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Localization of Molecular Halothane in Phospholipid Bilayer Model Nerve Membranes

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The molecular motion and distribution of the inhalation anesthetic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) in a phospholipid bilayer model nerve membrane preparation was studied using fluorine nuclear magnetic resonance. Bilayers containing stable free radicals at known depths were studied to measure possible localization of halothane within certain areas of the bilayer. Bilayer suspensions containing manganese ions in the aqueous phase were used to test the partition of halothane between the aqueous and lipid phases. It was found that halothane rapidly achieves complete exchange throughout the bilayer and the surrounding aqueous phase. The results provide experimental evidence against the formation of anesthetic clathrates hypothesized by Pauling and Miller in their theories of anesthesia. (Key words: Theories of anesthesia, membrane; Membrane, halothane; Anesthetics, volatile, halothane.)

AN UNDERSTANDING of the molecular basis of anesthetic action is necessary to provide better insight into the selection, use, and design of anesthetics. Prior to an investigation of the effect of anesthetics on protein-containing model membranes, it is necessary to determine whether an inhalation anesthetic, such as halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), is dispersed throughout the bilayer or localized in a particular region of the bilayer. Only with this information can one determine in subsequent experiments whether specific protein–anesthetic interactions do indeed exist.

The techniques of electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) applied to phospholipid bilayer model nerve membranes provide powerful investigative tools. Earlier studies with EPR have indicated that the internal fluidity of protein-free phospholipid bilayers is increased by inhalation anesthetics in a concentration-dependent manner[1,2] and that inhalation anesthetics have large effects on the fluid–gel phase transition temperatures of these bilayers.[3] Nuclear magnetic resonance has been used to demonstrate that anesthetics interact with proteins[4] and that they change the fluidity of lipid membranes.[5]

In an earlier study‡ we showed that fluorine nuclear magnetic resonance (^{19}F-NMR) is useful for observing the motion of a halothane molecule in a model nerve membrane. The ^{19}F-NMR spectrum of the fluorine on halothane is a doublet peak (fig. 1) caused by the additive or subtractive interaction of the fluorine nuclear spin with that of the adjacent proton. the present study is based on three phenomena observed in the ^{19}F-NMR spectrum of halothane. The first is that the so-called "chemical shift" of the doublet is influenced by the solvent environment of the halothane molecule. Thus, the spectrum of halothane dissolved in hexane, an environment like the interior of a phospholipid bilayer, would be easily distinguished from the spectrum of halothane in water (fig. 1). We proposed that if halothane dissolved in a lipid membrane so as to partition statically between the lipid bilayer and the surrounding aqueous environment, we would observe a spectrum of two doublets, one near the chemical shift due to hexane and the other at the

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‡ Trudell JR, Hubbell, WL, Milberg RM, Cohen EN, unpublished data.

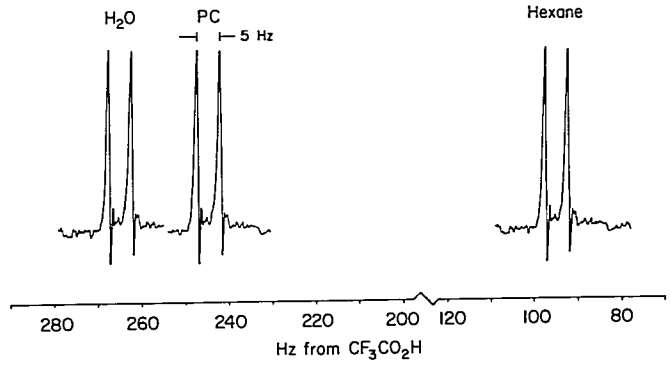

FIG. 1. ^{19}F-NMR spectrum, demonstrating the fluorine doublet centered at chemical shifts of 263, 242, and 93 Hz downfield of the fluorine of the trifluoroacetic acid reference when the halothane molecule is dissolved in water, a 20 per cent phosphatidylcholine suspension, or hexane, respectively. The single doublet of intermediate chemical shift in the case of the phosphatidylcholine suspension implies that halothane exchanges rapidly between the bilayer interior and the surrounding aqueous phase.

chemical shift due to water. The ratio of the areas of these doublets would yield the distribution coefficient. The clathrate theories of Pauling[6] and Miller[7] would suggest such a distribution. These theories hypothesized the formation of water structures surrounding anesthetic molecules. However, if the halothane molecules exchange rapidly between the membrane interior and the aqueous environment, a single doublet with a chemical shift intermediate between those for water and hexane would be observed.

The second phenomenon is that stable free radicals in close proximity to the fluorines broaden the halothane doublet. We are able to prepare phospholipid bilayers specifically labeled at various depths with a stable free radical. It is thus possible to observe whether any portion of the halothane molecules is localized in the water phase outside of the bilayer membrane, adjacent to the polar headgroup region, midway between the headgroup region and the bilayer center, or at the bilayer center itself.

The third phenomenon is that manganese ion broadens the fluorine doublet of halothane but is unable to enter the bilayer. Only those halothane molecules that are either in the aqueous phase or equilibrate rapidly between the bilayer interior and the aqueous phase will be broadened. Halothane molecules that remain in the bilayer will be observed as a sharp doublet proportional in size to their concentration.

Methods

A mixture containing 20 per cent by weight egg phosphatidylcholine in water was sonicated to produce a phospholipid vesicle suspension, as previously described.[2] Manganese ion was added to two 4-ml portions of this suspension to produce concentrations of 10^{-3} and 10^{-4} M manganese. In some experiments, 1 per cent of the egg phosphatidylcholine was replaced by a synthetic phospholipid containing a nitroxide spin label spaced 4, 8, or 16 methylene units from the carbonyl group of the C-14, C-17, or C-22 β-fatty acid chain. Vesicle suspensions that contained these spin labels at known distances from the exterior surface of the bilayer were then prepared.

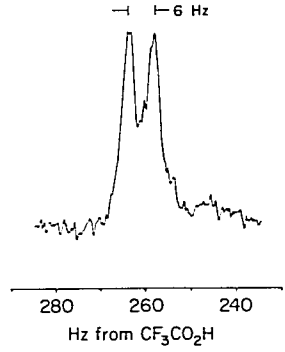

FIG. 2. The ^{19}F-NMR spectrum of halothane in a 20 per cent phosphatidylcholine suspension containing 10^{-4} M manganese. The broadening of the doublet is a measure of the rapid exchange of halothane between the bilayer interior and the surrounding aqueous phase.

Halothane was added to each of the above suspensions to produce a concentration of 0.4 per cent by weight. This concentration yields a halothane concentration in the lipid bilayer that is near that of neural tissue during clinical anesthesia.[2] The spectra of these suspensions were measured at 94.1 MHz on a Varian HA-100 spectrometer internally referenced to trifluoracetic acid in a separate coaxial capillary. Samples of 0.2 per cent by weight halothane in water and in hexane, and 0.4 per cent by weight halothane in a 20 per cent phosphatidylcholine suspension, were measured at 94.1 MHz on a Varian XL-100 Fourier transform spectrometer internally referenced to deuterium oxide.

Results

The ^{19}F-NMR spectrum of halothane is a doublet with a 5-Hz spacing. The doublet center was downfield of the fluorine of the trifluoracetic acid reference, at 263 Hz in water; at 242 Hz in a 20 per cent phosphatidylcholine bilayer suspension; and at 93 Hz in hexane (fig. 1). The entire fluorine signal was equally broadened (similar to fig. 2) in each of the three bilayer suspensions labeled with stable free radicals. The entire doublet signal was somewhat broadened in the phospholipid bilayer suspension containing 10^{-4} M manganese (fig. 2) and was completely broadened in the suspension containing 10^{-3} M manganese.

Discussion

The appearance of a single doublet with a chemical shift intermediate between water and hexane in the ^{19}F-NMR spectrum of halothane in a phospholipid bilayer implies that all the halothane in the suspension rapidly achieves equilibrium (on the NMR time scale) between the aqueous phase and the bilayer interior. The complete broadening of the fluorine doublet by 10^{-3} M manganese in the aqueous phase confirms this interpretation and shows that no more than a very small amount of halothane is permanently held in the bilayer. The equal broadening of the fluorine doublet by each of the three phospholipid bilayer suspensions containing strata of stable free radicals suggests that each halothane molecule not only rapidly exchanges between the aqueous and lipid phases but exchanges between the polar head groups on the exterior and the hydrocarbon chains at the center of the bilayer.

These results suggest that molecular theories of anesthesia must include not only a fluid and rapidly changing membrane[5] but also anesthetic molecules in rapid motion and exchange between the aqueous and phospholipid phases of nerve cell membranes. These findings are not consistent with the anesthetic clathrate theories of Pauling[6] and Miller[7] insofar as they postulate stable anesthetic–water or anesthetic–phospholipid interactions.

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Fluids and Electrolytes

HYPERGLYCEMIA AND CONGESTIVE HEART FAILURE Rapid increases in serum osmolality may occur with diabetes-induced hyperglycemia. A 39-year-old man had had diabetes mellitus since the age of 4 years. His course had been complicated by numerous episodes of ketoacidosis, as well as renal insufficiency, retinopathy, peripheral and autonomic neuropathy, peripheral vascular disease, and congestive cardiac failure. He was being treated with insulin, digoxin, furosemide, and a 1,500-calorie diet with 40 g protein and 2 g sodium. On the day prior to admission, he had consumed approximately 1,500 g of watermelon, half a cantaloupe, several pears, and other foods at a party. The next day, because of dyspnea on mild exertion, he was hospitalized. Blood glucose was 932 mg/100 ml, blood urea nitrogen 84 mg/100 ml, serum osmolality 338 mOsm/kg, and plasma ketones were negative. Serum sodium and potassium were normal, while chloride was 88 mEq/l and carbon dioxide content 25 mEq/l. The patient was dyspneic and using his accessory muscles of respiration. Hepatomegaly was present, as was peripheral pitting edema. He was treated with insulin but did not receive any cardiotonic drug. His condition improved rapidly and he was discharged on the third hospital day. It is postulated that his improvement was due largely to the movement of fluid from the extracellular to the intracellular space, and resulted only in small part from net fluid loss from the body. Although the author indicates that treatment of congestive heart failure with insulin alone is inadvisable in a patient with hyperglycemia and renal insufficiency, one should be aware that changes in blood glucose concentration (and therefore serum osmolality) may be significant in redistributing fluid volume. (*Axelrod L: Response of congestive heart failure to correction of hyperglycemia in the presence of diabetic nephropathy. N Engl J Med 293:1243–1245, 1975.*)