increased. Although we have recently suggested that changes in lateral phase separation behavior may be more important than changes in the fluidity of the bulk nerve membrane (9), the basic technique of directly equilibrating a model system with a partial pressure of an anesthetic would appear to offer many advantages.

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