Biophysical Note

The Molecular Freedom of Halothane in the Mixed Hydrate of Halothane and H_2S

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The mixed hydrate of H_2S and halothane was examined by nuclear magnetic resonance spectroscopy and was shown to possess a reorientational freedom of the halothane molecules, consistent with its proposed clathrate structure. (Key words: Molecular freedom; Halothane; Mixed hydrate of halothane.)

IN A PRELIMINARY STUDY[1] of the Pauling[2] theory of general anesthesia, the mixed hydrate of halothane and H_2S[3] was examined, using nuclear magnetic resonance (NMR) spectroscopy to determine the molecular freedom of the halothane molecules within their supposed clathrate cages.

Theory

The clathrate structures described by Pauling are low-density forms of ice which are stabilized by the inclusion within regular packing faults or cavities of nonpolar anesthetic molecules.

The technique of NMR involves the resonant absorption of energy between degenerate energy levels of the atomic nucleus that have been separated by immersion in a magnetic field. The ability of a magnetic field to separate degenerate energy levels of a nucleus depends on its magnetic moment; thus, although the ^{19}F and ^{1}H present in halothane are capable of resonating, their different magnetic moments result in different resonant frequencies.

The resonant absorption is not perfectly sharp, but occurs over a range of frequencies about the resonant value. This range of frequencies is measured in a simple resonance by the width of the resonant line shape, and is dependent on both the nuclear concentration and the type of nuclear motion. For stationary nuclei the line width is generally broad (approximately 5 to 10 gauss) and in the main is the result of magnetic dipole interaction of the nuclei. For rotating nuclei the line width is narrow because the dipole interaction is averaged to a very small value (approximately 25 milligauss) that is reduced effectively to zero if motion that is both translational and rotational is encountered. In the latter situation the line width is usually limited by factors other than the molecular motion of the specimen.

For further reading on NMR, references 4 and 5 are recommended. The former is more relevant for studies in solids, and is concerned with broad line NMR. The latter, however, concentrates on high-resolution NMR and, thus, would be of more use to those interested in studying liquids.

Method

A sample of the mixed hydrate of halothane and H_2S was prepared and spectra drawn for ^{19}F at temperatures indicated in figure 1. Saturation and time-constant effects on the line shape were tested for and found to be absent. Line width was measured by the distance of the maximum deflection on each graph. A parallel NMR study was made of a pure halothane sample over the same temperature range. The NMR spectrometer was a Varian DA/DP-60A wide-line unit.

Discussion

At 200 K two species of resonant fluorine were apparent, one with a width of approxi-

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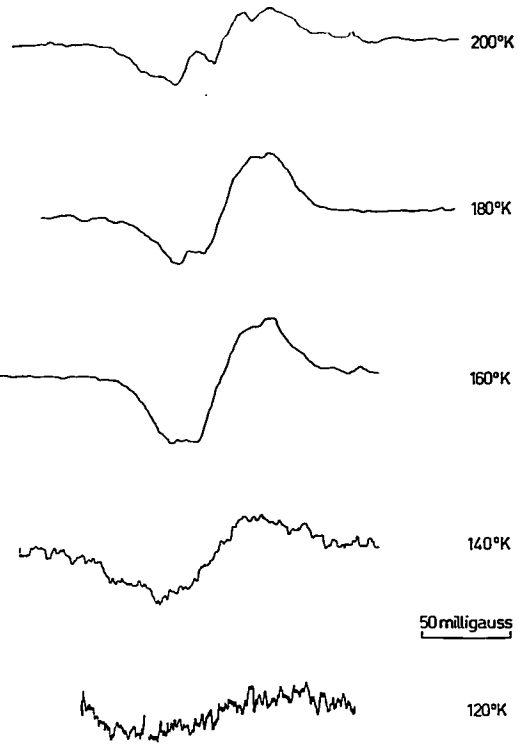

FIG. 1.

mately 15 milligauss, the other with a width of approximately 50 milligauss. The behavior of the narrow-line species is consistent with ^{19}F in liquid halothane that is not encaged and thus is capable of translational and rotational motion. Comparison with the spectrum from the pure halothane sample showed the 15 milligauss to be the limit of the spectrometer resolution and not a dipole broadening. As the temperature of pure halothane was lowered to 155 K and below (halothane freezes at 155 K), this line broadened and ultimately disappeared as the halothane molecules became fixed within the frozen structure, which disallowed both rotation and translation. The broad line, however, was unaffected by temperature until 120 K, when it is assumed that the interaction between the encased halothane and the protons of the cavity structure resulted in a slight broadening. This suggests that some of the halothane molecules were trapped within the clathrate cages and were free to rotate isotropically without translational motion throughout the structure.

These results also suggest that of the three possible environments available to a halothane atom the liquid and solid only are exchanging as the temperature is lowered. This is seen

from the temperature dependence of the narrow line (15 mg) and the independence of the broad line (50 mg). Were exchange occurring between the liquid and the clathrate, the narrow line would diminish in amplitude relative to the broad line rather than broaden to the width characteristic of a solid as was seen in this experiment.

Conclusion

The NMR evidence of the molecular freedom of halothane within the mixed hydrate of halothane and H_2S suggests a clathrate structure, a finding in agreement with previous results.[1, 3]

This study also suggests a technique to establish the existence of clathrate structures *in vivo*, provided sufficient sensitivity may be obtained. The existence of such structures is essential to the theory put forward by Pauling.[2]

References

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Drugs

HALOTHANE HEPATITIS Stimulation of lymphocytes, as measured by incorporation of ^{3}H-thymidine into their desoxyribonucleic acid, was observed in the presence of halothane in ten of 15 patients with halothane hepatitis, but not in healthy controls, patients with hepatic disease, or patients exposed to halothane who did not have hepatic damage. Lymphocytes of a patient with hepatic damage attributable to methoxyflurane were stimulated by methoxyflurane. Preliminary data indicate that sensitization is temporary. The plasma of the patients may contain a factor that inhibits lymphocytic stimulation. Australia antigen was not detected in the sera of the patients, but antimitochondrial antibodies seemed to correlate with lymphocytic stimulation. Stimulation of lymphocytes in the presence of halothane is helpful in the differential diagnosis of viral and halothane hepatitis, and indicates that in some patients the anesthetic may be a sensitizing agent with a pathogenetic role in the hepatic damage. (*Paronetto, F., and Pepper, H.: Lymphocyte Stimulation Induced by Halothane in Patients with Hepatitis Following Exposure to Halothane, New Eng. J. Med. 283: 277 (Aug.) 1970.*)

PROPRANOLOL AND PACEMAKER THRESHOLD The increasing use of implanted pacemakers and propranolol for the control of arrhythmias prompted a study of the effects of this drug on the stimulation threshold during electrical pacing. Five patients requiring pacing via transvenous electrodes were studied at various intervals following the intravenous administration of 1 mg, then 4 mg, and finally, 5 mg propranolol. A marked increase in energy threshold (product of mean voltage, mean current, and impulse duration required for appropriate pacing) followed administration of the drug, and the maximum did not appear to be reached within the 45-minute testing period. If propranolol is to be used in a patient with an implanted pacemaker, the initial doses should be small and the patient observed closely for loss of response to pacemaker stimulation. (*Kubler, W., and Sowton, E.: Influence of Beta-blockage on Myocardial Threshold in Patients with Pacemakers, Lancet 2: 67–68, 1970.*)