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Review

Insect TRP channels as targets for insecticides and repellents

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This review provides a brief overview of ion channels, then focuses on TRP channels, describing the properties and functions of the seven TRP channel classes found in insects. Finally, recent work showing that a heteromeric channel composed of Nanchung and Inactive vanilloid TRP (TRPV) channel subunits is the target of the selective feeding blockers pymetrozine and pyrifluquinazon is described. The possible utility of other TRP channels as targets of insecticides and repellents is also considered. © Pesticide Science Society of Japan

Keywords: transient receptor potential channel, insecticide, pymetrozine, repellent, nanchung, inactive.

Introduction

Transient Receptor Potential (TRP) channels are a large class of ion channels that play a critical signaling role in virtually all sensory modalities. While members of other classes of ion channels, specifically the voltage-dependent ion channels, ligand-gated ion channels and intracellular calcium-release channels (ryanodine receptors) have long been among the most successful insecticide targets, it was only recently discovered that a TRP channel was the long-sought target of certain insecticides known as feeding inhibitors, bringing this class of channels and sensory processes in general into focus as valid insecticide targets. It was also recently shown that the repellent citronellal activates TRPA1 channels. Because the ability to sense and respond to external and internal stimuli is critical to insect survival and propagation, TRP channels might offer more alternative targets for insect control.

1. Ion Channel Overview

An ion channel is an integral membrane protein containing a narrow, water-filled transmembrane pore that selectively conducts specific ions across the membrane, as well as extracellular and intracellular domains involved in opening and closing, or gating, of the channel. Ion channels are present in all cells and function in regulating cell volume, establishing a resting membrane potential, fluid transport and cell signaling.[1)]

Ion channels are formed as closely packed circular assemblages of four or more subunits or pseudo-subunits that span the plane of the lipid bilayer membrane and enclose the pore. There is also a small family of channels that form two such pores.[2)] Each (pseudo) subunit has four or more transmembrane α-helical segments that span the membrane, and there are normally a total of 20 or more of these transmembrane α-helical segments that, as well as forming the pore, may serve other functions depending on their location and sequences. The four to five helices surrounding the pore provide a hydrophilic lining to the pore, and also provide ion selectivity, while the outermost helices have hydrophobic residues that face and interact with the surrounding lipid bilayer to anchor the channel in the membrane. The other segments serve to hold the pore-lining helices in the correct positions and also to move them under the influence of appropriate signals to open and close the pore. Ions flow passively through open channels in both directions, without the input of metabolic energy. The net flux is down the ion's electrochemical gradient, which is a function of ion concentration and membrane potential.

The majority of channels involved in nerve signaling are selective for Na^+, K^+, Cl^- or Ca^{2+}, or are non-selective cation channels. Because of their electrochemical gradients, Na^+ and Ca^{2+} flow into the cell through open channels and are excitatory, because they depolarize the normally negative cell. In addition to depolarizing the cell membrane, Ca^{2+} ions directly regulate many intracellular processes. K^+ ions are at high concentrations inside cells, and flow outward through channels, making the inside of the cell more negative, which is inhibitory or stabilizing for the cell membrane potential. Electrical neutrality demands that the cations are balanced by an equal concentration of anions. Organic acids form the majority of anions inside the cell, whereas Cl^- ions are at a high concentration outside the cell, so Cl^- ions tend to flow into the cell through open chloride channels and are thus inhibitory. Nonselective cation channels gener-

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ally depolarize the cell, so are excitatory on an electrical basis and also due to the influx of Ca^{2+} ions they permit.

The ion channel concept originated with the landmark deconstruction of the nerve action potential by Hodgkin and Huxley, which they found was generated by distinct Na^+ and K^+ conduction pathways.[3–5] The existence of channels was confirmed with the 'patch clamp' technique.[6] The protein sequences of many ion channels are now known from genomic sequencing, and these proteins are now routinely expressed in heterologous systems that allow various types of measurements of channel function and structure. X-ray crystallographic structures of very few ion channels have been obtained to date, because of the difficulties in crystallizing these proteins outside of a lipid membrane. Only one insecticide binding site in an ion channel has been characterized by X-ray crystallography—the macrocyclic lactone binding site in the glutamate-gated chloride channel.[7] However, structural determination of ion channels by cryo-electron microscopy (cryo-EM) is increasing at a rapid rate and the cryo-EM structures of two TRP channels have already been published.[8,9]

Many toxins produced by plants, microorganisms, snakes, fish, scorpions, spiders and other animals act by disrupting specific ion channels. Ion channels are also important target sites for many insecticides; 9 of the 27 insecticide modes of action enumerated in the current version of the IRAC (Insecticide Resistance Action Committee) Mode of Action Classification are on ion channels (www.irac-online.org).

2. TRP Channels

The first TRP channel was identified in the *Drosophila trp* mutant, which has visual impairment and shows a shortened response to light in electroretinogram recordings. Once the *trp* gene product was identified as an ion channel, it was found that TRP channels are a large superfamily of ion channels that are conserved among all metazoans.[10] Furthermore, TRP channels are also present in protists, chlorophyte algae, choanoflagellates and fungi, where they function as chemo-, thermo- and mechanosensors. Thus, TRP channels arose early in the evolution of Eukaryotes, but have been secondarily lost in plants.[11,12]

The TRP superfamily contains approximately 30 members that are classified into seven families: TRPC (canonical), TRPA (ankyrin), TRPV (vanilloid), TRPN (nompC), TRPM (melastatin), TRPML (mucolipin) and TRPP (polycystic), based on sequence similarity.[13] The fourth letter in the family name refers to an aspect of one or more members of each family, but not necessarily of all members. For example, TRPC channels cluster with the canonical *Drosophila* TRP channel isolated from the *trp* mutant mentioned above, TRPA channels have multiple Ankyrin repeat motifs in the N-terminal cytosolic domain (although so do several other families), TRPV channels include some that are activated by vanilloids, and TRPN channels are related to the NOMPC (No Mechanoreceptor Potential) channel, which is important in mechanotransduction.[14]

TRP channels bear sequence similarity to the voltage-dependent ion channels, but have extended cytosolic C-terminal and N-terminal regions that are involved in their multimodal sensitivity to physical and chemical stimuli. Like some voltage-gated channels, TRP channel subunits have six transmembrane helices and assemble as tetramers.[15] In other voltage-gated channels, four pseudo-subunits, each containing six transmembrane helices, are linked together as domains of a single protein.[16]

TRP channels function in vision, thermosensation, olfaction, taste, hygrosensation, mechanosensation, intracellular signaling, synaptic transmission and ion homeostasis. Disruption of any of these functions could conceivably control at least some insects. Furthermore, TRP channels are often activated by chemicals that provide the sensory experience associated with their normal sensory function. For example, activation of the mammalian thermosensitive TRPV1 channel by capsaicin in hot peppers provides a sensation of heat,[17] while activation of TRPM8 channels by menthol gives a cool sensation.[18] Pungent irritants found in materials from horseradish to tear gas activate TRPA1 channels, informally known as wasabi receptors. TRPV1 and TRPA1 channels were the first TRP channels to have their structures solved by cryo-EM, and give insight into the binding of chemicals to these channels.[8,9] The affinity of capsaicin for TRPV1 channels is 70 nM,[19] which is in the range of commercial insecticides.

Drosophila melanogaster has 13 TRP channels, with at least one from each of the seven families, showing that all TRP channel families had evolved before the divergence of the insects. In a genomic survey of six insect species, all were found to have 13-14 TRP channels, including 2 TRPVs, 1 TRPN, 1 TRPM, 3 TRPCs and 1 TRPML. Three of the species in the survey had one TRPP channel, but the lepidopteran *Bombyx mori* and the hymenopterans *Apis mellifera* and *Nasonia vitripennis* had none. TRPP is present even in yeast and is thought to be the most ancient TRP channel family, so it was secondarily lost in at least some groups of insects.[20]

2.1. TRPC

In addition to the TRP channel itself, flies have a second TRPC family member, known as the TRP-like channel TRPL, which, like TRP, is involved in phototransduction and additionally in cold sensation.[13] TRP is a Ca^{2+}-selective channel, while TRPL, like most other TRP channels, is a non-selective cation channel, also conducting Ca^{2+}. Flies also have a third TRPC channel, TRPγ, which is a nonselective cation channel expressed in proprioceptive chordotonal organs and is essential for fine motor control but is not found in Johnston's organ, which is also a chordotonal organ, but specialized for gravity and sound perception.[21]

2.2. TRPP

The *Drosophila* TRPP channel Almost there (Amo) is located in the sperm flagellum and is essential for sperm entry into the female spermatheca.[22,23] Although disruption of fertilization might have limited insect control applications, these would likely not be enough to justify insecticide research. A homolog

of mammalian Polycystic Kidney Disease 2, Amo is also called PKD2 and has been found to play an as yet undetermined role in intracellular Ca^{2+} regulation during phagocytic clearance of apoptotic cells[24)]

2.3. TRPM

The single *Drosophila* TRPM channel is expressed in the Malpighian tubules and is essential for removal of Mg^{2+} from the hemolymph, with loss of function mutations leading to hypermagnesemia, associated with slowed larval development and developmental arrest in the prepupal stage. The effects were exacerbated with a high Magnesium diet.[25)] While other TRP channels affect senses and their disturbance could disrupt behavior, this is the only TRP channel with a directly lethal mutant phenotype and is therefore a candidate for targeting by insecticides. Attacking the TRPM channel in chewing herbivorous insects such as Lepidoptera and Coleoptera might be especially effective, because of the high Mg^{2+} content of their diets.

2.4. TRPML

In *Drosophila*, TRPML forms phosphatidylinositol 3,5-bisphosphate-activated cation channels in endolysosomal and plasma membranes.[26)] While mutants of TRPML display severe neurodegeneration and motor deficits associated with impairment of autophagic removal of damaged mitochondria, as well as accumulation of lysosome vesicles,[27)] it is unclear how rapidly these effects would incapacitate an insect if TRPML were attacked chemically.

2.5. TRPN

The *Drosophila* TRPN1 channel nompC (no mechanoreceptor potential C) is a candidate mechanotransduction channel in a variety of *Drosophila* mechanosensory neurons where high sensitivity is important. In the tips of mechanosensory bristles it is required for the sense of soft touch in adult flies.[28)] In larvae, which lack bristles, the body wall is tiled by dendritic arborizations of two classes of multidendritic mechanosensory neurons. In the class IV subset of these neurons, the piezo channel, which is not a TRP channel, transduces stronger, noxious touch stimuli, while in the class III neurons nompC transduces soft touch.[29)] NompC is also essential in the transduction of cuticular deformation by campaniform sensillae,[30)] in auditory transduction by Johnston's organ in adult *Drosophila*[31,32)] and in vibration detection by the lateral (pentascolopidial) chordotonal organs in *Drosophila* larvae.[33)] NOMPC is also present in the distal tip of the cilium of leg chordotonal organs, where it is likely to also be involved in sensory transduction for the proprioceptive sense.[34)] Because of its widespread importance in sensitive mechanosensory processes of touch, hearing and proprioception and the importance of these senses in social interactions and behavior, NOMPC is likely to be a valid insecticidal target. *nompC* null mutant flies are severely uncoordinated,[28,35)] and it is likely that chemical modification would have the same effect.

2.6. TRPA

Because of their small size and lack of thermoregulating ability, insects must sense temperature in order to seek microenvironments that are optimal for growth and reproduction and free from damaging extremes.[36,37)] *Drosophila* have four TRPA channels—TRPA1, Painless (Pain), Pyrexia (Pyx) and water witch (Wtrw). With the exception of Wtrw all of these are involved in thermosensation. *Drosophila* TRPA1, Pain and Pyx are known as thermoTRPs because they are directly gated by changes in temperature. Members of the *Drosophila* TRPV, TRPC and TRPP families also have roles in temperature sensing but are not thermoTRPs and are downstream of a primary temperature sensor.[37)]

The *Drosophila* TRPA1 channel is involved in nociception (detection of noxious touch), inflammation and sensitivity to noxious chemicals such as allyl isothiocyanate. *Drosophila* express at least four TRPA1 isoforms with differing N-terminal intracellular regions that confer different thermosensing properties.[37)] TRPA1-A opens at 26°C to sense temperatures in the comfortable range. TRPA1-D opens at 34°C, which is too low for it to directly mediate responses to noxious heat that occur above 39°C. Although TRPA1 is involved in the noxious heat response, this response also involves the other two thermoTRPs—Pain, which has a temperature activation threshold of 39 to 42°C, and Pyx, which opens at 46°C, but the individual roles of the three channels are unclear.[13,36)] TRPA1 is likewise required for avoidance of high-intensity light by larvae, by an unknown mechanism that may also involve a gustatory receptor.[37)] The B and C isoforms of TRPA1 are not temperature sensitive, but all forms are activated by allyl isothiocyanate.[37)]

The hymenopterans *Apis mellifera* and *Nasonia vitripennis* lack TRPA1 channels but instead have HsTRPAs (Hymenoptera specific TRPAs), which have evolved by duplication of *wtrw* genes and function as thermosensors to detect temperature increase.[20)] It has been proposed that TRPA1 was lost from Hymenoptera after HsTRPAs gained temperature sensitivity.[20)]

Sensing of temperature fluctuations by TRPA1 is also important in entraining circadian rhythms[39)] and in insect development.[40)] Furthermore, ectoparasites of warm-blooded animals such as mammals and birds use thermosensing dependent on TRPA1 channels to find hosts and to find optimal feeding sites on those hosts.[41,42)] In fact, plant-derived repellents carvacrol and alpha-terpineol that activate the varroa mite TRPA1L channel but not the honey bee HsTRPA channel repressed mite entry into brood cells in hives, showing some potential for the use of TRPA1-activating compounds in apiculture.[43)] TRPA1 operating downstream of -Gq/phopshoplipase C signaling cascade is also required in *Drosophila* for the avoidance of the repellent citronellal, whereas the *Anopheles gambiae* TRPA1 is directly activated by citronellal.[44)]

The roles of Pain and Pyx in detecting noxious heat have already been mentioned above. Both channels are also involved in gravity perception in chordotonal organs, as discussed below. Pain is also involved in mechanosensation of strong stimuli.[45)]

Because of their large surface to volume ratio, insects are par-

Fig. 1. African migratory locust, *Locusta migratoria*, 10 min after injection with 1 μL DMSO, left, or 1 μL DMSO containing 1 μg pymetrozine, right.

ticularly sensitive to humidity, and its detection is important for their survival. *wtrw* is expressed in specialized sensory hairs on the third segment of the *Drosophila* antenna and is required for sensing moist air[46] and for mechanosensory amplification in chordotonal organs (see below).

Some insects in the orders Hymenoptera, Coleoptera and Lepidoptera have an extra TRPA channel, TRPA5.[20,47] This channel has not been studied functionally, but its expression in antennae suggests that it might have an olfactory function.[47]

2.7. TRPV

The vanilloid-type TRP (TRPV) channels are the only TRPs that have been identified as targets of commercial insecticides. Insects have two TRPV channel subunits, Nanchung (Nan) and Inactive (Iav), which, in *Drosophila*, are co-expressed only in chordotonal neurons, where they form heteromers that are activated by the pyridine azomethine derivative insecticides pymetrozine and pyrifluquinazon.[48] Chordotonal organs are stretch receptors that occur in most joints in the body and are activated by stretch resulting from the relative movement of the articulating joint members.[49] In most joints, chordotonal organs report on joint angle and movement, providing the proprioceptive sense, but a chordotonal organ in the joint between the second and third antennal segments in flies, known as Johnston's organ, reports on movement of the antenna under the influence of gravity, wind and sound, so is involved in the senses of equilibrium, air movement and hearing.[32,50] In addition, many insects have tympanal hearing organs, in which a chordotonal organ transduces the vibrations of a thin cuticular membrane, for sound perception.[51]

Pymetrozine, commercialized in 1999, is a systemic insecticide with remarkable selectivity for plant-sucking insects, causing specific behavioral effects such as immediate removal of the stylets from the plant and cessation of further probing, without the knockdown or paralysis effects typical of other neuroactive insecticides. Pyrifluquinazon is an analog of pymetrozine with similar properties, introduced in 2007. Death from both of these insecticides is delayed and results from starvation as a consequence of inability to feed.[52–54] The first real clue to the mode of action of these insecticides was the observation that exposure of locusts to pymetrozine by feeding or injection caused them to hold their hind legs raised, with the tibiae extended (Fig. 1).[53] Using the feedback loop controlling the femorotibial joint as a model, it could be clearly shown that pymetrozine specifically activated the femoral chordotonal organ, which is normally activated by joint flexion. The nervous system, falsely registering the joint as flexed, reflexively activated the extensor muscle to extend the tibia.[55] The compound had a similar effect on chordotonal organs in the wing hinge and the tegula, but did not affect sensory hairs of the tegula, campaniform sensillae on the tibia and subcostal vein or a non-chordotonal stretch receptor in the wing hinge, leading to the conclusion that it had a selective action on all chordotonal organs.[55] This conclusion is further supported by the observations that pymetrozine also acts selectively on chordotonal organs of the bush cricket tympanum,[56] on Johnston's organ of adult *Drosophila* and on the pentascolopidial chordotonal organ of *Drosophila* larvae.[48]

Chordotonal organ neurons are specialized according to their function. As a transducer for sound, gravity and wind, the fly Johnston's organ is one of the most complex chordotonal organs, with five subsets of chordotonal neurons variously specialized to sense vibrations of different frequencies as well as static deflections due to wind and gravity.[50] Although the mechanisms of mechanotransduction in the various classes of Johnston's organ neurons are not clear, heteromeric Nan-Iav TRPV channels are required for the nerve response to mechanical stimulation in all of them,[32] and activation of these channels by pymetrozine and pyrifluquinazon activates Ca^{2+} signals in all Johnston's organ neurons as well as in the larval pentascolopidial neurons,[48] affirming the conclusion[55] that all chordotonal organs are affected by pymetrozine.

Other TRP channels are essential to only some chordotonal neurons. Whereas mutations in Iav or Nan silence chordotonal nerve function and disrupt both hearing and gravity perception,[57,58] nompC nulls have a specific defect in hearing.[31,32,58] NompC is also required in the function of mechanosensory bristles and other mechanosensory organs.[28] Two TRPC channels, TRP and TRPL, as well as the TRPA channel Wtrw are required for mechanosensory amplification in Johnston's organ,[59] whereas mutations in the TRPA channel genes *pain* and *pyx* disrupt gravity perception but not hearing.[58] These channels also have other sensory functions, as described above and summarized in Table 1. As mentioned above, the TRPC channel TRPγ is required in proprioceptive chordotonal neurons but not in Johnston's organ.[21]

While Iav and Nan occur together in chordotonal neurons as subunits of a heteromeric TRPV channel that is activated by pyridine azomethine insecticides, the individual distribution of these TRPs is broader. Iav is expressed without Nan in motor neurons, where it acts as a key regulator of synaptic develop-

Table 1. Insect TRP channels, their function, orders where they are lacking and their value as insecticide or repellent targets

Family	Channel	Function	Orders Lacking	Insecticide Target Value
TRPC	TRP	Vision, chordotonal mechanosensory amplification	None	Unknown
	TRPL	Vision, cold sense, chordotonal mechanosensory amplification	None	Unknown
	TRPγ	Proprioceptive chordotonal organs/fine motor control	None	Unknown
TRPP	Amo (PKD2)	Required in sperm flagellum for entry into spermatheca; phagocytic clearance of apoptotic cells	Lepidoptera, Hymenoptera	Unknown
TRPM	TRPM	Mg^{2+} excretion	None	Validated lethal, but depends on diet.
TRPML	TRPML	Locomotion, autophagy, clearance of apoptotic cells	None	Unknown
TRPN	NOMPC	Mechanotransduction channel for light touch in adult bristles and larval mdIII neurons, for hearing and proprioception in chordotonal neurons, and for cuticle deformation in campaniform sensillae	None	Unknown
TRPA	TRPA1	Noxious heat, strong touch, strong light, chemical irritants, comfortable temperatures	Hymenoptera (replaced by HsTRPA)	Repellent target (possibly bee-safe)
	Painless (Pain)	Noxious heat, strong touch, gravity sensing by chordotonal organs		Unknown
	Pyrexia (Pyx)	Noxious heat, gravity sensing by chordotonal organs		Unknown
	Water witch (Wtrw)	Moist air, mechanosensory amplification in chordotonal organs	None	Unknown
	TRPA5	Only in some Hymenoptera, Coleoptera and Lepidoptera–function unknown	Most	Unknown
TRPV	Nanchung (Nan)	Chordotonal organ (heteromer with Iav); dry air	None	Target of IRAC Group 9
	Inactive (Iav)	Chordotonal organ (heteromer with Nan); presynaptic resting $[Ca^{2+}]$ regulation	None	Target of IRAC Group 9

ment and function by influencing presynaptic resting Ca^{2+} concentration.[60] Nan is expressed in some multidendritic neurons of fly larvae[48] and in a class of sensory hairs on the third antennal segment of *Drosophila* that respond to dry air, and Nan is required for avoidance of dry air.[46] Because Nan and Iav do not seem to form functional homomers,[48] they might be expected to form functional heteromers with other partners in the other cells where they occur, but this has not been shown. Nevertheless, pymetrozine or pyrifluquinazon only evoked calcium signals in chordotonal neurons and not in larval multidendritic neurons or adult hygroreceptors that only express nan.[48]

Conclusion

TRP channels play a signaling role in all sensory modalities and can often be activated by chemicals. Specific actions of insecticides on TRP channels and sensory function has only recently become known, with the demonstration that the pyridine azomethine insecticides pymetrozine and pyrifluquinazon selectively activate insect TRPV channels. No other insecticides are known to act on TRP channels, and it is unclear which, if any, of the eleven or twelve other insect TRP channels would be good insecticide targets. Some repellents, such as citronellal, carvacrol and alpha-terpineol act on TRPA1 channels. Because this channel is lacking in hymenoptera, TRPA1 has been investigated as a suitable target for repelling honey bee parasites such as varroa mites.

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