

Anesthesiology  
1996; 85:385-92  
© 1996 American Society of Anesthesiologists, Inc.  
Lippincott-Raven Publishers

## Genetic Differences Affecting the Potency of Stereoisomers of Isoflurane

Phil G. Morgan, M.D.\* Marianne F. Usiak,† Margaret M. Sedensky, M.D.\*

**Background:** In previous studies, researchers demonstrated the ability of a variety of organisms and *in vitro* sites of anesthetic action to distinguish between stereoisomers of isoflurane or halothane. However, it was not shown whether organisms with differing sensitivities to stereoisomers of one volatile anesthetic are able to distinguish between stereoisomers of another. In this study, the responses of mutants of *Caenorhabditis elegans* to stereoisomers of isoflurane were determined for comparison to previous results in halothane.

**Methods:** Mutant strains of *C. elegans* were isolated and grown by standard techniques. The EC<sub>50</sub>s (the effective concentrations of anesthesia at which 50% of the animals are immobilized for 10 s) of stereoisomers of isoflurane and the racemate were determined in wild type and mutant strains of *C. elegans*.

**Results:** Wild type *C. elegans* and strains with high EC<sub>50</sub>s of the racemate were more sensitive to the (+) isomer of isoflurane by approximately 30%. The racemate showed a EC<sub>50</sub>s similar to the less potent isomer, the (–) form. In the strains with low EC<sub>50</sub>s, one strain showed no ability to differentiate between the stereoisomers, whereas two showed a 60% difference between the (+) and (–) forms.

**Conclusions:** The ability to distinguish between stereoisomers of isoflurane is associated with genetic loci separate from those that distinguish between stereoisomers of halothane. These results are consistent with multiple sites of action for these anesthetics. (Key words: Anesthetics, volatile: isoflurane. Animals, worms: nematodes. Genetic factors: mutations.)

IN previous studies, researchers examined the relative potencies of stereoisomers of volatile anesthetics. They

have used enantiomers of halothane or isoflurane on whole organisms,<sup>1-3</sup> on specific putative sites of anesthetic action,<sup>4,5</sup> or on specific regions of the mammalian brain<sup>6</sup> or heart.<sup>7</sup> The goals of such experiments have been twofold. First, if the isomers exhibit different potencies, then it is likely that a site of anesthetic action has a three-dimensional structure with which to differentiate the steric structures. Franks and Lieb state that such differences favor protein involvement at an anesthetic site of action<sup>4</sup> although it should be noted that phospholipids can also show stereoselectivity. A second goal is to determine if differences between stereoisomers confer advantages as an anesthetic on one of the stereoisomers.<sup>8</sup> These experiments indicate that at least some potential sites of anesthetic action have the ability to differentiate between stereoisomers of volatile anesthetics and therefore favor a protein as an anesthetic site of action. Such reports raise other interesting questions. First, if an organism or proposed site of action shows different responses to stereoisomers of one anesthetic, will it show differences to stereoisomers of other anesthetics? If volatile anesthetics have multiple sites of action, then the ability of an organism to differentiate between the stereoisomers of one anesthetic would not imply the ability to differentiate between the stereoisomers of another anesthetic. A second question is: do the differences in the potencies of volatile anesthetics tell us anything about the nature of the sites of action (other than the lipid-protein debate described previously<sup>1,2,4,5</sup>). To answer these questions, we studied a genetic pathway controlling anesthetic sensitivity described for *Caenorhabditis elegans*.<sup>9</sup>

We have isolated mutations in several genes giving altered sensitivity to a variety of volatile anesthetics when compared to the wild type strain, N2.<sup>10</sup> These mutations include *unc-79*, *unc-9*, *fc20*, *fc21*, and *fc34* and define multiple sites of action in *C. elegans*, a finding also noted in other organisms.<sup>11,12</sup> Such mutations are useful because they isolate the effects of volatile anesthetics on particular sites of action, and can be ordered in a pathway determining sensitivity to volatile

\* Associate Professor, Departments of Anesthesiology and Genetics, University Hospitals of Cleveland and Case Western Reserve University.

† Department of Neuroscience, Case Western Reserve University.

Received from the Department of Anesthesiology, University Hospitals of Cleveland and Case Western Reserve University, Cleveland, Ohio. Submitted for publication August 18, 1995. Accepted for publication April 18, 1996. Supported in part by the National Institutes of Health Awards #GM41385 and #GM45402 and Academic Anesthesiology, Inc.

Address reprint requests to Dr. Morgan: Department of Anesthesiology, University Hospitals of Cleveland, 11100 Euclid Avenue, Cleveland, Ohio 44106-5007. Address electronic mail to: pgm2@po.cwru.edu.



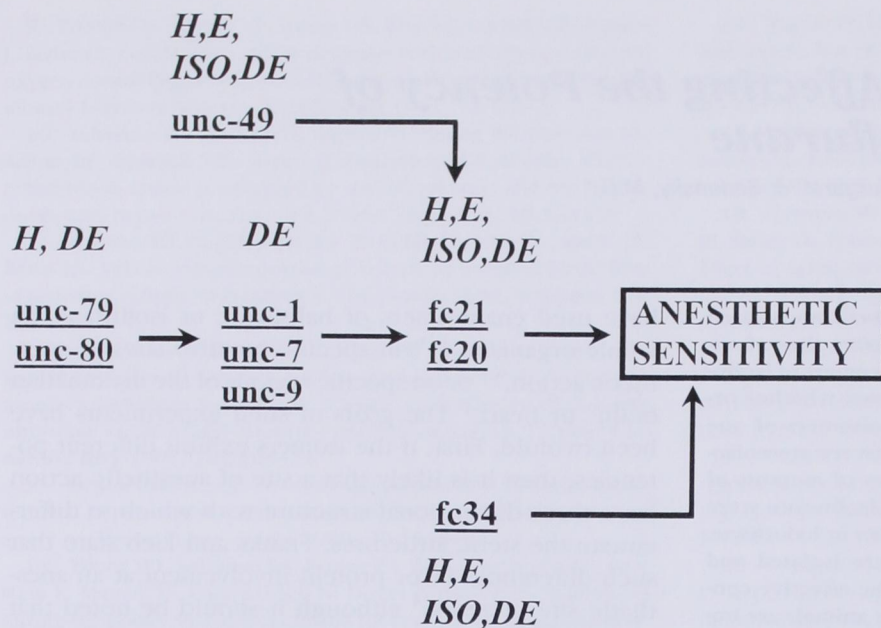


Fig. 1. A model genetic pathway for the control of sensitivity to volatile anesthetics in *C. elegans*. The anesthetics whose potencies are affected by the mutations are listed as capital letters above the mutations. This pathway indicates that *unc-79* and *unc-80* are the most upstream members of this pathway. The arrows indicate that *unc-79* (and *unc-80*) function through the activities of *unc-1* (and *unc-7* and *unc-9*). In turn, *unc-1* functions through *fc21* and *fc20*. *unc-49* and *fc34* are in separate pathways and intersect the main pathway as indicated by their arrows. An expanded discussion can be found in reference 9. E = enflurane; H = halothane; ISO = isoflurane; DE = diethylether.

anesthetics in *C. elegans* (fig. 1).<sup>9</sup> The products of these genes have not been determined.

Our previous work showed a strong stereospecific difference between the stereoisomers of halothane in the mutant *fc34*.<sup>2</sup> We found smaller differences for the wild type worms and several other mutants. No differences between stereoisomers were found for the very sensitive mutants *fc20* and *fc21*. Here we study the responses of the members of the pathway in figure 1 to stereoisomers of isoflurane. In addition, we studied the responses of *unc-49*, a mutant lacking the gamma aminobutyric acid (type A) receptor,<sup>13,14</sup> to the stereoisomers of isoflurane.

## Methods

### Nematodes

The methods for growing nematodes and performing genetic crosses was first described by Brenner<sup>15</sup> and expanded by Wood.<sup>16</sup> Certain strains were obtained from the Caenorhabditis Genetics Center (Minneapolis, MN), including N2, *unc-9*(*e101*), and *unc-49*(*e362*). The double mutant *unc-79*<sup>Δ</sup>*c-9* was constructed by standard techniques.<sup>15,16</sup>

‡ Bamber B, Jorgensen E: University of Utah, Salt Lake City, UT: personal communication.

### Anesthetics

Ninety-nine percent pure stereoisomers of isoflurane were donated by Rod Eckenhoff (Philadelphia, PA) and Anaquest (Madison, WI).

### Dose-response Curves

Nematodes were exposed to the volatile anesthetics in closed glass chambers. Anesthetic was injected in liquid form and allowed to vaporize. Initial injections for N2, *unc-9*, *unc-49*, *unc-79*, and *unc-79; unc-9* were estimated to be 20–30% below the EC<sub>50</sub> (the effective concentration of anesthetic at which 50% of the animals are immobilized for 10 s) of racemic isoflurane for N2, the nonmutated worm. The initial concentrations of anesthetic for *fc20*, *fc21*, and *fc34* were established at about 50% of the EC<sub>50</sub> of racemic isoflurane for *fc21*. The animals were exposed to one concentration for 2 h with agitation, during which time a steady-state response was reached. Experiments were carried out at room temperature, which was maintained at 22–3°C and checked before and during each experiment. Nematodes were then scored as immobile if they did not move for 10 s when observed through a dissecting microscope. After scoring the cultures for immobility, a sample of gas was taken from the chamber for measurement of the anesthetic concentration by gas chromatography. Either a second injection of anesthetic was added to the chamber to increase the anesthetic concentration, or some gas was withdrawn from the cham-



ber and replaced with air to decrease the anesthetic concentration. The chambers were then allowed to equilibrate again for 2 h. After scoring the culture for immobility and obtaining a second gas sample, the procedure was repeated a third time. After the third concentration point the chambers were opened and the nematodes were allowed to recover. In all cases, the animals fully recovered after returning to room air.

The technique of adding anesthetic to chambers already containing anesthetic differs from that used in most of our previous studies, but was described in the studies with isomers of halothane.<sup>2</sup> Previously, each concentration was obtained by a single injection of anesthetic into a chamber.<sup>9,10</sup> Control experiments were done using this procedure with racemic isoflurane and the strains of *C. elegans* used in this study. No difference in the EC<sub>50</sub>s or dose-response curves for these cultures in racemic isoflurane was noted between the two techniques.

#### Statistical Methods

EC<sub>50</sub>s and slope constants were determined using the technique of Waud.<sup>17</sup> Waud compares values from different curves using a normal distribution; thus, comparisons between different EC<sub>50</sub>s were done using analysis of variance.<sup>17,18</sup> Statistical methods are described in detail elsewhere.<sup>18,19</sup> Significance was defined as a  $P < 0.01$  to avoid type 1 errors. Dose-response curves were constructed using at least 30 concentration points, scoring at least 50 animals at each point. Each EC<sub>50</sub> then represents scoring at least 1,500 animals. Because the standard errors are dependent on the number of animals scored, they became quite small. The statistical approach of Waud assumes that each measurement is independent. In this study, we used the same animals for two to three measurements (as the doses of anesthetic were changed). We have previously constructed dose-response curves using a few (5–10) animals measured multiple times and found no difference in the EC<sub>50</sub>s from studies in which each animal is only studied once. Because these animals are isogenic, age synchronized and grown under identical conditions, they are all equally likely to move in the 10-s period used to determine immobility. Thus, repeated measurements are independent and this approach remains valid.

## Results

#### Effects on the Wild Type Strain, N2

The (+) and (–) forms of isoflurane differed in their potencies for the wild type *C. elegans*, N2. The (+)

form showed a 23% increase in potency compared with the (–) form. Interestingly, the racemate had an EC<sub>50</sub> similar to that of the less potent isomer, the (–) form (fig. 2 and table 1).

#### Effects of Isomers on the Suppressor *unc-79* and on *unc-9*

Both *unc-79* and the suppressor strain, *unc-9*, possess EC<sub>50</sub>s in racemic isoflurane identical to that of N2. The N2 pattern for enantiomers also was repeated in both *unc-9(e101)* and *unc-79(ec1)*. In each case the (+) isomer of isoflurane was more potent than the (–) isomer or the racemic mixture (table 1).

We also studied the effects of the isomers on double mutant, *unc-79<sup>+</sup>c-9*. The (+) form of isoflurane showed a 41% increase in potency compared with the (–) form and the racemate. Thus, the double mutant showed a larger difference between isomers than seen in N2, *unc-79*, or *unc-9* (table 1).

#### Effects of Isomers on *fc20* and *fc21*

Both *fc20* and *fc21* represent loss-of-function mutations, which unmask sites of anesthetic action more

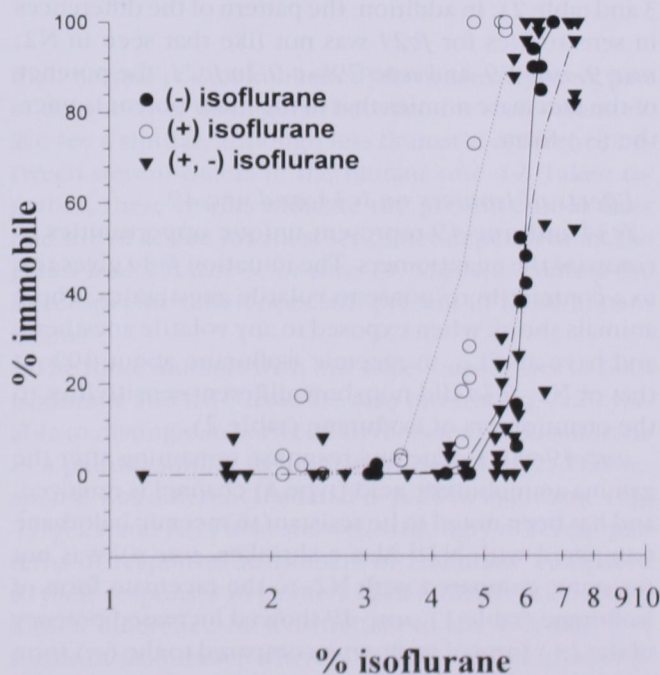


Fig. 2. Dose-response curves of N2 to the (+) and (–) isomers of isoflurane and the racemate. Note that the racemate is similar to the (–) isomer. Dose-response experiments were done as described in Methods. EC<sub>50</sub>s and slope constants were calculated using the techniques of Waud<sup>17</sup> and curves were constructed using the program Sigma Plot (Jandel Scientific, San Rafael, CA).



**Table 1. EC<sub>50</sub> Values of Isoflurane and the Racemate in Five Strains of *C. elegans***

Strain	(+) Isoflurane	(-) Isoflurane	Racemate
N2	4.6 ± 0.04	6.0 ± 0.06*	6.0 ± 0.04*
<i>unc-9</i>	4.3 ± 0.05	5.9 ± 0.08*	5.8 ± 0.04*
<i>unc-79</i>	4.1 ± 0.01	6.1 ± 0.08*	5.9 ± 0.05*
<i>unc-79; unc-9</i>	3.6 ± 0.06	6.1 ± 0.1*	6.1 ± 0.05*
<i>unc-49</i>	4.6 ± 0.08	6.3 ± 0.1*	6.4 ± 0.06*

Values are mean ± SE. These strains were grouped due to their higher EC<sub>50</sub> values in racemic isoflurane.

\* EC<sub>50</sub> different from that of (+) isoflurane,  $P < 0.01$ .

sensitive to all volatile anesthetics than seen in N2. As candidates for final common sites of action for these anesthetics, they also may present different relationships between the enantiomers of isoflurane.

The mutant *fc20* showed an increase in sensitivity to the (+) isomer compared with the racemate of isoflurane (table 2). This pattern is similar to that seen in N2 and the other high-dose requiring strains. *fc21*, conversely, demonstrated a 38% increase in sensitivity to the (+) isomer compared with the (-) isomer (fig. 3 and table 2). In addition, the pattern of the differences in sensitivities for *fc21* was not like that seen in N2, *unc-9*, *unc-79*, and *unc-79; unc-9*. In *fc21*, the potency of the racemate mimics that of the more potent isomer, the (+) form.

#### Effects of Isomers on *fc34* and *unc-49*

*fc34* and *unc-49* represent unique opportunities to compare the enantiomers. The mutation *fc34* gives rise to a contractile response to volatile anesthetics. These animals shrink when exposed to any volatile anesthetic and have an EC<sub>50</sub> in racemic isoflurane about 30% of that of N2. *fc34* did not show different sensitivities to the enantiomers of isoflurane (table 2).

*unc-49* will isolate any response remaining after the gamma aminobutyric acid (type A) channel is removed, and has been noted to be resistant to racemic halothane compared with N2.<sup>2</sup> Also a shrinker, *unc-49* was not resistant, compared with N2, to the racemate form of isoflurane (table 1). *unc-49* showed increased potency of the (+) form of isoflurane compared to the (-) form and the racemate, a pattern similar to that seen with N2 (table 1).

No significant differences were found in the slope constants between the enantiomers or the racemate in any strain and were similar to those reported earlier for the racemate.<sup>9,10</sup> In addition, dose-response curves

**Table 2. EC<sub>50</sub> Values of (+) and (-) Stereoisomers of Isoflurane and the Racemate in Three Strains of *C. elegans***

Strain	(+) Isoflurane	(-) Isoflurane	Racemate
<i>fc20</i>	2.4 ± 0.06	3.9 ± 0.1*	3.5 ± 0.05*
<i>fc21</i>	1.3 ± 0.07	2.1 ± 0.1*	1.2 ± 0.06
<i>fc34</i>	2.1 ± 0.07	2.3 ± 0.2	2.5 ± 0.1

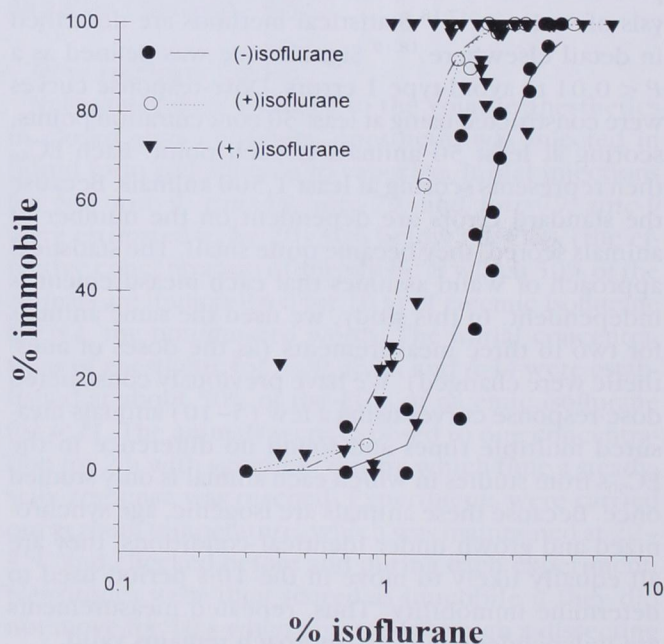
Values are mean ± SE. These strains were grouped due to their low EC<sub>50</sub> values in racemic isoflurane.

\* EC<sub>50</sub> different from that of (+) isoflurane,  $P < 0.01$ .

repeated 2 or 3 times in successive weeks and by 2 or 3 independent observers did not differ (data not shown).

## Discussion

N2, the wild type *C. elegans*, contains all the possible sites of volatile anesthetic action in *C. elegans* and serves as a control for animals in which potential sites of action have been removed or altered. The increase in sensitivity of N2 to the (+) isomer of isoflurane



**Fig. 3.** Dose-response curves of *fc21* to the (+) and (-) isomers of isoflurane and the racemate. Note that the racemate is similar to the (+) isomer. Dose-response experiments were done as described in Methods. EC<sub>50</sub>s and slope constants were calculated using the techniques of Waud<sup>17</sup> and curves were constructed using the program Sigma Plot (Jandel Scientific, San Rafael, CA).



## GENETICS AND STEREOSPECIFIC RESPONSES TO ISOFLURANE

compared with the (–) isomer and racemate was similar to the pattern of sensitivities for N2 to isomers of halothane. Two possibilities exist for the difference in potencies between the (+) and (–) forms of isoflurane in N2. An inhibitory site of action may be more sensitive to the (+) form; this would result in a greater inhibitory effect by the (+) isomer than by the (–) isomer and cause a leftward shift of the dose-response curve of the (+) isomer relative to the (–) isomer. The other possibility is that an excitatory site may be more sensitive to the (–) form; this would result in a greater excitatory effect by the (–) isomer and a net rightward shift of the dose-response curve of the (–) isomer relative to the (+) isomer. Of course, combinations of these two effects are possible.

Loss-of-function mutations may eliminate or unmask gene products that confer stereospecific differences in potency, resulting in specific changes in potency of stereoisomers of volatile anesthetics. Therefore, we tested such mutations from each step in the pathway from figure 1, along with *unc-49*, for sensitivities to stereoisomers of isoflurane.

As described in a previous article, the genes listed in figure 1 were ordered by their interactions with each other.<sup>9</sup> If two mutations work along separate pathways then an animal carrying both mutations will show characteristics of both mutations. If the mutations represent early and late steps in a common pathway, the doubly mutant animal will exhibit the behavior of the mutation that represents the later step. The earlier acting mutation requires the activity of the second step to exert its effect.

By the above criterion, mutations in the gene *unc-79* occupy the most upstream position in the pathway in figure 1. However, they do not affect sensitivity to isoflurane. We expected, therefore, that the pattern of differences seen in N2 with respect to enantiomers of isoflurane would be similar to that of *unc-79*. A similar argument can be made for the gene *unc-9*. The N2 pattern of sensitivity to the stereoisomers of isoflurane was repeated in *unc-79* and *unc-9* as well as the double mutant *unc-79; unc-9*. The pattern of sensitivities of a *unc-79* to isomers of isoflurane was different than that seen to isomers of halothane (fig. 4). We interpret these data to indicate that a gene product other than those eliminated in *unc-9* or *unc-79* is responsible for the difference in potencies of the (+) and (–) forms of isoflurane in N2. It is noteworthy that the difference between the enantiomers was increased in the double mutant. Such a result indicates that these two genes in

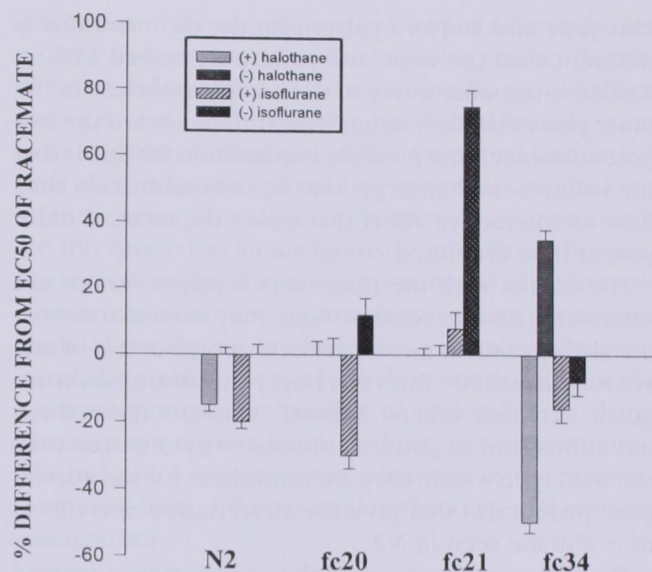


Fig. 4. The percent differences in  $EC_{50}$ s between the (+) and (–) isomers of halothane and isoflurane and the racemate for four strains of *C. elegans*. The zero line represents the responses to the racemates of the two anesthetics. N2 is the wild type strain. *fc20*, *fc21*, and *fc34* are three strains that show different patterns of responses to the isomers of halothane than to patterns of the isomers of isoflurane.

their normal (*i.e.*, nonmutated) form actually mask part of the ability to distinguish between the stereoisomers. We see a similar, although less dramatic difference between stereoisomers in the mutant *unc-49*. Taken together, these results indicate the presence of at least one site of action for anesthetics not dependent on the genes *unc-79*, *unc-9*, or *unc-49*. This site confers the stereospecific differences still present in these mutant strains.

The three mutants with low dose sensitivities to both isoflurane and halothane are also interesting. *fc20* was able to distinguish between stereoisomers of isoflurane in a pattern similar to the pattern seen in N2. This response was different than that of *fc20* to halothane (fig. 4). *fc21* and *fc34* also showed strikingly different patterns of responses to isomers of isoflurane compared to their responses to isomers of halothane. *fc21* shows a 60% difference in sensitivities to the (+) and (–) forms of isoflurane, whereas *fc34* is unable to distinguish between the isomers. This is in contrast to the effects of these two mutations on sensitivities to enantiomers of halothane (fig. 4). *fc34* showed a threefold difference in sensitivities to the stereoisomers of halothane whereas *fc21* had identical sensitivities to both enantiomers and the racemate of halothane. In isoflu-



rane *fc21* also shows a pattern for the racemate that is different than the expected pattern described earlier. The dose-response curve of the racemate is close to the more potent isomer, rather than shifted toward the less potent isomer. One possible explanation for this is that the isomers show synergy; that is, in combination they have an interactive effect that makes the mixture more potent than predicted.

The results with the three very sensitive strains are interesting for two reasons. First, they demonstrate that the ability to distinguish between stereoisomers of one volatile anesthetic does not imply the ability to distinguish between others. Second, because these three mutations lead to patterns of sensitivity different than the wild type worm, they are candidates for the normal gene product(s) that give rise to stereospecific effects of isoflurane seen in N2.

The different potencies of the enantiomers observed in this study are unlikely to be caused by differences in their metabolism, because we allow the animals to come to a steady-state response. However, the metabolism of isoflurane has not been measured in *C. elegans*. In studies by Eckenhoff, the amount of racemate halothane was decreased in *unc-79* and *unc-80* when compared to N2 at identical concentrations.<sup>§</sup> If decreased metabolism, even of only one isomer, caused the difference in sensitivities, one would expect the opposite result. It also seems unlikely that the varied responses of the mutant strains to stereoisomers of isoflurane all arise from different changes in metabolism. However, we cannot formally rule this out at this time.

As mentioned earlier, several researchers have studied the relative potencies of stereoisomers of volatile anesthetics. Franks and Lieb exposed an isolated neuron from the molluscan nervous system to stereoisomers of isoflurane.<sup>4</sup> They found that the (+) isomer was twice as potent as the (−) isomer at eliciting a response from a novel anesthetic activated K<sup>+</sup> channel. Jones and Harrison also recently showed that the (+) isomer of isoflurane produced greater increases in inhibitory postsynaptic currents than the (−) isomer in rat hippocampal neurons.<sup>6</sup>

Harris *et al.* studied the effects of stereoisomers of volatile anesthetics in whole animals.<sup>1</sup> They found a

20–40% increase in sleep time of the (+) isomer compared with the (−) isomer. However, insufficient drug precluded the determination of the EC<sub>50</sub>s. Lysko *et al.* determined minimum alveolar concentrations of isomers of isoflurane in rats.<sup>3</sup> They found a similar increase in the potency of the (+) isomer as did Harris *et al.*<sup>1</sup> They also noted that the EC<sub>50</sub> of the racemate was intermediate between the two isomers, a result differing from that found in *C. elegans*. It is interesting that in all these studies that have found a difference in the potencies of stereoisomers of isoflurane, the (+) isomer has been more potent than the (−) isomer. In general, we also find the (+) isomer to be more potent in *C. elegans*. However, in tadpoles, Firestone and colleagues were unable to identify a difference between stereoisomers of isoflurane.<sup>||</sup> Thus, while it is certainly possible to show a twofold or threefold difference in potencies between stereoisomers of volatile anesthetics, not all systems will exhibit different responses to the stereoisomers of each volatile anesthetic.

The previous studies indicate that the precise three-dimensional structure of a volatile anesthetic can change its potency, and that multiple sites of action exist. It seems reasonable to expect that each volatile anesthetic will fit in a site of action differently. Thus, steric differences in potency for one anesthetic should not necessarily imply differences for all of them. By isolating the effects of several components controlling sensitivity to volatile anesthetics, it may be possible to identify different types of interaction with stereoisomers. We chose mutations germane to each step in the genetic pathway affecting sensitivity (fig. 1) to examine differences among the stereospecific effects of different volatile anesthetics.

As a model, *C. elegans* has many outstanding features; however, it is not without its limitations. The normal animal is relatively resistant to volatile anesthetics in comparison to other species, with an EC<sub>50</sub> 5–10 times that of minimum alveolar concentrations in humans. This may represent an evolutionary adaptation of this ancient organism to living at cool temperatures and being exposed to organic compounds like alcohols and alkanes within its environment, or the choice of endpoint defining anesthesia in worms. In addition, the relative rank of potencies of isoflurane and enflurane is slightly different in worms than in humans. This difference is a result of the failure of mammals to adhere to the Meyer-Overton relationship in regard to the chemical isomers, enflurane and isoflurane. Enflurane is slightly more lipid soluble than isoflurane (oil–gas

§ Eckenhoff RG: Subcellular distribution of halothane in anesthetic sensitive mutants of *C. elegans* (abstract). FASEB J. 1991; 5: A868.

|| Firestone S, Ferguson C, Firestone L: Isoflurane's optical isomers are equipotent in *Rana pipiens* tadpoles (abstract). ANESTHESIOLOGY 1992; 77:A758.



partition coefficient of 96 *vs.* 91 for isoflurane), yet is less potent than isoflurane in humans, dogs, and mice.<sup>20</sup> Unlike their more complicated animals, *C. elegans* generates the rank order of volatile anesthetic potencies predicted by the Meyer-Overton relationship. Several studies indicate there are multiple sites of action for volatile anesthetics. It seems likely that enflurane has an excitatory effect in mammals that does not exist in *C. elegans*; this in turn causes its more perfect adherence to the Meyer-Overton relationship. Obviously, simple models are simpler than complex ones. Far from eliminating *C. elegans* as a model, we believe this strengthens it as a manageable system, without the complicating response to enflurane. Overall, we think that the drawbacks of *C. elegans* are outweighed by the animal's impeccable adherence to the Meyer-Overton relationship,<sup>21</sup> the reversibility of our anesthetic endpoint, the qualitative behavior of the animals in various anesthetic agents, and the maintenance of the cutoff effect.<sup>22</sup>

This study demonstrates two important points. As seen most clearly in the case of *fc34* (but also noted with *unc-79*), the ability to distinguish between stereoisomers of halothane does not imply the ability to distinguish between stereoisomers of isoflurane. In the case of *fc21*, we see the reverse effect: *fc21* can distinguish between the enantiomers of isoflurane but not halothane. These results strengthen the case that these volatile anesthetics do have specific steric constraints to their effects on an anesthetic site of action. The patterns of such differences also are consistent with our earlier genetic studies indicating at least two distinct sites of action for volatile anesthetics. These distinct sites, which change sensitivity to specific groups of anesthetics, also show different stereospecific responses to the different anesthetics. However, it should be remembered that it is the varied response of mutants that leads to this conclusion. The altered proteins in *fc34* and *fc21* lead to the difference in responses to different stereoisomers. Thus, the different altered proteins each have separate effects on these volatile anesthetics. Of course, such different sites may reside on one common protein target or even one phospholipid; however, they are separable at least by genetics. These results raise the possibility of identifying more ideal volatile anesthetics.<sup>8</sup>

A second important point can be found in the strains requiring high doses of anesthetics, specifically *N2*, *unc-9*, *unc-79*, *unc-49*, and *unc-79; unc-9*. With both halothane and isoflurane we see a similar pattern. The

potencies of the racemate are always similar to the less potent stereoisomer. Standard models predict that the dose-response curves of the racemates be about halfway between those of the isomers, but shifted from the more potent isomer toward that of the less potent isomer.<sup>23,24</sup> Our dose-response curves lie, in general, to the right of these predictions. Such a shift would conventionally be interpreted as an antagonistic interaction between the two isomers. This antagonism could arise from direct interaction or steric interference between the isomers. However, the model assumes that these drugs are mutually at their site of action.<sup>24</sup> In our opinion, it also assumes similar mechanisms of action by the drugs. Because we do not know if these assumptions hold, the rightward shift of curves generated by the racemate may arise from different modes of action, and not from antagonism.

The authors thank Ms. Susan Thomas for her technical assistance in the preparation and care of the *C. elegans* strains, and Ms. Sharon Peskin and Ms. DaLee Lambert for their critical reading and aid in the preparation of the manuscript; Sandor Kiss who isolated *fc34* and Helen Salz for her discussions concerning these mutations; Helmut F. Cascorbi for suggestions concerning the manuscript and ongoing work; and the faculty members of the Department of Anesthesiology at University Hospitals.

## References

1. Harris B, Moody E, Skolnick P: Isoflurane anesthesia is stereoselective. *Eur J Pharmacol* 1992; 217:215-6
2. Sedensky MM, Cascorbi HF, Meinwald J, Radford P, Morgan PG: Genetic differences affecting the potency of stereoisomers of halothane. *Proc Natl Acad Sci U S A* 1994; 91:10054-8
3. Lysko GS, Robinson JL, Castro R: The stereospecific effects of isoflurane isomers in vivo. *Eur J Pharmacol* 1994; 263:25-9
4. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. *Nature* 1994; 367:607-14
5. Franks NP, Lieb WR: Stereospecific effects of inhalational general anesthetic optical isomers on nerve ion channels. *Science* 1991; 254:427-30
6. Jones MV, Harrison NL: Effects of Volatile Anesthetics on the kinetics of inhibitory postsynaptic currents in cultured rat hippocampal neurons. *J Neurophysiol* 1993; 70:1339-49
7. Graf BM, Boban M, Stowe DF, Kampine JP, Bosnjak ZJ: Lack of stereospecific effects of isoflurane and desflurane isomers in isolated guinea pig hearts. *ANESTHESIOLOGY* 1994; 81:129-36
8. Moody E, Harris BD, Skolnick P: The potential for safer anaesthesia using stereoselective anaesthetics. *Trends Pharmacol Sci* 1994; 15:1387-91
9. Morgan PG, Sedensky MM: Mutations conferring new patterns of sensitivity to volatile anesthetics in *Caenorhabditis elegans*. *ANESTHESIOLOGY* 1994; 81:888-98
10. Morgan PG, Sedensky MM, Meneely PM: Multiple sites of action of volatile anesthetics in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 1990; 87:2965-8



11. Krishnan KS, Nash HA: A genetic study of the anesthetic response: Mutants of *Drosophila melanogaster* altered in sensitivity to halothane. *Proc Natl Acad Sci U S A* 1990; 87:8632-6
12. MacIver MB, Roth SH: Inhalational anesthetics exhibit pathway-specific and differential actions in hippocampal synaptic responses *in vitro*. *Br J Anaesth* 1988; 60:680-91
13. McIntire SL, Jorgensen E, Horvitz RH: Genes required for GABA function in *Caenorhabditis elegans*. *Nature* 1993; 364:334-7
14. McIntire SL, Jorgensen E, Kaplan I, Horvitz: The GABAergic nervous system of *Caenorhabditis elegans*. *Nature* 1993; 364:337-41
15. Brenner S: The genetics of *Caenorhabditis elegans*. *Genetics* 1974; 77:71-94
16. Wood WB: The Nematode *Caenorhabditis elegans*. New York, Cold Spring Harbor, 1988, pp 587-606
17. Waud DR: On biological assays involving quantal responses. *J Pharmacol Exp Ther* 1970; 183:577-607
18. Snedecor GW, Cochran WG: Statistical Methods, 6th ed. Ames, Iowa State University Press, 1968, pp 271-5
19. Gunther WC: Analysis of Variance. Englewood Cliffs, Prentice-Hall, 1964, pp. 31-50, 54-7
20. Franks NP, Lieb WR: Selective actions of volatile general anaesthetics at molecular and cellular levels. *Br J Anaesth* 1993; 71:65-76
21. Koblin DD: Mechanisms of Action in Anesthesia. 4th edition. Edited by Miller RD. New York, Churchill Livingstone, 1994, 67-100
22. Anton AH, Berk AI, Nicholls CH: The "anesthetic" effect of alcohols and alkanes on *Caenorhabditis elegans*. *Res Commun Chem Pathol Pharmacol* 1992; 78:69-83
23. Berenbaum MC: The expected effect of a combination of agents: the general solution. *J. Theor Biol* 1985; 114:413-31
24. Chou TC, Talalay P: Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzymol Regul* 1984; 22:27-55