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Effect of Anesthetics and a Convulsant on Normal and Mutant Caenorhabditis elegans

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The authors have developed a method for studying the action of volatile anesthetics in *Caenorhabditis elegans* (C.e.), a free living nematode. C.e. appears to be a useful model for the study of the influence of genetics on susceptibility to anesthetics. This worm has a small, completely defined nervous system, easily manipulated genetics, and a large number of nervous system mutants. Under normal conditions C.e. moves almost constantly. When exposed to anesthetics there is an initial phase of increased locomotion, followed by uncoordinated motion that progresses to immobility. Motion returns quickly when the nematodes are removed from the anesthetic. The authors called loss of locomotion "anesthesia." The ED_{50}s of various anesthetics with C.e. are as follows: methoxyflurane 0.45%, chloroform 1.25%, halothane 2.7%, enflurane 4.2%, isoflurane 5.6%, fluroxene 9.9%. The authors also studied the action of a convulsant, flurothyl, on C.e. Flurothyl has anesthetizing properties in these animals with an ED_{50} of 8.1%. No convulsant activity was noted. However, mixtures of halothane and flurothyl were antagonistic in their effects, while halothane and enflurane were additive. Furthermore, the authors isolated a mutant strain (HS1) of C.e. that shows altered responses to several anesthetics and a convulsant. HS1 is uncoordinated when not exposed to anesthetics. Like the normal strain (N_2) HS1 loses mobility when exposed to anesthetics. The ED_{50}s for various anesthetics in HS1 were as follows: methoxyflurane 0.04%, chloroform 0.52%, halothane 0.85%, isoflurane 4.9%, enflurane 6.0%, fluroxene 10.9%. When compared with the normal C.e., HS1 exhibits a marked increase in sensitivity to methoxyflurane, chloroform, and halothane. No alterations in sensitivity to isoflurane or fluroxene were noted. HS1 exhibited decreased sensitivity to enflurane. HS1 reverts to normal motion when exposed to low concentrations of flurothyl and shows decreased sensitivity to the anesthetic effects of flurothyl with an ED_{50} of 11.8%. The slope of the curve of the log ED_{50} *versus* the log oil/gas partition coefficient is steeper in mutant than in normal nematodes. (Key words: Anesthetics, volatile: enflurane; flurothyl; fluroxene; halothane; chloroform; isoflurane; methoxyflurane. Brain: convulsions. Potency, anesthetic. Theories of anesthesia.)

THE MECHANISM OF ACTION of volatile anesthetics remains a subject of conjecture and scientific study. The close correlation between the oil/gas partition coefficients and ED_{50}s of anesthetics with widely differing structures leads to the theory that anesthetics interact with a membrane region with specific properties.[1] In addition, the ability to reverse anesthetic action with hyperbaric pressures leads some authors to suggest a membrane expansion model.[2,3] Recently, however, Franks and Lieb[4] showed that the thermodynamic restrictions dictated by the properties of known anesthetics do not eliminate the possibility of a protein receptor. Genetic variation may arise in the synthesis either of a protein receptor or of a specific membrane component. Thus, mutants may be a tool for determining site and mode of action of volatile anesthetics.

Previous authors have shown differences in susceptibility to inhalation anesthetics in different strains of mice and fruit flies.[5,6] However, the neurologic and genetic complexity of these organisms makes elucidation of the locus and mode of action of anesthetics very difficult.

Caenorhabditis elegans (C.e.) is an organism with several advantages in a study of nervous system function. First, the adult nematode has a nervous system that has been completely mapped anatomically. Several authors, using electron microscopy, have reconstructed the detailed organization of the 302 neurons in the adult hermaphrodite.[7–10] In addition, recently published studies of the development of C.e. have traced the developmental genealogy of every cell in the adult worm.[11]

Second, the life cycle of C.e. is about three and one-half days at 20° C. Most adults are self-fertilizing hermaphrodites. Such hermaphrodites are quite advantageous for genetic analysis. Recessive genes segregate rapidly in these populations, leading to phenotypic expression of recessive gene products in homozygous individuals. Self-fertilization makes it easy to clone such homozygous mutants. In addition, a small number of adults are phenotypic males, which allows for genetic crossing. This makes it possible to identify a single gene product in a mutant responsible for altered susceptibility to anesthetics.

Third, many investigators are presently studying the genetics of C.e. Large numbers of nervous system mutants have been observed and genetically mapped.[12] These mutants include uncoordinated mutants, chemotactic mutants, and thermotactic mutants and are available for research.

We present here the responses of wild type C.e. to volatile anesthetics and a convulsant. In addition, we have observed a mutant strain of C.e. with altered responses to these same compounds.

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Materials and Methods

Nematodes

C.e. var. Bristol (strain N_2) were obtained from the laboratory of D. Hirsch, Boulder, Colorado. The strain was grown on agar plates containing nematode growth media (NGM) described below. *Escherichia coli* (strain OP_{50}) was used as food.

Bacteria

The OP_{50} strain of *E. coli* is a uracil-requiring derivative of strain BB originating from the MRC laboratory in Cambridge, England. We use this strain because the NGM plates contain little uracil, causing the *E. coli* to form a lawn adequate to support the nematodes but not so thick as to obscure visualization.[12] The OP_{50} stock cultures were grown routinely in H-broth.

Media

NGM was prepared by mixing 3 g NaCl, 2.5 g Difco Bacto-Peptone®, 17 g Difco Bacto-Agar® and 975 ml H_2O and autoclaving. Subsequently, 1 ml 1 M $CaCl_2$, 1 ml 2 mg/ml uracil, 0.5 ml 10 mg/ml cholesterol in 95% ethanol in H_2O, 25 ml 1 M KPO_4 buffer, *p*H 6.0, and 1 ml 1 M Mg SO_4 were added under sterile conditions. The NGM was then distributed to plastic Petri dishes (30 ml to 100 mm × 15 mm dishes, 10 ml to 60 mm × 15 mm dishes). OP_{50} cultures were added and allowed to form a uniform lawn before nematodes were added. H-Broth was prepared by adding 5 g NaCl, 1 g dextrose, 5 g Bacto-Peptone® (Difco), and 8 g Nutrient Broth® (Difco) to 980 ml H_2O and autoclaving the solution. KPO_4 buffer was prepared by adding 98 g KH_2PO_4 (anhydrous), 48 g K_2HPO_4 (anhydrous) to one liter of H_2O. The *p*H was adjusted to 6 and the solution autoclaved.

Mutations were induced using 40 mM ethyl methane sulphonate (EMS) in M9 buffer. M9 buffer was prepared by adding 6 g Na_2HPO_4, 3 g KH_2PO_4, 5 g NaCl, and 0.25 g $MgSO_4 \cdot 7H_2O$ to one liter of H_2O.

Dose–Response Curves

Chambers. Nematodes on NGM plates were placed in flat glass Pyrex® dishes, which were covered with a ground glass lid and sealed with C-clamps. Two steel needles were fitted through a hole in the side of the dishes, one for injection of anesthetic, the other for sampling atmospheric concentrations of anesthetics. Volatile anesthetics were injected in liquid form and allowed to evaporate to give the desired concentrations. Experiments were conducted at 20° C (±1° C). Concentrations of volatile anesthetics remained stable for 24 h in these chambers as shown by gas chromatography. Samples were assayed with a Beckman Chromatograph® (Model GC-72-5) against standards of known concentrations.

Organisms

Adult nematodes were placed on NGM plates and allowed to lay eggs for 12 h. The adult worms were then removed, leaving a closely synchronous population of eggs. The cultures were allowed to mature for 72 h at 20° C before exposure to anesthetic. The nematodes were viewed through a dissecting microscope (Wild M5A®) using a light source from below. To facilitate easy observation we selected plates containing 50–250 organisms for these studies.

Definition of Anesthesia

C.e. has three kinds of behavior. These are as follows: 1) maintenance of almost constant locomotion; 2) maintenance of a sinusoid form; and 3) an avoidance response to mechanical stimulation at their anterior end. All these behaviors are suppressed at equal concentrations of anesthetics. Since loss of locomotion is easier to quantify than loss of avoidance response, we defined loss of locomotion for ten seconds as "anesthesia." The nematodes also swing their heads and tails across the long axis of their bodies. This behavior is suppressed at anesthetic concentrations above those necessary to suppress locomotion.

We evaluated populations of C.e. after exposure to anesthetics for sufficient time to reach steady state. This varied with the anesthetics and was determined experimentally. The ED_{50} for an anesthetic was defined as the concentration of anesthetic required to immobilize 50% of the animals.

Definition of Lethal Dose

Nematodes were exposed to halothane, then removed and allowed to recover in air at 20° C for 8 h. The nematodes then were viewed through the microscope. Young adult nematodes, which were immobile for 30 s and had lost the normal sinusoid form, were scored as dead.

Mutagenesis

Adult nematodes were placed on NGM plates and allowed to lay eggs for 12 h. The adult worms were then removed. These cultures were allowed to mature for 30–36 h at 20° C until most nematodes were young adults. In the hermaphrodite at this time, differentiation has occurred between the sperm and egg cell lines.[12] Therefore, any mutagenizing event will affect only sperm or egg but not both.

The young adults were washed onto a 47-mm millipore filter and resuspended in EMS buffer for 4 h at 20° C. The exposed individuals were again filtered onto a 47-mm millipore filter and washed three times with M9 buffer. The filters were transferred to fresh agar plates and the organisms allowed to rest 1 h. Individual nematodes then were placed on agar plates containing OP_{50}; 100–500 individuals were transferred per mutagenesis.

Screening

The cultures grown from individual nematodes exposed to EMS were grown for two generations (about 5 days) at 20° C. When the majority of the individuals were young adults, the cultures were exposed to 2% halothane for 1 h. The nematodes were observed (magnification ×180), and any individuals not moving in 20 s of observation individually were transferred to fresh agar plates containing OP_{50}.

Statistical Methods

Regression curves from the dose response data were constructed using the least-squares method.‡ Values for ED_{50}s were calculated from the regression curves. Errors for the ED_{50}s were calculated from the standard errors of the corresponding slope and γ-intercept using the delta method in two dimensions.[13] *P* values for the ED_{50}s were calculated using Students's *t* test. Regression curves from ln ED_{50}s and ln O/G_as were constructed using the least-squares methods.‡

Results

Qualitative Actions of Volatile Anesthetics on C.e.

The wild type (N_2-strain) C.e. moves in a sinusoid motion, either forward or backward. When individual animals were observed for 10 s at 20° C consistantly, at least 98% of the nematodes moved. When an animal was exposed to volatile anesthetics, there was an initial phase of increased motion followed by slowing. The nematode progressed to a slow, uncoordinated movement, which was inefficient with regard to locomotion. Eventually, the nematode failed to move the long axis of its body, except for a swinging motion of the head and tail. Head and tail flicking ceased at slightly higher concentrations of anesthetics, usually 10–15% above the ED_{50} for loss of locomotion. A third type of motion, pharangeal pumping, was lost at about the same time as locomotion. We saw no such changes in animals placed in the chambers when no anesthetic was added.

Motion returned quickly when the nematodes were removed from the anesthetic, usually within 3–5 mins. We did not see residual effects after exposure to immobilizing levels of anesthetics. However, at two to three times ED_{50}, exposure to anesthetics was lethal. In addition, we were unable to see an effect when C.e. was exposed to decane, a result noted previously in higher animals.[2]

Effect of Age on ED

The age of C.e. from 24 h to 120 h after hatching did not affect the dose–response curve with enflurane. However, we found that the time of onset of anesthesia after exposure was age dependent. Younger nematodes were affected more rapidly than the larger adults. In addition, the younger nematodes also recover from the anesthetic effects sooner than the adults.

Quantitative Action of Anesthetics

We studied the quantitative response of the wild type (N_2 strain) to six anesthetics: methoxyflurane, chloroform, halothane, enflurane, isoflurane, and fluroxene. The dose response curves and the ED_{50} for these anesthetics are presented in figure 1 and table 1. The responses occur over a narrow range of concentrations for each anesthetic, and the curves closely fit straight lines. The shapes of the curves for each anesthetic were similar when normalized for the respective ED_{50}s. The ED_{50}s tended to increase as the oil/gas partition coefficients decreased and were larger than MAC in higher animals. We also found the ED_{50} of enflurane to be lower than that of isoflurane, whereas enflurane's MAC is higher than that of isoflurane.

Lethal Effects of Halothane

The dose–response curve for lethal doses of halothane paralleled the anesthetic dose–response curve. The LD_{50} for halothane was 6.8%, 2.5 times the ED_{50} of halothane. A particular developmental stage of C.e. known as a dauer larvae was resistant to these levels of halothane and showed 100% survival. These worms were easy to distinguish morphologically from the normal adult and were not included in the results.

Actions of Flurothyl

No convulsant activity, such as increased uncoordinated motion, was observed at any concentration of flurothyl. However, flurothyl did show anesthetic prop-

‡ NWA Statpak Multi-function Statistics Library Version 3.1, ii. 7, ii. 36. Least squares polynomial regression. Portland, Northwest Analytical Inc., 1983.

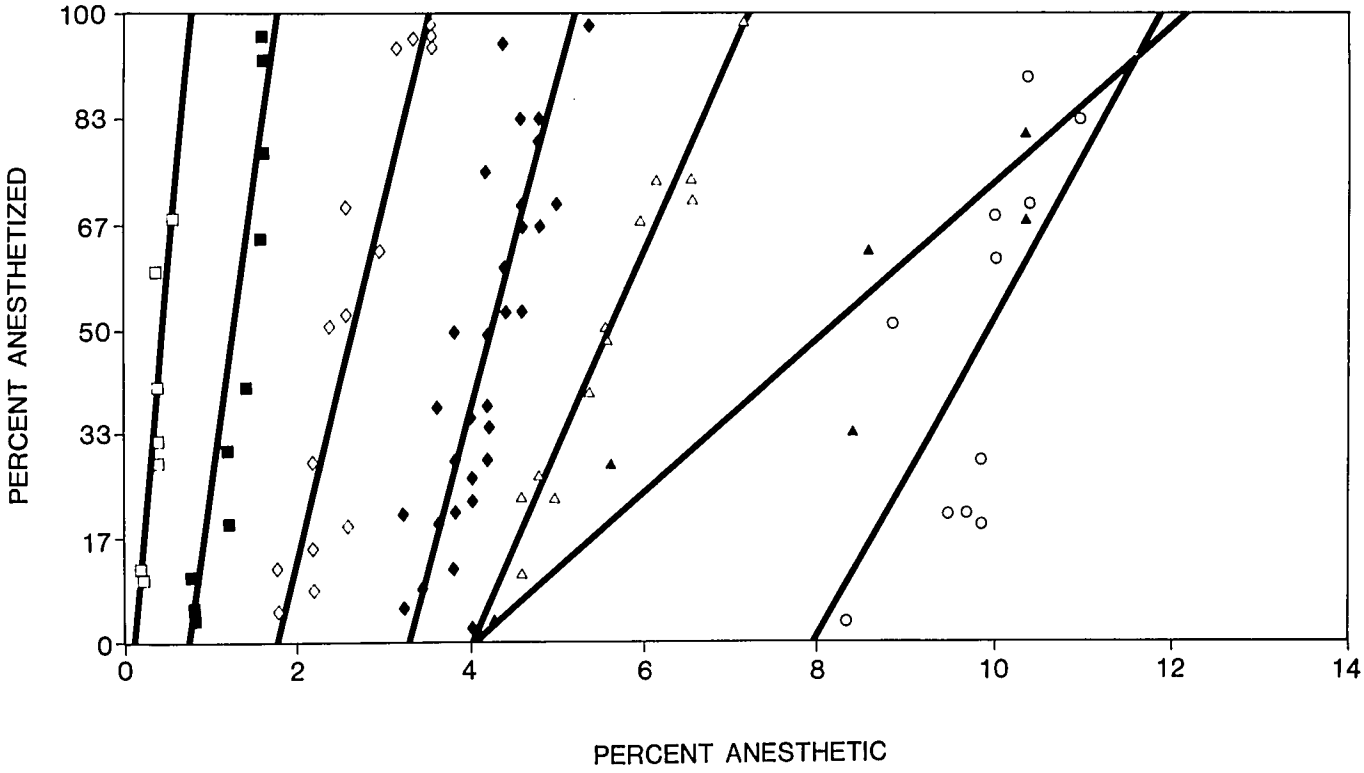

FIG. 1. Dose–response curves for six anesthetics and a convulsant. All values for anesthetic doses are volume % air. (□ = methoxyflurane; ■ = chloroform; ◇ = halothane; ◆ = enflurane; △ = isoflurane; ▲ = flurothyl; ○ = fluroxene.)

erties in C.e., with qualitative actions similar to the other anesthetics studied. The dose–response curve is shown in figure 1. In addition, the ED_{50} of flurothyl is listed with its oil/gas partition coefficient in table 1.

We also investigated the additive properties of flurothyl and halothane. As shown in table 2, the properties of these two agents are antagonistic in certain combinations. The anesthetic properties of halothane and enflurane are additive in this system.

STUDIES IN MUTANTS

We screened the second generation progeny of more than 1,000 nematodes exposed to EMS. Three strains were immobile in 2% halothane, a concentration not causing immobility in the wild type strain (N_2). Two strains remained immobile when removed from halothane. The third strain (HS1) regained mobility when removed from the anesthetic.

TABLE 1. ED_{50}s for Six Anesthetics and a Convulsant in C.e.

Anesthetic	ED_{50} (C.e.) (vol % ± SE) (20° C)	MAC (Dog) (37° C)	Oil/Gas Partition Coefficient (37° C)
Methox	0.45 ± 0.05	0.23 ± 0.03	970
Chloro	1.25 ± 0.08	0.77 ± 1	394
Haloth	2.70 ± 0.2	0.87 ± 0.12	224
Enflur	4.2 ± 0.2	2.2	98
Isoflu	5.60 ± 0.1	1.5 ± 0.26	98
Flurox	9.9 ± 0.8	6.0 ± 1.1	49
Flurot	8.1 ± 0.5	—	47

ED_{50}s for six anesthetics and a convulsant in nematodes compared with MAC in dogs and oil/gas partition coefficients.[16,17] Errors were calculated as described in the text. ED_{50}s were defined as concentrations of anesthetics causing 50% of observed nematodes to be immobile for 10 s.

TABLE 2. Effect of Mixtures of Flurothyl and Halothane in C.e.

Vol % Flurothyl	+	Vol % Halothane	Per Cent Anesthetized
0	+	3.1	46
1.7	+	3.1	20 ($P < 0.05$)
0	+	3.3	90
2.2	+	3.3	40 ($P < 0.01$
0	+	3.3	100
2.8	+	3.8	58 ($P < 0.01$)
1.7	+	0	0
2.2	+	0	0
2.8	+	0	0

Per cent of C.e. anesthetized with mixtures of halothane and flurothyl compared with C.e. anesthetized with flurothyl or halothane alone. *P* values were determined by analysis of variance and refer to the difference between the per cent anesthetized with both anesthetics *versus* the values for each anesthetic separately.

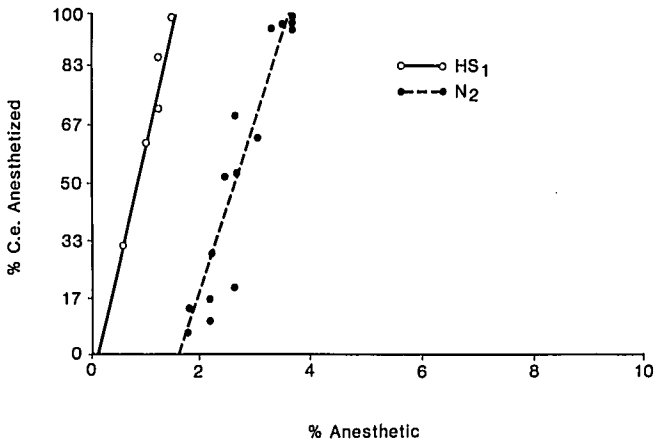

FIG. 2. Per cent C.e. anesthetized plotted *versus* volume per cent halothane in air. O—— O = HS1 strain; O - - O = N_2 strain.

Members of the strain HS1 were uncoordinated in room air at 20° C. They did not move in a sinusoid pattern as the N_2 strain did. Rather, their motion consisted of short bursts of locomotion, followed by pauses of several seconds. In addition, HS1 individuals were immobile for longer periods than members of the N_2 strain. Head and tail motion and pharyngeal pumping appeared unchanged from the N_2 strain. We discerned no morphologic difference between the N_2 and HS1 strains.

Qualitative Action of Anesthetics on HS1

The qualitative effects of anesthetics on the strain HS1 varied for the different anesthetics. When exposed to methoxyflurane, chloroform, or halothane, the nematodes showed no initial phase of increased activity. Enflurane, isoflurane, and fluroxene caused an initial increase in motion, though the motion remained uncoordinated. As noted previously in the N_2 strain, concentrations of anesthetics 10–15% above the ED_{50} for loss of locomotion in HS1 caused depression of head and tail movement.

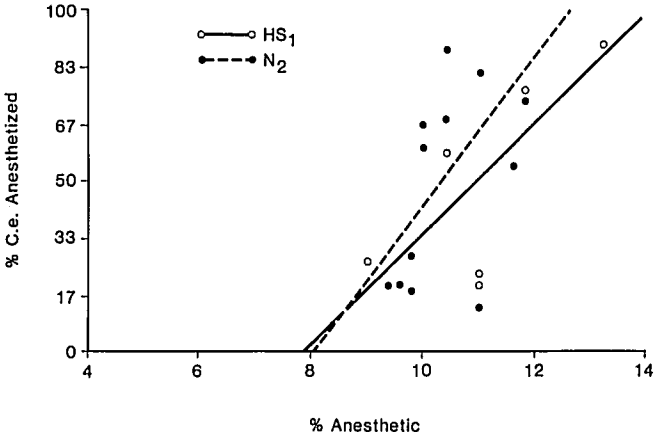

FIG. 3. Per cent C.e. anesthetized plotted *versus* volume per cent fluroxene in air. O—— O = HS1 strain; O - - - O = N_2 strain.

The time required to reach a steady state response with the HS1 strain was shorter than for the N_2 strain with methoxyflurane, chloroform, and halothane. Enflurane, isoflurane, and fluroxene had similar time courses for their effects in N_2 and HS1. Recovery from the effects of the anesthetics was slower in the HS1 strain than in the wild type. Typically, anesthetized HS1 nematodes began moving about 10 min after removal from the anesthetic.

The age of the nematodes showed a qualitative effect on the dose–response curves. The larval forms of the HS1 strain were mildly resistant to anesthetics when compared with the adult stage. We have not quantified this difference.

Quantitative Actions of Anesthetics on the HS1 Strain

We studied the response of the HS1 strain using the six anesthetics studied previously in the N_2 strain. The dose–response curves for methoxyflurane, chloroform, and halothane in the strain HS1 exhibited a leftward shift compared with the N_2 strain, reflecting an increase in sensitivity. The comparative dose response curves for halothane were representative of these three anesthetics and are shown in figure 2. The curves for isoflurane and fluroxene in the HS1 strain were not significantly shifted from those in the N_2 strain. The dose response curves for fluroxene are representative of this latter group and are shown in figure 3. The dose–response curve for enflurane in the mutant strain was shifted to the right, indicating a decrease in sensitivity to this anesthetic. The ED_{50}s for the HS1 and N_2 strains are listed in table 3. We have listed in table 4 the slopes and y-intercepts for each anesthetic with HS1 and N_2.

As in the N_2 strain, the responses occurred over a narrow range for each anesthetic, and the curves closely fit straight lines. The relative order of potencies of anesthetics may be altered in HS1 compared with N_2, though the variability in the ED_{50}s precluded a definite order between enflurane and isoflurane.

Actions of Flurothyl on the HS1 Strain

We have shown previously that flurothyl, a convulsant, has anesthetic properties in the N_2 strain. In the mutant strain, flurothyl, in low doses, changed the abnormal locomotion to normal sinusoid movement indistinguishable from that of the wild type C.e. At higher doses flurothyl was an anesthetic in HS1. The dose–response curves in the N_2 and HS1 strain are shown in figure 4.

TABLE 3. ED_{50}s for Six Anesthetics and a Convulsant in C.e.

Agent	ED_{50} (N_2) ± SE	ED_{50} (HS1) ± SE
Methox	0.45 ± 0.05	0.04 ± 0.01 ($P < 0.001$)
Chloro	1.25 ± 0.08	0.5 ± 0.1 ($P < 0.001$)
Haloth	2.7 ± 0.2	0.85 ± 0.1 ($P < 0.001$)
Enflur	4.2 ± 0.2	6.0 ± 0.6 ($P < 0.01$)
Isoflu	5.6 ± 0.1	4.9 ± 0.7 ($P > 0.1$)
Flurox	9.9 ± 0.8	10.9 ± 1.2 ($P > 0.1$)
Flurot	8.1 ± 0.5	11.8 ± 1.2 ($P < 0.02$)

The ED_{50}s are listed for the wild type strain (N_2) and a strain with altered sensitivities to volatile anesthetics (HS1). Errors were determined as described in the text.

The ED_{50}s and parameters for the curves are shown in tables 3 and 4, respectively. The HS1 strain showed marked resistance to the anesthetic effects of flurothyl when compared with the N_2 strain.

Discussion

Our data show that volatile anesthetics have anesthetizing properties in C.e. The qualitative actions of anesthetics on C.e. are similar to the effects in higher animals and include the following: 1) reversible loss of function; 2) initial increase in activity of C.e. when exposed to low concentrations of anesthetics; 3) sequential loss of function in C.e. after exposure to anesthetics; and 4) similar shapes of the dose–response curves.

The similarities between the effects of anesthetics in C.e. and higher animals indicate that the mechanism of action may be the same in each case. A ln-ln plot of MAC *versus* oil/gas partition coefficients approximates a straight line in higher animals.[1] A ln-ln plot of the ED_{50}s in C.e. *versus* oil/gas partition coefficients also approximates a straight line that parallels that of MAC in the dog. The line, however, is shifted to the right, reflecting the higher concentrations of anesthetics required in C.e. This shift is underestimated, since the oil/gas partition coefficients are measured at 37° C, while our experiments occurred at 20° C. This would cause approximately a 50–60% increase in the values for the oil/gas coefficients.[14] The difference between the ED_{50}s in C.e. and MACs in higher animals may reflect the different behavioral endpoints measured.

In higher animals, enflurane consistently has a higher ED_{50} than isoflurane, despite their similar oil/gas partition coefficients. Enflurane also has convulsant activity in these animals. Enflurane, therefore, has competitive effects, and higher levels of the anesthetic may be necessary to override the stimulation it produces.

In C.e. the relative potencies of isoflurane and enflurane are reversed. Enflurane may cause convulsant activity in higher animals by suppression of a normally inhibitory function. In nematodes such an inhibitory function may be lacking, or suppression of such a function may not be grossly observable. It is interesting that one such inhibitory system exists in nematodes, possibly involving GABA transmission.[15] During the organism's sinusoid motion, the muscle cells on the outside of each wave along the body are inhibited. Supression of this inhibitory function actually may lead to immobility by not allowing necessary relaxation. Studies measuring the effect of anesthetics on post neuromuscular junction membranes potentials may help answer this question.

We do find, as did Koblin *et al.*,[16] that flurothyl has anesthetizing properties. In light of the above situation

TABLE 4. Slopes and γ-Intercepts for Dose–Response Curves of C.e. and Several Anesthetics

Anesthetic	Slope (±SE)	γ-Intercept (±SE)	R
Methoxyflurane			
N_2	159 ± 21	−21 ± 9	0.92
Hs1	1088 ± 272	5 ± 17	0.89
Chloroform			
N_2	94 ± 10	−68 ± 12	0.94
HS1	124 ± 24	−15 ± 17	0.90
Halothane			
N_2	54 ± 5	−90 ± 16	0.93
HS1	79 ± 6	−17 ± 7	0.99
Ethrane			
N_2	45 ± 5	−162 ± 21	0.84
HS1	30 ± 7	−130 ± 44	0.90
Isoflurane			
N_2	31 ± 2	−121 ± 13	0.95
HS1	20 ± 4	−48 ± 27	0.82
Fluroxene			
N_2	24 ± 4	−187 ± 42	0.76
HS1	16 ± 5	−128 ± 57	0.78
Flurothyl			
N_2	12 ± 1	−47 ± 13	0.96
HS1	14 ± 4	−113 ± 48	0.83

The slopes and γ-intercepts are listed for the wild type strain (N_2) and a mutant (HS1). Values were determined as described in the text.

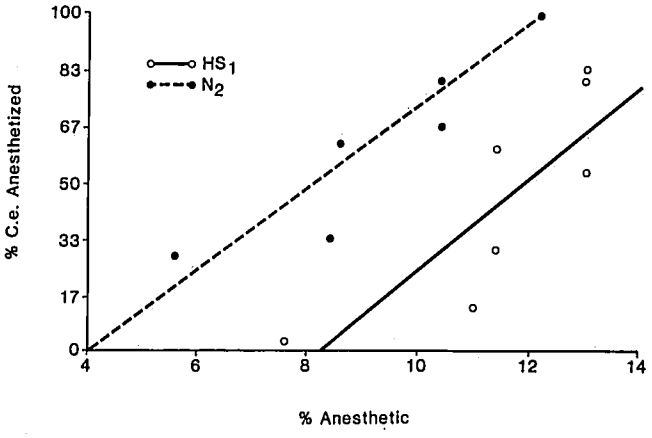

FIG. 4. Per cent C.e. anesthetized plotted *versus* volume per cent flurothyl in air. O——O = HS1 strain; O - - - O = N_2 strain.

with enflurane and isoflurane, it is interesting that flurothyl appears more potent than fluroxene in C.e., despite similar oil/gas partition coefficients. This difference may again reflect flurothyl's convulsant activity in higher animals. Our results also show that the anesthetic properties of flurothyl and halothane are antagonistic in C.e. Thus, flurothyl may have competing neurophysiologic effects with other anesthetics even in this simple neural system. The uncoordinated movement of the mutant strain is changed to normal movement in the presence of low concentrations of flurothyl. This may reflect a stimulating effect of flurothyl in C.e. that is not observable in the N_2 strain.

If HS1 were simply an inactive strain of C.e., we would expect the nematodes to be more sensitive to all anesthetics. Instead, the HS1 strain showed altered sensitivities that were related to the oil/gas partition coefficients of the anesthetics. HS1 showed the greatest percentage increase in sensitivity to methoxyflurane, which has the greatest O/G_a of the anesthetics we studied. As the O/G_a of the anesthetics decreased, the change in sensitivities of HS1 compared with N_2 also tended to decrease.

The log/log plot of the potencies and oil/gas partition coefficients of anesthetics in normal C.e. parallels similar plots in higher animals and has a slope of approximately -0.9. This slope increases to -1.6 in the mutant. To our knowledge, the mutant HS1 is the first animal in which this slope deviates significantly from -1. This may imply a change at the locus of action of volatile anesthetics.

The two strains need to be further studied to define the molecular basis of their differences. We intend to do this by two methods. First we will genetically map where the mutation has occurred. Since many uncoordinated mutations have been mapped, we may find HS1 maps onto a previously described genetic locus. Secondly, we intend to compare the membrane components of these two strains.

In summary, the above results indicate that C.e. is an excellent model for studying the mechanism of action of general anesthetics. The actions of anesthetics are reproducible and, in many ways, mimic previously observed results in higher animals. Enflurane and flurothyl, both of which have convulsant activity in higher animals, are more potent as anesthetics in C.e. than predicted by their oil/gas partition coefficients. No convulsant activity was seen in C.e. when exposed to flurothyl; flurothyl acted as an anesthetic in C.e. However, the combination of flurothyl and halothane showed antagonism between these two agents.

We also isolated a mutant strain of C.e. with altered sensitivities to anesthetics and flurothyl.

References

1. Meyer KH: Contribution to the theory of narcosis. Trans Faraday Soc 33:1062–1068, 1937
2. Mullins LJ: Some physical mechanisms in narcosis. Chem Rev 54:289–323, 1954
3. Matubayasi N, Ueda I: Is membrane expansion relevant to anesthesia? Mean excess volume. ANESTHESIOLOGY 59:541–546, 1983
4. Franks NP, Lieb WR: Molecular mechanisms of general anesthesia. Nature 300:487–493, 1982
5. Gamo S, Tanaka EN, Ogaki M: Alterations in molecular species of phosphatidylethanolamine between anesthetic resistant and sensitive strains of Drosophila Melanogaster. Life Sci 30: 401–408, 1982
6. Koblin DD, Dong DE, Deady JE, Eger EI II: Selective breeding alters murine resistance to nitrous oxide without alteration in synaptic membrane lipid composition. ANESTHESIOLOGY 52:401–407, 1980
7. Ward S, Thomson N, White JG, Brenner S: Electron microscopical reconstruction of the anterior sensory anatomy of the nematode Caenorpabditis elegans. J Comp Neurol 160:313–338, 1975
8. Ware RW, Clark D, Crossland K, Russell RL: The nerve ring of the nematode Caenorhabditis elegans: Sensory input and motor output. J Comp Neurol 162:71–110, 1975
9. Albertson DG, Thomson JN: The pharynx of Caenorhabditis elegans. Philos Trans Roy Soc London [Biol] 275:299–325, 1976
10. White JG, Southgate E, Thomson JN, Brenner S: The structure of the ventral nerve cord of Caenorhabditis elegans. Philos Trans Roy Soc London [Biol] 275:327–348, 1976
11. Sulsten JE: The embryonic cell lineage of the nematode Caenorhabditis elegans. Dev Biol 100:64–119, 1983
12. Brenner S: The genetics of Caenorhabditis elegans. Genetics 77: 71–94, 1974
13. Bishop YM, Fienberg SE, Holland PW: Discrete Multivariate Analysis Theory and Practice. Cambridge, MIT Press, 1980, p 493
14. Allot PR, Steward A, Flook V, Mapleson WW: Variation with temperature of the solubilities of inhaled anesthetics in water, oil and biological media. Br J Anaesth 45:294–299, 1973
15. Zuckerman BM: Nematodes as Biological Models, vol 1. New York, Academic Press, 1980, pp 166–167
16. Koblin DD, Eger EI II, Johnson BH, Collins P, Terrell RC, Speers L: Are convulsant gases also anesthetics? Anesth Analg 60:464–470, 1981
17. Eger EI II: Anesthetic Uptake and Action. Baltimore, Williams and Wilkins, 1974, pp 5, 82