

Review of octopamine in insect nervous systems

Tahira Farooqui

Department of Entomology, The Ohio State University, Columbus, OH, USA

Abstract: Octopamine (OCT) belongs to a group of compounds known as biogenic amines. OCT, a monohydroxylic analog of norepinephrine, is found in both vertebrate and invertebrate nervous systems. OCT is present in relatively high concentrations in the neuronal and non-neuronal tissues of most invertebrate species studied. However, OCT occurs as a trace amine in vertebrates where its physiological significance remains uncertain. OCT acts as a neurotransmitter, neuromodulator, and neurohormone in insect nervous systems where it prominently influences multiple physiological events. In the peripheral nervous system, OCT modulates the activity of flight muscles, peripheral organs, and most sense organs. In the central nervous system, OCT is essential for the regulation of motivation, desensitization of sensory inputs, arousal, initiation, and maintenance of various rhythmic behaviors, hygiene behavior, and complex social behaviors, including establishment of labor, as well as learning and memory. As a neurotransmitter, OCT regulates endocrine gland activity and controls the emission of light in the firefly lantern. As a neurohormone, OCT is released into hemolymph, transported to target tissues, and induces mobilization of lipids and carbohydrates, preparing insects for a period of extended activity or assisting recovery from a period of increased energy demand. OCT modulates hemocytic nodulation in nonimmune larvae and enhances phagocytosis as a neurohormone. OCT exerts its effects by binding to specific receptors belonging to the superfamily of G protein-coupled receptors and shares the structural motif of seven transmembrane domains. Activation of octopaminergic receptor types is coupled with different second messenger pathways depending on the species, tissue source, receptor type, and cell line used for expression of the cloned receptor. OCT-mediated generation of second messengers is associated with changes in cellular response, affecting insect behaviors. This review describes the roles of OCT in insect nervous systems at the behavioral and molecular levels.

Keywords: octopamine, octopamine receptor, biogenic amine, sympathomimetic amine, nervous systems, insects

Introduction

Octopamine (OCT) was first discovered in the salivary glands of *Octopus vulgaris*.¹ OCT is an invertebrate structural analog of vertebrate norepinephrine. It can be distinguished from norepinephrine by the absence of a hydroxyl group at position 3 of the phenol ring (Figure 1). In invertebrates, OCT induces and modulates signal transduction pathways similar to that of norepinephrine in vertebrates. OCT is present in high concentrations in the central and peripheral nervous systems of most invertebrate species, including insects, where it plays a multifunctional role.²⁻⁵ In contrast, only trace amounts of OCT have been reported in the central and peripheral nervous

Correspondence: Tahira Farooqui
Department of Entomology,
The Ohio State University,
Columbus, OH 43210, USA
Tel +1 614 488 0361
Fax +1 614 488 0361
Email farooqui.2@osu.edu

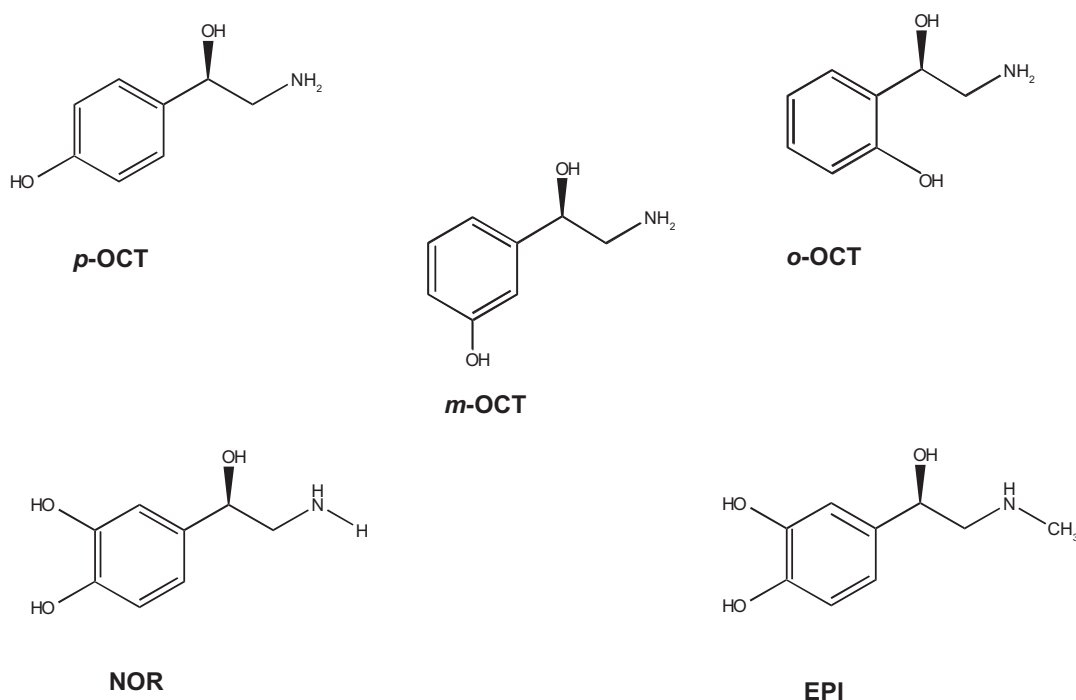


Figure 1 Chemical structures of octopamine isomers, norepinephrine, and epinephrine.

Abbreviations: *p*-OCT, para-octopamine; *m*-OCT, meta-octopamine; *o*-OCT, ortho-octopamine; NOR, norepinephrine; EPI, epinephrine.

systems of vertebrates.^{3–7} OCT is a sympathomimetic amine and known as a false neurotransmitter because it can be stored in vesicles replacing endogenous classical amines such as norepinephrine, dopamine, and serotonin.⁷ OCT is coreleased with other catecholamines, so many of its effects may be indirect, and the existence of octopaminergic receptors has not yet been established in vertebrates. Therefore, OCT seems to play no true physiological role in vertebrates.

OCT is found in high concentrations in the central and peripheral nervous tissues of insects where it serves as a neurotransmitter and a neuromodulator; however, when released in the hemolymph of insects it plays a neurohormonal role.^{2,4,5} Circulating levels of OCT are increased during “stressful” conditions, such as mobilization of lipids and sugars, so OCT is involved in adjusting an insect’s body for a period of extended activity or assisting in recovery from a period of increased energy demand.^{8–15} OCT produces a rapid increase in the circulating hemocyte population in response to bacterial challenge in some insects, such as the American cockroach, *Periplaneta americana*, and the beet armyworm, *Spodoptera exigua*.^{16,17} It is suggested that OCT mediates cellular immune responses such as hemocytic phagocytosis and nodule formation via eicosanoids during bacterial invasion in insects. As a neurotransmitter, OCT regulates emission in the light organ of the firefly and endocrine gland activity in other insects.^{18–21} As a peripheral neuromodulator, OCT

modulates the activities of skeletal and visceral muscles, other peripheral target organs including fat body, oviduct, heart, and sensory organs, and gregarization in locusts.^{22–26} As a centrally acting neuromodulator, OCT plays a major neuromodulatory role in regulating insect behaviors, such as rhythmic behaviors in locusts,^{3,27} locomotion and grooming in fruit flies,²⁸ dance and sting behavior in honeybees,^{29,30} sensitization and dishabituation of sensory input in locusts,^{31,32} discrimination of nestmates from non-nestmates in honeybees and fire ants,^{33,34} feeding behaviors of blowflies, cockroaches and honeybees,^{35–37} division of labor and foraging preference in honeybees,^{38,39} conditional courtship in fruit flies,⁴⁰ visual responses in locust and honeybees,^{31,41–44} learning and memory processes in honeybees, fruit flies, and crickets,^{45–49} and many others (Table 1).

Collective studies support the view that OCT orchestrates multiple physiological and behavioral processes by functioning as a neuromodulator, neurotransmitter, or neurohormone in insect nervous systems, prompting the whole organism to “dynamic action”. OCT plays important roles in the insect nervous system, and the main objective of this review is to update knowledge on OCT metabolism, classification of octopaminergic neurons, octopaminergic receptors, and OCT-mediated signaling, in the hope that this review may shed light on the molecular mechanism(s) underlying complex insect behaviors.

Table 1 Octopaminergic modulation of insect behaviors

Behavior	Insect	Reference(s)
Olfactory learning and memory	<i>Apis mellifera</i> , <i>Drosophila melanogaster</i> , and <i>Gryllus bimaculatus</i>	45–49
Sensitization and dishabituation	<i>Locusta migratoria</i>	31,32
Feeding response	<i>Phormia regina</i> , <i>Apis mellifera</i> , <i>Rhyarobia maderia</i>	35–37
Vision	Locusts and <i>Apis mellifera</i>	31,41–44
Aggression	<i>Drosophila melanogaster</i>	65
Motor control	<i>Locusta migratoria</i>	11–13
Locomotion and grooming	<i>Drosophila melanogaster</i>	28
Rhythmic behaviors	<i>Schistocerca gregaria</i> and <i>Manduca sexta</i>	3,27
Division of labor	<i>Apis mellifera</i>	38,39
Dance behavior	<i>Apis mellifera</i>	29
Discrimination of nestmates from non-nestmates	<i>Apis mellifera</i> and <i>Solenopsis invicta</i>	33,34
Sting response	<i>Apis mellifera</i>	30
Conditional courtship	<i>Drosophila melanogaster</i>	40
Gregarization (behavioral switch)	<i>Schistocerca gregaria</i>	26
Activity and energy metabolism of flight muscles, visceral muscle, peripheral organs, and sense organs	<i>Locusta migratoria</i> and <i>Acheta domesticus</i>	3,8,10–15,24
Ovulation	<i>Drosophila melanogaster</i>	61,64

Isomers

OCT exists in three different structural isomeric forms, ie, *para*- (*p*-), *meta*- (*m*-), and *ortho*- (*o*-), as shown in Figure 1. Each isomeric form exists as D(–) and L(+) enantiomers.^{50–53} However, the naturally occurring isomer of OCT is *p*-OCT in the octopus and other invertebrates, including insects, and is found in high concentration in the central nervous system, peripheral nervous system, and various other peripheral tissues.^{8,54,55} The (–)-enantiomer of *p*-OCT is the naturally occurring form in honeybees.⁵⁶ These findings support the original findings of Harmar and Horn who showed that the (–)-enantiomer of *p*-OCT is over 200 times more potent than the (+)-enantiomer in stimulating adenylyl cyclase activity in the cockroach brain.⁵⁷ The locust forewing stretch receptor has been reported to be more sensitive to D-OCT than to DL-OCT.⁵⁸ The *p*-isomer of OCT has approximately 500 times higher affinity for OCT than does the *m*-isomer, whereas tyramine exerts a nearly eight times lower affinity than *p*-OCT for the locust neuronal OCT receptor (OCT3), suggesting that *p*-isomers and *m*-isomers of OCT have

different affinities for receptors.⁵⁹ Collective evidence supports the presence of *p*-OCT in insects, and the *p*-, *m*-, and *o*-isomers in mammals.

Metabolism in insects

Biosynthesis

OCT biosynthesis from L-tyrosine is a two-step process in a *de novo* pathway (Figure 2A). In the first step, tyrosine is decarboxylated to tyramine by tyrosine decarboxylase.⁶⁰ In the second step, tyramine is hydroxylated on the β -carbon of the side chain to OCT by tyramine β -hydroxylase, a rate-limiting enzyme in the biosynthetic pathway.⁶¹ The enzymatic activities of tyrosine decarboxylase and tyramine β -hydroxylase depend on the availability and concentration of substrates and cofactors. Tyrosine decarboxylase requires tyrosine and pyridoxal phosphate, whereas tyramine β -hydroxylase requires tyramine, ascorbate, and copper to catalyze the reaction.⁶² The enzymatic activity and stability of these enzymes may also depend on transcriptional, translational, and post-translational modifications.⁶²

Tyrosine decarboxylase genes (*dTdc1* and *dTdc2*) and a tyramine β -hydroxylase gene (*T β h*) have been cloned from *Drosophila melanogaster*.^{61,63} *dTdc1* is expressed non-neurally, while *dTdc2* is expressed neurally. Tyramine β -hydroxylase has been found in all neurons and cells that synthesize OCT.⁷⁰ No detectable levels of tyramine and OCT are found in mutant (*Tdc2^{RO54}*) brains of *D. melanogaster*, and affected females are sterile due to egg retention but are not deficient in ovulation.⁶³ However, mutant flies (*T β h^{nM18}*) lacking neural OCT show a 10-fold increase in tyramine levels, but the females are deficient in ovulation.^{61,64} These findings suggest distinct and separable neural activities of OCT and tyramine. Reduction in OCT also decreases aggression in both males and females. In genetic rescue experiments, *Tdc1-Gal4*-driven tyramine β -hydroxylase expression has failed to rescue the aggression phenotype of mutant male flies (*T β h^{nM18}*). However, the combination of *Tdc1-Gal4* and *UAS-T β h* drivers rescued the deficiency in aggression, suggesting that the aggression phenotype in *T β h^{nM18}* mutants is the result of a tyramine β -hydroxylase deficiency in the central nervous system. The combination of *Tdc2-Gal4* and *Cha-Gal80* to drive tyramine β -hydroxylase expression in subesophageal ganglion neurons rescued the aggression phenotype in *T β h^{nM18}* mutants, indicating that OCT and a distinct subset of octopaminergic neurons in the subesophageal ganglion have functional importance in aggression.⁶⁵ Furthermore, tyramine has been reported to regulate transepithelial

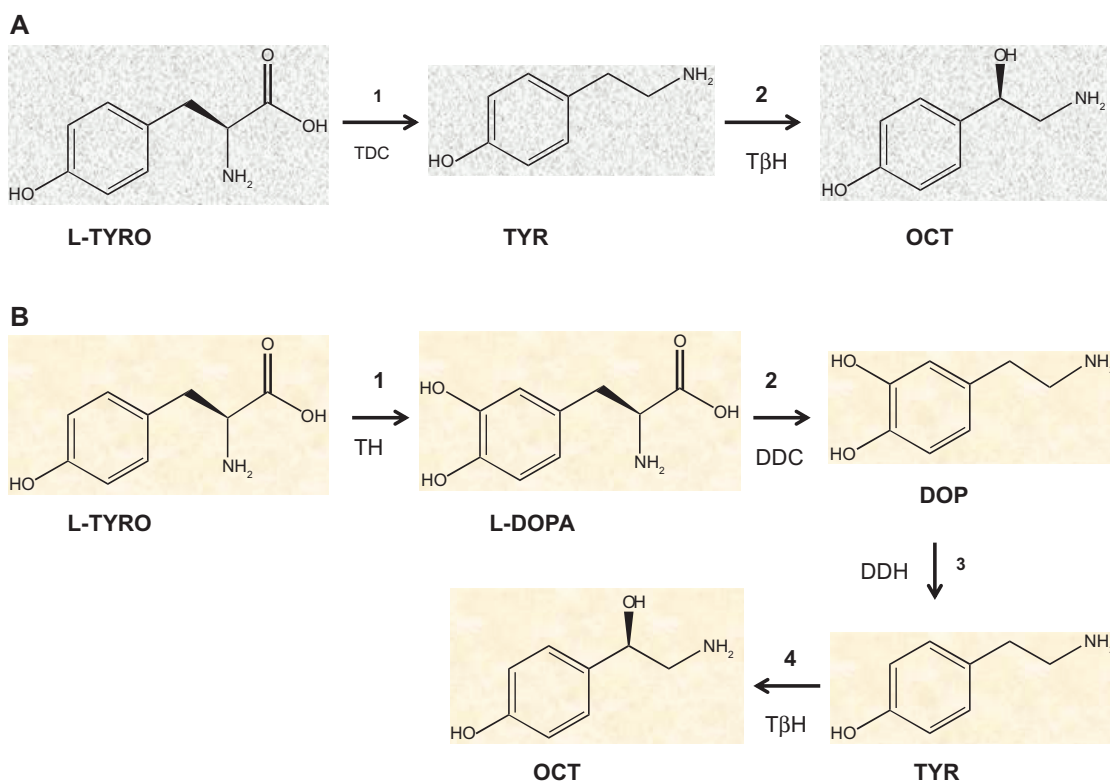


Figure 2 Biosynthetic pathways of octopamine in insects. **(A)** De novo pathway in which L-TYRO is decarboxylated to TYR by TDC (1). TYR is then hydroxylated to OCT by T β H (2). **(B)** Salvage pathway in which L-TYRO is hydroxylated to L-DOPA by TH (1). L-dopa is decarboxylated to DOP by DDC (2). DOP is then dehydroxylated to TYR by DDH (3), followed by conversion of TYR to OCT by T β H (4).

Abbreviations: L-TYRO, L-tyrosine; TYR, tyramine; TDC, tyrosine decarboxylase; OCT, octopamine; T β H, tyramine β -hydroxylase; L-DOPA, 3,4-dihydroxy phenylalanine; TH, tyrosine hydroxylase; DOP, dopamine; DDC, DOPA decarboxylase; DDH, dopamine dehydroxylase.

Cl⁻ conductance in Malpighian tubules of *D. melanogaster*.⁶⁶ The immunohistochemical staining of Malpighian tubules with an antibody against tyramine indicates that stellate cells are the sites of tyramine production, supporting the expression of *dTdc1* in non-neuronal tissue.^{63,66}

Salvage pathway

The salvage pathway may be an alternative pathway for OCT synthesis in insects (Figure 2B). This pathway was proposed 35 years ago.⁶⁷ Since then, it has been a subject of controversy on both logical and technical grounds.^{68–70} Later on, using improved histofluorescence technology, researchers have detected *p*-tyramine, *p*-OCT, and *p*-dopamine in the thoracic nervous system of the locust. Both octopaminergic and dopaminergic neurons have been reported to share common morphological features in the thoracic nervous system of the locust, as well as in the ventral nerve cord of the cricket.^{71,72} Theoretically, in the salvage pathway, the tyrosine decarboxylase reaction may be replaced by tyrosine hydroxylase that converts L-tyrosine to 3,4-dihydroxy-phenylalanine (L-dopa) via a hydroxylation reaction.⁷³ L-dopa is then decarboxylated to dopamine by dopamine decarboxylase.⁷⁴ Dopamine may then be subsequently

converted to tyramine by dopamine dehydroxylase,⁷⁵ followed by β -hydroxylation of tyramine to form OCT.

Hydroxylation of L-tyrosine to L-dopa followed by its decarboxylation to dopamine has been reported in the cerebral ganglion of the cockroach, *P. americana* L.⁷³ The presence of additional L-dopa results in increased OCT synthesis.^{76,77} These studies suggest that OCT and dopamine synthesis share a common first step in the hydroxylation of L-tyrosine to L-dopa in the salvage pathway (Figure 2B). The physiological relevance of this salvage pathway in insects is still not fully understood. However, it may promote the availability of tyramine during diminished levels of tyrosine decarboxylase at certain sites or stages of development in the central and/or peripheral nervous systems of insects.

Release, reuptake, and enzymatic inactivation

In insects, OCT is released into the extracellular space through exocytosis to modulate various metabolic activities. Using labeled OCT, it has been shown that OCT is released by depolarization through high potassium concentration or electrical stimulation.^{78–82} Once OCT is released into the

extracellular space, it binds to its postsynaptic receptors to elicit a physiological response. However, OCT release in the cytosol and reuptake is regulated by the presence of two types of transporters, ie, the transporter that carries OCT into secretory vesicles for storage by endocytosis and the transporter that mediates the reuptake of OCT following exocytosis. Both types of transporters play important roles, not only in the regulation of OCT homeostasis, but also in octopaminergic neurotransmission.

OCT is cleared rapidly from the extracellular space via a reuptake system involving membrane-bound transporters.^{83–86} Reverse-transcription polymerase chain reaction studies indicate that the OCT-type monoamine transporter is widely expressed in all insects, except in representatives of either Diptera (eg, *D. melanogaster*) or Hymenoptera (eg, *Apis mellifera*).⁸⁶ *D. melanogaster* utilizes less selective transporters for cationic amino acids or organic cations as an alternative mechanism for OCT transport.^{87,88} The OCT reuptake system in cockroaches and other insects is strongly inhibited by cocaine, an alkaloid isolated from coca plant (*Erythroxylum coca*) leaves. Cocaine exerts its insecticidal effect at naturally occurring concentrations in coca leaves by blocking OCT reuptake at octopaminergic end terminals in the insect brain, which results in increased OCT concentration in the synaptic cleft, leading to the potentiation of OCT-mediated responses.⁸⁹ The common occurrence of a phenolamine transporter amongst insects but lack of such a transporter in *D. melanogaster* and *A. mellifera* suggests species-specific existence of OCT reuptake systems, implying that OCT may be recycled at the synaptic cleft by alternative pathways. Further genetic and protein-based studies are required to understand the underlying role of transporters in the regulation of OCT release and reuptake systems in insects, because reuptake systems are considered to be an important target for synthesizing specific uptake inhibitors, which can act as novel insecticides.

Enzymatic inactivation of OCT in insects occurs due to amino terminal tagging of selective groups in its structure, and is catalyzed by specific enzymes (Figure 3). The main pathway of OCT inactivation in the central nervous system of the insect is via *N*-acetylation. This reaction is catalyzed by a cytoplasmic *N*-acetyltransferase, which acetylates the amino moiety of OCT, thereby converting it into *N*-acetyl-OCT, downplaying the mechanism of inactivation of oxidative deamination.^{3,67,90–94} Measurable *N*-acetyltransferase activity has been reported in the ventral nerve cord of the cockroach, the central nervous system of the tobacco hornworm and fruit fly, the firefly light organ, and the larvae of the

cattle tick.^{95–97} Some insects also utilize phenylethanolamine *N*-methyl transferase to convert OCT into syneprhine via an *N*-methylation reaction.^{3,98}

Other possible pathways for enzymatic inactivation of OCT may be conjugation reactions, such as β -alanine conjugation, γ -glutamylolation, and sulfate conjugation (Figure 3).^{3,94,96} The β -alanine conjugation reaction is catalyzed by ebony protein in *D. melanogaster*.⁹⁹ The presence of *N*- β -alanyl tyramine has been reported in the central nervous system of *Manduca sexta*, and *N*- β -alanyl-OCT as well as sulfated conjugates of OCT, dopamine, and serotonin in lobster neurons.⁹⁹ The ebony gene cloned from the *P. americana* brain shows homology with ebony sequences from *Anopheles gambiae*, *A. mellifera*, and *D. melanogaster*.¹⁰⁰ These studies support the occurrence of alanine conjugation as an alternative pathway for biogenic amine inactivation in the central nervous system of invertebrates, including insects. The γ -glutamylolation is catalyzed by γ -glutamyltransferase.^{3,94,100} This pathway has been observed in the horseshoe crab *Limulus* brain and eyes where γ -glutamyl OCT plays a role as an intracellular transmitter in the *Limulus* visual system.¹⁰¹ OCT inactivation via sulfation catalyzed by *O*-sulfotransferase has been reported in lobsters.¹⁰² *O*-sulfo-*N*-glutamyl OCT may either be synthesized directly via sulfation of *N*-glutamyl-OCT, a reaction catalyzed by an aryl sulfotransferase; or through a reaction catalyzed by γ -glutamyltransferase using OCT-*O*-sulfate as a substrate, a reaction previously suggested for serotonin catabolism in the mollusc, *Aplysia californica*.^{103,104} Based on these findings, it is suggested that β -alanine, glutamate, and sulfate conjugation reactions may be other major pathways for inactivation of biogenic amines in the insect nervous system (Figure 3). Lastly, monoamine oxidase (the key enzyme in the monoamine inactivation pathway in vertebrates) plays a minor role in inactivating OCT in the insect nervous system.⁹³

Octopaminergic neurons in the insect nervous system

The number of octopaminergic neurons in the insect nervous system varies considerably. The approximate number of neurons present in all ganglia of large insects is 108, and in small insects approximately 40–50 neurons. The distribution of octopaminergic neurons is well documented in insects including honeybees, fruit flies, blowflies, cockroaches, hawkmoths, and locusts.^{105–112} The best characterized group of neuromodulatory neurons in insects constitutes a unique group of unpaired efferent median neurons, the somata of which are located at the dorsal/ventral midline of the

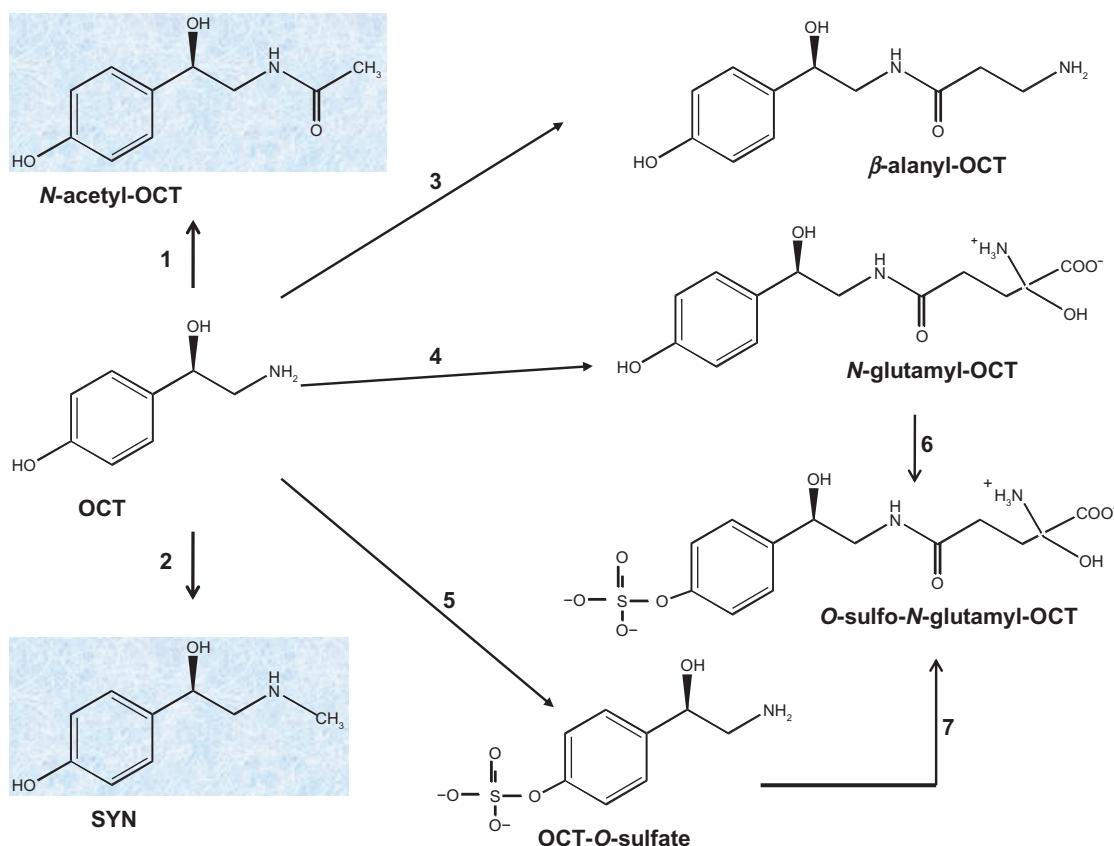


Figure 3 Enzymatic inactivation of octopamine in insects.

Notes: Enzymes involved in OCT degradative reactions are *N*-acetyltransferase (1), PNMT (2), ebony (3), γ -glutamyltransferase (4), *O*-sulfotransferase (5), aryl sulfotransferase (6), and γ -glutamyltransferase (7). Conversion of OCT to *N*-acetyl-OCT by *N*-acetylation is a major enzymatic pathway, whereas conversion of OCT to SYN has limited importance in inactivating OCT.

Abbreviations: PNMT, phenylethanolamine *N*-methyl transferase; OCT, octopamine; SYN, synephrine.

subesophageal ganglion, thoracic, and abdominal ganglia; these neurons are known as dorsal unpaired median or ventral unpaired median neurons.^{113–116} The classification of OCT-like immunoreactive neurons as clusters of cell bodies and perikarya within the cell body in the brain and the subesophageal ganglion has been reviewed elsewhere.^{106,107,111,117} Most unpaired dorsal/ventral efferent median neurons are octopaminergic.^{24,108,109} A subpopulation of the subesophageal ganglion dorsal/ventral unpaired median cells innervates most parts of the brain neuropils and is involved with specific activities and complex behaviors.^{105,118} The dorsal unpaired median and ventral unpaired median neurons also innervate sets of peripheral muscles, glands, and certain types of proprioceptors.¹¹⁹ The peripherally released OCT from dorsal/ventral unpaired median neurons modulates neuromuscular transmission, muscle contraction kinetics, muscle metabolism, and sensory sensitivity, and influences other properties of target organs. However, when released into the circulation, hemolymph OCT acts as a lipid mobilizing neurohormone during flight and long-lasting motor behaviors.^{8,119–121}

Recent immunocytochemical studies clearly demonstrate a fine and comparable distribution of octopaminergic neurons using antibody raised against OCT.^{106–108,111} Several well distinguished clusters of lateral cell bodies in the brain and many midline perikarya provide OCT-like immunoreactive processes to circumscribed regions of the subesophageal ganglion, antennal lobes, optic lobes, and protocerebrum neuropils in different insect species. The locations and projection patterns of OCT-immunoreactive neurons in the brain neuropils and subesophageal ganglion of different insects tested suggest some overlap with distinct differences in the distribution of OCT-immunoreactive processes, implicating common as well as highly specific targets among insect species.¹⁰⁷ Further evaluation will be helpful in recognizing additional neuromodulatory elements because some midline neurons show no OCT-like immunoreactivity on their dendritic processes but possess immunoreactive cell bodies. A previously reported overlap between the distribution of OCT-immunoreactive processes and expression of octopaminergic receptors in insect brain neuropils and in the

subesophageal ganglion¹¹¹ needs further confirmation by evaluating this overlap among various other insect species.

The modulatory roles of OCT-like neurons do not depend solely on their origin but also on the arborization patterns in the target organs where they release OCT. Several groups have investigated the sites of OCT release in the central and peripheral nervous systems and the origin, arborization, and modulatory roles of OCT neurons.^{94,105–124} It is timely to determine OCT levels, octopaminergic receptor subtype/density,

and neural activity/connectivity in specific brain neuropils to show the correlation with complex behaviors in insects.

Receptor classification

The original octopaminergic receptor classification was based on the pharmacological profiles of a range of physiological responses to OCT in an extensor tibiae muscle preparation of the locust.¹²⁵ According to this classification (Figure 4A), octopaminergic receptors in the insect were functionally

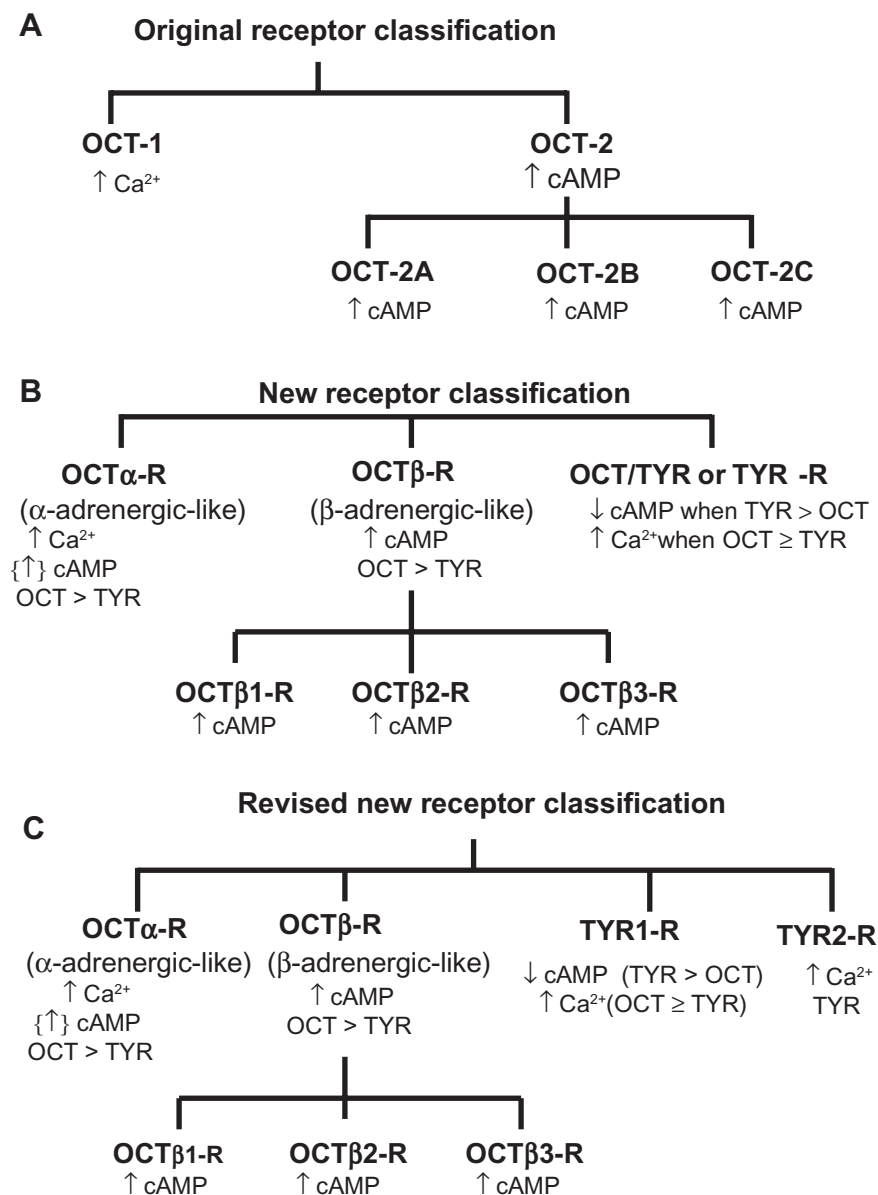


Figure 4 Classification schemes of octopaminergic receptors. **(A)** Original scheme receptor classification based on whole tissue responses, **(B)** new receptor classification based on the structural and signaling similarities of fruit fly cloned *Drosophila melanogaster* octopaminergic receptors with vertebrate adrenergic receptors, and **(C)** revised new receptor classification based on cloning and functional studies of second class of tyramineric receptors. Information is adapted from previous references.^{117,125,149–151}
Abbreviations: Ca²⁺, calcium; cAMP, cyclic adenosine monophosphate; ↑, increase; ↓, decrease. OCT, octopamine; TYR, tyramine.

classified into two main classes, ie, OCT-1 and OCT-2.¹²⁵ The OCT-1 class of receptors is associated with an increase in intracellular calcium Ca^{2+} levels, whereas the OCT-2 class of receptors is associated with an increase in intracellular cAMP levels.^{117,125–127} Based on this pharmacological difference, the OCT-2 class of receptors was initially divided into two subclasses (A and B). OCT-2A receptors are located on the presynaptic terminals of the slow motor neuron and modulate transmitter release, whereas OCT-2B receptors are located postsynaptically on the muscle and modulate the relaxation rate of twitch tension.¹²⁵ Subsequently, a third class, OCT-3, was pharmacologically characterized in the locust brain. OCT-3 is different from the peripheral octopaminergic receptors (1, 2A, and 2B) in terms of its rank order of affinities for selected antagonists and distribution in the insect brain.^{94,129,130} OCT-3 is referred to as OCT-2C due to similarities with OCT-2A and OCT-2B in coupling, with increased intracellular cAMP levels. This classification is based on second messenger changes induced in a variety of intact tissue preparations, so is considered to be problematic, particularly given the existence of more than one receptor subtype in the same tissue preparation.

Later on, progress in molecular cloning studies eased the identification of genes coding for octopaminergic and tyraminer-gic receptors in insects.^{26,131–148} Based on the structural and signaling similarities between cloned *D. melanogaster* octopaminergic receptors and vertebrate adrenergic receptors, Evans and Maqueira proposed a new classification.¹⁴⁹ According to this new classification (Figure 4B), octopaminergic receptors were grouped into three classes, ie, α -adrenergic-like (OCT α -R), β -adrenergic-like (OCT β -R), and octopaminergic/tyraminer-gic (OCT/TYR-R) or tyraminer-gic (TYR-R).¹⁴⁹ The OCT α -R class shows sequence homology with vertebrate α 1-adrenergic receptors. These receptors exert a higher affinity for OCT than tyramine and are coupled with an increase in intracellular Ca^{2+} concentration as well as a small increase in intracellular cAMP levels.^{137,138,141,143,145} The OCT β -R class shows sequence similarities with vertebrate β -adrenergic receptors, and activation of receptors in response to OCT specifically results in increased intracellular cAMP levels.^{144,149} The OCT β -R class is subdivided into several subclasses, which are pharmacologically different from each other.¹⁴⁴

The OCT/TYR-R or TYR-R class of receptors has structural and pharmacological similarities with vertebrate α 2-adrenergic receptors.¹⁴⁹ Depending on the preference of the agonist, these receptors can be stimulated by both

tyramine and OCT. The activation of OCT/TYR-R or TYR-R in response to tyramine is coupled with inhibitory G protein that inhibits adenylyl cyclase, reducing intracellular cAMP levels.^{132,133,139,140,148} However, receptor activation in response to OCT is coupled with an increase in intracellular Ca^{2+} release.^{135,136} Later on, Cazzamali et al cloned a gene (CG7431) from *D. melanogaster* and expressed it in Chinese hamster ovary cells or *Xenopus* oocytes.¹⁵⁰ This group reported that the expressed receptor encodes a protein that is specifically activated by tyramine, implying that it may belong to a new family of tyraminer-gic receptors.¹⁵⁰ In addition to CG7431, this group identified three more homologous genes (one from *D. melanogaster* (CG16766), and two tyramine-like receptor genes in the genomic databases (from the mosquito *A. gambiae* and the honeybee *A. mellifera*), and reported that all four tyramine-like receptors are phylogenetically distinct from the previously identified insect OCT/TYR-R or TYR-R class of receptors.¹⁵⁰

Huang et al cloned a cDNA from the nerve tissue of the *Bombyx mori* silk worm and expressed it in HEK-293 cells.¹⁵¹ This gene encodes a receptor protein (BmTAR2), which has considerably higher affinity for tyramine than other biogenic amines. BmTAR2 shows a tyramine-induced dose-dependent increase in intracellular Ca^{2+} levels (EC_{50} 11.6 nM), whereas OCT and dopamine increase intracellular Ca^{2+} levels only at high concentrations (>100 mM). The selective coupling to intracellular Ca^{2+} mobilization but no effect on intracellular cAMP concentration suggests that BmTAR2 may also belong to a new family of tyraminer-gic receptors.¹⁵¹ These findings favor a revision in the new receptor classification of Evans and Maqueira (Figure 4B),¹⁴⁹ by adding another subclass in the tyraminer-gic class of receptors as shown in Figure 4C.^{150,151}

Furthermore, the author has created a phylogenetic tree based on the comparison of 25 complete nucleotide sequences of insect octopaminergic and tyraminer-gic receptor genes by using the “Muscle” sequence alignment program.^{153–155} Based on nucleotide sequence homology in the phylogenetic tree, insect octopaminergic receptor sequences from the moth *M. sexta* (MsOA),¹⁴⁶ *D. melanogaster* (OAMB)¹³⁷ and splice variants (DmOA1A, and DmOA1B),¹⁴³ *A. mellifera* (AmOA1),¹³⁸ *P. americana* (PaOA1),¹⁴² *B. mori* (BmOAR1),¹⁴⁵ and locust *Schistocerca gregaria* (SgOct α R)²⁶ cluster together in the Oct α -R class, whereas *S. gregaria* (SgOct β R),²⁶ *B. mori* (BmOAR2),¹⁵² and *D. melanogaster* (DmOct β 1-R, DmOct β 2-R, DmOct β 3-R)¹⁴⁴ fall into the Oct β -R class (Figure 5). Tyraminer-gic receptor sequences from *A. mellifera* (Amtyr1),¹⁴⁸ *B. mori* (BmTAR1),¹⁴⁰ *Heliothis virescens*

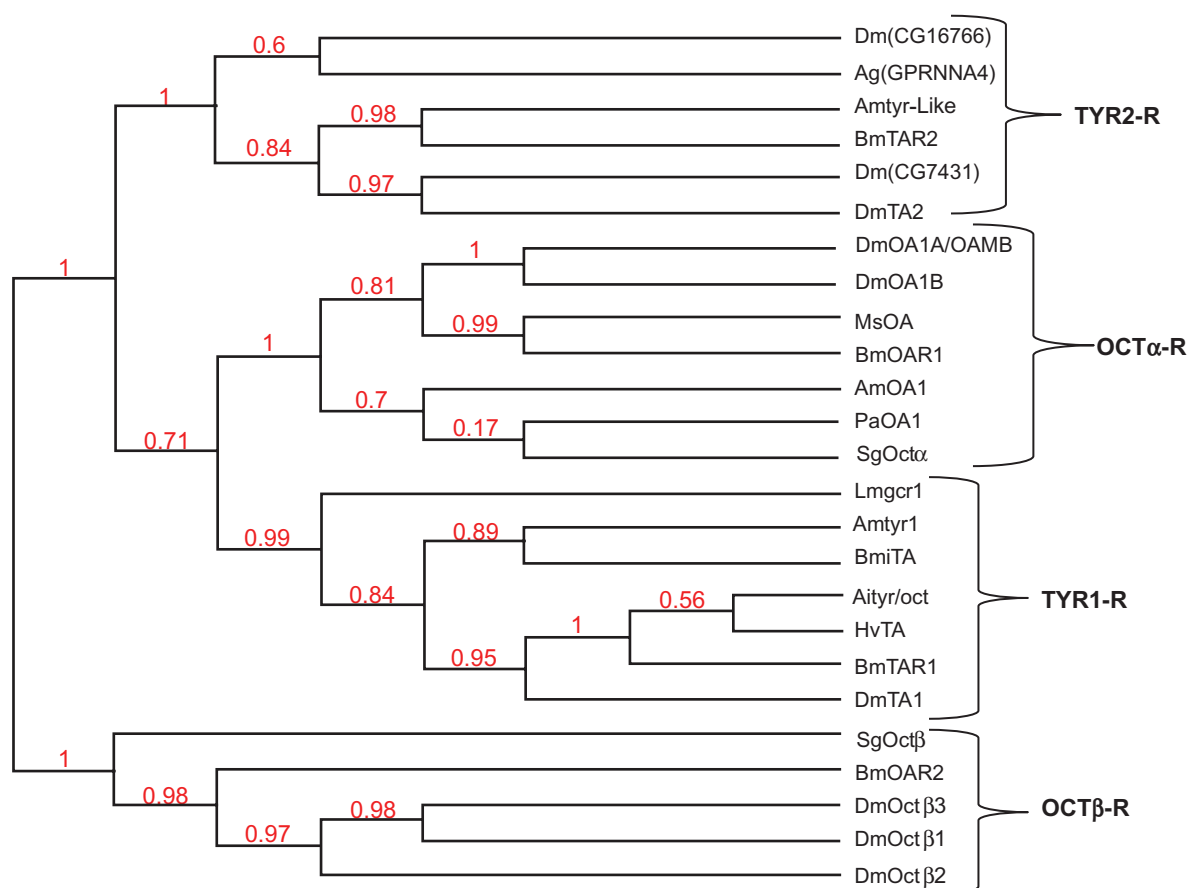


Figure 5 Phylogenetic tree comparison of insect octopaminergic and tyramineric receptors with respect to the new classification proposed by Evans and Maqueira.¹⁴⁹

Notes: Nucleotide sequences were aligned with Muscle (v3.7). After alignment, ambiguous regions containing gaps and/or poorly aligned were removed. The phylogenetic tree was constructed using the maximum likelihood method implemented in the PhyML program. The model (HKY85, statistical test aLRT) was used assuming an estimated proportion of invariant sites (of 0.057) and four gamma-distributed rate categories to account for rate heterogeneity across sites. The gamma shape parameter was estimated directly from the data (gamma 0.789), and the reliability for the internal branch was assessed using the aLRT test.^{153–155} NCBI Databank accession number of genes sequences: AmOA1, AJ547798; DmOAMB, AF065443; DmOA1A, AJ007618; DmOA1B, AJ007617; PaOA1, AY333178; BmOAR1, AB255163; MsOA, DQ840514; SgOCTα, GU237482; SgOCTβ, GU237483; BmOAR2, AB470228; DmOctβ1, AJ880687; DmOctβ2, AJ880689; DmOctβ3, NM_001038954; Amtyr1, AJ245824; Aityr/Oct, FJ640850; BmiTA, AJ010743; HvTA, X95606; Lmtyr (gcr1), X69520; BmTAR1, NM_001044039; DmTA1 (Tyr-Dro), X54794; DmTA2, AY03417; BmTAR2, AB462481; Dm(CG7431), NM_142395; Dm(CG16766), NM_142394; Amtyr-like, NM_001037318; and the genome of the malaria mosquito Ag(GPRNNA4), XM_309588).

Abbreviations: Am, *Apis mellifera*; Dm, *Drosophila melanogaster*; Pa, *Periplaneta americana*; Ms, *Manduca sexta*; Sg, *Schistocerca gregaria*; Bm, *Bombyx mori*; Hv, *Heliothis virescens*; Ai, *Agrotis ipsilon*; Bm, *Bombyx mori*; Lm, *Locusta migratoria*; Ag, *Anopheles gambiae*; NCBI, National Center for Biotechnology Information; OCT, octopamine; TYR, tyramine.

(HvTA),¹³⁴ *Agrotis ipsilon* (Aityr/OCT),¹⁴¹ *Locusta migratoria* (Lmtyr1),¹³⁹ and the cattle tick *Boophilus microplus* (BmiTA)¹⁴⁷ fall into the Tyr1-R subclass. However, *B. mori* (BmTAR2),¹⁵¹ *D. melanogaster* (DmTA1 or Tyr-Dro),¹³² *D. melanogaster* (DmTA2, DmCG7431, DmCG16766),¹⁵⁰ *A. mellifera* (Amtyr-like),¹⁵⁰ and *A. gambiae* genome sequences (GPRNNA4)¹⁵⁰ cluster together in the TYR2-R subclass (Figure 5). This phylogenetic tree further supports applicability of the new Evans and Maqueira classification scheme, except that there are two subclasses in the tyramineric receptor class.

Collectively, based on information obtained from pharmacological and functional studies of expressed receptor proteins and phylogenetic tree analysis of nucleotide sequences,

it is logical to accept revision of the new receptor classification by including an additional subclass in the tyramineric class of insect receptors.

Structural and function of octopaminergic and tyramineric receptors

Similar to adrenergic receptors in mammals, insect octopaminergic and tyramineric receptors belong to the superfamily of G protein-coupled receptors, which share a structural motif of seven transmembrane domains (TM 1-7) to mediate signal transduction in response to an agonist.^{156–160} The N-terminus (NH₂) of biogenic amine receptors is located

extracellularly and the C-terminus (COOH) intracellularly. The N-terminal domain often contains several consensus sites for N-linked glycosylation.^{156,161} The TM 1-7 in G protein-coupled receptors is linked by three extracellular loops (EL-1 to EL-3) and three intracellular (IL-1 to IL-3) loops. The signature residues, such as an aspartate (D) residue in TM3, serine (S) residues in TM5, and a phenylalanine (F) residue in TM6, are conserved in all biogenic amine receptors and contribute to ligand binding.¹⁵⁶⁻¹⁶⁰ The α -adrenergic-like OCT α -R class of receptors is coupled to both G_s and G_q proteins, inducing release of the intracellular second messengers, cAMP and Ca^{2+} . A point mutation study performed in BmOAR1, the α -adrenergic-like OCT receptor, has reported that residues such as D103 in TM3, S198 in TM5, and tyrosine (Y) in TM6 are involved in OCT binding and activation of this receptor through electrostatic or hydrogen bond interactions, but S202 does not participate in this process.¹⁶² The wild-type BmOAR1 exhibits significant stereoselectivity for OCT enantiomers in cAMP production and binding affinity, but not in the Ca^{2+} signaling response.¹⁶³ However, Y to F mutation (Y412F) in BmOAR1 abolishes discrimination between OCT enantiomers in binding affinity and does not evoke any cAMP signaling response, suggesting that Y412 may act as a molecular switch to regulate distinct G protein or multiple G protein couplings.¹⁶³

Other amino acids that maintain the structure and function of G protein-coupled receptors include: a chain of aspartate, arginine, and tyrosine at the cytoplasmic interface of TM3, which is involved in receptor coupling to G protein; two cysteines, one in EL-1 and the other in EL-2, which are involved in forming the disulfide bridge that stabilizes the receptor; and 1-3 cysteine (C) residues in the cytoplasmic tail which may be involved in post-translational modification of receptors with long-chain fatty acids.^{158,160,164} The insertion of palmitic acid (a 16C saturated fatty acid) occurs at one or more cysteine residues on the intracellular side of G protein-coupled receptors in the plasma membrane through a thioester linkage, and this post-translational modification is known as protein palmitoylation.¹⁶⁵ The thioester bond formed between the palmitate and the cysteine is cleavable, so the palmitoylation state of a receptor can be used to regulate its activity.¹⁶⁶ It has been reported that, in rare cases, other lipids can also be attached to G protein-coupled receptors, allowing palmitoylation to occur on residues other than cysteine.¹⁶⁷ It is likely that, similar to many G protein-coupled receptors, most insect octopaminergic and tyraminergeric receptors may undergo palmitoylation, but the enzymatic mechanism involved in palmitoylation remains elusive.

The conserved serine residues in TM5 of adrenergic receptors are believed to interact with the hydroxyl groups of the catecholamine ring to produce hydrogen bonding.^{168,169} These residues are separated by intervening amino acid residues.¹⁷⁰ A multiple sequence alignment of insect octopaminergic and tyraminergeric receptor sequences produced by ClustalW (2.0.12)¹⁷¹ shows that the conserved serine residues in TM5 are separated by a chain of alanine, leucine, and glycine in the Oct α -R class. Similar to the OCT α -R class, separation is achieved by three intervening amino acid residues in the TYR2-R class but the leucine residue in the alanine-leucine-glycine chain is replaced with methionine (Figure 6). In contrast, two serine residues are separated by either one or two intervening amino acids in the OCT β -R and TYR1-R classes (Figure 6). Such differences in the amino acid chain and in the number of intervening residues may depend on the variation in agonist binding affinity with different receptor subtypes due to coupling capacities with second messenger systems via different G proteins (G_s , G_i , and G_q) involving different signaling enzymes such as adenylyl cyclase, protein kinase C, and phospholipase C.^{160,170}

Octopamine and tyramine-mediated signaling

Similar to other biogenic amines, octopamine and tyramine signaling is mediated through binding to distinct receptors that belong to a family of metabotropic G protein-coupled receptors (Figure 7). The second messengers include Ca^{2+} , cAMP, inositol-1,4,5-trisphosphate, and diacylglycerol, depending on species, tissue source, receptor type, and cell line used for the expression of cloned receptor.^{117,160} The interaction of octopamine with OCT α class of receptors (OCT α -R) is coupled with an increase in intracellular Ca^{2+} levels as well as a relatively small increase in levels of intracellular cAMP in response to octopamine (Figure 7).^{117,137,138,142,143} The ligand binding to Oct α -R class is coupled with activation of phospholipase C via the G_q family of G proteins.^{117,137} Phospholipase C enzyme hydrolyzes phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate and diacylglycerol. Inositol 1,4,5-trisphosphate binding to its receptor in the endoplasmic reticulum results in the opening of Ca^{2+} channels, allowing Ca^{2+} release into the cytoplasm. Diacylglycerol and Ca^{2+} activate protein kinase C which regulates the physiological response by phosphorylating various signaling proteins and ion channels. However, the activation of Oct α -R by octopamine stimulates adenylyl cyclase via the stimulatory G proteins (G_s), inducing the production of intracellular cAMP levels, in turn stimulating

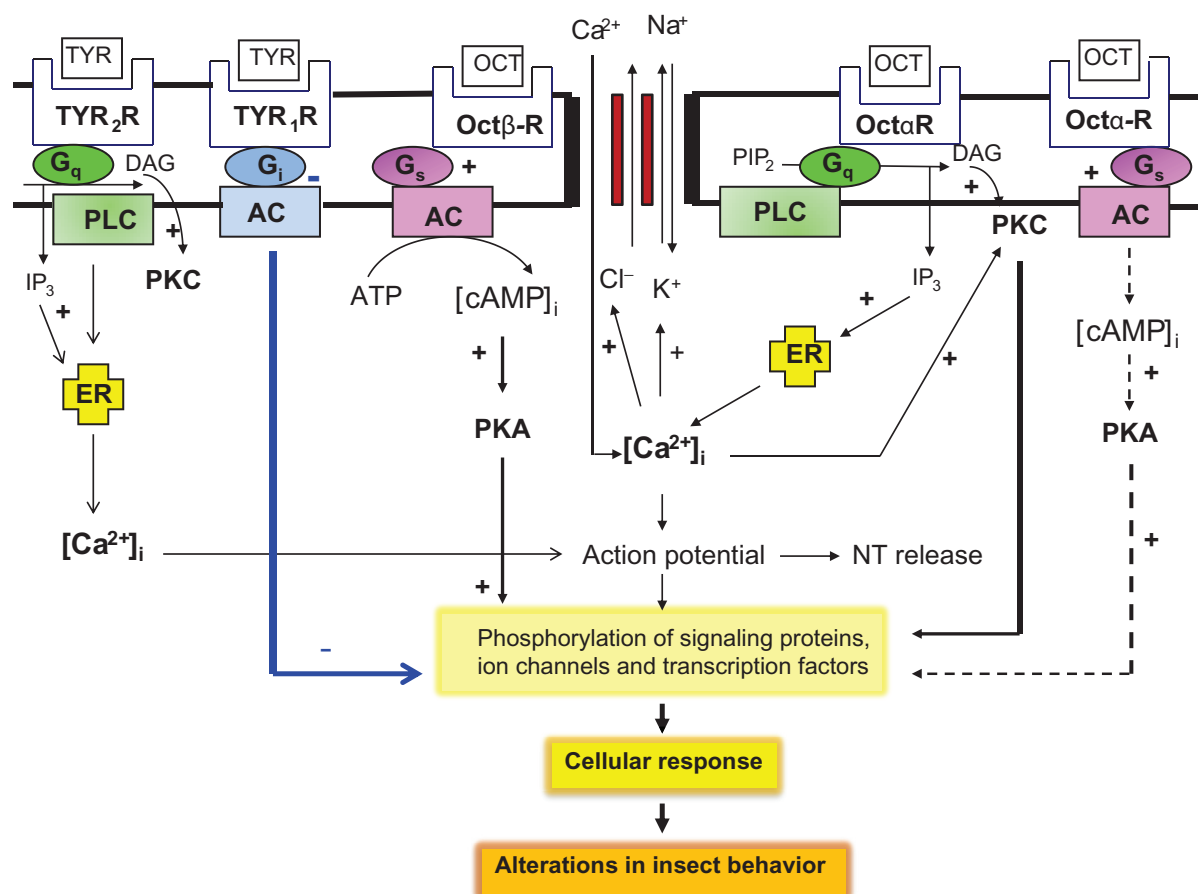


Figure 7 Hypothetical overview of the octopamine and tyramine receptors and second messenger pathways involved in insect signaling.

Notes: The interaction of OCT with Octα-R stimulates PLC via the G_q protein (G), inducing the generation of DAG and inositol 1,4,5-trisphosphate from PIP₂ followed by the release of intracellular calcium Ca²⁺ from the ER. Ca²⁺ and DAG then activate protein kinase C. When cloned Octα-R is expressed in a cell line, it is coupled with adenylyl cyclase activation that results in relatively small increase in cAMP, which is represented by a dashed line. The interaction of OCT with Octβ-R activates adenylyl cyclase via stimulatory G_s protein generating cAMP that stimulates PKA. Both protein kinase C and PKA elicit a variety of cellular responses by phosphorylating different signaling proteins at serine and threonine residues, regulating their activities. The interaction of OCT with TYR₁-R stimulates PLC via G_q protein and is coupled with an increase in intracellular Ca²⁺ levels. The dual role of TYR₁-R in response to TYR and OCT suggests that different agonists induce different receptor conformations, which can allow receptor coupling with different second messenger pathways, associated with selective functions in the cell. TYR specifically interacts with TYR₂-R class, which is coupled with an increase in intracellular Ca²⁺ levels. Activation of protein kinase C and PKA regulate the phosphorylation of signaling proteins, ion channels, and transcription factors, regulating cellular response, and altering insect behavior.

Abbreviations: ER, endoplasmic reticulum; OCT, octopamine; PLC, phospholipase C; DAG, diacylglycerol; PKA, protein kinase A; TYR, tyramine.

neurons, removal of tyramine from the synaptic cleft by the uptake system, and the action of tyramine on specific post-synaptic receptors in the nervous tissue. Release and uptake of tyramine in insects may modulate many physiological and behavioral changes related to insect behaviors.^{151,175,176}

Conclusion

Octopamine is widely distributed in the insect nervous system. It affects several aspects of insect physiology and behavior by acting as a neurotransmitter, a neuromodulator, and a neurohormone. The octopaminergic system of insects (invertebrates) and noradrenergic system of vertebrates are homologous. However, octopaminergic and noradrenergic

systems seem to be restricted to invertebrate and vertebrate physiology, respectively. Octopamine is released by octopaminergic neurons. The binding of octopamine to octopaminergic receptors is coupled with the activation of specific G proteins, which leads to transient changes in concentrations of intracellular second messengers. Further advances in molecular dissection and detailed analysis of octopaminergic signaling in insect nervous systems by using reverse molecular genetic techniques (RNA interference), DNA microarrays, and comparison of genome sequencing in more insects may aid in elucidating the molecular mechanism underlying octopamine-mediated physiological processes and behavioral changes in insects. The existence of distinct

tyraminergetic neurons and receptors in the insect nervous system indicate that tyramine can also act as an independent transmitter, at least in insects. It will be interesting to investigate the TYR2 receptor class further as soon as genome sequences become available for other insects. Greater pharmacological and functional screening of octopaminergic and tyraminergetic receptors may also aid in developing specific, potent, and efficacious agonists and antagonists, which may be important when developing specific insecticides.

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The author reports no conflicts of interest in this work.

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