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Multiple sites of action of volatile anesthetics in *Caenorhabditis elegans*

(genetics/mutations/anesthesia)

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ABSTRACT **The mechanism and site(s) of action of volatile anesthetics are unknown. In all organisms studied, volatile anesthetics adhere to the Meyer–Overton relationship—that is, a ln–ln plot of the oil–gas partition coefficients versus the potencies yields a straight line with a slope of −1. This relationship has led to two conclusions about the site of action of volatile anesthetics. (*i*) It has properties similar to the lipid used to determine the oil–gas partition coefficients. (*ii*) All volatile anesthetics cause anesthesia by affecting a single site. In *Caenorhabditis elegans*, we have identified two mutants with altered sensitivities to only some volatile anesthetics. These two mutants, *unc-79* and *unc-80*, confer large increases in sensitivity to very lipid soluble agents but have little or no increases to other agents. In addition, a class of extragenic suppressor mutations exists that suppresses some altered sensitivities but specifically does not suppress the altered sensitivity to diethyl ether. There is much debate concerning the molecular nature of the site(s) of anesthetic action. One point of discussion is whether the site(s) consists of a purely lipid binding site or if protein is involved. The simplest explanation of our observations is that volatile anesthetics cause immobility in *C. elegans* by specifically interacting with multiple sites. This model is in turn more consistent with involvement of protein at the site(s) of action.**

More than a century has passed since the introduction of volatile anesthetics, yet the site and mechanism of action of volatile anesthetics remain unknown. Meyer (1) and Overton (2) showed that the potency of volatile anesthetics depended solely on their oil solubilities, despite widely varying chemical structures. This relationship is termed the Meyer–Overton relationship. A second characteristic of anesthetics is that, in general, they are directly additive in their effects (3). These findings have led to two main conclusions about the sites of action of volatile anesthetics. (*i*) Volatile anesthetics work at a single lipophilic site to cause anesthesia. (*ii*) The site of action has properties similar to the lipid used to determine the oil–gas partition coefficient. In addition, the number of anesthetic molecules required at the site of action to cause anesthesia is a constant regardless of the anesthetic used (4). The identification of the site of action of these agents would seem to be a prerequisite for understanding their mode of action.

The nematode *Caenorhabditis elegans* appears to be a good model for the study of volatile anesthetics (5). The wild-type strain N2 responds to volatile anesthetics in a manner similar to all other species including humans: it follows the Meyer–Overton relationship (5) and exhibits additivity between anesthetics (P.G.M. and M.S., unpublished results). The behavioral pattern during exposure to anesthetics resembles that of more complex organisms. At low doses of anesthetic the animals become "excited," moving more than animals not exposed to anesthetics. As the anesthetic concentrations are increased, the animals next become very uncoordinated, and at higher doses they are immobilized. When removed from the anesthetics the nematodes quickly regain mobility and return to their normal phenotype. Mutations in two genes, *unc-79* and *unc-80*, confer altered responses to volatile anesthetics, in addition to an altered motor phenotype. When not in the presence of anesthetics, both these mutants are described as "fainters." Their motion consists of short periods of normal motion, followed by an apparent refractory period lasting a few seconds during which their locomotion ceases (6, 7). At least one allele of *unc-79* has been shown to be suppressed by an amber suppressor and thus represents a loss-of-function allele. These mutants have increased sensitivity to one group of anesthetics (thiomethoxyflurane, methoxyflurane, chloroform, and halothane; group 1), decreased sensitivity to a second group (enflurane and flurothyl; group 2), and no change in sensitivity to a third group (isoflurane and fluroxene; group 3). In addition, both mutations confer a mild increase in sensitivity to diethyl ether, the sole member of group 4. This increase is quantitatively different than the increase seen in group 1 (Fig. 1). We postulated that these mutations affect the site of action of volatile anesthetics (7). We have screened 105 uncoordinated mutants in *C. elegans*, in addition to screens of newly mutagenized nematodes, and have not identified other single-gene mutations that alter responses to volatile anesthetics (P.G.M. and M.S., unpublished results). Thus, it appears that the mechanisms controlling the response of *C. elegans* to volatile anesthetics will be genetically tractable and involve relatively few genes with major effects.

Another method for identification of gene products affecting the site of action of volatile anesthetics is the study of gene interaction (6, 8). If several gene products function together at a particular site, then mutations in a second gene may alter or compensate for mutations in an initial gene (9). The phenotypes of double mutants could provide valuable information about such interactions. We have studied a class of genetic interaction known as indirect suppression.

Mutations in *unc-79* and *unc-80* are more sensitive to halothane than is the wild type. Sedensky and Meneely (6) described mutations in the gene *unc-9* that suppressed the abnormal response of *unc-79* and *unc-80* to halothane. The double mutants, *unc-79;unc-9* and *unc-80;unc-9*, exhibit normal sensitivity to halothane. *unc-9* alone also has normal sensitivity to halothane and, when not in the presence of anesthetics, has a motion described as kinked. The mechanism by which *unc-9* exerts its effects is unknown. The effect of *unc-9* on *unc-79* and *unc-80* had only been studied for halothane. In this article we describe the effect of *unc-9* on the responses of *unc-79* and *unc-80* to nine anesthetics. We

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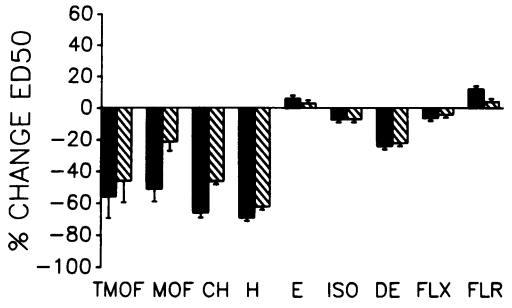

FIG. 1. Change in the ED_{50} value of *unc-79* (solid bars) and *unc-80* (hatched bars) compared to those of the wild-type strain, N2, in nine anesthetics. The zero line represents the normal response—i.e., that of N2, to each agent. Data are from ref. 8. TMOF, thiomethoxyflurane; MOF, methoxyflurane; CH, chloroform; H, halothane; E, enflurane; ISO, isoflurane; DE, diethyl ether; FLX, fluroxene; FLR, flurothyl. ED_{50} values are percent volumes at standard temperature and pressure and refer to the concentrations of anesthetic in air at which 50% of animals are immobile.

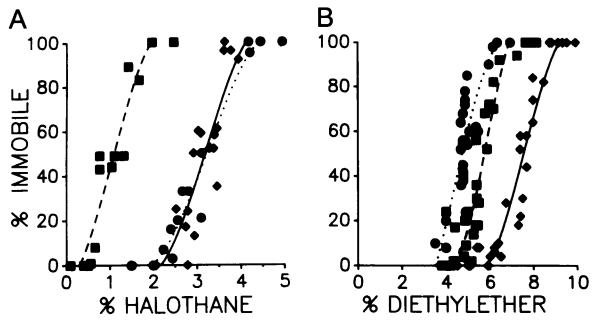

FIG. 2. (*A*) Dose–response curves of wild-type N2 (◆) and mutant *unc-79* (■) and *unc-79;unc-9* (●) *C. elegans* in halothane. The curves compare the percent nematodes immobilized to the percent volumes (vol %) of halothane. Dose–response curves were obtained as described in ref. 8. This figure was previously published in ref. 8 and is presented here for comparison with data in *B*. (*B*) Dose–response curves of wild-type N2 (◆), *unc-79* (■), and *unc-79;unc-9* (●) in diethyl ether.

also describe here the identification of two suppressors of *unc-79* and *unc-80*. These mutants, *unc-1* and *unc-7*, have uncoordinated phenotypes very similar to that of *unc-9*, described as kinked or curly (10). *unc-7* was initially studied because an allele, *nc933*, was mistakenly thought to be an *unc-9* allele. *unc-1* (*e538*) was originally studied as a negative kinked control for some *unc-9* experiments, only to demonstrate suppression activity itself. We determined the effects of these suppressors on the response of *unc-79* and *unc-80* to halothane. We then studied the effects of the *unc-1* and *unc-7* mutations on the responses of *unc-79* to a representative anesthetic from each of the four groups described above. We postulated that if these mutations alter the site(s) of anesthetic action, then there may exist differences in the quality or quantity of suppression for different anesthetics. Such differences may give insight to the nature of the site of action of volatile anesthetics in *C. elegans*.

Previous work has also shown that four classes of mutations exist in the *unc-1* gene (11). Two of these classes are recessive and have a kinked phenotype, and two are dominant and have a coiled phenotype as heterozygotes. Since one allele functioned as a suppressor, we studied a representative allele from each class to determine if each class functions as a suppressor for the response of *unc-79* to halothane.

METHODS

Nematodes. Certain strains were obtained from the *Caenorhabditis* Genetics Center in Columbia, MO. Method for raising, handling, and performing genetic crosses with *C. elegans* have been described elsewhere in detail (12).

Dose–Response Curves. Procedures for exposing nematodes to anesthetics, scoring their responses, and measuring anesthetic concentrations have also been described in detail (8). Concentrations of anesthetics were expressed in percent volume (vol %) at standard temperature and pressure in air. ED_{50} values were the percent volumes at which 50% of the animals were immobile for greater than 10 sec.

Anesthetics. Thiomethoxyflurane was kindly provided by E. I. Eger II (Department of Anesthesia, University of California, San Francisco). Methoxyflurane, chloroform, halothane, enflurane, isoflurane, diethyl ether, and fluroxene were commercial products. Halothane was supplied by Halocarbon Products (Hackensack, NJ). Enflurane, isoflurane, and flurothyl were supplied by Anaquest (Madison, WI). Diethyl ether was supplied by Fisher.

Statistical Methods. Regression analysis, ED_{50} values, and standard errors were calculated using the methods described by Waud (13). For each anesthetic, the ED_{50} values of all four strains were compared using an analysis of variance to see if they satisfied the null hypothesis ($P < 0.05$). If this analysis indicated that a difference existed within the group, we compared the individual mean values of each strain. Comparison of ED_{50} values for the different strains was performed by Tukey's method for multiple comparisons (14). Significance was defined as $P < 0.01$. The increased stringency was used to avoid type I errors (detecting a difference when none actually existed). Variances for the differences between ED_{50} values were calculated by adding the variances of each ED_{50} value involved.

Table 1. Effect of *unc-9* on the ED_{50} values of *unc-79* and *unc-80*

	ED_{50}, vol %					
Anesthetic	N2	*unc-9*	*unc-79*	*unc-79;unc-9*	*unc-80*	*unc-80;unc-9*
TMOF	0.11 ± 0.02	0.11 ± 0.03	0.05 ± 0.02	0.11 ± 0.03	0.07 ± 0.03	0.10 ± 0.04
MOF	0.58 ± 0.02	0.54 ± 0.03	0.28 ± 0.05	0.58 ± 0.02	0.46 ± 0.03	0.55 ± 0.03
CHL	1.47 ± 0.02	1.63 ± 0.02	0.50 ± 0.03	1.59 ± 0.02	0.80 ± 0.02	1.58 ± 0.02
H	3.18 ± 0.04	3.15 ± 0.05	0.98 ± 0.02	3.28 ± 0.05	1.20 ± 0.02	2.98 ± 0.05
E	5.89 ± 0.08	5.96 ± 0.08	6.24 ± 0.07	5.96 ± 0.08	6.06 ± 0.07	6.05 ± 0.1
ISO	7.18 ± 0.07	6.6 ± 0.1	6.67 ± 0.08	6.53 ± 0.1	6.14 ± 0.07	6.56 ± 0.1
DE	7.5 ± 0.1	5.6 ± 0.1	5.70 ± 0.06	4.8 ± 0.1	5.84 ± 0.06	4.9 ± 0.1
FLX	10.8 ± 0.1	9.9 ± 0.1	10.1 ± 0.1	8.8 ± 0.1	10.4 ± 0.1	9.4 ± 0.1
FLR	14.3 ± 0.1	14.8 ± 0.1	15.9 ± 0.1	15.0 ± 0.2	14.9 ± 0.1	14.5 ± 0.2

Data are mean ± SE. Wild-type strain N2 and mutant *unc-9* are included for comparison. Dose–response curves were constructed as described (8). TMOF, thiomethoxyflurane; MOF, methoxyflurane; CH, chloroform; H, halothane; E, enflurane; ISO, isoflurane; DE, diethylether; FLX, fluroxene; FLR, flurothyl.

Table 2. Effect of *unc-1(e719)* and *unc-7(e5)* on the ED_{50} values of *unc-79(ec1)* in four anesthetics

Anesthetic	ED_{50}, vol %					
	N2	*unc-79*	*unc-1*	*unc-79;unc-1*	*unc-7*	*unc-79;unc-7*
H	3.2 ± 0.05	1.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.15 ± 0.05	3.10 ± 0.05
E	6.6 ± 0.1	7.1 ± 0.1	6.5 ± 0.1	6.2 ± 0.1	6.8 ± 0.1	6.6 ± 0.1
ISO	7.2 ± 0.1	6.7 ± 0.1	7.0 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	7.0 ± 0.1
DE	6.8 ± 0.1	5.3 ± 0.1	6.2 ± 0.1	5.5 ± 0.1	6.6 ± 0.1	5.3 ± 0.1

Data are mean ± SE. Effect of *unc-1(e719)* on the ED_{50} values for four anesthetics in two mutant strains with altered sensitivity to volatile anesthetics. *unc-1(e719)* was obtained from the *Caenorhabditis* Genetics Center. Abbreviations are in Table 1.

RESULTS

Table 1 lists the ED_{50} values (mean ± SE) for six strains (N2, *unc-79*, *unc-80*, *unc-9*, and the double mutants *unc-79; unc-9* and *unc-80;unc-9*) in nine volatile anesthetics. *unc-79* and *unc-80* show increased sensitivity to all four anesthetics in group I (thiomethoxyflurane, methoxyflurane, chloroform, and halothane) (7). However, the double mutants *unc-79;unc-9* and *unc-80;unc-9* have normal sensitivity to these agents. Thus, *unc-9* suppresses the increased sensitivity of *unc-79* and *unc-80* to all agents in group I. *unc-79* shows mild resistance to anesthetics in group II (enflurane and flurothyl). *unc-9* also suppresses this mild resistance; i.e., the double mutant *unc-79;unc-9* has normal sensitivity to these agents. *unc-79* and *unc-80* have normal sensitivities to anesthetics in group III (isoflurane and fluroxene) and *unc-9* had no effect on their response to these agents. *unc-79* and *unc-80* exhibit mild increases in sensitivity to diethyl ether. *unc-9* does not suppress this response. In fact, the double mutants *unc-79;unc-9* and *unc-80;unc-9* are more sensitive to diethyl ether than are the single mutants (Table 1 and Fig. 2).

To determine if *unc-1* and *unc-7* were suppressors of *unc-79* and *unc-80*, we exposed strains containing *unc-1* or *unc-7*, instead of *unc-9*, to a representative from each of the four groups of anesthetics. Table 2 lists the data for the strains *unc-1(e719)*, *unc-79;unc-1*, *unc-7(e5)*, and *unc-79;unc-7*. Table 3 lists the equivalent data for the strains involving *unc-80*—that is, *unc-1*, *unc-80;unc-1*, *unc-7*, and *unc-80;unc-7*. Both *unc-1* and *unc-7* suppress the increased sensitivities of *unc-79* and *unc-80* to halothane. Neither *unc-1* nor *unc-7* suppress the increased sensitivities of *unc-79* and *unc-80* to diethyl ether. The resistance of *unc-79* to enflurane is also suppressed by both *unc-1* and *unc-7*, whereas these mutations exert no significant effect on the sensitivity of *unc-79* or *unc-80* to isoflurane. Thus, the pattern of suppression for *unc-1* and *unc-7* is the same as for *unc-9*.

The effect of each of the four classes of *unc-1* mutations (11) on *unc-79* is shown in Table 4. The double mutants were screened in 2% halothane, a concentration that immobilizes 100% of either *unc-79* or *unc-80* but leaves N2 unaffected (6, 7). Each strain was scored for percent immobilized at this concentration. The data show that both classes of *unc-1* that are recessive in their kinked phenotype (represented by *e114* and *e580*) are also recessive suppressors of *unc-79* in halothane. The classes that are dominant in coiled phenotype (represented by *n494* and *n774*) are not suppressors, as heterozygotes, of *unc-79* in halothane. However, the representative of class 4, *n774*, exhibits a kinked phenotype as a homozygote, and, as a homozygote, it is a suppressor of *unc-79* in halothane. The representative of class 3, *n494*, remains coiled as a homozygote. As a homozygote *n494* does not suppress the altered sensitivity of *unc-79* to halothane. Thus, the ability to suppress *unc-79* in halothane follows exactly the kinked phenotype in *unc-1*. As with *unc-9* and *unc-7*, none of the *unc-1* mutants alone alters halothane sensitivity.

DISCUSSION

We have characterized mutations in three genes that act as indirect suppressors of the responses of *unc-79* and *unc-80* to volatile anesthetics. Each of the suppressors followed the same pattern. Each mutation alone showed a normal response to the anesthetics tested. *unc-1*, *unc-7*, and *unc-9* individually suppress the increased sensitivity of *unc-79* and *unc-80* to halothane. In addition, each one suppresses the decreased sensitivity of *unc-79* to enflurane. None of the suppressors altered the normal response of *unc-79* to isoflurane. In contrast to the suppressing activities noted above, however, none of the three mutations suppressed the increased sensitivity of *unc-79* to diethyl ether. We must also note that *unc-1* and *unc-7* were studied because of their resemblance to *unc-9* and not picked up on independent screens for suppressors. We have also tested other kinked mutants, *unc-2* and *unc-42*, and found that they do not suppress *unc-79*. (P.G.M. and M.S., unpublished results). Thus, although the kinked phenotype correlates with the capability of *unc-1* to suppress *unc-79*, the kinked phenotype alone is not sufficient to confer the capability to suppress *unc-79*. It should be noted that the "kinked" phenotype may arise from a variety of neurological defects.

We have suggested (7) that *unc-79* and *unc-80* respond to volatile anesthetics as if multiple classes of volatile anesthetics exist. The three suppressors in this study affect the responses of *unc-79* and *unc-80* to some anesthetics but not to others. They also alter both increased sensitivities to some anesthetics and decreased sensitivities to others. We think

Table 3. Effect of *unc-1(e719)* and *unc7(e5)* on the ED_{50} values of *unc-80(e1272)*

Anesthetic	ED_{50}, vol %					
	N2	*unc-80*	*unc-1 (e719)*	*unc-80;unc-1*	*unc-7*	*unc-80;unc-7*
H	3.2 ± 0.05	1.2 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	3.15 ± 0.05	3.3 ± 0.1
E	6.6 ± 0.1	6.7 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.8 ± 0.1	6.6 ± 0.1
ISO	7.2 ± 0.1	7.0 ± 0.1	7.0 ± 0.1	7.0 ± 0.1	6.9 ± 0.1	6.9 ± 0.1
DE	6.8 ± 0.1	5.5 ± 0.1	6.2 ± 0.1	5.2 ± 0.2	6.6 ± 0.1	5.1 ± 0.2

Data are mean ± SE. Effect of *unc-7* on the ED_{50} values of four anesthetics, for two mutant strains with altered sensitivity to volatile anesthetics. *unc-7* was obtained from the *Caenorhabditis* Genetics Center. Abbreviations are in Table 1.

Table 4. Suppression of *unc-79* by various alleles of *unc-1*

unc-1 allele	Class	Heterozygote phenotype	Suppression	Homozygote phenotype	Suppression
e114	I (r)	Wild type	−	Kinked	+
e580	II (r)	Wild type	−	Kinked	+
n494	III (d)	Coiled	−	Coiled	−
n774	IV (d)	Coiled	−	Kinked	+

r, Recessive; d, dominant. Effect of four classes of *unc-1* on *unc-79*. Alleles of *unc-1*(*e114*, *e580*, *n494*, and *n774*) were kindly provided by Carl Johnson (Cambridge NeuroSciences).

that these results are best explained if multiple classes of volatile anesthetics exist.

At the turn of the century Meyer (1) and Overton (2) independently noted that the potency of volatile anesthetics was related to their lipid solubility. This relationship has proved true in all organisms studied to date, including wild-type *C. elegans* (7). The Meyer–Overton model for the action of volatile anesthetics implies that all such agents act by perturbing a site with certain lipophilic qualities. This model has been interpreted to further imply that all volatile anesthetics work at an identical site, with potencies depending only on lipophilic properties of the anesthetic. However, if such were the case, an increase in sensitivity to one anesthetic should be accompanied by similar changes to other anesthetics as well. For *unc-79* and *unc-80* we see such is not the case; these mutants exhibit large increases in sensitivity to some anesthetics and not to others. Further, in this study, we now show that one class of suppressors are able to suppress the altered sensitivities of *unc-79* and *unc-80* to some anesthetics but not to others.

The simplest explanation of these data is that all volatile anesthetics do not work at an identical site in *C. elegans*. We think at least three different sites must be affected in this organism (Table 5). We should note that we are using the term "site" in a general sense. The different sites may actually be one locus that is perturbed in different manners by different anesthetics. Thus, different sites may refer to different types of interactions at only one location or may refer to physically different loci (or a combination of these possibilities). These different interactions may then be separable by mutation. One such site appears to be altered strongly by the mutations *unc-79* or *unc-80* and suppressed by mutations in *unc-1*, *unc-7*, or *unc-9*. A second site is also altered by *unc-79* or *unc-80* but is not suppressed by *unc-1*, *unc-7*, or *unc-9*. A third site is unaffected or only slightly affected by the mutations *unc-79* or *unc-80*. We are hesitant to separate sites based on the mild resistance of *unc-79* to enflurane and flurothyl compared to the normal response of this mutant to isoflurane and fluroxene. Further work is necessary to determine if two separate sites are identified by these differences.

Table 5. Three sites of action of volatile anesthetics in *C. elegans*

Site	Gene products affecting site	Anesthetic affected
A	*unc-79*, *unc-80*, *unc-9*, *unc-7*, *unc-1*	Halothane (group 1)
B	None identified	Isoflurane and enflurane (groups 2 and 3)
C	*unc-79*, *unc-80*	Diethyl ether (group 4)

Characteristics of three sites of action of volatile anesthetics in the nematode *C. elegans*. It is possible that site B may be subdivided based on the mild resistance of *unc-79* to enflurane and flurothyl and the ability of *unc-9* to suppress this resistance. We have elected not to make this distinction until genes strongly affecting such sites are identified.

Volatile anesthetics do have multiple effects in clinical practice, and these effects vary with different agents. For example, at equipotent anesthetic doses, halothane depresses respiration more than diethyl ether or fluroxene (15); enflurane is known to depress the seizure threshold, whereas halothane does not (16). Many other clinical differences (i.e., skeletal muscle relaxation, cardiac contractility, or vasomotor tone) exist between these agents (17, 18). This certainly implies that volatile anesthetics interact with many different sites. Recent neurophysiologic studies have also clearly documented that anesthetics have effects at different sites (19, 20). However, these other effects do not follow the Meyer–Overton relationship. Ethanol and the benzodiazepines have been correlated with altered type A γ-aminobutyric acid receptors (21, 22). Altered sensitivity to volatile anesthetics has not been correlated with different channel or receptor mutations. It is unlikely that these volatile anesthetics work by mechanisms identical to those of ethanol and the benzodiazepines.

Other causes of the alterations seen in these mutants are possible. It may be that the mutations, *unc-79* and *unc-80*, cause a structure, not initially sensitive to volatile anesthetics, to become sensitive and produce immobility at low doses of very-lipid-soluble anesthetics. We feel this possibility is unlikely since *unc-79* and *unc-80* also cause a decrease in sensitivity to enflurane and fluroxene. Since we will always measure the sensitivity of the most sensitive site, we must have altered the site originally sensitive to the last two agents in a manner decreasing their sensitivity. A *de novo* site sensitive to very-lipid-soluble agents would not be expected to cause this change in responses to enflurane and flurothyl.

A second possibility is that the sites of action are not altered, but the number of sites is changed in the mutants, thereby producing a greater effect for a given concentration of anesthetic. Such a change will still help identify a type of site or an interaction affected primarily by the very-lipid-soluble agents (group I). We feel this possibility is unlikely to be caused by a loss-of-function mutation [such as the amber mutation of *unc-79* (*ec1*)].

A third possibility is that the metabolism of the very-lipid-soluble agents is specifically decreased in *unc-79* and *unc-80*. Such a change, if a large percentage of the agents are rapidly metabolized under normal conditions, could lead to changes in apparent sensitivity by increasing the available agent at the site of action. Such large percentages of metabolism are not seen in other organisms, and the concentration of anesthetic in the atmosphere over the nematodes is constant over several hours (P.G.M. and M.S., unpublished data). Such a specific change in metabolism must also account for the altered phenotype of *unc-79* and *unc-80* when not exposed to volatile anesthetics and for the decrease in sensitivity to enflurane and flurothyl. Finally, the most-lipid-soluble agents vary greatly in structure. Two, thiomethoxyflurane and methoxyflurane, are ethers; one, chloroform is a halogenated single-carbon alkane; and one, halothane, is a halogenated two-carbon alkane. It is unlikely that a single enzymatic change would alter the metabolism of all three types of agents. Thus, we doubt this explanation for

these data. Each of these possibilities seems unlikely but cannot be ruled out formally at this time.

How then does one resolve the altered responses seen here with the Meyer–Overton relationship? We believe the answer may be that anesthetics affect a family of structurally similar channels or regions in the cell membrane. Since many chemically and voltage-gated channels exist is similar membrane environments, it is not unreasonable that they may have similar structures that function within a lipid moiety. Several chemically gated channels have large regions with a high degree of amino acid homology (23). The clinical differences among anesthetics may then reflect the preference of certain anesthetics for distinct members of such a family. However, the similarities between members of a family of channels may put them all at risk for disruption by any volatile anesthetic, at only slightly different concentrations. These small differences in sensitivity may be lost on a log–log plot; thus, hidden within a straight-line log–log plot multiple sites may be functioning. The correlation between lipid solubility and potency, interpreted as function at a single site, may actually arise from the function of volatile anesthetics at different, but similar, sites.

This information may cause a change in the strategies used to determine the neurophysiologic basis of anesthetic action. Instead of searching for one type of channel disrupted by all anesthetics in a pattern following the Meyer–Overton relationship, we suggest that a family of similar channels is more likely involved. These results do not entirely rule out the possibility of multiple membrane regions of similar fatty acid, cholesterol, etc., packing that contain differences sufficient to confer preferences for different volatile anesthetics. However, we believe that, given this type of specificity for different anesthetics, a more likely model involves interaction with a protein in a lipid moiety such as that suggested by Franks and Leib (24).

Such a model may explain one of our observations. We have identified mutants with increased sensitivity to anesthetics but have been unable to identify single gene mutations conferring resistance to halothane (P.G.M. and M.S., unpublished results). If multiple sites are available as targets to cause immobility in *C. elegans*, then we will measure the effect on the most sensitive one. If we make only one site resistant by mutagenesis, we may merely unmask a second one that displays a sensitivity to anesthetics only slightly different from the original site. However, by searching for multiple mutants with two or more resistant sites, perhaps strains resistant to an anesthetic can be identified. Using *unc-9* as an initial mutation, we have now identified strains with a second mutation conferring resistance to halothane. Neither mutation individually alters sensitivity to halothane.

In summary, we have identified a class of genes that suppresses the abnormal response of *unc-79* and *unc-80* to halothane and other anesthetics but specifically does not suppress the response to diethyl ether. The simplest explanation of these data is that volatile anesthetics cause immobility in *C. elegans* by interacting with more than one site and that a pure lipid binding model is unlikely.

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