nitrile and 1% trifluoroacetic acid (TFA), the extract was centrifuged, and the supernatant was lyophilized. The material was resuspended in 0.1% TFA and extracted with chloroform:methanol (2:1). The aqueous phase was lyophilized, resuspended in 0.1% TFA, and fractionated by P-30 gel filtration (Bio-Rad, Richmond, CA). Fractions were assayed for antimicrobial activity by a radial diffusion plate assay against *Escherichia coli* D31 and *Candida tropicalis* [M. Zasloff, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5449 (1987)]. The active antimicrobial fractions were purified by reversed-phase (C_{18}) HPLC (Poly LC, Columbia, MD) followed by strong cation exchange HPLC (Poly LC). Salt was removed from each fraction with a C_{18} Sep-pak cartridge (Waters, Milford, MA), and the fractions were then dried overnight and assayed for antimicrobial activity against *E. coli* D31 and *C. tropicalis*.

10. The mass of the purified peptide was 4627.5 daltons by electrospray mass spectrometry, consistent with the size and amino acid composition of a β-defensin. Amino acid analysis was also consistent with a β-defensin. The sequence of ~25 amino acids from the COOH-terminus was determined after digestion of the purified peptide with trypsin, reduction and alkylation of the cysteine residues, and chromatographic separation of the peptide fragments. Microsequencing of individual fragments was performed by standard Edman degradation. The peptide sequence NKGICVPIRCPG-SMRQIGTCLGAQVK (*27*) was confirmed and completed by cloning of the full-length complementary DNA from a bovine tongue epithelial library.
11. Minimal inhibitory concentrations (MICs) were assessed in 96-well microtiter plates. Microorganisms were grown at log phase in 0.25× trypticase soy broth at a density of 1×10^5 cells per milliliter. For each organism, dilutions of peptide ranging from >500 to 1 μg/ml were tested. MICs were calculated on the basis of the lowest concentration of peptide that inhibited overnight growth.
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13. A polymerase chain reaction (PCR)–based strategy was designed to amplify a probe from tongue poly(A)$^+$ RNA. Assuming conservation of the signal sequence in defensin families (*6*), we used a PCR primer (5′-ATGAGGCTCCATCACCTG-3′) corresponding to the first six amino acids of the signal sequence of TAP (MRLMML) for the sense strand. Assuming nucleotide identity corresponding to amino acids in the COOH-termini of LAP and TAP (VKCC), we designed a degenerate (1:8) antisense primer [5′-ACAGCATTT-TAC(TC)TG(ACGT)GC-3′] with fully degenerate divergent amino acids (AQ). PCR conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 1 min, 58°C for 2 min, and 72°C for 3 min, followed by 72°C for 15 min. The products were purified on gels and subcloned into Bluescript vector that had been modified to accept PCR products [D. Marchuk, M. Drumm, A. Saulino, F. S. Collins, *Nucleic Acids Res.* **19**, 1154 (1991)]. The PCR product was sequenced and used as a probe to identify clones from a bovine tongue λZap II cDNA library (Stratagene, La Jolla, CA) constructed from 2 μg of bovine anterior tongue poly(A)$^+$ RNA. Inserts were selected by size from 0.1 to 3 kb. Phages (0.5×10^6) were plated over 10 plates; there were ~100 positive plaque-forming units per plate. Nylon membranes (Genescreen II, DuPont Biotechnology Systems, Boston, MA) were used for plaque lifts. Six phages were isolated after successive rounds of plaque purification. The inserts were ligated into pBluescript vectors by in vivo excision and sequenced by dideoxy sequencing from T3 and T7 primers. The final cDNA sequence was derived from sequencing of multiple clones and their complementary strands.
14. W. S. Young III, E. Mezey, R. E. Siegel, *Neurosci. Lett.* **70**, 198 (1986). Three tongues with visible, naturally occurring sores were selected from recently killed cows. The lesions and surrounding epithelia were fixed with 4% paraformaldehyde in phosphate-buffered saline, embedded in paraffin, and mounted on sialanated slides, which were then subjected to standard in situ hybridization methods for RNA probes. Probes were prepared from the full-length LAP cDNA, which was subcloned into Bluescript and linearized with Sma I and Kpn I for sense and antisense transcripts, respectively. Hybridization was performed overnight at 42°C with 2×10^6 cpm per slide, after which slides were washed at 65°C with 1× standard saline citrate (SSC) containing 0.1% β-mercaptoethanol. Slides were exposed to Kodak NTB-2 emulsion for ~30 days, counterstained with hematoxylin and eosin, and photographed. Additional slides of the lesions were also hybridized with full-length RNA probes for α-tubulin (sense and antisense).
15. Adult bovine epithelial tissue specimens were obtained from five cows and frozen in liquid nitrogen. Fetal tongue RNA was pooled from five fully formed, third-trimester fetuses (Moyer Packing Co.). Calf tongue RNA was prepared from 4-month-old calves (Marcho Farms, Souderton, PA). Frozen epithelial tissue was extracted with guanidinium isothiocyanate [J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutler, *Biochemistry* **13**, 5294 (1979)]. Cows differ from humans in having a four-part stomach that comprises the rumen, reticulum, omasum, and abomasum (proximally to distally). The transverse segment of bovine colon is identified as the spiral colon. For the poly(A)$^+$ RNA blot, oligo(dT) columns were used to isolate mRNA or bovine poly(A)$^+$ RNA was obtained from Clontech (Palo Alto, CA). Poly(A)$^+$ RNA (4 μg) from each tissue was subjected to electrophoresis on a 6.7% formaldehyde gel (Fig. 4A, lanes 1 to 6). Approximately 15 μg of total RNA was used per lane for tissue distribution (Fig. 4B) and developmental (Fig. 4A, lanes 7 to 9) studies. The RNA was transferred under neutral conditions to Zetabind membranes (Bio-Rad). The LAP 48-nucleotide oligomer was 5′-CCTCCTGCAGCA-TTTTACTTGGGCTCCGAGACAGGTGCCAATCT-GTCT-3′. Hybridization was performed overnight at 42°C in 6× SSC, 5× Denhardt's solution, 20% formamide, yeast RNA (200 μg/ml), and 0.5% SDS. The probe was end-labeled with ^{32}P by polynucleotide kinase to a specific activity of 1×10^8 cpm/μg. A full-length bovine α-tubulin cDNA was randomly primed to a specific activity of 0.5×10^9 cpm/μg. The blots were washed at 65°C with 1× SSC containing 0.1% SDS for LAP probe hybridizations, and at 65°C with 0.1× SSC containing 0.1% SDS for α-tubulin probe hybridizations.
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27. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

28. We thank W. Lane (Harvard University Microchemistry Facility, Cambridge, MA) for performing peptide characterization and microsequencing; C. Bevins (Children's Hospital of Philadelphia, Philadelphia, PA) for bovine α-tubulin cDNA; and C. A. Janeway Jr., T. Uzzell, A. Shinnar, T. Williams, P. Molinoff, and L. Jacob for critical reviews of the manuscript.

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Modulation of Serotonin-Controlled Behaviors by G_o in *Caenorhabditis elegans*

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Seven transmembrane receptors and their associated heterotrimeric guanine nucleotide–binding proteins (G proteins) have been proposed to play a key role in modulating the activities of neurons and muscles. The physiological function of the *Caenorhabditis elegans* G protein G_o has been genetically characterized. Mutations in the *goa-1* gene, which encodes an α subunit of G_o ($G\alpha_o$), cause behavioral defects similar to those observed in mutants that lack the neurotransmitter serotonin (5-HT), and *goa-1* mutants are partially resistant to exogenous 5-HT. Mutant animals that lack $G\alpha_o$ and transgenic animals that overexpress $G\alpha_o$ [*goa-1(xs)* animals] have reciprocal defects in locomotion, feeding, and egg laying behaviors. In normal animals, all of these behaviors are regulated by 5-HT. These results demonstrate that the level of G_o activity is a critical determinant of several *C. elegans* behaviors and suggest that G_o mediates many of the behavioral effects of 5-HT.

Changes in environmental conditions or physiological status often produce global changes in the behavior of animals. Two sets of experimental results suggest that seven transmembrane receptors (7-TMRs) and G proteins play a pivotal role in the modulation of behavior. First, synaptic signals produced by 7-TMRs are well suited to the task of promoting long-term changes in behavior because G proteins typically regulate the activities of neurons and muscles by means of a cascade of second messengers (*1*). Second, neurotransmitters that act on 7-TMRs (that is, metabotropic agonists) are potent modulators of many behaviors (*2*). However, the specific receptors and G proteins that mediate the response to a particular agonist are often not known.

We have genetically analyzed signaling by the metabotropic agonist 5-HT in *Caenorhabditis elegans*. It has been proposed that

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5-HT acts as a neurohormone that regulates many behaviors of C. *elegans* (3–6). We screened for 5-HT signaling mutants in a two-step process. Because mutants that lack 5-HT move hyperactively (7), we first isolated hyperactive mutants. We then determined whether the hyperactivity could be corrected by exogenous 5-HT.

Two mutations in a previously uncharacterized gene, *n363* and *n1134*, had been described as conferring resistance to 5-HT (8). We confirmed these results, finding that *n363* and *n1134* animals are extremely hyperactive and that this hyperactivity is resistant to exogenous 5-HT. Because it may encode an effector of 5-HT signaling, we cloned the gene defined by these mutations.

We mapped the *n363* and *n1134* mutations to a 0.2–map unit interval on chromosome I (Fig. 1A). The *n363* mutation corresponds to a deletion of greater than 13 kb (Fig. 1B), which spans the *goa-1* gene (9). The *n1134* mutation is a missense mutation in codon 1, and the predicted *n1134* protein corresponds to a four-residue NH_2-terminal truncation of $G\alpha_o$, as a result of initiation of translation at Met^5 (Fig. 1C). Unlike the wild-type protein, the NH_2-terminus of the *n1134* protein does not fit the consensus for NH_2-terminal myristoylation (*10*). Because nonmyristoylated $G\alpha_o$ subunits fail to associate with both $\beta\gamma$ subunits and membranes, the *n1134* protein is likely to be defective for $G\alpha_o$ function (*11*). Thus, both *n363* and *n1134* correspond to mutations in the *goa-1* gene, which implies that defects in $G\alpha_o$ cause hyperactive locomotion and resistance to 5-HT.

Does G_o mediate the 5-HT modulation of other behaviors? Four behaviors of C. *elegans* (locomotion, defecation, feeding, and egg laying) are known to be regulated by 5-HT. We used several criteria to determine whether G_o mediates the effects of 5-HT on these behaviors. First, we examined the expression pattern of the *goa-1* gene to determine if the cells underlying these behaviors express $G\alpha_o$ (Fig. 2) (*12*). Second, we examined *goa-1* and *goa-1(xs)* animals for defects in these behaviors (*13*, *14*). And third, we tested *goa-1* mutants for sensitivity to 5-HT and other metabotropic agonists (*15*). Our results suggest that G_o mediates the effects of 5-HT on locomotion, defecation, and probably egg laying (Table 1).

We further explored the role of G_o in regulating locomotion by comparing the behaviors of *goa-1* and *goa-1(xs)* animals (Fig. 3A). The comparative rates of locomotion were *n363* = *n1134* > wild type > *goa-1(xs)*. These experiments demonstrate that the endogenous level of $G\alpha_o$ activity regulates locomotion. The hyperactive locomotion of *goa-1* mutants (both alleles) was partially resistant to exogenous 5-HT (and other serotonergic agonists) but was sensitive to quinpirole, a dopamine agonist (Fig. 4A). The simplest interpretation of these experiments is that 5-HT, signaling by means of $G\alpha_o$, inhibits the activity of the ventral cord motor neurons (which express $G\alpha_o$, Fig. 2A) thereby inhibiting locomotion. However, because $G\alpha_o$ is expressed in many other neurons as well (Fig. 2F), we could not conclusively determine the site of 5-HT action in modulating locomotion.

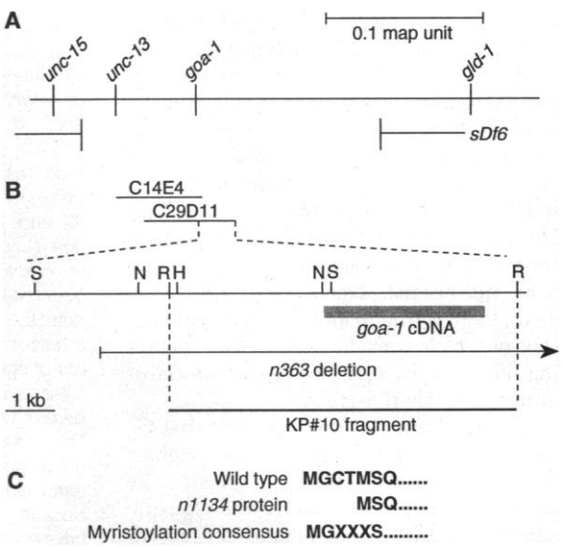

Fig. 1. The mutations *n363* and *n1134* are in the *goa-1* gene (*25*). **(A)** These mutations genetically map in the interval bounded by *unc-13* and *gld-1* on chromosome I. **(B)** Restriction map of *goa-1*. The mutation *n363* corresponds to a deletion starting approximately 5 kb 5′ of exon 1 and extending more than 5 kb 3′ of the last exon of *goa-1*. The *n363* polymorphism is detected by the cosmids C14E4 and C29D11. The right-hand end point of the *n363* deletion has not been determined. The KP#10 plasmid (used for overexpression studies) contains a 7-kb Eco RI fragment that spans *goa-1*. Enzymes are indicated as follows: H, Hind III; N, Nco I; R, Eco RI; and S, Sph I. **(C)** The *n1134* mutation is a G-to-A transition in codon 1, which eliminates a Nco I site. The predicted protein product of *n1134* does not fit the consensus for NH_2-terminal myristoylation (*10*).

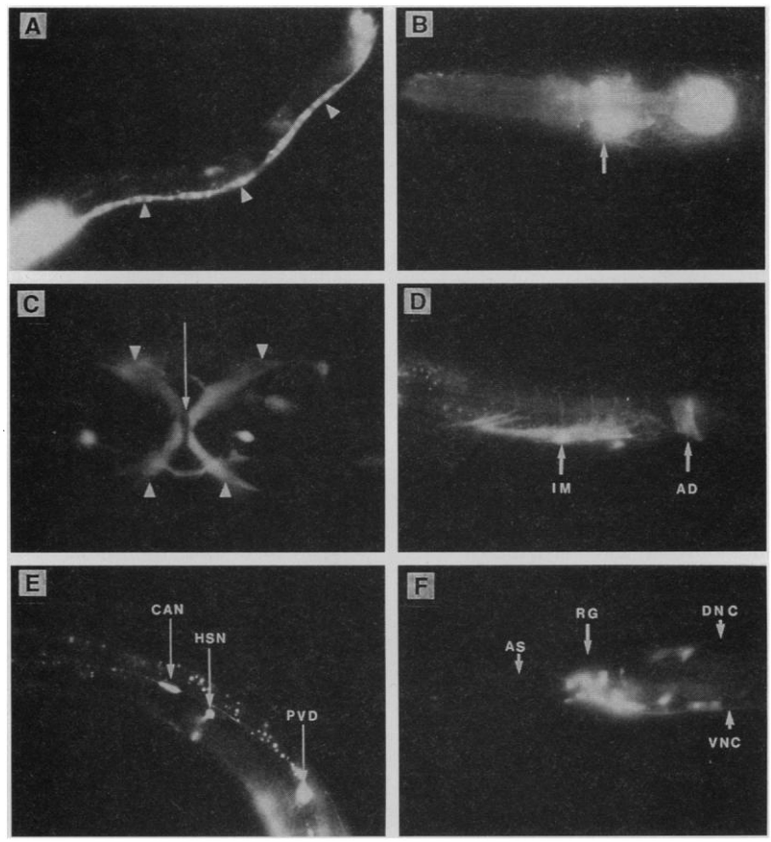

Fig. 2. Expression of $G\alpha_o$ (*12*). **(A)** Ventral cord motor neurons (arrow heads). **(B)** Pharyngeal muscles and nerve ring neuropile (arrow). **(C)** Vulva muscles (arrow heads). Arrow indicates the vulva opening. **(D)** Enteric muscles: the intestinal muscles (IM) and the anal depressor muscle (AD). The anal sphincter muscle also stains but is out of the plane of focus. **(E)** CAN, HSN, and PVD neurons. **(F)** Anterior sensory tracts (AS), ring ganglia (RG), and dorsal and ventral nerve cords (DNC and VNC).

The expression of $G\alpha_o$ in the pharyngeal muscles and in pharyngeal neurons is shown in Fig. 2B. The pharyngeal muscles pump food into the intestine and hence are essential for viability. Mutant animals pump food more slowly than wild-type animals (Fig. 3B) and, consequently, have the characteristic pale appearance of starved animals. Conversely, *goa-1(xs)* animals pump actively in the absence of food, a condition where normal animals pump slowly (Fig. 3B). Because 5-HT normally stimulates pharyngeal pumping (*4*, *16*), we wondered whether the pumping defect of *goa-1* mutants might also reflect defects in serotonergic signaling. However, we found that 5-HT stimulated the pharyngeal pumping of *goa-1(n363)* and wild-type animals equally well (Fig. 4B). Thus, $G\alpha_o$, acting in either pharyngeal neurons or muscles, regulates pharyngeal pumping, probably in response to a neurotransmitter other than 5-HT.

Several lines of evidence suggest that G_o regulates egg laying: Eggs in the hermaphrodite uterus are expelled through the vulva by the contractions of the vulva muscles, the serotonergic HSN motor neurons are presynaptic to the vulva muscles, and 5-HT stimulates egg laying (*5*, *17*, *18*). Because $G\alpha_o$ is expressed in both the vulva muscles and the HSN neurons (Fig. 2, C and E), we analyzed the effects of $G\alpha_o$ expression on egg laying. We found that mutant animals lay eggs hyperactively, whereas *goa-1(xs)* animals lay eggs poorly (Fig. 3, C and D). Octopamine and dopamine are known to inhibit egg laying; therefore, we wondered whether they might signal by means of G_o (*4*). The *goa-1(n363)* mutation had no effect on inhibition of egg laying by octopamine and quinpirole (Fig. 4C). To determine whether lack of $G\alpha_o$ activity in the vulva muscles is sufficient to cause hyperactive egg laying, we examined the egg laying behavior of *goa-1; egl-1* double mutants. Mutations in the *egl-1* gene cause the HSN neurons to die (*5*). Because the *goa-1(n363); egl-1* mutants continue to lay eggs hyperactively (*19*), we conclude that $G\alpha_o$ acts in the vulva muscles to inhibit egg laying. Because both 5-HT and *goa-1* mutations stimulate vulva muscle contractions (and hence egg laying), *goa-1* mutations may cause constitutive 5-HT signaling in the vulva muscles.

During defecation, intestinal contents are expelled through the anus by contraction of the enteric muscles (that is, the intestinal, anal sphincter, and anal depressor muscles) (*20*). Although $G\alpha_o$ is expressed in the enteric muscles (Fig. 2D), we observed no defects in the enteric muscle contractions of either mutant or transgenic animals (*19*). It has been shown that 5-HT inhibits enteric muscle contractions (*21*); therefore, we wondered whether $G\alpha_o$ is required for 5-HT regulation of the enteric muscles. We found that the enteric muscle contractions of wild-type animals are more potently inhibited by 5-HT than are those of *goa-1(n1134)* animals (Fig. 4D). These results suggest that $G\alpha_o$ mediates the inhibitory effects of 5-HT on defecation.

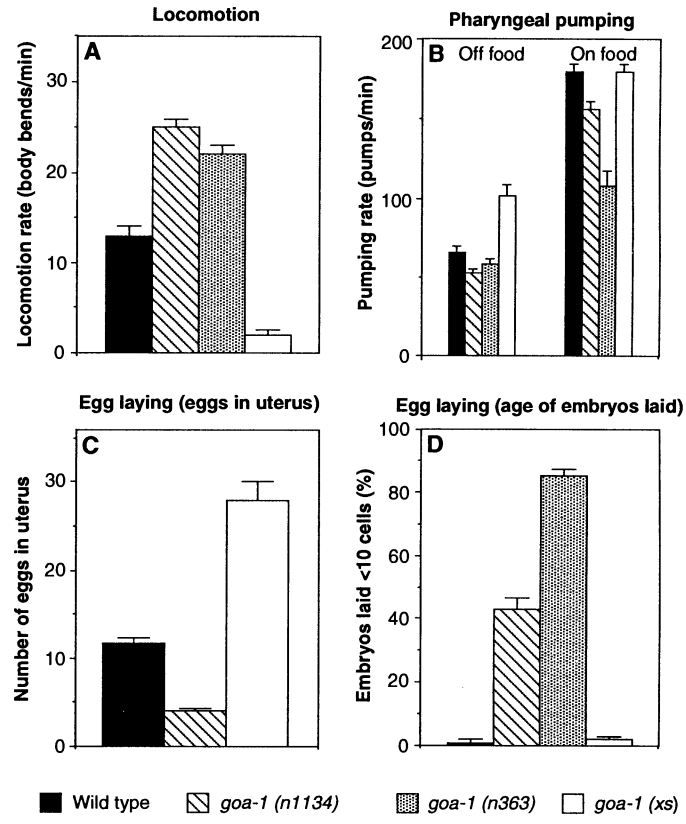

Fig. 3. The dose of $G\alpha_o$ activity modulates several behaviors. Behaviors were quantitated as described (*13*). Genotypes are as indicated by the legend. Error bars indicate the SEM. (**A**) Locomotion rate: *goa-1* > wild type > *goa-1(xs)*. (**B**) Pharyngeal pumping rate: without food, *goa-1(xs)* > wild type = *n1134* = *n363*; with food, wild type = *goa-1(xs)* > *n1134* > *n363*. (**C** and **D**) Egg laying: *goa-1* mutants laid eggs hyperactively, whereas *goa-1(xs)* animals were defective for egg laying, as assessed by the number of eggs in the uterus (C) and the age of embryos when laid (D). Eggs retained in the uterus of *goa-1(n363)* mutants was not reported because these animals have greatly reduced fertility compared with *goa-1(n1134)* animals (average broods 36 and 150, respectively).

Which neurotransmitters signal via G_o? All of the behaviors regulated by G_o are also regulated by 5-HT, and *goa-1* mutants are resistant to the effects of 5-HT on locomotion and defecation (Fig. 4, A and D). In contrast, we consistently failed to find defects in signaling by other agonists (that is, octopamine and dopamine) in *goa-1* mutants, which suggests that the effects on 5-HT signaling are relatively specific (Fig. 4). Although we cannot exclude the possibility that other neurotransmitters signal via G_o, it seems plausible that most of the behavioral effects of $G\alpha_o$ might arise from changes in signaling by 5-HT. Because 5-HT is probably acting as a neurohormone in *C. elegans* (*3*), the widespread expression of $G\alpha_o$ is not surprising.

The mechanisms by which G_o regulates downstream effectors are not known. For other G proteins, signaling occurs via both the α and the βγ subunits (*22*). If $G\alpha_o$

Table 1. Summary of $G\alpha_o$ expression behavioral effects. Cells expressing *goa-1* were determined as described (*12*), and they were identified by their anatomical positions and morphologies (*18*, *26*). The behaviors of wild-type animals treated with 5-HT and of untreated *goa-1* and *goa-1(xs)* animals were analyzed as described (*13*). The sensitivity of mutant animals to 5-HT was determined as described (*15*). The signaling subunit refers to the subunit predicted to mediate 5-HT signaling in each behavior. ND, not determined; NA, not applicable.

Behavior	Cells expressing $G\alpha_o$	Behavioral effect of			5-HT sensitivity of *goa-1* mutants	Signaling subunit
		5-HT	*goa-1*	*goa-1(xs)*		
Locomotion	Motor neurons*	Inhibit	Stimulate	Inhibit	Resistant	$G\alpha_o$
Feeding	Pharyngeal muscles and neurons	Stimulate	Inhibit	Stimulate	Sensitive	NA
Egg laying	Vulva muscles	Stimulate	Stimulate	Inhibit	ND	$G_o\beta\gamma$?
Defecation	Enteric muscles†	Inhibit	Normal	Normal	Resistant	$G\alpha_o$

*Many neurons express $G\alpha_o$ including ventral cord motor neurons (*12*). †Enteric muscles are the intestinal, anal sphincter, and anal depressor muscles.

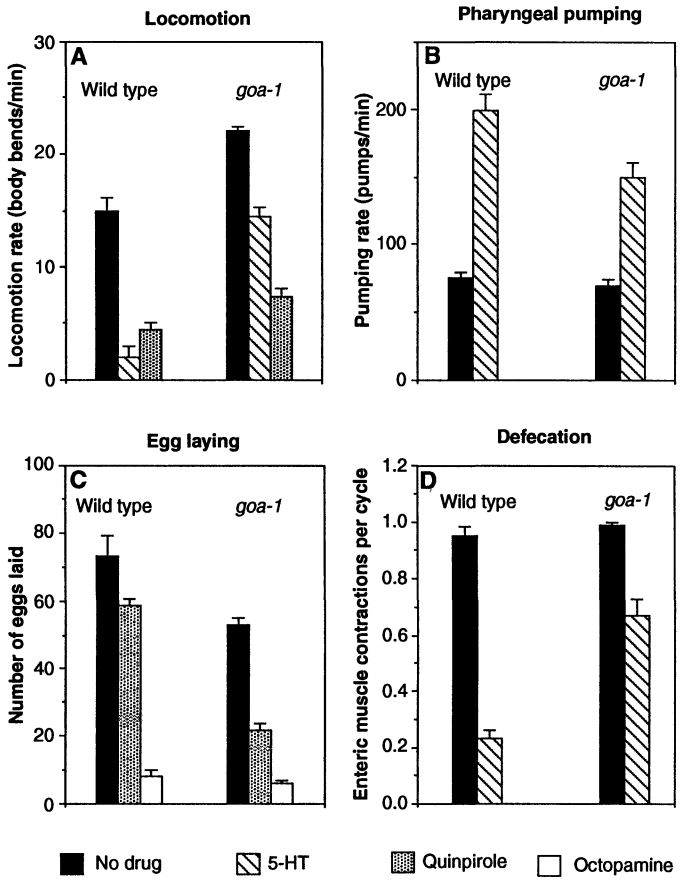

Fig. 4. Sensitivity of *goa-1* mutants to metabotropic agonists (*15*). Drug treatments are indicated by the legend. Error bars indicate the SEM. (**A**) Locomotion of *goa-1(n363)* mutants is partially resistant to 5-HT but fully sensitive to quinpirole. (**B**) 5-HT stimulates the pharyngeal pumping of *goa-1(n363)* and wild-type animals equally well. (**C**) Egg laying of *goa-1(n363)* mutants is fully sensitive to quinpirole and octopamine. Stimulation by 5-HT of egg laying was not analyzed because of the hyperactive egg laying phenotype of *goa-1* mutants. (**D**) Enteric muscle contractions of *goa-1(n1134)* mutants are partially resistant to 5-HT.

signals, then mutations in *goa-1* should block signaling, whereas if βγ signals, then mutations in *goa-1* should produce constitutive signaling. Our results are consistent with the following model for G_o function. Locomotion and defecation are inhibited by 5-HT via $G\alpha_o$, and *goa-1* mutants are defective for 5-HT signaling in these tissues. In contrast, 5-HT stimulates vulva muscle contractions via βγ, and *goa-1* mutants have constitutive 5-HT signaling in the vulva muscles. Alternatively, because βγ subunits facilitate phosphorylation of 7-TMRs by receptor kinases (*23*), defects in $G\alpha_o$ might produce constitutive phosphorylation and hence constitutive desensitization of 5-HT receptors. However, constitutive desensitization of 7-TMRs in *goa-1* mutants would produce widespread agonist resistance, whereas we observed resistance only to 5-HT (Fig. 4). Thus, our results are most consistent with the model that 5-HT signals via G_o, thereby modulating locomotion, defecation, and probably egg laying. Our results lend further support to the proposal that signals produced by 7-TMRs and G proteins engender global changes in behavioral states.

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12. For expression studies, two hybrid genes containing 5′ transcriptional control regions of *goa-1* fused to the coding region of the green fluorescent protein (GFP) gene of *Aequorea victoria* were constructed as follows: for plasmid KP#13, a 3-kb Hind III–Nco I fragment from the cosmid C14E4 (after blunting the Nco I site with mung bean nuclease) was subcloned into the GFP expression plasmid TU#61; for plasmid KP#14, a 6-kb Sph I–Cla I fragment derived from C14E4 was cloned into TU#63, which fuses *goa-1* codons 1 to 15 to the GFP coding sequence [M. Chalfie, Y. Tu, G. Euskirchen, W. W. Ward, D. C. Prasher, *Science* **263**, 802 (1994)]. Similar expression patterns were observed in transgenic animals prepared with *rol-6* and *lin-15* as transformation markers [C. Mello, J. Kramer, D. Stinchcomb, V. Ambros, *EMBO J.* **10**, 3959 (1991); L. Huang, P. Tzou, P. Sternberg, *Mol. Biol. Cell* **5**, 395 (1994)]. Pharyngeal muscles and HSNs stain only with KP#14 transgenes. Expressing neurons include: sensory neurons ALM, AVM, IL1, OLQ, OLL, PHA, PVD, and PVM; motor neurons SMD, HSN, RIM, RMD, and RMG; interneurons BDU, CAN, RID, PVC, and SIA; and the pharyngeal neuron NSM. Many neurons were not identified because of confounding expression by the pharyngeal and enteric muscles. The head mesodermal cell and the spermatheca also express *goa-1*. Similar expression studies were also performed by J. Mendel *et al.* [*Science* **267**, 1652 (1995)].
13. For behavioral studies, animals at room temperature on petri dishes containing *Escherichia coli* as a food source were analyzed as follows: locomotion, body bends per minute; feeding, contractions of the terminal bulb of the pharynx per minute (*16, 24*); egg laying, the age of embryos when laid (estimated by counting the number of embryonic cells) and the number of eggs in the uterus; and defecation, enteric muscle contractions per defecation cycle (*20*).
14. For overexpression studies, transgenic animals expressing KP#10 (1 μg/ml, Fig. 1B) were prepared with either *rol-6* or *dpy-20* (50 μg/ml) as transformation markers. The *dpy-20*–derived *goa-1(xs)* strains were used to analyze locomotion (Fig. 3A).
15. Behaviors of animals were quantitated after treatment with metabotropic agonists as follows: locomotion, 2 hours of exposure to 6.5 mM 5-HT or quinpirole (1 mg/ml); pharyngeal pumping, 60 min on 6.5 mM 5-HT; enteric muscle contractions, 10 to 16 hours on 3.2 mM 5-HT; and egg laying, eggs laid in 90 min on quinpirole (0.4 mg/ml) or octopamine (20 mg/ml). With the exception of feeding, all behaviors were assayed in the presence of *E. coli*.
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