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Title: Anesthetic Action: Support for a Multiple Site Hypothesis Using Haar Transformed Nuclear Magnetic Resonance Spectra

Authors: Douglas C. Dorrough, Ph.D.

Affiliation: Departments of Anesthesiology and Biochemistry, School of Medicine, Loma Linda University, Loma Linda, California

Introduction

Since its first proposal in the open literature, the unitary hypothesis of anesthetic action has undergone several modifications: from a simple statement that anesthetics produce their results by acting upon the lipoids of the nerve cell [1], the hypothesis has been gradually refined to state that the temporally primary site of volatile anesthetic action is the phospholipid system of the neurolemma [2]. Essentially, the hypothesis states that the increased fluidity of membrane phospholipids produced by anesthetics, with the consequent increased thermal motion, enhances intramembraneous pressure to the extent that the conformational changes required of membrane-bound enzymes associated with synaptic transmission are inhibited [3,4,5].

Reported here are some results that indicate: 1) the molecular activity of volatile anesthetics upon the lipid structures of model membranes is more complex than anticipated by the unitary hypothesis; 2) the indicated complexity involves, among other things, biochemically multiple sites of anesthetic action. These results were obtained by use of a recently developed nuclear magnetic resonance (NMR) system involving a pulsed mode Haar transform [6,7]. The new system gives an increased resolution of from 25% to 30% over the conventional pulsed mode Fourier transform method [8].

Materials and Methods

β,γ - Dipalmitoyl - L - α - lecithin (DPL) from Calbiochem was checked for purity by thin-layer chromatography, $CHCl_3$ - CH_3OH - H_2O (65:25:4 v/v/v) and a polarimeter. Further purity was not required. Chloroform was purchased from Matheson, Coleman and Bell and deuterated chloroform (99.80%) was obtained from Fabrique par CEA. The deuterium-oxide (D_2O, 99.8) solvent utilized was produced by Stohler Chemicals, Incorporated. Stabilized halothane was obtained from Ohio Medical Products. A sonicated lecithin dispersion in D_2O was produced with a Braun Sonic 1510, using a standard probe and a 300 ml cooling cell. Sonication times ranged from eight to fifteen minutes. A nonsonicated suspension of lecithin in D_2O was obtained by repeated passage of a mixture of the two through a 22 gauge needle followed by shaking in a Vortex mixer.

The PNMR and ^{31}P-NMR spectra were produced by a Varian XL-100-15 spectrometer operated in a pulsed Haar transform mode by an interfacing INTERDATA 832 computer. Both sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) and tetramethylsilane (TMS) were used as internal references. Orthophosphoric Acid (O-H_3PO_4, 85%) was used as an external reference.

Conclusions/Discussion

Haar Transform studies [8] allows for peak assignment and peak changes to two proton systems that have been considered till now to be too rigid [9]: the acetate and the -NMe_3. Consistent shifting of these peaks together with equally consistent changes in intensity and line width with the addition of either halothane or chloroform was observed. In each case, the change is unique to the agent added. Thus, chloroform will affect one of the peaks and not the other; while halothane produces the opposite effect. A corresponding change in high resolution ^{31}P-NMR, Haar transformed, spectra of deuterated DPL in the presence of both chloroform and halothane was also observed.

Under three different pressure conditions, the above experiments were repeated while varying the membrane -D_2O dispersion temperature about that of its phase transition value (41°C) [10]. These variables were introduced both with and without the agents being considered. Changes in the sharpness of the NMR lines, due to changes in the motion of the proton groupings did occur. However, spectral locations remained the same with line width and intensity changes matching those established by other investigators for changes in temperature. It would therefore appear that differential "binding" sites for the agents involved obtains.

References

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