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Letter

Contributions of dipole moments, quadrupole moments, and molecular polarizabilities to the anesthetic potency of fluorobenzenes

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Abstract

Previous studies have emphasized the role of molecular polarizability and electric moments, especially dipole and quadrupole moments, in binding of drugs to sites of action. A recent publication of ED50s that prevent response to a noxious stimulus for eight fluorobenzenes has made it possible to compare anesthetic potency with ab initio Hartree–Fock calculations of molecular polarizability as well as dipole and quadrupole moments. Fluorobenzenes provide a stringent test of the role of electric moments in anesthetic potency because individual dipole moments range from 0 to 2.84 debye (D) while the quadrupole moment of benzene is large and negative (-30×10^{-40} C m^2), that of hexafluorobenzene is large and positive (30×10^{-40} C m^2), and that of 1,3,5-trifluorobenzene is nearly zero. We found that anesthetic potency of fluorobenzenes was not affected by the presence of either dipole or quadrupole moments. This result is surprising because fluoroalkanes and fluorocycloalkanes are most potent when half fluorinated and are usually not anesthetics when perfluorinated. The results suggest that electrostatic interactions are not important for binding of fluorobenzenes at sites of anesthetic action and that these sites are different from those that bind conventional anesthetics.

Keywords: Dipole; Quadrupole; Polarizability; Anesthesia; Fluorobenzene; Drug binding

1. Introduction

Previous studies have emphasized the role of molecular polarizability and electric moments, especially dipole and quadrupole moments, in binding of drugs to sites of action. Special interest has been given to aromatic–aromatic and cation–aromatic interactions because of the importance of the corresponding aromatic and cationic amino acids in protein stability [1,2]. The highly directional nature of the axial quadrupole moment of the fluorobenzenes leads to predic-

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tions of the structure of binary homomeric and heteromeric complexes of benzene and hexafluorobenzene that have been verified experimentally [3–6]. For example, the 20-fold lower potency of the pentafluorophenyl analog of angiotensin II was suggested to result from reversal of direction of the molecular quadrupole of native angiotensin II. The assumption was made that both drugs are held in the same partially constrained binding site [7].

Recent publication of the anesthetic potencies of fluorine-substituted benzenes [8] has made it possible to compare these potencies with the molecular polarizability, dipole and quadrupole moment of each gas. The potencies are expressed as the partial pressure (atm) in the alveoli that prevents response to a noxious stimulus in 50% of a population of rats; the minimum alveolar concentration (MAC). The series of molecules includes benzene, 1-fluorobenzene, 1,2-difluorobenzene, 1,4-difluorobenzene, 1,3,5-trifluorobenzene, 1,2,4-trifluorobenzene, pentafluorobenzene, hexafluorobenzene, toluene and perfluorotoluene [8]. This series provides a stringent test of the importance of electric moments in anesthetic potency because individual dipole moments range from 0 to 2.84 debye (D) while the quadrupole moments even change sign. Both experiments and computations show that the molecular quadrupole of benzene is large and negative (-30×10^{-40} C m^2), that of hexafluorobenzene is large and positive (30×10^{-40} C m^2), and 1,3,5-trifluorobenzene is nearly zero [4,5].

We also considered the molecular polarizability of each fluorobenzene because this is the leading term in calculating both charge-induced dipole and London dispersion interactions [9,10]. These two interactions are likely to be important for binding of fluorobenzenes at their sites of action. This is especially true for 1,3,5-trifluorobenzene that, at least at the molecular level, was predicted to have neither charge, dipole, nor quadrupole interactions with a binding site [1].

Our recent investigations of single site-directed mutations revealed significant changes in anesthetic potency in glutamate receptors [11] and alterations in the carbon chain length 'cutoff' in alkanol potency in GABA receptors [12]. We and others [13,14] have interpreted these changes in terms of specific steric requirements in the anesthetic binding sites. As a result, we included the steric properties of molecular volume and maximum atom center to atom center length in this study.

In the present study electronic and steric properties were calculated and then compared with two indices of anesthetic potency; the MAC values and MAC times the octanol/gas partition coefficient. The latter value predicts anesthetic concentration in a slightly polar hydrophobic phase and forms the basis of the Meyer–Overton theory of narcosis [15,16].

2. Material and methods

The fluorobenzenes were modeled with the Builder module of Insight II (MSI, San Diego, CA) and then the structures were optimized with Discover 97 (MSI). The structures were further optimized by ab initio Hartree–Fock calculations with Turbomole (MSI) using the 6-31g** basis set. Finally, polarizabilities and electric moments were calculated with Turbomole. We calculated the quadrupole tensors from the second moments given by Turbomole by applying, for example, $Qxx = (3XX - RR)/2$ where $RR = XX + YY + ZZ$. We calculated a scalar value (Q) for the axial quadrupole moment of the axially symmetric molecules (benzene, 1,3,5-trifluorobenzene and hexafluorobenzene) by applying $Q = Qzz = -2Qxx = -2Qyy$ [3,7]. The values for axial quadrupole moments calculated as described agreed well with experimental values [3,4]. For example, we calculated Q for hexafluorobenzene to be $+31.5$ C $m^2 \times 10^{-40}$ and the experimental value is $+(32 \pm 2)$ C $m^2 \times 10^{-40}$ [3]. A nearly identical result ($+31.66$ C $m^2 \times 10^{-40}$) was obtained with Gaussian 94 using the 6-311 + G basis set (Gaussian, Inc., Pittsburgh, PA). Including electron correlation at the MP2 level of theory with the 6-31g** basis set (Turbomole, MSI) changed the result only slightly to $+31.65$ C $m^2 \times 10^{-40}$ and was not done for other molecules because of greatly increased computation time. Molecular volumes and maximum lengths, atom

Table 1
Electronic moments and molecular polarizabilities of fluorobenzenes and fluorotoluenes

Molecule	Dipole (D)	Quadrupole (Q)	Polarizability (au)	MAC (atm)	MAC X oct/gas (atm)	Volume (A^3)	Length (A)
Benzene	0	−28.9	48.1	0.0101 + 0.0016	4.43	104.9	5.2
1-Fluorobenzene	1.68	NAS	53.9	0.0112 + 0.0006	6.02	112.5	5.2
1,2-Difluorobenzene	2.84	NAS	54.3	0.0061 + 0.0008	3.77	119.2	5.2
1,4-Difluorobenzene	0	NAS	47.3	0.0064 + 0.0000	3.27	119.1	5.4
1,3,5-Trifluorobenzene	0	1.8	57.9	0.0222 + 0.0015	5.53	124	5.2
1,2,4-Trifluorobenzene	1.61	NAS	54.6	0.0097 + 0.0019	4.77	124	5.4
Pentafluorobenzene	1.59	NAS	59.0	0.0125 + 0.0002	4.05	138.8	5.4
Hexafluorobenzene	0	31.5	49.2	0.0161 + 0.0016	3.23	147.3	5.4
Toluene	0.27	NAS	65.0	0.0045 + 0.0006	6.93	126.2	5.9
Perfluorotoluene	0.69	NAS	76.0	Convulsant	Convulsant	177.5	6.3

The dipole moments (D), quadrupole moments (C $m^2 \times 10^{-40}$), and molecular polarizabilities (au) were calculated as described in Section 2. Quadrupole moments are shown for only those molecules with axial symmetry; other molecules do have molecular quadrupole tensors but they cannot be represented as scalar quantities because they are not axially symmetric (NAS). The minimum alveolar concentrations (MAC in atm) that prevents response to a noxious stimulus in 50% of a population of rats and the product of MAC times the corresponding octanol/gas partition coefficients (atm) are from Fang et al. [8]. Molecular volumes (A^3) and maximum lengths (A), atom center to atom center, were calculated as described in Section 2.

center to atom center, were calculated with Spartan (Wavefunction, Inc., San Diego, CA).

3. Results

Table 1 shows that anesthetic potency (MAC) remained remarkably constant for the entire series of fluorobenzenes [8]. This surprising finding is in contrast to previous studies with fluoroalkanes and cyclofluoroalkanes in which the highest potencies (lowest MAC) were found for half-fluorinated molecules [17]. For example, in the fluoromethane series the MAC values of CH4, CH2F2 and CF4 are 8, 0.7 and 66 atm, respectively [17]. All higher perfluoroalkanes [17] and perfluorocycloalkanes [8] have no anesthetic potency [18].

Inspection of Table 1 reveals no correlation between both dipole or quadrupole moments and MAC values. Multiplication of MAC values by the corresponding octanol/gas partition coefficients provided the predicted membrane concentrations but did not improve the relationships. The lack of effect of molecular electric moments is striking (Fig. 1). For example, 1,2-difluorobenzene has the largest dipole moment (2.8 D) but is equipotent to 1,4-difluorobenzene with no dipole moment. Moreover, benzene, hexafluorobenzene, and 1,3,5-trifluorobenzene are nearly equipotent despite great variation in quadrupole moments.

The Meyer–Overton hypothesis proposed that anesthesia occurs when the anesthetic concentration in a non-polar region of a membrane is 30–50 mmol of anesthetic per mmol of membrane [15,16]. This hypothesis predicts that MAC times the octanol/gas partition coefficient should be a constant. In the case of conventional anesthetics that value is 2.8 (slope 0.98, $r^2 = 0.98$) [19]. However, for the fluorobenzenes studied here that mean value is 4.38. Thus, these compounds are approximately half as potent as conventional anesthetics according to the Meyer–Overton hypothesis.

The calculated polarizabilities shown in Table 1 are all very similar. It may be that this small range of polarizabilities resulted in the corresponding similarities in anesthetic potencies. The calculated polarizabilities were compared graphically with both MAC values and oil/gas partition coefficients but no correlation was observed.

4. Discussion

Anesthetic drugs have long been considered to have non-specific effects in a non-polar mem-

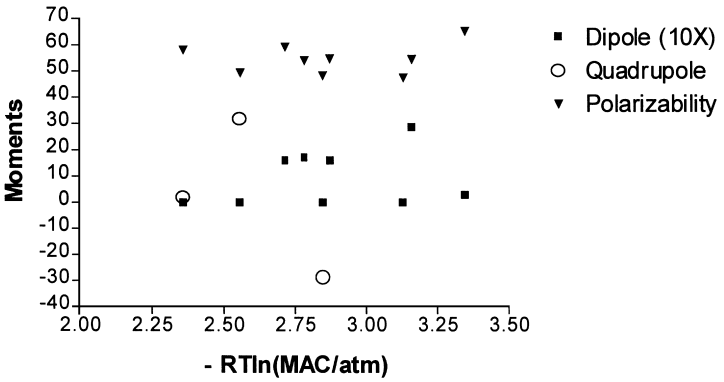

Fig. 1. Relative anesthetic potency expressed as $-RT \ln(MAC/atm)$ (kcal/mole) vs. dipole moments (debye $\times 10$), quadrupole moments for the three molecules with axial symmetry ($Cm^2 \times 10^{-40}$), and molecular polarizabilities (au). Relative anesthetic potency increases from left to right and relative binding energy should increase as an approximately linear function of dipole moment or polarizability. Thus, if anesthetic potency were a function of either dipole moments or polarizability their respective graphs would have a positive slope of one.

brane phase [19–21]. However, more recent studies have implicated a protein site for anesthetic action [22]. There is a growing consensus that the sites of anesthetic action are unique [18] and specific [11–13,23]. In contrast to non-polar hydrophobic phases, protein binding sites offer a wide range of electrostatic interactions with anesthetic drugs [24]. In this study we have examined the role of molecular polarizability as well as dipole and quadrupole moments in determining the anesthetic potency of fluorobenzenes.

We conclude that in the case of the fluorobenzenes, the presence of dipole and quadrupole moments does not affect their anesthetic potency (Fig. 1). This conclusion is supported when these electronic properties are compared with either anesthetic MAC values or with the predicted solubility in a moderately polar membrane phase (MAC $\times$ octanol/gas partition coefficient) [8].

In a recent study of a model binding site for the anesthetic action of noble gases we suggested that a charged group in the binding site contributed substantial binding energy through a charge-induced dipole term [25]. This result agreed with a previous thermodynamic study that suggested a charge in the binding site was essential for binding of noble gases to anesthetic sites of action [26]. However, the present results suggest that there is no charged group in the binding sites for the fluorobenzenes because either charge-dipole or charge-quadrupole interactions could contribute as much as -8.1 or -5.0 kcal/mol more binding energy [7], respectively, compared to 1,3,5-trifluorobenzene that possesses neither a dipole nor a quadrupole.

The lack of a clear correlation between anesthetic potency and either molecular polarizabilities, dipole moments, or quadrupole moments (Table 1 and Fig. 1) suggests that it is unlikely that there is a charged group in the binding sites relevant to anesthetic action. Therefore we suggest that London dispersion energy is the major contributor to binding of fluorobenzenes to sites of anesthetic action.

It is tempting to propose that these molecules all bind non-specifically to a large hydrophobic cavity [27]. This is not the case, however, molecular structure and volume are very important because addition of a single trifluoromethyl group to hexafluorobenzene to form perfluorotoluene creates a potent convulsant [8]. Perfluorotoluene is devoid of anesthetic potency when administered alone and does not affect the MAC of the conventional anesthetic desflurane when they are administered together [8]. The fluorobenzenes, in contrast, show additive potency with desflurane (i.e. 50% MAC of hexafluorobenzene + 50% MAC of desflurane = 1 MAC for preventing response to a noxious stimulus). Previous studies have shown a dependence of anesthetic potency

on molecular volume [12,28] and chain length [13,14]. Perfluorotoluene has significantly greater volume and maximum length compared to other members of this series (Table 1).

We conclude that production by fluorobenzenes of an anesthetic state, as defined by lack of response to a noxious stimulus, results from an interaction at non-polar hydrophobic sites. The properties of those sites are quite distinct from those of sites where alkanes [13,28] and cycloalkanes [14] produce an anesthetic state. Furthermore, the abrupt loss of anesthetic potency in transforming hexafluorobenzene to perfluorotoluene suggests that there are considerable steric constrains in these sites.

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