Anesthetic and Convulsant Properties of Aromatic Compounds and Cycloalkanes: Implications for Mechanisms of Narcosis

Zexu Fang, MD*, James Sonner, MD*, Michael J. Laster, DVM*, Pompi Ionescu, MD*, Lori Kandel, BS*, Donald D. Koblin, PhD, MD*, Edmond I Eger II, MD*, and Michael J. Halsey, DPhil†

*Department of Anesthesia, University of California, San Francisco, California, and †Nuffield Department of Anaesthetics, Oxford, England

We examined the anesthetic and convulsant properties of 16 unfluorinated to completely fluorinated aromatic compounds, having six to nine carbon atoms (e.g., benzene to 1,3,5-tris(trifluoromethyl)benzene), and four cycloalkanes (cyclopentane to cyclooctane). Benzene, fluorobenzene, toluene, *p*-xylene, ethylbenzene, and cyclopentane caused excitation (twitching, jerking, and hyperactivity), and three aromatic compounds (perfluorotoluene, *p*-difluorotoluene and 1,3,5-tris(trifluoromethyl)benzene) and three cycloalkanes (cyclohexane, cycloheptane, and cyclooctane) produced convulsions. Cyclooctane and 1,3,5-tris(trifluoromethyl)benzene were nonanesthetics. Except for nonanesthetics and perfluorotoluene (too toxic to test for anesthetic potency), all compounds produced anesthesia or decreased the minimum alveolar anesthetic concentration of desflurane. Aromatic compounds were more potent and lipid-soluble than *n*-alkanes (data from previous report) and cycloalkanes. All three series increasingly disobeyed the Meyer-Overton hypothesis as molecular size increased. For a particular number of carbons (e.g., cyclohexane, *n*-hexane, and benzene), the deviation was cycloalkanes ≥ normal alkanes > aromatic compounds. These results suggest that molecular shape (including "bulkiness") and size provide limited clues to the structure of the anesthetic site of action.

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Our previous studies of anesthetic mechanisms of action investigated whether *n*-alkanes obey the Meyer-Overton hypothesis (1) (that affinity for lipids [e.g., nonpolar regions of the membrane] predicts anesthetic potency [e.g., that minimum alveolar anesthetic concentration (MAC) times the olive oil/gas partition coefficient equals a constant]) (2,3). We found that this hypothesis applied to smaller *n*-alkanes, wherein MAC times the olive oil/gas partition coefficient gave values similar to those obtained with conventional anesthetics. However, the values increased with increasing carbon chain length. That is, the increasing anesthetic potency associated with increasing carbon chain length was less than predicted from the concurrent increases in lipid solubility (1). We considered [as suggested long ago by Mullins (4)] that increasing carbon chain length increases the deviation from the Meyer-Overton hypothesis, because the larger *n*-alkanes exceed the size of, or do not conform to, the site of anesthetic action. Regarding other forms of alkanes, previous studies reveal that unsaturated bonds increase anesthetic potency (e.g., ethylene [$CH_2{=}CH_2$] is more potent than ethane [$CH_3{-}CH_3$]) (5), suggesting that factors beyond size and conformation may influence the interaction of molecules with the site of anesthetic action.

In this study, we extend our observations to cyclic and aromatic compounds. Our intent was to delineate further the influence of molecular size, shape, and the presence of aromatic bonds on anesthetic (and convulsive) potency and on the Meyer-Overton hypothesis. Some of these compounds have been studied previously. Henderson and Lucas (6) demonstrated the valuable anesthetic properties of cyclopropane 60 years ago. Virtue (7) reported that cyclopentane and cyclohexane produced excitement and anesthesia, but did not determine solvent or MAC values.

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Address correspondence to Edmond I Eger II, MD, Department of Anesthesia, S-455, Box 0464, University of California, San Francisco, CA 94143-0464.

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Although Burns et al. (8,9) did not obtain solvent values, they examined the anesthetic effects of several fluorinated aromatic compounds in a manner that permits a comparison with MAC values. Neither Virtue nor Burns et al. used their data to test the Meyer-Overton hypothesis.

Methods

Compounds

Aromatic compounds and cyclic alkanes were purchased from Aldrich, Inc. (Milwaukee, WI); Fairfield, Inc. (Blythewood, SC); Fisher Scientific, Inc. (Pittsburgh, PA); PCR, Inc. (Gainsville, FL); or Sigma Chemical Co. (St. Louis, MO). Their purities were benzene (99%); fluorobenzene (99%); *o*-difluorobenzene (99%); *p*-difluorobenzene (99%); 1,2,4-trifluorobenzene (>97%); 1,3,5-trifluorobenzene (>97%); pentafluorobenzene (99%); hexafluorobenzene (99%); toluene (99%); perfluorotoluene (>97%); methylpentafluorobenzene (>97%); 1,3,5-tris(trifluoromethyl)benzene (>97%); *m*-xylene (99%); ethylbenzene (99%); *o*-xylene (99%); *p*-xylene (99%); cyclopentane (99%); cyclohexane (99%); cycloheptane (98%); and cyclooctane (99%).

Animals

We studied the anesthetic and convulsant properties of these 16 aromatic compounds and 4 cycloalkanes, and the kinetics of 15 aromatic compounds and 4 cycloalkanes in rats. With the approval of the Committee on Animal Research of the University of California at San Francisco, we studied 204 male, 350–550 g, specific-pathogen-free Sprague-Dawley rats from Charles River Laboratories (Saratoga, CA). Before study, each rat was caged individually and had continuous access to standard rat chow and tap water. Each rat was exposed to a 12-h light/dark cycle.

Exposure Chambers and Determination of the Minimum Inspired Concentration (MIC) Required to Suppress Movement in Response to Stimulation

As previously described (1), four rats were placed in individual plastic cylinders having an internal diameter of 6.25 cm and a length of 29 cm. The "tail" end of each cylinder had a rubber stopper with multiple holes. The tail and a rectal temperature probe passed through these holes, and the tail was secured with tape to a plastic extension of each cylinder. Three pairs of subcutaneous stimulating platinum needle electrodes were placed under the skin of the tail at successively distal locations, and the external continuation of each electrode was taped to the tail. The rat was free to move but not able to turn around, and the secured tail prevented it from exiting through the open ("head") end of the cylinder.

Each cylinder containing a rat prepared as previously described was inserted into a slightly larger individual plastic chamber. Both ends of the chamber were equipped with rubber stoppers. A catheter for sampling inspired gas in the chamber and another tube for delivery of gases traversed the stopper at the head end of the chamber. The stopper at the tail end of the chamber was pierced by a tube for exit of gases. To form a closed circle system, the tubes leading to the head ends of the four chambers were connected with a demand oxygen supply (bypass flow approximately 50 mL/min); the tubes at the tail ends were connected to a carbon dioxide absorbent canister containing 200 g of fresh soda lime; and, completing the circle, the absorbent canister was connected to a circulating fan that led to the head-end tubes and demand system input. To permit delivery of compounds, we added a "T" connector with a metal stopcock between the carbon dioxide absorber and the tail end of the four chambers.

After completing these preparations, using the gas delivery tube at the head end of the chamber, we flushed the system with oxygen for 10 min or until the oxygen concentration was 95% or higher (as determined using a Pauling meter), then closed the system. Each test compound was introduced separately in sequentially increasing concentrations. For each concentration, a 20-min equilibration period was allowed, after which we observed the rats for movement. We began at a concentration that always permitted movement in response to stimulation. If the rats moved normally or appeared wakeful, the concentration in the system was increased by 10%–20% until spontaneous movement disappeared. If the rats appeared to be asleep, we applied an electrical stimulation (10) and noted whether the rats moved. The electrical stimulation was a biphasic current of 15 V, with each impulse of 6.5 ms duration and a frequency of 50 Hz applied to one of three pairs of electrodes placed in the tail. Stimulation was first applied to the most distal pair of electrodes, later to more proximal electrodes. After observing movement of the forelimbs or legs, we increased the concentration in the system by 10%–20%. These stepwise increases in concentration were continued until no animal moved in response to stimulation.

We measured the concentrations of test compounds in inspired gas samples using gas chromatography. We noted the inspired concentrations bracketing responsiveness and unresponsiveness to electrical stimulation (MIC). For each animal, we determined the highest inspired concentration permitting movement and the lowest concentration preventing movement; MIC was the average of these two concentrations. As

will be detailed herein, for several of the test compounds, MIC values could not be used as surrogate measures of alveolar or arterial partial pressures of test compounds. Further studies were required to translate MIC into measures of alveolar or arterial partial pressures (MAC; see herein). We maintained rectal temperatures between 36°C and 39°C by external application of heat or cold. Oxygen was measured (Pauling meter) at intervals and was maintained at 50% or greater. Carbon dioxide (infrared analysis) was measured at the same intervals and never exceeded 0.5%.

Because of cyclooctane's relatively low vapor pressure and high blood solubility, we considered the possibility that our failure to produce anesthesia by inhalation with this compound might have resulted from inadequate absorption. Accordingly, we also administrated 11.4 g/kg of cyclooctane in olive oil by intraperitoneal injection and conducted additivity studies with desflurane. The partial pressure of cyclooctane in blood was determined from an aortic blood sample (taken at the end of that study) using gas chromatography.

Additivity Studies

If the test compound alone did not produce anesthesia, we performed "additivity" studies to determine whether the agent decreased the MAC required to suppress movement for desflurane in rats. Control MAC values for rats for desflurane were determined approximately 1 wk before additivity studies. These control studies differed from those previously described by our use of tail clamp rather than electrical stimulation (thus eliminating the need for a tube within a tube) and by our directing a continuous flow of oxygen containing desflurane through the circle system (that produced a semiclosed rather than a closed system). The test compound subsequently (approximately 1 wk later) was given in combination with desflurane, using electrical stimulation in the closed system described herein. If the compound did not decrease the MAC values for desflurane at the highest tolerated partial pressure of the test compound (usually not the saturated vapor pressure, because the saturated pressure often proved lethal when applied in combination with desflurane), we considered the compound to be a nonanesthetic.

All nonanesthetics also caused twitching and jerking at lower concentrations and tonic-clonic seizures (convulsions) at higher concentrations. We use "weak," "moderate," and "strong" as terms describing our subjective impression of the strength of the convulsion. Thresholds for induction of convulsive activity of these and transitional compounds were obtained in a manner similar to that used to determine MIC for the test compounds, except that electrodes were not inserted into the tails of rats. The MIC for convulsive activity was calculated as the average of the concentrations just permitting and preventing convulsions.

Determination of Blood Partial Pressures and Partition Coefficients

If the olive oil/gas partition coefficient of the test compound was as low as that for conventional anesthetics (e.g., as desflurane), we presumed that the inspired compound concentration after a 20-min equilibration period represented the alveolar concentration, and "MIC" would be taken to represent MAC. Otherwise, the inspired compound concentration after a 20-min equilibration period would not represent the alveolar concentration. Using separate sets of rats (three to four rats for each test compound), we therefore determined inspired and arterial partial pressures of all test compounds, except 1,3,5-tris(trifluoromethyl)benzene, a nonanesthetic, and moderate convulsant. Arterial blood was drawn anaerobically into heparinized syringes from a previously implanted arterial catheter, and inspired gas was drawn simultaneously. Gas was analyzed directly by gas chromatography.

The blood partial pressures and partition coefficients between blood and gas of test compounds were determined as previously described (11,12). We used the ratio of arterial blood partial pressure: to inspired partial pressure times the previously determined MIC value to give the MAC value for either anesthesia or convulsions.

Catheterization of the Femoral Artery

After anesthetizing rats with desflurane, we made an inguinal incision and exposed the femoral artery. The artery was then bathed in 1% lidocaine to prevent vasospasm. A 22-gauge catheter was inserted in the artery and connected to a sampling port on the rubber stopper at the tail end of the chamber. Clot formation was prevented by injecting 0.2 mL of Hespan containing heparin (10 U/mL). The catheter was sutured in place, and the wound was closed. The rat was further prepared as previously described, excluding electrodes and stimulation, and was given the same exposure to each of the test compounds [except 1,3,5-tris(trifluoromethyl)benzene]. When the inspired concentration and exposure time approached those applied in the determination of MIC, we obtained an inspired gas sample from the chamber and withdrew 3–10 mL of arterial blood, using a calibrated syringe pretreated with heparin.

Hyperbaric Chamber

Because 1,3,5-tris(trifluoromethyl)benzene has a low vapor pressure (0.013 atm), we determined the convulsive threshold to this compound in helium and

Table 1. Convulsions with Test Compounds

Compound	*n*	MIC for convulsions (atm)	MAC for convulsions (atm)
Perfluorotoluene	4	0.00074 ± 0.0000	0.00035 ± 0.00000
1,3,5-Tris(trifluoromethyl)benzene	4	0.014 ± 0.002	0.014 ± 0.002
p-Difluorobenzene	2/9[a]	0.0061/0.0100	0.0046/0.0076
Cyclohexane	4	0.023 ± 0.000	0.022 ± 0.000
Cycloheptane	4	0.0067 ± 0.0015	0.0046 ± 0.0010
Cyclooctane	4	0.0037 ± 0.0004	0.0015 ± 0.0002

Values are presented as mean ± SD.
MIC = minimum inspired concentration; MAC = minimum alveolar anesthetic concentration.
[a] Convulsions occurred in two of nine tested rats. MAC values are derived from the MIC values by multiplying MIC by P_a/P_I (P_a/P_I = ratio of the arterial to inspired partial pressure of the test compound as determined in separate studies [see Methods and Table 4]).

oxygen in a hyperbaric chamber. This permitted us to achieve higher vapor pressures, because the higher thermal conductivity of the helium permitted higher temperatures in the chamber without corresponding increases in the rat temperature. Each rat was placed in a transparent plastic cylinder with rectal temperature probe and stimulating electrodes as previously described. Studies were conducted with sets of two rather than four rats. Two rats in their individual cylinders were placed in a 3.4-L pressure chamber equipped with soda lime and a fan for air circulation. The chamber was flushed with oxygen to produce an exiting concentration in excess of 97%. The chamber then was sealed and pressurized to 2 atm to test for leakage. If no leakage occurred, we decreased the pressure to 1 atm, injected the test compound, and pressurized the chamber to 5 atm with helium. After equilibration for 20 min, we measured the total pressure and determined the concentration in the chamber by gas chromatography. If the rats did not convulse, we increased the concentration of tested compound by 10%–30%. MIC for convulsion was calculated as noted for the regular chamber, taking into account the increased pressure.

Solubility in Saline, Olive Oil, and Octanol: Vapor Pressure

Saline/gas, olive oil/gas, and octanol/gas partition coefficients of all the aforementioned compounds at 37°C were measured using standard techniques (11–13). Vapor pressures (at room temperature) were measured as previously described (1).

Results

Three aromatic compounds [perfluorotoluene, *p*-difluorobenzene, and 1,3,5-tris(trifluoromethyl)benzene] and three cycloalkanes (cyclohexane, cycloheptane, and cyclooctane) produced convulsions (Table 1). In the pressure chamber at 5.0 atm, 1,3,5-tris(trifluoromethyl)benzene was a moderate convulsant (MIC for convulsion, 0.014 ± 0.002 atm; $n = 4$). This drug did not decrease the MAC for desflurane and is thus a nonanesthetic (Table 2). Perfluorotoluene was a strong convulsant (MIC for convulsions, 0.00074 ± 0.00000 atm; $n = 4$) and was toxic (killing all tested rats [$n = 8$]), with the lethal concentration equaling 0.0026 atm or higher (0.0032 atm killed half the rats and 0.0056 atm killed all of the rats), despite the presence of 0.04–0.06 atm desflurane. Accordingly, we did not determine MAC for perfluorotoluene. Although *p*-difluorobenzene was a convulsant and an anesthetic (MAC = 0.0064 atm), it caused only weak convulsions in two of nine rats tested. The remaining seven rats displayed twitching and jerking movements in the absence of desflurane. Cyclooctane was a strong convulsant that produced convulsions in rats in the presence of 0.061 atm of desflurane, but had no effect on anesthesia (and thus was a nonanesthetic). Although benzene, fluorobenzene, toluene, *p*-xylene, ethylbenzene, and cyclopentane all produced excitation (twitching, jerking, and hyperactivity; Table 3), all were anesthetics (Table 4). Similarly, the eight-carbon aromatic compounds *o*-xylene, *m*-xylene, *p*-xylene, and ethylbenzene were anesthetics, as were the cycloalkanes smaller than cyclooctane (Table 4).

For the compounds in which MAC could be measured, MAC correlated with lipophilicity in a manner similar to that found previously for conventional anesthetics and *n*-alkanes (Table 5; Figs. 1 and 2). MAC correlated inversely with lipophilicity, as defined by the olive oil/gas partition coefficient (Fig. 1) or the octanol/gas partition coefficient (Fig. 2). The values for aromatic compounds and for cycloalkanes (*c*-alkanes) were from the present study. The values for conventional anesthetics (14) and for normal alkanes (values were for even-numbered *n*-alkanes from ethane to decane) were from our previous work (15,16). Regression analysis for the combined data showed a good correlation (r^2 of 0.86–0.88), but the slope deviated from 1.00, being 1.27 for olive oil and 1.15 for octanol. If the regression analysis were applied just to the conventional anesthetics, the respective slopes were 1.08 and 0.98, with r^2 values of 0.93 and 0.98. Values for conventional anesthetics lay below the line, values for the aromatic compounds

Table 2. Anesthetic Potencies of Test Compounds When Administered with Desflurane

Compound	*n*	Control desflurane MAC (atm)	Experimental desflurane $MAC_{des+test}$ (atm)[a]	Partial pressure of test compound (atm)[b]	MAC of test compound (atm)[c]
1,3,5-Tris(trifluoromethyl)benzene	4	0.080 ± 0.006	0.082 ± 0.006	0.012 ± 0.000	NA
Cyclohexane	3	0.069 ± 0.000	0.037 ± 0.000	0.021 ± 0.000	0.045 ± 0.000
Cycloheptane	4	0.069 ± 0.000	0.043 ± 0.001	0.0074 ± 0.0002	0.014 ± 0.003
Cyclooctane	4[d]	0.073 ± 0.007	0.078 ± 0.000	0.00081 ± 0.00000	NA

Values are presented as mean ± SD.
MAC = minimum alveolar anesthetic concentration; des = desflurane; NA, nonanesthetic.
[a] Desflurane MAC in the presence of the test compound.
[b] Partial pressure of test compound in the presence of desflurane.
[c] Calculated MAC of test compound.
[d] Cyclooctane was a strong convulsant, even in the presence of 0.061 atm of desflurane: the MIC for convulsion was 0.005 ± 0.000 atm (n = 4). When administrated alone, the MIC for convulsion was 0.0037 ± 0.000 atm. Data for cyclooctane are drawn from two experiments. In one experiment, three rats moved at 0.069 ± 0.000 atm of desflurane and at 0.003 ± 0.0004 atm of cyclooctane; all three rats had repeated convulsions. In the other experiment, the three rats did not move at 0.076 ± 0.000 atm of desflurane and at 0.006 ± 0.000 atm of cyclooctane, and no convulsions occurred in any rat.

Table 3. Excitation with Test Compounds

Compound	*n*	Excitation (blood partial pressure; atm)
Benzene	4	Jerking at 0.0074 atm; twitching at 0.0086 atm
Fluorobenzene	3	Twitching at 0.007–0.010 atm
Toluene	4	Hyperactivity at 0.003–0.0043 atm
p-Xylene	4	Strong tremors at 0.0009 ± 0.0000 atm
Ethylbenzene	4	Twitching at 0.00113 atm
Cyclopentane	4	Twitching at 0.0026 atm

Table 4. Anesthetic Potency and Kinetics of Aromatic Compounds and Cycloalkanes

Compound	*n*	MIC (atm)	*n*	P_a/P_I[a]	B/G P.C.	MAC (atm)[b]
Benzene	4	0.0171 ± 0.0028	3	0.590 ± 0.076	10.4 ± 1.6	0.0101 ± 0.0016
Fluorobenzene	4	0.0161 ± 0.0009	3	0.694 ± 0.233	11.4 ± 1.0	0.0112 ± 0.0006
o-Difluorobenzene	4	0.0121 ± 0.0017	3	0.502 ± 0.088	9.16 ± 1.53	0.0061 ± 0.0008
p-Difluorobenzene	4	0.0084 ± 0.0000	3	0.755 ± 0.078	7.38 ± 0.81	0.0064 ± 0.0000
1,2,4-Trifluorobenzene	4	0.0124 ± 0.0025	4	0.784 ± 0.106	5.78 ± 0.63	0.0097 ± 0.0019
1,3,5-Trifluorobenzene	4	0.0258 ± 0.0018	4	0.861 ± 0.207	3.10 ± 0.44	0.0222 ± 0.0015
Pentafluorobenzene	4	0.0142 ± 0.0002	3	0.878 ± 0.270	3.23 ± 0.67	0.0125 ± 0.0002
Hexafluorobenzene	4	0.0175 ± 0.0018	4	0.921 ± 0.216	2.46 ± 0.78	0.0161 ± 0.0016
Toluene	4	0.0090 ± 0.0012	3	0.500 ± 0.063	13.8 ± 2.3	0.0045 ± 0.0006
Methylpentafluorobenzene	4	0.0078 ± 0.0004	3	0.822 ± 0.188	5.40 ± 0.19	0.0064 ± 0.0003
o-Xylene	4	0.0064 ± 0.0005	4	0.184 ± 0.027	41.3 ± 3.0	0.00118 ± 0.00009
m-Xylene	4	0.0071 ± 0.0005	3	0.195 ± 0.041	41.5 ± 4.6	0.00139 ± 0.00010
p-Xylene	4	0.0078 ± 0.0004	3	0.194 ± 0.011	33.9 ± 1.1	0.00151 ± 0.00007
Ethylbenzene	4	0.0070 ± 0.0005	4	0.172 ± 0.018	18.2 ± 1.9	0.00121 ± 0.00009
Cyclopentane	4	0.0586 ± 0.0043	4	0.901 ± 0.021	1.74 ± 0.05	0.053 ± 0.004
Cyclohexane	3	0.0448 ± 0.0000	3	0.930 ± 0.050	1.65 ± 0.09	0.042 ± 0.000
Cycloheptane	4	0.0196 ± 0.0039	5	0.697 ± 0.026	5.20 ± 0.19	0.0137 ± 0.0027

Values are presented as mean ± SD.
MIC = minimum inspired concentration; P_a/P_I = ratio of the arterial to inspired partial pressure of the test compound as determined in separate studies; B/G P.C. = blood/gas partition coefficient; MAC, minimum alveolar anesthetic concentration.
[a] The P_a/P_I ratio was used to correct the MIC value to a MAC value.
[b] Corrected MAC values [MAC = (MIC) (P_a/P_I)] were lower than the MIC for anesthesia in these 14 aromatic compounds and three cyclic alkanes, because P_a/P_I differed significantly from 1.0.

tended to lay on the line, and values for cycloalkanes lay above the line.

Discussion

Some of the present results confirm those of Burns et al. (8,9) and Virtue (7). Burns et al. reported that 0.028 atm pentafluorobenzene and 0.002–0.035 atm hexafluorobenzene caused anesthesia, and perfluorotoluene was toxic at 0.005–0.009 atm. (It killed all test mice.) Virtue found that 0.0156 atm cyclopentane and 0.0182 atm cyclohexane produced anesthesia. These partial pressures are lower than those we obtained and may have resulted from the use of different

Table 5. Comparison of MAC × Oil/Gas Versus MAC × Octanol/Gas

Compound	Saline/gas partition coefficient	Oil/gas partition coefficient	MAC × oil/gas (atm)	Octanol/gas partition coefficient	MAC × octanol/gas (atm)
Benzene	2.89 ± 0.13	565 ± 25	5.65	443 ± 9	4.43
Fluorobenzene	2.29 ± 0.021	661 ± 34	7.38	539 ± 30	6.02
o-Difluorobenzene	3.06 ± 0.13	853 ± 41	5.18	621 ± 4	3.77
p-Difluorobenzene	2.41 ± 0.10	654 ± 18	4.16	515 ± 15	3.27
1,2,4-Trifluorobenzene	1.80 ± 0.03	621 ± 6	6.03	491 ± 27	4.77
1,3,5-Trifluorobenzene	0.72 ± 0.03	335 ± 2	7.44	249 ± 10	5.53
Pentafluorobenzene	0.74 ± 0.03	393 ± 9	4.89	326 ± 13	4.05
Hexafluorobenzene	0.40 ± 0.01	251 ± 4	4.05	200 ± 7	3.23
Toluene	2.28 ± 0.16	2170 ± 140	9.77	1540 ± 100	6.93
Perfluorotoluene	0.159 ± 0.005	356 ± 7		298 ± 5	
Methylpentafluorobenzene	0.43 ± 0.02	1470 ± 100	9.43	1100 ± 22	7.05
o-Xylene	3.21 ± 0.36	10,800 ± 1330	12.7	5670 ± 200	6.69
m-Xylene	1.92 ± 0.16	7780 ± 390	10.8	4240 ± 150	5.89
p-Xylene	1.96 ± 0.10	6300 ± 860	9.51	4500 ± 320	6.80
Ethylbenzene	1.87 ± 0.06	5450 ± 680	6.59	3590 ± 200	4.34
1,3,5-Tris(trifluoro)benzene	0.0130 ± 0.0005	264 ± 7		233 ± 6	
Cyclopentane	0.088 ± 0.002	139 ± 4	7.35	145 ± 2	7.67
Cyclohexane	0.211 ± 0.031	385 ± 22	16.0	396 ± 7	16.5
Cycloheptane	0.075 ± 0.004	2780 ± 130	37.8	1820 ± 90	24.8
Cyclooctane	0.054 ± 0.003	7010 ± 270		7420 ± 260	
n-Hexane[a,b]	0.0093 ± 0.0002	148 ± 7	5.18	223 ± 9	7.80
Mean value (present data)			9.69 ± 7.91		7.16 ± 5.46

Values are presented as mean ± SD.
Other data are obtained from the present study.
MAC = minimum alveolar anesthetic concentration.
Values for MAC × the olive oil/gas partition coefficients exceed those for MAC × the octanol/gas partition coefficients. Although the use of the octanol/gas partition coefficient may decrease the deviation from the Meyer-Overton hypothesis, a significant deviation remains, particularly for the cycloalkanes. For conventional anesthetics, MAC × the oil/gas partition coefficient = 1.8, and MAC × the octanol/gas partition coefficient = 2.8 (14).
[a] Olive oil/gas or *n*-octanol/gas partition coefficient from Taheri et al. (16).
[b] MAC from Liu et al. (15).

indices to define anesthesia. Neither Virtue nor Burns et al. determined the lipid solubilities of these compounds.

Olive oil solubilities and anesthetic potencies increased from cyclopentane through cycloheptane (Tables 4 and 5). Although these trends parallel those for *n*-alkanes from pentane to heptane (1), data for the cycloalkanes result in a deviation from the Meyer-Overton hypothesis at a lower carbon number than with *n*-alkanes (Table 5; Figs. 1 and 2). Similarly, the potencies for convulsions increased from cyclohexane to cyclooctane (Table 1). This tendency to excitation exceeds that found for *n*-alkanes containing the same number of carbons (1) (e.g., in that earlier study, although hexane and heptane caused tremors, neither caused convulsions). Taken in combination, these results might be interpreted to suggest that convulsive properties antagonize and increase the requirement for anesthesia (i.e., increase MAC). This might explain the greater deviation from the Meyer-Overton hypothesis at a given carbon number. However, our previous work suggested that excitatory activity does not appreciably increase anesthetic requirement (17). For example, as shown in the present study, the convulsive activity of nonanesthetics, such as 1,3,5-tris(trifluoromethyl)benzene, neither increases nor decreases the MAC of desflurane.

The order of anesthetic potencies for the six-carbon unfluorinated compounds in three series is benzene (0.0101 atm) > normal hexane (0.0350 atm) ≥ cyclohexane (0.042 atm) (Ref. 1 and the present study). As noted earlier for the effect of unsaturation in the *n*-alkane series (5), the aromatic compound benzene was more potent than *n*-hexane and cyclohexane. For conventional anesthetics, MAC times the olive oil/gas partition coefficient equals 1.8 atm, and MAC times the octanol/gas partition coefficient equals 2.8 atm (14). Benzene, *n*-hexane, and cyclohexane were less potent than would be predicted from their solubilities in olive oil or *n*-octanol. However, benzene deviated less from its predicted value than did *n*-hexane and cyclohexane (Table 5; Figs. 1 and 2). Three possibilities may explain the closer fit by benzene. First, the aromatic structure may increase anesthetic potency relatively more than with the *n*-alkane or cycloalkane, compensating for the greater lipid solubility. Second, benzene might be toxic, thereby producing an artifactually low MAC value. However, rats survived without apparent ill effects for 24 hours after testing with

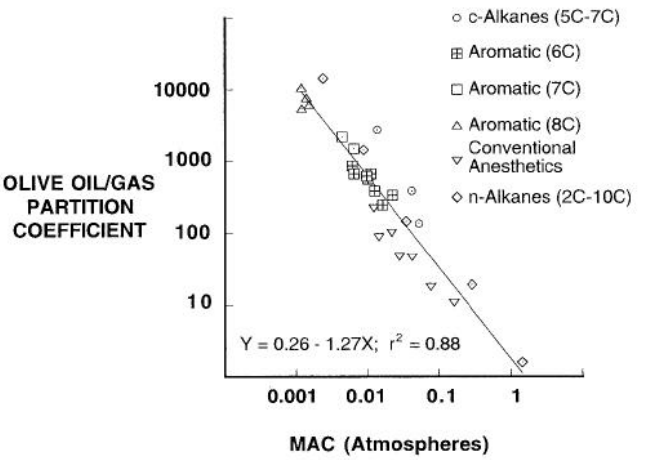

Figure 1. Minimum alveolar anesthetic concentration (MAC) correlates inversely with lipophilicity, as defined by the olive oil/gas partition coefficient. Values for aromatic compounds and for cycloalkanes (*c*-alkanes) are from the present study. Values for conventional anesthetics (14) and for normal alkanes (values are for even-numbered *n*-alkanes from ethane to decane) are from our previous work (15,16). Regression analysis for the combined data show a good correlation (r^2 of 0.88), but the slope of 1.27 deviates from 1.00. In contrast, application of the regression analysis to just the conventional anesthetics gives a slope of 1.08, with an r^2 value of 0.93. Values for conventional anesthetics lie below the line, values for the aromatic compounds tend to lie on the line, and values for cycloalkanes lie above the line.

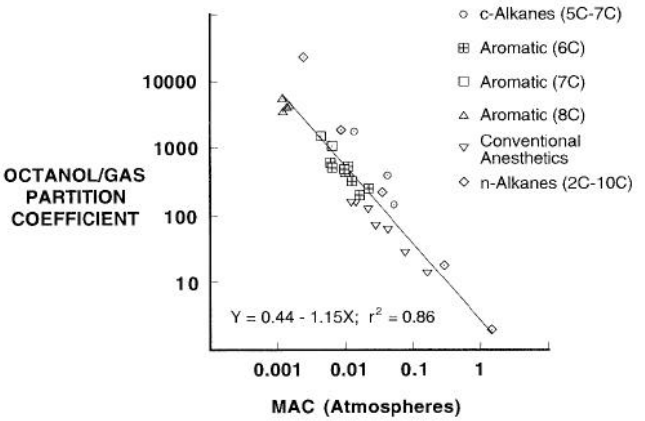

Figure 2. Minimum alveolar anesthetic concentration (MAC) also correlates inversely with lipophilicity, as defined by the octanol/gas partition coefficient. Sources for values are given in the legend to Figure 1. Regression analysis for the combined data shows as good a correlation for octanol as for olive oil (r^2 of 0.86), but, as with olive oil, the slope deviates from 1.00, being 1.15. In contrast, if the regression analysis is applied just to conventional anesthetics, the slope for octanol is 0.98, with an r^2 value of 0.98. Values for conventional anesthetics lie below the line, values for aromatic compounds tend to lie on the line, and values for cycloalkanes lie above the line.

benzene. Third, excitation of the central nervous system might antagonize anesthetic effects. *N*-Hexane causes tremors (1), and cyclohexane produces convulsions, whereas benzene causes only moderate excitation. The different degrees of excitation may explain why cyclohexane was the least potent of these three six-carbon compounds. In conflict with that explanation, we find that the convulsive activity of nonanesthetics, such as 1,3,5-tris(trifluoromethyl)benzene, neither increases nor decreases the MAC of desflurane.

Halogenation of benzene with 1, 2, 3, 5, or 6 fluorine atoms produced no consistent change in MAC values, lipid solubilities, or Meyer-Overton values, suggesting that—for this aromatic structure—the addition of fluorine atoms does not affect anesthetic properties. This differs from results with *n*-alkanes, wherein maximum potencies and lipid solubilities are produced when the molecule is half-fluorinated, especially when fluorination is distributed over the entire molecule (18).

P-difluorobenzene causes weak convulsions, whereas *o*-difluorobenzene does not cause convulsions. Similar results were found for *p*-xylene versus *o*- or *m*-xylene, possibly reflecting the relative position of the fluorides or methyl groups in these compounds. Excitation may result where fluorine atoms or methyl groups are symmetrically arranged at the ends of the molecule. This is consistent with data for other compounds [e.g., note the convulsant properties of flurothyl (19)].

Compared with benzene, both toluene and methylpentafluorobenzene (compounds with seven carbons) have higher lipid solubilities and are more potent anesthetics (Tables 4 and 5). The three xylenes and ethylbenzene with eight carbons showed the same progression when compared with toluene and methylpentafluorobenzene. Consistent with the larger size/greater number of carbon atoms, these compounds deviated more than smaller aromatic compounds (fluorinated benzenes) from the Meyer-Overton hypothesis (Table 5).

The 15 aromatic compounds with saline/gas partition coefficients over 0.15 had appreciable blood/gas partition coefficients, and thus P_a/P_I values less than 1 (Table 4). (P_a/P_I is the ratio of the arterial to inspired partial pressure of the test compound as determined in separate studies.) Correcting the MIC values to MAC by taking the product of MIC $\times$ P_a/P_I brought the Meyer-Overton products (MAC times lipid solubility) closer to those obtained with conventional anesthetics. However, despite this improvement, deviation from the value predicted from conventional anesthetics remained significant and substantial (Table 5; Figs. 1 and 2).

Although the olive oil/gas partition coefficient of 1,3,5-tris(trifluoromethyl)benzene was 264, it did not change the desflurane MAC of control rats and thus is a nonanesthetic. As true of other nonanesthetics, 1,3,5-tris(trifluoromethyl)benzene was a convulsant (convulsant 50% effective dose of 0.014 atm). Of the compounds studied, this had the largest molecular weight, and its size and symmetrical fluorination may have contributed to its convulsive and nonanesthetic actions.

Present data are consistent with previous studies in showing that the Meyer-Overton hypothesis is not tenable in its original form. We believe that a given anesthetic action (e.g., suppression of movement in response to a noxious stimulus) results from an effect on a specific site through a specific mechanism (20). Our data indicate that the anesthetic state, as defined by immobility, results from more than an interaction with a hydrophobic site. We have argued that affinity to water also may play a role (20). Present wisdom suggests that anesthesia may result from stereospecific interactions of anesthetics with specific protein sites (e.g., see Refs. 21 and 22). However, it is difficult to understand how the diverse sizes and shapes of anesthetic molecules in the present and previous studies can be reconciled with such a concept.

In summary, aromatic compounds were more potent and lipid-soluble than *n*-alkanes or cycloalkanes. All three series increasingly disobeyed the Meyer-Overton hypothesis as molecular size increased; for a particular number of carbons (e.g., cyclohexane versus *n*-hexane versus benzene), the deviation was cycloalkanes $\geq$ normal alkanes $>$ aromatic compounds. Thus, the cyclic structure (aromatic compound and cycloalkanes) versus the straight-chained structure did not consistently affect the deviation. One might conjecture that the cyclic shape decreased potency and increased the deviation from the Meyer-Overton hypothesis, but that unsaturation increased potency and partially compensated, thereby, for the deviation. Despite such qualitative speculations, our results suggest that molecular shape (including "bulkiness") and size provide only limited clues to the structure of the anesthetic site of action.

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