Insect toxins – selective pharmacological tools and drug / chemical leads

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Abstract

Insect toxins comprise a diverse array of chemicals ranging from small molecules, polyamines and peptide toxins. Many target nervous system and neuromuscular ion channels and so rapidly affect the behaviour of animals to which the toxin is applied or injected. Other modes of action have also been identified. Wasps, bees, flies, beetles and ants generate a rich arsenal of channel-active toxins, some of which offer selective pharmacological probes that target particular ion channels, while others act on more than one type of channel. Philanthotoxins from the digger wasp have been fruitful in adding to our understanding of ligand-gated ion channels in the nervous system and at neuromuscular junctions. Fire ants produce the toxic alkaloid solenopsin, a molecule which has stimulated attempts to generate synthetic compounds with insecticidal activity. Apamin from bee venom targets calcium-activated potassium channels which can in turn control the release of neuropeptides. Melittin, another bee venom component, is a membrane-acting peptide. The saliva of the assassin bug contains toxins that target the voltage-gated calcium channels of their insect prey. Certain beetles produce diamphotoxin, a haemolytic peptide toxin with traditional use as an arrow poison and others generate leptinotarsin with similar properties. Mastoparan is a powerful peptide toxin from wasp venom. Its toxic actions can be engineered out leaving a potent antimicrobial molecule of interest. Here we describe the modes of action of such compounds and evaluate their potential as neuroactive pharmacological tools and candidate lead molecules for insect control and as therapeutic candidates with potential antimicrobial, antiviral and anti-cancer applications.

Philanthotoxins

The polyamine, philanthotoxin-433 (Fig.1), is the most active toxin isolated from the venom of the Egyptian digger wasp, *Philanthus triangulum,* which rapidly paralyses its prey [1]. The toxin acts on several important cation-permeable ligand-gated ion channels (LGICs), notably L-glutamate-gated ion channels (GluRs) and nicotinic acetylcholine receptors (nAChRs) [2–4]. The low specificity of natural PhTX-433 is the main obstacle to its candidacy for insecticide development. Nonetheless, structure-activity studies have been pursued with the aim of enhancing their potency and specificity toward particular ligand-gated ion channels.

Philanthotoxin's actions on ligand gated ion channels

Early studies showed that insect nAChRs are less sensitive than GluRs to philanthotoxin [5, 6]. Benson and colleagues [7] compared the actions of natural PhTX-433 and 33 synthetic analogues of philanthotoxin on nAChRs of isolated locust thoracic ganglion cell bodies, cockroach sensory nerve-giant axon synapses and GluRs of housefly neuromuscular junctions. The authors discovered that replacing region III of PhTX-343 with an aromatic group and the region II secondary amine by a methylene group enhanced selectivity for nAChRs over GluRs. Region III elongation and lipophilic substitution resulted in the most active analogues with 75-fold and 8-fold increase in potency over locust nAChRs and housefly GluRs respectively [7].

Studies on vertebrate excitatory ligand-gated ion channels such as nAChRs and GluRs have also proved informative. It was proposed that inhibitory actions of PhTX-433 mainly related to the number of nitrogen atoms in the polyamine chain and their likely interactions with negatively charged or polar amino acids of the channel's cation-selective pore [8] and to a lesser extent on the head group which anchors the toxin to the extracellular entrance of the channel [9] .

The PhTX-12 analogue (Fig. 1) was more effective on muscle-type of nAChRs than on several types of GluRs [10], hence a series of analogues (PhTX-7 to PhTX-11) was generated to investigate the role of the distance between the head region and terminal amine group. PhTX-11 was the only synthetic analogue with higher potency than PhTX-12 on muscle-type nAChRs [11] but no strong evidence for receptor selectivity was obtained.

However, PhTX-83 (Fig. 1), in which the amino group near the head region was replaced by a methylene, is a potent and selective inhibitor of L-glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) [8]. PhTX-56 is highly potent and specific for Ca^{2+} permeable-subtypes of AMPARs. It showed 20-fold increase in inhibitory activity

compared to PhTX-83 when tested on homomeric GluR1 AMPARs. In addition, PhTX-56 potency was 500-fold and 1000-fold lower on homeric GluR5 kainic acid receptors (KainateRs) and GluR2-containing AMPARs respectively [12].

Recently, modifications in region II (the polyamine moiety) of PhTX-343 have been explored [13]. A straight conformation has been suggested for region II of PhTX-343 (Fig. 1) due to the presence of three protonated amine groups, while the absence of the two secondary amine groups (PhTX-12) indicates a fully-folded conformation [14]. In 2014, Franzyk and colleagues showed that incorporating a cis cyclopropane structure to this region of PhTX-343 causes a semi-folded conformation and this increased its activity on the rat GluA1 flop subtype of AMPARs, whereas the *trans* isomer remains linear and shows higher activity than *cis* [15]. These findings may offer a new approach to the development of selective compounds for excitatory ligand-gated ion channel subtypes based on the various semi-folded conformations that the PhTX molecule can adopt, thereby influencing molecular interactions with different amino acids of the channel pore.

Findings for PhTX-analogues with changes in the aromatic head group have often been more difficult to interpret, albeit with certain notable exceptions that lead to strong subtype-selectivity. For example, replacing the tyrosyl group in PhTX-343 derivatives with N-cyclohexyl-L-alanine [XX paper by Cha]) led to the generation of six synthetic analogues. All of these show higher potency and selectivity towards human muscle nAChRs, while mostly similar inhibition activities were obtained on AMPA receptors [16]. In contrast, the blocking actions of PhTX-343 derivatives on both NMDARs and nAChRs were increased significantly by replacing the butyryl group with 2-phenylacetyl [10].

Kachel and colleagues investigated the inhibition of different neuronal nAChR subunit combinations as well as of embryonic muscle receptors expressed in *Xenopus laevis* oocytes [4]. The α3β4 nAChR subtype was most sensitive to PhTX-343 (IC_{50} = 12 nM at −80 mV) with α4β4, α4β2, α3β2, α7 and α1β1γδ being 5, 26, 114, 422 and 992 times less sensitive. The embryonic muscle receptor α1β1γδ was most sensitive to PhTX-12 with α4β4, α4β2, α3β2 and α7 being 3, 3, 26 and 49 times less sensitive [4]. PhTX-343 inhibition was strongly voltage-dependent for all subunit combinations except α7, whereas this was not the case for PhTX-12 for which weak voltage dependence was observed. The authors concluded that PhTX-343 mainly acts as an open-channel blocker of nAChRs with strong subtype selectivity [4].

Further work is clearly needed but there are grounds for hoping that PhTXs could serve as lead compounds for highly potent and selective inhibitors of cation selective ligand-gated ion channels of both mammals and insects.

Solenopsins

Solenopsin A (Fig. 2) is an alkaloid present in the venom of fire ants of the genus *Solenopsis*, renowned for their painful bites [yy]. The molecule contains a piperidine ring substituted with a methyl group together with an extended hydrophobic chain. Solenopsin A is the major toxic component of the fire ant venom. It is likely that this component of the venom accounts for cardiorespiratory failure in individuals who receive multiple fire ant stings [zz].

Solenopsin was first synthesized By Habermann in 1998 [aa] and other chemically related piperidines have since been found in the venom. Solenopsin inhibits angiogenesis *in vitro* via an action on the phosphoinositide 3-kinase (PI3K) signalling pathway [17] and also inhibits neuronal nitric oxide synthase (nNOS) [ref]. Synthetic piperidine derivatives, the cis- and cis-trans mixtures of both 2-methyl-6-undecyl piperidine and 2-methyl-6-tridecyl piperidine have been explored as potential insecticides. Although not insecticidal in whole insect toxicity tests, activity was detected on insect neuronal nAChRs [BB] which are established insecticide targets [CC DD EE]. Recently Solenopsin analogues have been explored for the potential treatment of human psoriasis [18].

Apamin

Apamin (Fig. 3) is a small peptide containing 18 amino acids present in honeybee venom. It makes up about 2% of dry bee venom and acts by blocking small conductance, calcium-activated potassium channels (SK channels). These channels are present in a wide range of excitable and non-excitable cells [ref]. They account for the after-hyperpolarisations that follow the action potential and are important in regulating repetitive firing in neurons. Three distinct subclasses exist, of which the SK2 and SK3 channels are apamin-sensitive whereas SK1 is apamin-insensitive [ref]. Thus, apamin is a potential lead molecule of interest and shows a degree of selectivity in its actions on SK channel subtypes. Check 3 types in insects and verts

Melittin

Melittin (Fig.3) is a small amphiphilic peptide from the venom of the honeybee *Apis mellifera* containing 26 amino acids, with a hydrophobic N terminus and a hydrophilic C-terminus. It contributes strongly to the allergic properties of bee venom. It disrupts the phospholipids of cell membranes and causes lysis. Potential antimicrobial, antiviral and anti-cancer applications have been explored and these are reviewed in detail by Moreno and Giralt [19].

Assassin bug toxin (Insect toxin)

The peptide toxins produced by the assassin bug (sometimes known as the kissing bug) is used to immobilise and digest their prey – typically insect larvae and crickets. The toxic saliva of the assassin bugs contains a complex cocktail of small and large peptides with roles in prey-immobilisation, pre-digestion of prey and defence against competitors and predators. Assassin bug toxins are small peptides with disulfide bonding, targeting calcium channels [20] and showing homology to the calcium channel blockers the omega-conotoxins [21] isolated from marine cone snails.

One of these small peptide toxins, known as Ptu1 from *Peirates obscurus* contains 34 amino acid residues and is cross-linked by 3 disulfide bridges (Fig.3). It targets the N-type calcium channels of vertebrates [ref]. It is much less effective on either the L-type or P/Q-type calcium channels, thereby showing calcium channel subtype selectivity [ref]. Other assassin bug toxins are: *Agriosphodrus dohrni* toxin Ado1 and the *Isyndus obscurus* toxin Iob1 [refs].

Diamphotoxin and Leptinotarsin

Diamphotoxin is produced by beetles of the genus Diamphidia [22]. It is produced by both larvae and adults. It is hemolytic and cardiotoxic in its actions. For example, it increases the permeability of red blood cell membranes disrupting the normal ionic composition of the cell. Although without toxic actions on neurons its hemolytic effect can be lethal. It has been deployed as an arrow poison for hunting game, where it paralyses the muscles of large mammals hunted with arrows tipped with this toxin. From leaf beetles species of the genus Leptinotarsa, a peptide toxin, leptinotarsin, similar to that of the assassin bug toxin has been obtained [23].

Mastoparan

Mastoparan (Fig. 3) is a small amphiphilic peptide toxin from wasp venom composed of 14 amino acids. Its actions vary depending on the type of cell with which it interacts but often involves, for example, the triggering of exocytosis leading to the secretion of histamine from mast cells, 5-hydroxytryptamine release from blood platelets and the discharge of

catecholamines from chromaffin cells [24]. When in a phospholipid environment, mastoparan structurally resembles activated G protein receptors and the resulting signalling cascade leads to release of intracellular inositol trisphosphate with the resulting calcium influx [ref].

Mastoparan inhibits all developmental forms of *Trypanosoma cruzi*, the parasite responsible for Chagas disease [25]. It also shows potent antimicrobial activity. In 2016, Irazazabal and colleagues found that the inclusion of an arginine and an isoleucine residue at positions 5 and 8, respectively, reduced the toxicity of mastoparan, but retained its antimicrobial activity thereby turning it into a potential drug candidate for the control of infectious diseases [26].

Many other insect toxins are known and include the peptide sarcotoxins. A sarcotoxin was first isolated in 1983 from the haemolymph of the fleshfly *Sarcophaga peregrina*. Sarcotoxins have antibacterial properties. It appears that the most toxic insect venom is that of the harvester ant *Pogonomyrmex maricopa*.

Conclusions

Although only a small selection of insect toxins have been discussed in this brief overview, it is apparent that some, including the philanthotoxins, apamin and the assassin bug toxin, are proving to be excellent pharmacological tools with selectivity to particular ion channel and receptor subtypes. Others, such as melittin and mastoparan, solenopsins, have potential therapeutic roles either in their natural state or following some re-engineering to further enhance their utility.

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Figure legends

Fig 1. Chemical structure of the *Philanthus triangulum* venom polyamine component, PhTX-433, molecular weight 435. The numerals 433 denote the number and sequence of the methylene groups separating the amine groups. Philanthotoxins with varying degrees of receptor subtype specificity for mammalian ligand-gated ion channels are also illustrated. A similar enumeration system accounts for the names of the other philanthotoxins in the series. Key regions of the PhTX-433 molecule are highlighted with Roman numerals. Region I = ammonium group, region II = thermospermine, region III = butyryl and region IV = tyrosine.

Fig 2. Chemical structure of the Solenopsin A, a component of the venom of fire ants of the genus *Solenopsis.*

Fig. 3 Amino acid sequences of the peptide insect toxins Apamin, Mastoparan, Mellitin and the Assassin bug toxin. Brackets are used to show disulphide bridges.

Fig. 4 Schematic to show how the actions of venom and salivary gland components can be tested in either cell-based plate assays or physiological assays to search for toxins or toxin-inspired small molecules with activity. ‘Hits’ in such screens can then be explored for their potential for further optimisation towards a useful pharmacological probe or a therapeutic candidate.