Title: IS MEMBRANE EXPANSION RELEVANT TO ANESTHESIA? 1. VOLUME OF ANESTHETICS

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Introduction: By using volume functions of membranes and anesthetics to elucidate the molecular mechanisms of anesthesia, two opposing reports were published last year. Franks and Lieb (1) used solution densimetry and reported that the volume of halothane does not differ in pure liquid state, in aqueous phase, or in membranes. In contrast, Kita et al (2) reported that the volumes of halothane and methoxyflurane in pure liquid form are larger than those in the aqueous phase and smaller than those in lipid membranes or olive oil measured by dilatometry. In both reports, the derivation of volume was not thermodynamically rigorous, partly because of the involvement of the two-phase system. The present study was undertaken to clarify the discrepancies by comparing the volume of anesthetics in well-defined single-phase media using a straightforward derivation of the partial molal volume from the data obtained by high-precision solution densimetry.

Methods: Halothane, enflurane and isoflurane contain water to varying degrees. The water was removed by passing the anesthetics through an anhydrous aluminum oxide (Fluka) column several times. The stabilizer in halothane is removed by this procedure. Complete absence of water from the preparations was confirmed by infrared spectroscopy. Decane was treated similarly. Water was purified (specific resistance 16 Mohm·cm) by a Milli-Q system (Millipore) and was pipetted into a 60 ml glass ampule. The mass of water in the ampule was measured by weighing the ampule by a semi-micro balance (Mettler) to $1 \cdot 10^{-5}$ g. Aqueous solutions of anesthetics were prepared by adding the water-free liquid anesthetics with a microsyringe in the glass ampule and the added mass was again measured by weighing. The opening of the ampule was flame-closed and incubated at 25 C for 3 days with shaking. Decane solutions were prepared in a similar manner. Liquid density was measured by a Mettler-Paar oscillation densimeter DMA60/DMA601HT at 25.00 ± 0.005 C. The density data were converted to the apparent and partial molal volumes according to the standard method in thermodynamics.

Results: The partial molal volumes of anesthetics in water and decane at infinite dilution, together with the molal volumes of their liquid state, are shown in the following table (cm^3/mol at 25.00 C).

	Halothane	Enflurane	Isoflurane
Pure Liquid	106.32_8	121.98_9	123.66_4
In Water	95.0	102.6	107.5
In Decane	114.5	135.1	135.4

Discussion: Although Seeman (3) reported that the volume occupied by anesthetic molecules in the hydrophobic domain of cell membranes is one order of magnitude larger than the van der Waals volume, Trudell (4) argued about the validity of the estimation and reported that they are about the same. Apparently, the difference is small and experimental demonstration has been difficult. Recent sophistication of the Anton-Paar oscillation densimeter made the estimation of the small volume change possible. Franks and Lieb (1) advocated that membrane expansion is irrelevant to anesthesia, based on their findings that 1) expansion of the volume of membranes is negligible, and 2) the volume of halothane is same in the aqueous phase and in membranes. Their report, however, violates the general rule that hydrophobic molecules decrease volume when transferred into water from the pure liquid state. Our result is in agreement with the dilatometry data reported by Kita et al (2). The term "volume expansion" is often used without rigorous definition. In thermodynamic terms, the volume change expressed by ΔV refers to the excess volume. The ΔV value is zero when anesthetic molecules migrate from the aqueous phase into the membrane and expand the membrane by the volume they occupied in water. Present results show positive ΔV for the transfer of anesthetic molecules from aqueous phase to lipid phase. Since an equilibrium state (such as the depth of anesthesia) must shift to the smaller volume under high pressure, the pressure-reversal of anesthesia can be explained by the positive excess volume described above. Contrary to the claim of Franks and Lieb (1), there is no need to assume a specific binding site for anesthetics.

References:
1. Franks NP, Lieb WR: Nature 292:248-251, 1981
2. Kita Y, Bennett LJ, Miller KW: Biochim. Biophys. Acta 647:130-139, 1981
3. Seeman P: Pharmacol Rev 24:583-655, 1972
4. Trudell JR: Biochim Biophys Acta 470:509-510, 1977