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THE INTRAMEMBRANE LOCATION OF ALCOHOL ANESTHETICS*

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SUMMARY

In order to identify the chemical nature of the membrane receptor sites involved in general anesthesia the apparent free energy of binding of methylene groups of *n*-alcohols in anesthetic systems was evaluated and compared with data from model systems. It was concluded that the sites may consist either of (i) the non-polar portions of lipids, (ii) a non-polar region of a lipoprotein made up of non-polar portions of the protein and lipid moieties or (iii) protein. However, if the site consists only of protein it is possible that this material undergoes a ligand-induced conformation change on binding the anesthetic. Some reasons for the use of caution in employing temperature effects to identify the chemical nature of the receptor sites are outlined.

INTRODUCTION

The chemical nature of the site of membrane action of general anesthetics is unknown. It has been suggested to consist of protein or a protein–water interface[1–4] or lipid[5–7]. It has been shown recently that there is a close correlation between the anesthetic properties of phenols, aliphatic alcohols and steroids with their ability to protect erythrocytes against hypotonic hemolysis[8] suggesting that the chemical nature of the site of action may be similar. To obtain more information about its nature the apparent free energy of adsorption per methylene group on erythrocyte membranes and in various anesthetic systems was evaluated. It is concluded that either a lipid, a lipoprotein or a protein site is compatible with the data. However, if the site contains protein, this material may undergo a ligand-induced conformational change on binding the alcohol.

METHODS

The free energy of adsorption per methylene group was evaluated assuming the extent of anesthesia or protection against hemolysis is directly proportional to the number of molecules adsorbed at the sites responsible for these phenomena. The standard free energy of adsorption of the alcohols at these active sites, ΔF°, is[9]

$$\Delta F^\circ = -2.303\, RT \log K = -2.303\, RT \log \frac{\theta}{(1-\theta)a} \qquad (1)$$

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where K is the alcohol–receptor affinity constant, a the activity of alcohol in the aqueous phase, θ the fraction of narcosis-active sites occupied by adsorbate, R the gas constant and T the absolute temperature. The standard free energy of adsorption per methylene group, ΔF_t°, was taken as the difference in ΔF° for two homologues differing in length by a single CH_2 group, hence as

$$\Delta F_t^\circ = 2.303\, RT \log \frac{a_i}{a_{i+1}} \qquad (2)$$

where a_i and a_{i+1} refer to the activity of the ith and ith + 1 homologues, respectively. The standard state for the alcohol in solution was taken as the hypothetical unit mole fraction solution which has the enthalpy properties of the infinitely dilute solution. For the alcohol on the membrane the standard state was taken to be that where θ equals 1 but where the adsorbate has the properties it would possess at an extremely low level of surface coverage. In practice ΔF_t° was evaluated, assuming the activity of the alcohol equaled its mole fraction, from the least-mean-square slope of the linear portion of plots of the logarithm of the mole fraction of alcohol required to cause anesthesia or to inhibit hemolysis to a given extent *versus* the number of carbon atoms of the alcohol.

The use of mole fraction terms instead of activities in the above evaluation seems to be justifiable. For C_2 to C_4 alcohols at the concentrations of interest activity coefficients for the water in binary water–alcohol solutions are apparently 1 (ref. 10), hence that for the alcohol component is probably also very close to 1. Activity coefficients are not available for the higher alcohols but can be estimated as also being close to unity if deviations from ideal behavior of such aliphatic amphiphilic compounds are ascribed to dimer formation resulting from hydrophobic bonding[11]. On this basis, estimating the free energy of dimerisation as —600 cal/mole per CH_2 group[12], the amount of dimer may be computed as less than 1 % that of monomer. The validity of this calculation is supported by data for the amount of dimer and quadramer in solutions of sodium dodecanoate. At a mole % of $6.5 \cdot 10^{-4}$ (36 mM) at 25° less than 1 % of this compound is calculated to be aggregated[11]. Most of the data employed for the alcohols in the anesthetic and erythrocyte hemolysis systems are for shorter chain lengths at even lower concentrations, hence deviations from ideality due to solute–solute interactions may be expected to be even smaller.

The presence of salt in the aqueous phase of the biological systems would be expected to increase the activity coefficients of the alcohols over the values in pure water. Using solubility data for *n*-alcohols in water and in Ringers solution at 30° (ref. 13) activity coefficients in the presence of salt could be estimated as being 4.3 % higher for butanol and increasing to 9.7 % for heptanol. Since ΔF_t° is computed from a slope which involves the logarithm of alcohol activity, ignoring the effect of salt leads to an error of 5.4 % or less, a quantity which is considered to be insignificant in the present analysis.

The above method for evaluating ΔF_t° has elements in common with that of MEYER AND HEMMI[5]. It differs from others[14,15] in choosing the standard state of the anesthetic to be the hypothetical mole fraction unity solution instead of the pure liquid or solid narcotic. Using this latter procedure results in each alcohol having a different standard state with the result that interpretation of comparisons among homologues may become somewhat ambiguous, particularly for the lower members.

For instance, using ethanol and *n*-butanol as examples, it becomes necessary to interpret in molecular terms the difference in free energy in taking ethanol from pure ethanol and *n*-butanol from pure butanol and transferring them to the membrane at the concentration at which narcosis occurs. This is difficult to do since the two alcohols are in different molecular environments when in their pure liquid states. The use of the mole fraction unity solution coupled with the evaluation of free energies for the non-polar portions only may obviate such difficulties to a large extent and also facilitates comparisons with model systems.

RESULTS

Several representative curves of the logarithm of alcohol concentration required to cause a given degree of anesthesia or to inhibit erythrocyte hemolysis to a given extent as a function of the number of carbon atoms are shown in Fig. 1. They are characterised by a non-linear portion for the lower members, usually up to *n*-propanol or *n*-butanol followed by an apparently linear portion. In some instances a second non-linear portion appears with *n*-octanol or *n*-nonanol as shown in Fig. 1A for the inhibition of reflex responses in tadpoles. In terms of Eqn. 2 and the assumption that the physiological or anti-hemolytic effects observed depend on θ this behavior means that initially ΔF_t° varies as chain length increases then assumes a constant value and subsequently may begin to vary again. A possible reason for the variation with

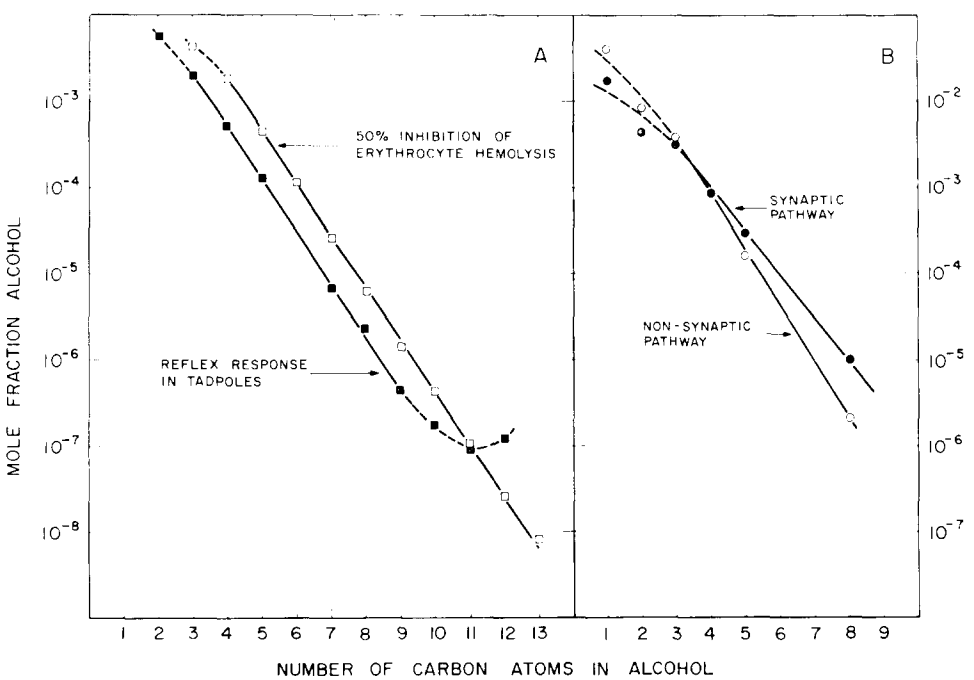

Fig. 1. A. Log alcohol concentration required to suppress the reflex response in tadpoles and to produce a relative inhibition of 50% of hypotonic hemolysis of human erythrocytes. The dashed portions indicate the regions taken to be non-linear. B. Log alcohol concentration required to suppress the transmission of nerve impulses in cats travelling along different pathways. The dashed portions indicate the regions taken to be non-linear.

the lower alcohols could be the presence of head group effects[16–18] on the free energy of alcohol uptake. Binding of the alcohol by the receptor sites may involve removal, either partially or completely, of water surrounding the aliphatic chain. The polar head group may perturb the structure of water in its immediate vicinity for a distance equivalent to that spanned by several CH_2 residues with a concomitant dependance of ΔF_t° upon the distance between the head group and methylene groups.

The ΔF_t° values obtained from the linear portions are summarised in Table I for thirteen anesthetic systems. A notable fact about these systems is their diversity since they include examples ranging from bacterial to mammalian tissues or intact organisms. The ΔF_t° for the inhibition of hypotonic hemolysis by alcohols is also included in Table I. The average of the ΔF_t° values for the erythrocyte and anesthetic systems together is —815 cal per mole of CH_2 groups and —813 cal/mole for the anesthetic systems alone. The values for the anesthetic systems range from —637 to —1080 cal/mole.

The free energy of transfer per mole of CH_2 groups from water to three different non-polar environments is shown in Table II. These values range from —750 to —883 cal/mole.

The temperature at which the anesthesia experiments were carried out is

TABLE I

FREE ENERGY OF BINDING AND STANDARD ERROR PER MOLE OF METHYLENE GROUPS

No. denotes the number of points used to compute the slope.

System	$-\Delta F_t^\circ$ *(cal)*	*No.*	*Temp.*
Narcosis of frog ventricle[19]	924 ± 77	5	20°*
Narcosis of tortoise heart[20]	882	2	18°
Reversible suppression of luminescence in bacteria[20]	857 ± 27	5	20°
Reversible suppression of reflex response in tadpoles[20]	815 ± 81	6	20°*
Immobilisation of *Barnacle nauplii*[21]	794 ± 8	4	18.5°*
Suppression of response of frog gastrocnemius muscle to electrical stimulus[20]	753 ± 18	4	17°
Narcosis of *Gobio fluviatilis*[22]	748 ± 55	4	15°
Guinea-pig gut contractility[23]	722 ± 15	5	33°
Reversible inhibition of respiration of goose erythrocytes[20]	721 ± 71	4	20°*
Reversible suppression of spontaneous contractions of isolated frog heart[20]	705 ± 4	5	20°*
Paramecium mobility[23]	637 ± 29	5	20°*
Reversible suppression of transmission of nerve impulses in cats[24]			
synaptic pathway	924 ± 16	4	36°
non-synaptic pathway	706 ± 14	4	36°
Reversible suppression of spontaneous movement in tadpoles[20]			
6-day-old	1080	2	18°
12-day-old	931	2	18°
40-day-old	833 ± 10	5	18°
83-day-old	792 ± 48	3	18°
Inhibition of erythrocyte hemolysis (ref. 8; P. SEEMAN, personal communication):			
25% inhibition	857 ± 35	10	22°
50% inhibition	809 ± 8	10	22°
75% inhibition	803 ± 11	10	22°

* Assumed value.

TABLE II

FREE ENERGY OF TRANSFER OF METHYLENE GROUPS TO NON-POLAR ENVIRONMENTS

System	$-\Delta F_t^\circ$ (*cal*)	*Temp.*
Adsorption at a light petroleum–water interface[25]	820	20°
Solubility of liquid hydrocarbons (pentane to octane) in water[26]	883 ± 23*	25°
Triolein–water partition coefficients of alcohols[27]	750 ± 20*	25°

* Standard error.

shown in Table I. In most instances where it was not cited in the original reference it was assumed equal to 20°. Because of the differences in temperature among some systems, the uncertainty in others, and the fact that ΔF_t° would be a function of temperature, a detailed statistical analysis of the significance of the differences in ΔF_t° was considered to be inappropriate. However, it is noteworthy that in two instances where a similar temperature was employed, the suppression of nerve impulses in cats travelling by different pathways, the differences in ΔF_t° are significant at the 1 % level.

DISCUSSION

The ΔF_t° values in the anesthetic and hemolysis systems are close to those in model systems where CH_2 groups are transferred from water to non-polar environments. This correlation suggests that the non-polar residues of the alcohols in the hemolysis and in the anesthetic systems are also in a non-polar environment. Furthermore, the similarity of ΔF_t° in the anesthetic and hemolysis systems, the latter being apparently a purely membrane phenomenon[28], coupled with the correlation between the anesthetic and inhibition of hemolysis effects of various compounds[8] supports the view that anesthesia involves membranes[29].

Since membranes generally contain appreciable amounts of lipid[30], the non-polar site could consist of cholesterol and of the fatty acid residues of membrane phosphatides. Another possibility is that it consists of non-polar residues of the protein and lipid components of a membrane lipoprotein. A third possibility is that the site consists entirely of protein and is, presumably, made up primarily of non-polar side chains. In this latter instance it is possible to describe a feature the receptor molecules could be expected to possess. Thus, in general, where ΔF_t° for the combination of CH_2 groups with proteins can be determined or estimated, the values are lower than those in anesthetic systems, ranging from about —560 cal down to about —100 cal (refs. 31–34). Larger values have been found: —650 cal for chymotrypsin–fatty acid tyrosyl ester interactions[35], —650 cal for lipoxygenase–aliphatic alcohol interactions[31] and —1100 cal for hydrocarbon binding to β-lactoglobulin A (ref. 36). However, chymotrypsin undergoes a conformational change on combination with inhibitors[37] and this might occur also when β-lactoglobulin combines with hydrocarbons[3,38]. Aliphatic alcohols inhibit lipoxygenase by a mechanism involving non-competative inhibition, hence there is the possibility here too of a ligand-induced conformational change. The ΔF_t° in such coupled systems would be expected to contain terms due

to hydrophobic interactions as well as to the linked conformation change[39]. Thus, for ΔF_t° in the biological systems to lie in the range of —637 to —1080 cal and the binding site be located on a protein, the protein could be expected to undergo a ligand-induced conformation change.

Since the ΔF_t° values indicate that the receptor sites have a relatively high affinity for non-polar materials, gaseous general anesthetics, which are essentially non-polar materials, would be expected to bind at these same sites and may possess their physiological effects for this reason. If these sites involve only proteins the general anesthetics may cause them to undergo a conformation change as suggested by other workers[3,40,41] and seems plausible for the aliphatic alcohols. The fact that such structural changes can occur has been shown in studies of globular protein–anesthetic interactions[3,42].

Anesthetics such as inert gases which do not form hydrogen bonds in aqueous solution have been suggested to act through the formation of hydrates. In contrast, polar materials such as alcohols which can form hydrogen bonds were suggested to act in some other way[1]. The present analysis, by emphasising the apparent linear dependence of anesthetic potency on chain length, suggests that hydrogen bond forming ability, of the alcohols at any rate, is not of prime importance.

Because the ΔF_t° values for alcohol methylene groups are compatible with at least three different structures for the membrane binding sites it is evident that free energy data alone cannot be used to distinguish between them unequivocally. Therefore, the question of whether or not the use of another thermodynamic parameter, the enthalpy (ΔH), would provide greater discrimination was considered. It was concluded that there may be a limitation in the use of this parameter. The reason is that in using enthalpy data a comparison must be made between the effects of temperature on the potency of the anesthetic in a biological system on the one hand and on the effects of temperature on a model system involving the anesthetic on the other. This comparison is based on the assumption that the relevant interactions in the two systems are sufficiently similar. Oil–water partitioning of anesthetics has been used as a model in some instances[43,44] and hydrophobic bonding in proteins[12] may be considered as another possibility. However, the enthalpy of solution of non-polar compounds in water, a process which has similar thermodynamic parameters to that for transfer or partitioning between a non-polar solvent and water, may change sign with temperature. This occurs with benzene and butane[45], two molecules similar in size to some general anesthetics, at about 26°, a temperature where anesthesia experiments have been carried out[43,44]. Moreover, ΔH for hydrophobic bonding has been predicted to change sign as temperature increases[12]. The temperature at which the change in sign occurs in a particular system may be expected to depend on the nature of the intermolecular interactions involved. Therefore, in the case of non-polar anesthetics the anesthetic–receptor interactions over a given temperature interval may be generally similar to those in a particular model yet sufficiently different to make ΔH differ in sign.

In the case of polar molecules such as alcohols their amphiphilicity may introduce an additional complication. Molecules of this sort may reside in the receptor site such that the hydroxyl residue is in contact with the aqueous phase while the hydrocarbon portion is immersed or 'buried' in a non-polar region. The enthalpy of interaction of an alcohol with the receptor would include a contribution from the

hydroxyl group and this quantity and its variation with temperature would have to be determined before the effects of temperature on anesthesia could be rationalised.

It is noteworthy in connection with the general argument presented above that there is an instance where the potency of an anesthetic passes through a minimum at about 20° as temperature varies from 4 to 28° (butyl acetate immobilisation of *Barnacle nauplii*)[21].

A rigorous discussion of the differences in ΔF_t° among the various anesthetic systems must await identification of the chemical nature of the receptors, especially since it is conceivable that they need not be the same in every tissue or organism. However, it is noteworthy that if the site involves the non-polar portions of membrane lipids arranged in a lamellar array the differences could depend, in part, on the nature of the fatty acid residues of the phosphatides. This possibility arises from the fact that the interaction of phosphatides and cholesterol in monolayers depends on the fatty acid components of the former[46]. Consequently, the free energy of penetration of alcohols would also be expected to depend on their nature. Another possible reason for the differences is that ΔF_t° is probably a composite quantity containing contributions from changes induced in the membrane structure somewhat remote from the receptor site. For instance, if the binding site is a protein, the conformation change undergone by the protein may cause changes in the arrangement of the lipids associated with it. Alternately, if the site consists of lipids, changes might be induced in nearby proteins.

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