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Abstract

Plants have evolved beneficial and protective mechanisms including the production of essential oils. Essential oils are the odiferous component of plant extracts, which give plants a variety of unique properties. Essential oils are composed of various terpenoid compounds, particularly monoterpenoids and related aromatic compounds, along with sesquiterpenoids. A variety of terpenoids have been shown to have a toxic effect against insects. It is thought that this toxic action occurs through a neurological mechanism of action. Octopamine receptors and tyramine receptors are G-Protein-Coupled Receptors (GPCRs) primarily found in invertebrates, including insects. GPCRs have been a popular target for pharmaceutical development but not for agrochemical development. A summary of insect octopaminergic and tyraminergic systems is discussed.

Disciplines

Entomology | Neuroscience and Neurobiology

Chapter 4

G-Protein-Coupled Receptors (GPCRs) as Biopesticide Targets: A Focus on Octopamine and Tyramine Receptors

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Plants have evolved beneficial and protective mechanisms including the production of essential oils. Essential oils are the odiferous component of plant extracts, which give plants a variety of unique properties. Essential oils are composed of various terpenoid compounds, particularly monoterpenoids and related aromatic compounds, along with sesquiterpenoids. A variety of terpenoids have been shown to have a toxic effect against insects. It is thought that this toxic action occurs through a neurological mechanism of action. Octopamine receptors and tyramine receptors are G-Protein-Coupled Receptors (GPCRs) primarily found in invertebrates, including insects. GPCRs have been a popular target for pharmaceutical development but not for agrochemical development. A summary of insect octopaminergic and tyraminergic systems is discussed.

The Need for Safe and Effective Insecticides

The growing world population, which is estimated to be around 9 billion by 2050, is placing growing demands on agriculture. The agrochemical and animal health industries are trying to discover new methods to control economically devastating pests, like insects and ticks, along with the diseases these organisms are capable of vectoring. Discovery of agrochemicals and

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veterinary external-parasiticides has become difficult in a changing landscape of agricultural practices characterized by increased public and governmental scrutiny and demands. Such stipulations for agrochemicals include the discovery of compounds having characteristics of decreased toxicity to non-target vertebrate and invertebrate organisms, along with decreased environmental contamination. While significant advances have been made in reducing the use rate and environmental impact of conventional synthetic pesticides, biopesticides do not share an equal amount of the market (*1*). Additionally, biologically-based technology to aid in controlling agricultural pests still lacks public acceptance, and is not as globally accepted outside of the United States. Further restraints on agrochemical development include increased product costs and time to get a product to market (*1*). Currently, agricultural pests are controlled by over 900 types of chemistry that have over 100 mechanisms of action (*2*). However, even with this vast chemistry and mechanisms of action there is still a desideratum for new mechanisms of action. It is important to note that new mechanisms of action, along with new chemistry, are only successful with proper pesticide use and the use of integrative approaches to pest control.

New Agrochemical Targets: G-Protein-Coupled Receptors (GPCRs)

G-Protein-Coupled Receptors (GPCRs) are membrane-bound receptors, which are involved in the sensing of extracellular signals. In turn, the extracellular signal is internalized to result in some physiological or cellular response. This very nature of GPCRs allows them to be highly "druggable" targets, and they have been widely exploited by the human pharmaceutical industry. It is estimated that as much as 50% of all human pharmaceuticals target GPCRs, which indicates their vast importance to normal cellular and physiological functions and their susceptibility to pathological conditions (*3*). However, GPCRs historically have not been a dominant force in the agrochemical market. Recently, there has been growing interest in the discovery of agrochemicals targeting GPCRs (*3*–*6*).

Several ligands can activate GPCRs; here we will focus on biogenic amines as ligands for GPCRs, specifically tyramine and octopamine, and their importance to invertebrate function, particularly in relation to insects. Another significant class of ligands that are capable of activating GPCRs are neuropeptides. The physiological importance of neuropeptides in *D. melanogaster* has been recently reviewed (*7*). Since GPCRs are important to the pharmaceutical industry, there have been several systems developed to study GPCRs, which have also been previously reviewed (*3*, *4*, *6*, *8*).

Octopamine and Tyramine Synthesis

Octopamine and tyramine are biogenic monoamines that are found in the nervous system of arthropods, including ticks and insects. Octopamine and tyramine were originally identified in the salivary glands of the octopus (*9*). Octopamine and tyramine are catecholamines like dopamine, norepinephrine

(noradrenaline) and epinephrine (adrenaline). Other biogenic amines include the indolamines, such as, serotonin or 5-hydroxytryptamine (5-HT) and histamine. Catecholamines use the amino acid tyrosine as the backbone for synthesis, as shown in Figure 1. Briefly, tyramine is the rate-limiting product in the formation of octopamine. Tyramine is produced by the decarboxylation of the amino acid tyrosine via tyrosine decarboxylase (*10*). Tyramine can also be synthesized from a dopamine metabolite; however, this is not believed to be a major synthetic route (*11*). Tyramine is further acted upon by tyramine-β-hydroxylase to form octopamine (*12*).

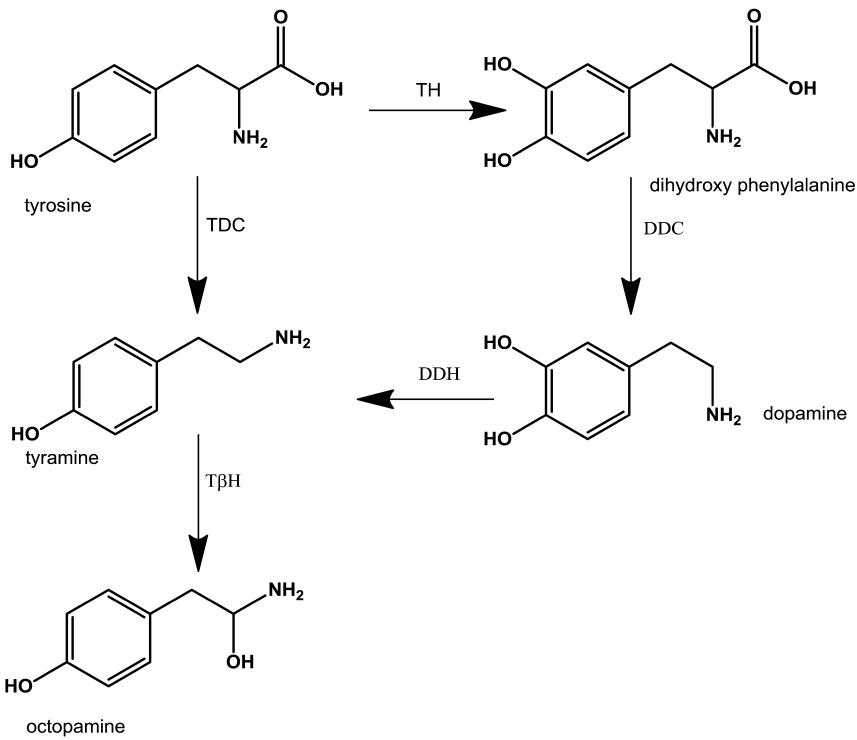

Figure 1. The amino acid, tyrosine, is vital to the synthesis of tyramine and octopamine. Tyramine is synthesized when tyrosine decarboxylase (TDC) converts tyrosine to tyramine. Octopamine is synthesized when tyramine-β-hydroxylase (TβH) converts tyramine to octopamine. It is possible that tyramine can be synthesized from a dopamine pathway when dihydroxy phenylalanine is synthesized from tyrosine via tyrosine hydroxylase (TH). Dihydroxy phenylalanine is converted to dopamine via DOPA decarboxylase. Dopamine is converted to tyramine via dopamine dehydroxylase (DDH).

Signal Transduction of Octopamine and Tyramine

Octopamine and tyramine are released from various portions of the insect's nervous system (*11*, *13*). Octopamine and tyramine's physiological functions are realized when octopamine or tyramine binds to its specific membrane-bound receptors. In turn, the receptor internalizes this original chemical message into a biochemical cascade via the production of second messenger(s), which ultimately results in a cellular response. Octopamine and tyramine primarily activate the superfamily GPCRs. Specifically, octopamine and tyramine activate rhodopsin-like metabotropic GPCRs. GPCRs are sometimes referred to as heptahelical receptors or serpentine receptors; this is because the receptor transverses the cell membrane seven times (7-TM). The seven transmembrane regions of GPCRs are connected by three extracellular loops and three intracellular loops. Residues in several octopamine receptors and several tyramine receptors have been shown to be important in ligand binding and receptor function, which has been discussed in a recent review (*14*). Receptor activation allows for the recruitment of a heterotrimeric intracellular G-protein, which are composed of an α -subunit, β-subunit and a γ-subunit.

The original classification of octopamine receptors was based on second messenger production in various invertebrate tissues. However, the advent of molecular biology has allowed for a comprehensive approach to octopamine receptor classification, now including tyramine receptors as a separate entity. The new classification system is based on sequence homology with the mammalian adrenergic receptors and signaling properties (*15*). That is, octopamine receptors are classified based on sequence similarities and the production of specific second messenger pathways realized during receptor activation. The α-adrenergic-like octopamine receptor (OctαR) shares a sequence homology with the mammalian α-adrenergic receptor(s). Activation of OctαR results in an increase of the intracellular calcium ($[Ca^{2+}]_i$) concentration, which is liberated from intracellular calcium stores, like the endoplasmic reticulum or the sarcoplasmic reticulum, via the activation of the inositol pathway. β-adrenergic-like octopamine receptors (OctβRs) share sequence homology with the mammalian β-adrenergic receptor(s). Activation of OctβRs results in the increase of the intracellular concentration of cyclic adenosine monophosphate (cAMP), via activation of the membrane-bound enzyme adenylate cyclase. It is not unusual for OctαRs and OctβRs to respond to either octopamine or tyramine at different concentrations; this is probably due to structure similarity between octopamine and tyramine. Ligand-agonist coupling or ligand-trafficking, which is peculiarized as the activation of different second-messenger pathways based on the ligand, has been reported for octopamine and tyramine at a single receptor (*16*). Ligand-agonist coupling is commonly found with the octopamine/tyramine or tyramine receptor, which were later classified as tyramine-1 receptors (TAR1). When octopamine activates these receptors, it can result in an increase of the intracellular concentration of calcium. When tyramine activates TAR1, it can result in an inhibitory effect on adenylate cyclase, decreasing the intracellular concentration of cAMP. It is now accepted that tyramine is the preferred ligand of TAR1 (*14*, *17*). Recently, tyramine-2 receptors (TAR2) have been identified, which are also specifically activated by

tyramine, versus octopamine, and have been shown to result in the release of calcium from intracellular stores (*18*, *19*). Bayliss *et al.* has proposed a third class of tyramine receptors (TAR3), which have a different pharmacological profile, and result in an increase of intracellular cAMP, when heterologously expressed in Chinese hamster ovary (CHO-K1) cells. Additionally, TAR3 seems to be specific to *Drosophila melanogaster*, where it is expressed in the crop and eye of the adult flies and the hindgut in larvae (*17*). The signal transduction pathways for these GPCRs are shown in Figure 2.

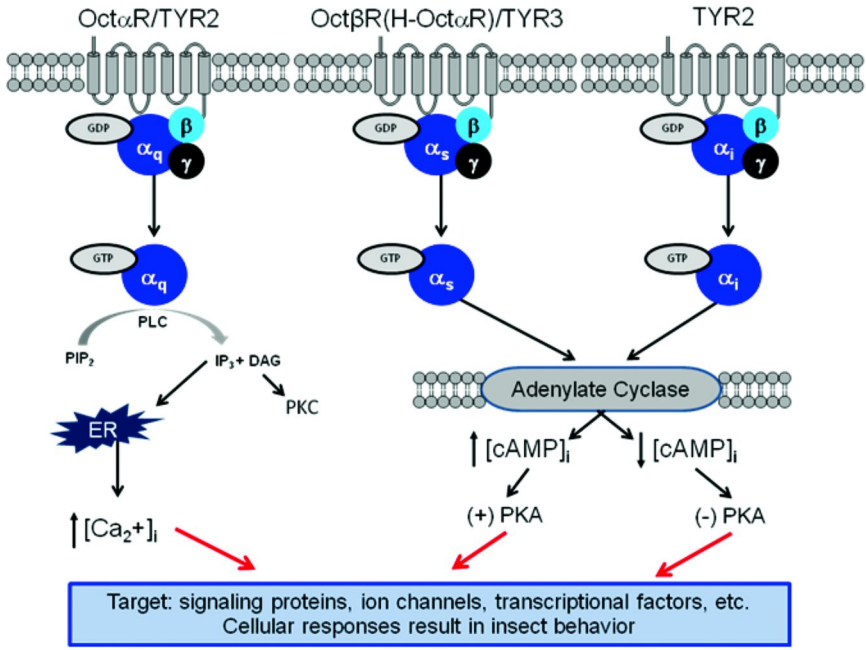

Figure 2. Signal transduction of tyramine and octopamine G-protein-coupled receptors (GPCRs). Here the cellular biochemical pathway is shown for the α-adrenergic-like octopamine receptor (OctαR), the β-adrenergic-like octopamine receptor (OctβR), the Type-1 tyramine receptor (TAR1), the Type-2 tyramine receptor (TAR2) and the Type-3 tyramine receptor (TAR3). Activation of cellular biochemical pathways results in an insect behavior or function. Abbreviations: GDP, guanosine diphosphate; GTP, guanosine triphosphate; PLC, phosphoinositide phospholipase C; PIP_2, phosphatidylinositol-4,5-bisphosphate; IP3, inositol-1,4,5-trisphosphate; DAG, diacyl glycerol; PKC, protein kinase C; cAMP, cyclic adenosine monophosphate; PKA, protein kinase

Ligand-gated ion channels, like GPCRs, are transmembrane ion channels and are involved in the flow of ions into or out of a cell upon the binding of a ligand or chemical message. Recently, ligand-gated chloride channels that are preferentially activated by tyramine have been identified in *Caenorhabditis elegans,* Cel LGC-55 (*20*), and in *Haemonchus contortus*, Hco-LGC-55 (*21*). Cel-LGC-55

appears to act on neck muscles to suppress head oscillation and promote backward movement or reversal behavior in *C. elegans* (*20*). Hco-LGC-55 has been shown to be expressed in all life stages of the parasite; expression may be reduced in the adult male (*21*).

Octopamine and Tyramine: Diverse Physiologically Active Biogenic Amines

There is a plethora of studies examining the physiological importance of octopamine and its receptors in various invertebrates; this has been the topic of several excellent reviews (*11*, *14*, *15*, *22–24*), and therefore, will not be discussed here. Tyramine and its receptors, on the other hand, have not had as much research attention. This is largely because tyramine was initially thought to only be the biosynthetic precursor to octopamine. Therefore, we will focus on the advances made in understanding the physiological role of tyramine through a brief review of the literature.

Insects undergo differential behavioral states using semiochemicals, and this is extended to the complex interactions of social insects, like the honey bee, *Apis mellifera*. Previous studies have indicated neurohormonal and neuromodulatory effects on honey bee behavior to aid in the support of social hierarchy in the bee hive (*25–29*). Previous studies have indicated that the honey bee queen uses pheromones, which are produced and released from the mandibular gland and/or the Dufur's gland, to maintain a reproductive hierarchy in the colony (*30*). The concentration of pheromones produced in the mandibular gland is high in the queen bee, but low in the worker bees (*31*, *32*). Recently, tyramine has been shown to result in reproductive dominance over the fertility of the bee. Specifically, tyramine has been shown to be involved in ovary development, and pheromone production and secretion; specifically, a pheromone that is consistent with a queen (*33*). Tyramine did not have effects opposite of octopamine, which had been thought to be a major role of tyramine in insects (*24*). Instead octopamine appears to be involved in cast differentiation and the production of specific worker pheromones (*26*, *27*, *33*).

Insects are able to respond to environmental cues via a variety of chemosensory organs. The molecular mechanism of odor reception in insects has been recently reviewed (*34*). While octopamine and tyramine may not be the original sensing signals, they are involved in the neuronal modulation of the signal. A *D. melanogaster* mutant has been identified as having an olfactory defect resulting in behavioral changes (reduced avoidance). It was determined that this reduced avoidance was a result of a p-element upstream of the type-1 tyramine receptor (TAR1); this decreased the expression of the tyramine receptor. This indicates that tyramine has a role in modulation of *D. melanogaster* sensory processing (*35*). Mutation of the tyramine-β-hydroxylase (TβH) gene results in an abnormally low concentration of octopamine with a high concentration of tyramine. The decreased level of octopamine results in a poor locomotion phenotype in *D. melanogaster*. For instance, TβH mutant larvae were described as being slow and "pausing", compared to wild-type, described as a decrease

in linear translocation; this phenotype was recovered by feeding the larvae octopamine (*36*). Tyramine was able to decrease flight, possibly via a central motor pattern generator, in honey bees; this is an opposite effect of octopamine (*37*). Tyramine has also been shown to affect egg laying, reversal movement and head oscillations in *C. elegans* (*38*). It is important to note that more sensory behavior effects, head oscillations and reversal movement, were observed via the effects at the chloride-gated tyramine receptor (LGC-55) (*20*).

Octopamine has previously been reviewed to have effects in the reproductive system of insects (*11*); however, tyramine also has such a role. It has been demonstrated that there are tyraminergic innervations in the *Locusta migratoria* oviduct muscles. Tyramine was shown to increase the amplitude of excitatory junctional potentials and hyperpolarize the oviduct muscle; this effect was seen at low concentrations of tyramine (*39*). Tyramine has also been reported to have an effect on other types of muscles, specifically, muscles involved in insect flight. In *D. melanogaster*, tyramine has been shown to inhibit flight initiation at high concentrations (*40*).

Botanical Insecticides

Botanical insecticides, such as pyrethrum, rotenone, neem and plant essential oils, have been used for over 150 years in the United States; however, some botanical insecticides have been used for thousands of years in other countries (e.g. China, Egypt, Greece and India). Essential oils can be characterized as lipophilic liquids, which when isolated from the plant, display a strong odor. Essential oils function as plant secondary metabolites, which means they are not involved in the primary metabolism of the plant but still serve a variety of functions; for instance they can deter herbivorial feeding (*41*, *42*). Essential oils are commonly obtained via steam distillation from various plant tissues/organs or plant foliage under a variety of conditions (*41–43*). Essential oils are a complex mixture of different chemistries including various types of terpenes/terpenoids and related aromatic terpenoid compounds. Here we will use "terpene" interchangeably with "terpenoid". Since botanical compounds, like essential oils, are widely found in everyday items, like cosmetics and fragrances, food additives and pharmaceuticals, they are generally believed to have minimal mammalian toxicity (*44*). Some essential oils and essential oil components are found on the United States Environmental Protection Agency's exempt lists (25b and 4a). Additionally, some essential oils are Generally Recognized As Safe (GRAS), according to the Food and Drug Administration (FDA).

Essential oil terpenoids are synthesized from isoprene units, which are the five-carbon building blocks of terpenoids. The coupling of these isoprene units can lead to structures that have 5 – 40 carbons. Here, we focus on terpenoid structures composed of two isoprene units, monoterpenoids (10-carbons), and terpenoid structures composed of three isoprene units, sesquiterpenoids (15-carbons). The carbon backbone of terpenoids is further targeted by a variety of enzymes that give terpenoids diverse characteristics. For instance, terpenoids can be cyclic or acyclic, and they can contain a variety of heteroatoms to create

alcohols, aldehydes, ketones, esters, epoxides, ethers, and acids (*45–47*). Not all terpenoids are aliphatic; some related aromatic terpenoids are synthesized from the shikimic acid pathway, which is the pathway that plants commonly use to synthesize aromatic amino acids. In particular, phenylalanine and tyrosine are responsible for the phenylpropane/phenylpropene units that are the building blocks for the aromatic compounds found in essential oils (*46*).

Terpenoid Mechanism of Action: Focus on Octopamine and Tyramine Receptors

An understanding of the mechanism of action of insecticidal activity of essential oils, and their terpenoids, will aid in the integration of these compounds into a pest control strategy. While several studies have indicated that these compounds have a neurotoxic mechanism of action (*48*, *49*), it is possible that several targets or mechanisms are involved, both inside and outside of the insect's nervous system. Several studies have been performed assessing different mechanisms of neurotoxic action. These studies included the compound's ability to inhibit the enzyme acetylcholinesterase, leading to an increased concentration of acetylcholine in the synaptic cleft (*50–55*). Another study evaluated the ability of essential oil components to affect chloride conductance by altering the γ-aminobutyric acid ($GABA_A$) receptor (*56*). Additionally, different modulations of the $GABA_A$ receptor along with physicochemical properties, to predict successful modulation of this $GABA_A$ receptor, have been described (*57*, *58*). Recently, binding at the house fly (*Musca domestica*) nicotinic acetylcholine receptor (nAChR) has been reported (*59*). In addition to GABA and nAChR receptors, essential oil terpenoids have also been reported to have an effect at other ion channels. Specifically, essential oil terpenoids have been reported to inhibit transient receptor potential (TRP) channels, which are important sensory channels in humans (*60*).

Essential oil toxicity may be attributed to the multi-functionality of octopamine, and now tyramine, to insect physiology. Application of essential oil terpenoids may result in hyperactivity, hyperextension of extremities and abdomen, knockdown, which can be followed by death. Homogenate of the American cockroach (*Periplaneta americana*) nervous system resulted in an increase of cAMP upon terpenoid application, leading the author to suggest that toxicity was mediated via the octopaminergic system in the insect's nervous system (*49*). Later studies performed in *Helicoverpa armigera* homogenate also showed an increase of cAMP (agonistic activity) from abdominal dermal tissue with the application of several essential oil terpenoids, which was blocked by the octopamine receptor antagonist, phentolamine (*61*). A cloned α-adrenergic-like octopamine receptor (Pa oa1) has been described from the American cockroach (*49*) and an α-adrenergic-like octopamine receptor (OAMB) from *D. melanogaster* (*62*). When these octopamine receptors were expressed in human embryonic kidney (HEK-293) cells they resulted in an increase of the intracellular concentration of cAMP and calcium, which is peculiar since these both are OCTαR's and should signal via the inositol pathway (increase in

intracellular calcium). This may be an artifact of the heterologous expression system or this may indicate the ability of essential oils to recruit different G-proteins, activating multiple second messenger pathways (*23*). When eugenol, a plant essential oil monoterpenoid, was applied to HEK-293 cells expressing Pa oa1 it decreased the basal level of cAMP. Application of trans-anethol to HEK-293, expressing OAMB, resulted in an increase of the cellular concentration of cAMP (*62*). However, little effect was reported on the calcium response (*62*). Essential oil activity has also been reported on a cloned tyramine receptor, from *D. melanogaster* that was expressed in *Drosophila S2* cells. Here, a strong calcium response, along with a decrease of forskolin-stimulated cAMP was observed with the addition of tyramine (*63*). In addition to *in vitro* heterologous expression studies performed for the *D. melanogaster* tyramine receptor; *in vivo* studies were also performed to determine the toxicity of essential oil terpenoids against *D. melanogaster* (*63*). The aromatic monoterpenoid, thymol, which was the only tested monoterpenoid that resulted in an increase of the basal level of cAMP, and an increase of the intracellular concentration of calcium resulted in the lowest mortality (*63*). Thymol's stereoisomer, carvacrol, which had a strong calcium response against this *D. melanogaster* tyramine receptor, had similar mortality to thymol (*63*). These results indicate a correlation between tyramine receptor activity and insect mortality.

Conclusion

GPCRs have diverse physiological functions within invertebrates, including insects, mites, ticks and nematodes; however, they are an underutilized target for agrochemical development. Since GPCRs are a widely utilized target for the pharmaceutical industry there are several screening systems available, which can be applied to insects and ticks, to study GPCRs. The use of botanically-based insecticides may allow for the development of efficacious biopesticide products or serve as the starting material for safer arthropod and nematode control programs. Hopefully GPCRs will be exploited as targets for insecticides or acaricides in the future.

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