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Molecular Connectivity and Structure-Activity Relationship of General Anesthetics

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SUMMARY

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Molecular connectivity indices were calculated for 28 inhalation anesthetics. An excellent correlation is obtained between the relative anesthetic potency and the molecular connectivity index ${}^0\chi^v$ in addition to the charge Q_H on the polar hydrogens. This quantitative structure-activity relationship accounts for and quantifies previous observations on general anesthetics. Our structure-activity relationship is discussed briefly in relation to theories of general anesthesia.

INTRODUCTION

The potency of anesthetic gases has been numerically related to several physical properties, such as boiling point, solubility, partition coefficients, molar refraction, van der Waals equation constants, and molar volumes. Much of this work has been recently reviewed (1). These relationships have been used to elaborate molecular theories of the mechanism of anesthesia. These physical properties are a result of the structure of the gases; thus their study reveals only parallel behavior in magnitudes, not a structure-activity relationship. A true structure-activity study, by this standard, has not been made to date.

We have recently developed a method of quantitating the topological structure of organic molecules, which we call molecular connectivity (2). The method derives numerical descriptors in which are encoded information about the number and kind of atoms and their bonding relationship to each other. This method has produced very significant correlations with all the physical properties noted above, as well as others (2-8). In addition, molecular connectivity has been successfully used to arrive at structure-activity relationships with many classes of biological agents (2, 3, 9).

The method lends itself ideally to initiation of a structure-activity study of anesthetic gases.

METHODS

A brief description of the calculation of molecular connectivity indices is given here (for a thorough description, see ref. 2). The structure of a molecule can be reflected by a set of numbers, which we call χ terms. The terms are computed from a

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hydrogen-suppressed formula or graph of the molecule.

The simplest term designated ${}^0\chi$ and called a connectivity χ, is computed by the equation

$$ {}^0\chi = \sum (\delta_i)^{-1/2} \qquad (1) $$

where the sum is over all non-hydrogen atoms in the molecule and δ_i is a number assigned to each atom, reflecting the number of atoms connected to it. The nature of the atom is not considered for the simple connectivity terms. The first-order ${}^1\chi$ is computed by the equation

$$ {}^1\chi = \sum (\delta_i \delta_j)^{-1/2} \qquad (2) $$

where the sum is over all connections or edges in the hydrogen-suppressed graph. Atoms i and j are formally bonded.

In general, extended terms of χ, ${}^m\chi_p$, are computed for linear paths (P) of m bonds by the equation

$$ {}^m\chi_p = \sum_{j=1}^{N_s} \left[\prod_{i=1}^{m+1} \delta_i \right]_j^{-1/2} \qquad (3) $$

where N_s is the number of distinct paths with m edges.

Terms describing nonlinear arrangements of bonds, such as clusters of three bonds and circuits (or rings), are computed in an analogous way.

The specific treatment of heteroatoms and unsaturation requires further differentiation of atom connectivity beyond simple adjacency. This level of treatment leads to valence χ terms, χ^v. For this calculation, the valence δ^v values are assigned on the basis of the expression

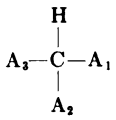
$$ \delta^v = Z^v - h_i \qquad (4) $$

where Z^v is the number of valence electrons and h_i is the number of attached hydrogen atoms.

In the case of halogen atoms, the use of Eq. 4 leads to identical values of δ^v for each halogen, hence redundant values of χ^v. At this time we must depart from the nonempirical δ^v values and utilize an empirical assignment of δ^v, based on fitting of the χ^v for halogen-containing molecules to molar refraction data (2, 8). Sulfur was treated in a similar fashion (2).

The nature of χ can be briefly summarized by stating that it is a simply computed number (or numbers) which is a weighted count of bonds and connected sets of bonds. The weighting is based on the connectivity of each atom in a bond formula or graph of the molecule.

Although quantum mechanics will provide accurate charges for the polar hydrogens, the calculations are lengthy and involved. Because our aim is to elaborate easily usable, quantitative structure-activity relationships, we have derived the following charge attribution:



for $A_i \neq H$

$$ A = \sum_{A_i=1}^{i} (B_i - B_C) \qquad (5) $$

where B_i and B_C are the Pauling electronegativities (10) of atoms i and carbon C, respectively. Then we approximate that

1. For $A > 1.0$, $Q_H = +0.10$
2. $0.5 \leqq A \leqq 1.0$, $Q_H = +0.05$
3. $A < 0.5$, $Q_H = 0.00$

We have ranked polar hydrogens in three categories and given approximate charges to each category. Charges derived by quantum mechanics are available for some of the anesthetic gases (11, 12), and we have used them to elaborate and check our charge attribution. Halothane and methoxyflurane were at the border between categories 1 and 2; because of adjacent CF_3 and CF_2 groups, respectively, they were given $Q_H = +0.10$. For acetylene, which cannot be handled by this technique, the available CNDO[2] charges (12) put it in the $Q = +0.05$ range.

The experimental anesthetic pressures chosen were the ED_{50} pressures (ATA) for righting reflex of mice determined by Miller *et al.* (13, 14). At this point molecular connectivity cannot deal with atomic

[2] The abbreviation used are: CNDO, complete neglect of differential overlap; ATA, anesthetic ED_{50} pressures.

molecules; thus noble gases were not considered. The diatomic molecules H_2 and N_2 were also omitted. Since the quality of the experimental data is not uniform, the correlations were divided into two groups. The first, considered by Miller *et al.* (14) to represent superior experimental data, is shown in Table 1, and the second, containing all the experimental data, in Table 2.

A complete set of connectivity indices was calculated for the molecules shown in Table 2 and statistically analyzed with an IBM REGR program to find the best set of variables. Also included in the statistical analysis were the logarithm of the molecular weights and the attributed charge (Q_H) on the most polar hydrogen (or hydrogens) of the molecule.

RESULTS AND DISCUSSION

The statistical analysis has shown that the single best variable to correlate with activity is ${}^0\chi^r$, and the best set of two variables is ${}^0\chi^r$ and Q_H.

The following equations have been formulated from the data in Table 1. The letter p in log $1/p$ is the effective anesthetic pressure (ATA) in atmospheres; n is the number of data points used in deriving the equations; R is the correlation coefficient, and s is the standard deviation. The 95% confidence intervals are shown in parentheses.

$$\log 1/p = 0.694\ (\pm 0.06)\,{}^0\chi^r - 0.793\ (\pm 0.15) \quad (6)$$
$$R = 0.958,\ s = 0.388,\ n = 14,\ F = 134.2,\ p < 0.001$$

$$\log 1/p = 22.8\ (\pm 4.7)\,Q_H - 0.282\ (\pm 0.26) \quad (7)$$
$$R = 0.812,\ s = 0.791,\ n = 14,\ F = 23.3,\ p < 0.001$$

$$\log 1/p = 0.548\ (\pm 0.06)\,{}^0\chi^r + 8.28\ (\pm 2.2)\,Q_H - 0.797\ (\pm 0.10) \quad (8)$$
$$R = 0.982,\ s = 0.267,\ n = 14,\ F = 148.8,\ p < 0.001$$

Equation 6, with one variable, already accounts for 92% (R^2) of the variance in log $1/p$, while Eq. 8 accounts for 96% (R^2). Table 3 includes activity predictions using Eq. 8, and these results indicate satisfactory ranking.

The equations formulated from the data in Table 2 are

$$\log 1/p = 0.571\ (\pm 0.06)\,{}^0\chi^r - 0.638\ (\pm 0.16) \quad (9)$$
$$R = 0.881,\ s = 0.496,\ n = 28,\ F = 90.4,\ p < 0.001$$

TABLE 1

Correlation of anesthetic ED_{50} pressures (ATA) for righting reflex of mice, using most reliable experimental data

Anesthetic	log $1/p$		$\lvert\Delta \log 1/p\rvert$	${}^0\chi^r$	Q_H
	Observed[a]	Calculated			
CF_4	−1.24	−1.01	0.23	−0.394	0.00
C_2F_6	−1.19	−0.98	0.21	−0.342	0.00
C_3F_8	−1.16	−0.96	0.20	−0.289	0.00
SF_6	−0.75	−1.24	0.49	−0.813	0.00
N_2O	−0.18	−0.10	0.08	1.264	0.00
$CH_2{=}CH_2$	−0.15	−0.02	0.13	1.414	0.00
CF_2Cl_2	0.40	0.55	0.15	2.461	0.00
CH_3CClF_2	0.60	0.44	0.16	2.257	0.00
c—C_3H_6	0.80	0.37	0.43	2.121	0.00
$CHClF_2$	0.80	0.76	0.04	1.334	0.10
Et—O—Et	1.52	1.71	0.19	3.822	0.05
$CHCl_3$	2.08	2.33	0.25	4.189	0.10
$CF_3CClBrH$	2.11	2.00	0.11	3.595	0.10
$CHCl_2CF_2OCH_3$	2.66	2.47	0.19	4.446	0.10

[a] From ref. 14.

TABLE 2

Correlation of anesthetic ED_{50} pressures (ATA) for righting reflex of mice

Anesthetic	log 1/p		|Δ log 1/p|	$^0\chi^r$	Q_H
	Observed[a]	Calculated			
CF_4	−1.24	−1.00	0.24	−0.394	0.00
C_2F_6	−1.19	−0.98	0.21	−0.342	0.00
C_3F_8	−1.16	−0.95	0.21	−0.289	0.00
SF_6	−0.75	−1.21	0.46	−0.813	0.00
CH_4	−0.66	−0.81	0.15	0.000	0.00
N_2O	−0.18	−0.18	0.00	1.264	0.00
$CH_2{=}CH_2$	−0.15	−0.11	0.04	1.414	0.00
$CH_3{-}CH_3$	−0.11	0.18	0.29	2.000	0.00
C_3H_8	0.05	0.54	0.49	2.707	0.00
C_2H_2	0.15	0.28	0.13	1.155	0.05
$CH_3{-}CHF_2$	0.35	0.78	0.43	1.130	0.10
$CH_3CH{=}CH_2$	0.40	0.33	0.07	2.284	0.00
CF_2Cl_2	0.40	0.41	0.01	2.461	0.00
$CH_3{-}CClF_2$	0.60	0.31	0.29	2.257	0.00
$c{-}C_3H_6$	0.80	0.24	0.56	2.121	0.00
$CHClF_2$	0.80	0.88	0.08	1.334	0.10
$CFCl_3$	0.82	1.12	0.30	3.888	0.00
CH_3F	0.85	0.60	0.25	0.776	0.10
CH_3Cl	0.85	0.80	0.05	2.204	0.05
CH_3I	1.15	1.39	0.24	4.430	0.00
$CH_3{-}CH_2Cl$	1.40	1.15	0.25	2.911	0.05
$CH_3{-}CH_2Br$	1.40	1.02	0.38	3.691	0.00
Et—O—Et	1.52	1.60	0.08	3.822	0.05
CH_2Cl_2	1.52	1.25	0.27	3.115	0.05
$CH_3{-}CHCl_2$	1.59	1.68	0.09	3.985	0.05
$CHCl_3$	2.08	2.30	0.22	4.189	0.10
$CF_3CClBrH$	2.11	2.00	0.11	3.595	0.10
$Cl_2CHCF_2OCH_3$	2.66	2.42	0.24	4.446	0.10

[a] From refs. 13 and 14.

$$\log 1/p = 15.6\ (\pm 3.8)Q_H + 0.07\ (\pm 0.20) \quad (10)$$

$$R = 0.627,\ s = 0.817,\ n = 28,\ F = 16.9,\ p < 0.001$$

$$\log 1/p = 0.496\ (\pm 0.04)^0\chi^r + 10.3\ (\pm 1.3)Q_H - 0.807\ (\pm 0.09) \quad (11)$$

$$R = 0.966,\ s = 0.278,\ n = 28,\ F = 173.3,\ p < 0.001$$

Equations 9 and 11 account for 78% (R^2) and 93% (R^2), respectively, of the variance in log $1/p$. The inclusion of the variable Q_H is statistically significant at the 99.9% level, based on the proper statistical test [$F(1, 25) = 58$]. Furthermore, $^0\chi^r$ and Q_H do not significantly correlate ($R = 0.282$).

Let us now put these equations to test on the qualitative structure-activity relationships which have been accumulated through research on anesthetics (15, 16). Table 3 shows the effects of halogen substitution on anesthetic activity and our calculated activities. Although the experimental values are not in the same units as the calculated ones, we can clearly see that the equations we have derived account for the following structure-activity observations. (a) Halogenation of hydrocarbons and ethers increases potency in the order $F < Cl < Br < I$. (b) Fluorination usually decreases potency, boiling point, and flammability and increases the stability of adjacent halogen atoms. (c) Compounds substituted as dibromides and dichlorides tend to be more potent than their monosubstituted analogues. The dibromo compounds are more potent than the dichloro derivatives. (d) As shown in Tables 1 and 2, the low activity of perfluoro compounds is well accounted

TABLE 3

Effect of halogen substitution on anesthetic potency

Compound	Calculated log $1/p^a$	MAC[b]	Compound	Calculated log $1/p^a$	MAC	Compound	Calculated log $1/p^a$	MAC
		vol %			*vol %*			*vol %*
CF_3CH_2F	0.20	40	CF_3CHF_2	0.01	50	CF_3CHF_2	0.01	50
CF_3CH_2Cl	0.57	8	CF_3CHClF	0.79	15	CF_3CHCl_2	1.57	2.7
CF_3CH_2Br	1.00	2.8	CF_3CHBrF	1.22	5	$CF_3CHClBr$	2.00 (2.11)[c]	0.8
CF_3CH_2I	1.79	1.25	CF_3CHIF	2.01	2	CF_3CHBr_2	2.43	0.4

[a] Calculated from Eq. 8.

[b] MAC, Minimum alveolar concentration in volume % from ref. 16.

[c] Experimental value from ref. 14.

for by our equations, and this also quantifies the observation that complete hydrogen substitution by fluorine in the molecules usually produces a physiologically inert compound. (e) Q_H for the polar hydrogens accounts well for the observations that compounds containing an asymmetric end carbon seem to produce good anesthesia (e.g., —CHFCl, —CHFBr, and —CHClBr) and, to a lesser degree, that one or more hydrogen atoms in the molecule are necessary for effective central nervous system depression.

Let us now try to interpret the equations that we have derived in relation to the theories of anesthesia and the best previously reported property-activity relationships.

The best correlation of activity with physical properties is with olive oil/anesthetic gas partition coefficients (14). The correlation coefficient R is greater than 0.99, and the standard deviation is very low, as can be seen in Fig. 2 of ref. 14. This correlation contains 21 anesthetics for which superior experimental activity data are available. The same types of animals were used; standard error or 95% confidence limits are given; the results of several laboratories are compared; and the corrected pressure (fugacity) is used to account for nonideal gases. It is this set of superior experimental data that has been used in Table 1 and gives a correlation coefficient of 0.982 for a two-variable equation.

Hansch *et al.* (17) also obtained good results in the correlation of activity with octanol/water partition coefficients (P) tion coefficients (14, 17) emphasize the importance of lipid solubility for anes- and a polar factor for hydrogens (I). With these two variables, for 30 molecules the correlation coefficient was 0.947 and the standard deviation 0.438. If we extract from their list the set of superior experimental data that comes from ref. 14 described above, Hansch's two-variable correlation has the following characteristics: $R = 0.923$, $s = 0.624$, and $n = 18$. It is generally accepted that a model which properly describes the system should give improved correlation statistics with better data; such is the case with the connectivity approach presented here, but, strangely, not with Hansch's partitioning model.

To check further whether molecular connectivity gives superior correlations with oil/water partition coefficients, we have also calculated the regression equations for the set of molecules that we have in common with Hansch's set (17). For a set of 23 molecules, Hansch's two-variable regression gives $R = 0.910$, $s = 0.434$, and with only the logarithm of the partition coefficient (log P), $R = 0.319$, $s = 0.967$; for our two variables we obtain $R = 0.967$ and $S = 0.266$, and with only the connectivity term, $R = 0.864$ and $s = 0.514$. Both ${}^0\chi^r$ alone and ${}^0\chi^r$ with Q_H give better correlations than log P alone and log P with I.

${}^0\chi^r$ does not correlate with the logarithm of the oil/water partition coefficient ($R = 0.398$ for $n = 24$) for these anesthetics. This absence of correlation rules out the possibility that ${}^0\chi^r$, in this case, is only another way of computing oil/water partition coefficients.

The correlations of activity with partithetic activity and indicate the importance of both dispersion forces and polar forces (including hydrogen bonding). These correlations would support the Meyer-Overton hypothesis: "Narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell" (18).

Another view of anesthetic potency involves a modified version of Mullins' original model, which has been called the critical volume hypothesis (19–21). It states: "Anesthesia occurs when the volume of a hydrophobic region is caused to expand beyond a certain critical amount by the absorption of molecules of an inert substance. If the volume of this hydrophobic region can be restored by changes of temperature or pressure, then the anesthesia will be removed."

These two hypotheses can be summarized in two equations:

Meyer-Overton "mole fraction theory":

$$P_{50} \cdot x_2 = \text{constant} \quad (12)$$

Miller-Mullins "volume fraction theory":

$$P_{50} \cdot x_2 \cdot \bar{V}_2 = \text{constant}$$

where P_{50} is the ED_{50} pressure of the anesthetic in the atmosphere, x_2 is the mole fraction solubility in the lipid phase (or some analogue of it, such as olive oil), and $\bar{V}_2$ is the partial molar volume of the anesthetic in the lipid phase, in milliliters per mole.

Cavity surface areas (3) and molecular volumes[3] have been correlated quite well with molecular connectivity indices, and this can provide a basis for the good correlation betwen anesthetic activity and ${}^0\chi^r$. The role which ${}^0\chi^r$ plays in this correlation parallels the volume of the molecules; thus our findings would support the "critical volume fraction theory" and the suggestions presented by Cohen *et al.* (22). But ${}^0\chi^r$ reflects more than just the size of the molecule, which, incidentally, does not correlate very well with anesthetic activity. It is consistent with our earlier work that ${}^0\chi^r$ reflects the volume expansion caused by the anesthetic gases, which contains the volume of the molecule itself plus the contribution to expansion of more specific dipole effects of the halogens, the ether oxygens, and the π bonds. For example, these dipole effects could lead to the perturbation of some important hydrogen bonds and cause expansions (23–27).

For molecules containing a polar hydrogen, we have used their attributed charge Q_H. These polar hydrogens are considered implicitly in the connectivity indices, but because molecular connectivity is a property of the whole molecule it does not explain very localized properties of the molecules (in its present stage of elaboration, and especially for hydrogens, at least). Experimentally, these polar hydrogens were found to be better hydrogen bond donors than aliphatic C—H (28). Thus these polar hydrogens can also contribute to the volume expansion caused by the anesthetics because of their hydrogen bonding ability and their ability to perturb existing hydrogen bonds.

Davies *et al.* (29) have given a quantitative interpretation of phase effects in anesthesia. They fitted the anesthetic potencies of 45 halogenated hydrocarbons to a simple phase distribution model. Their model approximates the activity coefficient at infinite dilution as a function of the dominant intermolecular interactions in the given phase. For their series, containing only halogenated hydrocarbons, their function reduces to an empirical balance between van der Waals interactions and hydrogen bond donor properties. Gas/liquid partition coefficients were estimated, based on the additive effect of substituent groups upon a parent compound. The scale for estimating the acidity of hydrogens was adopted, counting a halogen on a carbon atom attached to the acidic hydrogen as unity, while the influence of halogens acting throgh each carbon atom is reduced successively by a factor of 3.

An analogy can be drawn between the correlation of Davies *et al.* and ours. Their van der Waals interactions term, depicted by gas/liquid partition coeffi-

[3] L. B. Kier, unpublished observations.

cients, corresponds to our ${}^0\chi^r$ term. Also their scheme for estimating the acidity of hydrogens depicts hydrogen bonding ability as our Q_H term does.

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