THE SITE OF ACTION OF GENERAL ANESTHETICS – A CHEMICAL APPROACH

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Dedicated to Professor Josef Paldus on the occasion of his 70th birthday.

Recently Urban (*Br. J. Anaesth.* **2002**, *89*, 167) and Trudell (*Br. J. Anaesth.* **2002**, *89*, 32) assessed the present state of the art in anesthesiological research. This article is an attempt to add to the discussion some ideas from the chemist's point of view. General anesthesia is a matter of molecular associations. Among the intermolecular interactions that can be involved, weak hydrogen bonding and van der Waals forces are believed to be most important. A pluralistic view is proposed, thereby different anesthetics can choose different interactions in conformity with their chemical structure. This can involve proteins, lipids, and sugars. Special attention is given to glycoproteins and glycosphingolipids. A review with 90 references.
Keywords: Anesthesia; Weak hydrogen bonds; Protein–carbohydrate interactions; Glycoproteins; Glycoconjugates; Glycosphingolipids; *Ab initio* calculations.

For many years the unitary way of thinking dominated discussions on the mechanisms of anesthesia. The reason for this was confidence in the Meyer–Overton rule. Indeed, general anesthetics are usually lipid-, not water-soluble and there is a correlation between lipid solubility and anesthetic potency. This in itself, however, does not reveal the mechanisms of anesthesia. In later years an even better correlation was found between anesthetic potency and the effect of anesthetics on proteins, based mainly on the firefly experiments. For both lipid and protein theories, the mechanisms were presumed to involve intermolecular interactions at hydrophobic sites. It is not intended here to review this epoch. From the immensity of the pertaining literature I am citing only the critical reviews of the lipid era by Miller[1] and Ueda et al.[2] and the review of the protein era by Franks and Lieb[3].

There is another fact about anesthetics certainly as important as their lipid solubility. It is the enormous variety of their molecular structure.

Some of them are nonpolar like rare gases or paraffinic hydrocarbons, others contain polar groups like alcohols, ketones, halohydrocarbons, and many others. In view of these facts chemical intuition leads to the assumption that the site of anesthetic action must be amphiphilic to allow both for polar and nonpolar interactions. Following this idea we put forward a pluralistic theory of anesthesia[4–8].

A great deal of progress has been made in recent years in anesthetic research. It became possible to have a close look at the chemistry of the nerve cell, in particular that of the synapse. It is not intended here to review this field either. I am citing only the assessment of the synaptic basis of general anesthesia by Richards[9] and the articles of Krasowski and Harrison[10], Belelli et al.[11], Pocock and Richards[12], and Mihic et al.[13]

In addition to lipid solubility and the great variety of the molecular structure of anesthetics the third great fact is that anesthetic action proceeds without breaking and formation of covalent or electrovalent bonds; it is a matter of changes in intermolecular associations.

Then the next question is: what are the intermolecular associations that are involved? This is the subject of the subsequent discussion. Recently Urban[14] and Trudell[15] reviewed the present situation in anesthesiological research. This article is an attempt to introduce some ideas concerning the possible sites of action of general anesthetics and the intermolecular interactions that can be involved, as viewed by a chemist.

Hydrogen Bonding and van der Waals Forces

Intermolecular forces are essentially of two kinds: van der Waals or hydrogen bonding (cf. Zahradník and Hobza[16]). All of them play an important role in the living material; they ensure the right conformation of biological macromolecules on which their functioning depends. They entail both polar and nonpolar interactions. Nature uses hydrogen bonds (H bonds) whenever a certain degree of stability, but not rigidity, is needed with a degree of specificity. The energies (enthalpies) of H bonds range from about one to about fifty kilocalories (4 to 200 kJ). The energy of weak H bonds is almost entirely electrostatic in origin while the very strong ones may have a high amount of covalent character. Very strong H bonds seldom occur in living bodies, but the weaker ones are ubiquitous, they are essential in nucleic acids, proteins, sugars, and of course water, on which life is built. Because of their electrostatic character they are fairly long distance, proportional to r^{-1} where r is the r(XY) distance in X–H···Y. They can reach 3 or 4 Å. Many anesthetics also contain proton donors or acceptors or both. The

widely used halohydrocarbon anesthetics are the best example. Chloroform, halothane, methoxyflurane, isoflurane, enflurane, all contain the so-called acidic hydrogen[17–20]. They can form weak H bonds. We could show by combined quantum mechanical-thermodynamic calculations[21,22] that although weak, they can seriously perturb the free-association equilibrium in stronger H bonds of the O–H···O or N–H···O=C type which are the most important for the living organism. Halothane also contains a Br atom which is highly polarizable and most of the others contain Cl atoms; they can all play some role in associations. It could be shown, however, that this is secondary to H bond association due to the acidic hydrogen[6]. These conditions can be studied in model systems by infrared spectroscopy. There is a smooth relationship between the anesthetic potency of these molecules and the extent to which they perturb the free/association ratio in stronger H bonds[4,5,23–25].

Now, since anesthesia is a reversible phenomenon, the associations which are perturbed must be weak so that the regular order can be restored by thermal fluctuations. Van der Waals associations and weak H bonds must be involved, of the order of 4 to 9 kJ or less. In recent years such H bonds received considerable attention. A book by Desiraju and Steiner[26] is entirely devoted to them and it contains a chapter on biologically important weak H bonds. So do the recent books by Jeffrey and Saenger[27], Jeffrey[28] and the one, theoretical, by Scheiner[29]. They all contain a wealth of references to previous work.

The most important in this respect are H bonds formed by CH groups. These were first identified in crystals by Sutor[30,31]. A great deal of progress was made by Allerhand and Schleyer[32]. A book by Green[33] summed up existing knowledge on these at an early stage. Objections against the concept of C–H···X hydrogen bonds were definitely defeated by Taylor and Kennard[34]. From then on the field has known rapid progress. Desiraju[35] overviewed C–H···O H bonds in crystals. Steiner[36] presented neutron diffraction data on C–H···O interactions involving amino acid C_α–H. Numerous other cases of H bonds of the C–H···O and C–H···N types have been described by Desiraju and Steiner[26].

Such bonds can be attractive or repulsive depending on the balance of the exchange repulsion and attractive electrostatic terms in the expression of the H bond energy. The first case of a repulsive bond was described by Sandorfy and coworkers[4] in the case of the $–CHF_2$ group. Many others became known later ("Blue shifting" H-bonds)[37–42]. In the present context weak, attractive H bonds are the most important. Many such bonds exist in proteins and in sugars. It is then pertinent to point out that similar weak H

bonds are formed by halothane type and some other anesthetics. Thus the possibility that these weak H bonds formed by anesthetics replace or perturb existing C–H···O or C–H···NH bonds reversibly, must be considered.

Steiner and Saenger[43] examined the role of C–H···O H bonds in the coordination of water molecules, in particular their implication in the structural biology of proteins involving internal water molecules. CH donors participate in the coordination of water molecules, mainly when not enough OH or NH donors are available. Water is everywhere in biological systems and it often mediates H bonds where space requirements prevent the formation of direct H bonds by OH or NH donors.

Let us remember what Huggins[44] said 68 years ago about these weak H bonds: "These interactions have similar energies and geometries to those of van der Waals complexes and are distinguished from them by evidence of a directional involvement of the A–H bonds." Among the eligible proton acceptors a privileged place should be given to π-acceptors. These are available in aromatic ring containing amino acids, tryptophan, tyrosine, and phenylalanine. The proton donors could be OH, NH, SH, or CH groups. That aromatic molecules can act as weak proton acceptors has been known for many years[45–47]. In the biological context they were introduced by Levitt and Perutz[48], Perutz[49], Wahl and Sundaralingam[50], Burley and Petsko[51,52], and Desiraju and Steiner[26]. The most recent assessment known to the writer is by Steiner and Koellner[53]. These weak H bonds could again be replaced or perturbed by other weak H bonds formed by incoming anesthetics; even van der Waals interactions may suffice for this.

It should be remembered that neurotransmitters contain OH and/or NH groups. This cannot be due to chance. The H bonds formed by these groups can determine the positioning of neurotransmitters at the synapse. The proton acceptors may or may not be to aromatic amino acids, according to cases.

Lemieux[54] stressed the importance of water in saccharide recognition by proteins. As he put it: "Like a chaperon, water accompanies the reactants in their search for each other." Atwood et al.[55] provided X-ray diffraction evidence for aromatic π hydrogen bonding to water. Hanessian et al.[56] gave a striking example for molecular recognition and self-assembly by weak H bonding. Berger and Egli[57] discussed the role of C–H···OH bonds in the organization of nucleic acid tertiary structure. Burley and Petsko[51,52] stressed the importance of aromatic-aromatic interactions in protein stability and function.

Protein–Saccharide Interactions

In glycoproteins a sugar entity is covalently bound to the protein, in lectins oligosaccharides are bound to the protein by H bonds and van der Waals interactions. Saccharides have an immense potential for physiological recognition processes because of their great structural variety. They possess both hydrophobic rings and polar OH groups, so they are amphiphilic and can be receptors for many kinds of molecules.

A recent, highly informative review has been provided by Kiessling et al.[58] They pointed out that aromatic amino acid side chains interact with bound sugars in many structures. A great deal of progress has made in oligosaccharide research in recent years. Weis and Drickamer[59] noted that aliphatic protons of the sugar rings bear a small positive charge which could lead to weak interactions with the π-cloud of aromatic residues. Then there are interactions between amino acid residues and saccharide OH groups and indirect H-bonds mediated by water molecules[58]. Aromatic amino acid side chains were found to interact with bound sugars in many structures determined by X-ray crystallography.

Recently the author[60] made the proposal that oligosaccharides associated with proteins could be targets for anesthetics either at their hydrophobic rings or at their OH groups. There is no reason for giving exclusivity to proteins and lipids in our search for the site of general anesthesia. In view of the many weak intermolecular associations in which oligosaccharides can participate it is logical to expect that anesthetics can interfere with many of these and in a reversible way.

Glycoconjugates at the Synapse

Next we have to consider the conditions at the synapse. An enlightening review was given by Gurd[61]. He pointed out that the nerve terminal and synapse contain high concentrations of glycoproteins. Already Rambourg and Leblond[62] demonstrated an enrichment of saccharide-containing material in the region of the synaptic cleft. Pfenninger[63] extended these works. Among others the nicotinic acetylcholine receptors, a glutamate binding protein and the opiate receptor are known to be glycosylated. Lectins are also present at the synapse[64,65] (for a recent assessment, see Sharon and Lis[66]). Among the many pertaining publications the author would like to mention the recent book by Sharon and Lis[66] and the papers by Zanetta and coworkers[65], Lis and Sharon[64], Gurd[61], and Margolis and Margolis[67]. As Gurd[61] states, “the oligosaccharide groups of synaptic glycoproteins are lo-

cated within the synaptic cleft, so that changes in sugar composition will alter the general molecular environment of the cleft". It seems to follow that oligosaccharides of glycoproteins or lectins are possible targets for attack by anesthetics.

Glycoproteins are also present in synaptic vesicles. They are filled with neurotransmitters which are liberated when a nerve impulse reaches the synapse. The release of neurotransmitters is preceded by Ca^{2+} release. Whether or not these events can be perturbed by anesthetics does not seem to be firmly established. Carlson[68] reviewed existing knowledge on synaptic vesicle glycoproteins.

The nature of protein–saccharide interactions was thoroughly studied by Quiocho[69,70] on the arabinose-binding protein–sugar complex. He stressed that all atoms of both sugar anomers interact with the protein via H bonds and van der Waals contacts, that H bonds are the major force in the stability of protein–sugar complexes and that the H bonds are distributed equally between two types: five neutral–neutral and five neutral-charged H-bonds. Lys 10 is engaged in multiple interactions; its ammonium side chain makes van der Waals contacts or a very weak H bond with two anomeric hydroxy groups. These could be prime targets for anesthetics.

As both Lis and Sharon[64] and Quiocho[69] comment, a widely occurring interaction is the stacking of a monosaccharide on a side chain of an aromatic amino acid. As stated above this is due to the π-electron cloud of the aromatic rings and the weak proton donor property of the aliphatic CH links of the sugar. Many anesthetics could compete with these weak bonds.

Johnson et al.[71] overviewed protein–oligosaccharide interactions in lysozyme, phosphorylase, and amylases. Their results lead to similar conclusions concerning polar and nonpolar protein–sugar interactions and possible perturbations by anesthetics.

Eckenhoff[72,73], Eckenhoff and Johansson[74], Johansson et al.[75,76], and Manderson and Johansson[77] demonstrated by using a number of techniques, in particular the fluorescence of tryptophan in the typical case of halothane that volatile anesthetics bind to proteins at cavities in close proximity to tryptophan residues.

Glycosphingolipids (GSL)

In one of the recent publications of the writer a brief mention was made of glycosphingolipids[60]. Sugars can be linked to lipids as well as to proteins. Glycosphingolipids are present in significantly higher proportions in nerve cells than in other cells. In particular, gangliosides, sialic acid containing

glycosphingolipids are abundant in the brain[78–80]. They interact with membrane proteins and could modulate the function of receptors. They have potent signalling properties. An associated glycosphingolipid can alter the conformation and the activity of a specific protein.

Glycosphingolipids consist of ceramide (sphingosine and fatty acid) and a saccharide residue linked to and oriented perpendicularly to ceramide. Except for sphingomyelin, sphingolipids do not contain phosphate groups and instead of an ester group they contain an amide group and one or more additional OH groups. A great deal of present knowledge on GSL is contained in a volume published in the Annals of the New York Academy of Sciences[78–81].

As Pascher[82] who contributed much of the progress in this field pointed out: "The amide group of the ceramide, which serves as a link between the hydrocarbon chains, has a basic significance for the conformation of the entire molecule." Sphingolipids are amphiphilic containing both polar and nonpolar parts. Both polar interactions and hydrophobic effects are instrumental in the formation of protein–saccharide complexes. The configuration of the planar amide group, which connects the two hydrocarbon chains in the ceramide part of GSL is an important factor in determining the conformation of the whole molecule[83]. In particular the hydrogen atom of the amide nitrogen participates in a three-center (bifurcated) intramolecular H bond. One of the bonds is directed towards the oxygen of the fatty acid hydroxy group, the other towards the oxygen of the glycosidic linkage. This is a conformation determining interaction[84–86]. Since bifurcated H bonds are usually weaker than normal H bonds, this could be again a relatively easy target for anesthetics. If, for example, one of the bonds is broken this can have a profound influence on the conformation of the whole GSL and, as a consequence, on the interaction of the GSL with its protein partner. As Pascher and Sundell[83] remarked, due to the shovel shape of the molecule, the sugar residue is not in packing contact with its own ceramide part but with those of neighbouring molecules. Indeed, as shown subsequently by Nyholm et al.[85] "by abolishing the intramolecular H bond between the amide NH group and the glycoside oxygen the galactose ring changes its orientation from layer-parallel to layer-perpendicular". If certain anesthetics can affect this H bond in a GSL located in the vicinity of a neuroreceptor, this could perturb the functioning of the nervous system.

Many GSL contain a sialic acid component of which a great variety exists. They are a family of nine-carbon carboxylated sugars usually found as terminal monosaccharides of animal oligosaccharides. Saccharides influence

the conformation of glycoproteins, and because of their great structural diversity, may serve as recognition determinants[87–90]. Sialic acids greatly contribute to these effects. Now, sialic acid (*N*-acetylneuraminic acid) also contains an amide group and sugar and alcoholic OH groups. Whether or not the amide group is involved in a weak intramolecular bifurcated H bond may depend on cases. In addition, some of the many H bonds formed by the OH groups, some of them mediated by water molecules, could also be weak and could be perturbed by anesthetic molecules. This possibility has not yet been explored to the author's knowledge.

CONCLUSIONS

Since anesthesia is an interference with the normal functioning of the nervous system, all that could perturb the nervous system could be conducive to anesthesia. Since general anesthesia is a matter of perturbation of intermolecular associations, the mechanisms of anesthesia must involve H bonds and van der Waals contacts, in particular weak H bonds. As to the site of action of anesthesia, believed to be at the synapse, this may involve, in addition to proteins and lipids, glycoproteins, or glycolipids, in particular glycosphingolipids. In view of the great number of perturbable sites that all these macromolecules possess, one is led to believe that many sites are simultaneously perturbed during anesthetic action. All this would require experimental proof.

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