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The *N*-Methyl-D-aspartate Receptor Inhibitory Potencies of Aromatic Inhaled Drugs of Abuse: Evidence for Modulation by Cation-π Interactions

Douglas E. Raines, Fredrick Gioia, Robert J. Claycomb, and Renna J. Stevens

Department of Anesthesia and Critical Care, Massachusetts General Hospital, Boston, Massachusetts

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ABSTRACT

Benzene and several close structural analogs are inhaled drugs of abuse with general anesthetic activity. By virtue of their π electron clouds, they may engage in attractive electrostatic interactions with cationic atomic charges on protein targets. In this study, we tested the hypothesis that inhaled drugs of abuse inhibit human *N*-methyl-D-aspartate (NMDA) receptors with potencies that correlate with their abilities to engage in cation-π interactions. Electrophysiological techniques were used to define the NR1/NR2B NMDA receptor inhibitory concentrations of volatile benzene analogs, and computer modeling was used to quantify their abilities to engage in cation-π interactions and their molecular volumes. In addition, each compound's octanol/gas partition coefficient (a measure of hydrophobicity) was quantified. All 18 compounds inhibited human NR1/NR2B NMDA receptors reversibly and in a concentration-dependent manner. NMDA receptor inhibitory potency correlated strongly with the ability to engage in cation-π interactions, weakly with hydrophobicity, and was independent of molecular volume. This is consistent with the hypothesis that cation-π interactions enhance the binding of inhaled drugs of abuse to the NMDA receptor and suggests that the receptor binding site(s) for these drugs possesses significant cationic character.

More than a century ago, Meyer and Overton observed that compounds induce anesthesia with potencies that correlate strongly with their oil solubilities, implying that hydrophobic interactions are the major determinants of anesthetic potency (Meyer, 1899; Overton, 1901). This correlation, termed the Meyer-Overton Correlation, is most commonly interpreted to mean that anesthetics induce anesthesia via interactions with hydrophobic protein domains, a view supported by numerous studies demonstrating that anesthetics alter the function of proteins with potencies that correlate strongly with both their hydrophobicities and in vivo anesthetic potencies (Wood et al., 1991; Firestone et al., 1994; Mihic et al., 1994; Zimmerman et al., 1994; Raines et al., 2002). However, it has been suggested that other interactions also make important contributions toward defining anesthetic potency (Eckenhoff and Johansson, 1997; Johansson and Zou, 1999). In particular, electrostatic interactions (e.g., hydrogen bonding interactions) between anesthetics and their receptor targets have recently been suggested to modulate the agonist-enhancing actions of anesthetics on $GABA_A$ and nicotinic acetylcholine receptors and the inhibitory actions of anesthetics on firefly luciferase (Abraham et al., 1991; Moss et al., 1991; Raines et al., 2001, 2003).

Volatile aromatic compounds such as benzene and toluene are found in a variety of household and commercial products where they are commonly used as solvents or as major components of adhesives and cleaning fluids. Some may also be inhaled to produce dizziness, learning and memory impairment, and even unconsciousness, and thus they are general anesthetics (Neal and Robson, 1966; Massey and Jackson, 1973; Fang et al., 1996; Balster, 1998). By virtue of their π electrons, these inhalants may engage in electrostatic interactions with protein residues that possess cationic atomic charge (Mecozzi et al., 1996). Such interactions (cation-π interactions) can have strengths that are comparable with or stronger than that of a typical hydrogen bond (several kilocalories per mole) and have become increasingly recognized as an important molecular force contributing to the binding of ligands to receptor targets (Zhong et al., 1998; Beene et al., 2002; Manderson and Johansson, 2002; Watts, 2002; Zacharias and Dougherty, 2002). Consequently, cation-π interactions may be important determinants of the receptor potencies of volatile aromatic inhalants.

The NMDA receptor is a calcium-permeable ligand-gated

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; atm, atmospheres.

ion channel that mediates excitatory synaptic transmission in the brain. It is thought to play an important role in synaptic plasticity, memory, and motor coordination and has been implicated in a number of pathological conditions, including Alzheimer's and Parkinson's diseases, Huntington's chorea, schizophrenia, and epilepsy (Chase et al., 2000; Coyle et al., 2002; Kemp and McKernan, 2002). The NMDA receptor is also considered to be an important target of a number of inhaled and noninhaled anesthetics such as nitrous oxide, xenon, cyclopropane, ethanol, and ketamine that dose dependently and reversibly inhibit NMDA receptor-mediated currents at physiologically relevant concentrations (Peoples and Weight, 1995; Peoples et al., 1997; Yamakura and Harris, 2000; Raines et al., 2001). Benzene and alkylbenzenes also potently inhibit NMDA receptor function and induce anesthesia, suggesting that aromatic inhalants may produce their physiological effects, at least in part, by inhibiting NMDA receptor function (Cruz et al., 1998, 2000).

The purpose of this study was to test the hypothesis that cation-π interactions are important molecular forces governing the NMDA receptor inhibitory potencies of volatile aromatic inhalants. We used electrophysiological techniques to define the NMDA receptor inhibitory potencies of volatile aromatic inhalants and quantified each inhalant's octanol/gas partition coefficient (a measure of anesthetic hydrophobicity) experimentally and cation-π binding energy and molecular volume computationally for use as molecular descriptors for structure-activity analysis.

Materials and Methods

Determination of Solvent/Gas Partition Coefficients

Buffer/Gas Partition Coefficient. For each inhalant, a stock solution of buffer containing inhalant (0.05–1 mM) was prepared. Three to 7 ml of stock solution was added to a calibrated gastight 10-ml syringe via a three-way stopcock. Room air (gas) was added to the syringe to bring the total volume of buffer and gas to 10 ml, and the stopcock was closed. The stock solution and gas were shaken for 1 to 2 h at room temperature (20–22°C) to reach equilibrium. The concentrations of inhalant in the stock solution before and after equilibration with gas were determined spectrophotometrically (Table 1).

The partition coefficient (λ) was calculated as follows:

$$\lambda = \frac{V_{gas}}{V_{stock}} \cdot \frac{C_2}{(C_1 - C_2)} \quad (1)$$

where V_{gas} and V_{stock} are the volumes of gas and stock solution, respectively, in the syringe, and C_1 and C_2 are the inhalant concentrations of the stock solution before and after, respectively, equilibration with gas.

Octanol/Gas Partition Coefficients. For each inhalant, a stock solution of octanol containing inhalant (0.05–1 mM) was prepared. Two milliliters of stock solution were added to a 2-liter glass bottle that was immediately sealed with a Teflon-coated cap. The stock solution and gas were shaken at room temperature for 1 to 2 h to reach equilibrium. The concentrations of inhalant in the stock solution before and after equilibration with gas were determined as described above, and the octanol/gas partition coefficient was calculated using eq. 1.

Molecular Modeling

An electrostatic potential map for each inhalant was generated and cation-π binding energy calculated using MacSpartan Pro V1.01 (Wavefunction Inc., Irvine, CA) on an Apple MacIntosh G4 computer. Geometry optimization was performed using ab initio molecular orbital calculations (Hartree-Fock, 6-31G** basis set). The cation-π binding energy was defined as the negative binding energy of a generic cation probe (Na^+) to the inhalant's π electron cloud as described previously (Mecozzi et al., 1996). Molecular volumes were similarly obtained from ab initio molecular orbital calculations.

Oocyte Expression

Xenopus frogs were maintained and treated in accordance with regulations specified by the Massachusetts General Hospital Animal Care Committee (Boston, MA). Before surgery, frogs were anesthetized with 0.2% tricaine (ethyl-*m*-aminobenzoate) and hypothermia. Ovary lobes were excised via a small laparotomy incision and placed in OR-2 solution (82 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.6). After a 1-h incubation period in collagenase D (1 mg/ml in OR-2) to separate oocytes from connective tissue, stage 4 and 5 oocytes were selected for injection with mRNA encoding the NR1 and NR2B subunits of the NMDA receptor. Oocytes were injected with 5 to 10 ng of mRNA encoding for each subunit at a ratio of 1:1 and incubated in microtiter wells for at least 24 h in ND-96 buffer (96 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 0.8 mM $MgCl_2$ and 10 mM HEPES, pH 7.6) containing 5 U/ml of penicillin and 5 μg/ml of streptomycin at 17°C before electrophysiological studies.

TABLE 1
Partition coefficients, NMDA receptor IC_{50} values, and molecular volumes of volatile aromatic inhalants
Data are presented as mean ± S.D.

Inhalant	$\lambda_{buffer/gas}$	$\lambda_{octanol/gas}$	IC_{50}[a]	Hill Coefficient	Molecular Volume
			atm		$Å^3$
Benzene	4.9 ± 0.3	940 ± 50	$1.1 \pm 0.1\times10^{-3}$	1.2 ± 0.1	86.6
Toluene	4.8 ± 0.3	1830 ± 110	$5.6 \pm 0.3\times10^{-4}$	1.0 ± 0.1	103.8
o-Xylene	7.0 ± 1.0	9860 ± 300	$7.8 \pm 0.4\times10^{-4}$	0.82 ± 0.1	119.6
m-Xylene	4.1 ± 0.2	8430 ± 400	$1.9 \pm 0.2\times10^{-3}$	0.67 ± 0.06	119.7
p-Xylene	3.0 ± 0.7	5300 ± 210	$7.4 \pm 1.1\times10^{-4}$	0.72 ± 0.04	119.7
Ethylbenzene	3.5 ± 0.1	5450 ± 310	$1.1 \pm 0.2\times10^{-3}$	0.75 ± 0.10	119.8
Fluorobenzene	4.7 ± 0.3	930 ± 70	$8.1 \pm 0.6\times10^{-4}$	0.96 ± 0.06	92.1
o-Difluorobenzene	4.7 ± 0.2	1290 ± 130	$8.8 \pm 0.8\times10^{-4}$	0.94 ± 0.05	97.6
p-Difluorobenzene	4.4 ± 0.2	1230 ± 80	$4.8 \pm 0.2\times10^{-3}$	1.5 ± 0.1	97.6
1,3,5-Trifluorobenzene	2.1 ± 0.3	520 ± 40	$5.1 \pm 0.5\times10^{-3}$	1.0 ± 0.1	103.0
1,2,4-Trifluorobenzene	2.8 ± 0.6	950 ± 60	$5.9 \pm 0.8\times10^{-3}$	0.8 ± 0.1	101.0
Pentafluorotoluene	1.0 ± 0.1	1970 ± 50	$3.4 \pm 0.9\times10^{-2}$	1.0 ± 0.2	128.1
Pentafluorobenzene	1.1 ± 0.1	640 ± 10	$4.3 \pm 0.1\times10^{-2}$	1.0 ± 0.1	114.0
Perfluorobenzene	0.84 ± 0.03	390 ± 10	$9.2 \pm 0.8\times10^{-2}$	0.75 ± 0.04	119.4

[a] IC_{50} (atm) = IC_{50} (millimolar)/[(44.614)($\lambda_{buffer/gas}$)].

Electrophysiology

All experiments were performed at room temperature. Oocytes were placed in a 0.04-ml chamber and impaled at the animal pole with two capillary glass electrodes filled with 3 M KCl and possessing open tip resistances of 0.2 to 2 MΩ. Oocytes were voltage clamped at –50 mV using a GeneClamp 500B amplifier (Axon Instruments, Foster City, CA). Oocytes were perfused with buffer (96 mM NaCl, 2 mM KCl, 2 mM $BaCl_2$, and 10 mM HEPES, pH 7.6) at a rate of 4 ml/min. Buffer perfusion was controlled using a six-channel valve controller (Warner Instruments, Hamden, CT), interfaced with an Axon Digidata card, and driven by a personal computer using Axon's pClamp 8.0 software. The perfusion apparatus was made from gastight glass syringes and Teflon tubing to minimize absorptive and evaporative loss of inhalants. In parallel experiments, gas chromatographic analysis of solutions exiting the perfusion system and entering the oocyte chamber indicated that such loss was <15%. Inhalants were purchased from Aldrich Chemical Co. (Milwaukee, WI). NMDA and glycine was from Sigma-Aldrich (St. Louis, MO).

In each experiment, a control current was first obtained by perfusing the oocyte with buffer containing 100 μM NMDA (+ 10 μM glycine) for 30 s and measuring the peak current response. The effect of inhalant was then assessed after a 5-min recovery period by perfusing the oocyte with buffer containing inhalant for 60 s and then switching to buffer containing inhalant and 100 μM NMDA (+ 10 μM glycine) for 30 s and measuring the peak current response. After another 5-min recovery period, the control current was measured again to assure reversibility. The control peak current was quantified as the average of the two control experiments.

Data Analysis

Aqueous inhalant concentrations were converted to partial pressures using buffer/gas partition coefficients. Concentration-response curves were generated by plotting the peak current in the presence of anesthetic normalized to that in its absence (control). Each data point on all curves represents the mean of at least three measurements obtained using different oocytes and the error bars indicate the standard deviation from the mean. Data points were fit to a Hill equation using Igor Pro 4.01 (Wavemetrics Inc., Lake Oswego, OR) in the following form:

$$I_{peak} = \frac{IC_{50}^{n}}{IC_{50}^{n} + [Drug]^{n}} \quad (2)$$

where I_{peak} is the normalized peak current in the presence of inhalant, IC_{50} is the concentration of inhalant that reduces the peak current by one-half, and n is the Hill coefficient.

Results

All 14 volatile aromatic inhalants inhibited NMDA-elicited currents reversibly and in a concentration-dependent manner. Representative traces demonstrating current inhibition by benzene, 1,3,5-trifluorobenzene, and perfluorobenzene are shown in Fig. 1, A, B, and C, respectively. In each figure part, the first and last traces are the control currents recorded upon pulsing *Xenopus* oocytes expressing NR1/NR2B NMDA receptors with agonist. The middle trace demonstrates the NMDA receptor inhibitory actions of 3×10^{-3} atm benzene (Fig. 1A), 6.1×10^{-2} atm 1,3,5-trifluorobenzene (Fig. 1B), or 7.8×10^{-2} atm perfluorobenzene (Fig. 1C) when applied before and during the agonist pulse. The concentration-response curves for NMDA receptor inhibition by these three inhalants are shown in Fig. 1D. At the highest concentrations studied, benzene and 1,3,5-trifluorobenzene inhibited nearly all of the agonist-elicited current (90 ± 3 and 94 ± 2%, respectively), whereas perfluorobenzene inhibited only 47 ± 6% of the current even at the highest attainable concentration (i.e., saturation). A fit of the data in Fig. 1D to eq. 1 yielded IC_{50} values for benzene, 1,3,5-trifluorobenzene, and perfluorobenzene of $1.1 \pm 0.1 \times 10^{-3}$, $5.1 \pm 0.5 \times 10^{-3}$, and $9.2 \pm 0.8 \times 10^{-2}$ atm, respectively. The respective Hill coefficients derived from these fits were 1.2 ± 0.1, 1.0 ± 0.1, and 0.75 ± 0.04.

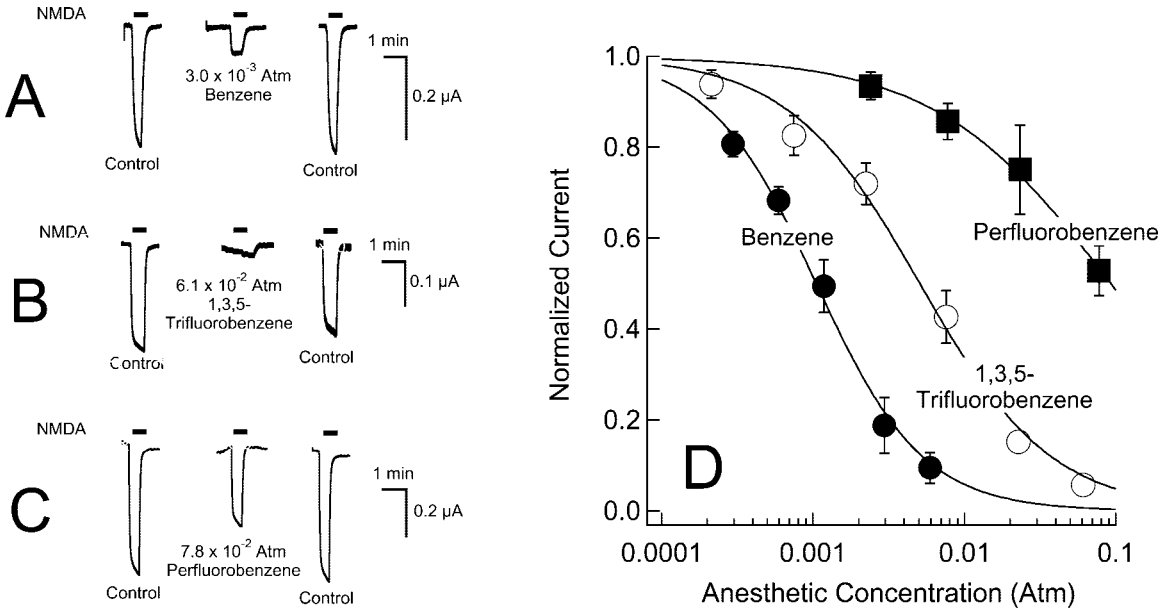

Fig. 1. Inhibition of human N21/NR2B NMDA receptors by aromatic inhalants. Representative current traces in the presence of benzene (A), 1,3,5-trifluorobenzene (B), and perfluorobenzene (C). A to C, first and last traces are the control currents recorded upon pulsing *Xenopus* oocytes expressing NR1/NR2B NMDA receptors with agonist, and the middle trace demonstrates the inhibitory action of the inhalant. D, inhalant concentration-current response curves for benzene, 1,3,5-trifluorobenzene, and perfluorobenzene. Each point is the mean of at least three measurements using three different oocytes, and the errors bars indicate the standard deviations. The curves are the best fits of the data to eq. 2, and the results of these fits are given in Table 1.

Table 1 lists the NMDA receptor IC_{50} values, Hill coefficients, and partition coefficients of all 14 volatile aromatic inhalants evaluated in this study. IC_{50} values ranged from $5.6 \pm 0.3 \times 10^{-4}$ atm (toluene) to $9.2 \pm 0.8 \times 10^{-2}$ atm (perfluorobenzene), a range of 164-fold, and the Hill coefficients averaged 0.94 ± 0.22. The hydrophobicities of these inhalants, as reflected by their octanol/gas partition coefficients ($\lambda_{octanol/gas}$), ranged from 389 ± 9 (perfluorobenzene) to 8430 ± 400 (*m*-xylene). Where hydrophobicity is the principle determinant of inhibitory potency, then log ($1/IC_{50}$) and log ($\lambda_{octanol/gas}$) are predicted to be strongly correlated. Although the regression analysis of our data revealed that the correlation between log ($1/IC_{50}$) and log ($\lambda_{octanol/gas}$) reached statistical significance ($p = 0.044$), it also indicated that inhalant hydrophobicity was a relatively poor predictor of NMDA receptor inhibitory potency ($r^2 = 0.30$; Fig. 2A).

Previous studies have shown that general anesthetics act on many protein targets, including the NMDA receptor, with potencies that depend upon their size (Wood et al., 1993; Peoples and Weight, 1995; Mascia et al., 1996; Wick et al., 1998; Jenkins et al., 2001; Peoples and Ren, 2002). Commonly, potency increases with anesthetic molecular volume because molecular volume often correlates with hydrophobicity (Abraham et al., 1991). However in some cases, the potencies of larger, more hydrophobic anesthetics are less than would be predicted from the extrapolation of the potencies of smaller, less hydrophobic ones (a phenomenon termed "cutoff"), presumably because steric hindrance reduces anesthetic binding to a site that is limited in size. In our group of inhalants, we observed no relationship between inhalant molecular volume and NMDA receptor inhibitory potency (Table 1). For example, although perfluorobenzene's molecular volume is not different from those of ethylbenzene and the three xylene isomers (~119 Å^3), its potency is approximately 2 orders of magnitude lower. Similarly, whereas benzene has the smallest molecular volume (86.6 Å^3) and ethylbenzene has one of the largest volumes (119.8 Å^3), their NMDA receptor inhibitory potencies are not different (1.1×10^{-3} atm). Figure 2B plots log ($1/IC_{50}$) versus molecular volume for the entire group of aromatic inhalant to demonstrate the lack of correlation between the inhibitory potencies and molecular volumes of the inhalants studied ($p = 0.19$; $r^2 = 0.14$).

Using quantum mechanical modeling, we also characterized the electrostatic potential surfaces of these inhalants and quantified their abilities to engage in cation-π interactions (Fig. 3). In this figure, we used a color scale ranging from +20 to –20 kcal/mol to emphasize the electrostatic potential differences in the aromatic ring regions. Blue was used to signify an electrostatic potential equal to or more positive than +20 kcal/mol and red to indicate a potential equal to or more negative than –20 kcal/mol. Inspection of this figure reveals that the electrostatic potential surfaces of these inhalants vary greatly. Benzene and its alkyl-substituted analogs possess regions of highly negative electrostatic potential over their aromatic rings, reflecting the high electron density of their π systems. Because fluorine groups withdraw electron density, the successive addition of fluorine onto the aromatic ring progressively reduces the negative electrostatic potential of the π system, and thus the ability to engage in cation-π interactions.

Benzene and its five alkyl-substituted analogs possess similarly high abilities to engage in cation-π interactions (29 ± 1 kcal/mol; Fig. 3) and similarly low NMDA receptor IC_{50} values ($1.0 \pm 0.5 \times 10^{-3}$ atm; Table 1). Conversely, perfluorobenzene cannot engage in cation-π interactions and has an IC_{50} that is nearly 2 orders of magnitude higher than those of benzene and its alkyl-substituted analogs. Figure 4 plots log ($1/IC_{50}$) versus cation-π interaction strength and demonstrates that the relationship is linear and the correlation highly significant ($r^2 = 0.85$; $p < 0.0001$), suggesting that attractive cation-π interactions are important determinants of NMDA receptor inhibitory potency.

We further explored the role that cation-π interactions might play in modulating NMDA receptor inhibitory potency

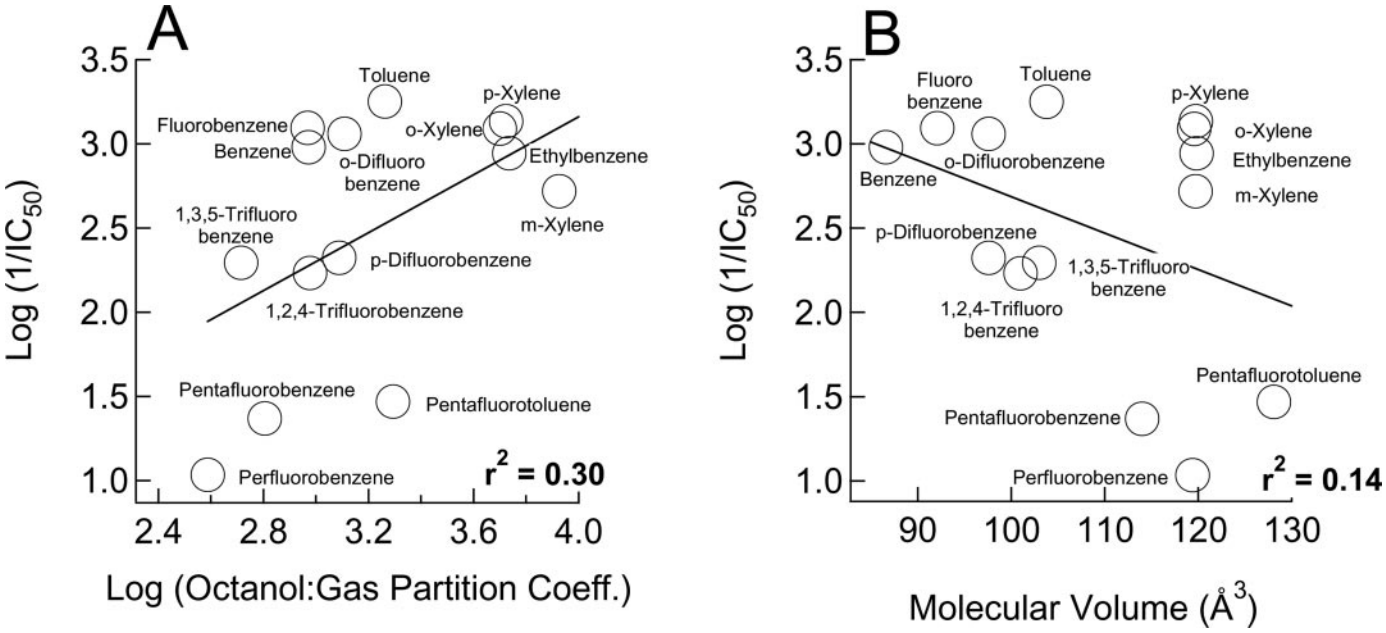

Fig. 2. Correlation between the NMDA receptor inhibitory potencies of aromatic inhalants and their oil/gas partition coefficients (A) or molecular volumes (B). In A and B, the line was derived from linear least-squares analysis. In A, $r^2 = 0.30$ and $p = 0.044$; in B, $r^2 = 0.19$ and $p = 0.14$.

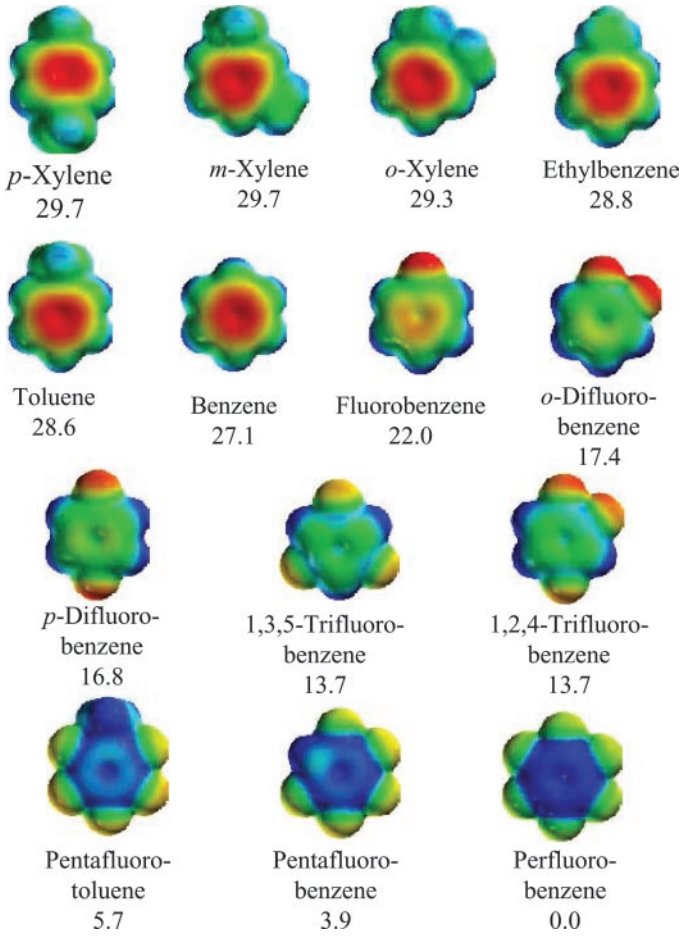

Fig. 3. Electrostatic potential surfaces of aromatic inhalants and their cation-π interaction strengths. Geometry optimization was performed using ab initio molecular orbital calculations (Hartree-Fock, 6-31G** basis set). The cation-π binding energy was defined as the negative binding energy of a generic cation probe (Na^+) to each inhalant's π electron cloud as described previously (Mecozzi et al., 1996). A color scale ranging from +20 to –20 kcal/mol to emphasize the electrostatic potential differences in the aromatic ring regions. Blue was used to signify an electrostatic potential equal to or more positive than +20 kcal/mol and red to indicate a potential equal to or more negative than –20 kcal/mol.

using a second set of volatile compounds: cyclohexene, 1,4-cyclohexadiene, 4-methyl-1-cyclohexene, and 1-methyl-1,4-cyclohexadiene. These compounds are structurally similar to benzene and toluene, but vary in the extent of their π-electron system, and thus their predicted abilities to engage in cation-π interactions. Ab initio molecular orbital calculations confirmed this prediction because the cation-π interaction strengths of these compounds in kilocalories per mole were 28.6 (toluene), 27.1 (benzene), 24.9 (1-methyl-1,4-cyclohexadiene), 24.2 (1,4-cyclohexadiene), 19.1 (4-methyl-1-cyclohexene), and 18.8 (cyclohexene). Electrophysiological studies revealed that all of these compounds reversibly inhibited NMDA receptor-mediated currents in a concentration-dependent manner. The inhibitory potencies ranged nearly 2 orders of magnitude, and the relationship between log (IC_{50}) and the cation-π binding energy was linear and the correlation highly significant ($r^2 = 0.97$, $p = 0.0004$; Fig. 5A). Conversely, the potencies of these compounds did not correlate significantly with either their hydrophobicities ($r^2 = 0.22$, $p = 0.355$; Fig. 5B) or their molecular volumes ($r^2 = 0.14$, $p = 0.86$; Fig. 5C).

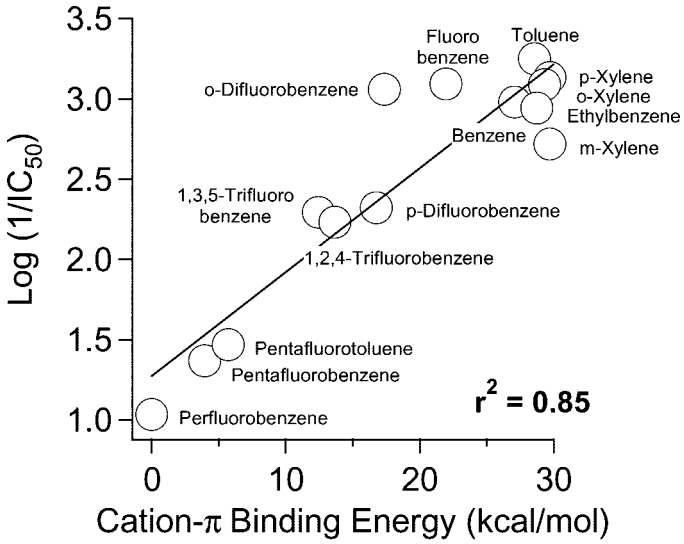

Fig. 4. Correlation between the NMDA receptor inhibitory potencies of aromatic inhalants and their abilities to engage in cation-π interactions. The line was derived from linear least-squares analysis. There is a strong correlation between an inhalant's NMDA receptor inhibitory potency and its ability to engage in a cation-π interaction ($r^2 = 0.85$; $p < 0.0001$).

We then evaluated the dependence of NMDA receptor inhibitory potency on NMDA and glycine concentrations. We chose benzene (4.6×10^{-3} atm) and perfluorobenzene (0.11 atm) as representative aromatic inhalants having high and low inhibitory potency, respectively. Currents were measured upon activation by 100 μM NMDA and 10 μM glycine, 1000 μM NMDA and 10 μM glycine, or 100 μM NMDA and 100 μM glycine. As shown in Fig. 6, 10-fold increases in either NMDA or glycine did not significantly reduce the inhibitory potencies of these inhalants, indicating that the inhalants do not inhibit NMDA receptors by competing with NMDA or glycine for binding.

Discussion

Clinical anesthetics and inhaled drugs of abuse representing a wide range of chemical classes, including alkanes, ethers, and even noble gases, may be inhaled to produce a constellation of behaviors (e.g., unconsciousness, amnesia, analgesia, and immobility in response to noxious stimulation) that are collectively referred to as the state of anesthesia (Fang et al., 1997; Koblin et al., 1998; Sonner et al., 1998; Zhang et al., 2000). Although the receptors responsible for the production of the anesthetic state are not known with certainty, the $GABA_A$, glycine, and NMDA receptors are generally considered to be among the most likely candidates (Franks and Lieb, 1994). The predominant actions of drugs with anesthetic activity are to enhance inhibitory $GABA_A$ and glycine receptor function and/or to inhibit excitatory NMDA receptor function (Mascia et al., 1996; Orser et al., 1997; Zimmerman et al., 1994; de Sousa et al., 2000; Hollmann et al., 2001).

Studies to define the relationship between the structure of anesthetic drugs and their receptor activities have commonly used members of the homologous series of normal alcohols (Wood et al., 1991; Peoples and Weight, 1995; Wick et al., 1998). In this series, alcohol hydrophobicity and molecular volume increase steadily with alkyl chain length, allowing

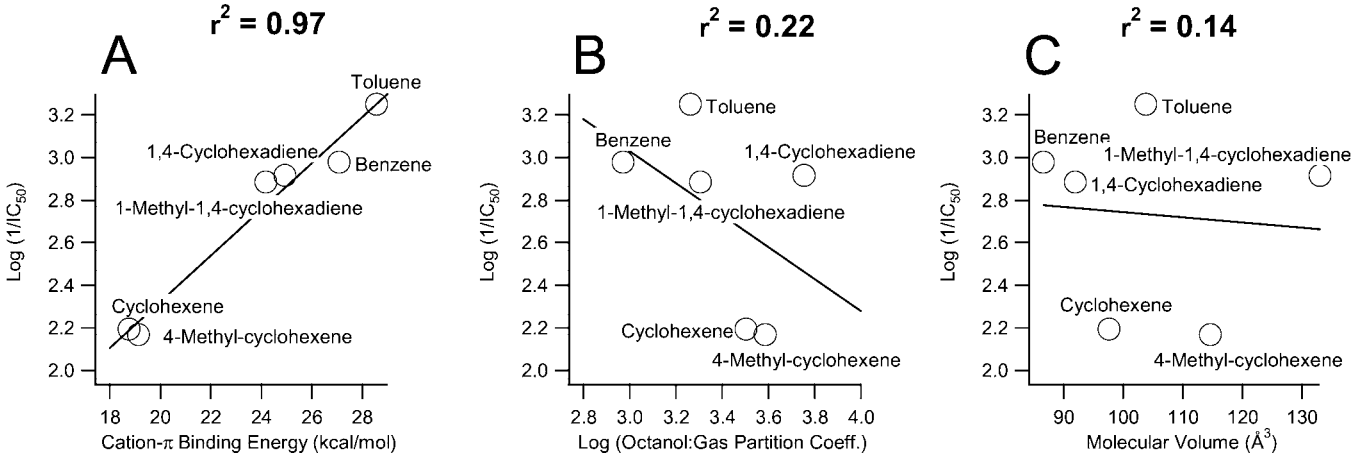

Fig. 5. Correlation between the NMDA receptor inhibitory potencies of volatile compounds and their abilities to engage in cation-π interactions (A), oil/gas partition coefficients (B), or molecular volumes (C). In A to C, the line was derived from linear least-squares analysis. In A, $r^2 = 0.97$ and $p = 0.0004$; in B, $r^2 = 0.22$ and $p = 0.355$; and in C, $r^2 = 0.14$ and $p = 0.86$.

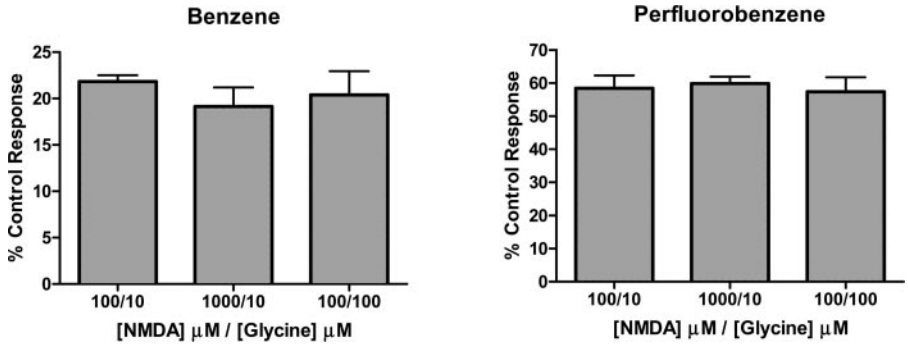

Fig. 6. Inhibition of NMDA receptors by benzene or perfluorobenzene does not vary with NMDA or glycine concentrations. The data represent the mean and standard deviations obtained using five oocytes. Currents were measured upon activation by 100 μM NMDA and 10 μM glycine, 1000 μM NMDA and 10 μM glycine, or 100 μM NMDA and 100 μM glycine.

one to readily assess the importance that hydrophobic and steric interactions play in modulating potency. In the NMDA receptor, inhibitory potency increases upon ascending the series from methanol to heptanol (Peoples and Weight, 1995). Cut-off is observed beyond heptanol because octanol is less potent than heptanal, and the potencies of higher homologues are so low that even saturated solutions fail to produce any detectable inhibition. Similarly in $GABA_A$ and glycine receptors, the potentiating actions of normal alcohols increase with alkyl chain length before reaching a cut-off between decanol and dodecanol, a pattern of activity suggesting that hydrophobic interactions enhance alcohol binding affinity and hence potency, whereas steric interactions reduce them (Wick et al., 1998).

In addition to hydrophobic and steric interactions, electrostatic interactions between drugs and their protein targets have been proposed to be important forces modulating potency (Abraham et al., 1991; Moss et al., 1991; Eckenhoff and Johansson, 1997; Manderson and Johansson, 2002; Raines et al., 2003). Among the strongest electrostatic interactions that may occur between a volatile drug and a protein binding site are hydrogen-bonding and cation-π interactions. Hydrogen-bonding interactions may occur between a donor molecule that contains an "acidic" hydrogen and another molecule that contains an electronegative acceptor atom (most commonly oxygen or nitrogen in biological systems). A hydrogen atom is made acidic (i.e., develops a significant partial positive electrostatic charge) if it is bonded to an atom that also bonds electron-withdrawing groups. Because many general anesthetics possess oxygen atoms (a hydrogen bond acceptor) and/or acidic hydrogen (a hydrogen bond donor), many anesthetics can hydrogen-bond to protein targets. Cation-π interactions are potentially strong attractive electrostatic interactions that occur between a π-electron cloud and an atom that carries a full or partial positive charge. Although none of the inhaled anesthetics currently used contain aromatic groups, a number of inhaled drugs of abuse with anesthetic activity do. Because hydrogen-bonding and cation-π interactions can provide several additional kilocalories per mole of energy to stabilize protein binding, they can enhance the binding affinities of aromatic inhaled drugs by more than an order of magnitude.

Studies to examine the nature of the interactions modulating anesthetic binding to the firefly luciferase enzyme (a soluble protein model for studying such interactions) provide experimental support for the importance of electrostatic interactions (Abraham et al., 1991). These studies demonstrated that the enzyme's binding site for anesthetics, although possessing significant hydrophobic character, can accept a hydrogen bond from an anesthetic about as well as water can. Thus, anesthetics representing a range of chemical classes inhibit this enzyme with potencies that depend upon their hydrogen-bonding abilities. High resolution X-ray crystallographic studies of halogenated alkane binding to insulin dimers has provided direct structural evidence that favorable electrostatic interactions are strong enough to enhance binding affinity (Gursky et al., 1994). Manderson and Johansson (2002) showed that substituting tyrosine for tryptophan in a synthetic four-α-helix bundle reduces the binding affinity of the inhaled anesthetics chloroform and halothane

6- and 3-fold, respectively, without significantly changing the protein's overall structure. They concluded that interactions between the positive end of the anesthetic's dipole and the tryptophan's π-electron cloud enhance binding affinity. Similarly, high-resolution NMR and photolabeling studies reveal that inhaled anesthetics can interact specifically with tryptophan residues in gramacidin A (Tang et al., 2000). However, Lui et al. (2002) were unable to detect such interactions between halothane and tryptophan in human serum albumin suggesting that the importance that cation-π interactions play in defining inhaled anesthetic binding affinity varies with the target protein studied due to the specific physicochemical properties of the anesthetic binding site.

We have previously shown that electrostatic interactions can also modulate the potencies with which anesthetics act on ligand-gated ion channels. For example, general anesthetics enhance agonist action on *Torpedo* nicotinic acetylcholine receptors with potencies that correlate with their abilities to form hydrogen bonds (Raines and Claycomb, 2002). Similarly, nonhalogenated alkanes, which are distinguished from most other anesthetics by their inability to engage in electrostatic interactions, enhance agonist action on $GABA_A$ receptors with potencies that are an order of magnitude lower than would be predicted by their hydrophobicities (Raines et al., 2003). However, electrostatic interactions do not seem to modulate anesthetic potency in all receptor systems as halogenated and nonhalogenated alkanes act on $\alpha_4\beta_2$ neuronal nicotinic acetylcholine receptors with potencies that correlate highly with their hydrophobicities and not their electrostatic properties (Raines et al., 2002).

In the present study, we tested the hypothesis that cation-π interactions can modulate the NMDA receptor inhibitory potencies of inhaled drugs of abuse. We used compounds whose abilities to engage in cation-π interactions vary depending upon either the identity of the substituent groups on the aromatic ring or the degree of conjugated unsaturation. The presence of strongly electron-withdrawing fluoro groups on aromatic rings or incomplete unsaturation substantially reduced the cation-π binding energy as determined by ab initio quantum mechanical calculations. Our studies reveal that volatile aromatic drugs inhibit NMDA receptor-mediated currents with potencies that are highly correlated with their abilities to engage in cation-π interactions. This is consistent with an important role for cation-π interactions in modulating the inhibitory potencies of volatile aromatic compounds. Our findings also suggest that it is possible to predict the NMDA receptor inhibitory potencies of novel aromatic compounds using molecular modeling by defining their abilities to engage in cation-π interactions. Such an approach could lead to the rapid screening of available compounds or the design of new ones that inhibit NMDA receptors with high potency.

The compounds that we studied have NMDA receptor potencies that range by 164-fold (Table 1). If this were due to differences in anesthetic binding affinity to the NMDA receptor, then this would represent a difference in binding energy between toluene and perfluorotoluene, the most and least potent anesthetics, respectively, of 3.0 kcal/mol. This value is much lower than the binding energy calculated using molecular modeling, which assumes that anesthetics interact with a full positive charge in a vacuum. It is, however, similar to the 2.4 and 4 kcal/mol values estimated for the cation-π interactions that modulate cationic agonist binding to a binding site tryptophan on nicotinic and serotonergic receptors, respectively (Beene et al., 2002). Solvation can reduce the effective cationic charge and thus the electrostatic contribution to the binding energy by a factor of 80 (based on the dielectric constant of 80 for water). The contribution to the binding energy may also be reduced if the cation-π interaction involves a partial cationic charge (i.e., the positive end of a permanent or inducible dipole) rather than a full charge.

We also assessed the contribution that anesthetic hydrophobicity and molecular volume make toward defining NMDA receptor inhibitory potency. For the 14 aromatic inhalants studied, the correlation between NMDA receptor inhibitory potency and inhalant's hydrophobicity reached statistical significance, but it was not as strong as that observed between inhibitory potency and cation-π binding energy. Thus, hydrophobic interactions may explain the activity (albeit at very high concentrations) of perfluorbenzene, which cannot engage in cation-π interactions at all. For the second group of compounds in which the extent of conjugated unsaturation varies (Fig. 5B), no significant correlation between NMDA receptor inhibitory potency and hydrophobicity was observed. Although there was some trend toward decreasing inhibitory potency with increasing molecular volume (Figs. 2B and 5C), this trend was not statistically significant and cutoff at large molecular volume was not observed.

In summary, we used electrophysiological and computational approaches to assess the importance that cation-π interactions play in defining the NMDA receptor inhibitory potencies of inhaled drugs that possess π electrons. Our studies show that inhaled drugs inhibit NR1/NR2B NMDA receptors with potencies that are correlated strongly with their abilities to engage in cation-π interactions, weakly with their hydrophobicities, and are independent of their molecular volumes. This is consistent with the hypothesis that cation-π interactions enhance the binding of these inhaled drugs to the NMDA receptor and suggests that the binding site(s) for these drugs possesses significant cationic character.

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Address correspondence to: Dr. Douglas E. Raines, Department of Anesthesia and Critical Care, Massachusetts General Hospital, 55 Fruit St., Boston, MA 02114. E-mail: draines@partners.org