Biophysical Chemistry 22 (1985) 249–254
Elsevier

BPC 01007

THE EFFECT OF ANESTHETICS ON HYDROGEN BONDS

AN INFRARED STUDY AT LOW ANESTHETIC CONCENTRATIONS

René BUCHET and Camille SANDORFY

Département de Chimie, Université de Montréal, Montréal, Québec H3C 3V1, Canada

Received 4th April 1985
Accepted 10th May 1985

Key words: Hydrogen bonding; Hydrophobic interaction; Anesthetic; Infrared spectroscopy; N-Ethylacetamide

It is shown that a striking parallelism exists between the anesthetic potency of general halocarbon anesthetics and their influence on the hydrogen bond association constants in N-H...O=C type hydrogen bonds, important for shaping the ion channels. It is further shown that the effect of potent anesthetics (which contain an acidic hydrogen) on the free/associated ratio in such hydrogen bonds is still significant at clinical anesthetic concentrations. It is argued that the results are in keeping with a pluralistic theory of anesthesia based on both hydrophobic and polar interactions.

1. Introduction

Current theories of anesthesia are based on the assumption that anesthetics exert their action in the hydrophobic parts of neuronal membranes (for reviews see refs. 1–5).

In previous publications of our laboratory, however, attention has been drawn to a property of anesthetics which might be relevant to the problem: they alter the free/associated ratio in H-bonds in favor of the free or less associated species [6–12]. This has been shown to be the case for N-H...O=C, N-H...N, O-H...O, S-H...S, etc., type H-bonds and for general anesthetics of the halofluorocarbon type but also for barbiturates, lidocaine, tetracaine, and other types of anesthetics [13]. General anesthetics such as halothane ($CF_3CHClBr$), or methoxyflurane (CH_3OCF_2-$CHCl_2$) provide striking examples of this effect.

As a result of our numerous model studies we have proposed a pluralistic theory of anesthesia: while weak anesthetic action might be obtained through hydrophobic interactions only, the action of potent anesthetics must involve polar interactions, in particular the dissociation and formation of H-bonds [8–10]. The main tool used in these studies was infrared spectroscopy [6–10]. The experimental results were corroborated by quantum-chemical calculations [11,12].

Two objections could be made to our work. First, much (but not all) of our initial spectra had been recorded at low temperatures in order to magnify H-bond breaking effects. This objection has been countered by the room temperature overtone work of Trudeau et al. [9]. Second, the anesthetic concentration was, in most (but not all) cases, much higher than under clinical conditions. The present paper intends to counter this latter argument.

2. Outline of the work

A solution of *N*-ethylacetamide (NEA) in CCl_4 was chosen as a model system with a number of general halocarbon anesthetics. The concentration of the latter was varied, making it possible to compute approximate values of the H-bond associ-

ation constants for equilibria of the type:

$$\text{N-H}\ldots\text{O=C} + \text{CHCl}_3 \rightleftharpoons \text{CCl}_3\text{C-H}\ldots\text{O=C} + \text{NH} \quad (1)$$

Halocarbons containing no acidic hydrogen also exert an influence on H-bond equilibria but a much weaker one. Therefore, a number of anesthetics, some containing an acidic hydrogen and some not, were tried. The possession of the association constants made it possible to extrapolate to very low anesthetic concentrations.

The N-H...O=C H-bond formed by NEA is also typical of proteins and peptides. Thus, in a qualitative sense, it can represent the H-bonds which are necessary to ensure the right structure and dimensions of the ion channels. These are located in proteins spanning the neuronal membrane; the proper functioning of the nervous system depends on them.

In order to estimate association constants the following equilibria were considered:

$$\text{N} + \text{N} \rightleftharpoons \text{N}_2 \qquad K_1 = \frac{[\text{N}_2]}{[\text{N}]^2} \quad (2)$$

and

$$\text{N}_2 + \text{A} \rightleftharpoons \text{NA} + \text{N} \qquad K_2 = \frac{[\text{NA}][\text{N}]}{[\text{N}_2][\text{A}]} \quad (3)$$

where N represents NEA and A anesthetic.

The concentration of NEA was 70 mM throughout. At this concentration NEA exists almost exclusively in the monomeric or dimeric form (50–50%), higher associations being negligible. Eq. 2 was therefore considered sufficient to take account of the self-association of NEA. Higher associations are possible between NEA and anesthetics; however, this is considered unlikely at the given concentrations.

The self-association constant, K_1, was obtained by using a nonlinear least-square minimization method (ref. 14 and references cited therein) with eqs. 2 and 4:

$$A = \varepsilon l[\text{N}] \quad (4)$$

where A denotes the absorbance of the monomer, ε its molar absorption coefficient, l the cell length (0.101 cm) and [N] the molar concentration of the monomer.

The constant K_2 was obtained through eqs. 2, 3 and 5:

$$A = \varepsilon l\{[\text{N}] + [\text{NA}]\} \quad (5)$$

where A, as above, represents the absorbance of the free NH stretching band and [NA] the concentration of the NEA-anesthetic complex. The latter contains an H-bond formed by the acidic hydrogen of the anesthetic and the carbonyl group of the amide, so that the NH becomes free. Nonlinear least-square minimization was used [14]. Only the concentration of the anesthetics was varied.

3. Experimental

NEA (Eastman Chemical Co.) was distilled under vacuum. Spectrograde CCl_4, $CHCl_3$, CH_2Cl_2 (all from American Chemical Ltd.), $CHBr_3$ (Fisher Scientific Co.), and *n*-decane (Phillips Petroleum Co.) were purified according to the procedures described by Perrin et al. [15]. Freon-11 ($CFCl_3$) and freon-114-*b*-2 (CF_2BrCF_2Br) (from Du Pont) were purified by distillation. Halothane (Ayerst Laboratories), methoxyflurane (Abbott Laboratories) and enflurane (Ohio Medical of Canada, Inc.) and the other anesthetics (P.C.R.) were used without further purification. C^2HCl_3 (99.8%) was obtained from the Cambridge Isotope Laboratories.

A Perkin-Elmer model 621 infrared spectrometer was used with a resolution of the order of 2 cm^{-1} with 0.101 cm cells.

4. Results

The bands in the infrared spectrum of NEA have been assigned previously [16,17]. A part of the spectrum is shown in fig. 1. The band at 3460 cm^{-1} belongs to the free NH vibration of *trans*-NEA and the weak shoulder at about 3418 cm^{-1} to that of *cis*-NEA. The broad band centered at 3300 cm^{-1} belongs to the stretching vibration of the associated NH group of *trans*-NEA while its

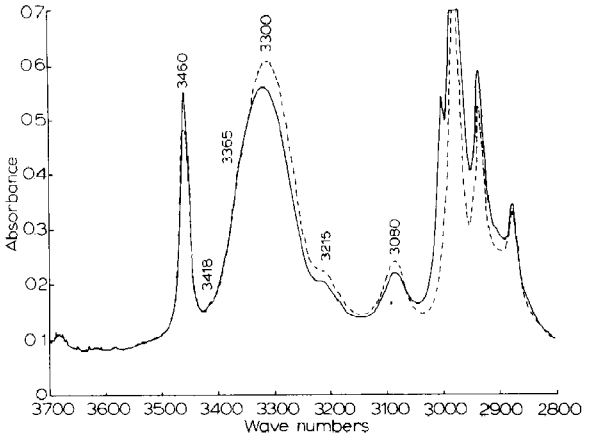

Fig. 1. Part of the infrared absorption spectrum of 0.07 M NEA (— — —) and of 0.07 M NEA+0.046 M halothane (———) in CCl_4.

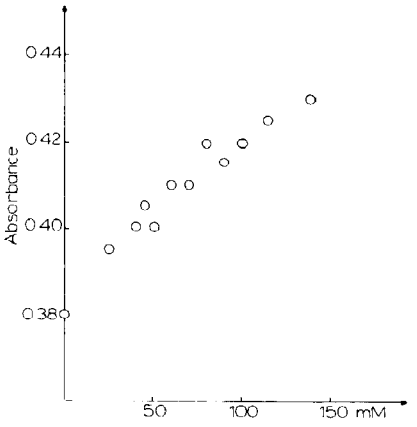

Fig. 2. Variation of the intensity of the free NH stretching vibration (3460 cm^{-1}) of NEA as a function of halothane concentration (in CCl_4). The concentration of NEA was kept constant at 0.07 M.

shoulder at about 3365 cm^{-1} is due to the first overtone of the amide I vibration which is at 1688 cm^{-1}. The band at 3215 cm^{-1} was assigned to the corresponding band of the associated NH group of *cis*-NEA [17] or to the summation band of the amide I and amide II vibrations [16]. This is of no consequence for the present study. The band at 3080 cm^{-1} is the first overtone of the amide II band (1550 cm^{-1}); its intensity is boosted by Fermi resonance with the 3300 cm^{-1} band [17]. In fig. 1 the spectrum of a 70 mM solution of NEA in CCl_4 is compared to the spectrum of the same solution with 46 mM halothane added (solid line). As expected, in presence of halothane, the free NH band at 3460 cm^{-1} increases and the association band at 3300 cm^{-1} decreases in intensity, showing that a part of the N-H...O=C hydrogen bonds have been dissociated.

In fig. 2 the variation of the intensity of the free NH band at 3460 cm^{-1} with anesthetic concentration is illustrated. Although the changes are, of course, not as spectacular as those at low temperatures or at high anesthetic concentrations, they are readily observable. From these we computed the dissociation constant K_2 (eq. 3) for a number of anesthetics and also K_3, the NEA-anesthetic association constant:

$$K_3 = K_1 K_2 = \frac{[NA]}{[N][A]} \tag{6}$$

For the self-association constant of NEA we obtained 18 ± 3 M^{-1}; this value and the molar absorption coefficient for the 3460 cm^{-1} band, 110 ± 10 M^{-1} cm^{-1}, were used in the calculations. The concentration of NEA was held constant at 70 mM while the concentration of the anesthetic was varied from 0.1 to 0.5 M. The results are presented in table 1. Table 1 includes log AD_{50} and $\log(1/P)$ values which are considered as measures of the anesthetic potency. The former gives the vapor concentration of the anesthetic at which half of the experimental animals are in a state of narcosis, in the latter P is the anesthetic pressure expressed in atmospheres at which the righting reflex of half of the mice is suppressed. [1]

The parallelism between anesthetic potency and the constants K_2 and K_3 is evident. The weak anesthetics in the upper part of table 1 have zero or low K_2 and K_3 values. These are hydrocarbons or halocarbons containing no acidic hydrogen. CH_2Cl_2 is an example of a moderately strong anesthetic. The potent anesthetics shown in the lower part of table 1, like $CHCl_3$ halothane and

Table 1

Equilibrium constants K_2 and K_3 of NEA with different anesthetics in CCl_4

The anesthetic potencies log AD_{50} and log$(1/P)$ are shown for comparison. $T = 21 \pm 1°C$.

Sample	K_2	K_3 (M^{-1})	log AD_{50}	log$(1/P)$
$CH_3(CH_2)_8CH_3$	≈ 0	≈ 0		
$CH_3(CH_2)_3CH_3$	≈ 0	≈ 0		
$CFCl_3$	≈ 0	≈ 0		
CF_3CCl_3	≈ 0	≈ 0		
$CBrF_2CBrF_2$	0.005 ± 0.001	0.09 ± 0.02		
$CHCl{=}CCl_2$	0.013 ± 0.004	0.23 ± 0.07		
$CHBrCl_2$	0.014 ± 0.002	0.25 ± 0.04		
CH_2Cl_2	0.015 ± 0.004	0.27 ± 0.07	0.49 [18]	1.52 [19]
$CF_2BrCBrH_2$	0.018 ± 0.005	0.32 ± 0.09		
$CHCl_2CF_2OCH_3$	0.026 ± 0.005	0.47 ± 0.09		2.66 [19]
$CHBr_3$	0.028 ± 0.006	0.50 ± 0.11		
$CHClFCClH_2$	0.032 ± 0.006	0.57 ± 0.11		
$CHIFCClF_2$	0.035 ± 0.005	0.63 ± 0.09		
$CHClFCF_2OCF_2H$	0.039 ± 0.006	0.70 ± 0.11		
$CHCl_3$	0.041 ± 0.005	0.74 ± 0.09	−0.25 [20]	2.08 [19]
$CDCl_3$	0.042 ± 0.005	0.76 ± 0.11		
$CHClFCHClF$	0.043 ± 0.007	0.77 ± 0.13	−0.60 [18]	
$CHCl_2CClF_2$	0.047 ± 0.006	0.84 ± 0.11	−0.22 [18]	
$CHClFCBrF_2$	0.049 ± 0.006	0.88 ± 0.11	0.11 [18]	
$CHBrClCF_3$	0.060 ± 0.011	1.08 ± 0.20	−0.16 [18]	2.11 [19]

methoxyflurane have high K_2 and K_3 values. They all contain an acidic hydrogen.

In the case of the *N*-methylacetamide/$CHCl_3$ system, the association constants were determined several years ago by ^{1}H-NMR spectroscopy by Takahashi and Li [21] who obtained $K_3 = 0.46$ M^{-1} at 16°C. Our value is 0.74 ± 0.09 M^{-1} at 21°C for the NEA/$CHCl_3$ system.

5. Discussion

The hydrophobic theory of anesthesia stems from the Meyer-Overton rule which is a smooth relationship between lipid solubility and anesthetic potency. However, one of our previous spectroscopic studies [9] indicated that such a relationship also exists between H-bond breaking ability and anesthetic potency. The present study now provides us with such a relationship with H-bond association constants which are based on infrared intensities measured at a series of different concentrations. This shows that lipid solubility is not the only quantity having a good correlation with anesthetic potency.

But do anesthetics affect H-bonds at clinical concentrations? Fig. 3 is an attempt to answer this question. In fig. 3, the concentration of a moderately strong anesthetic, CH_2Cl_2, a strong anesthetic $CHCl_3$ and an even stronger anesthetic, $CF_3CHClBr$ (halothane) are plotted against the percentage of dissociated H-bonds, computed using the constants K_2 and K_3. The concentrations vary from 0 to 25 mM. The changes produced in the H-bond equilibrium at these low concentrations are small but significant. For example, 10 mM halothane dissociates about 1% of the H-bonds in dimeric NEA. Would this be sufficient to affect the functioning of the nervous system? It is believed that it would, for two reasons. First, there is much more orientation in a cell membrane than in a CCl_4 solution making it easier for an anesthetic to enter into associations. Second, it seems to be a logical thought that blocking even a fraction of the ion channels could temporarily block the functioning of the nervous system.

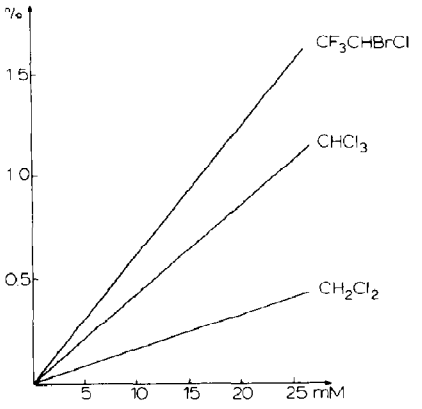

Fig. 3. Dissociation (in %) of dimeric NEA as a function of the concentration of the anesthetics: $CF_3CHBrCl$, $CHCl_3$ and CH_2Cl_2.

In the work presented here an N-H...O=C hydrogen bond has been used as a model. This type of H-bond is highly characteristic of proteins. Now, the ion channels are formed in proteins. Thus, the alteration of the free/associated ratio in the H-bonds that are needed to give the channel its proper structure and dimensions could be the main action of a potent anesthetic such as $CHCl_3$, halothane, methoxyflurane and many others. A strong experimental indication for this has already been provided by Urry and his co-workers [22,23] by measuring the current across model gramicidin A ion channels. Infrared spectroscopic evidence for this in the same system (in the presence of water and lipid) has recently been obtained by Buchet et al. [24].

The Meyer-Overton relationship shows, however, that anesthetics must first enter the lipid part of the membrane before they can act upon ion channels. Their disordering effect exerted therein could in itself be a cause of a moderate degree of anesthetic action by indirectly affecting ion channels. Whether the actual site of action is located in the brain or elsewhere in the organism would make no difference in this respect.

All this, we believe, is in line with our proposed pluralistic theory: weak anesthetic action is possible through hydrophobic interactions only but strong anesthetic action necessitates, in addition, polar interactions and, in particular, a change of the free/associated ratio in certain H-bonds in the ion channels.

Other H-bonds might also be affected. These could be, among others, ester carbonyl/water or ester carbonyl/cholesterol H-bonds. A suggestion to this effect has been made previously [25]. Still another possibility is the amide carbonyl/water or cholesterol H-bond in sphingolipids present in neuronal membranes [25]. In this respect we can mention the work by Ueda and co-workers [26] who have shown that general anesthetics weaken the H-bonds between water and membrane lipids, releasing bound interfacial water. They suggested that this is related to the molecular mechanisms of anesthesia.

An unexpected result has been obtained in the case of methoxyflurane. The latter possesses a higher anesthetic potency than either $CHCl_3$ or halothane, yet the association constants K_2 and K_3 place it within the range of moderately strong anesthetics. However, it should be borne in mind that we are using the free NH stretching vibration in evaluating these constants. Now, the methoxy group with its oxygen lone pair can serve as a proton acceptor for the free NH groups. So, while the molecule acts as a proton donor by its acidic hydrogen producing free NH groups, the methoxy group decreases their number. This then leads to a low apparent value of the association constants even though the H-bond breaking ability of this anesthetic is actually high.

6. Conclusions

It clearly appears from the above results that H-bond association constants in N-H...O=C type H-bonds are affected by the presence of general anesthetics. Such H-bonds, typical of proteins, play an important role in determining the structure of ion channels on which the functioning of the nervous system depends. It has been shown that an evident parallelism exists between anesthetic potency and these association constants. Furthermore, it could be ascertained that this effect is still significant at low (clinical) anesthetic concentrations.

These results are in line with a pluralistic theory

of anesthesia. By this we mean that weak anesthetic action might be obtained by hydrophobic interactions between an anesthetic and the lipid part of the neuronal membrane affecting the ion channels indirectly. For strong anesthetic action, however, anesthetics must also influence the ion channels directly through altering the free/associated ratio in H-bonds on which their structure depends.

Acknowledgements

Financial assistance from the Natural Sciences and Engineering Research Council of Canada is acknowledged. R.B. is indebted for a graduate scholarship from the World University Service of Canada.

References

1 K.W. Miller and E.B. Smith, in: A guide to molecular pharmacology-toxicology, part 2, ed. R.M. Featherstone (Marcel Dekker, New York, 1973) p. 427.
2 M.J. Halsey, in: Anesthetic uptake and action, ed. E.I. Eger (Williams and Wilkins, Baltimore, 1974) p. 45.
3 J.C. Miller and K.W. Miller, in: Physiological and pharmacological biochemistry, ed. H.K.F. Blaschko (Butterworths, London, 1975) p. 33.
4 R.D. Kaufman, Anesthesiology 46 (1977) 49.
5 D.D. Denson, Chemtechnology 8 (1977) 446.
6 T. Di Paolo and C. Sandorfy, J. Med. Chem. 17 (1974) 809.
7 T. Di Paolo and C. Sandorfy, Can. J. Chem. 52 (1974) 3612.
8 C. Sandorfy, Anesthesiology 48 (1978) 357.
9 G. Trudeau, K.C. Cole, R. Massuda and C. Sandorfy, Can. J. Chem. 56 (1978) 1681.
10 G. Trudeau, P. Dupuis, C. Sandorfy, J.M. Dumas and M. Guérin, Curr. Chem. 93 (1980) 91.
11 P. Hobza, F. Mulder and C. Sandorfy, J. Am. Chem. Soc. 103 (1981) 1360.
12 P. Hobza, F. Mulder and C. Sandorfy, J. Am. Chem. Soc. 104 (1982) 925.
13 M. Guérin, J.M. Dumas and C. Sandorfy, Can. J. Chem. 19 (1980) 2080.
14 R. Buchet, L. Beauvais and C. Sandorfy, J. Biomol. Struct. Dyn. 2 (1984) 221.
15 D.D. Perrin, W.L.F. Armareqo and D.R. Perrin, Purification of laboratory chemicals, 2nd edn. (Pergamon Press, Oxford, 1980).
16 F. Fillaux and C. de Lozé, J. Chim. Phys. 69 (1972) 36.
17 R.L. Jones, Spectrochim. Acta 23A (1967) 1745.
18 R.H. Davies, R.D. Bagnall and W.G.M. Jones, Int. J. Quantum Chem. Quantum Biol. Symp. 1 (1974) 201.
19 K.W. Miller, W.D. Paton, E.B. Smith, Nature 206 (1965) 574.
20 B.H. Robbins, J. Pharmacol. Exp. Ther. 86 (1946) 197.
21 F. Takahashi and N.C. Li, J. Phys. Chem. 69 (1965) 2950.
22 R.J. Bradley, D.W. Urry, G. Parenti-Castelli and G. Lenaz, Biochem. Biophys. Res. Commun. 101 (1981) 963.
23 D.W. Urry, G.M. Venkatachalam, K.U. Prasad, R.J. Bradley, G. Parenti-Castelli and G. Lenaz, Int. J. Quantum Chem. Quantum Biol. Symp. 8 (1981) 385.
24 R. Buchet, C. Sandorfy, T.L. Trapane and D.W. Urry, to be published.
25 P. Mercier, C. Sandorfy and D. Vocelle, J. Phys. Chem. 87 (1983) 3670.
26 T. Yoshida, H. Okabayashi, K. Takahashi and I. Ueda, Biochim. Biophys. Acta 772 (1984) 102.