Characterization of GAR-2, a Novel G Protein-Linked Acetylcholine Receptor from *Caenorhabditis elegans*

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Abstract: We have previously identified two G protein-linked acetylcholine receptors (GARs), GAR-1 and GAR-3, in the nematode *Caenorhabditis elegans*. Whereas GAR-3 is a homologue of muscarinic acetylcholine receptors (mAChRs), GAR-1 is similar to but pharmacologically distinct from mAChRs. In the current work we isolated a new type of GAR using *C. elegans* genome sequence information. This receptor, named GAR-2, consists of 614 amino acid residues and has seven putative transmembrane domains. Database searches indicate that GAR-2 is most similar to GAR-1 and closely related to GAR-3/mAChRs. The overall amino acid sequence identities to GAR-1 and GAR-3 are ∼32 and ∼23%, respectively. When GAR-2 was coexpressed with the G protein-activated inwardly rectifying K⁺ (GIRK) channel in *Xenopus* oocytes, acetylcholine was able to evoke the GIRK current in a dose-dependent fashion. Oxtremorine, a classical muscarinic agonist, had little effect on the receptor, indicating that GAR-2 is pharmacologically different from mAChRs but rather similar to GAR-1. GAR-2 differs from GAR-1, however, in that it showed virtually no response to muscarinic antagonists such as atropine, scopolamine, and pirenzepine. Expression studies using green fluorescent protein reporter gene fusion revealed that GAR-2 is expressed in a subset of *C. elegans* neurons, distinct from those expressing GAR-1. Together with our previous reports, this study demonstrates that diverse types of GARs are present in *C. elegans*. Key Words: GAR-2—G protein-linked acetylcholine receptor—Muscarinic acetylcholine receptor—*Caenorhabditis elegans*—*Xenopus* oocyte expression—Green fluorescent protein fusion.


Acetylcholine (ACh) is known to be a major neurotransmitter in both vertebrates and invertebrates. In humans, ACh has been implicated in various nervous functions such as contraction of skeletal muscles, emotion, perception, cognition, learning, and memory. Two distinct types of cholinergic neurotransmission exist: a rapid form mediated by nicotinic ACh receptors (nAChRs) and a slow form mediated by muscarinic ACh receptors (mAChRs). Whereas nAChRs are pentameric proteins that act as ligand-gated ion channels, mAChRs are single polypeptides with seven transmembrane domains that mediate signal transduction by activating G proteins and intracellular effector molecules.

The functional roles of ACh in the nervous system are diverse, and this functional diversity stems largely from the molecular diversity of both nAChRs and mAChRs. Gene cloning studies have shown that vertebrate nAChRs are encoded by multiple α and non-α subunit genes. Various combinations of these subunits could yield many subtypes of nAChRs with different channel properties (McGehee, 1999). For vertebrate mAChRs, five subtypes (m1–m5) are encoded by separate genes (Bonner et al., 1987, 1988). The mAChR subtypes exhibit tissue-specific expression and mediate distinct biochemical responses (Kubo et al., 1986a,b; Peralta et al., 1987, 1988).

*Caenorhabditis elegans* is considered to possess most of the fundamental components of vertebrate synapses and thus may serve as a model for investigating the molecular mechanisms underlying synaptic function. Genetic analyses have identified many *C. elegans* genes that are involved in cholinergic neurotransmission. Two
Drugs

Acetylcholine, (±)-isoproterenol hydrochloride, 5-hydroxytryptamine (5-HT) creatine sulfate, oxotremorine free base, carbamylcholine chloride (carbachol), atropine sulfate, (−)-scopolamine hydrochloride, and pirenzepine dihydrochloride were purchased from Sigma.

Isolation of gar-2 cDNA

The wild-type N2 strain of C. elegans was maintained and grown on NGM agar plates seeded with Escherichia coli strain OP50. Total RNA was isolated from a mixed population of C. elegans using the Tri reagent (Sigma). RT-PCR was performed using the RNA PCR kit (Takara) by following the procedure recommended by the manufacturer. PCR primers used are as follows: AF3, 5'-GCTGGAATGCTGGAAAGATGCCGGTTGCA-3' (the underlined sequence CT was changed from the sequence GG to create a BglII site); and AR1, 5'-TAATAAGATCTATGGCAATCAACTCTTCG-3' (a BglII site created is underlined). The PCR products were digested with BglII and ligated into pBluescript that had been digested with BamHI. The nucleotide sequences of the PCR products were determined by the dideoxynucleotide chain termination method, using the Cy5 AutoRead sequencing kit and ALFExpress DNA sequencer (Pharmacia). To determine the 5' end of the gar-2 cDNA, RT-PCR was performed using spliced leader 1 (SL1) or 2 (SL2) as a forward primer and a gene-specific primer AR2 as a reverse primer. The RT-PCR products were ligated into pBluescript and sequenced as described above. The AR2 sequence is 5'-GTGTCGTCCCAAATGATGAG-3'.

Electrophysiological analysis

The cRNAs for GAR-2, pig m2 mAChR, rat GIRK1 channel, human β2-adrenergic receptor, human cystic fibrosis transmembrane conductance regulator (CFTR), and mouse 5-HT2C receptor were prepared by in vitro transcription using the Megascript kit (Ambion). Preparation of Xenopus oocytes and electrophysiological recordings were performed as described by Lee et al. (1999).

Expression of GAR-1:GFP and GAR-2::GFP

To examine the expression pattern of GAR-1, we constructed a fusion gene between the upstream region of gar-1 and the green fluorescent protein (GFP) reporter gene. To obtain the gar-1 upstream region, a cosmid DNA (C15B12; kindly provided by Dr. A. Coulson) containing the gar-1 gene was amplified with primers MF-5UT and MR-5UT. The PCR product, containing ~2.6 kb of the upstream region and the first 16 codons of gar-1, was digested with PstI and BamHI and then inserted into the C. elegans GFP expression vector pPD95.75 (kindly provided by Dr. A. Fire). This construct was named pPD-gar1, and the junction sequences were confirmed by DNA sequencing.

To examine the expression pattern of GAR-2, we constructed a fusion gene between gar-2 and the GFP reporter gene. This construct, which we named pPD-gar2I, contains ~2.7 kb of the upstream region, ~4.6 kb of the first intron, and the first 65 codons of gar-2. To obtain this sequence, a cosmide DNA (F47D12; kindly provided by Dr. A. Coulson) was amplified with two primer pairs, AF-SUT/AR5UT and AF-5UT/AR5. The PCR product, containing ~2.6 kb of the upstream region and the first 16 codons of gar-1, was digested with PstI and BamHI and then inserted into the C. elegans GFP expression vector pPD95.75 (kindly provided by Dr. A. Fire). This construct was named pPD-gar1, and the junction sequences were confirmed by DNA sequencing.
BamHI site created is underlined); and AR5, 5'-ATTGGATC-CTCGAATGCTGTGTACACCGGATA-3' (a BamHI site created is underlined). PCR was performed in a total volume of 50 µl containing 1× plaque-forming unit (pfu) DNA polymerase buffer, 200 µM each deoxynucleotide triphosphate, 2.5 units of cloned pfu DNA polymerase (Stratagene), 50 pmol of each primer, and 100 ng of cosmid DNA. Cycling conditions were 95°C for 45 s, 55°C for 45 s, and 72°C for 6 min (16 min for the PCR with AF-5UT/AR5) for 25 cycles, followed by a 20-min extension at 72°C. pPD-gar1 or pPD-gar2I DNA was microinjected into the gonads of C. elegans, and the transformed progeny were analyzed by confocal (Bio-Rad) or conventional (Carl Zeiss) fluorescence microscopy.

RESULTS

Cloning of gar-2 cDNA

Recently, the complete genome sequence of C. elegans has been reported (C. elegans Sequencing Consortium, 1998). Three genes (C15B12.5, F47D12.2, and C53A5.12) have been predicted to encode putative GARs by computer analysis of the genome sequence. We have previously reported that C15B12.5 encodes a novel GAR (GAR-1) (Lee et al., 1999), whereas C53A5.12, together with Y40H4A.1, encodes a homologue of mAChRs (GAR-3) (Hwang et al., 1999). From the sequence analysis of a cosmid clone F47D12 (GenBank accession no. U22831), we hypothesized that F47D12.2 and F47D12.1, predicted to be two separate genes by computer analysis, may encode a single polypeptide that is similar to GAR-1. To verify this, we performed RT-PCR using F47D12.2-specific oligonucleotide (AF3) as a forward primer and F47D12.1-specific oligonucleotide (AR1) as a reverse primer (Fig. 1, top). From the several PCR products obtained, we were able to isolate a cDNA clone whose nucleotide sequence matches the corresponding genomic sequence. This cDNA, which we designated gar-2, is expected to encode a polypeptide of 614 amino acids with an estimated molecular mass of 69,959 daltons. Comparison of the cDNA sequence with the corresponding genomic sequence reveals that the gar-2 gene spans ~11 kb and contains 10 introns in the coding region (Fig. 1, bottom).

GAR-2 contains seven putative transmembrane domains and two conserved cysteine residues (Cys79 and Cys160) for possible disulfide linkage formation, indicating that GAR-2 has structural features of G protein-linked receptors. GAR-2, however, differs from most G protein-linked receptors in that it carries an extremely short amino-terminal extracellular domain of five amino acid residues and lacks consensus sites for N-linked glycosylation at the amino terminus (Fig. 1, top). To examine whether the proposed ATG initiation codon is authentic, we attempted to determine the 5' end sequence of the gar-2 mRNA. It has been known that many C. elegans mRNAs are trans-spliced at their 5' ends to a 22-nucleotide spliced leader, SL1 or SL2. The gar-2 gene possesses a potential 3' splice acceptor sequence (TTGCAG) 43 bp upstream of the putative ATG initiation codon, raising the possibility of trans-splicing. To test this possibility, we performed RT-PCR using SL1 or SL2 as a forward primer and a gar-2-specific oligonucleotide (AR2) as a reverse primer. When SL1 was used, a band of the expected size of 612 bp was observed. Sequence analysis of this PCR product confirmed that gar-2 has an SL1 sequence at the 5' end (Fig. 1, top). This also strongly suggests that the proposed ATG codon is the start signal for protein synthesis.

Amino acid sequence comparison of GAR-2 with other GARs

Database searches indicate that GAR-2 is most similar to GAR-1. The overall amino acid sequence identity to GAR-1 is ~32% (Fig. 2). When a highly variable third intracellular loop (i3 loop) region is excluded, the identity goes up to 45%. GAR-2, like GAR-1, shows a relatively low degree of sequence identity to GAR-3, the C. elegans mAChR. The amino acid sequence identity between GAR-2 and GAR-3, excluding the i3 loop region, is ~28%. The amino acid sequence identities of GAR-2 to human mAChR subtypes, excluding the i3 loop region, are 27–35%: 32% to human m1, 35% to human m2, 27% to human m3, 33% to human m4, and 30% to human m5. It was noted that GAR-1 and GAR-2 exhibit a high degree of sequence identity in the membrane-proximal segments of the i3 loop. As these segments of mAChRs are known to be important for proper G protein recognition and activation (Wess et al., 1995), it is possible that GAR-1 and GAR-2 share a certain degree of functional similarity.

Site-directed mutagenesis analyses of mAChRs have shown that 11 amino acid residues are critically involved in muscarinic ligand binding (Wess et al., 1995). Indeed, these amino acid residues are conserved among mAChRs, including GAR-3 (Fig. 2). Of the 11 conserved amino acid residues, five are different in GAR-2; four are different in GAR-1 (Fig. 2). Four of the five different amino acid residues in GAR-2 are identical to the four different ones in GAR-1, suggesting that ligand binding properties of GAR-2 may be similar to those of GAR-1. The phylogenetic tree shown in Fig. 3 also indicates that GAR-2 is more closely related to GAR-1 than to other GARs. Taken together, GAR-1 and GAR-2 may share a common ancestor and constitute a novel family of GARs distinct from the mAChR family.

GAR-2 couples to activation of GIRK1 channel in Xenopus oocytes

GAR-1 has been shown to couple to the GIRK1 channel (Lee et al., 1999), which is known to be activated by the GsGoi family in Xenopus oocytes. Here we tested whether gar-2 cDNA encodes a functional receptor that can stimulate the GIRK1 channel. We expressed GAR-2 and GIRK1 by injecting their cRNAs into Xenopus oocytes. Replacement of ND96 solution with high potassium (hK) solution generated an inward current (IhK) in oocytes (Fig. 4A). The amplitude of IhK in water-injected

J. Neurochem., Vol. 75, No. 5, 2000
oocytes (89.0 ± 6.4 nA, n = 5) was similar to that in oocytes expressing GAR-2 alone (93.0 ± 9.7 nA, n = 5). The current amplitude was larger in oocytes expressing GIRK1 alone (235.0 ± 20.0 nA, n = 5). Treatment with 1 μM ACh of oocytes expressing both GAR-2 and GIRK1 gave rise to an additional increase of inward current (484.3 ± 55.8 nA, n = 14). This GAR-2/GIRK1-mediated current was reproducibly evoked by a repeated application of ACh. In control experiments, ACh had little if any effect on the inward current in oocytes expressing either GAR-2 alone (n = 5) or GIRK1 alone (n = 5). As shown in Fig. 4A, the ACh-induced, GAR-2/GIRK1-mediated inward current increased in a dose-dependent manner (EC50 of ~35 nM), although it decreased at a concentration of ACh >10 μM. These results indicate that GAR-2 activates the GIRK1 channel in Xenopus oocytes, probably via the Gia/Go family of G proteins.

**FIG. 1.** Top: Nucleotide sequence and deduced amino acid sequence of the C. elegans GAR-2. Sequences of SL1 and primers (AF3, AR2, and AR1) used for RT-PCR experiments are indicated. Seven putative transmembrane domains are underlined. This sequence has been deposited in GenBank under accession no. AF272738. Bottom: Genomic organization of the gar-2 gene. Black boxes indicate exons; lines indicate introns. The first and fifth introns are unusually long (~4.6 and ~2.3 kb, respectively). ATG, translation initiation codon; TGA, translation termination codon.
We also examined whether GAR-2 couples to other G proteins, besides the G\(_i\)/G\(_o\) family. We focused on the CFTR, a Cl\(^{-}\) channel activated by cyclic AMP-dependent protein kinase after stimulation of G\(_s\) (Uezono et al., 1993), and the Xenopus endogenous Cl\(^{-}\) channel activated by inositol 1,4,5-trisphosphate after stimulation of G\(_q\) or G\(_o\) (Lubbert et al., 1987). When GAR-2 cRNA was coinjected into oocytes with CFTR cRNA, 1 mM ACh treatment elicited only a weak CFTR current (71.4 \pm 14.4 \text{nA}, n = 18) and virtually no transient Cl\(^{-}\) current (20.0 \pm 11.7 \text{nA}, n = 18; Fig. 4B). By contrast, activation of the \(\beta_2\)-adrenergic receptor with 1 mM isoproterenol generated a robust CFTR current (1,009.1 \pm 114.9 \text{nA}, n = 11). Activation of the 5-HT\(_2C\) receptor by 1 mM 5-HT also produced a distinctive transient Cl\(^{-}\) current (2,787.5 \pm 413.4 \text{nA}, n = 4). These analyses indicate that GAR-2 does not couple to G\(_s\), G\(_q\), or G\(_o\) effectively. Taken together, these results demonstrate that the cloned gar-2 cDNA encodes a functional ACh receptor that couples to a G protein, most likely of the G\(_i\) family, in Xenopus oocytes.

Pharmacological comparison of GAR-2 with m2 mAChR

We compared the pharmacological properties of the GAR-2 receptor with those of pig cardiac mAChR (m2), which is also known to couple to the activation of the GIRK1 channel (Kubo et al., 1993). Oocytes expressing GAR-2 and m2 mAChR were co-injected with CFTR cRNA, and ACh-mediated currents were measured in the presence or absence of forskolin or pertussis toxin (PTX). The results showed that GAR-2 and m2 mAChR share similar pharmacological properties, with both receptors being inhibited by the muscarinic antagonists atropine and SC-59589. However, the sensitivity to these antagonists varied between the two receptors, with GAR-2 being more sensitive to the muscarinic antagonist pirenzepine (PZP)

![FIG. 2. Amino acid sequence comparison of the three C. elegans GARs. Amino acid residues that are identical to GAR-2 are highlighted in gray. Putative transmembrane domains (I–VII) are indicated. Amino acid residues reported to be important for muscarinic ligand binding (Wess et al., 1995) are denoted by asterisks. Cysteine residues that are likely to be engaged in disulfide bond formation are indicated by arrows. Sequences were aligned using the Clustal X program. The amino acid sequences of GAR-1 and GAR-3 were taken from Lee et al. (1999) and Hwang et al. (1999), respectively.](image-url)

![FIG. 3. Phylogenetic tree of GARs. The three C. elegans GARs and some representative mAChRs were aligned using Clustal X with default parameters. The phylogenetic tree was drawn using TREEVIEW (Page, 1996). The tree was rooted by using the human histamine H3 receptor as an outgroup. Bootstrap values obtained with 1,000 replicates are shown at the nodes. The protein sequences were taken from public databases with the indicated accession numbers: Hm, human mAChR (Hm1, X52068; Hm2, M16404; Hm3, U29589; Hm4, M16405; and Hm5, M80333); Xm, Xenopus mAChR (Xm4, P30544); Dm, Drosophila mAChR (Dm1, M27495); and HistamineH3, human histamine H3 receptor (AF140538).](image-url)
pig m2 mAChR and the GIRK1 channel produced the inward current (310.0 ± 21.7 nA, n = 14) by treatment with 1 μM ACh (Fig. 5A2 and B2). The potent muscarinic agonist oxotremorine (1 μM) failed to elicit the GIRK current in oocytes expressing GAR-2 and the GIRK1 channel (n = 5; Fig. 5A1, center), whereas treatment with oxotremorine of oocytes expressing m2 mAChR and the GIRK1 channel induced the current (n = 4) as with ACh (Fig. 5A2, center). Furthermore, the GIRK current produced by another muscarinic agonist, carbachol (1 μM), was only 39.2 ± 2.3% (n = 4) of that produced by ACh in GAR-2 (Fig. 5A1, right), whereas this value was much higher in the m2 receptor (81.5 ± 1.7%, n = 5; Fig. 5A2, right). Next we examined the effects of antagonists on the ACh-induced GIRK current activated by the two receptors. Atropine (1 μM), scopolamine (1 μM), and pirenzepine (1 μM) were not able to reduce the current mediated by GAR-2 (Fig. 5B1). By contrast, all these antagonists inhibited the GIRK current mediated by pig m2 mAChR by 98.7 ± 0.8 (n = 5), 97.4 ± 0.8 (n = 5), and 61.4 ± 5.7% (n = 5), respectively (Fig. 5B2). These data show that GAR-2 exhibits pharma-

FIG. 5. Pharmacological comparison of GAR-2 and pig m2 mAChR. A: Specificity of the agonists oxotremorine (OXO) and carbachol (CCH). B: Specificity of the antagonists atropine, scopolamine, and pirenzepine. GIRK1 cRNA (2.5 ng) was injected either with GAR-2 cRNA (2.5 ng; A1 and B1) or with pig m2 mAChR cRNA (2.5 ng; A2 and B2). Drugs were present in the bathing solution during the times indicated by bars. ND, ND96 solution. All the current traces were recorded at a holding potential of −70 mV.
macological characteristics that are different from those of mammalian mAChRs.

Expression pattern of GAR-2::GFP

We examined the expression pattern of GAR-2 during development using a GAR-2::GFP construct (see Materials and Methods). GAR-2 was expressed in a subset of neurons from the folding stage during embryogenesis through adulthood (data not shown). This observation is consistent with our RT-PCR results showing the presence of gar-2 mRNA throughout development (data not shown). We found that GAR-2 was expressed in some head neurons with ciliated endings (Fig. 6C and D), which are putative sensory neurons, in many cells of the ventral cord (Fig. 6F and H), which are considered to be motor neurons, and in the hermaphrodite-specific neuron (HSN) motor neurons (Fig. 6J), which innervate vulval muscles in the hermaphrodite.

We also examined the expression pattern of GAR-1::GFP and observed that GAR-1 was expressed in a subset of neuronal cells different from those expressing GAR-2. GAR-1 was expressed in some head neurons with ciliated endings (Fig. 6A) and in the nucleus periventricularis magnocellularis (PVM) neuron (Fig. 6B).

DISCUSSION

We have previously reported the identification of two GARs in C. elegans, termed GAR-1 and GAR-3. Whereas GAR-3 is a C. elegans mAChR (Hwang et al., 1999), GAR-1 is a new type of GAR that is pharmacologically different from conventional mAChRs (Lee et al., 1999). Here we characterized a third type of GAR, designated GAR-2. Amino acid sequence analysis suggests that like GAR-1, GAR-2 does not belong to any known mAChR subtypes and therefore may constitute a novel family of GARs. Taken together, three different types of GARs appear to mediate the slow form of cholinergic neurotransmission in C. elegans.

By comparing electrophysiology, pharmacology, and expression of the three GARs, we found some similarities and differences among them. First, both GAR-1 and GAR-2, when expressed in Xenopus oocytes, activate the GIRK1 channel. On the other hand, GAR-3 activates the endogenous Cl− channel in Xenopus oocytes. These results imply that the signal transduction pathway mediated by GAR-3 is distinct from those mediated by GAR-1 and GAR-2. Second, GAR-1 and GAR-2 are different from GAR-3 in that muscarinic ligands (such as oxotremoreline, atropine, scopolamine, and pirenzepine) do not work on GAR-1 and GAR-2 as effectively as on GAR-3. GAR-2 is still distinguishable from GAR-1 because GAR-2 is not inhibited at all by muscarinic antagonists (atropine, scopolamine, and pirenzepine) that block GAR-1 to some extent. Third, RT-PCR experiments show that all the three GARs are expressed throughout development, from embryonic to adult stage. GFP expression studies indicate, however, that GAR-1 and GAR-2 are expressed in different types of neuronal cells.

Unlike other GARs, GAR-2 contains no consensus site for N-linked glycosylation at its amino terminus. Nevertheless, GAR-2 was able to activate the GIRK1 channel in response to ACh. This observation suggests that N-linked glycosylation may not be required for the physiological function of GARs. Indeed, it has been shown that elimination of potential N-linked glycosylation sites from the m2 mAChR by site-directed mutagenesis does not affect cell surface localization, ligand binding, stability, or functional coupling of the receptor (van Koppen and Nathanson, 1990). The lack of N-linked glycosylation is not unique to the C. elegans receptor but is also found in some mammalian G protein-linked receptors. Human and rat α2β-adrenergic receptors, for example, have been shown to possess no putative site for N-linked glycosylation (Lomasney et al., 1990; Zeng et al., 1990).

Database searches indicate that most C. elegans genes for G protein-linked receptors contain introns in the coding region, whereas the genes for human G protein-linked receptors are predominantly intronless (Gentles and Karlin, 1999). This seems to be also true for GARs. Nucleotide sequence comparison between the cDNA and the genomic DNA indicates that the gar-2 gene contains 10 introns in the coding region. Two other GAR genes in C. elegans, gar-1 and gar-3, are also shown to have introns in the coding region (Hwang et al., 1999; Lee et al., 1999). By contrast, no intron has been found in the coding region of human GAR (that is, mAChR) genes. Although the neurophysiological significance of this genomic divergence between C. elegans and humans is not fully understood, the introns of the C. elegans receptor genes appear to play two important roles. First, the introns may contain information essential for proper cell-specific expression of the receptors. When we compared the expression patterns of the two GFP constructs with and without the first intron of gar-2, more neurons were observed to express the GFP construct with the first intron. For example, the GFP construct without the first intron, which contains ~2.7 kb of the upstream region and the first eight codons of gar-2, was not expressed in the motor neurons of the ventral cord (data not shown). In the case of gar-1, however, the introns may not be involved in cell-specific expression: inclusion of all the introns of gar-1 did not significantly alter the expression pattern (data not shown). Second, the introns of the C. elegans receptor genes seem to be crucial for generating multiple receptor isoforms from single genes. We have previously shown that three functional GAR-1 isoforms are generated by alternative use of introns of the gar-1 gene (Park et al., 2000). Our preliminary data also indicate that three and two mRNA species are synthesized from the gar-2 and gar-3 genes, respectively. It seems likely that alternative splicing serves as a general mechanism for diversifying GARs in C. elegans.

The expression of GAR-2 in various cell types (Fig. 6) may reflect the involvement of the receptor in diverse behaviors of C. elegans. For instance, the expression of
FIG. 6. Expression patterns of GAR-1 (A and B) and GAR-2 (C–J). A: Confocal image of GAR-1::GFP expression in the head region of a larva. B: GAR-1 expression in the PVM neuron of an adult. C: Confocal image of GAR-2::GFP expression in the head region of a larva. D: Confocal image of GAR-2::GFP expression in the head region of an adult. The animal is laid on the slide 90° twisted from that in C. E and F: GAR-2 expression in the ventral region of an adult shown by (E) Nomarski optics image and (F) GFP fluorescence image. G and H: GAR-2 expression in the tail region of a larva shown by (G) Nomarski optics image and (H) GFP fluorescence image. I and J: GAR-2 expression in the HSN motor neurons of an adult shown by (I) Nomarski optics image and (J) GFP fluorescence image. Bar = 10 μm.
GAR-2 in the HSN motor neurons (Fig. 6J) may indicate that GAR-2 participates in egg-laying behavior because these neurons play an important role in egg laying (Trent et al., 1983; Desai and Horvitz, 1989). We plan to determine the roles of GAR-2 in C. elegans behaviors by generating and analyzing loss-of-function mutants in gar-2.

Many neurotransmitter receptors act not only as postsynaptic signal transducers but also as presynaptic signal modulators. Our GFP expression data indicate that GAR-1 and GAR-2 might be present in some putative sensory neurons. As many synaptic inputs on sensory neurons are considered to be presynaptic, it is possible that GAR-1 and GAR-2 function as autoreceptors and/or heteroreceptors; that is, these receptors are located at the presynaptic site of the sensory neurons and regulate the release of neurotransmitters. In vertebrates, several lines of evidence have shown that mAChRs can work presynaptically. In the electric organ of Torpedo ocellata activation of mAChRs results in the inhibition of ACh release (Michaelson et al., 1979), suggesting the role of mAChRs as negative feedback regulators. Axoplasmic transport of mAChRs in dog splenic nerve (Laduron, 1980) also supports the presynaptic function of the receptors. In addition, immunocytochemistry indicates the presynaptic localization of mAChRs in the rat striatum and hippocampus (Hersch et al., 1994; Rouse et al., 1999). The detailed molecular mechanisms underlying presynaptic control of neurotransmitter release are not known, and the C. elegans system might provide a convenient model for the study.

This study, combined with our previous reports (Hwang et al., 1999; Lee et al., 1999), shows that three types of GARs (GAR-1, GAR-2, and GAR-3) are present in C. elegans. Whereas GAR-3 appears to be the mAChR homologue, homologues of GAR-1 and GAR-2 have not been reported in other organisms. It is not clear at present whether GAR-1 and GAR-2 are uniquely found in C. elegans. It is possible that the existence of these receptors may have been overlooked in other species because potent mAChR-specific agents such as atropine, scopolamine, and oxotremorine are not very effective on these receptors. Further investigation is needed to explore this possibility.

Acknowledgment: We thank Dr. Alan Coulson for providing cosmid clones and Dr. Andrew Fire for providing GFP vectors. This study was supported by grant 1998-019-D00157 to N.J.C. and B.-K.K. from the Korean Ministry of Education and grant 98-NF-04-05-A-01 to J.L. from the Korean Ministry of Science and Technology. Y.-S.L. and S.S. were supported by BK21 Research Fellowships from the Korean Ministry of Education.

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