Biochimica et Biophysica Acta, 649 (1981) 125–128
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71545

CORRELATION OF GENERAL ANESTHETIC POTENCY WITH SOLUBILITY IN MEMBRANES

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(Received March 24th, 1981)
(Revised manuscript received August 24th, 1981)

Key words: Anesthetic potency; Membrane solubility; Partition coefficient

Recently (Franks, N.P. and Lieb, W.R. (1978) Nature 274, 339–342) it has been claimed that the traditional correlation between anesthetic potency and vegetable oil solubility breaks down when the alkanols are compared to other volatile anesthetics. Lately, however, new information on the partitioning of anesthetics into lipid bilayers has become available. In this report the potency of twenty-one structurally diverse anesthetic agents is shown to correlate well with their ability to partition into phosphatidylcholine bilayers. Thus the original Meyer-Overton oil solubility hypothesis accomodates a wider range of anesthetics, including alkanols, volatile and gaseous agents, and barbiturates, when lipid bilayer solubility is substituted for oil solubility.

At the turn of the century Meyer and Overton, simultaneously but independently noted a correlation between anesthetic potency and olive oil solubility [1,2]. This correlation has stood the test of time and accurately predicts the potencies of gaseous and volatile anesthetics differing in potency by up to four orders of magnitude [3,4]. Meyer [5] assumed explicitly that olive oil was a model for the solubility properties of cellular lipids. However, later workers have not ruled out the possibility that the hydrophobic site modeled by olive oil is actually a suitable region in some protein. Recently theoretical arguments have been advanced which suggest that the anesthetic site might have substantial hydrophilic character [6]. Another study showed that the *n*-alkanols deviated systematically from other agents in the olive oil correlation but fitted a correlation using the octanol partition coefficient [7]. This study concluded that because of the polarity of octanol the physiological site of action of general anesthetics probably involves protein rather than the lipid region of some excitable membrane. On the other hand we note that the polar head group of the phosphatidylcholine molecule represents about 40% of its mass, so that it is not self-evident that the correlation with octanol solubility points uniquely to a protein as the site of action of anesthesia.

The most direct way of resolving the problem of interpreting the octanol correlation would be to measure the solubility of anesthetics in models that are of greater physiological relevance. The work of Seeman and co-workers [8] established a good correlation between the concentration at which many agents block nerves and their red cell/buffer partition coefficients, but since the red cell contains roughly equal quantities of lipid and protein by weight these data do not resolve the issue at hand. A better test would be provided by comparing general anesthetic potency with the partitioning behaviour of anesthetics into purely lipid bilayers. Such a test has only been performed with gaseous and volatile anesthetics [9] but not with the critical alkanols. However sufficient data have now accumulated from the efforts of

several groups to enable a much wider comparison to be made.

Partition coefficients for twenty one anesthetics in phosphatidylcholine bilayers are available in the literature. No other membrane has been examined in such detail. Although the phosphatidylcholine used by different workers varies with respect to the degree of saturation of the acyl chains (see legend to Fig. 1), this is known to have little effect on the partition coefficient [10,11]. Fig. 1 shows the correlation between the partitioning into phosphatidylcholine bilayers and anesthetic potency for twenty one agents, including alcohols, fluorocarbons, halocarbons, barbiturates, an inert gas and a ketone. Although the data are of heterogenous origin a remarkedly good correlation is obtained covering four orders of magnitude in dose for this widely diverse group of compounds. The line in Fig. 1 was fitted by the method of least squares which yielded a correlation coefficient, r, of -0.965 and a slope of -1.15 ± 0.072 (S.D.). This slope is not significantly different from -1 ($P > 0.05$) as is required by the theory. In contrast to the situation in olive oil [7], the alcohols (open circles) do not systematically deviate from this excellent correlation. In this respect the phosphatidylcholine solubility correlation is more successful than the traditional oil solubility correlation.

For membranes of other compositions there is less extensive partition coefficient data, but that available for thirteen agents covering three orders of magnitude in potency in phosphatidylcholine/cholesterol (2 : 1) bilayers and for thirteen agents covering three orders of magnitude in potency in the red cell membrane are to a first approximation consistent with our findings in phosphatidylcholine bilayers (see legend to Fig. 1 for details). Thus the presence or absence of cholesterol and/or protein apparently does not affect the correlation.

Although the correlation with lipid solubility in Fig. 1 is most persuasive, it still does not allow one to rule out the possibility that anesthesia may result from specific protein-anesthetic interactions. This model would be satisfied for instance if the appropriate lipid/protein partition coefficient were approximately one for all agents examined. While a direct approach to this problem is difficult because the exact site or sites of general anesthetic action remain undefined (for reviews see Refs. 4, 8 and 12), anesthetic-protein interactions might be expected to exhibit greater structural selectivity than anesthetic-lipid interactions. Thus examples of distinct structural requirements for anesthetic potency would provide a further test of theories of anesthesia.

The only case of apparent structural specificity for which the appropriate data is available at present is in fact consistent with the lipid hypothesis [13]. This is the case of the so called cut-off in potency in the alkanols (for a review see Ref. 12). As one ascends this series potency increases steadily until dodecanol, but tridecanol is only a partial anesthetic and tetradecanol and higher homologues exhibit no potency at all. Thus although it has been suggested that alkanols can specifically interact with the lipid bilayer [14] the sharp cut-off in potency turns out to be caused simply by a decrease in the membrane partition coefficient [13,15]. Fig. 2 shows that for the lower alcohols the maximum achievable concentrations in membranes (i.e. the product of saturated aqueous solubility and membrane buffer partition coefficient) is two orders of magnitude higher than that required to achieve anesthesia. This margin decreases slowly to decanol, after which successive additions of methylene groups cause a precipitious fall. By tetradecanol the maximum achievable concentration has become an order of magnitude lower than that required to cause anesthesia. Thus the loss of anesthetic potency that occurs in the higher members of the series is accurately predicted by the membrane aqueous phase partition coefficient. The presence or absence of protein apparently does not affect such predictions to a first approximation since they are valid both for biomembranes and lipid bilayers. Once again we cannot rule out that a similar correlation might be seen with some protein-alcohol interactions, but Figs. 1 and 2 taken together clearly restrict this probability.

Whether limitations in membrane solubility can also explain the cut-off in potency seen in the normal hydrocarbons cannot be assessed at present for lack of appropriate partition coefficient data but such an explanation appears likely [16].

The limited solubility of the longer chain alcohols calls into question many earlier spectroscopic studies which now appear to have been carried out under supersaturated conditions [13,17].

In conclusion, it should be evident that while the extension of the olive oil solubility correlation to

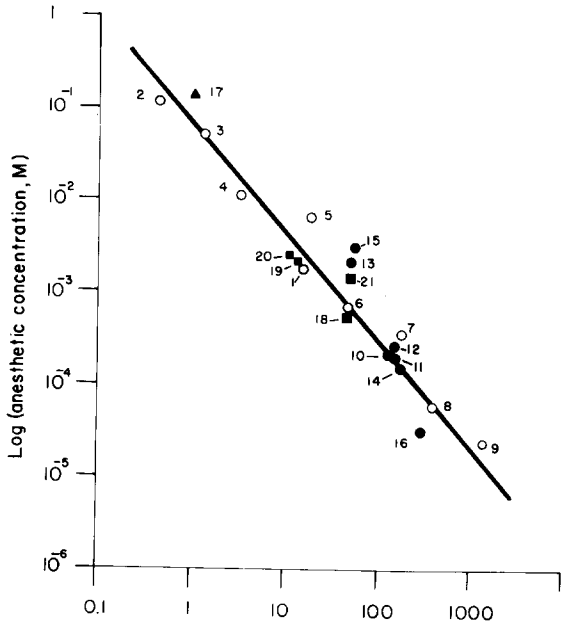

Fig. 1. For twenty one diverse agents there is a correlation between partitioning into phosphatidylcholine bilayers and the equilibrium anesthetic concentration (ED_{50}) in water bathing aquatic animals. The partition coefficient is defined as the equilibrium solute concentration per unit volume in the lipid phase divided by the equilibrium solute concentration in the aqueous phase. Symbols denote different animals; circles are tadpoles, squares are newts and triangles are frogs, all at room temperature. The alcohols are in open symbols, the rest are filled. The anesthetic data are from Pringle et al. [13] (compounds 2–4, 6 and 8); Meyer and Hemmi [18] (compounds 5, 7 and 9); Kita et al. [19] (compounds 1, 10 and 11); Miller and Ambalavanar (unpublished) (compounds 12 and 13); Lee-son et al. [20] (compounds 14–16); Meyer [21] (compound 17); Smith [22] (compounds 18–21). Partition coefficient data in dimyristoyl phosphatidylcholine: Katz and Diamond [23] (compounds 1–4 and 17). Partition coefficient data in egg phosphatidylcholine: Jain [24] (compounds 5–9); Colley and Metcalfe [25] (compound 1); Miller and Yu [26] (compound 14); Pang et al. [27] (compound 15); Korten et al. [28] (compound 16). Partition coefficient data in egg phosphatidylcholine: 4% phosphatidic acid: Smith et al. [9] (compounds 10–13 and 19–21); Miller et al. [10] (compound 18). Where tested dimyristoyl phosphatidylcholine, egg phosphatidylcholine and egg phosphatidylcholine: 4% phosphatidic acid bilayers have almost identical partition coefficients at room temperature [10,11,26]. Partition coefficients for the barbiturates were corrected in order to correspond to the pH (7.4) at which the anesthetic determinations were made. Anesthetics are referred to as follows: 1, benzyl alcohol; 2, ethanol; 3, propanol; 4, butanol; 5, pentanol; 6, hexanol; 7, heptanol; 8, octanol; 9, nonanol; 10, halothane; 11, methoxyflurane; 12, isoflurane; 13, fluroxene; 14, pentobarbital; 15, phenobarbital; 16, thiopental; 17, acetone; 18, cyclopropane; 19, xenon; 20, carbon tetrafluoride; 21, sulfur hexafluoride. Addition of 33 mol% cholesterol to the phosphatidylcholine bilayers did not change this correlation for those compounds for which data was available (compounds 1; 10–16; 18–21 and urethane). Anesthetic and phosphatidylcholine : cholesterol/aqueous phase partition coefficient data for urethane are available from Meyer [5] and Pang et al. [27], respectively.

The correlation was also not changed when partitioning into red cells was considered. Partition coefficient data: for compounds 1, 5–9 from Seeman [8]; for compound 10, Smith et al. [9]; for compound 14, 15, and 16, Korten et al. [28,29]; for compound 21, Power and Stegall [30]. Anesthetic and partition coefficient data for nitrogen is available from Smith [27] and Stegall [30], respectively.

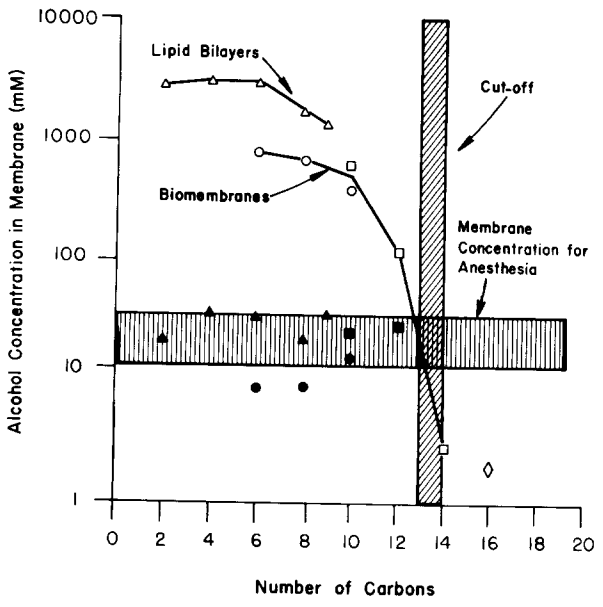

Fig. 2. The cut-off in potency for long chain alcohols is accounted for by membrane solubility. The partition coefficient for hexadecanol was determined after the method of Heap et al. [31]. The saturated aqueous concentration of nonanol is from Kinoshita et al. [32]. Sources for the rest of the data are given in Pringle et al. [13]. Symbols refer to different membranes. Triangles are phosphatidylcholine, diamond is egg phosphatidylcholine/cholesterol (2 : 1), circles are red cells and squares are intestinal brush border membranes. Open symbols are maximum alcohol concentration in membrane at saturation; filled symbols refer to anesthetic concentrations. Over the range of aqueous concentrations used, deviations from Henry's law are assumed to be minimal.

phospholipid bilayers provided here does not allow additional mechanistic insights, if Myer had been armed with this data many subsequent ambiguities in interpretation might have been avoided. Although the data presented here are consistent with the lipid hypothesis it remains possible that other models, as yet untested, might do as well.

This work was supported in part from a U.S. National Institute for General Medical Sciences Grant (GM-25911) and in part by joint funding from the U.S. Office of Naval Research and the Naval Medical Research and Development Command through O.N.R. contract N 00014-756-0727. K.W.M. is a Research Career Development Awardee of the National Institute of General Medical Sciences (GM 00199).

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