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PHYSICAL PARAMETERS OF THE ANESTHETIC SITE

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Summary

Theoretical consideration of the interaction between induced dipoles and a field of force composed of ionic, dipole and London forces are presented. The analysis as applied to the phenomena of general anesthesia allows characterization of the anesthetic site with three physical parameters: (1) the cohesive energy of the site, E_c , (2) the adhesive contribution to the enthalpy of solution, A , and (3) the Barclay-Butler B coefficient. Their values are 13694 cal/mol, 299 (cal/mol)^{1/2} and 0.00237 K⁻¹, respectively. All these values are much higher than the measured values of these same parameters, using noble gases, in dimyristoyl phosphatidylcholine bilayers above their phase transition temperature and the bulk organic liquid, benzene.

The relatively large values of the adhesive term and Barclay-Butler B coefficient imply that the anesthetic site is quite hydrophilic in nature in the sense that it likely possesses a formal charge. The large value of the cohesive energy of the site suggests that the site may either be a boundary lipid or a protein (which we cannot distinguish between) but it is definitely not, for these gases, like a simple lipid bilayer above its transition temperature or like benzene.

An extension of the molecular interpretation of selectivity, that includes other interactions besides the London-Van der Waals type is presented. The selectivity (at constant temperature) is determined by the adhesive term and the Barclay-Butler B coefficient. Using this analysis we found that synapses but not axons exhibit the same selectivity as the anesthetic site. This implies that the synapse is clearly the model to study in attempting to elucidate molecular mechanisms of anesthesia.

Introduction

A number of inert gases are known to cause narcosis in animals by perturbing the normal activity of their central nervous system [1]. Current models of

anesthesia favor a membrane as the site of anesthetic action [2]. As inert gases, such as xenon, can promote anesthesia in animals it is clear that covalent or hydrogen bond formation of the anesthetic with the site is not necessary to produce this effect. It is, therefore, probable that narcosis is due to a physical rather than a chemical interaction of the anesthetic with the site. This view is supported by the correlation between anesthetic potency and molar refraction, polarizability, solubility in olive oil, boiling points of the anesthetic agents, and Van der Waals a and b constants [3].

The nature of the site where the inert gases partition has been the subject of much speculation in recent years. The anesthetic site has been, at one time or another, suggested to be modeled by such chemically and structurally different materials as water, olive oil, benzene, carbon disulfide, lipid bilayers, n -octanol and various proteins [4]. In this context we, in this paper, describe a method to compare some physical and thermodynamic properties of the anesthetic site with model systems, such as benzene and lipid bilayers. The physical parameters obtained from the theory are the cohesive energy of the site, the Barclay-Butler B coefficient and the adhesive contribution to the enthalpy of solution of a molecule from the gas phase to the site.

We have found that substantial differences exist between those parameters found for the anesthetic site and for benzene and lipid bilayers above their phase transition temperature. However, much better agreement exists between the anesthetic site and a lipid bilayer below its phase transition temperature. The results of our analysis suggest that interactions other than simple London types are present in the absorption of the anesthetic to the site. We conclude that the anesthetic site is a "boundary lipid" or a protein. At the present we cannot distinguish between these two possibilities.

Theory

Consider the distribution of an anesthetic agent between two phases, one of which is a gas phase and the other the anesthetic site. At equilibrium * we have from general thermodynamic considerations [6]:

$$\Delta\mu^0 = \mu_L^0 - \mu_g^0 = RT \ln \frac{p}{X} \quad (1)$$

Where μ_L^0 is the standard chemical potential of the agent at the anesthetic site, μ_g^0 its standard chemical potential in the gas phase, p its partial pressure in the gas phase and X its mole fraction at the anesthetic site. We assume the solution in the anesthetic site to be dilute [2]. According to Overton's rule anesthesia will occur when solutes reach a certain concentration at the anesthetic site [2]. Thus Eqn. 1 becomes

$$P_{an} = X e^{\Delta\mu^0/RT} = X e^{\Delta H^0/RT} e^{-\Delta S^0/R} \quad (2)$$

where P_{an} is the anesthetic pressure for a given agent. Since the solution is dilute, the mole fraction is proportional to the concentration and Overton's rule can be expressed in terms of mole fractions.

* It is agreed that general anesthesia is an equilibrium process [7].

The standard enthalpy of a solute in dilute solutions can be expressed quite generally as an algebraic sum of all energy changes that are involved in the process of solution. These energies are cohesive energies holding the solvent molecules together and opposing solution, adhesive energies arising from interactions between solvent and solute. In regard to anesthesia, the anesthetic site (L) will be the solvent and the solute will be the anesthetic gas (g).

The potential energy, V , between an inert gas molecule, with spherical symmetry and zero permanent dipole moment with an ion, a permanent dipole and a molecule like itself can be expressed [8] as

$$V = - \underbrace{\left(\frac{(ze)^2}{2Dr}\right) \left(\frac{k\alpha_g}{r^3}\right)}_{\text{ion-induced dipole}} - \underbrace{\left(\frac{2\mu^2}{D^2r^3}\right) \left(\frac{k\alpha_g}{r^3}\right)}_{\text{dipole-induced dipole}} - \underbrace{\left(\frac{k\alpha_L}{r^3}\right) \left(\frac{k\alpha_g}{r^3}\right)}_{\text{induced dipole-induced dipole}} \quad (3)$$

z is the number of formal charges in the site; e , the charge of an electron; μ , permanent dipole moment; D , dielectric constant, α_g , polarizibility of anesthetic gas; α_L , polarizibility of anesthetic site; k , constant; r , the distance between site and agent molecules. Or

$$V = - \left(\frac{(ze)^2}{2Dr} + \frac{2\mu^2}{D^2r^3} + \frac{k\alpha_L}{r^3} \right) \left(\frac{k\alpha_g}{r^3} \right) \quad (4)$$

From Eqns. 3 and 4 it is clear that if the anesthetic site is devoid of all interactions except the London-Van der Waal's type then

$$V = - \frac{k\alpha_L}{r^3} \cdot \frac{k\alpha_g}{r^3}$$

Since all the terms in Eqn. 4 represent attraction between site and agent we would expect weaker interactions when the site is hydrophobic. Also

$$\frac{k^2\alpha_g^2}{r^6} = a_g \quad (5)$$

where a_g is the Van der Waals constant a for the anesthetic agent [5].

It has been previously shown by Hildebrand [6] and others that under these situations, the energy of vaporization of the gas U_g^v can be written

$$(U_g^v)^{1/2} \alpha \left(\frac{k^2\alpha_g^2}{r^6} \right)^{1/2} = \frac{k\alpha_g}{r^3} \quad (5a)$$

Substituting Eqn. 5 into Eqn. 4 we obtain

$$V = - \left\{ C \left(\frac{(ze)^2}{2Dr} + \frac{2\mu^2}{D^2r^3} + \frac{k\alpha_L}{r^3} \right) \right\} (U_g^v)^{1/2} \quad (6)$$

where C is a constant.

Now, the standard enthalpy of solution, ΔH^0 , can be expressed as follows:

$$\Delta H^0 = \underbrace{E_c}_{\text{cohesive (opposes solution)}} - \underbrace{A(U_g^v)^{1/2}}_{\text{adhesive (promotes solution)}} \quad (7)$$

where E_c is the cohesive energy of anesthetic site and A is the value in $\{ \}$ in Eqn. 6.

It is noteworthy to mention that if ΔH^0 is measured and plotted against $(U_g^v)^{1/2}$, experimentally known quantities, the slope will yield the adhesive contribution and the intercept the cohesive energy of the site.

The standard entropy and enthalpy of solution are not independent of each other. They are related by the famous Barclay-Butler relation [9],

$$\Delta S^0 = -a + B\Delta H^0 \quad (8)$$

the meaning of which was discussed in the literature and its applicability to membranes demonstrated where a and B are temperature-dependent constants [20].

From Eqns. 2, 6, 7 and 8 we obtain

$$P_{an} = X \exp[(E_c - A(U_g^v)^{1/2}) \cdot (1 - BT) + aT/RT]$$

or

$$RT \ln P_{an} = RT \ln X + aT + E_c(1 - BT) - A(1 - BT)(U_g^v)^{1/2} \quad (9)$$

According to our model, we predict that a graph of $RT \ln P_{an}$ vs. $(U_g^v)^{1/2}$ should be a straight line whose slope, $-A(1 - BT)$, reflects only parameters of the site. Should the sites * be different in different species (or the same species) this would be reflected in the above as a change in slope.

The theory mentioned here is similar in its main features to other theories of solution and its applicability range and limitation are discussed elsewhere [10].

A test of the theory

Based on the previous assumptions regarding interactions between site and agent and the applicability of the Overton rule we predicted a linear relation between the square root of the energy of vaporization of the agent and its anesthetic potency.

In Fig. 1 we plot RT times the logarithm of the anesthetic pressure producing loss of righting reflex in mice against the square root of the molar energies of vaporization of different agents, $(U_g^v)^{1/2}$. The values of $(U_g^v)^{1/2}$ were taken from Hildebrand and Scott [6]. Values of anesthetic pressures are from Miller et al. [11] who studied the pressures for the loss of righting reflex in mice reported by many sources and arrived at a "best" value. Strongly hydrogen bonding anesthetics and completely fluorinated compounds with the exception of SF_6 were omitted. The reason for this selection is that the theory, as presently developed, applies only when the anesthetic molecules behave like noble gases [8]. Should this not be the case, Eqn. 9 would not hold and the linear relation described in Fig. 1 is not expected [6]. The reasons being that the energy of vaporization for hydrogen bonding agents are much higher than what is suggested by Eqn. 5a and therefore the particular linear dependence shown in Fig. 1 is not expected to hold for such molecules.

Fluorocompounds are known to behave differently to noble gases and chloroform and therefore are not expected to have the same type of interaction

* We define site as the structure whose interaction with the anesthetic agent results in anesthesia.

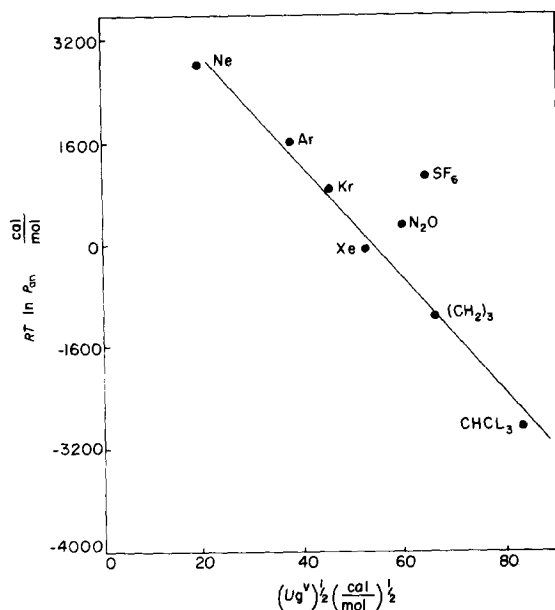


Fig. 1. Graph of $RT \ln P_{an}$ times the logarithm of the pressure necessary to abolish the righting reflex in mice [11] vs. the square root of energy of vaporization of the anesthetic gas [6]. The points are fitted by the straight line that refers to Eqn. 10 in the text.

with a given solvent as does the noble gases or chloroform. SF_6 was introduced to Fig. 1 only to demonstrate the expected deviation of fluorocarbons rather than their place in the correlation. Similar deviations are found for other fluoromolecules.

The graph in Fig. 1 shows a good correlation between $RT \ln P_{an}$ and $(U_g^v)^{1/2}$, supporting the model used to describe anesthesia of noble gases. The straight line in Fig. 1 is fit by the equation

$$RT \ln P_{an} = 4736 - 87.56 (U_g^v)^{1/2}; r^2 = 0.956 \quad (10)$$

Upon examining Eqns. 5, 5a and 9 we would also expect to obtain a relation between $\ln P_{an}$ and $a_g^{1/2}$ where a_g is the Van der Waal's a constant for the anesthetic gas. Indeed, such a relation is described by Koski et al. [12] who used similar data for P_{an} . Koski et al. [12] also developed a model to describe this dependence. Their model resembles ours in some aspects and differs in others. First, their model tacitly assumes that the anesthetic agent absorbs to the site without modifying the site itself, whereas we assume the site to be modified during absorption. This difference will not be reflected in the slope of Fig. 1 but will change the meaning of the intercept. Second, they did not include entropic contributions to absorption and therefore to narcosis in their model. Finally, and most importantly, the interpretation of the slope and intercept of Fig. 1 as reflecting site characteristics were ignored.

It is noteworthy that both U_g^v and a are temperature dependent. Consequently the extrapolation of these "constants" from boiling point temperatures to physiological temperatures may lead to errors and deviations from the cor-

relation described. However, we believe that the use of these values is justified by the agreement established between theory and experiment when used to describe solubilities in bulk hydrocarbons and phospholipid bilayers.

Comparison of anesthetic effects in different animals

It has been observed when measuring the partition coefficient of non-electrolytes from water into a particular solvent and then comparing these values with those obtained by measuring the partition coefficient of these same non-electrolytes into a different solvent that a systematic relationship exists between these two solvent systems. In fact, Collander [13] demonstrated that upon measuring the partition coefficients of a group of solutes, of comparable acidity, into different solvents and comparing the results to each other (e.g. *n*-octanol/water compared to benzene/water) a linear relation exists between the logarithms of the partition coefficients in the different systems as seen below

$$\log K_{i,y} = S_{x,y} \log K_{i,x} + r_{x,y} \quad (11)$$

where $K_{i,y}$ or $K_{i,x}$ is the partition coefficient of solute *i*, between solvent *x* or *y* and water*; $S_{x,y}$ and $r_{x,y}$ are constants for the particular solvents *x* and *y*. Such a relation holds also when one of the systems compared is a biological system. The slope of Eqn. 11 is a measure of the relative selectivity of the system [14]. The more selective one system is over the other the greater the differences between their partition coefficients of different solutes.

Correlations of this kind were used often in the literature to characterize membranes and to evaluate mode of action of drugs [2] and to compare biological systems and models [14]. The physical meaning of selectivity when small non-polar molecules are absorbed by hydrophobic solvents is seen in Eqn. 12. Here we see that the selectivity coefficient obtained upon comparing two solvents is dependent on the contribution of the solvents to adhesion of the solute and to the Barclay-Butler relation equation [8]; that is, the selectivities will be reflected by the slope of the line in Fig. 2. Explicitly the selectivity constant is given by the relation

$$S_{x,y} = \frac{A_x(1 - B_x T)}{A_y(1 - B_y T)} \quad (12)$$

where *x* and *y* specify two different solvents. The theory is consistent with the data for dimyristoyl phosphatidylcholine membranes (above their transition temperature) as well as for bulk hydrocarbons. Since we assumed a similar theory would also apply to general anesthetics, the selectivity constant will reflect differences in site structure when the anesthetic site is compared to well characterized materials.

To determine if various species have similar sites we compared minimum alveolar concentrations [16] in various animals with minimum alveolar concentration in man. The minimum alveolar concentration is proportional to the

* $K_{i,y}$ could also be the partition coefficient between the gas and the solvent in which case *K* can be expressed as a reduced pressure (i.e. P_{an}/P_0^g). It is also possible to multiply Eqn. 11 by *RT* and compare the free energies of transfer.

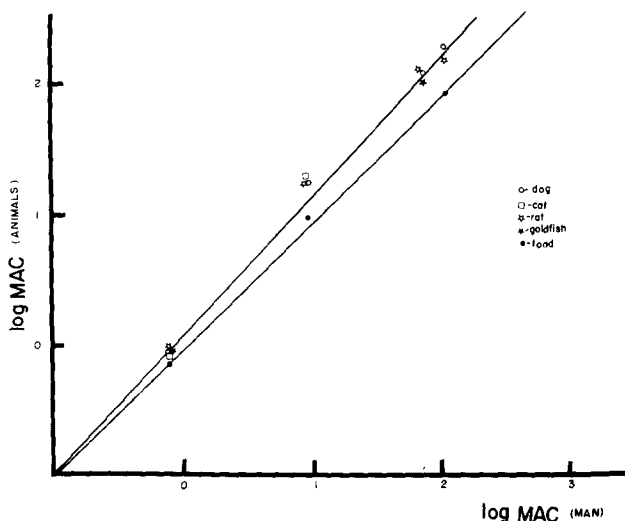


Fig. 2. Comparison of the minimum alveolar concentration of anesthetics (MAC) in man compared to other animals (dog, cat, rat, toad and goldfish). Slopes in the range 0.9–1 are shown.

anesthetic pressure, which in turn is a measure of the standard chemical potential (see note to eqn. 11). Thus, for a given effect, the mol fraction of anesthetic in the site is constant, and consequently the chemical potential, as shown in Fig. 3, will differ from the standard chemical potential by an additive constant. This will not alter the value of $S_{x,y}$ upon comparing two different solvent systems.

In Fig. 2 comparison between minimum alveolar concentrations in dog, cat, rat, toad and goldfish are compared with those in man. Minimum alveolar concentrations used in Fig. 2 are those given by Eger [16]. It is easy to see that the selectivity is close to 1 (ranging from 1.03 in toad to 0.93 in dog and cat). Also the intercept in Fig. 2 shows that the values are not simply proportional, but are nearly identical. In view of the approximations in theory and experimental errors in this correlation it can be taken as an indication that the sites are similar in all animals tested. It is possible that these correlations are not sensitive enough to reflect changes in anesthetic sites from one species or system to another. To demonstrate that selectivity coefficients can and do deviate from a slope of 1 we compare in Fig. 3 solubilities of noble gases in dimyristoyl phosphatidylcholine at 30°C and benzene at 25°C with $RT \log$ minimum alveolar concentrations in man. Selectivity coefficients of 0.64 and 0.99, were found in dimyristoyl phosphatidylcholine and benzene, respectively. Although Fig. 3 is a comparison between free energies and Fig. 2 is a comparison between log minimum alveolar concentrations, the log minimum alveolar concentrations are proportional to the free energies. Moreover, if Overton's rule is assumed, it is a unique function of the free energy.

Thermodynamic functions of anesthetics

In order to compare several systems it is beneficial to know not only chemical potentials but also other thermodynamic parameters such as the entropy and

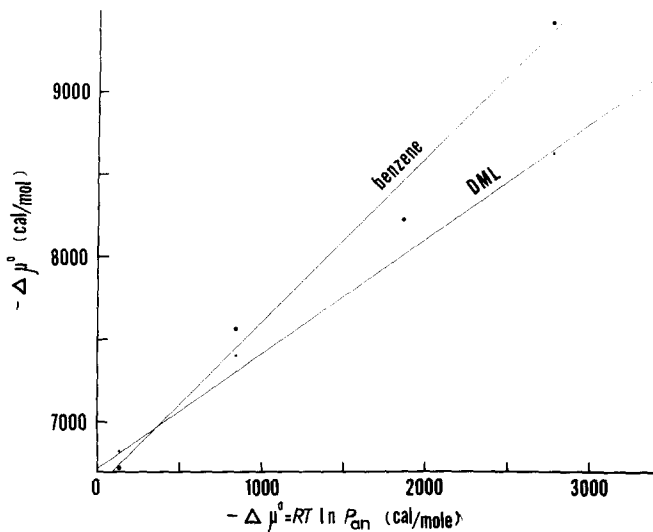


Fig. 3. The free energies of transfer of the noble gases into benzene (●) at 25°C and dimyristoyl phosphatidylcholine (DML) (■) at 30°C vs. RT times the logarithm of the minimum alveolar concentration in man [16]. For the abscissa, Overton's rule was assumed, so that the $\Delta\mu^0$ in the figure differs from the actual $\Delta\mu^0$ by an additive constant, $-RT\ln X$. The slope of the line for benzene is 0.99 indicating that benzene has the same selectivity as the anesthetic site. The slope of the line for dimyristoyl phosphatidylcholine is 0.64 indicating that this lipid above its phase transition temperature does not have the same selectivity as does the anesthetic site. Data for dimyristoyl phosphatidylcholine is obtained from an article in preparation by Y. Katz.

enthalpy. These functions can be obtained by measuring temperature dependence of anesthetic pressure and is available in the literature [17,18,19]. The enthalpy of solution can be expressed in terms of cohesive and adhesive contributions as is shown in Eqn. 7. Hence, by knowing the cohesive and adhesive

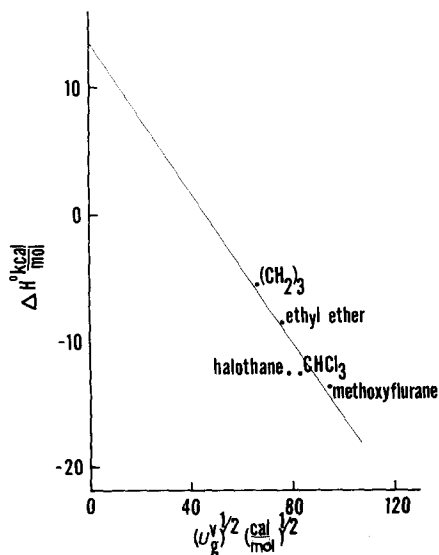


Fig. 4. A graph of the enthalpy of solution, ΔH^0 , as determined by different investigators [17,18,19] for anesthetic gases versus the square root of energy of vaporization of these gases [6]. The straight line in the figure refers to Eqn. 14 in the text.

TABLE I

System property	Loss of righting reflex	Benzene	Dimyristoyl phosphatidyl- choline ($T > T_m$)	Dimyristoyl phosphatidyl- choline ($T < T_m$)
Selectivity	1	1	0.64	n.a.
Cohesive energy (cal/mol)	13 694	5510	4600	11 250 ^a
Adhesive contribution (cal/mol) ^{1/2}	299	138	119 ^c	n.a.
Barclay-Butler B constant (K^{-1})	0.00237	0.0013	0.00178 (0.00226) ^b	(0.00296) ^b

^a Calculated from $E_T \approx E^C + \Delta H_m$; ΔH_m from ref. 30.

^b From ref. 20 (for non-electrolytes).

^c Data from Y. Katz (in preparation), n.a., data not available.

contributions separately it is then possible to calculate enthalpy and, conversely from measured enthalpies, it is possible to extract the site parameters for cohesion and adhesion since under these conditions we have two equations with two unknowns.

The cohesive and adhesive parts of the enthalpy of solution were calculated from Eqn. 7 using experimentally determined values of ΔH^0 [17,18,19] and $(U_g^v)^{1/2}$ from Hildebrand and Scott [6]. Fig. 4 presents the data in graphical form. A least-square fit to the data yields the following equations:

$$\Delta H^0 = 13102 - 297 (U_g^v)^{1/2}; \quad r^2 = 0.82 \quad (13)$$

$$\Delta H^0 = 13694 - 299 (U_g^v)^{1/2}; \quad r^2 = 0.92 \quad (14)$$

Eqn. 13 is the equation for the data with the point included for halothane and Eqn. 14 without halothane. Eqn. 14 gives a better fit, consequently we will use the values obtained in Eqn. 14. This difference will not change any of our conclusions. We note that we had some latitude in choosing values of ΔH^0 in the literature. The values of the cohesive and adhesive contributions for the anesthetic site are considerably higher than the values for dimyristoyl phosphatidylcholine above its transition temperature or benzene at 25°C (see Table I). This point will be discussed in more detail later.

Estimation of the Barclay-Butler constants for anesthesia

From Fig. 1 and the calculated site parameters obtained from Eqn. 14 we can calculate a value of the Barclay-Butler B coefficient. From Eqn. 10 we found $A(1 - BT) = 87.6$ (cal/mol)^{1/2} and combining the above with the value of A obtained from Eqn. 14 we obtain a value of $B = 0.00237^\circ K^{-1}$ for the Barclay-Butler B coefficient. Even without considering the physical meaning of this constant, it is clear that it can be used to characterize sites and illustrate differences between them. Different constants for sites implies that the sites differ from each other. The inverse statement is naturally false.

The value of B of the anesthetic site obtained using ΔS^0 and ΔH^0 values of the noble gases, are considerably higher than the B values of benzene and dimyristoyl phosphatidylcholine above its phase transition temperature (Table I).

This value is also higher than the B value obtained for dimyristoyl phosphatidylcholine above its phase transition temperature ($B = 0.00226 \text{ K}^{-1}$) as determined by partitioning non-electrolytes, rather than noble gases into dimyristoyl phosphatidylcholine [20]. Again, for non-electrolytes the value of B for dimyristoyl phosphatidylcholine below is transition temperature ($B = 0.00296 \text{ K}^{-1}$) is greater than the value for the anesthetic site. These results are consistent with the molecular interpretation of B values. Generally, the lower the B value the more non-polar the solvent. Thus as expected, noble gases partition into a more hydrophobic environment than non-electrolytes which seem to accumulate at the interfacial region of the bilayer. The great differences in the B values clearly distinguish between regions in the bilayer. In this context the anesthetic site is a relatively hydrophilic environment.

Comparison of effects of anesthetics on narcosis and on peripheral nerve

Experiments using anesthetics on peripheral nerve were done not only for pharmacological reasons but also to determine if this preparation would be a good model for the anesthetic site [21,22]. It was shown that anesthetic agents interfere with nerve conductance, and at high concentrations block it totally. Hence, we compared the effects of anesthetics on producing narcosis with those blocked on nerve conductance. Then using Eqn. 11 in much the same way as we did in Figs. 2 and 3 we may obtain a selectivity coefficient to see if these two "solvent" systems are the same.

If the cellular structures involved in narcosis and in nerve conductance were similar we would expect to have not only the same selectivity patterns in both cases but also the same slope as in Fig. 1. This is equivalent to demanding that a

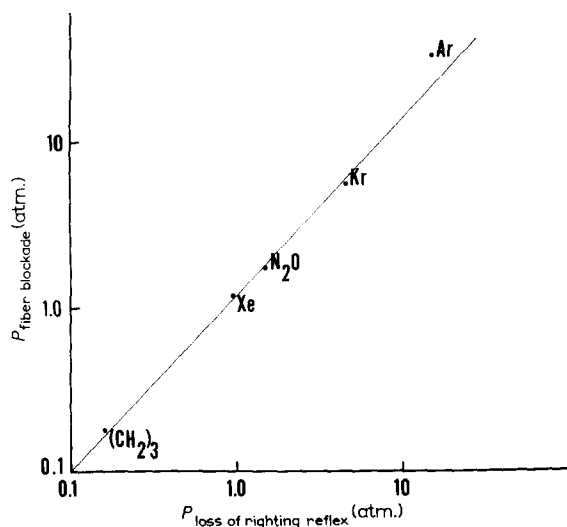


Fig. 5. This figure is a selectivity graph that compares the pressure necessary to cause blockage of 50% of the axons [21,22] vs. the pressure necessary to cause a loss of righting reflex in mice [11]. The straight line has a slope of 0.86 and is fitted by Eqn. 15 in the text and shows that these axons do not exhibit the same selectivity as does the anesthetic site.

log vs. log plot of anesthetic pressures for loss of righting reflex (RR) (in mice) versus pressure causing nerve blockade will give a straight line with a slope of 1, or equivalently that $d\log P_{RR}/d\log P_{\text{nerve block}} = 1$. Such a plot is shown in Fig. 5. The slope is 0.86 indicating that loss of righting reflex and nerve conduction blockage occur at different anesthetic sites. The deviation of the value of the slope from 1 is larger than could be accounted for by experimental errors since errors needed to cause such a deviation have to be over an order of magnitude due to the use of logarithmic scales.

The equation for the straight line is

$$\log P_{\text{axon}} = -0.906 + 0.86 \log P_{RR}; \quad r^2 = 0.97 \quad (15)$$

It is possible, however, to obtain a slope of 1 when using the average values of the E_{50} data obtained by Richards and White [23], who measured the depression of synaptic transmission in the dentate gyrus and olfactory cortex. Fig. 6 shows a graph of the logarithms of pressure necessary to abolish the rolling reflex in mice, P_{RR} , vs. the pressure necessary to cause a given depression of synaptic conduction.

The general expression for the relationship between P_{RR} and P_{synapse} is

$$\log P_{RR} = a_0 + a_1 \log P_{\text{synapse}} \quad (16)$$

The solid lines in Fig. 6 we described by following equations:

$$\text{Dentate gyrus:} \quad \log P_{RR} = 0.167 + 0.973 \log P_{\text{dg}}; \quad r^2 = 0.970 \quad (17)$$

$$\text{Olfactory cortex:} \quad \log P_{RR} = 0.083 + 0.990 \log P_{\text{oc}}; \quad r^2 = 0.999 \quad (18)$$

Such agreement implies that the anesthetic molecules cause their physiological action by perturbing conduction in the synapse rather than by impairing

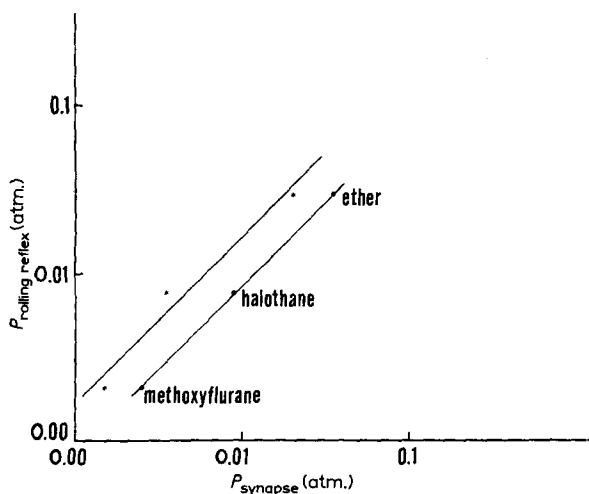


Fig. 6. The selectivity of the synapse as revealed by plotting the pressure necessary to abolish the rolling reflex [11] vs. the pressure necessary to cause a specific depression in the Dentate gyrus (*) and the olfactory cortex (●) [23]. The straight lines are given by Eqns. 17 and 18 in the text and they have slopes of 0.97 and 0.99, respectively. The values of these slopes indicate that these synapses have the same selectivity as does the anesthetic site.

axonal conduction. This of course, is not a new suggestion [2], as it has been shown that synapses are much more sensitive to anesthetics than axons. Richards and White [23] have suggested that this could be the result of decreasing the output of the transmitter from the presynaptic terminal or by decreasing the sensitivity of the post-synaptic membrane to the transmitter or both.

Solvent models system of anesthesia properties of the anesthetic site

A correlation found by Overton [24] between oil solubility of anesthetic agents and their anesthetic potency was interpreted as indicating that the anesthetic site is in the membrane and that membranes have hydrophobic regions. This was followed by works on other bulk solvent model systems having properties in common with the anesthetic site [4] as well as model membranes. Consequently a theory was proposed according to which anesthetic agents cause anesthesia by modifying the lipid bilayer structure of a biological membrane [25]. In this section we will test these proposals using the relations developed above.

The theory developed above for the action of certain general anesthetics is an extension of a theory that was developed and tested for solubility of noble gases in hydrophobic solvents and phospholipids. A common feature for both theories is the demand that comparable systems will have the same selectivity coefficients. Another common feature is that both theories make predictions about the cohesive energy and the solvent dependent part of the adhesive energy and the value of these energies as characterizing the solvent system is stressed. Thirdly, each solvent is characterized by its Barcley-Butler coefficients correlating the entropies and enthalpies of solution in the given solvent.

A comparison between the standard chemical potential of solubility of the noble gases neon, argon, krypton and xenon in benzene and dimyristoyl phosphatidylcholine and the logarithm of the pressure needed to cause loss of righting reflex in mice is depicted in Fig. 3. The $\ln P_{an}$ values were multiplied by an RT factor to facilitate comparison. The standard chemical potential of solubility is defined as $\Delta\mu^0 = RT\ln P/X$. Whenever X is kept constant, as was assumed to be the case in narcosis, the change of $\ln P_{an}$ will reflect the change in $\Delta\mu^0$. As was discussed previously this is certainly a good approximation for a fixed temperature. Similar systems are expected to have a similar change in $\Delta\mu^0$ values in passing from one solute to the other. Since different units are involved in the comparison (and also the ommittance of the constant factor $RT\ln X$ in the values for the loss of righting reflex) one has to expect different values on the abscissa and the ordinate. However, the difference is by a constant and does not affect the magnitude of the number except for an additive constant. Comparable systems have to show a slope of 1, whereas systems differing in properties will have slopes deviating from 1. The comparison in Fig. 3 gives a slope of 0.99 when benzene solubilities are compared to loss of righting reflex in mice by the same solutes, whereas for dimyristoyl phosphatidylcholine the slope is 0.64.

Other parameters that can be used as basis for comparison are: (1) the cohesive energies of the site and of model solvents and (2) the solvent's (site or model solvent) contribution to adhesion between the solvent and the solute

anesthetic. These parameters are characteristic of the solvent and as such can be used to compare solvent systems. The cohesive energy is related to other physical properties of the solvent, such as surface tension and compressibility and the adhesive contribution as it affects the selectivity of the system.

Table I shows the comparative values of the adhesive, cohesive and Barclay-Butler B coefficient; of the anesthetic site, benzene and dimyristoyl phosphatidylcholine above and below its transition temperature. It is clear that the cohesive energy of the anesthetic site (13694 cal/mol) is considerably higher than that for, the organic liquid, benzene (5510 cal/mol) and for dimyristoyl phosphatidylcholine above its transition temperature (4600 cal/mol). However, we note that it is considerably closer to the value of dimyristoyl phosphatidylcholine below its transition temperature (11250 cal/mol).

The sites' contribution to adhesion of $299 \text{ (cal/mol)}^{1/2}$ is considerably higher than the value of benzene $(138.5 \text{ cal/mol})^{1/2}$ and for dimyristoyl phosphatidylcholine above its transition temperature $(119 \text{ cal/mol})^{1/2}$.

The Barclay-Butler B coefficient, which characterizes the site, of 0.00237 K^{-1} , as previously stated, is much greater than the B coefficient that describes the site where anesthetics partition in dimyristoyl phosphatidylcholine above its phase transition or in benzene, but is at a somewhat higher value than the place where non-electrolytes partition into dimyristoyl phosphatidylcholine above its phase transition. This, of course, is a relatively hydrophilic site.

From these comparisons we may say the anesthetic site for non-polar gases has the following characteristics. It has a selectivity much like benzene, a cohesive energy much like a lipid bilayer below its transition temperature and from the Barclay-Butler B coefficient a relatively hydrophilic environment, one that is somewhat more polar than that which non-electrolytes see when they partition into dimyristoyl phosphatidylcholine above its transition temperature but less polar than the environment that non-electrolytes experience below the transition region. This environment is considerably more polar than the environment the anesthetic molecules will experience in benzene.

As the selectivities for benzene and the anesthetic site are similar benzene is a good model solvent to use to test for anesthetic potency [26]. However, as the cohesive energies of the site and benzene are considerably different, and as it is the cohesive energy which determines physical properties of the material one cannot use benzene or other simple organic liquids to obtain information about the physical properties of the anesthetic site.

The relatively large value of the adhesive contribution to the enthalpy of solution of a noble gas to the anesthetic as compared to benzene and dimyristoyl phosphatidylcholine (above T_m) rules out the possibility that the interaction only involves dispersion forces. This can be seen in Table I where the one finds that the values of the adhesive contributions for noble gases into benzene and dimyristoyl phosphatidylcholine (above T_m), which are primarily due to dispersion forces, are considerably lower than the adhesive contribution for the anesthetic site.

Nor can the interaction of the noble gases with a permanent dipole account for the approximately 100% increase in the adhesive contribution. The dipole-induced dipole interaction can contribute up to 20% of the interaction energy between noble gases and permanent dipoles [8]. Rather, it is likely that the

large value of the adhesive contribution is due to an ion-induced dipole interaction. This interaction is the only one of sufficient strength to account for the large value of the adhesion.

Finally, we would like to offer some speculations as to the nature of the anesthetic site. Two possibilities exist, which to our minds are currently indistinguishable. The first is that the anesthetic absorbs into the boundary lipids [27] of this protein(s) that are responsible for the change in permeability that will eventually lead to the physiological state called anesthesia. The reason for choosing the boundary lipids is that they are relatively immobilized in comparison with other lipids not associated with proteins, but not as restricted as lipids below their phase transition. In this regard they should have a higher cohesive energy than non-associated lipids. As a result of this high cohesive energy (which opposes solubility) one might not expect the noble gases to penetrate as "deep" into the bilayer as they would if the lipids were not bound. Consequently the gases will see a more hydrophilic environment, which could account for the high value of the Barcley-Butler *B* coefficient that was obtained. The partition of the anesthetic gases into boundary lipids was recently suggested by others for different reasons [28].

A second possibility would be that the gases partition into a protein molecule [29]. In many proteins, the non-polar amino acids are very tightly packed and proteins usually carry a net formal charge.

The actual nature of the site will of course be determined by experiment.

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