Goα Regulates Volatile Anesthetic Action in *Caenorhabditis elegans*

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ABSTRACT

To identify genes controlling volatile anesthetic (VA) action, we have screened through existing *Caenorhabditis elegans* mutants and found that strains with a reduction in Go signaling are VA resistant. Loss-of-function mutants of the gene *goa-1*, which codes for the α-subunit of Go, have EC_{50}s for the VA isoflurane of 1.7- to 2.4-fold that of wild type. Strains overexpressing *egl-10*, which codes for an RGS protein negatively regulating *goa-1*, are also isoflurane resistant. However, sensitivity to halothane, a structurally distinct VA, is differentially affected by Go pathway mutants. The RGS overexpressing strains, a *goa-1* missense mutant found to carry a novel mutation near the GTP-binding domain, and *eat-16(rf)* mutants, which suppress *goa-1(gf)* mutations, are all halothane resistant; *goa-1(null)* mutants have wild-type sensitivities. Double mutant strains carrying mutations in both *goa-1* and *unc-64*, which codes for a neuronal syntaxin previously found to regulate VA sensitivity, show that the syntaxin mutant phenotypes depend in part on *goa-1* expression. Pharmacological assays using the cholinesterase inhibitor aldicarb suggest that VAs and GOA-1 similarly downregulate cholinergic neurotransmitter release in *C. elegans*. Thus, the mechanism of action of VAs in *C. elegans* is regulated by Goα, and presynaptic Goα-effectors are candidate VA molecular targets.

THE synapse is the probable arena where general anesthetics depress neuronal function (Pocock and Richards 1991, 1993; Franks and Lieb 1994). In part due to technical limitations, most studies of anesthetic action have focused on postsynaptic putative targets such as $GABA_A$ receptors (Franks and Lieb 1994; Mihic *et al.* 1997); however, presynaptic transmitter release is also inhibited by clinical concentrations of volatile anesthetics (VAs) and various molecular mediators for this effect have been proposed (Zorychta and Capek 1978; Takenoshita and Takahashi 1987; Kullmann *et al.* 1989; Miao *et al.* 1995; Perouansky *et al.* 1995; Schlame and Hemmings 1995; MacIver *et al.* 1996; Nishikawa and MacIver 2000). Thus, *in vitro* studies have revealed several potentially relevant molecular mechanisms for general anesthesia. Which, if any, are operant *in vivo* is unknown. Genetic investigations are a means toward identifying gene products controlling anesthetic action *in vivo*.

Few genes have been identified that regulate sensitivity to clinical concentrations of anesthetics (Krishnan and Nash 1990; Tinklenberg *et al.* 1991; Leibovitch *et al.* 1995; Gamo *et al.* 1998; Quinlan *et al.* 1998; van Swinderen *et al.* 1999). Most of these "anesthesia" genes, which include Drosophila *shaker* (Jan *et al.* 1977; Ganetzky and Wu 1982; Wu *et al.* 1983), the not yet molecularly identified halothane-resistant *har* genes (Nishikawa and Kidokoro 1999), and *Caenorhabditis elegans* genes coding for SNARE proteins (van Swinderen *et al.* 1999), are thought to regulate synaptic transmitter release. In *C. elegans* reduction-of-function mutations in genes coding for synaptic vesicle machinery proteins drastically alter VA sensitivity. In general, these mutants, which all have reduced cholinergic synaptic transmission, were VA hypersensitive; however, a splice-site mutant in syntaxin that similarly reduced neurotransmitter release was highly VA resistant. Thus, an indirect effect of reduction of syntaxin function could not explain the VA resistance; rather, the data suggest that the mutant protein directly alters VA binding or efficacy and implicates syntaxin or syntaxin-binding proteins as potential anesthetic targets (van Swinderen *et al.* 1999).

To better understand the presynaptic VA mechanism in *C. elegans*, we have begun to examine the effect on VA sensitivity of genes known to regulate transmitter release. Go has been shown to regulate negatively synaptic transmission in vertebrates in part by its βγ-subunit directly binding to and inhibiting non-L type Ca^{2+} channels (Dolphin *et al.* 1993; Hescheler and Schultz 1994; Sudhof 1995; Zhang *et al.* 1996). Go regulation of some presynaptic Ca^{2+} channel subtypes appears to require syntaxin bound to the channel (Stanley and Mirotznik 1997). Mutants disrupting Go signaling have been isolated in *C. elegans* (Mendel *et al.* 1995; Segalat

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et al. 1995; Koelle and Horvitz 1996; Hajdu-Cronin *et al.* 1999; Nurrish *et al.* 1999). The behavioral and pharmacological phenotypes of null alleles of *C. elegans goa-1*, which codes for the neuronally expressed Goα-subunit, are indeed consistent with a disinhibition of transmitter release (Mendel *et al.* 1995; Segalat *et al.* 1995; Miller *et al.* 1999; Nurrish *et al.* 1999). In *C. elegans*, the presynaptic mechanisms of Go are mediated in part by inhibition of a Gqα, phospholipase C β, diacylglycerol pathway that stimulates transmitter release at cholinergic motor neuron terminals. Here, we test the hypothesis that Go regulates VA action and find that an intact Go signaling pathway is required for normal sensitivity to VAs, that *goa-1(rf)* mutants antagonize the anesthetic phenotype of syntaxin mutants, and that Go regulates the sensitivity of two structurally distinct VAs differently.

MATERIALS AND METHODS

Strains and culture conditions: *C. elegans* mutant strains were obtained from the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health National Center for Research Resources, and from several laboratories whose research is referenced in this work. Strains were grown as described (Brenner 1974) on nematode growth media (NGM) agar in uncrowded conditions (200–300 animals per plate). The following strains and alleles were used in this work. Wild-type worms were N2 (var. Bristol). Mutant strains used for this work are as follows:

LGI: *goa-1(n363)*, *goa-1(sy192)*, *goa-1(n1134)*, *goa-1(pk62)*, *eat-16(sy438)*, *eat-16(ad702)*
PS3479: *goa-1(sy192);dpy-20(e1282);syEx352[pJMGO;pMH86]*
PS3480: *goa-1(sy192);dpy-20(e1282);syEx353[pJMGOHS; pMH86]*
MC21: *goa-1(n363);syEx352*
LGIII: *unc-64(md130)*, *unc-64(js21)*, *unc-64(md1259)*
LGIV: *dpy-20(e1282)*
LGV: *egl-10(md176)*, *egl-10(n480)*
LGX: *sag-1(sy428)*, *sag-1(sy429)*, *sag-1(nu199)*, *sag-1(md1777)*, *lin-15(n765)nIs51[egl-10(+)lin-15(+)]*, *lin-15(n765)nIs54 [egl-10(+)lin-15(+)]*.

Note that *sag-1* is also known as *dgk-1*. By standard methods (Huang and Sternberg 1995), *goa-1;unc-64* double mutant strains (loopy Unc phenotypes) were isolated from the brood of Unc animals segregating from *goa-1/+;unc-64(rf)/+* heterozygotes. The presumed double mutant genotypes were confirmed by crossing with N2 males and finding the presence of both single mutant phenotypes in the F_2 progenies. To assay heterozygous *goa-1* mutants, N2 males were mated to *goa-1; dpy-20(e1282)* hermaphrodites, and their non-Dpy progeny were scored. All assays were performed on well-fed young adult animals at room temperature (21°–23°). For egg-laying, body bend, and aldicarb assays, staged adults, ~24 hr after being selected as L4 larvae, were used.

Transformation rescue: For rescue of the hyperactive and anesthetic-resistant phenotypes of *goa-1(sy192)* and *goa-1 (n363)*, *goa-1(sy192);dpy-20(e1282)* was transformed by gonadal injection (Mello and Fire 1995) with pMH86 (15 ng/μl) and either pJMGO (5 ng/μl) or pJMGOHS (5 ng/μl) along with pBluescript$^+$ (180 ng/μl) as carrier DNA. pMH86 contains a full-length wild-type copy of *dpy-20* genomic DNA including its native promoter inserted into pBluescript. pMH86 rescues the Dpy phenotype of *dpy-20(e1282)*. pJMGO contains a full-length wild-type copy of *goa-1* genomic DNA including its native promoter inserted into pBluescript$^+$. pJMGOHS contains a full-length wild-type copy of *goa-1* genomic DNA minus its native promoter inserted into pPD49.78, which has the heat-shock-inducible promoter from the hsp 16-2 gene (Stringham *et al.* 1992; Mello and Fire 1995). Non-Dpy F_1 transformants were chosen, and stably transformed lines were examined for phenotypic rescue. For rescue of *goa-1(n363)*, *n363/+* males were crossed with *dpy-20(e1282);syEx352* hermaphrodites, and Egl hermaphrodite F_1 progeny (presumed *syEx352*-containing animals) were picked onto individual plates. Non-Dpy Egl F_2 progeny were picked from plates that also segregated loopy/hyperactive Dpy progeny. Strains that segregated both loopy/hyperactive and Egl nonhyperactive in an ~1:1 ratio for multiple generations were kept for testing and presumed to have the genotype *goa-1(n363);syEx352*. We were unable to isolate loopy/hyperactive Dpy progeny from Egl animals despite multiple attempts, suggesting that *n363*, *e1282*, and *syEx352* are synthetic lethal or at least have unfavorable growth characteristics. This question, however, was not directly tested. For induction of *goa-1(+)* expression in PS3480, agar plates containing young adult animals grown at 20° were placed in a 34° oven for 4 hr and then the animals were allowed to recover for 2 hr at 20° prior to behavioral testing.

Behavioral assays and statistics: *Anesthetic assays:* The dispersal assay as described previously was used to measure the locomotion defects produced by VAs (Crowder *et al.* 1996; van Swinderen *et al.* 1997). Between 300 and 500 nematodes were washed off NGM plates with 1 ml of S-basal (Sulston and Brenner 1974) into plastic Eppendorf tubes. The animals were subsequently washed twice more with S-basal and once with distilled water by allowing most to settle and immediately removing the supernatant. Following the last wash in water, the animals were resuspended in 100 μl of distilled water and immediately aliquoted in 10-μl samples containing 50–100 worms onto the center of a dispersal assay plate, which is a 9.5-cm NGM plate seeded at its edge 1–2 days prior to the assay with a thin ring of OP50 *Escherichia coli* (Brenner 1974). The preparations were then placed into sealed glass chambers and exposed to various concentrations of anesthetic. VAs were delivered as described previously (Crowder *et al.* 1996; van Swinderen *et al.* 1997). We have previously shown that with this delivery method VAs rapidly disrupt *C. elegans* behaviors including locomotion with steady state effects reached within 10 min (Crowder *et al.* 1996). As soon as the 10-μl water aliquot dried (typically in 5 min), the preparation was briefly shaken to induce the animals to unclump and begin dispersing. The assay was then allowed to run its course untouched for 45 min. Then, the fraction of animals having reached the bacterial ring divided by the total was scored as the dispersal index. Concentration/response data were fitted by nonlinear regression to estimate EC_{50}s (the *e*ffective VA *c*oncentration for a *50%* reduction in the dispersal index), slopes, and standard errors of the estimate using the equation: $y = \min + (\max - \min)/(1 + (x/x^{50})^{-k})$ where y = the dispersal index; min = the minimum dispersal index, which was assumed to be zero for all curves because all approached zero; max = the average of the maximum dispersal indices for that strain; x = [VA]; x^{50} = the EC_{50}; and k = the slope of the curve. The EC_{50} was used as the measure of the VA sensitivity of the strain. Significant resistance or hypersensitivity of a strain's EC_{50} was determined relative to the wild-type strain N2 by simultaneous curve fitting (Waud 1972; DeLean *et al.* 1978; van Swinderen *et al.* 1997).

For transformation rescue experiments, dispersal assays were performed as described above with animals washed from plates segregating both Dpy or non-Egl (nontransformed)

and non-Dpy or Egl (transformed) progeny. Dispersal indices were calculated independently for transformed and nontransformed animals with both genotypes present on the same dispersal plates.

Aldicarb assay: Aldicarb is an inhibitor of cholinesterases that paralyzes *C. elegans* due to excess acetylcholine accumulating at the synapse producing a hypercontracted state (Nguyen *et al.* 1995; Miller *et al.* 1996). Loss-of-function mutations in genes coding for neurotransmitter release machinery confer resistance to aldicarb, presumably by reducing transmitter release and counteracting the effect of aldicarb (Rand and Nonet 1997). The effect of VAs on the aldicarb sensitivity of wild-type and mutant strains was tested as described previously (van Swinderen *et al.* 1999). Briefly, 20–30 young adult animals were placed onto seeded NGM plates containing a specific dose of aldicarb mixed in the agar. After 4 hr of exposure, the animals were picked into a circle of 0.5-cm diameter on the same aldicarb plate. This preparation was then exposed, in triplicate, to various concentrations of VA as described above, including a no-anesthetic control. The fraction of animals that crawled completely out of the circle after 1 hr was tallied as the movement index. Different mutant strains required different aldicarb concentrations in order to paralyze the majority of animals. To allow comparison of the different strains, we used an aldicarb concentration where the movement index was 5–20% of that in the absence of VAs. The significance of differences between movement indices was determined by a two-tailed Student's *t*-test.

Egg-laying assays: Young adult hermaphrodites were placed individually on standard culture plates and eggs were harvested at 15-min intervals. The number of cells in each freshly laid egg were counted using Nomarski optics. Eggs having eight or fewer cells were classified as early. For all genotypes, eggs were harvested from at least 10 hermaphrodites.

Locomotion assays: To calculate forward locomotion rate and the frequency of spontaneous backing in the absence of VAs, staged adult hermaphrodites were observed under conditions that maximize forward locomotion and minimize other behaviors: 200 μl of a fresh 5-ml OP50 *E. coli* culture was spread over the entire surface of a 60-mm NGM plate preincubated at 20°. Immediately after drying plates were covered and used within 2 hr. Animals were transferred to these thin lawn plates without adding extra food and were left undisturbed for 3 min prior to observation. Seconds elapsed per sine wave (counting anterior flexing just posterior to the pharynx) and the number of times that backing was initiated were recorded over a 2-min period using a keystroke recorder written by Hou-Pu Chou and Chieh Chang. For the body bends/minute in Table 2, only forward waves were recorded and the clock was paused during a reversal; waves preceding or following a reversal were not included. Entries for each animal were averaged and converted to waves and reversals per minute. For each genotype, the mean ± standard deviation value of several animals (n = 25–28) is reported. For the body bends/minute in Table 4, again only forward waves were recorded but the clock was not paused for reversals; this methodological difference was used because of the locomotion phenotypes of *unc-64(rf)* mutants, which unlike *goa-1(lf)* mutants tend to pause their movement before or after reversals for extended periods (n = 10 animals/genotype). The significance of differences between values was determined by a two-tailed Student's *t*-test.

Sequence analysis of sy192: Five overlapping fragments spanning the *goa-1* coding region were amplified in both wild-type and *sy192* genomic DNA in three independent reactions using long range PCR (Boehringer Mannheim, Indianapolis). Primer sequences are available on request. The products of the PCR reactions were pooled and subcloned into pGEM (Promega, Madison, WI). Three independent plasmids for each fragment were sequenced by the Caltech DNA sequencing facility on an automated sequencing machine (Applied Biosystems, Foster City, CA). Mismatches were resequenced on the opposite strand.

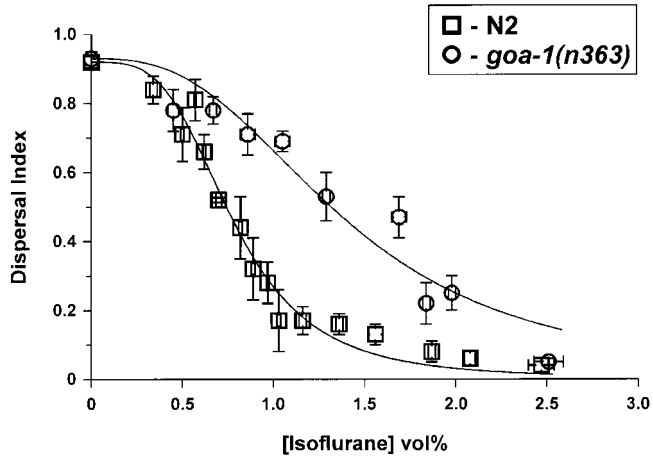


Figure 1.—Isoflurane concentration-response curves for the wild-type N2 and *goa-1(n363)* strains. The dispersal index, the fraction of animals moving in 45 min from the center to the edge of a 9.5-cm agar pad rimmed with bacteria, is plotted against isoflurane concentration to estimate the sensitivity of the strains to anesthetic. The EC_{50} for N2 in isoflurane is 0.75 ± 0.02 vol%. For *goa-1(n363)*, the EC_{50} is 1.40 ± 0.06 vol%. *goa-1(n363)* is significantly resistant by simultaneous curve-fitting methods (Waud 1972; DeLean *et al.* 1978; van Swinderen *et al.* 1997) compared to N2 ($P < 0.01$). Data points for similar isoflurane concentrations are pooled, and the mean ± SEM of the concentrations and dispersal indices are represented by horizontal and vertical error bars, respectively.

RESULTS

***goa-1* mutants are resistant to isoflurane:** The VA isoflurane disrupts normal locomotion in *C. elegans* (Crowder *et al.* 1996; van Swinderen *et al.* 1997). The EC_{50} or isoflurane concentration that half maximally disrupts coordinated movement in the wild-type strain N2 as measured by a dispersal assay (see materials and methods) is 0.75 ± 0.02 vol% (0.4 mm aqueous concentration), which is similar to the 0.31 mm concentration required to anesthetize humans (Franks and Lieb 1993). We measured the isoflurane sensitivity of four different *goa-1* loss-of-function mutants and found them all to be significantly resistant compared to N2 (Figure 1; Table 1) The EC_{50} for *goa-1(n363)*, which carries a null mutation deleting the entire *goa-1* coding region (Segalat *et al.* 1995), is 1.40 ± 0.06 or 87% higher than N2. Two other *goa-1(lf)* alleles, *goa-1(n1134)* (Segalat *et al.* 1995) and *goa-1(pk62)* (Mendel *et al.* 1995), are similarly isoflurane resistant. A fourth G_o α-subunit mutation, *goa-1(sy192)* (Mendel *et al.* 1995), was even more resistant to isoflurane, with an EC_{50} of 1.77 ± 0.13 vol% isoflurane.

***goa-1* allelic variation for halothane sensitivity:** Halo-

TABLE 1

Anesthetic phenotypes of *goa-1(lf)* mutants

Strain	Native DI[a]	Halothane EC_{50} (vol%)[b]	Slope[c]	Isoflurane EC_{50} (vol%)[b]	Slope[c]
N2 (wild type)	0.90 ± 0.01	0.42 ± 0.03	1.82 ± 0.24	0.75 ± 0.02	3.50 ± 0.33
goa-1(sy192)	0.98 ± 0.01	1.07 ± 0.04*	2.88 ± 0.38	1.77 ± 0.13*	2.44 ± 0.49
goa-1(n363)	0.95 ± 0.01	0.48 ± 0.02	3.17 ± 0.48	1.40 ± 0.06*	2.76 ± 0.35
goa-1(n1134)	0.96 ± 0.02	0.47 ± 0.05	3.40 ± 0.90	1.29 ± 0.06*	2.67 ± 0.45
goa-1(pk62)	0.66 ± 0.08	0.45 ± 0.05	3.50 ± 1.1	1.37 ± 0.08*	3.90 ± 0.64

* $P < 0.01$, significantly resistant to the anesthetic when compared to wild-type curve by simultaneous curve fitting (DeLean *et al.* 1978).

[a] Dispersal index without VAs (mean ± SEM; $n > 3$).

[b] Estimated VA concentration where effect is half-maximal ± standard error of the estimate; replicate experiments were pooled and refitted by the logistic equation as described in MATERIALS AND METHODS.

[c] The slope of the curve used to estimate the corresponding EC_{50}; for each VA none of the slopes are significantly different.

thane is a structurally distinct volatile anesthetic that is ~1.5-fold more potent than isoflurane in both *C. elegans* and humans (Franks and Lieb 1994; Crowder *et al.* 1996), with an EC_{50} of 0.42 ± 0.03 vol% in the N2 wild-type strain. We measured the halothane sensitivity of the four *goa-1* alleles (Table 1). Surprisingly, only *goa-1 (sy192)* was resistant to halothane with an EC_{50} >2-fold that of wild type (Figure 2). The other three alleles had halothane EC_{50}s virtually identical to N2.

To evaluate the allelic variation of *goa-1* mutants in their halothane sensitivity, we determined the genetic behavior and molecular identity of the *sy192* allele and compared that to *n363* and *n1134*. Previously, all *goa-1* alleles have been shown to have abnormal egg-laying and locomotion behaviors (Mendel *et al.* 1995; Segalat *et al.* 1995). Reduction-of-function mutations in *goa-1* cause eggs to be laid at an abnormally early stage of development; *goa-1(rf)* also confers a hyperactive movement phenotype. We measured egg laying and two aspects of locomotion in the *n363*, *n1134*, and *sy192* alleles. As reported previously (Mendel *et al.* 1995; Segalat *et al.* 1995), the three tested *goa-1(rf)* alleles lay eggs at an earlier stage than does the wild-type strain (Table 2). Forward speed is increased significantly in the null mutants but to a much greater degree in *sy192* (Table 2). The frequency of change from forward to backward locomotion (reversal of direction) is also increased significantly in all of the *goa-1* alleles. Unlike *n363* and *n1134*, which are recessive, *sy192* acts in a dominant fashion against both egg laying and forward locomotion (Table 2).

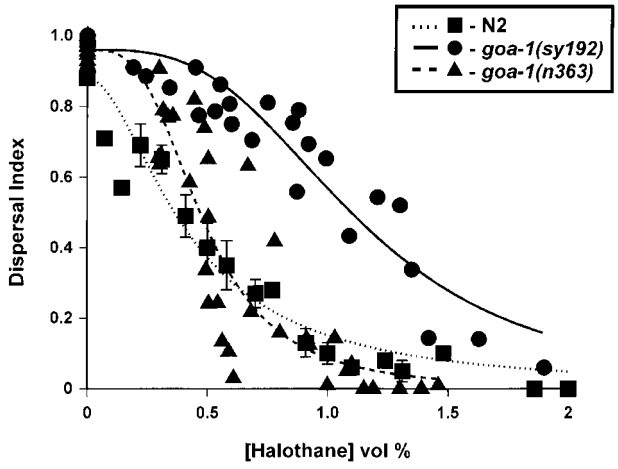


Figure 2.—Halothane concentration-response curves for the N2, *goa-1(sy192)*, and *goa-1(n363)* strains. *goa-1(sy192)* is significantly resistant to halothane ($P < 0.01$) compared to the wild-type strain N2 by simultaneous curve fitting, but *goa-1 (n363)* is not. Data points for similar isoflurane concentrations are pooled for N2, and the mean ± SEM of the concentrations and dispersal indices are represented by horizontal and vertical error bars, respectively.

To help us understand the differences in phenotypes and genetic behavior between *sy192* and the other *goa-1* alleles, *goa-1(sy192)* was sequenced. Sequencing of the *goa-1(sy192)* gene and comparison to the previously published wild-type sequence (Lochrie *et al.* 1991) identified a single missense mutation (ACT–ATT) that converts the threonine at position 330 to isoleucine. This is a relatively unconserved residue near the C-terminal end of the protein not previously shown to be essential for Go function. As mentioned above, *n363* has an 11-kb deletion of the entire coding region and upstream sequences of the *goa-1* gene (Segalat *et al.* 1995); thus, it is a null mutation by molecular criteria. *n1134* carries a missense mutation in the consensus sequence for N-terminal myristoylation (Segalat *et al.* 1995). Myristoylation has previously been shown to be required for function of G protein α-subunits (Mumby *et al.* 1990; Linder *et al.* 1991; Denker *et al.* 1992a). Thus, *n1134* would be expected to behave as a null mutation. Indeed, *n1134/n363* heterozygotes are phenotypically indistinguishable from *n1134* homozygotes consistent with

TABLE 2

Behavioral phenotypes of *goa-1* mutants

Strain	Egg laying[a] % early eggs	Movement	
		Body bends/minute[b]	Reversals/minute[c]
N2 (wild type)	10 (60)	24.9 ± 4.5 (26)	0.8 ± 0.7 (26)
goa-1(n363)	100 (20)	27.3 ± 3.8* (25)	4.7 ± 0.9* (25)
goa-1(n1134)	100 (38)	29.9 ± 4.4* (28)	3.5 ± 1.3* (28)
goa-1(sy192)	96 (56)	40.1 ± 3.7† (27)	5.0 ± 1.0* (27)
n363/+	19 (74)	26.9 ± 3.3 (26)	0.6 ± 0.6 (26)
n1134/+	13 (119)	27.6 ± 5.9 (26)	0.6 ± 0.6 (26)
sy192/+	92 (72)	44.4 ± 3.1† (27)	1.2 ± 0.8 (27)

[a] Newly laid eggs with eight or fewer cells were defined as early. No. of eggs scored is given in parentheses.
[b] Average body bends/minute over a 2-min assay period ± SD. *, significantly different from wild type ($P < 0.05$). †, significantly different from N2, *n363*, and *n1134* ($P < 0.0001$). No. of hermaphrodites scored is given in parentheses.
[c] Average no. of times backward movement was initiated/minute over a 2-min assay period ± SEM. *, significantly different from wild type ($P < 0.0001$). No. of hermaphrodites scored is given in parentheses.

n1134 being a functional null allele of *goa-1* (data not shown). Thus, the phenotypes of *n363* not *sy192* are most likely to represent the null phenotypes of the *goa-1* locus. The genetic (lack of haploinsufficiency of *n363*), behavioral, and molecular data suggest that the *sy192* mutation acts in a dominant negative fashion and produces a phenotype even more severe than the null phenotype.

Transformation rescue of *goa-1(lf)* VA resistance: The isoflurane resistance of four strains carrying independently generated reduction-of-function mutations in *goa-1* is very strong evidence that the known mutations in *goa-1* are indeed responsible for that phenotype. However, the halothane resistance phenotype was unique to *goa-1(sy192)*. Thus, we attempted transformation rescue of the halothane resistance phenotype of *sy192*. *goa-1 (sy192);dpy-20(e1282)* was transformed with an extrachromosomal array carrying the wild-type *goa-1* and *dpy-20* genes. Non-Dpy (*sy192;e1282* mutants carrying the array) animals had halothane EC_{50}s fourfold less than their Dpy sibs (*sy192;e1282* mutants that lost the array) and about fivefold less than untransformed *goa-1 (sy192)* (Figure 3, A and B). The isoflurane resistance of *sy192* was similarly rescued by *goa-1(+)* expression (Figure 3B). The *syEx352* array was crossed into *goa-1 (n363)* without *dpy-20(e1282)*, and *goa-1(n363);syEx352* (array-containing animals were identified by their Egl phenotype) was tested for rescue of isoflurane resistance. *n363;syEx352* animals had fourfold lower isoflurane EC_{50}s than their non-Egl siblings (Figure 3B). Finally, we asked whether the halothane resistance of *sy192* could be rescued if *goa(+)* expression was delayed until the adult stage at the same time as the anesthetic assays. To answer this question, we drove expression of *goa-1(+)* with a heat-shock-inducible promoter. Indeed, heat-shocked non-Dpy animals (carrying the array) were twofold more sensitive to halothane than non-heat-shocked controls (Figure 3B). Heat-shock of nontransformed *goa-1(sy192)* did not alter its halothane resistance (data not shown).

VA resistance by other genes in the *goa-1* pathway: To define the upstream and downstream GOA-1 signaling molecules that regulate VA action, we measured the VA sensitivity of mutants with altered GOA-1 signaling (Table 3). *egl-10* codes for an RGS (regulator of G protein signaling) protein acting upstream of *goa-1* (Koelle and Horvitz 1996). RGS proteins inhibit G protein activity by catalyzing the hydrolysis of the G protein α-subunit to its inactive GDP-bound state (Dohlman and Thorner 1997). Overexpression of *egl-10* results in behavioral defects similar to those caused by *goa-1(lf)* (Koelle and Horvitz 1996). *egl-10(nIs51)* and *egl-10 (nIs54)*, which both carry integrated multicopy *egl-10* transgenes, are resistant to both halothane and isoflurane (Table 3). Therefore, for halothane sensitivity, overexpressers of *egl-10* phenocopy the missense *goa-1(sy192)* mutant not the null mutants. Two reduction-of-function *egl-10* mutants were also tested, *egl-10(md176)* and the behaviorally less severe *egl-10(n480)*. Both alleles show a statistically insignificant decrease in halothane and isoflurane sensitivity.

The *sag* (*s*uppressors of *a*ctivated *G* protein) genes function opposite to and downstream of or parallel to *goa-1* (Hajdu-Cronin *et al.* 1999). Reduction-of-function mutations in the *sag* genes suppress the nearly paralyzed *goa-1(gf)* phenotype of *goa-1(syIs17)*, which carries integrated copies of a heat-shock-inducible *goa-1* gain-of-function construct (Mendel *et al.* 1995). We hypothesized that one or both of these *sag(rf)* mutants might be resistant to VAs. We measured the VA sensitivity of these two *sag* strains in the *goa-1(syIs17)* background at the permissive room temperature, thereby testing the *sag* mutations in the absence of activated *goa-1*. None of the *sag-1(rf)* alleles tested (also known

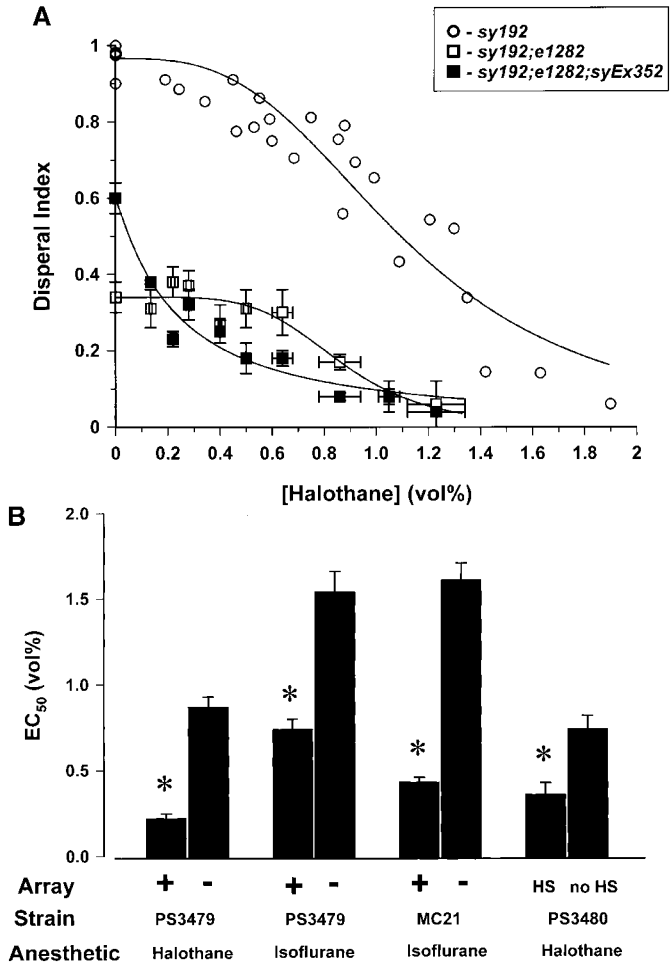


FIGURE 3.—Transformation rescue of *goa-1(rf)* VA resistance phenotypes. (A) Concentration-response curves for *goa-1(sy192); e1282* with and without the rescuing array: *syEx352[goa-1(+);dpy 20(+)]*. The curve for *goa-1(sy192)* is shown for comparison. (B) Summary of VA sensitivities of array- and nonarray-containing rescued strains. The genotypes of the strains are as follows: PS3479, *goa-1(sy192); dpy-20(e1282); syEx352[goa-1(+); dpy-20(+)]*; MC21, *goa-1(n363);syEx352*; PS3480, *goa-1(sy192); dpy-20(e1282); syEx353[pHS::goa-1(+); dpy-20 (e1282)]*. For PS3479 and PS3480, the array was assumed to be present in non-Dpy animals and absent in Dpy animals. For MC21, Egl animals were scored as having the array. Anesthetic sensitivity was measured by the dispersal assay with six- to eight-point dose-response curves using the dispersal assay with one alteration: the dispersal indices were scored with the observer blinded to the anesthetic concentration. The number of dispersal assays per strain were: PS3479 (halothane), 4; PS3479 (isoflurane), 2; PS3480, 2; MC21, 2; *sy192*, 4. The anesthetic sensitivities of transformed and untransformed siblings were measured on the same dispersal assay plates simultaneously. For heat-shock experiments, heat-shocked and non-heat-shocked animals were grown and tested in parallel. EC_{50}s (slopes) for the curves were: PS3479 (halothane) array +: 0.22 ± 0.03 (1.1 ± 0.17), array −: 0.87 ± 0.06 (4.2 ± 1.3); PS3479 (isoflurane) array +: 0.74 ± 0.06 (2.1 ± 0.23), array −: 1.54 ± 0.12 (3.7 ± 1.1); MC21 (isoflurane) array +: 0.43 ± 0.03 (2.1 ± 0.39), array −: 1.61 ± 0.10 (3.8 ± 1.0); PS3480 (halothane) HS: 0.36 ± 0.07 (2.3 ± 0.86), no HS: 0.74 ± 0.08 (3.7 ± 1.8). *, significantly different from nonarray or non-HS sibs, $P < 0.01$ by simultaneous curve fitting.

as *dgk-1)* are halothane or isoflurane resistant; however, both alleles of *eat-16* (after isolation *sag-2(sy438)* was shown to be allelic to a previously identified *eat-16* mutation) are resistant to both halothane and isoflurane (Table 3). Thus, a relatively specific aspect of GOA-1's function, which is suppressed by *eat-16(rf)* but not by *sag-1(rf)*, mediates its effect on VA action.

As discussed above, Goα has been shown in vertebrate neurons to regulate presynaptic Ca^{2+} channels in a syntaxin-dependent manner (STANLEY and MIROTZNIK 1997). *unc-64* codes for the only known *C. elegans* neuronal syntaxin and profoundly regulates VA sensitivity (VAN SWINDEREN *et al.* 1999). Two *unc-64(rf)* alleles are VA hypersensitive and one, *unc-64(md130)*, is VA resistant; the halothane and isoflurane EC_{50}s of these alleles differ by 7- and 30-fold, respectively. We have hypothesized that *unc-64(md130)* confers its resistance by somehow blocking the binding or transduction of binding of VAs whereas the hypersensitive syntaxin mutants are altering VA sensitivity indirectly (VAN SWINDEREN *et al.* 1999). To examine the relationship between Goα and syntaxin in regulating VA sensitivity, we generated double mutant strains carrying both *goa-1(n363)* and each of the three *unc-64(rf)* mutations. Double mutant strains with *goa-1(n363)* and either of the two VA hypersensitive *unc-64* mutations had anesthetic phenotypes similar to *goa-1(n363)* (Table 4); that is, they were resistant to isoflurane and normally sensitive to halothane. However, the anesthetic phenotype of *n363* in combination with the VA-resistant *unc-64(md130)* mutation was qualitatively similar to *md130* (*i.e.*, halothane and isoflurane resistant). However, quantitatively the isoflurane resistance of the double mutant was intermediate between that of either single mutant. In other words, the isoflurane resistance phenotypes are not additive; rather, the high-level resistance of *unc-64(md130)* is in part suppressed by *goa-1(n363)*.

Anesthetic resistance is not secondary to behavioral hyperactivity: We questioned whether the VA resistance phenotypes were influenced by the native dispersal of the animals in the absence of anesthetics. In the absence of anesthetic, some of the VA-resistant strains perform better than N2 in air (Tables 1, 2, and 3), raising the possibility that their resistance was an artifact of their hyperactivity. To examine this issue directly, we altered the assay in two ways and tested the most hyperactive of the strains, *goa-1(sy192)*. First, we shortened the time allowed for dispersal from 45 min to 25 min, thereby increasing the difficulty of the task and increasing the assay's sensitivity to locomotion defects. This modification brings the native dispersal index of *goa-1(sy192)* down to ~70%, below that of N2 in the standard assay. Second, we lengthened the animals' preassay exposure to anesthetics from 5 min to 15 min by increasing the initial spotting volume (see MATERIALS AND METHODS) to 20 μl. This second modification allowed the anesthetic more time to take effect in case these mutants managed to get a "head start" before being fully affected by

TABLE 3

Anesthetic phenotypes of mutations in the *goa-1* pathway

Strain	Native DI[a]	Halothane EC_{50} (vol%)[b]	Isoflurane EC_{50} (vol%)[b]	Molecular lesion[c]
N2	0.90 ± 0.01	0.42 ± 0.03	0.75 ± 0.02	Wild type
egl-10(nIs51)	0.99 ± 0.01	1.06 ± 0.04*	2.25 ± 0.18*	Transgene gf
egl-10(nIs54)	0.89 ± 0.04	1.14 ± 0.09*	1.43 ± 0.07*	Transgene gf
egl-10(md176)	0.50 ± 0.04	0.45 ± 0.12	0.43 ± 0.07	Rearrangement-null
egl-10(n480)	0.80 ± 0.04	0.52 ± 0.01	0.81 ± 0.04	Weak missense lf
sag-1(sy428)[d]	0.47 ± 0.03	0.28 ± 0.02	0.58 ± 0.05	Strong lf
sag-1(sy429)	0.62 ± 0.03	0.44 ± 0.04	0.88 ± 0.07	rf
sag-1(nu199)	0.85 ± 0.05	0.36 ± 0.04	0.78 ± 0.11	rf
sag-1(md1777)	0.84 ± 0.02	0.45 ± 0.05	0.67 ± 0.06	rf
eat-16(sy438)	0.90 ± 0.03	0.73 ± 0.04*	1.20 ± 0.07*	Missense rf
eat-16(ad702)	0.90 ± 0.03	1.10 ± 0.04*	1.33 ± 0.06*	Missense rf

* $P < 0.01$, significantly resistant to the anesthetic when compared to wild-type curve by simultaneous curve fitting (DeLean *et al.* 1978).

[a] Dispersal index without VAs (mean ± SEM; $n > 3$).

[b] Estimated VA concentration where effect is half-maximal ± standard error of the estimate; replicate experiments were pooled and refitted by the logistic equation as described in MATERIALS AND METHODS.

[c] gf, gain-of-function; rf, reduction-of-function; lf, loss-of-function.

[d] *sag-1* is also known as *dgk-1*.

the drug. However, this seemed unlikely since we have shown previously that VAs rapidly produce their behavioral effects reaching a steady state within 10 min (Crowder *et al.* 1996). The halothane EC_{50} of *sy192* is not decreased significantly by either modification of the assay (EC_{50} @ 25 min = 0.93 ± 0.02; EC_{50} w/ 20-μl drop = 1.17 ± 0.05). Moreover, not all VA-resistant strains are hyperactive. *eat-16(sy438)*, *eat-16(ad702)*, and *egl-10(nIs54)* performed similarly to N2 in the absence of anesthetics, yet all are resistant to both halothane and isoflurane. *goa-1(pk62)*, *goa-1(sy192);dpy-20(e1282)*, and the double mutant *n363;md1259* disperse significantly less than N2 in air yet each is halothane and/or isoflurane resistant. Finally, the *goa-1* alleles *n363* and *n1134* disperse as well as *sy192*, yet neither is halothane resistant. Further, although not hyperactive by the dispersal assay, *sag-1/dgk-1(rf)* alleles have been noted to be hyperactive in other locomotion assays (Hajdu-Cronin *et al.* 1999; Miller *et al.* 1999; Nurrish *et al.* 1999), yet these strains are not anesthetic resistant (Table 3). Therefore, hyperactivity is neither necessary nor sufficient to produce VA resistance.

TABLE 4

Phenotypes of *goa-1;unc-64* double mutants

Strains	Halothane EC_{50} vol%[a]	Isoflurane EC_{50} vol%[a]	Native DI	BBM	MI on aldicarb	
					0.1 mM	0.5 mM
N2	0.42 ± 0.03	0.75 ± 0.02	0.90 ± 0.01	18.6 ± 0.84	0.79 ± 0.02	0.03 ± 0.03
goa-1(n363)	0.43 ± 0.03	1.46 ± 0.06[b]	0.95 ± 0.01[b]	18.6 ± 1.08	0.05 ± 0.02[b]	0 ± 0
unc-64(js21)	0.17 ± 0.03[b]	0.13 ± 0.04[b]	0.64 ± 0.04[b]	4.8 ± 0.82[b]	0.81 ± 0.04	0.70 ± 0.01[b]
unc-64(md1259)	0.17 ± 0.10[b]	0.18 ± 0.02[b]	0.38 ± 0.05[b]	10.2 ± 1.25[b]	0.72 ± 0.02	0.23 ± 0.04
unc-64(md130)	1.29 ± 0.12[b]	4.36 ± 0.36[b]	0.64 ± 0.02[b]	12.1 ± 1.08[b]	0.95 ± 0.05	0.65 ± 0.10[b]
n363;js21	0.44 ± 0.06[c]	1.15 ± 0.14[b,c]	0.79 ± 0.06	15.4 ± 1.11[c]	0.93 ± 0.02[b,d]	0.26 ± 0.05[b,c]
n363;md1259	0.39 ± 0.04[e]	1.52 ± 0.13[b,e]	0.70 ± 0.05[b,e]	18.8 ± 1.16[e]	0.48 ± 0.05[b,d]	0.01 ± 0.01
n363;md130	0.97 ± 0.09[b,d]	2.20 ± 0.23[b,d,f]	0.75 ± 0.04[b,f]	17.6 ± 1.99[f]	0.60 ± 0.06[d,f]	0 ± 0[f]

Native DI ± SEM, dispersal index in the absence of anesthetic; BBM ± SEM, body bends/minute; MI ± SEM on aldicarb, fraction moving after incubation on 0.1 or 0.5 mM aldicarb agar pads. $P < 0.01$ set as significance threshold for all comparisons.

[a] EC_{50}s ± standard error of the estimate by dispersal assay and compared by simultaneous curve fitting (DeLean *et al.* 1978). All other values are means ± SEM and compared by two-sided *t*-test assuming unequal variances.

[b] Different from N2.

[c] Different from *js21*.

[d] Different from *n363*.

[e] Different from *md1259*.

[f] Different from *md130*.

Anesthetic resistance is not secondary to an increase in cholinergic neurotransmission: Since VAs have been shown to decrease cholinergic neurotransmission in *C. elegans* (van Swinderen *et al.* 1999), disruption of *goa-1* signaling might indirectly confer VA resistance simply by elevating neurotransmitter release. To examine this question, we made use of the drug aldicarb. Aldicarb is an acetylcholinesterase inhibitor that produces a hypercontracted paralysis in *C. elegans* due to accumulation of acetylcholine at the neuromuscular junction (Rand and Nonet 1997). Mutants with defects in presynaptic machinery, for example, syntaxin mutants, have shown that decreases in transmitter release confer aldicarb resistance (Nguyen *et al.* 1995; Miller *et al.* 1996; Saifee *et al.* 1998). As had been shown previously (Miller *et al.* 1999; Nurrish *et al.* 1999), *goa-1 (n363)* (Table 4) and the other three *goa-1* alleles have an increase in cholinergic neurotransmission as evidenced by their aldicarb hypersensitivity; 0.1 mm aldicarb produced nearly 100% paralysis in *goa-1(lf)* compared to 20% for wild type. Likewise, *egl-10(nIs51)* (Figure 4) and *egl-10(nIs54)* [movement index (MI) @ 0.1 mm = 0.01 ± 0.02] were aldicarb hypersensitive; however, *egl-10(md176)*, a reduction-of-function allele (Table 3), was not aldicarb resistant (MI @ 0.5 mm = 0.026 ± 0.01 compared to N2 @ 0.5 mm = 0.03 ± 0.03). This result is consistent with the finding that *egl-10(md176)* is not VA hypersensitive.

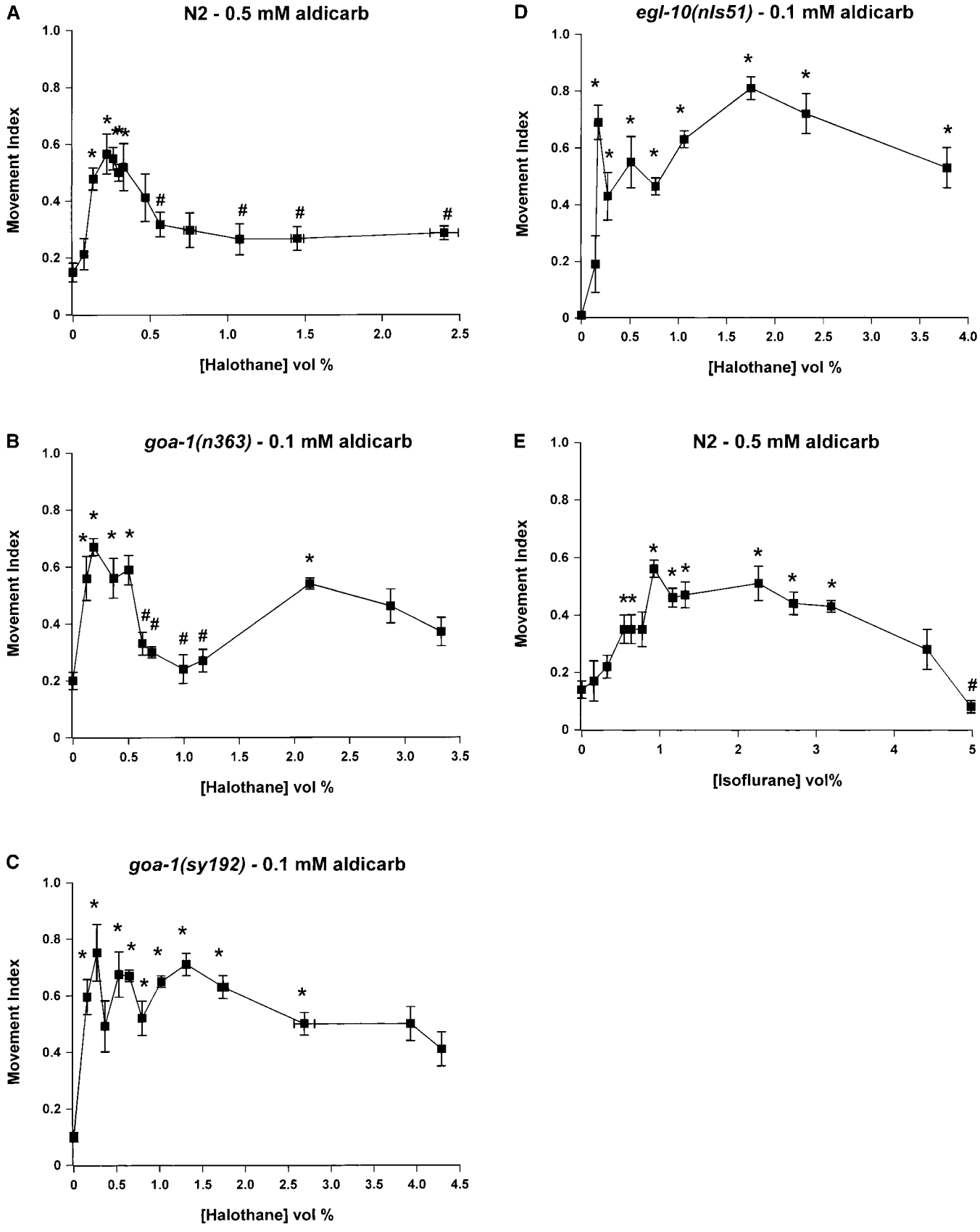
While aldicarb hypersensitivity and increased transmitter release generally correlate with VA resistance, there are exceptions. Downstream of *goa-1*, both *eat-16(ad702)* and *sag-1(sy428)* strains are, as had been shown previously (Miller *et al.* 1999; Nurrish *et al.* 1999), aldicarb hypersensitive (MI @ 0.1 mm: *eat-16 (ad702)* = 0.09 ± 0.02; *sag-1(sy428)* = 0 ± 0); however, *eat-16(ad702)* is VA resistant while *sag-1(sy428)* is not. We also measured the aldicarb sensitivity of the *goa-1 (n363);unc-64(lf)* double mutants (Table 4). We suspected given their locomotion behaviors that these VA-resistant mutants might have cholinergic transmission similar to that of wild type. Indeed, both *goa-1(n363); unc-64(md1259)* and *goa-1(n363);unc-64(js21)* had aldicarb sensitivities similar to that of N2 and much less than that of *goa-1(n363)* (Table 4); thus, increased cholinergic transmission is neither sufficient nor necessary to produce VA resistance. Levamisole is a direct acetylcholine receptor agonist used to identify postsynaptic effects of *C. elegans* mutants. Neither *goa-1(n363)* or *goa-1 (sy192)* differed from wild type in their levamisole sensitivity or in VA effects on levamisole sensitivity (data not shown). This further supports a presynaptic site of action for *goa-1* in regulating VA sensitivity.

To further examine this issue, we looked at VA effects on aldicarb sensitivity in various genetic backgrounds (Figure 4). As shown previously (van Swinderen *et al.* 1999), halothane and isoflurane significantly reduce the potency of aldicarb in paralyzing wild-type animals (Figure 4, A and E). This result suggests that VAs reduce cholinergic neurotransmission. None of three mutants in the *goa-1* pathway that were tested [*goa(n363)*, *goa-1 (sy192)*, and *egl-10(nIs51)*] blocked or reduced halothane-induced aldicarb resistance (Figure 4). However, the concentration/response curves did differ among the strains at higher halothane concentrations. In the wild-type and *goa-1(n363)* strains, the fraction of animals moving on aldicarb significantly decreases above 1 vol% halothane (Figure 4, A and B). In other words, halothane appears to lose its ability to confer aldicarb resistance at higher concentrations. However, the movement index on aldicarb of the halothane-resistant strains, *goa-1* (*sy192*) and *egl-10(nIs51)*, does not decrease at higher halothane concentrations (Figure 4, C and D). The movement indices of *goa-1(n363)* and N2 were significantly different ($P < 10^{-8}$) from *goa-1(sy192)* and *egl-10 (nIs51)* in the 0.5–1.5 vol% halothane concentration range. Unlike halothane, isoflurane did not have a bimodal effect on aldicarb sensitivity; rather, isoflurane induces aldicarb resistance that does not decrease until very high concentrations (Figure 4E). These results suggest that halothane has a secondary effect on neurotransmission not shared by isoflurane that is blocked in the halothane-resistant *goa-1(sy192)* and *egl-10(gf)* strains.

DISCUSSION

Several mutants in the *goa-1* signaling pathway were shown to be VA resistant. We tested two anesthetics,

Figure 4.—VAs induce resistance to the acetylcholinesterase inhibitor aldicarb. Wild-type or mutant *C. elegans* were exposed to concentrations of aldicarb that paralyzed an average of 80–95% of adult animals. Subsequently, the paralyzed animals were exposed to various concentrations of halothane or isoflurane, and the fraction of moving animals (MI) was scored after 1 hr. Each point represents 3–13 measurements at similar anesthetic concentrations; the mean ± SEM of the anesthetic concentration is indicated by horizontal error bars; the mean ± SEM of the MI at each anesthetic concentration is plotted. (A) Halothane effects on aldicarb sensitivity in N2. *, significantly different from MI for 0 halothane baseline. #, significantly different from pooled peak MI (defined as those from 0.22 to 0.47 vol% halothane). (B) *goa-1(n363)* is wild type for halothane-induced aldicarb resistance. *n363*, *sy192*, and *nIs51* are hypersensitive to aldicarb in the absence of halothane; thus, a lower aldicarb concentration is used. *, significantly different from MI for 0 halothane baseline. #, significantly different from pooled peak MI (defined as those from 0.12 to 0.50 vol% halothane). (C) The aldicarb sensitivity of *goa-1(sy192)* responds differently than wild type to halothane. *, significantly different from MI for 0 halothane baseline. (D) *egl-10(nIs51)* is similar to *sy192* in its response to halothane on aldicarb. *, significantly different from MI for 0 halothane baseline. (E) Isoflurane effect on aldicarb sensitivity in N2. *, significantly different from MI for 0 isoflurane baseline.


A
N2 - 0.5 mM aldicarb
Movement Index
[Halothane] vol %
B
goa-1(n363) - 0.1 mM aldicarb
Movement Index
[Halothane] vol %
C
goa-1(sy192) - 0.1 mM aldicarb
Movement Index
[Halothane] vol %
D
egl-10(nls51) - 0.1 mM aldicarb
Movement Index
[Halothane] vol %
E
N2 - 0.5 mM aldicarb
Movement Index
[Isoflurane] vol%

halothane and isoflurane, and found that some *goa-1* mutants were resistant only to isoflurane (*n363*, *n1134*, *pk62*) whereas other mutations in *goa-1* pathway genes conferred resistance to both isoflurane and halothane [*goa-1(sy192)*, *egl-10(gf)*, *eat-16(rf)*]. The different anesthetic sensitivities of the strains, the results from testing of the *goa-1(n363);unc-64(rf)* double mutants, and the aldicarb experiments suggest and exclude potential mechanisms whereby GOA-1 controls VA action in *C. elegans*.

Is GOA-1 a VA target? By genetic and molecular criteria, both *goa-1(n363)* and *goa-1(n1134)* eliminate *goa-1* activity (Segalat *et al.* 1995). Thus, not surprisingly, these two alleles behaved similarly in all of our anesthetic and behavioral assays. These *goa-1* nulls were found to be significantly resistant to isoflurane's effect on locomotion but to be similar to wild type for halothane sensitivity. The absence of GOA-1 protein then appears not to be sufficient for conferring halothane resistance. Thus, while the α-subunit of G_o clearly regulates anesthetic action, it cannot be halothane's primary target. In the case of isoflurane, *goa-1(null)* is much less resistant than the syntaxin mutant *unc-64(md130)*; thus, if GOA-1 is an isoflurane target it cannot be the only one. Rather, the effect of GOA-1 signaling on VA sensitivity is more plausibly indirect.

One possible indirect mechanism of GOA-1 regulation of VA action is through its negative effects on transmitter release. However, the resistance produced by mutations that reduce GOA-1 activity cannot be explained simply by counteracting the action of VAs on transmitter release. Neither hyperactivity nor aldicarb hypersensitivity is necessary or sufficient for VA resistance. Further, none of the *goa-1* pathway mutants blocks the activity of clinical concentrations of halothane against aldicarb-induced paralysis. Thus, *goa-1* does not appear to regulate VA action either by altering the levels of neurotransmitter independent of VAs or by blocking the effect of VAs on transmitter levels. However, it is possible that these mutants disrupt transmitter release in a way not detected by the pharmacological assay. Aldicarb sensitivity is an indicator of steady state levels of acetylcholine and does not directly measure the magnitude or coordination of individual synaptic events that would mediate the locomotion disrupted by VAs. Addressing these questions will require physiological techniques only recently developed in *C. elegans* (Raizen and Avery 1994; Goodman *et al.* 1998; Richmond *et al.* 1999).

A clue to GOA-1's anesthetic regulatory activity is provided by the phenotypes of *goa-1(sy192)* and the *egl-10* overexpressing strains. These mutants are both halothane and isoflurane resistant. Further, in the absence of anesthetics, *sy192* has a locomotion phenotype more severe than that of the null mutants. Thus, loss of the α-subunit of Go does not appear to eliminate completely the activity of the GOA-1 pathway. We speculate on the basis of the function of EGL-10 and nature of the mutation in *sy192* that the additional phenotypes of *sy192* and *egl-10(gf)* could be due to dominant negative effects on the βγ-subunit of Go or of some other G protein that regulates locomotion, egg laying, and anesthetic action. *egl-10* encodes an RGS protein thought to negatively regulate wild-type GOA-1 by increasing the GTPase activity of the GOA-1 subunit (Koelle and Horvitz 1996; Koelle 1997); thus, *egl-10(gf)* mutants should increase levels of αGDP and recruit βγ-subunits away from their target substrates (Dohlman and Thorner 1997). Likewise, the location of the *sy192* mutation suggests that *sy192* may alter the equilibrium of G protein signaling by also reducing βγ activity. Although the residue altered in *sy192* is not highly conserved, it is the first residue of the carboxy-terminal α-helix and lies immediately adjacent to a highly conserved region involved in nucleotide binding (Noel *et al.* 1993). These regions have been implicated in receptor-mediated GDP release and G protein activation (Denker *et al.* 1992b; Rasenick *et al.* 1994). Substitution at position 330 could reduce nucleotide exchange, locking the mutant α-subunits in a GDP-bound state and competing with wild-type Goα-subunits for receptors and/or βγ-subunits. Alternatively, excess or mutant Goα-subunit could act against another G protein-coupled receptor or an RGS protein. A dominant negative action of the *sy192* product against the EAT-16 RGS protein would increase the activity of the Gq pathway and would also be consistent with our results.

Downstream mediators of GOA-1 anesthetic regulatory activity: Reduction-of-function mutations in *egl-30*, which codes for a homolog of $G_{q\alpha}$, are lethargic, aldicarb resistant, and epistatic to *goa-1(lf)*. Thus, G_q and G_o function antagonistically in regulating locomotion and transmitter release in *C. elegans*, G_q functioning downstream of or parallel to G_o (Hajdu-Cronin *et al.* 1999; Lackner *et al.* 1999; Miller *et al.* 1999). Components of the G_q signaling cascade include the products of *eat-16* and *sag-1/dgk-1*. *sag-1/dgk-1* codes for a diacylglycerol kinase ortholog that reduces the activity of diacylglycerol (DAG), which is likely to mediate, at least in part, stimulation of transmitter release by EGL-30 Gq_α (Nurrish *et al.* 1999). *eat-16* encodes an RGS protein that acts to negatively regulate EGL-30 $G_{q\alpha}$ (Hajdu-Cronin *et al.* 1999). Our findings that *eat-16(rf)* but not *sag-1/dgk-1(rf)* mutants are VA resistant suggests that G_q regulates VA sensitivity but through second messengers either distinct from or in addition to DAG.

Of the many proteins whose function is regulated by G_o/G_q, we favor syntaxin and/or syntaxin-binding proteins as most likely to be VA targets. As mentioned in the Introduction, syntaxin mutant alleles have a >30-fold difference in their isoflurane sensitivity that cannot be explained by differences in the effects of the mutations on syntaxin's normal function (van Swinderen *et al.* 1999). Thus, syntaxin and syntaxin-binding proteins including calcium channels, SNAP-25, VAMP, UNC-13, UNC-18, and synaptotagmin are all candidate VA targets. Because of the severity of their locomotion defects,

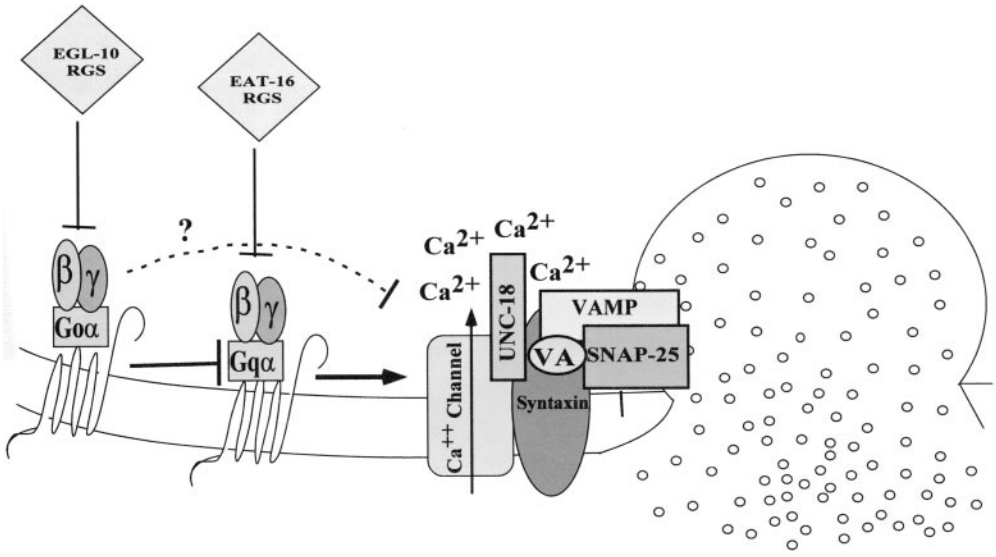


FIGURE 5.—Model for volatile anesthetic action at presynaptic terminals in *C. elegans*. Depicted is a schematic of a presynaptic terminal with a single synaptic vesicle releasing neurotransmitter and the gene products found to regulate sensitivity to clinical concentrations of VAs in *C. elegans* as reported here and previously (VAN SWINDEREN *et al.* 1999). The relationship between proteins is based on epistasis experiments reported here and elsewhere (as cited in the text). VAs are shown as binding to the SNARE complex (syntaxin, SNAP-25, and VAMP) although other binding targets (N/P-type calcium channels or UNC-18 in complex with syntaxin) are also reasonable. Arrows indicate positive regulation; lines capped by bars indicate negative regulation.

we have not been able to test the anesthetic sensitivity of strains with severe mutations in the latter three genes. We have tested mutant alleles of both L-(*egl-19*) and non-L-type (*unc-2*) calcium channels without finding a strongly resistant strain (data not shown). The existing weak reduction-of-function mutants of SNAP-25 and VAMP are hypersensitive to anesthetic (VAN SWINDEREN *et al.* 1999). While these results do not rule out any of the gene products as VA targets because the mutations either are not nulls or move too poorly to test, we have no direct genetic data showing that any one of these proteins is required for VA sensitivity. Given that UNC-13 is a DAG binding protein (LACKNER *et al.* 1999) capable of dissociating UNC-18 n-Sec1 from UNC-64 syntaxin (SASSA *et al.* 1999), the lack of an effect of the *sag-1/dgk-1* mutations on VA sensitivity suggests that *unc-13* and *unc-18* may not regulate VA action, or they do so in a DAG-independent manner.

Our working hypothesis prior to our findings reported here was that VAs bound to the SNARE complex and disrupted its function in mediating vesicle fusion and transmitter release and that the *unc-64(md130)* product somehow prevented the binding or effect of binding of VA to the SNARE complex. Here, we found that the high-level isoflurane resistance of *unc-64 (md130)* is in part dependent on the presence of normal GOA-1 activity. Thus, the syntaxin-mediated and GOA-1-mediated mechanisms of regulating VA action are not additive. How might GOA-1 regulate VA action if the VA target is indeed the SNARE complex? A working model of VA action in *C. elegans* is shown in Figure 5. The SNARE complex is hypothesized as the VA binding site in this pathway on the basis of arguments given above. Other syntaxin-binding proteins such as UNC-18 n-Sec1 or N-type calcium channels, either in complex with syntaxin or not, are also reasonable VA targets. G_o either directly or indirectly through inhibition of G_q regulates VA sensitivity and the resistance produced by *unc-64(md130)*. One potential mechanism for G_o/G_q's regulation of VA sensitivity is by altering phosphorylation states of the presynaptic transmitter release machinery. Phosphorylation is known to affect the association of syntaxin with other synaptic proteins including SNAP-25, mUNC-18, and N-type calcium channels (FUJITA *et al.* 1996; SHIMAZAKI *et al.* 1996; YOKOYAMA *et al.* 1997). Thus, phosphorylation could alter both the effect of VAs on synaptic proteins and the ability of the *unc-64(md130)* product to interact with the VA target and block VA binding or its effect. Future experiments will be aimed at demonstrating that one or more proteins that function downstream of GOA-1, such as those that form the SNARE complex, are indeed VA targets and at understanding how the *goa-1* pathway regulates VA sensitivity.

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