

Weak Intermolecular Associations and Anesthesia

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THE right structure and functioning of biologic macromolecules are ensured by a great number of intermolecular associations, hydrogen bonds, and van der Waals associations. Because general anesthesia must be reversible its action is likely to consist in the replacement or perturbation of weak hydrogen bonds or van der Waals associations by others involving the anesthetic. A qualitative understanding of general anesthesia can be based on this idea.

Hydrogen bonds (H-bonds) are ubiquitous in nature. It is no overstatement to say that life would not be possible without them. Water would not be liquid at ambient temperature; water, proteins, nucleic acids, and saccharides that are the main constituents of living bodies would not have their specific structures that enable them to fulfill their functions. Nature uses H-bonds whenever a certain degree of stability is needed as well as versatility. All this naturally applies to the constituents of the nervous system. Moreover, as anesthesia represents an interference with the normal functioning of the nervous system, it would be surprising if H-bonds were not involved with the mechanism of anesthesia. This does not mean, however, that other types of intermolecular associations are not involved.

This view took a long time to become widespread. The fact that general anesthetics are usually lipid and not water soluble and that there is a correlation between lipid solubility and anesthetic potency (the Meyer-Overton rule) focused the attention of researchers on nonpolar interactions in the hydrophobic parts of the lipid cell membrane and, later, of proteins. Excellent critical reviews on this evolution are available.¹⁻³

The most important fact for the present discussion is that general anesthesia occurs through an interplay of intermolecular interactions without proper chemical reactions, although certain anesthetics metabolize after exerting their action. So the question is this: what kind of intermolecular interactions are involved? At this point one must remember the extreme variety of the chemical structure of anesthetics. Nearly all molecules have some anesthetic potency. Among these are rare gases, saturated hydrocarbons, alcohols, ethers, ketones, and halo-

carbons, among others. A chemist could hardly admit that they all exert their action by the same "unitary" mechanism. The differences should be looked for in the nature of intermolecular interactions that are involved. A pluralistic theory was proposed.⁴⁻⁵

Present research tends to locate the site of action of anesthetics in the neurotransmitter receptors at the synapse.⁶⁻⁹ The receptors are proteins, possibly glycoproteins or lectins.¹⁰ The structure of these macromolecules and their conformations that are adapted to their functions are ensured by a great number of intermolecular interactions, van der Waals or H-bonding. Some anesthetics, like xenone can be supposed to use only van der Waals interactions where the main factor is polarizability. However, most of them possess atoms or groups that can act as proton donors or acceptors, or both. This is why the role of H-bonding seems to be inevitable.

A typical example are the widely used anesthetics which are halocarbons containing the "acidic hydrogen" like chloroform, halothane, methoxyflurane, isoflurane, enflurane, and others.¹¹ Such molecules can form H-bonds as weak proton donors. Evidence has been provided by infrared spectroscopic methods that these weak H-bonds can alter significantly the free/H-bonded equilibrium even in much stronger H-bonds of the O-H...O or N-H...O types.¹²⁻¹⁶ The reasons for this were investigated by quantum chemical-thermodynamic calculations.¹⁷⁻¹⁸ A correlation was found between the H-bond bond breaking ability of acidic hydrogen containing halocarbons and their anesthetic potency.¹⁴

The idea that the formation and perturbation of H-bonds plays a role in the mechanism of general anesthesia was advocated in a series of publications from the author's laboratory.¹²⁻¹⁸ More recently Brockerhoff *et al.*¹⁹ and Abraham *et al.*²⁰ assessed the role of H-bonding in the mechanism of general anesthesia.

Then the next question is this: what type of H-bonds are likely to be affected by anesthetics? There exists a great variety of H-bonds; their energy (enthalpy) varies from <1 to approximately 50 kcal/M. From the point of view of the living organism the weaker ones are the most important. Anesthetic action must, of course, be reversible, so the perturbation that it entails must fall into the range of thermal fluctuations or other weak—van der Waals—interactions. Because weak H-bonds are long range interactions falling off with r^{-1} , they are still significant at H...X distances of 3.0 or 3.5 Å.²¹ They have energies (enthalpies) on the order of 1 or 2 kcal/M.

Jeffrey and Saenger devote a chapter to weak H-bonding interactions in biologic systems: "Weak Hydrogen-Bonding Interactions Formed by C-H Groups as Donors

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and Aromatic Rings as Acceptors.”²¹ These are, indeed, among the H-bonds most eligible to play a role in the mechanism of anesthesia.^{22–26} Desiraju and Steiner more recently devoted their book to weak H-bonds.²⁷ They give thorough consideration to C-H...O and C-H...N interactions, X-H... π and other weak H-bonds. An immense variety of all these exists in the living organism. Such bonds are expected to be reversibly perturbed by all kinds of anesthetic molecules.

Thornton et al. (Singh and Thornton²⁸ and Mitchell et al.²⁹) obtained important results on amino/aromatic interactions in proteins. They did find amino/aromatic H-bonds but also, more frequently, stacked ring structures. This they explained by the “potential of structures for extra conventional hydrogen bonding, to either protein or solvent.” The energy in stacked structures is of electrostatic origin with some π to π overlap according to cases; it could also be perturbed by anesthetics. Gursky et al.³⁰ studied stereospecific dihaloalkane binding in insulin crystals and concluded that both dispersion and polar interactions must contribute significantly to the binding enthalpy and stereospecificity.

Burley and Petsko^{31,32} studied aromatic-aromatic and amino-aromatic interactions as a mechanism of protein structure stabilization. Berger and Egli³³ examined the role of C-H...O H-bonds. Atwood et al.³⁴ provided X-ray diffraction evidence for aromatic π H-bonding to water. Kiessling et al.³⁵ pointed out that aromatic amino acid side chains interact with bound sugars in numerous structures. Weis and Drickamer³⁶ noted that aliphatic protons of the sugar ring bear a small partial positive charge that could lead to weak interactions with the π -cloud of aromatic residues. Protein-carbohydrate recognition is an important mechanism in physiologic recognition processes. The many hydroxy groups in carbohydrates can form H-bonds with amino acid residues. Some of these interactions are mediated by water molecules.³⁵ A very great number of such weak bonds ensure the right structure of biologic macromolecules. The author made the proposal³⁷ that oligosaccharides associated with proteins could be targets for anesthetics both at their hydrophobic rings and at their OH groups.

It is significant that neurotransmitters all contain OH or NH groups prone to form H-bonds. On the other hand, neurotransmitter receptors also contain numerous groups that are proton donors or acceptors.

Recently Eckenhoﬀ et al.^{38,39} examined the binding of volatile anesthetics to proteins by using a number of techniques, in particular the fluorescence of tryptophan. Volatile anesthetics bind to proteins at cavities in proximity to tryptophan residues. Even if the distance is approximately 10 Å the H-bond could be mediated by water molecules.

Johansson et al.^{40–44} provided experimental support to the effect that small anesthetic molecules can bind in cavities formed between α -helices. They examined the

structural requirement for an inhalational anesthetic binding site on a protein target and the way in which they alter protein function in the central nervous system.

All this does not mean that H-bonds are the only weak associations that are involved with the mechanism of general anesthesia. Hydrophobic, van der Waals interactions are also important in determining the right conformations of biologic macromolecules. Rare gases or saturated hydrocarbons can be expected to affect mainly these. Alcohols were shown to act at the water-lipid interface of the nerve cell membranes.⁴⁵ However, many anesthetics are amphiphilic and could affect both H-bonds and van der Waals associations. Halothane, in particular, has a bromine atom that can undergo associations. To a lesser extent this can also be said about chlorine atoms. It could be shown, however, that this tendency is weaker than the tendency to H-bond formation.¹⁶ The mechanism is expected to consist in the reversible replacement or perturbation of weak existing H-bonds or van der Waals associations by other weak H-bonds or van der Waals associations involving the anesthetic. This would explain the reversibility of general anesthesia as well as the great variety of molecules having anesthetic potency.

Whether the anesthetic severs an existing H-bond or only induces changes in its geometry (distance, angle) could depend on cases. A relatively slight perturbation could be enough to induce conformational changes in proteins. The perturbation might affect H-bonds or van der Waals contacts.

In a recent article on molecular modeling of anesthetic interactions, Trudell and Bertaccini⁴⁶ pointed out that the nicotinic acetylcholine receptor requires cholesterol for proper functioning. Jones and McNamee,⁴⁷ Corbin et al.,⁴⁸ Rankin et al.,⁴⁹ and Mercier et al.⁵⁰ studied the relation between the self-association of cholesterol and its association with ester and amide groups, as well as the extent to which acidic hydrogen containing anesthetics can interfere with these. Cholesterol has not only a large hydrophobic core but also an OH group that is highly associative. Among the possible H-bonds there is one that is quite weak, the ester-cholesterol O-H...O = C bond. It could be an easy target that anesthetics could perturb reversibly. This seems to be still another possibility.

Conclusions

All this requires experimental support. It appears, however, that the following points can be made. Weak H-bonds and van der Waals contacts contribute to ensure the right conformation of proteins. Therefore severing or perturbing such interactions could induce conformational changes in proteins that in turn, could cause anesthesia. Anesthetics are likely to perturb several of these to exert their action. Both polar and nonpolar

interactions can be important. Different anesthetics might affect different weak interactions in accordance with their own chemical structure. The action of every anesthetic, neurotransmitter, and receptor needs special consideration; "unitary" views should not be imposed.

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