J Mol Model (2007) 13:313–318
DOI 10.1007/s00894-006-0145-8

ORIGINAL PAPER

Molecular surface electrostatic potentials and anesthetic activity

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Received: 8 October 2005 / Revised: 31 July 2006 / Published online: 22 September 2006
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Abstract General anesthetics apparently act through weak, noncovalent and reversible interactions with certain sites in appropriate brain proteins. As a means of gaining insight into the factors underlying anesthetic potency, we have analyzed the computed electrostatic potentials $V_S(\boldsymbol{r})$ on the surfaces of 20 molecules with activities that vary between zero and high. Our results are fully consistent with, and help to interpret, what has been observed experimentally. We find that an intermediate level of internal charge separation is required; this is measured by Π, the average absolute deviation of $V_S(\boldsymbol{r})$, and the approximate window is $7<\Pi<13$ kcal mol^{-1}. This fits in well with the fact that anesthetics need to be lipid soluble, but also to have some degree of hydrophilicity. We further show that polyhalogenated alkanes and ethers, which include the most powerful known anesthetics, have strong positive potentials, $V_{S,max}$, associated with their hydrogens, chlorines and bromines (but not fluorines). These positive sites may impede the functioning of key brain proteins, for example by disrupting their normal hydrogen-bond patterns. It has indeed been recognized for some time that the most active polyhalogenated alkanes and ethers contain hydrogens usually in combination with chlorines and/or bromines.

Keywords Halogenated ethers and hydrocarbons · Anesthetic activity · Electrostatic potentials · Hydrogen and halogen bonding

Proceedings of "*Modeling Interactions in Biomolecules II*", Prague, September 5th–9th, 2005.

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Introduction

One of the most striking features of general anesthetics, as pointed out recently by Sandorfy, [1] is the remarkable diversity of compounds that have been found to have at least some potency: inert gases, CO_2, N_2, HC≡CH, $H_2C{=}CH_2$, cyclopropane, N_2O, alkanes, aldehydes and ketones, $CHCl_3$ and other halogenated methanes and ethanes, SF_6, both unsubstituted and halogenated ethers, etc. Thus it does not seem likely that any particular type of chemical reactivity will explain anesthesia; indeed, the desired function requires that the interactions be weak and readily reversible, which suggests some van der Waals sort of intermolecular association.

Consistent with this is the idea that, among anesthetics, activity correlates with lipid solubility; this came from the work of Meyer [2] and Overton, [3] more than a century ago. This has continued to be an important element of anesthesia theory [4–14]. However lipid solubility is, in general, necessary but not sufficient for anesthetic potency; some compounds, such as C_2F_6, are lipid soluble but show little or no activity [11, 12].

In fact, a study of halogenated ethers showed that a certain level of aqueous phase affinity is also required [12]. Thus both polarity and nonpolarity appear to be factors, which is consistent with the argument that the sites of anesthetic activity must be able to accommodate both types of interactions [1, 15–21].

As was already pointed out, the fact that such a wide array of molecular types produces at least some degree of general anesthesia indicates that this is not linked to some particular features of chemical composition or structure. What is apparently important is the overall long-range effect that the molecule produces, i.e. how the receptor site "perceives" it. This recognition is achieved by means of the

electrostatic potential of the anesthetic molecule, and this is accordingly the property upon which we focus in this paper.

Molecular electrostatic potentials

The electrostatic potential $\mathbf{V}(\mathbf{r})$ that the electrons and nuclei of a molecule create in the surrounding space is given rigorously by Eq. (1):

$$\mathbf{V}(\mathbf{r}) = \sum_{\mathrm{A}} \frac{Z_{\mathrm{A}}}{|\mathbf{R}_{\mathrm{A}} - \mathbf{r}|} - \int \frac{\rho(\mathbf{r}')\mathrm{d}\mathbf{r}'}{|\mathbf{r}' - \mathbf{r}|} \tag{1}$$

Z_{A} is the charge on nucleus A, located at $\mathbf{R}_{\mathrm{A}}$, and $\rho(\mathbf{r})$ is the electronic density.

$\mathbf{V}(\mathbf{r})$ is a physical observable, which can be determined experimentally, by diffraction methods, [22–24] as well as computationally. Regions of negative $\mathbf{V}(\mathbf{r})$ are usually associated with the lone pairs of electronegative atoms, the π electrons of unsaturated hydrocarbons, and strained C–C bonds [25].

The analysis of $\mathbf{V}(\mathbf{r})$ has proven to be an effective approach for interpreting and quantifying noncovalent interactions, [24, 26–28] which are primarily electrostatic in nature. For this purpose, we compute $\mathbf{V}(\mathbf{r})$ on the molecular "surface," which we define, following Bader et al., [29] as the 0.001 electrons/bohr^3 contour of the electronic density $\rho(\mathbf{r})$. This surface potential is labeled $V_{\mathrm{S}}(\mathbf{r})$.

We characterize $V_{\mathrm{S}}(\mathbf{r})$ by means of several statistical quantities, [26–28] including its most positive and negative values, $V_{\mathrm{S,max}}$ and $V_{\mathrm{S,min}}$, and its average absolute deviation, Π, [30].

$$\Pi = \frac{1}{\mathrm{n}} \sum_{i=1}^{n} \left| V_S(\mathbf{r}_i) - \overline{V}_S \right| \tag{2}$$

In Eq. (2), the summation is over the points of a grid covering the surface, and $\overline{V}_S$ is the average of $V_{\mathrm{S}}(\mathbf{r}_{\mathrm{i}})$ over this grid,

$$\overline{V}_S = \frac{1}{\mathrm{n}} \sum_{i=1}^{n} VS(\mathbf{r}_i) \tag{3}$$

$V_{\mathrm{S,max}}$ and $V_{\mathrm{S,min}}$ have been found to correlate with hydrogen-bond-donating and -accepting tendencies, [31] respectively, while Π has been shown to be a measure of internal charge separation, [30] which is found even in molecules with zero dipole moment, e.g. CO_2, *para*-dinitrobenzene, etc. The magnitude of Π typically ranges between about 2–3 kcal mol^{-1} for alkane hydrocarbons and 20–25 kcal mol^{-1} for highly polar molecules such as H_2O. $V_{\mathrm{S,max}}$ and Π will have key roles in our analysis.

Procedure

We have optimized the geometries and computed the electrostatic potentials, at the Hartree-Fock 6–31G* level, for a group of 20 molecules with anesthetic activities ranging from none to high. Most of them are halogenated alkanes or ethers, which have been a primary focus of synthesis and testing in the last fifty years, [11, 12, 32–35] and they include the currently most-widely-used general anesthetics.

A common measure of anesthetic activity, which we will cite when available, is the minimum alveolar anesthetic concentration (MAC), which is the least amount needed to produce no response in rats to electrical stimulation [12]. The lower the MAC, the more powerful is the anesthetic.

It should be noted that $V_{\mathrm{S}}(\boldsymbol{r})$, $V_{\mathrm{S,max}}$ and Π are given in units of kcal mol^{-1}. Strictly speaking, the units of the electrostatic potential should be energy/charge. However it is nearly universal practice to express $\mathbf{V}(\mathbf{r})$ as simply an energy, commonly kcal mol^{-1}. Thus it can be interpreted as the interaction energy of the system with a unit positive charge placed at the point $\mathbf{r}$.

Results and discussion

In Table 1 are listed the 20 molecules included in this study, along with their computed Π, surface areas and $V_{\mathrm{S,max}}$ values. All local maxima of $V_{\mathrm{S}}(\boldsymbol{r})$ are given except those associated with interior carbons. Also presented in each case is the MAC or other available information concerning anesthetic activity. The molecules are arranged in order of decreasing Π.

We will look first at the overall electrostatic potentials on the surfaces of some of the molecules in Table 1. Figure 1 shows $V_{\mathrm{S}}(\boldsymbol{r})$ for $CHCl_3$. As would be anticipated, there is a strong positive potential encompassing the hydrogen, with $V_{\mathrm{S,max}}$=36.7 kcal mol^{-1}. Perhaps more surprisingly, there is also a smaller and weaker positive region on the outermost portion of each chlorine, centered about the intersection of its surface with the C–Cl axis. These $V_{\mathrm{S,max}}$ are 14.5 kcal mol^{-1}.

Such halogen positive regions, to which has been given the name "σ-holes," are found on some covalently-bonded chlorines, more often and more positive on bromines and iodines, but not (to our knowledge) on fluorines [36–40]. They have been invoked as the explanation for "halogen bonding," which is a noncovalent interaction (somewhat analogous to hydrogen bonding) between a covalently-bound halogen on one molecule and a negative site on another [38, 39, 41–44]. It is the presence of a positive σ-hole that is believed to make this possible. The strength of halogen bonding increases in the order Cl<Br<I.

Table 1 Computed properties and observed anesthetic activities

Molecule	Π (kcal mol^{-1})	$V_{S,max}$[a] (kcal mol^{-1})	Surface area (A^2)	Anesthetic Activity[b]
$CF_3CH_2OCH{=}CH_2$ (fluroxene)	12.7	29.4 (H), 23.7 (H)	not available	moderate
CH_3OCF_2CHFBr	12.2	29.0 (H), 23.3 (H), 13.3 (Br)	150.5	0.0069
$CH_2FOCH(CF_3)_2$ (sevoflurane)	12.0	37.2 (H), 29.0 (H)	160.1	moderate
N_2O	11.6	24.4 (N)	65.5	low
$CH_3OCF_2CHCl_2$ (methoxyflurane)	11.5	29.9 (H), 23.6 (H), 9.2 (Cl)	not available	0.0027
CHF_2OCF_2CHFCl (enflurane)	11.3	36.4 (H), 34.5 (H), 13.0 (Cl)	154.4	0.022
$CHF_2OCHFCF_3$ (desflurane)	11.1	33.3 (H), 33.3 (H)	140.7	0.0464
$CHF_2OCHClCF_3$ (isoflurane)	10.4	35.8 (H), 33.5 (H), 14.1 (Cl)	153.1	0.0145
$CF_3CHBrCl$ (halothane)	8.24	37.7 (H), 22.3 (Br), 15.3 (Cl)	137.7	high
$CF_3OCHFCF_3$	8.21	36.6 (H)	145.1	1.96
$CHCl_3$ (chloroform)	8.21	36.7 (H), 14.5 (Cl)	119.7	moderate
$CHF_2OCClBrCF_3$	8.19	37.4 (H), 23.3 (Br), 16.2 (Cl)	169.2	0.0151
$CHF_2OCCl_2CF_3$	7.78	36.3 (H), 21.2 (Cl), 20.6 (Cl)	166.5	0.0982
$CH_3OCH_2CH_3$	7.57	11.4 (H)	116.4	—
$(CH_3CH_2)_2O$	6.63	10.9 (H)	137.4	moderate
CF_3CF_3	4.52	—	109.1	inactive
$CF_2ClOCFClCF_3$	4.19	21.0 (Cl), 20.8 (Cl)	172.7	inactive
$CF_2ClOCF_2CClF_2$	4.07	20.9 (Cl), 20.1 (Cl)	175.4	inactive
CH_4	2.68	7.6 (H)	59.8	inactive
CH_3CH_3	2.14	6.2 (H)	83.4	inactive

[a] The $V_{S,max}$ values for interior carbons are not reported.
[b] Anesthetic activity as measured by the minimum alveolar concentration (MAC) [12] when available; otherwise, activities are listed as high, moderate, low or inactive [7, 12, 35, 45, 52].

The existence and positive strength of a σ-hole on the halogen X in a molecule RX depend upon (a) the degree of *sp* hybridization of the *s* lone pair of X, and (b) the relative electronegativities of R and X [39, 40]. Thus the introduction of electron-withdrawing substituents into R, such as NO_2, F, Cl, CN, etc., may cause a σ-hole to appear where it had not been before (F_3CCl vs H_3CCl), or may strengthen an already-existing one (F_3CBr vs H_3CBr) [39]. This can be seen in Fig. 2, which is the $V_S(\boldsymbol{r})$ for halothane, $CF_3CHBrCl$, a widely-used general anesthetic [45]. The presence of the strongly electron-attracting CF_3 increases the $V_{S,max}$ of the chlorine σ-hole to 15.3 kcal mol^{-1}, with a stronger one on bromine, as expected; $V_{S,max}$(Br)=22.3 kcal mol^{-1}. The hydrogen is also more positive, $V_{S,max}$(H) = 37.7 kcal mol^{-1}.

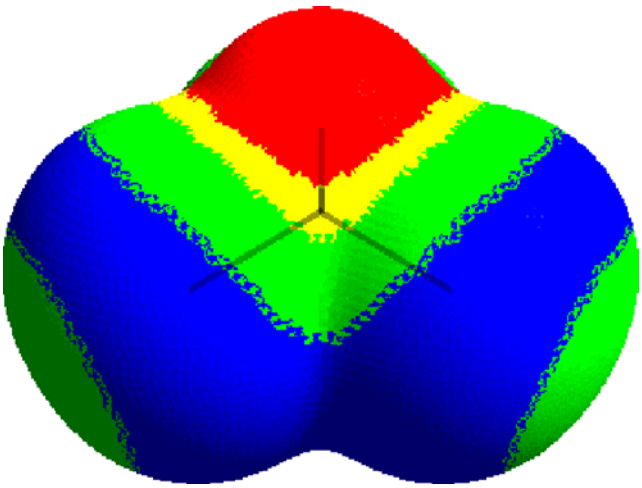

Fig. 1 The computed HF/6-31G* electrostatic potential, in kcal mol^{-1}, on the 0.001 electrons/bohr^3 surface of $CHCl_3$. The color ranges are: red, more positive than 25; yellow, between 15 and 25; green, between 0 and 15; blue, between −10 and 0. The strongly positive (red) potential at the top corresponds to the hydrogen. Each chlorine has a less positive (green) region on its outermost surface

In contrast, the fluorines in C_2F_6 are negative, with no σ-holes (Fig. 3). We attribute the failure of fluorine to have σ-holes to its high electronegativity and the significant *sp* hybridization of its unshared *s* electrons [39, 40].

The data in Table 1 indicate that some degree of internal charge separation is needed for anesthetic activity. All those molecules with Π<5 kcal mol^{-1} are inactive; these include C_2F_6 and two fully halogenated methyl ethyl ethers, $CF_2ClOCFClCF_3$ and $CF_2ClOCF_2CClF_2$. On the other hand, even the most powerful anesthetics in the table have Π<13 kcal mol^{-1}. Since Π varies, for most molecules, between 2 and 25 kcal mol^{-1}, it seems that a reasonable degree of anesthetic potency requires an intermediate level of internal charge separation, roughly in the range 7–13 kcal mol^{-1}. This quantifies the conclusion reached experimentally [12] (through studies of aqueous and lipid affinities) that both polar and nonpolar features are needed [1, 15–21].

Figure 3 brings out the relatively low level of charge separation in anesthetically-inactive C_2F_6, which results in a Π value of only 4.52 kcal mol^{-1}. The comparison with

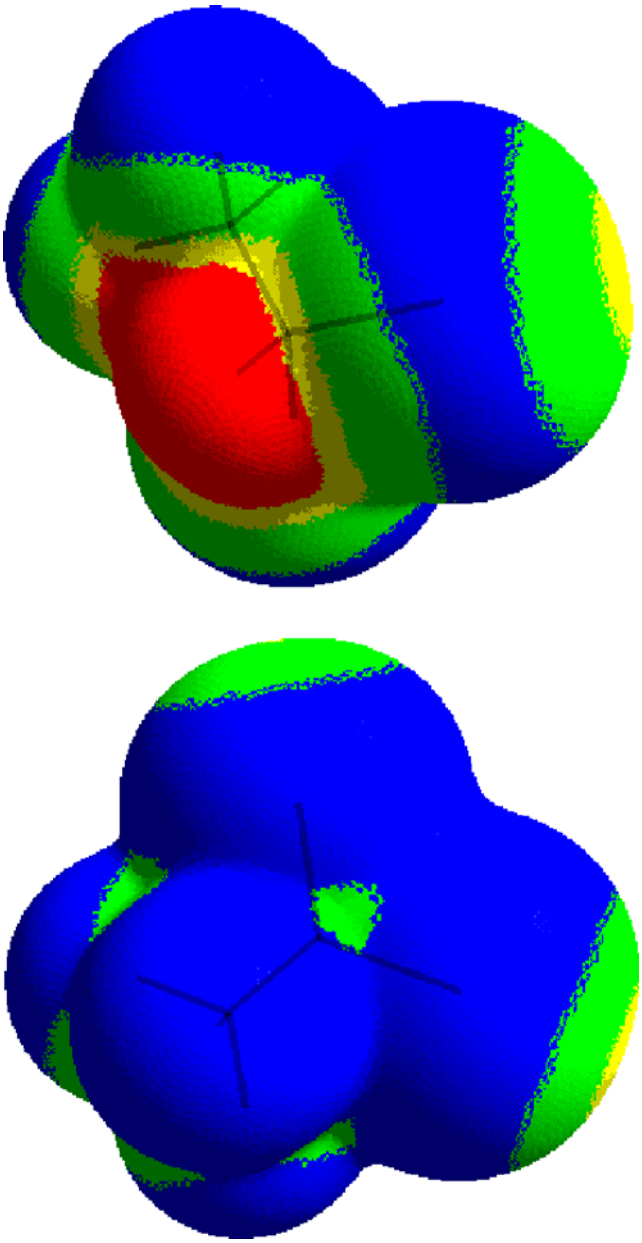

Fig. 2 The computed HF/6-31G* electrostatic potential, in kcal mol^{-1}, on the 0.001 electrons/$bohr^3$ surface of halothane, $CF_3CHBrCl$. The color ranges are: red, more positive than 25; yellow, between 15 and 25; green, between 0 and 15; blue, between −10 and 0. In the top view, the strongly positive (red) potential is due to the hydrogen; the yellow and green positive regions at the right on the outermost bromine surface. In the bottom view, the weakly positive (green) potential at the top is on the chlorine; the stronger yellow and green regions at the right are on the bromine

$CHCl_3$ (Fig. 1, Π=8.21 kcal mol^{-1}) and halothane (Fig. 2, Π=8.24 kcal mol^{-1}) is striking.

However Π is not the only factor to consider, as can be seen from the low activity of N_2O, which has a suitable Π of 11.6 kcal mol^{-1}. More than thirty years ago, [34, 35] and again more recently, [12, 46] it was observed in studies of halogenated ethers that the best anesthetics among them

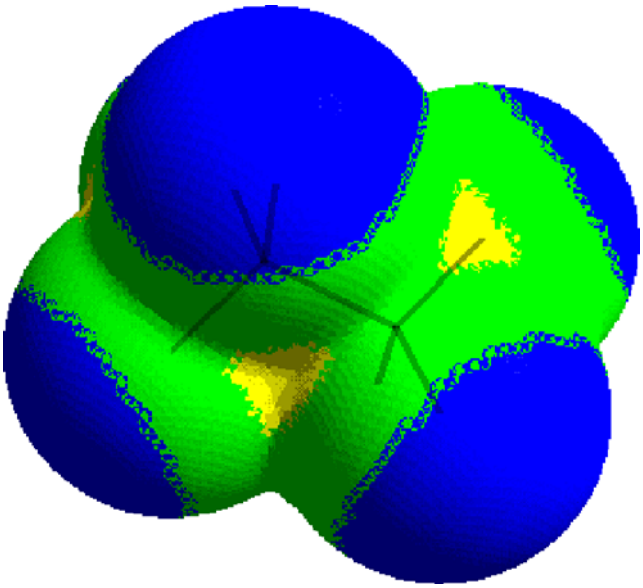

Fig. 3 The computed HF/6-31G* electrostatic potential, in kcal mol^{-1}, on the 0.001 electrons/$bohr^3$ surface of C_2F_6. The color ranges are: yellow, between 15 and 25; green, between 0 and 15; blue, between −10 and 0. The hemispheres corresponding to the fluorines are completely negative

had one or two hydrogens in conjunction with at least one chlorine or preferably bromine. Table 1 suggests that the key point here is the positive sites that are provided by the hydrogens and the σ-holes of the chlorines and bromines. All of these can be seen in Fig. 2 for halothane. The greater anesthetic effectiveness of bromine relative to chlorine can be understood to be due to its typically more positive σ-hole, in comparable circumstances. Two hydrogens and no chlorines or bromines is also a possibility (desflurane, Fig. 4). The fluorines in these molecules do not have

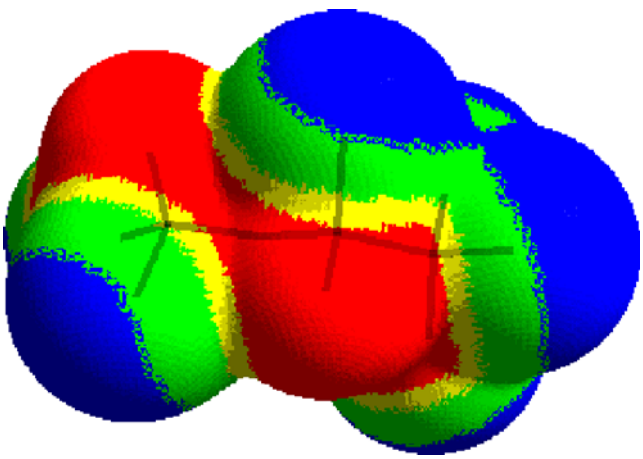

Fig. 4 The computed HF/6-31G* electrostatic potential, in kcal mol^{-1}, on the 0.001 electrons/$bohr^3$ surface of desflurane, $CHF_2OCHFCF_3$. The color ranges are: red, more positive than 25; yellow, between 15 and 25; green, between 0 and 15; blue, between −10 and 0. The two strongly positive (red) potentials are associated with the hydrogens

σ-holes, but they make the other portions more positive through their electron-withdrawing capacities.

Nitrous oxide, N_2O, may appear not to fit the picture that has been presented in terms of hydrogens and halogens. However it does have a fairly strong $V_{S,max}$, 24.4 kcal mol^{-1}, associated with the central nitrogen. It is also a small molecule, with a computed surface area of 65.5 A^2, which is less than half of most of the molecules in Table 1. Thus it may be that, at the high concentrations that are required for this weak anesthetic, [7] more of these small molecules can interact simultaneously with the receptor, and thus give the effect of two or more positive sites on a larger molecule.

Why are the positive sites important? How does the anesthetic exert its effect? It has been suggested that anesthetics impede the normal functioning of key brain proteins, [1, 44, 47] for example by perturbing their conformations. Halogenated hydrocarbons and ethers such as those in Table 1 with Π values between 7 and 13 kcal mol^{-1} could do this by disrupting the existing hydrogen bond structure of the protein, achieving this by means of their own strongly positive hydrogens as well as their chlorines and bromines that are capable of halogen bonding. It has indeed been shown, in extensive studies by Sandorfy et al., [48–51] that halogen bonding can compete and interfere with hydrogen bonding. Nitrous oxide could presumably bring about an analogous result, if present in sufficient concentration, via the positive potential associated with its central nitrogen.

We recognize that this interpretation does not apply to some weak anesthetics such as xenon. It may be that the high polarizability of the latter is a factor. Many years ago, Wulf and Featherstone [52] and Koski et al. [53] did find correlations, primarily for rather weak anesthetics, between potency and the van der Waals constant a, which is a function of polarizability.

Trudell and Bertaccini have recently discussed various concepts and modeling studies relating to anesthetic-receptor interactions [21]. In this context, it is relevant to mention the warnings that current molecular dynamics force fields may not adequately treat halogen interactions, [38] as is already recognized to be the case for semi-empirical computational procedures [54, 55].

Summary

Key features of the computed electrostatic potentials $V_S(\boldsymbol{r})$ on the surfaces of a group of 20 molecules, primarily halogenated alkanes and ethers, are fully consistent with experimental observations of factors relating to their anesthetic activities, and help to explain these observations. First, the internal charge separation of the molecule, as measured by the average absolute deviation of $V_S(\boldsymbol{r})$, should be in a range intermediate between non- and highly polar, roughly $7<\Pi<13$ kcal mol^{-1}. This presumably allows the molecule to reach the appropriate site and to position itself properly. Second, the molecule needs at least two strongly positive potentials, $V_{S,max}$. It is hypothesized that these allow it to impede the normal functions of key proteins in the brain.

Acknowledgement We would like to thank Mr. Alfred Politzer for his assistance with this work.

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