

Insect GPCRs and TRP Channels: Putative Targets for Insect Repellents

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SYNOPSIS

Many insects such as mosquitoes cause life-threatening diseases such as malaria, yellow fever and West Nile virus. Malaria alone infects 500 million people annually and causes 1-3 million death per year. Volatile insect repellents, which are detected through the sense of smell, have long been used to protect humans against insect pests. Antifeedants are non-volatile aversive compounds that are detected through the sense of taste and prevent insects from feeding on plants. The molecular targets and signaling pathways required for sensing insect repellents and antifeedants are poorly understood.

Transient Receptor Potential (TRP) Ca²⁺-permeable cation channels exist in organisms ranging from *C. elegans* to *D. melanogaster* and *Homo sapiens*. *Drosophila* has 13 family members, which mainly function in sensory physiology such as vision, thermotaxis and chemotaxis. G protein-coupled receptors (GPCRs) initiate olfactory signaling cascades in mammals and in nematodes *C. elegans*. However, the mechanisms of G protein signaling cascades in insect chemosensation are controversial.

In this review, I will discuss the putative roles of G protein-coupled receptors (GPCRs) and Transient Receptor Potential (TRP) channels as targets for insect repellents.

	Compounds
Insect Repellents	Synthetic : DEET Natural : Citronellal, Geraniol
Olfactory attractants	CO ₂ , 1-octen-3-ol
Pheromones	cis-vaccenyl acetate (cVA)
Antifeedants	Bitter compounds

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Key Words: GPCRs; TRP channels; olfaction; taste; insect repellents

OVERVIEW

Taste and smell are critical sensory modalities for survival since insects need to discriminate safe from noxious foods and toxic odors. Most insects are deleterious for plants. Thus, plants produce compounds to protect them from insect pests. Some chemicals are volatile repellents, which deter insects from landing on the plant. Other chemicals are non-volatile compounds, referred to as antifeedants, which inhibit the propensity for insects to feed on plants. Insect pests can detect repellents through the sense of smell and antifeedants through sense of taste.

Studying insect repellents and antifeedants is very important since they can reduce insect borne disease (repellents) or protect crops from damage (antifeedants). Insects are the vectors for many infectious diseases such as malaria, yellow fever and West Nile virus. For example, *Anopheles gambiae* is the vector for malaria and *Aedes aegypti* is the vector for Dengue fever and yellow fever. Malaria alone infects an estimated 500 million people annually and causes 1 million deaths per year¹. Moreover, the control of insect pests is important for decreasing crop destruction. Insect pests contribute to loss of fourteen percent of total crops. About \$200 billion per year in the United States and \$2 trillion worldwide are lost to insect destruction². Nevertheless, the molecular targets and signaling pathways required for sensing insect repellents and antifeedants are poorly understood. In this review, I will discuss the putative roles of G protein-coupled receptors (GPCRs) and Transient Receptor Potential (TRP) channels as targets for insect repellents.

DIVERSE STRATEGIES OF CHEMOSENSORY-BASED INSECT CONTROL

Diverse chemosensory-based strategies have been used in attempts to control insect pests³. Olfactory attractants, such as CO₂ or 1-octanol are used to lure insects to a trap. Pheromones have also been used effectively as trapping agents or to disrupt mating. Volatile insect repellents, which are detected through the sense of smell, have long been used to protect humans against insect pests. The most commonly-used synthetic repellent is DEET. However, there are some limitations. These include low potency, short range, and ineffectiveness against bees and certain mosquitoes⁴. Also, it cannot be applied to some synthetic fabrics and leather.

Thus, the use of plant-derived essential oils is widely considered as alternatives for synthetic insect repellents such as DEET. Citronellal, geraniol and camphor are among the most popular plant derived insect repellents⁵. Citronellal is commonly used in lotions, sprays and candles to repel mosquitoes and reduce the incidence of insect bites. Geraniol also shows repellency against a wide range of biting insects, including mosquitoes, flies,

Table 1. Chemosensory based insect control

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Olfactory attractants	CO ₂ , 1-octen-3-ol
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Antifeedants	Bitter compounds

fire ants and ticks⁶. Their repellent efficiencies against mosquitoes have been verified.

Antifeedants are non-volatile aversive compounds that are detected through the sense of taste and prevent insects from feeding on plants. However, they do not necessarily repel insects. An antifeedant may cause an insect to starve to death, since it inhibits feeding, but it may not cause the insect to move away from the source. Many plant-derived natural compounds such as aristolochic acid can be used as antifeedants³. Most of the chemosensory-based control strategies described here were not based on the molecular understanding of insect chemosensation. Thus, further understanding of insect olfaction and taste would contribute to identify molecular targets for insect pest control.

DROSOPHILA OLFACTORY SYSTEM

Drosophila serves as an excellent model organism to investigate the mechanisms underlying olfaction. The antenna and maxillary palp are the main sensory organs for sensing volatile compounds in *Drosophila*⁷. Each antenna and maxillary palp is covered with hair-like sensilla. And each sensillum houses around 1-4 olfactory receptor neurons (ORNs) that are surrounded by support cells. The antenna has three distinct morphological and functional types of sensilla: club-shaped basiconic sensilla, long and pointed trichoid sensilla and short coeloconic sensilla⁸. The different sensilla are distributed in a highly stereotyped pattern. Large basiconic sensilla are clustered at the medial-proximal side of the antenna, while trichoid sensilla are clustered at the lateral-distal edge. Small basiconic and coeloconic sensilla are distributed in the middle region on the antenna. The maxillary palp has a simpler structure than the antenna and contains only one class of basiconic sensilla. The antenna has about 410 olfactory sensilla, whereas about 60 olfactory sensilla cover the maxillary palp⁸. In total, there are between 1,100-1,250 ORNs in each antenna and 120 ORNs in the maxillary palp⁹. All ORNs express a unique combination of olfactory receptors (ORs) and project axon into a single olfactory glomerulus in the antennal lobe (AL), which is the functional homolog of mammalian olfactory bulb.

Drosophila olfactory receptors (ORs) were identified using computational and molecular approaches^{10,11}. There are 62 ORs

transcribed from 60 OR genes. ORN expresses up to three single ligand-binding Ors, along with an olfactory co-receptor, Orco (previously known as Or83b). Orco is expressed in most ORNs and is required for trafficking and functioning of ORs in *Drosophila*¹². Odorants pass through the pores in the sensillum, interact with ORs on the membranes of sensory dendrites from ORNs and induce action potential in these neurons.

Single sensillum recordings, which measure the frequencies of action potentials in ORNs, are used to define the olfactory profiles of basiconic and coeloconic sensilla on the antenna and maxillary palp¹³⁻¹⁵. Specific ORs are assigned to specific sensilla types, which have specialized ligands. Basiconic sensilla are specialized to detect food odors, whereas tricoic sensilla appear to detect pheromones^{16,17}. The coeloconic sensilla are specialized for detecting water vapor, ammonia and putrescine¹⁸. In addition to ORs, ionotropic glutamate receptors (IRs) sense odorants¹⁹.

DROSOPHILA GUSTATORY SYSTEM

Unlike mammalian taste, which is mediated through taste buds on the tongue, the taste organs in fruit flies are distributed over multiple body parts. Flies detect tastants using taste bristles (sensilla) distributed at the tip of the proboscis (labella), legs and wings²⁰. Each labellum on the proboscis has 31 taste bristles, each containing 2-4 gustatory receptor neurons (GRNs), one mechanosensory neuron and three accessory cells. GRNs in the proboscis have a single dendrite that extends to the tip of the sensilla and project an axon into the subesophageal ganglion (SOG) region of the brain²¹.

The three types of taste sensilla on the labellum are categorized by length (long; l-type; intermediate, i-type; and short, s-type)²². The four kinds of GRNs in s-type and l-type sensilla show responses to four taste modalities: sugar (S cell), water (W cell), low concentrations of salt (L1) and high salt concentration and bitter compounds (L2). Bitter compounds also inhibit activity of the S cells and W cells. The i-type sensilla have only two GRNs. The first GRN responds to sugars and low salt, while the second GRN responds to bitter compounds and high salt²³. Each sensilla has relatively different responses to sugar and bitter compounds. In general, the l-type sensilla produce stronger responses to sugars, while the i- and s-type sensilla respond to bitter compounds strongly. Flies also detect tastants with gustatory sensilla in their legs and wing margins²⁴. Each leg has 30 to 50 taste sensilla and each sensillum contains around two to four GRNs. The wing margin houses 40 taste sensilla, each containing four GRNs.

Drosophila GRs were identified through analysis of genomic DNA sequences²⁵. There are 68 GRs in *Drosophila*²⁶. Gr5a and Gr66a are expressed in a very large number of non-overlapping

GRNs. Gr66a is broadly expressed in bitter-responsive GRNs, whereas Gr5a broadly marks sugar-responsive GRNs. Genetic ablation experiments for Gr5a or Gr66a expressing neurons suggest that these GRNs are required for sensing bitter compounds and sugars, respectively^{21,27-29}.

TRANSIENT RECEPTOR POTENTIAL (TRP) CHANNELS IN CHEMOSENSATION

TRP channels are non-selective cation channels conserved from worms to flies and humans³⁰. The first TRP channel member, TRP, was identified by characterizing a *Drosophila* mutant with a defect in visual transduction^{31,32}. *Drosophila* has 13 TRP channel family members and these channels function in various sensory modalities such as vision, gravitaxis and thermotaxis. For example, TRPA subfamily members, TRPA1, Pain and Pyrexia participate in the detection of hot temperatures³³⁻³⁵. At least five TRP channels are required for gravity sensation or sound sensation³⁶⁻³⁸.

Several TRP channel family members in *C. elegans* and mammals participate in chemosensation. In *C. elegans*, the TRPV family member, OSM-9 functions in sensing odorants³⁹. In addition, mutant worms lacking TRPC channel are impaired in their response to nicotine⁴⁰. In mammalian organisms, several thermo-sensitive TRP channels also function in the detection of noxious or pungent chemicals. For example, TRPA1 is chemically activated by pungent compounds such as wasabi, garlic (allicin), mustard oils (isothiocyanates), and cinnamon oil (cinnamaldehyde)⁴¹.

Drosophila TRP channels also function in chemosensation. One of the most commonly used plant-derived insect repellents is citronellal. Citronellal is used commonly in lotions, sprays, and candles to repel mosquitoes, fleas, and ticks. A recent study indicates that *Drosophila* TRPA1 is required for avoiding the volatile insect repellent citronellal. The response to citronellal relied on a G protein (Gq)/PLC signaling cascade rather than direct detection of citronellal by TRPA1⁴². In contrast to fly TRPA1, *Anopheles gambiae* TRPA1 was directly and potently activated by citronellal. In addition to olfactory function, *Drosophila* TRPA1 was required in gustatory receptor neurons for the behavioral and electrophysiological responses to the antifeedant, aristolochic acid⁴³. TRPA1 was not activated directly by aristolochic acid, but was coupled to a PLC signaling cascade. *Drosophila* TRPA1 is also expressed in GRNs and can be directly activated by AITC, the pungent component in wasabi⁴⁴. Another TRPA family member, Painless is also expressed in GRNs and is required for avoidance to AITC⁴⁵. However, it is not known whether Painless senses wasabi in GRNs. It is also unclear whether Painless senses wasabi directly or through a signaling pathway.

GPCRS SIGNALING IN OLFACTION

G protein-coupled receptors (GPCRs) constitute a large superfamily of seven transmembrane proteins that sense molecules outside the cell and activate signal transduction pathways⁴⁶. GPCRs interact with a family of heterotrimeric GTP binding proteins, referred to as G proteins (Gs, Gi/o, and Gq). Ligand-induced activation of GPCRs induces a conformational change of the receptor and triggers G proteins that in turn regulate the activity of other effector molecules. In general, Gs subunits regulate the activation of adenylyl cyclase, resulting in an increased synthesis of cAMP. The Gi/o class of G proteins activates phosphodiesterases, which leads to the inhibition of stimulated cAMP. Finally, Gq subunits couple to phospholipaseC β (PLC β), thereby producing diacylglycerol (DAG) and inositol triphosphate (IP). DAG activates protein kinase C whereas IP mobilizes intracellular Ca²⁺. *Drosophila* encodes about 117 canonical GPCRs, whereas more than 1,000 GPCRs are encoded in vertebrates and *C. elegans*⁴⁷.

GPCRS SIGNALING IN OLFACTION

There is broad evidence that GPCRs initiate olfactory signaling cascade in mammals and the nematode. Mammalian ORs are GPCRs, which bind to odorants in the ciliary membrane. This activates a G protein that, which in turn, stimulates adenylyl cyclase (AC). cAMP directly gates cyclic nucleotide-gated (CNG) ion channels causing an odorant-induced inward current carried by Na⁺ and Ca²⁺ ions⁴⁸.

Currently, the mechanisms underlying insect olfactory signaling is controversial. Our understanding of the molecular components required for sensing odorants in insects is limited primarily to Ors and IRs. Although *Drosophila* ORs were widely assumed to be GPCRs, several studies suggest that insect ORs have a topology opposite to conventional GPCRs. Moreover, fly ORs have no amino acid homology to mammalian and *C. elegans* ORs⁴⁹⁻⁵². In addition, insect ORs do not have conventional binding sites for G proteins. One report concludes that ORs are strictly ligand-gated channels⁵³. In contrast, another study suggests that ORs are both ligand gated channels and GPCRs⁵⁴.

In one study, expression of ORs in heterologous systems⁵⁵ indicates that ORs and Orco form a non-selective cation channel and are activated by odorants to produce fast ionotropic response, which does not require G proteins. However, a second study indicates that G protein signaling cascades mediate the slower metabotropic pathway, which leads to the production of second messengers, and activation of Orco. According to this model, the ionotropic pathway functions at higher odorants concentrations, whereas the metabotropic pathway amplifies signal at low odorants concentrations in a G protein-dependent

manner. These data suggest that canonical GPCRs may initiate G proteins signaling cascades in ORNs for odorants sensation.

Despite the controversy as to whether or not ORs can capable of functioning as GPCRs, G proteins are expressed in ORNs⁵⁶. Gs and Gq are enriched in the dendrites of ORNs, suggesting that G protein might function in olfactory transduction⁵⁷⁻⁶¹. A fly mutant disrupting the dGq gene encoding the Gq α subunit shows reduced responses to odor stimulation⁵⁸. The odor-induced response was further attenuated by mutations in plc21C, a gene encoding a PLC β .

G PROTEINS SIGNALING CASCADE IN TASTE SENSATION

Mammalian sweet, bitter and umami taste all depend on PLC signaling and the TRPM5 channel^{62,63}. The specificity for different bitter and sweet tastants is mediated by GPCRs. Two classes of taste GPCRs have been identified: T1Rs and T2Rs⁶⁴. T1R family members consist of three receptors: T1R1, T1R2 and T1R3 and function as heterodimers. T1R1 + T1R3 and T1R2 + T1R3 mediate umami taste and the sweet taste, respectively^{65,66}. In addition, T2Rs, which consist of about 30 members in mammals, mediate bitter taste⁶⁷.

It is not clear whether insects use single signaling mechanism to sense all tastants in GRNs. It has been proposed that *Manduca sexta* use multiple transduction pathways that functions in response to bitter compounds, such as aristolochic acid and caffeine^{68,69}. The molecules whose roles in *Drosophila* taste are best understood are GRs. These proteins have no homology with mammalian GPCRs. A recent report suggests that *Bombyx mori* GR-9 (BmGR-9), and its *Drosophila* homolog, Gr43a, constitute a ligand-gated ion channel⁷⁰. It is still controversial whether the *Drosophila* GRs are cation channels. As is the case for insect olfaction, the *Drosophila* gustatory system appears to employ G protein-mediated signaling pathways. Mutations disrupting *Gas*, *Gaq*, *G γ 1*, or phospholipid signaling caused partial reductions in the trehalose response^{44,71}. In addition, *Gao* functions in the detection of sucrose, glucose, and fructose in *Drosophila*⁷². However, there was no report suggesting that PLC was involved in an insect taste signaling cascade. The fact that mammalian taste is mediated by a single PLC/TRPM5 transduction pathway raises the possibility that insects might use PLC and TRP channel signaling for taste sensation.

POTENTIAL MOLECULAR TARGETS FOR INSECT REPELLENTS

The identification of receptors for insect repellents would provide new opportunities for insect pest control. Specifically, these receptors could be used to perform high-throughput chemical

screens for activators of the receptors, which might serve as new, highly potent insect repellents. However, the direct molecular targets for insect repellents are poorly understood. A recent study indicates that olfactory receptors are targets for the most commonly used repellent, DEET (N,N-diethyl-m-toluamide). However, the mode of action of DEET is still controversial. It has been proposed that DEET does not act directly as a volatile repellent but inhibits olfactory responses to attractive compounds^{73,74}. Another study suggests that ORNs in the mosquito, *Culex quinquefasciatus*, respond directly to DEET⁷⁵.

TRP channels and GPCRs represent additional candidate targets for insect repellents. Recent study suggests that mosquito TRPA1 is directly and potently activated by insect repellent citronellal⁴². It raises the exciting possibility that high-throughput screens for novel activators of mosquito TRPA channel might lead to the discovery of improved repellents to control insect-borne disease. In addition, G protein signaling cascades participate in some aspects of chemosensation in ORNs and GRNs^{57-61,71,76}. Thus, GPCRs might function as putative targets for insect repellents.

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