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Review

Dopamine receptors in *C. elegans*

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Abstract

Dopamine regulates various physiological functions in the central nervous system and the periphery. Dysfunction of the dopamine system is implicated in a wide variety of disorders and behaviors including schizophrenia, addiction, and attention-deficit hyperactivity disorder. Medications that modulate dopamine signaling have therapeutic efficacy on the treatment of these disorders. However, the causes of these disorders and the role of dopamine are still unclear. Studying the dopamine system in a model organism, such as *Caenorhabditis elegans*, allows the genetic analysis in a simple and well-described nervous system, which may provide new insight into the molecular mechanisms of dopamine signaling. In this review, we summarize recent findings on pharmacological and biochemical properties of the *C. elegans* dopamine receptors and their physiological role in the control of behavior.

Keywords: Dopamine; Dopamine receptor; *C. elegans*; Behavior; Genetics

Contents

1. Introduction

Dopamine is a major neurotransmitter in the mammalian central nervous system and regulates neuroendocrine functions, locomotor activity, cognition, and emotion. The dopamine system has been extensively studied because dysfunction of this system is linked to various pathological conditions including Parkinson's disease, schizophrenia, Tourette's syndrome, and drug addiction. Unraveling the signaling pathways involved in the dopamine system may provide a better understanding of the mechanisms underlying these disorders and may help identifying novel therapeutic targets.

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Dopamine is synthesized from tyrosine by two sequential reactions. Tyrosine hydroxylase catalyzes the conversion of tyrosine to L-DOPA (3,4-dihydroxyphenyl-L-alanine), and L-DOPA is converted to dopamine by aromatic L-amino acid decarboxylase. The synthesized dopamine is incorporated into synaptic vesicles through the vesicular monoamine transporter and then released from synaptic terminals. The dopamine signal is transmitted through dopamine receptors on the cell surface. Five dopamine receptors have been cloned and characterized in mammals, all of which are G protein-coupled, seven transmembrane (TM) receptors (Missale et al., 1998; Vallone et al., 2000). Based on their sequence similarities, pharmacological profiles, and biochemical properties, they are divided into two subfamilies, D1-like receptors and D2-like receptors. The D1-like receptor subfamily consists of dopamine D1 and D5 receptors, while the D2-like receptor subfamily consists of dopamine D2, D3, and D4 receptors. D1-like receptors, upon agonist binding, activate adenylyl cyclase through coupling to stimulatory G protein $G_s\alpha/G_{olf}\alpha$ subunits, which in turn increase the intracellular cyclic AMP concentration. By contrast, D2-like receptors couple to $G_i\alpha/G_o\alpha$ subunits to inhibit the activation of adenylyl cyclase. Dopamine receptors can also couple to other signal transduction pathways, such as the regulation of calcium and potassium channel activity and the release of arachidonic acid. However, through which signaling pathway dopamine modulates various behaviors in vivo requires further characterized.

Caenorhabditis elegans is a desirable model organism to study the molecular mechanisms of the nervous system (Riddle et al., 1997). The nervous system of *C. elegans* consists of only 302 neurons, of which the positions and synaptic connections have been well described (White et al., 1986). The simple nervous system allows for the identification of the individual neurons involved in various behaviors and specific physiological functions. The complete genome information (*C. elegans* sequencing consortium, 1998), powerful forward and reverse genetic approaches (Riddle et al., 1997), and a number of behavioral assays (Rankin, 2002) are available to study in *C. elegans*. Because of these features, studies in *C. elegans* have revealed numerous novel genes that function in the nervous system. Genetic analysis of the dopamine system in *C. elegans* could provide new insight into in vivo regulation and signaling of this system by revealing novel components.

Direct detection of dopamine by formaldehyde-induced fluorescence (FIF) has revealed eight dopaminergic neurons in the nervous system of *C. elegans* hermaphrodites: two ADE neurons, four CEP neurons, and two PDE neurons (Sulston et al., 1975; for the description of *C. elegans* neurons, see White et al., 1986 and http://www.wormbase.org/). These neurons are likely sensory neurons because they have ciliated endings in the cuticle (Perkins et al., 1986; Ward et al., 1975; White et al., 1986). Six additional dopaminergic neurons exist in the sex-specific structures of male animals: three pairs of ray sensory neurons, R5A, R7A, and R9A. The *C. elegans* genome contains several orthologues of mammalian genes known to be involved in dopamine synthesis and signal transduction (for review, see Wintle and Van Tol, 2001). Some of these genes have been functionally characterized and indeed possess properties similar to their mammalian counterparts. Mutant animals deficient in some of these genes have been isolated and characterized. Mutants for tyrosine hydroxylase, GTP cyclohydrolase (required for tyrosine hydroxylase function), aromatic L-amino acid decarboxylase, and vesicular monoamine transporter display abnormal dopamine FIF staining (Duerr et al., 1999; Lints and Emmons, 1999; Sulston et al., 1975), indicating that the genes coding for those proteins actually participate in dopamine synthesis and transport. The dopamine transporter has been identified and characterized in *C. elegans* and, as in mammals, the dopamine transporter and the tyrosine hydroxylase are both expressed in the dopaminergic neurons (Fig. 1; Lints and Emmons, 1999; Nass et al., 2002). Similar to the mammalian form, the *C. elegans* orthologue dopamine transporter has been shown to mediate neurotoxin-induced degeneration of dopaminergic neurons (Jayanthi et al., 1998; Nass et al., 2002). Exogenous application of dopamine on *C. elegans* inhibits locomotion and egg laying (Schafer and Kenyon, 1995; Weinshenker et al., 1995), and the mutant strains deficient in dopamine have defects in food sensing (Duerr et al., 1999; Sawin et al., 2000), showing that dopamine controls various behaviors in *C. elegans*. These findings establish that a functional dopamine system exists in *C. elegans*. In this paper, we review recent progress in *C. elegans* dopamine system research, with special emphasis on pharmacological and genetic analyses of *C. elegans* dopamine receptors.

2. Dopamine receptor genes of *C. elegans*

To date, two dopamine receptors have been cloned and functionally characterized from *C. elegans*: the D1-like

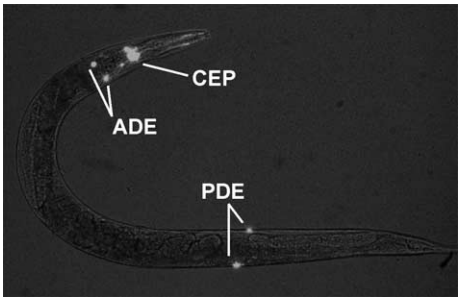

Fig. 1. Dopaminergic neurons in *C. elegans*. Dopaminergic neurons are visualized by GFP expression under the promoter of *C. elegans* dopamine transporter (*dat-1*). Animals carrying *dat-1*::GFP was provided by Dr. Randy Blakely.

receptor DOP-1 (F15A8.5; Sanyal et al., 2004; Suo et al., 2002) and the D2-like receptor DOP-2 (K09G1.4; Suo et al., 2003). Cloning of cDNAs for DOP-1 and DOP-2 reveal that they contain seven transmembrane domains (TM) and some characteristic features of G protein-coupled receptors, such as the DRY sequence at the end of TM III, consensus phosphorylation sites for protein kinases, and consensus *N*-glycosylation sites. These receptors also contain amino acid residues thought to be important for dopamine binding: an aspartic acid residue in TM III and two serine residues in TM V (Livingstone et al., 1992; Pollock et al., 1992). DOP-1 shows higher homology with D1-like receptors than D2-like receptors, and the highest homology was found with insect D1-like receptors, such as *Drosophila melanogaster* DmDOP1 (Gotzes et al., 1994; Sugamori et al., 1995) and *Apis mellifera* AmDOP1 (Blenau et al., 1998). DOP-2 shows higher homology with D2-like receptors and the highest homology with the *Drosophila melanogaster* D2-like receptor DD2R (Hearn et al., 2002). The gene for DOP-1 contains eight introns and at least three splice variants are generated by alternative splicing in the regions coding the third intracellular loop and C-terminal tail. This differs from the mammalian D1-like receptor genes, which do not contain introns in their coding sequences (Missale et al., 1998; Vallone et al., 2000). DOP-2 also has two splice variants that differ in the length of the third intracellular loop. This form of splice variation is also found in mammalian dopamine D2 receptors (Dal Toso et al., 1989; Giros et al., 1989; Monsma et al., 1989).

Phylogenetic analysis of predicted G protein-coupled receptors have revealed the presence of two additional putative dopamine receptors in *C. elegans*: predicted genes T14E8.3 and C52B11.3. T14E8.3 has a high homology with D2-like receptors and appears to be most closely related to DOP-2. C52B11.3 shows a high homology with the insect dopamine receptor DAMB. DAMB is an invertebrate-specific dopamine receptor cloned from *D. melanogaster* (Feng et al., 1996; Han et al., 1996) and *A. mellifera* (Mustard et al., 2003). When expressed in mammalian cells, T14E8.3 and C52B11.2 exhibited dopamine-induced inhibition and activation of cAMP formation, respectively (M. Sugiura, S. S., and S. I., manuscript in preparation), indicating that these two genes encode functional dopamine receptors. It is possible that additional novel dopamine receptors that have no obvious sequence similarity with any known dopamine receptors exist in *C. elegans*. Such receptors are unlikely to be identified by homology-based searches, but may be isolated in genetic screens for dopamine-related behaviors. An example of this is the genetic screen for mutants defective in serotonin-controlled inhibition of locomotor activity in *C. elegans* identified the serotonin-gated chloride channel (Ranganathan et al., 2000) that has no sequence similarity to mammalian genes.

3. Pharmacology and signal transduction of *C. elegans* dopamine receptors

Characteristic features of mammalian D1-like receptors are the high-affinity binding to benzazepines (e.g., SCH23390: *R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) and the stimulation of cAMP formation through $G_s\alpha$. In contrast, mammalian D2-like receptors show high affinity binding to butyrophenones, like spiperone, and activate $G_i\alpha$ to inhibit cAMP formation. The pharmacological and biochemical properties of DOP-1 and DOP-2 have been determined by their heterologous expression in mammalian cells or in *Xenopus* oocytes (Table 1). There are no established *C. elegans*-derived cell lines that are suitable for heterologous expression.

DOP-1 and DOP-2 were expressed in COS-7 cells to determine their ligand binding properties (Suo et al., 2002, 2003). [^{125}I]iodo-LSD binds to DOP-1 and DOP-2, with a dissociation constant Kd ~3 and ~7 nM, respectively. Competition binding studies show that both DOP-1 and DOP-2 have higher affinities for dopamine than other biogenic amine neurotransmitters (serotonin, norepinephrine, tyramine, and octopamine). Several known dopaminergic antagonists have the ability to displace [^{125}I]iodo-LSD, but the dissociation constants for DOP-2 show no clear correlation with either those of mammalian D1-like or D2-like receptors. In addition, both DOP-1 and DOP-2 show no high-affinity binding to [^{3}H]SCH23390 and [^{3}H]spiperone, the typical high-affinity radioligands for mammalian D1- and D2-like receptors, respectively.

DOP-1 expressed in COS-7 cells mediates dopamine-stimulated [^{35}S]GTP-γ-S binding and is sensitive to cholera toxin (Sanyal et al., 2004). When DOP-1 is coexpressed with the G protein-activated inwardly rectifying potassium channel Kir3.2 and bovine or *C. elegans* $G_s\alpha$ subunits in *Xenopus* oocytes, DOP-1 facilitates robust channel activation, while coexpression of DOP-1 and Kir3.2 with *C. elegans* $G_o\alpha$ does not result in channel activation. Furthermore, DOP-1 stimulates cAMP production in CHO cells. These observations clearly show that this receptor is functionally related to mammalian D1-like receptors. Unexpectedly, Both the specific mammalian D1-like receptor agonist SKF38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol) and the antagonist SCH23390 act as agonists of DOP-1, albeit at high concentrations, and stimulate cAMP formation. In addition, the dopamine D2 antagonists, butaclamol and haloperidol, and the dopamine D1/D2 antagonist flupentixol display inverse agonist activity by reducing the ligand-independent activity of DOP-1. Whether the endogenous DOP-1 receptor displays any constitutive activity is unknown.

The dopamine-induced activation of DOP-2 expressed in CHO cells inhibits forskolin-stimulated cAMP formation (Suo et al., 2003) which is a typical feature of D2-like receptors. Butaclamol, which has high affinity for this receptor in ligand-binding assays, blocks the dopamine-

Table 1
Pharmacological properties of invertebrate dopamine receptors

D1-like receptors		
DOP-1 (*C. elegans*)[a]	agonist	SKF38393, SCH23390
	inverse agonist	Butaclamol, haloperidol, flupentixol
DmDOP1 (*Drosophila*)[b]	agonist	6,7-ADTN, NPA, pergolide, SKF81927, lisuride, L-DOPA, SKF82526, SKF38393, apomorphine
	antagonist	Butaclamol, SCH23390, flupentixol, spiperone, chlorpromazine, clozapine, haloperidol
AmDOP1 (*Apis*)[c]	binding	Lisuride>chlorpromazine >flupentixol>spiperone >butaclamol >6,7-ADTN >SCH23390>haloperidol[d]
	agonist	6,7-ATDN
	antagonist	Flupentixol, butaclamol, spiperone, SCH23390
D2-like receptor		
DOP-2 (*C. elegans*)[e]	binding	Butaclamol>clozapine >SCH23390>haloperidol >spiperone>chlorpromazine >sulpride[d]
	antagonist	Butaclamol
DD2R (*Drosophila*)[f]	Agonist	Bromocriptine, NPA, 6,7-ADTN, apomorphine, SKF82958
	Antagonist	Butaclamol, flupentixol

SKF38393: (±)-1-Phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol. SCH23390: *R*-(+)-7-Chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine. 6,7-ADTN: (±)-2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene. NPA: *R*(−)-Propylnorapomorphine. SKF81927: *R*-(+)-6-Chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine L-DOPA: 3,4-dihydroxyphenylalanine. SKF82526: (*R*)-6-Chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol. SKF82958: (±)-6-Chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine.

[a] (Sanyal et al., 2004).
[b] (Gotzes et al., 1994; Sugamori et al., 1995).
[c] (Blenau et al., 1998; Mustard et al., 2003).
[d] Listed as order of affinity.
[e] (Suo et al., 2003).
[f] (Hearn et al., 2002).

induced stimulation of DOP-2, while other neuroleptics do not block the receptor, which may be due to their low affinities for DOP-2. There is essentially no difference in pharmacological and signaling properties between the splice variants of DOP-1, or the DOP-2 variants.

While DOP-1 and DOP-2 show the highest affinity for dopamine compared to other endogenous amines in *C. elegans* and are linked to signal transduction pathways typical of mammalian D1-like and D2-like receptors, respectively, their pharmacological properties are distinct from those of mammalian dopamine receptors. In general, the affinities of the tested dopaminergic ligands are lower for *C. elegans* receptors than for their mammalian counterparts. In addition, as mentioned above, there is a case of a mammalian antagonist acting as an agonist of DOP-1 (SCH23390). Dopaminergic ligands have been designed for their high affinity and efficacy on mammalian dopamine receptors. Because the binding and selectivity of these compounds are not only dependent on the dopamine "binding pocket", there is no evolutionary pressure to conserve the affinity for these synthetic compounds. Therefore, it is not surprising that the pharmacological properties of the receptors from distantly related species show only small similarities. Dopamine receptors cloned from other invertebrates, such as *Drosophila* DmDOP1 (Gotzes et al., 1994; Sugamori et al., 1995), DD2R (Hearn et al., 2002), and *A. mellifera* AmDOP1 (Blenau et al., 1998; Mustard et al., 2003), also show low affinities for mammalian dopaminergic ligands.

4. Physiological functions of dopamine and dopamine receptors in *C. elegans*

4.1. Dopamine-modulated behaviors in C. elegans

Exogenous application of dopamine has various effects on *C. elegans* behavior. When incorporated into growth media, dopamine inhibits locomotion, egg laying, defecation, and feeding of the wild-type animal. These behavioral effects are blocked by the mammalian D2-like receptor antagonist haloperidol (Schafer and Kenyon, 1995; Weinshenker et al., 1995). Antagonists of mammalian D2-like receptors increase egg laying and defecation of the *egl-2*(n683) mutant, which is defective in these behaviors due to a gain of function mutation of the potassium channel encoded by this gene (Reiner et al., 1995). This suggests that these behaviors are under negative control by endogenous dopamine signaling. However, because *C. elegans* dopamine receptors show distinct pharmacological properties from mammalian receptors, it is possible that some of the effects of these antagonists are not mediated by blocking dopamine signaling.

Wild-type *C. elegans* animals slow their movement upon entering a bacterial lawn, their food source (a.k.a. basal slowing response). This adaptive behavior is thought to be a strategy to maximize foraging by allowing the animals to stay close to their food source. The vesicular monoamine transporter mutant *cat-1*, in which dopamine and serotonin are absent from the nerve terminals, is shown to be defective in this slowing response (Duerr et al., 1999). Sawin et al. (2000) showed that the dopamine deficient mutants *cat-2* (tyrosine hydroxylase mutant) and *cat-4* (GTP cyclohydrolase mutant, which also has decreased serotonin levels) are also defective in the basal slowing response. The slowing response is rescued in these mutant animals when exogenous dopamine is supplied in the media. A series of experiments in which neurons postsynaptic to the known dopaminergic neurons are eliminated by laser ablation suggests that dopamine-mediated regulation of this specific behavior may be at least in part through neurohormonal

activity, rather than solely synaptic transmission (Sawin, 1996). It has also been shown that dopaminergic neurons sense a tactile attribute of bacteria to elicit the slowing response and probably release dopamine in the presence of bacteria. Starved animals, which are kept in the absence of food for 30 min, exhibit stronger slowing response upon reentering the food source (enhanced slowing response). *cat-4* mutant animals are defective in the enhanced slowing response and are rescued by exogenous serotonin, indicating that this starvation-induced behavior is regulated by serotonin signaling (Sawin et al., 2000).

Dopamine, together with glutamate, is also implicated in the area-restricted search (ARS) behavior of *C. elegans* (Hills et al., 2004). Animals turn more frequently in the presence of food, and as time since the last food encounter increases, the turning frequency decreases. This behavior allows the animal to increase the time spent at sources of abundant food by restricting the size of the search area, and to extend the search to a larger area when the food supply is exhausted. The turning behavior in *C. elegans* includes changing the direction from forward to backward (reversal) and sharp head to tail turns (omega turns). Hills et al. (2004) have shown that the turning frequency of *C. elegans* significantly decreases after 30 min in the absence of food. The disruption of the dopamine system, either by ablation of dopaminergic neurons, *cat-2* mutation, or dopamine receptor antagonist treatment, eliminated ARS behavior. However, exogenous application of dopamine to the absence of food increased the turning frequency. Mutations in the glutamate receptor subunits GLR-1 and GLR-2 and the vesicular glutamate transporter EAT-4 also disrupt ARS behavior, indicating that glutamate signaling plays a role in ARS. The *eat-4* mutant animals and double mutants for *glr-1* and *glr-2* do not respond to exogenous dopamine, suggesting that dopamine, released in response to food, modulates glutamate signaling to regulate the turning frequency either by modulating glutamate release presynaptically or by regulating the activity of glutamate receptors postsynaptically.

4.2. Expression patterns and functions of dopamine receptors

The expression pattern of *dop-1* has been examined with reporter constructs in which the regulatory region of *dop-1* has been fused to the green fluorescent protein gene (GFP; Table 2; Sanyal et al., 2004; Tsalik et al., 2003). Two independent studies using different lengths of the regulatory sequences show GFP expression in different sets of neurons. The reporter gene fusions used in these studies contained 1.7 to 4.1 kb upstream sequences as well as some of the exons and introns, but no sequences downstream of the *dop-1* gene. The next predicted gene is about 15 kb upstream from *dop-1*. Therefore, the expression patterns presented in both studies may be incomplete and may require immunohistochemical approaches. However, in both studies, GFP expression is seen in the mechanosensory neurons that are required for the light body touch avoidance (Chalfie et al., 1985) and the tap response (Wicks and Rankin, 1995) namely, ALM, AVM, and PLM neurons (expression in ALM neurons is found in both studies).

Disruption of *dop-1* gene expression by RNA-mediated interference (RNAi) has been conducted in whole-genome analysis of G protein-coupled receptors (Keating et al., 2003). Although RNAi is generally less effective in neuronal cells, it serves as a powerful tool when dealing with a large number of genes due to easier and faster disruption of genes than isolating the individual mutants. In preliminary general screening, RNAi of *dop-1* results in slower movement, poor reversal, and poor touch response which is interesting because the mechanosensory neurons control these behaviors. However, these defects were not examined in detail.

Sanyal et al. (2004) isolated a mutant line that has a 2.4 kb deletion in the *dop-1* gene. This *dop-1* mutant does not show defects in locomotion, egg laying, and the slowing response to food. Because *dop-1* is expressed in the mechanosensory neurons, the ability of *dop-1* mutant animals to respond to mechanical stimuli has been examined. However, *dop-1* mutants respond normally to light body touch. ALM, AVM, and PLM are also shown to be required for the response to a nonlocalized mechanical stimulus, or tap, administered to the culture plate. Adult animals respond to the tap primarily with a reversal response which is mediated largely by ALM and AVM neurons, or

Table 2
Expression patterns of the dopamine receptors and other genes

Gene	Region in fusion (kb)[a]	Expression pattern
dop-1	−4.1~+4.6	ALM, ALN, AVM, PLN, PVQ, RIS, some unidentified neurons in head, excretory gland, head muscle, amphid sheath/socket cells[b]
	−1.7~+1.9	ALM, AUA, PHC, PLM, RIB, RIM, some unidentified neurons in head[c]
dop-2	−4.4~0	PDA, RIA, RID, SIA, SIB, some unidentified neurons in head[b]
	−3.5~+2.2	ADE, CEP, PDE, some unidentified neurons in head and tail[d]
eat-4	−2.2~+0.2	ADA, ALM, ASH, ASK, AUA, AVJ or AIN, AVM, FLP, IL1, LUA, OLL, OLQ, PLM, PVD, PVR[e]
glr-1	−5.3~+4.3	AVA, AVB, AVD, AVE, PVC, AIB, RMD, RIM, SMD, AVG, PVQ, URY[f]
glr-2	−5.2~+6.0	AVA, AVD, AVE, PVC, RMD, AIA, AIB, AVG, RIG, RIA, RIR (?), M1[f]
mec-7	−0.9~0	ALM, ALN, AVM, FLP, PLM, PVM[g]

[a] Genomic regions that are included in the reporter fusions: indicated as the relative position to the translation start position.
[b] (Tsalik et al., 2003).
[c] (Sanyal et al., 2004).
[d] (Suo et al., 2003).
[e] (Lee et al., 1999).
[f] Brockie et al., 2001.
[g] Hamelin et al., 1992.

less frequently by moving forward at a higher speed ("acceleration") which is mediated by PLM neurons. Repeated presentation of the tap stimulus to wild-type animals results in a decrease in the frequency of reversals and reversal distance due to habituation (Rose and Rankin, 2001).

The *dop-1* mutant and wild-type animals respond with similar high rates of reversals to the first tap. Wild-type animals show a decreased reversal rate within 20 to 30 taps at an interstimulus interval of 10 s, while *dop-1* mutant animals habituate significantly faster and show already a large reduction in reversal frequency within 3 to 5 taps. The normal habituation to repetitive tap stimuli is rescued by injection of the wild-type *dop-1* gene into *dop-1* mutant animals. The site of action of *dop-1* for the tap habituation response involves the mechanosensory neurons because the normal habituation is rescued by expression of DOP-1 under control of the *mec-7* promoter that mediates expression in the mechanosensory neurons including ALM, AVM, and PLM (Table 2). *cat-2* mutant animals also display an enhanced habituation phenotype which further confirms that dopamine signaling is involved in this behavior. ALM, AVM, and PLM neurons are not postsynaptic to known dopaminergic neurons, but rather project to them: ALM synapses on CEP, AVM on ADE, and PLM on PDE (White et al., 1986). This suggests that the action of dopamine in this behavior is not through synaptic transmission but through neurohormonal activity. It is possible that the dendritic release of dopamine via the dopamine transporter on the dopaminergic neurons (Falkenburger et al., 2001) may play a role in this form of nonsynaptic neurotransmission. All of these tap habituation experiments have been done in the presence of food which is thought to stimulate dopaminergic neurons and consequently activate the DOP-1 receptor in wild-type animals. It is unknown whether the differences in habituation between wild-type and *dop-1* or *cat-2* mutant animals are absent on bacteria-free plates.

Interestingly, *eat-4* mutant animals also display enhanced habituation to repeated taps (Rankin and Wicks, 2000), similar to that which is observed for *dop-1* mutant animals. In addition, *eat-4* is required for the function of glutamatergic neurons and is expressed in glutamatergic neurons including the mechanosensory neurons, ALM, AVM, and PLM (Table 2; Lee et al., 1999). Taken together with the result on the dopamine-mediated control of ARS, these observations suggest that dopamine and *dop-1* may regulate glutamate release at the mechanosensory neurons to modulate the probability of turning, and consequently, may affect both basal and tap-induced reversal frequency (Fig. 2).

DOP-2 has only recently been recognized as a dopamine receptor in *C. elegans* and, as of yet, little is known about its functional role. The expression pattern of *dop-2* has been examined by a reporter gene fusion approach (Table 2). When animals were injected with the GFP fusion construct using the 4.4 kb upstream region of the *dop-2* gene, expression is observed in RIA, SIA, SIB, RID, PDA, and some other unidentified head neurons (Tsalik et al., 2003). A GFP reporter using a region extending from 3.5 kb upstream of the initiation codon to exon 4 mediates expression in all dopaminergic neurons and several additional neurons, which may include RIA, SIA, SIB, RID, and PDA neurons (Suo et al., 2003). This suggests that sequences downstream from the initiation codon contain regulatory elements required for the expression in dopaminergic neurons. Because *dop-2* is expressed in dopaminergic neurons, this receptor may function as an autoreceptor and regulate the synthesis and the release of dopamine. In mammals, the dopamine D2 receptor functions as an autoreceptor and regulates dopamine release and the activity of the dopamine transporter (Cass and Gerhardt, 1994; L'hirondel et al., 1998; Meiergerd et al., 1993; Mercuri et al., 1997). Because the dopaminergic neurons in *C. elegans* contain a similar complement of dopamine system proteins as seen in mammalian dopaminergic neurons, *dop-2* may fulfill a similar regulatory role as an autoreceptor in *C. elegans*.

In a genome-wide RNAi analysis of the 16,757 *C. elegans* genes, the putative dopamine receptor T14E8.3 was identified as a fat regulatory gene (Ashrafi et al., 2003). When T14E8.3 is disrupted by RNAi, the fat content in the intestine (the fat storage organ for nematodes) is reduced. However, *cat-2* mutants were not isolated in this screen, which may be due to the low sensitivity of neurons to RNAi. Mutant animals for *dop-2*, T14E8.3 and C52B11.3, have not yet been characterized. Because *dop-1* mutant did not show defects in behaviors that are thought to involve

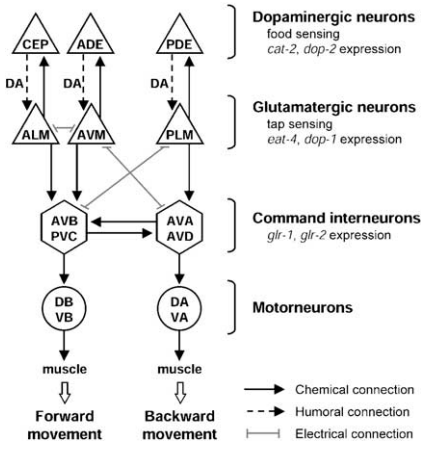

Fig. 2. A simplified diagram of the neural circuit controlling basal and tap-induced reversal frequency. Dopaminergic neurons, CEP, ADE, and PDE, sense a tactile attribute of bacteria which serve as food source for *C. elegans*. Glutamatergic mechanosensory neurons, ALM, AVM, and PLM, sense the nonlocalized mechanical stimuli, tap. Dopamine (DA) is hypothesized to work as a neurohormone to activate DOP-1 expressed in the mechanosensory neurons because the dopaminergic neurons do not synapse on these neurons. Command interneurons, AVA, AVB, AVD, and PVC, that express the glutamate receptors GLR-1 and GLR-2, regulate motorneurons, DA, DB, VA, and VB, to control the direction of movement.

dopamine signaling (i.e., egg laying, defecation, and the slowing response to food), these other dopamine receptors may play a role in these behaviors.

5. Conclusion

Analysis of the *C. elegans* dopamine system has revealed a remarkable conservation with the mammalian dopamine system. Nevertheless, while *C. elegans* has functional orthologues of both D1- and D2-like dopamine receptors, the dopamine receptors of *C. elegans* show distinct pharmacological properties from that of mammalian receptors. However, it is noteworthy that the dopamine-mediated modulation of mechanosensory plasticity, food sensing, and the ARS to maximize foraging may reflect a general role of dopamine in modulating adaptive responses as seen for the mammalian dopamine system. Dysfunction of adaptive dopamine responses may involve various disorders including addiction (Beninger and Miller, 1998; Berke and Hyman, 2000), schizophrenia (Seeman, 1987), and attention-deficit hyperactivity disorder (Seeman and Madras, 1998; Faraone et al., 2001). Furthermore, considering that both the glutamatergic and dopaminergic systems are implicated in schizophrenia (Laruelle et al., 2003; Tsai and Coyle, 2002), the possible role of dopamine in the regulation of glutamate signaling in *C. elegans* is of particular interest. The *C. elegans* dopamine system may also serve as a model system for Parkinson's disease (reviewed in Nass et al., 2001; Nass and Blakely, 2003). Parkinson's disease is characterized by the progressive and irreversible loss of dopaminergic neurons. Specific uptake of environmental toxins through the dopamine transporter and the "over-accumulation" of α-synuclein due to genetic mutations are hypothesized to be the causes of the neurodegeneration (Dauer and Przedborski, 2003). Recent studies have shown that the uptake of the neurotoxin 6-hydroxydopamine through the dopamine transporter (Nass et al., 2002) or overexpression of α-synuclein (Lakso et al., 2003) causes the specific loss of dopaminergic neurons in *C. elegans*. This large number of similarities of the *C. elegans* dopamine system with the mammalian system suggests that *C. elegans* can serve as a model system to study the molecular basis of dopamine-related behaviors and disorders, and that the application of powerful genetic techniques available in *C. elegans* may provide new insight into the molecular mechanisms of dopamine signaling.

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