The Sophisticated Peptide Chemistry of Venomous Animals as a Source of Novel Insecticides Acting on Voltage-Gated Sodium Channels

Peigneur Steve and Tytgat Jan
Laboratory of Toxicology, University of Leuven (K.U.Leuven)
Belgium

1. Introduction

Arthropods pests are considered as a global health treat since they are responsible for the transmission of several new and reemerging human diseases such as malaria, dengue and yellow fever (mosquitoes), Lyme disease, ehrlichiosis and tularemia (ticks). Furthermore, arthropods pests destroy 20-30% of the world’s food supply every year. Evidently, modern agriculture still depends strongly on the use of insecticides. The majority of insecticides used today act on one of the following neuronal targets: acetylcholinesterase, the nicotinic acetylcholine receptor, the γ-aminobutyric acid (GABA)-gated chloride channel and the voltage-gated sodium channel (Na$^+_V$). This review will focus on Na$^+_V$ channels as possible targets for insecticides.

For over 50 years, synthetic pyrethroids, which are analogous of the natural occurring insecticidal components of Chrysanthemum flowers, have been used as classical industrial pesticides. The public awareness of the health hazards and environmental damage, due to their low insect-selectivity, has complicated the use of these pesticides. More importantly, the intensive use of DDT and other pyrethroids has facilitated the development of resistance against these compounds. The so-called knockdown resistance, in which insects have reduced their sensitivity towards pyrethroids by point mutations in their target site, heralded the loss of major classes of insecticides. As a consequence, there is an urgent need for new, potent and insect-selective insecticides. A need that could be fulfilled by insecticidal neurotoxins derived from venomous animals.

2. The sodium channel structure and function

Voltage-gated sodium channels (Na$_V$ channels) are transmembrane protein complexes constituted of an α-subunit of approximately 260 kDa which can be associated with up to four auxiliary β-subunits (β1-4) of 30 to 40 kDa. The pore-forming α-subunit alone is sufficient to obtain sodium current, however co-expression of β-subunits modifies expression level, kinetics and voltage dependence of channel gating (Yu & Catterall, 2003). The α-subunit is organized in four homologous domains (DI-IV). Each domain contains six putative transmembrane segments (S1-S6) connected by extracellular or intracellular loops.
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(fig. 1A). The S4 segments are the most conserved segments and they contain a basic residue, either lysine or arginine, in every third position. These positive charged S4 segments are believed to function as voltage sensors. They transport gating charges by moving outward upon membrane depolarization and as such initiating the voltage dependent activation which results in the opening of the channel. The selectivity filter and pore are formed by the transmembrane segments S5 and S6 together with the re-entrant segments that are part of the loop which connects the S5 and S6 of each domain. Folding of the domains in a clockwise orientation, in which domain I and IV are in close proximity of each other, leads to the formation of the outer vestibule and the selectivity filter (Catterall, 2000; Chanda & Bezanilla, 2002). The short intracellular linker that connects the domains III and IV contains a highly conserved sequence of three hydrophobic residues (isoleucine, phenylalanine and methionine) or IFM motif. Sodium channel inactivation is mediated by this hydrophobic motif since it serves as an inactivation gate crucial for causing fast inactivation by binding to a receptor. This inactivation gate receptor is located near or within the intracellular mouth of the sodium channel pore. It has been shown that several residues in the intracellular loop that connects III-S4-S5 and in the loop connecting IV-S4-S5 are contributing to the inactivation gate receptor (Dong, 2007; Yu & Catterall, 2003).

Nine different mammalian sodium channel isoforms have been cloned, characterized and functionally expressed. These sodium channel isoforms exhibit distinct expression patterns in skeletal and cardiac muscle tissues and in the central and peripheral nervous systems (Goldin, 1999). Nav1.1, Nav1.2, Nav1.3 and Nav1.6 are expressed in the central nervous system (CNS), whereas Nav1.7, Nav1.8 and Nav1.9 are predominantly expressed in the peripheral nervous system (PNS). Nav1.4 is expressed in skeletal muscles, while Nav1.5 is also known as the cardiac muscle isoform. The functional and pharmacological diversity of the mammalian Nav channels is primarily resulting from the expression of multiple genes (Goldin et al., 2000). The selective expression of different sodium channel genes significance the specialized function of sodium channels in various mammalian tissues and cell types (Yu & Catterall, 2003). Their specialized function results from the fact that each mammalian sodium channel α-subunit isoform features distinct electrophysiological properties such as unique gating kinetics (Dong, 2007; Goldin, 2001).

Voltage-gated insect sodium channels closely resemble their mammalian counterparts in electrophysiology, ion conductance and also in overall structure. However, they do differ in amino acid sequence and therefore in their pharmacological diversity and flexibility (Zlotkin, 1999). Furthermore, the insect-selective action of pesticides such as pyrethroids and DDT and moreover, the high specificity for either mammalian or insect NaV channels displayed by neurotoxins from scorpion, spider and sea anemone venoms have evidenced the existence of a pharmacological distinction between mammalian and insect NaV channels.

3. Structural comparison between mammalian and insect NaV channels

Two genes putatively encoding for NaV channels, DSC1 and para, were isolated from Drosophila melanogaster. Later on, it was shown that DSC1 encodes for a Ca2+-selective cation channel and not for a sodium channel. The first insect NaV channel encoding gene, para, was identified from a genomic DNA library in studies using mutants with a temperature sensitive paralytic phenotype (Loughney et al., 1989). The gene has been cloned and upon functional expression in Xenopus laevis oocytes it was demonstrated that para indeed encodes for a voltage-gated sodium channel (DmNaV1) (Feng et al., 1995; Warmke et al., 1997).
Fig. 1. Molecular structure and the neurotoxin receptor sites of Na\textsubscript{V} channels. (A) Schematic two-dimensional representation of the functional α-subunit and the auxiliary β-subunits. (B) Identification of known neurotoxin receptor sites on Na\textsubscript{V} channels. Green circles represent the outer (EEDD) and inner (DEKA) rings of amino acid residues that form the ion selectivity filter and the proposed neurotoxin receptor site-1. In the case of receptor sites 3 and 4, only areas where there is more than a five-fold increase in binding affinity are highlighted. Insect-selective spider toxins are highlighted in red text. Figures were adapted from (Nicholson, 2007).
Screening of the *Drosophila* genome revealed that no other genes were similar to *para*, suggesting that *para* is the only gene encoding the sodium channel in *Drosophila* and presumably also in other species (Littleton & Ganetzky, 2000). Electrophysiological recordings in different insect neurons have shown the presence of distinct sodium currents, suggesting the existence of sodium channels with differing properties (Defaix & Lapied, 2005; Grolleau & Lapied, 2000). Recent studies have shown that the occurring heterogeneity in sodium channel properties in insects is generated by extensive alternative splicing and RNA editing of the *para* gene transcript (Dong, 2007). Thus, it seems that insects rely on alternative splicing and RNA editing to produce a functional diversity of sodium channels, whereas mammals depend on multiple sodium channel genes to generate channels with unique gating properties (Dong, 2007; Yu & Catterall, 2003). An increasing number of orthologous *para* genes have been identified in agricultural and medical important insect species (Soderlund & Knipple, 2003). Unfortunately, only partial cDNA clones could be obtained in most cases. Up to date, the full length cDNA clones for only 3 orthologous *para* genes have been identified: in the housefly *Musca domestica* (Vssc1), the German cockroach *Blattella germanica* (BgNaV) and the mite *Varroa destructor* (VmNaV) (Dong, 1997; Ingles et al., 1996; Wang et al., 2003). All 3 genes generate voltage-dependent sodium currents when heterologously expressed in oocytes (Du et al., 2009; Ingles et al., 1996; Tan et al., 2002b). A functional characterization of 20 BgNaV splice variants demonstrated that, similar to *para*, RNA editing and alternative splicing results in an astonishing diversity of NaV channels with distinct expression levels and varying kinetics of gating.

An insecticidal counterpart of the mammalian auxiliary β-subunit has also been identified in the Drosophila genome. The temperature-induced paralysis locus E (*TipE*) encodes for a small transmembrane protein consistent of two transmembrane segments which are connected by a large extracellular loop and intracellular amino and carboxyl termini (Dong, 2007; Feng et al., 1995). *TipE* increases the sodium peak current, alters the kinetics of fast inactivation and changes the pharmacology of DmNaV1 when it is co expressed with this para α-subunit (Warmke et al., 1997). Furthermore, *TipE* plays an important assisting role in the trafficking of the α-subunit from the endoplasmic reticulum to the membrane and the incorporation in the membrane (Moore et al., 2000).

Four *TipE* homologs have been characterized in *D. melanogaster* (TEH1-4) (Derst et al., 2006). Similar to *TipE*, coexpression of TEH1-3 in oocytes results in increased sodium peak currents. Furthermore, it was shown that TEH1 shifts the voltage-dependent inactivation and it alters the rate of recovery from inactivation of DmNaV1. TEH1 is only expressed in the central nervous system while TEH2-4 are widely expressed in both neuronal and non-neuronal tissues (Derst et al., 2006). This might imply that these TEH auxiliary subunits are involved in specific regulation of sodium channels in wide variety of insect cells (King et al., 2008). An orthologous *TipE* gene has been cloned from the *Musca domestica* (Vssc β) (Lee et al., 2000). Up to date, no ortholog has been identified in the German cockroach. However, it has been shown that co-expression of *TipE* with BgNaV or with Vssc1 in both cases resulted in an enhanced expression in oocytes (Lee et al., 2000; Tan et al., 2002a). These results suggest that the auxiliary subunits are functionally conserved among different insect species. A recent study has shown that that the channel affinity of the conotoxin MrVIB, a mammalian sodium channel blocker isolated from the cone snail *Conus marmoreus*, is strongly influenced by the co expression of β-subunits (Wilson et al., 2011).

Their high conservation among different insect species, their wide expression in distinct tissues together with the observation that they are involved in the neurotoxin-channel...
interaction raises the intriguing question whether the insect auxiliary subunits might represent interesting new phyla-selective targets for neurotoxin insecticides.

4. Rationale for the use of insect Na\textsubscript{V} channels as targets for insecticides

Na\textsubscript{V} channels mediate the increase in sodium permeability during the initial rapidly raising phase of the action potential making them a crucial component in the generation and propagation of action potentials in neurons and most electrically excitable cells. Because of this key role in the excitability of biological systems, Na\textsubscript{V} channels are one of the foremost targets of venomous animals. These venoms are complex cocktails that have evolved to paralyze or to kill arthropod preys. Mutations and positive selection have lead to an optimization of the venom compounds which resulted in toxins able to act highly specific and very potently upon their target.

Further justification for the use of Na\textsubscript{V} channels as targets for the development of novel insecticides is delivered by the pharmacological flexibility of Na\textsubscript{V} channels. This arises for instance from the existence of a large number of sodium channel binding sites (fig; 1B). To date, seven neurotoxin binding sites have been identified, potentiating a broad diversity of insecticidal targets. Although the identification and characterization of the distinct receptor sites were mainly determined using vertebrate preparations, similar receptor sites have been shown for insect neuronal membranes (Gordon \textit{et al.}, 2007). The large number of Nav channel binding sites is much more than thus far described for other possible ion channel targets such as Ca\textsubscript{V} and K\textsubscript{V} channels. Several studies using insect-selective toxins from different venomous animals have underlined that even though there is an overall structural similarity with the mammalian isoforms, insect Na\textsubscript{V} channels do exert a distinct pharmacological diversity compared to their mammalian counterparts. Furthermore, the allosteric coupling of the binding sites provides cooperative aspects which possess far-reaching practical, economical and ecological agro-technical implications (Nicholson, 2007; Zlotkin, 1999). As such, a synergistic mixture, constituted of 2 insecticides acting on distinct but allosterically coupled sites, will allow a significant decrease in the required dosages and concentrations of the 2 constituting insecticides and thus reducing production costs. Moreover, the combinatorial use of insecticides will delay the onset of resistance (Nicholson, 2007; Zlotkin, 1999).

It should be critically noted that there is no evidence to reason why Na\textsubscript{V} channel are a more suitable target for developing new insecticides compared to other ion channels such as Ca\textsubscript{V} or K\textsubscript{V}. Given the fact that Na\textsubscript{V} and Ca\textsubscript{V} channels belong to the same superfamily of structural related voltage-gated ion channels, it is most likely that insect Ca\textsubscript{V} channels present a similar broad panel of potential binding sites for insecticide development. Certainly since at least 3 distinct classes of insect Ca\textsubscript{V} channels have been identified (Jezierski \textit{et al.}, 2000). However, due to a lack of functionally cloned insect Ca\textsubscript{V} channels and a limited number of accessible and well-characterized insect neuron preparations, does the number of potential new insecticides acting on Ca\textsubscript{V} channels remain scarce (King \textit{et al.}, 2008).

5. Toxins isolated from venomous animals acting selective on insect Na\textsubscript{V} channels

Venomous animals such as scorpions, spiders and sea anemones have become medicinal chemists of unprecedented skills by evolving their peptide chemistry and
neuropharmacology in order to develop components that insure complete shut down of the nervous system of their arthropod prey. Peptide neurotoxins that target ion channels are abundantly represented in these venoms. Specifically toxins acting on NaV channels provide venomous animals the possibility to induce a rapid paralysis upon envenomation. These toxins, thanks to their insect selectivity, are potential new lead compounds for the development of a novel generation insecticides. All insect-selective neurotoxins characterized to date are selectively binding to three of the seven known neurotoxin sites on insect NaV channels. Therefore these sites can be considered as potential insecticide targets (King et al., 2008).

5.1 Spider venoms

Over 42,000 species of spiders have already been described worldwide (Platnick, 2000). They are divided in 110 families which are classified in the order Araneae within the Arachnida class, a group that also includes scorpions, mites and ticks. Araneae comprise three suborders: the non-venomous Mesothelae, the Mygalomorphae or tarantulas and the Araneomorphae. Only a minority of spider species are capable of inflicting clinically significant or sometimes fatal envenomations. These species include the latrodectism or widow spiders, the loxoscelism or recluse spiders and certain mygalomorph such as the Australian funnel web and mouse spiders (Billen et al., 2008; Isbister & White, 2004). Among the large number of species described, only of about 100 species has the venoms been studied. Venom composition is highly species-specific and varies depending on factors such as sex, nutrition, natural habitat and climate (Kuhn-Nentwig et al., 2004; Mebs, 2002). Spiders have optimized their venoms as complex, chemical mixtures containing a variety of biological active substances serving the general purposes for both attacking (killing or paralyzing prey) and protecting (defending against competitors) (Mebs, 2002; Vassilevski et al., 2009). These venoms can be divided into two groups based on the character of their function: neurotoxic and necrotic or cytolytic. This review will only focus on the neurotoxic group and more specific on the neurotoxins acting on NaV channels. The complete molecular diversity of spider venoms has recently been very well reviewed in (Vassilevski et al., 2009).

Spider neurotoxins channels are low molecular weight polypeptides. Even though peptides devoid of disulfide bridges have been reported, the majority of these neurotoxins are small disulfide rich peptides, containing 6 to 12 cysteine residues (Billen et al., 2008; Pimenta & De Lima, 2005). Spider neurotoxins which target NaV channels are in general peptides compromising 31 to 41 amino acid residues which are cross-linked by three to four disulfide bridges. Depending on their different cysteine arrangements and structural characteristics, these peptides can be divided into two distinct structural motif families: the inhibitory cysteine knot (ICK) motif and the disulfide-directed β-hairpin (DDH) scaffold (fig. 2) (Craik et al., 2001; Norton & Pallaghy, 1998).

Both panels schematically represent the structural motifs of the cystine-knot folding and possible addition of the third β-sheet. β-sheets are shown as gray arrows and disulfide bridges connecting cysteine residues are shown as dark gray lines with roman numerals. The dark arrow (β1) in the right panel represents the additional β-sheet not always present in ICK spider toxins. Figure was adapted from (Nicholson, 2007).

The majority of spider toxins contain the ICK motif. It is interesting to note that peptides with the ICK motif can be found in very diverse sources such as animals, plants, fungi and even viruses. The following arrangement of disulfide bonds is observed in all peptides.
belonging to this structural family: C\textsubscript{1}-C\textsubscript{4}, C\textsubscript{2}-C\textsubscript{5}, C\textsubscript{3}-C\textsubscript{6}. These ICK containing peptides are further characterized by a \(\beta\)-hairpin and by the presence of a so-called 'knot': the first two disulfide bridges (C\textsubscript{1}-C\textsubscript{4}, C\textsubscript{2}-C\textsubscript{5}) form a spatial ring which is penetrated by the third disulfide bridge (C\textsubscript{3}-C\textsubscript{6}) (Craik et al., 2001; Vassilevski et al., 2009). Even within a structural motif family are the biological activities diverse. Spider neurotoxins possessing the ICK motif can target proton-gated, voltage-gated or mechanosensitive channels while others exert a haemagglutination activity, protease inhibition or AMP activity (Bulet & Stocklin, 2005). The DDH scaffold is believed to be the ancestral motif from which the ICK motif has evolved. This structural motif is characterized by an arrangement of disulfide bridges as follows: C\textsubscript{1}-C\textsubscript{3}, C\textsubscript{2}-C\textsubscript{4}. Such a disulfide pattern implies that there are only two mandatory disulfide bridges that form the bulk of the hydrophobic core and that there is a formation of \(\beta\)-hairpins which are stabilized by these two conserved disulfide bonds (Vassilevski et al., 2009).

![Structural motifs found in insect-selective spider toxins.](image)

Fig. 2. Structural motifs found in insect-selective spider toxins.

Spider neurotoxins interact with Na\textsubscript{V} channels either by binding to site 1, blocking the sodium current or by modulating the kinetics of gating by binding to site 3 and 4 (King et al., 2008). Furthermore, a Na\textsubscript{V} channel targeting family of 56-61 residue insecticidal polypeptides has been isolated from the primitive weaver spider Diguentia canities. Their exact interaction site with the sodium channel still remains unknown although binding studies have shown that it is unlikely that they bind to site 3 (Nicholson et al., 2004). Further electrophysiological studies are awaiting in order to elucidate the binding site of these interesting insecticidal spider neurotoxins (Nicholson, 2007).

Environmental stress and evolution has led to hypermutational optimization of spider toxins resulting in mini-libraries of toxin variants allowing spiders to target slightly different versions of the same ion channel or receptor in distinct insect species (Gilles et al., 2002). Therefore spider venoms contain pre-optimized insecticidal toxins, which are readily available for investigation to isolate potential lead compounds in the search for new insecticides (Nicholson, 2007).
5.2 Scorpion venoms

Scorpions belong to the most ancient group of animals on earth as they have been roaming this planet for more then 400 million years. More than 1500 different species have been described and they are classified in the order Scorpionidae within the Arachnida class (Bosmans & Tytgat, 2007b). Approximately 50 species are known to cause clinical significant injuries out of which 25 are potentially fatal to humans. A scorpion sting might be trivial causing local pain only, but may also produce a very complex symptomatology of envenoming such as neurological, respiratory and cardiovascular collapse. Almost all of the lethal scorpions, except the Hemiscorpius species, belong to the family of the Buthidae. Dangerously venomous scorpions tend to have lean, delicate pincers, thin bodies and thick tails, as opposed to the large, bulky pincers, thick bodies and thin tails possessed by the non-lethal scorpions (Mebs, 2002). The lethal members of the Buthidae family include the genera of Androctonus (Northern Africa to Southeast Asia), Centruroides (Southwest of Northern and Central America), Leiurus (Middle East and Northern Africa), Mesobuthus (Asia), Parabuthus (Southern and Western Africa), Buthus (Mediterranean) and Tityus (Caribbean, Central and Southern America).

Scorpion venoms are multi component mixtures containing an unprecedented molecular diversity of pharmacologically active components such as enzymes, nucleotides, lipids, mucoproteins, biogenic amines, polypeptides and other unknown substances. The most characterized components of scorpion venoms are neurotoxins which have evolved towards a specific and specialized bioactivity aimed at efficient and successful capture of prey or defense against predators. Scorpion neurotoxins specifically target voltage-gated ion channels such as Na\textsubscript{v}, K\textsubscript{v} or Ca\textsubscript{v} and other cellular receptors such as the ryanodine receptor (Zamudio et al., 1997). Similar to spiders, is the composition of scorpion venom highly differing between species and are factors such as sex, habitat and local conditions determining for venom specificity (Bosmans & Tytgat, 2007b; Possani et al., 1999). For example, scorpion neurotoxins targeting Na\textsubscript{v} channels are much more represented in the venom from scorpions belonging to the dangerous Buthidae as compared to the venom of harmless species such as Pandinus. Moreover, the overall toxicity of scorpion venom to humans has mainly been attributed to the activity of neurotoxins affecting Na\textsubscript{v} channels (Martin-Eauclaire et al., 2005).

In general, scorpion neurotoxins acting on Na\textsubscript{v} channels are single chain polypeptides composed of 58-76 amino acids and cross-linked by four disulfide bridges. They posses a highly conserved core formed by an α-helix and two to three strands of β-sheet structural motifs, stabilized by the three intermolecular disulfide bridges (Bosmans & Tytgat, 2007b). The scorpion structural motif families compromise the βαββ family and the βααβα family which are all stabilized by the four disulfide bridges (Mouhat et al., 2004). Both structural motif families belong to the structural cysteine-stabilized α-helix and β-sheet (CSαβ) superfamily. Peptides belonging to the CS superfamily exhibit relatively diverse biochemical and biological functions. However, in most cases are these peptides sharing a common function as defenders of their host, as seen in animals (e.g. scorpion toxins), plants (e.g. defensins) and microorganisms (e.g. antifungal defensins). The extensive distribution of this common motif throughout diverse organisms highlights that this relatively stable and versatile scaffold has the potential to tolerate insertions, deletions and substitutions within the structure (Zhu et al., 2005).

According to their pharmacological profile, scorpion neurotoxins acting on Na\textsubscript{v} channels can be divided into two major groups: α-toxins and β-toxins. Upon binding at neurotoxin...
receptor site 3, α-toxins modulate the Nav channel by slowing down the fast inactivation. β-toxins modulate the activation process by binding to neurotoxin site 4. According to their preference for mammalian or insect Nav channels, α-toxins further subdivided into three groups: (i) the classical α-toxins with a preference for mammalian Nav channel isoforms; (ii) the α-like toxins which are active on both the insect and mammalian Nav channels; (iii) the insect α-toxins which are capable of discriminating with high affinity between insect and mammalian Nav channels and thus serve as potential candidates for the development of insecticides (Billen et al., 2008; Gordon et al., 2007; Gurevitz et al., 1998).

Similar to the α-toxins are the β-toxins also classified into 3 groups according to their pharmacological properties, exemplified by their preference for mammalian or insect sodium channels: (i) Mammalian-selective β-toxins are highly toxic to mammals (Martin et al., 1987); (ii) β-like toxins are capable of competing for binding sites on both insect and mammalian Nav channels; (iii) Insect-selective β-toxins fail to exert any affinity whatsoever for mammalian sodium channels, even in very high concentrations (de Dianous et al., 1987).

Exactly this complete lack of mammal activity combined with their strong insect specificity and potency makes these insect-selective β-toxins interesting lead compounds in the design of new insecticides (Gurevitz et al., 2007). The insect-selective β-toxins can be further subdivided into excitatory and depressant toxins according to the symptoms they evoke in vivo. Injection of excitatory toxins induces a fast repetitive activity of motor nerves which results in a reversible contraction paralysis (Billen et al., 2008; Zlotkin et al., 1985). These excitatory toxins differ from the other β-toxins because there is one disulfide bridge differently located and furthermore they do display extra secondary structural elements (Gurevitz et al., 1998; Rodriguez de la Vega & Possani, 2005). The depressant toxins cause a transient contraction followed by a slow depressant and flaccid paralysis (Karbat et al., 2007; Zlotkin et al., 1991). Remarkably, insect-selective depressant β-toxins are given the opportunity to affect mammalian Nav channels when these channels are excited by a long preconditioning depolarizing prepulse. This potential prerequisite to affect mammalian Nav channels possibly contributes to the observed lack of toxicity of depressant toxins towards mammals.

Predictions suggest that up to 100 000 distinct polypeptides are present in all known scorpion species. At this moment, only 1% thereof has been biochemical and functional characterized (Possani et al., 1999). The high number of yet unknown peptides together with the strong phyla specificity exerted by their neurotoxins validates scorpion venoms as a promising source for novel Nav channel targeting insecticides.

### 5.3 Sea anemone venoms

Sea anemones are ocean dwelling, solitary animals belong to the phylum Cnidaria. There are over 1400 species described, enclosing more then 45 families which are grouped into the order Actiniaria within the Anthozoa class. Sea anemones are widely distributed around the world as they can be found from the poles to the equator. Most sea anemones live in tidal zones and in shallow water. Sea anemones generally do represent a serious risk for humans as stings usually only cause mild reactions such as inflammation, pain and edema. However, certain tropical species can deliver a more painful sting in which case often also necrosis is observed (Oliveira et al., 2009). Sea anemone venoms are a known pharmacological treasure of biological active compounds. The venom can be divided into two proteic groups of compounds: (i) pore-forming toxins such as hemolysins and actinoporins; (ii) neurotoxins (Beress & Beress, 1975). These neurotoxins are acting upon a
diverse panel of ion channels such as TRPV1, NaV, Kv and acid-sensing channels (Diochot et al., 2003, 2004). Of these different toxins, those that target NaV channels are the best studied group with more then 100 known toxins (Bosmans & Tytgat, 2007a). In contrast, no more than 12 Kv channel toxins have been characterized to date. Since the beginning of last century sea anemones have been studied with an increasing interest. Although a number of sea anemone toxins have been isolated and characterized, these animals remain poorly studied in comparison with other venomous animals such as scorpions, spiders, cone snails or snakes. Based on structural differences and activity profile, the sea anemone toxins targeting NaV channels have been subdivided into 3 groups or types (Honma & Shiomi, 2006). Type 1 and 2 toxins are polypeptides composed of 46-49 amino acid residues, stabilized by the connection 6 cysteine residues forming 3 disulfide bridges. Both type 1 and 2 toxins are believed to have evolved from the same ancestral gene (Ishida et al., 1997). Consequently, type 1 and 2 toxins share the conservation of the six half-cysteines as well as several other residues thought to play a role in biological activity. Nevertheless, from a immunological point of view are these toxins distinguishable since no cross-reactivity occurs (Norton, 1991). Type 1 toxins are characterized by a core of four strands of anti-parallel β-sheets connected by two or three loops (Salceda et al., 2007). Type 3 sea anemone toxins comprise 4 peptides. These toxins contain 30-32 amino acid residues, stabilized by 4 disulfide bridges (Honma & Shiomi, 2006). All 3 types of sea anemone NaV channel toxins interact with site 3, hereby altering the inactivation kinetics. The validation of sea anemone as a potential source for new neurotoxin derived insecticides based on the existence of crustacean selective peptides in these sea anemones might seem peculiar. However, from an evolutionary point of view, it is understandable why we can encounter potential insecticidal toxins in the venom of sea anemones even when insects and sea anemones will never encounter one another (Bosmans & Tytgat, 2007a). It has been proposed that the ‘Pancrustacea’ taxon comprises all crustacean and hexapods (Bosmans & Tytgat, 2007a). It should hereby be noted that the class Insecta is comprised in the phyla Hexapoda which is a subphylum of the phylum Arthropoda. A monophyletic ‘Pancrustacea’ taxon has been supported by many molecular studies (Nardi et al., 2003; Shultz & Regier, 2000). In these studies is most of the subphylum Crustacea paraphyletic with respect to insects. Therefore it can be concluded that insects are descendents from crustacean ancestors and thus by definition can crustacean-selective toxins be considered as insect-selective toxins. Based on these peculiar evolutionary and pharmacological arguments have sea anemone venoms earned there status of promising source for new pesticides.

6. Neurotoxin receptor sites of NaV channels as molecular targets of insect-selective toxins

6.1 Insect-selective inhibiter of the sodium conductance: site 1 toxins?

The amino acid residues that form the neurotoxin receptor site 1 are primarily located in the pore loop which is formed by the membrane dipping part of the connecting loop between S5 and S6 of each domain (Catterall et al., 2007). This site is occupied by the water soluble heterocyclic guanidine tetrodotoxin (TTX) which is isolated from the tissue of at least 40 different species of puffer fish but it can also be found in mollusks, crabs, octopus and frogs (Catterall et al., 2007; Hwang et al., 1991). TTX exerts its strong toxicity and high fatality, also towards humans, by binding within the inner pore of the channel, physically occluding the ion pathway. Based on their sensitivity to TTX have the mammalian isoforms been divided
into TTX-sensitive (Nav1.1-Nav1.4, Nav1.6 and Nav1.7) or TTX-insensitive (Nav1.5, Nav1.8, Nav1.9) (Narahashi, 2008).

Fig. 3. Effects of site 1 toxins on insect Na_V channels. Panel represents the effects of site 1 toxins on insect Na_V channels expressed in Xenopus laevis oocytes. The arrow indicates the steady-state condition after application of site 1 toxin. Binding upon site 1 causes an inhibition of the sodium conductance (indicated by the arrow). Currents were evoked by 100 ms depolarizations to 0 mV, from a holding potential of -90 mV.

Besides TTX, it has also been shown that μ-conotoxins, neurotoxin peptides isolated from Cone snail species, bind to a micro site within the neurotoxin receptor site 1 (Stephan et al., 1994). Even though these μ-conotoxins are known to be highly selective and potent towards mammalian sodium channel isoforms, little investigation has been done regarding their activity towards insect sodium channels. No insect selective μ-conotoxin has been described yet. However, a recent study has reported that the δ-conotoxin TxVIA, which binds to receptor site 6 on Na_V channels, shows insecticidal activity when injected into lepidopteran (cabbage moth) and dipteran (house fly) larvae, suggesting that Cone snail neurotoxins
might have an interesting insecticidal potential (Bruce et al., 2011). For example, the terrestrial molluscan crop pest, *Deroceras reticulatum* or grey field slug, is the most damaging molluscan crop pest in the UK. Annual applications of pellets containing over 250 tonnes of active ingredients are estimated to cost approx. 34 million euro per annum (Bruce et al., 2011). As such it can be reasoned that Cone snail species, which have other mollusks as natural competitors, could be a yet unexplored source of neurotoxin-derived novel insecticides.

### 6.1.1 Site 1 toxins from spider venom

Huwentoxins and hainantoxins, isolated from the Chinese bird spiders *Ornithoctonus huwena* and *Ornithoctonus hainana* respectively, all belong to the same family of spider toxins. They are constituted of 33-35 amino acids, cross-linked by 3 disulfide bridges and folded according to the ICK motif. Both huwentoxins and hainantoxins are found to target Nav channels (King et al., 2008). Hainantoxin-I (HNTX-I) causes an inhibition of the sodium conductance without alteration of channel inactivation kinetics or of the voltage dependence of steady-state activation (Billen et al., 2008). HNTX-I stabilizes the channels in the inactivated state as demonstrated by the observed hyperpolarizing shift in the voltage dependence of steady-state inactivation. Electrophysiological studies have shown that HNTX-I displays a 15 fold-selectivity for insect Nav channels over the mammalian channel rNav1.2. HNTX-I does not show any affinity for other mammalian Nav channel isoforms and is therefore an interesting lead in the development of insecticides (Li et al., 2003).

Huwentoxin IV and hainantoxin III-V are also capable of reducing the sodium conductance but unlike HNTX-I show these toxins affinity for both mammals and insects (Xiao & Liang, 2003). These spider toxins possibly exert there activity through an interaction with site 1 although this remains to be determined.

It has been claimed that this group of toxins is the first family of spider polypeptides capable of selectively blocking Nav channels by occupying site 1. However, studies have evidenced that huwentoxin IV binds at site 4, trapping the voltage sensor of domain II in its inward position rather than interacting with site 1 or a distinct binding site within the extracellular pore region. Characterization of the huwentoxin IV interaction revealed that this toxin fails to induce any modification on the activation and steady-state inactivation, making it electrophysiological distinguishable from the previously described HNTX-I induced effects (Xiao et al., 2008). Therefore competitive radioligand binding studies are required to confirm for each of these toxins the exact interaction site with the voltage-gated sodium channel. Notwithstanding this lack of structural data, it can be concluded that these spider toxins represent the first family to selectively block the sodium conductance and, moreover, HNTX-1 can be seen as the first insect-selective Nav channel inhibiting peptide isolated from spider venom (Nicholson, 2007).

To date, no insect-selective neurotoxins capable of inhibiting the sodium conductance have been isolated from the venoms of scorpions or sea anemones.

### 6.2 Insect-selective gating modifiers of inactivation: site-3 toxins

The neurotoxin receptor 3 is mainly localized at the extracellular loop connecting the segments S3 and S4 from domain IV. It is believed that other parts of the channel such as the extracellular loops between the S5 and S6 of domain I and IV also contribute significantly to channel recognition and binding of site 3 toxins (Bosmans & Tytgat, 2007a). The voltage sensors of each domain will normally move outward under influence of the electric field.
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Fig. 5. Effects of site 3 toxins on insect Na\textsubscript{V} channels. Panel represents the effects of site 3 toxins on insect Na\textsubscript{V} channels expressed in *Xenopus laevis* oocytes. The asterisks indicate the steady-state condition after application of site 3 toxin. Binding upon site 3 causes such an extreme slowing down of inactivation that channels simply do not inactivate anymore. The result is a massive influx of Na\textsuperscript{+} ions. Currents were evoked by 100 ms depolarizations to 0 mV, from a holding potential of -90 mV.

when the membrane is depolarized (Hille, 2001). Upon binding at site 3 these toxins trap the voltage sensing segment S4 of domain IV in its inward position. As such they prevent the normal outward movement of these voltage sensors and herewith the conformational changes necessary for fast inactivation (Catterall *et al.*, 2007).

Summarized, toxin binding at neurotoxin receptor 3 affects the coupling of activation and inactivation, resulting in a slowing down or inhibition of the fast inactivation (fig. 5). Several studies have demonstrated the functionally relevant structural differences in insect and mammalian receptor site 3 regions (Gordon *et al.*, 1996). Although more structure-function work is necessary to completely unravel these structural differences, these phyla depending differences do support the arguments to target site 3 in the search for new Na\textsubscript{V} channel acting insecticides (Cohen *et al.*, 2006).

Magi 2  
\[ \text{CMGYDIECNENLPCCKHRLCVCETSGYWWYKRKYCRPIK} \]

Tx4 (6-1)  
\[ \text{CGDINAAKEDCDCCGYTTACDCYWSKSCCKCREAIVYTAPKKLTC} \]

PnTx4 (5-5)  
\[ \text{CADINGACKSDCCGDSVTDCYWSDSCKCRESNFKIGMAIRKKE} \]

PnTx4-3  
\[ \text{CGDINAAKEDCDCCGYTTACDCYWSSSCKCREAIVYTAPKKLTC} \]

Fig. 6. Insect-selective site 3 toxins isolated from spider venom.

Comparison of the amino acid sequences of insect-selective spider toxins which target site 3. Identical residues are boxed in grey, cysteines are shown in red.
6.2.1 Site 3 toxins from spider venom

Several insect-selective spider neurotoxins are known to bind at site 3. Six spider neurotoxins (Magi 1-6) have been isolated from the Japanese funnel-web spider Macrothele gigas (Corzo et al., 2003). Magi 1-4 compete for site 3 on insect Na\textsubscript{V} channels with the well-characterized scorpion toxin Lqh\textalpha\textit{IT} in radioligand binding experiments. Magi 2, which is composed of 40 amino acid residues and possesses the ICK fold, shares little sequence homology with any other spider toxin. It is an insect-selective toxin as it induces paralysis in insects while it is devoted of any activity on mammals. Like other spider toxins acting on site 3, Magi 2 still awaits delineation of its structure-function relationship in order to elucidate the key residues involved in the insect-selectivity and potent channel modulation of this toxin (Nicholson, 2007). The venom of the South American ‘armed’ spider Phoneutria nigriventer has been extensively studied as this spider accounts for the majority of spider envenomations in Brazil (Borges et al., 2009). Several insecticidal peptides have been isolated from the venom of P. nigriventer. One of them, a 48 residue long polypeptide with 5 disulfide bonds called PnTx4(6-1), was shown to exhibit its insecticidal activity through potent modulation of Na\textsubscript{V} channels. Moreover, this toxin binds with high affinity to site 3 of insect channels but fails to bind at the mammalian Na\textsubscript{V} channel isoforms rNa\textsubscript{V}1.2 and rNa\textsubscript{V}1.4, even in high concentrations (de Lima et al., 2002). Furthermore, two other toxins from P. nigriventer, PnTx4(5-5) and PnTx4-3, have shown to be highly insecticidal towards houseflies and cockroaches. At the same time, these toxins display no toxicity towards mammals. It has been shown that PnTx4(5-5) acts on NMDA-subtype of glutamate receptors and that PnTx4-3 inhibits glutamate intake possibly through interaction with Na\textsubscript{V} channels (Borges et al., 2009). Indeed, because of the high homology in sequence between these two toxins and PnTx4(6-1) it can be hypothesized that both toxin exert their insect-selective activity at least in part through an interaction with Na\textsubscript{V} channels.

Other spider neurotoxins which bind to site 3 are the \delta-atracotoxins, isolated from the venom of Australian funnel-web spiders. Although \delta-atracotoxins modulate insect Na\textsubscript{V} channels with high potency, they are of less interest for the development of new insecticides due to there almost equal affinity for both mammalian and insect Na\textsubscript{V} channels (Nicholson et al., 2004). Therefore this group of spider toxins will not be discussed in this review.

Magi 2 and PnTx4(6-1) are two examples of a growing group of potent insect-selective Na\textsubscript{V} channel toxins from spiders, validating both spider venoms as source of insecticidal peptides and site 3 as a potential target of novel peptide derived insecticides.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{insect-selective-site-3-toxins.png}
\caption{Insect-selective site 3 toxins isolated from spider venom.}
\end{figure}

Comparison of the amino acid sequences of insect-selective scorpion toxins which target site 3. Identical residues are boxed in grey, cysteines are shown in red, disulfide-bonding pattern is indicated above sequences.
6.2.2 Site 3 toxins from scorpion venom

The insect α-toxins isolated from scorpion venoms are potently active on insects and at the same time importantly weak or not active on mammals. Mutagenesis studies and toxin crystal structure determinations have shed light upon the structural basis which provides these toxins the ability to discriminate between insect and mammal Na\textsubscript{V} channels (Guan \textit{et al.}, 2004). The insect α-toxin Lqh\textsubscript{α}IT, isolated from the scorpion \textit{Leiurus quinquestriatuus hebraeus}, is the most potent and best characterized insecticidal scorpion toxin up to date (Gordon \textit{et al.}, 2007). This peptide is 64 amino acids long and contains 8 cysteins. Similar to all scorpion neurotoxins acting on Na\textsubscript{V} channels, Lqh\textsubscript{α}IT belongs to the CS\textsubscript{α}ǃ structural superfamily (Tugarinov \textit{et al.}, 1997). It serves as a marker toxin for site 3 and is widely used as radiolabeled ligand in binding studies.

More recently, Bj\textsubscript{α}IT was isolated from the black scorpion \textit{Hotentota judaica}. This toxin is an insect-selective toxin acting on site 3 of insect Na\textsubscript{V} channels in nM range. Even a tenfold higher concentration displays only weak activity on the mammalian sodium channel isoform Na\textsubscript{V}1.2 (Arnon \textit{et al.}, 2005).

Phaiodotoxin is a new 72 amino acid long peptide with a disulfide bond pattern which is unique because of the position of the 2 cysteines forming the fourth bridge. This toxin, isolated from the Mexican scorpion \textit{Anuroctonus phaidactylus}, shares only 49\% homology with any known scorpion toxin (Valdez-Cruz \textit{et al.}, 2004). Interestingly, phaiodotoxin acts as an insect-selective toxin with a unique alteration of Na\textsubscript{V} channel gating. It causes a negative shift in the voltage dependence of activation and, at the same time, a positive shift in the voltage dependence of inactivation. Both alterations of gating together results in an increased ‘window current’ by 225\%, which is thought to be the cause of its high toxicity toward insects.

Fig. 8. Insect-selective site 3 toxins isolated from scorpion venom.

Comparison of the amino acid sequences of insect-selective sea anemone toxins which target site 3. Identical residues are boxed in grey, cysteines are shown in red, disulfide-bonding pattern is indicated above sequences.

6.2.3 Site 3 toxins from sea anemone venom

In comparison with scorpion and spider toxins, are sea anemone toxins poorly studied. However, their remarkable insect over mammalian specificity has been noticed already a long time ago (Schweitz, 1984). It was evidenced that the toxin ATX-I, isolated from the wax sea anemone \textit{Anemonia sulcata}, could display preferential toxicity for crabs over mice (Norton, 1991). Since it has been described on a genetic level that there is a link between...
crustaceans and insects, one could hypothesize that ATX-I consequently has potential as insect-selective toxin (Boore et al., 1998). Another toxin from the same sea anemone, ATX-II is toxic to both insect and mammalian Na\textsubscript{v} channels. However, its binding affinity for cockroach neuronal membranes is very high whereas its binding affinity for rat brain synaptosomes is low (Gordon et al., 1996). Furthermore, ATX-II binds with extreme high potency to site 3 of insect Na\textsubscript{v} channels, resulting in a strong slowing down of the fast inactivation (Warmke et al., 1997). Sh-I from Stichodactyla gigantea and CgII from Condylactis gigantea were found to be moderately toxic to insects but were essentially non-toxic to mammals (Salgado & Kem, 1992).

Two toxins BgII and BgIII, both from Bunodosoma granulifera, have been extensively studied. These sea anemone toxins were studied for their activity on cloned mammalian and insect Na\textsubscript{v} channels, dorsal root ganglia and rat brain synaptosomes (Bosmans et al., 2002; Goudet et al., 2001). Furthermore were these toxins also thoroughly tested in mice (Loret et al., 1994). These studies revealed that both BgII and BgIII have a preference for insect channel. However, BgII has a 100-fold higher potency on insect channels compared to mammalian channels, BgIII only exert a 5-fold difference in potency of insect over mammal. This dissimilarity in insect-selectivity between both peptides is remarkable in this way that BgII and III only differ from each other in one amino acid, namely an aspargine to aspartate at position 16 (Bosmans et al., 2002).

Another justification for the interest in sea anemone toxins as potential insecticides can be found in the fact that these toxins have a devastating effect on the inactivation of insect channels. Upon binding at site 3, sea anemone toxins cause such an extreme slowing down of inactivation that channels simply do not inactivate anymore (fig. 5) (Bosmans & Tytgat, 2007a).

Fig. 9. Structural model of insect-selective site 3 toxins.
Insect-selective sea anemone toxins and insect-selective scorpion toxins, represented by ATX-I and LqhIT1, respectively. Both toxins exhibit their insecticidal activity by binding upon site 3. PDB ID number:1ATX and 2YEO.

### 6.3 Insect-selective gating modifiers of activation: site-4 toxins

Toxin binding at site 4 causes a shift in the voltage dependence of activation towards more hyperpolarized membrane potentials and reduces the peak sodium current amplitude (Cestele et al., 2006; Vijverberg et al., 1984). The shift in voltage dependence of Na\textsubscript{V} channel activating causes channels to open at, or close to, the resting potential. This increase in open channel probability leads to repetitive firing and consequently increases the influx of Ca\textsuperscript{2+} into the nerve terminals resulting in an increased frequency of miniature excitatory junctional potentials (King et al., 2008). The alterations in channel gating are believed to be a direct result of toxin binding at site 4 leading to a trapping of the voltage sensor in its outward, activated position (Cestele et al., 2001, 2006). The receptor site 4 has been primarily defined to specific residues in the extracellular loops connecting the S1-S2 and S3-S4 segments of domain II (Catterall et al., 2007). However, using the scorpion β-toxin Tz1 (Tityus zulianus) it was shown that three residues in the pore loop of domain III are determining for the specificity of β-toxin for different sodium channel isoforms (Leipold et al., 2006). A recent report showed that specific mutations in the voltage sensor of domain III enhance the binding of site 4 toxins to S4 of domain II providing evidence for the involvement of the domain III voltage sensor in the action mechanism of toxins acting on site 4 (Song et al., 2011).

![Fig. 10. Effects of site 4 toxins on insect Na\textsubscript{V} channels. Panel represents the effects of site 4 toxins on insect Na\textsubscript{V} channels expressed in *Xenopus laevis* oocytes. Toxin binding upon site 4 results in a shift in the voltage dependence of activation towards more negative membrane potentials, causing channels to open at potentials they normally remain closed. Currents were, from a holding potential of -90 mV, evoked by 100 ms depolarizations ranging from -90mv to 65 mV in 5 mV increments.](www.intechopen.com)
Fig. 11. Insect-selective site 4 toxins isolated from spider venom.

Comparison of the amino acid sequences of insect-selective spider toxins which target site 4. Identical residues are boxed in grey, cysteines are shown in red, disulfide-bonding pattern is indicated.

6.3.1 Site 4 toxins from spider venom

Insect-selective gating modifiers of activation have been isolated from spider venom. A highly selective site 4 toxin, Bs1 has been isolated from the venom of the Mexican therapсид spider Brachypelma smithii. Although with low potency, Bs1 could significantly shift the voltage-dependence of activation of insect channels whereas it did not affect mammalian Na\textsubscript{V} channel isoforms (Corzo et al., 2008). Another group of insect-selective site 4 spider toxins is constituted by the \(\delta\)-palutoxins (\textit{Paracelotes luctuosus}), curtatoxins (\textit{Hololena curta}), \(\mu\)-agatoxins (\textit{Agelenopsis aperta}) and the recently characterized \(\beta/\delta\)-agatoxins (\textit{Agelena orientalis}) (Billen et al., 2010; Corzo et al., 2000; Stapleton et al., 1990). All peptides belonging to this group are structurally related as they are composed of 36-37 residues and cross-linked by 4 disulfide bridges forming an ICK motif (Nicholson, 2007). Little is known about the mechanism of action of the curtatoxin but the highly homologous \(\mu\)- and \(\beta/\delta\)-agatoxins and \(\delta\)-palutoxins have been well studied. It was reported that the \(\mu\)-agatoxins could shift the voltage-activation curve towards more hyperpolarized potentials. However, these toxins also slowed down the inactivation process of the sodium channels, resulting in a non-inactivating persistent current (Adams, 2004). The same observations were made for the \(\beta/\delta\)-agatoxins and a thoroughly electrophysiological characterization of the action of these agatoxins was performed. It was concluded that agatoxins induce a bell-shaped voltage-dependent modulation of both the activation and the inactivation of insect Na\textsubscript{V} channels, suggesting no strict correlation between the toxin binding site and its effect on channel gating (Billen et al., 2010). The insect-selectivity of the \(\delta\)-palutoxins was designated based on studies showing that these toxins potently modulate insect Na\textsubscript{V} channels but fail to exert any affinity for the mammalian Na\textsubscript{V} channel isoform Na\textsubscript{V}1.2 (Ferrat et al., 2005). Mutagenesis studies have confirmed that \(\delta\)-palutoxins contain the same pharmacological determining key residues as the well studied site 4 toxin Bj-xtrIT. Furthermore, the \(\delta\)-palutoxins compete with this depressant \(\beta\)-toxin Bj-xtrIT for site 4 but they fail to displace the binding of \(\alpha\)-toxin LqhaIT from site 3 (Corzo et al., 2005). Notwithstanding herewith, these toxins act as insect-selective modulators of sodium channels by slowing down the inactivation, a modulation typically seen upon toxin binding at site 3 (Corzo et al., 2000). This remarkable difference in mode of action provides novel perspectives about the structural relatedness of receptor site...
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3 and 4. Therefore, the topological distinction between these two sites should be questioned. The structural belief that site 3 and 4 rather belong to one extended macrosite merit plausibility as the δ-palutoxins revealed that modulation of inactivation can be achieved by binding to a site which was until now, believed to be associated with alteration on channel activation (Nicholson, 2007).

![Fig. 12. Insect-selective site 4 toxins isolated from scorpion venom.](image)

Comparison of the amino acid sequences of insect-selective scorpion toxins which target site 4. Identical residues are boxed in grey, cysteines are shown in red. The disulfide-bonding pattern is indicated above the sequences of the depressant toxins and under the sequences of the excitatory toxins, respectively.

### 6.3.2 Site 4 toxins from scorpion venom

Similar to spiders are scorpions also capable of producing toxins which recognize the neurotoxin receptor site 4. Toxins belonging to the class of insect-selective β-toxins are long chain peptides composed of 58 to 76 amino acids, cross-linked by four disulfide bridges. They belong to the structural superfamily of cysteine stabilized α/β motif containing proteins. The insect-selective β-toxins can be further subdivided into excitatory and depressant toxins according to the symptoms they evoke in vivo. AahIT from *Androctonus australis* Hector, LqqIT1 from *Leiurus quinquestriatus quinquestriatus*, Bj-xtriIT isolated from *Hotentota judaica* and BmKIT from *Buthus martensi* Karsch belong to excitatory insect-selective β-toxins (Billen et al., 2008; Froy et al., 1999; Zlotkin et al., 1985). These excitatory toxins differ from the other β-toxins because there is one disulfide bridge differently located and furthermore they do display extra secondary structural elements (de la Vega & Possani, 2007). AahIT induces repetitive firing of action potentials as a result of activation of NaV channels at lower membrane potentials, explaining the typical contractile paralysis observed in insects injected by excitatory insect-selective β-toxins such as AahIT. The depressant toxins cause a transient contraction followed by a slow depressant and flaccid paralysis (Karbat et al., 2007; Zlotkin et al., 1991). Current clamp experiments have shown that peptides belonging to this group suppress the evoked action potentials as a result of strong depolarization of the membrane, causing an inability of axons to generate action potentials (Strugatsky et al., 2005). Representatives of this group are LqqIT2 from *Leiurus quinquestriatus quinquestriatus*, BjIT2 from *Buthotus judaicus* and the highly potent and insecticidal toxins lqh-dprIT3 and LqhIT2, isolated from the scorpion *Leiurus quinquestriatus hebraeus* (Zlotkin et al., 1993). It is interesting to note that LqqIT2 did not only cause a
hyperpolarizing shift in the activation of channels, it also affected the inactivation and the ion selectivity (Bosmans et al., 2005). Therefore, the high insecticidal action of this toxin may be attributed to a combined modification of gating kinetics, resulting in Na\textsubscript{V} channels that open at more negative membrane potentials and inactivate not normally and thus, display a significantly increased open probability. Remarkably, insect-selective depressant β-toxins are given the opportunity to affect mammalian channels when these channels are excited by a long preconditioning depolarizing prepulse. The same phenomenon is observed in the case of simultaneous binding of an α-toxin upon site 3 (Cohen et al., 2007). As such, it can be seen that the presence of depressant β-toxins in the scorpion venom may still significantly contribute to the toxicity towards mammals.

![Fig. 13. Structural models of site 4 toxin.](image)

Insect-selective spider and scorpion toxins, represented by µ-Aga1, BmKIT (excitatory toxin) and lqhIT2 (depressant toxin), respectively. Nevertheless these toxins are structurally differing from each other, they all do exert their insecticidal activity through binding upon site 4. PDB ID number: 1EIT, 1WWN and 2I61.

### 6.4 Insect-selective unidentified Na\textsubscript{V} channel interactions: novel site toxins?

Although it has been determined for many spider toxins what the exact site of interaction with the insect Na\textsubscript{V} channel is, there are still several insect-selective toxins awaiting further
structure-function and electrophysiological characterization. For example, the exact target site of ACTX-Hi:OB4219, a 38 residue long peptide isolated from the venom of the funnel-web spider *Hadronyche infensa* orchid Beach, has not yet been identified. Determination of its NMR structure revealed that this toxin contains 4 disulfide bridges and is folded accordingly the ICK motif with a triple-stranded antiparallel β-sheet (Rosengren et al., 2002). Interestingly, the cysteine pattern and loop sizes of ACTX-Hi:OB4219 are identical to the μ-agatoxins and to other site 4 spider toxins such as the curtatoxins and δ-palutoxins. However, despite the identical fold is the sequence similarity between ACTX-Hi:OB4219 and these toxins very low. Therefore are electrophysiological and radioligand binding studies required to determine if ACTX-Hi:OB4219 is exerting its insecticidal activity by acting on Na\textsubscript{V} channels and if so to identify the exact interaction site with the channel.

![ACTX-Hi:OB4219, DTX9.2, and Magi 6 amino acid sequences](image)

**Fig. 13.** Insect-selective toxins isolated from spider venom binding on unknown sites.

Comparison of the amino acid sequences of insect-selective spider toxins which are still awaiting the designation of their binding site. Identical residues are boxed in grey, cysteines are shown in red. The disulfide-bonding pattern is indicated for ACTX-Hi:OB4219.

DTX9.2, 10 and 11 constitute a family of 56-61 residue long insecticidal peptides, isolated from the venom of the primitive weaving spider *Diguentia canities* (Krapcho et al., 1995). In vivo studies have shown that these toxins do not affect mice when injected intraperitoneal or intracerebroventricular. Because they do cause progressive spastic paralysis in tobacco budworms, can these spider toxins be considered as potential novel insecticides. DTX9.2 caused depolarizations of cockroach axons and repetitive potential discharges in housefly larvae neuromuscular and sensory nerve preparations. TTX blocked these actions, suggesting the involvement of Na\textsubscript{V} channel in the mechanism of action of DTX9.2 (Bloomquist et al., 1996). Radioligand binding studies have indicated that it is unlikely that DTX9.2 interacts with site 3 and thus are further structure-function studies necessary to identify the precise target of these interesting insect-selective toxins (Nicholson, 2007).

An ICK motif possessing peptide of 69 residues named Magi 6, from *Macrothele gigas*, induces flaccid paralysis in insects with an even higher potency than the earlier described site 3 toxin Magi 2. However, in contrast to Magi 2, does the site of action for Magi 6 remain unknown. Competitive binding studies indicated that Magi 6 does not compete for sites 3, 4 or 6, neither on insect or mammalian Na\textsubscript{V} channels (Corzo et al., 2003). The rapid and strong lethality of Magi 6 when injected in insects, is suggestive of a strong antagonist action on the insect nervous system. Therefore it seems that Magi 6 targets a, for now, unknown site on Na\textsubscript{V} channels. Further structure-function studies are awaiting to determine if this is true for Magi 6 or that this toxin is rather interacting with another receptor.
7. The application of insect-selective toxins in the development of novel insecticides

There are 2 main issues that complicate further intensive use of classical industrial pesticides such as DDT and pyrethroids: firstly the growing public awareness of the health hazards and environmental damage caused by conventional agrochemical pesticides has resulted in a general public condemnation regarding the use of these agents (Dong, 2007; Hassan et al., 1990). Secondly, the widespread use of the classical pesticides, together with their limited number of nervous system targets, has resulted in a far-reaching resistance among arthropod populations responsible for the transmission of many human diseases or among pest species involved in major devastation of crops (Nicholson, 2007; Zlotkin et al., 2000). Together, both issues have led to a situation were the current demand of new pesticides is focusing on the one hand on insect-selective agents, minimizing the risk for human health and, on the other hand on potent agents, minimizing the influence on the environment.

Fulfilling this urgent need for new, potent and insect-selective insecticides by insecticidal neurotoxins derived from venomous animals implicates the requirement of formulations which provide these peptide toxins to reach their targets within the circulatory system of insects.

In nature, spiders bite and scorpions and sea anemones sting to deliver the venom directly in the bloodstream of their prey or offender, allowing the neurotoxins to get in direct contact with the Nav channels. Peptide toxins and polypeptides in general do not penetrate the insect gut and are little to not at all resistant against the proteolytic environment of the insect digestive system. As such does the industrial use of these neurotoxins as insecticidal agents only has prospects depending on the development of means to guarantee a sufficient delivery of peptide toxins to their targets. Therefore a lot of effort has been done to improve the bioavailability of peptides in general and insecticidal peptide toxins specifically. Several strategies are used to overcome the difficulties associated with peptide bioavailability and thus improve the insecticidal potency of neurotoxins.

To implement the insecticidal capacity of polypeptides the following toxin modifications have been proven to increase the bioavailability: (i) Incorporation of the toxin in baculoviruses; (ii) Incorporation of toxins in plants; (iii) Chemical approaches to increase toxin stability and resistance against proteolysis.

7.1 Incorporation of insect-selective toxins in recombinant baculoviruses: biopesticides

One approach to develop environmentally friendly measures to face the highly resistant insect species is the release of baculoviruses that have been genetically engineered to express insecticidal neurotoxins. Exactly because of their safety and insect specificity are insect baculoviruses of great interest as enhancer of neurotoxin bioavailability. The infectivity of baculoviruses is limited to a few closely related species within a single insect family, favoring these viruses as environmental safe insecticides. Their great advantage arises from the fact that baculoviruses do not replace the natural predators, as is the case with chemical insecticides, but rather complements these predators (Zlotkin et al., 2000).

From an evolutionary point of view have these viruses adapted themselves for self-preservation and propagation (Zlotkin, 1999). These baculoviruses have developed a strategy as such to keep their host alive as long as possible allowing a maximal progeny production (Bloomquist et al., 1996). This strategy of acting as slow as possible, and in the
mean time permitting the host insect larvae to feed continuously on the crops, is of course a major drawback in an efficient pesticide employment. However, this drawback has been overcome by genetically engineering of baculoviruses to potentiate their insecticidal activity and efficacy. The improved efficacy results from a significant reduced time that the viruses allow their host to feed itself (Bonning & Hammock, 1996). This enhancement of the insecticidal activity is achieved by engineering nucleopolyhedroviruses in such a way that their natural insect pathogenicity is combined with the expression of insect-selective neurotoxins (Gershburg et al., 1998). As such a 30-40% improved insecticidal activity is achieved (Chejanovsky et al., 1995).

The gene encoding for a insect-selective neurotoxin is subcloned into a transfer vector plasmid, which is then cotransfected with the parental baculovirus DNA into an appropriate insect cell line so that the neurotoxin encoding gene is transferred to the baculovirus by homologous recombination (Zlotkin et al., 2000). Insect-selective neurotoxins from spiders, scorpions and sea anemones have been used to the construction of recombinant baculoviruses. Nevertheless, currently there is a limited use of recombinant baculoviruses expressing insect-selective neurotoxins mainly due the lack of well-characterized toxins to be selected from as potential candidates for incorporation into the baculovirus genome. Therefore it is still essential to explore the large pharmacological libraries contained within various venomous animals (Nicholson, 2007).

Even though the insecticidal efficacy of sea anemone and scorpion toxins is higher compared to spider toxins, it should be noted that spider Nav channel toxins do posses some structural features which favors them as candidates for recombinant expression. Spider toxins are smaller in size and because of the smaller number of disulfide bridges, are they more likely to fold correctly. Furthermore, all characterized insect-selective spider toxins up to date are composed of the ICK motif. This conserved scaffold provides spider toxins a highly chemical stability and a strong resistance to a denaturizing and proteolytic environment (Nicholson, 2007; Norton & Pallaghy, 1998). Three spider toxins active on insect Nav channels have been successfully incorporated into baculovirus genome: µ-Aga IV from Agelenopsis aperta, DTX9.2 from Dignentia canities and Ta1TX-1 from the hobo spider Tegenaria agrestis (Hughes et al., 1997; Tomalski et al., 1989). Genes encoding for DTX9.2 or Ta1TX-1 were inserted into the Autographa californica multiple nuclear polyhedrosis baculovirus (AcMNPV) (Krapcho et al., 1995). AcMNPV is a baculovirus of wide interest as it is known to infect a variation of important lepidopterous pests (McCutchen et al., 1991). The efficacy of the recombinant baculoviruses expressing these insect selective spider toxins were evaluated in three important lepidopteran insect pests, Trichoplusia ni Hubner, Spodoptera exigua and Heliothis virescens. It was demonstrated that both DTX9.2 and Ta1TX-1 expressing baculoviruses reduced the host feeding time up to 40% and caused a reduction in host survival time ranging from 18% to 25%. Interestingly, the DTX9.2 expressing virus stopped the feeding faster while the Ta1TX-1 virus killed the hosts faster, suggesting that DTX9.2 is more promising in effective reducing crop damage (Hughes et al., 1997).

Similar results were obtained when the site 4 spider toxin, µ-Aga IV was expressed by AcMNPV. Infection of lepidopterous larvae with these recombinant baculoviruses resulted in a dramatic improvement of insecticidal efficacy, increasing killing time up to 50% (Prikhod’ko & Miller, 1996). The first insect-specific toxin gene to be transferred into a baculovirus was the highly insecticidal excitatory scorpion ß-toxin AaIT (Androctonus australis Hector) (Stewart et al., 1991). Lepidopterous larvae when infected with an AaIT expressing recombinant baculovirus such as BmNPV-AaIT, have a strongly reduced...
survival time and hence damage a significant lower amount of host plants compared to wild type viruses. The expressed insect-selective neurotoxin found in the circulation of the infected insects is characterized by the following important properties: (i) Chemical identity with native toxin; (ii) The expressed toxin causes the same physiological symptoms in vivo as the injected native one. In the case of AaIT this is the typical contractile paralysis observed in insects injected by excitatory insect-selective β-toxin. (iii) Expressed neurotoxins display an enhanced affectivity, designated as toxin potentiation and depicted by the fact that the expressed toxin requires a much lower hemolymph titer than the injected native one to cause similar paralysis (Maeda, 1989). It was assumed that this potentiation was the obvious consequence of the insecticidal pharmacokinetic-targeting cooperativity of both the recombinant baculovirus and its expressed scorpion toxin AaIT. However, later studies have indicated that the toxin potentiation observed for expressed toxins is the outcome of two phenomena. Firstly, the continuously toxin expression by the virus infected tracheal epithelia, ramifying within the insect and as thus creating a baculovirus conduit, hereby constituting a pursuing overall supply of newly generated toxin to its specific target namely the insect Na\textsubscript{v} channels. Secondly, the translocation of the toxin gene into the central nervous system of the insects provides the expressed toxin a free pathway to the target site which is inaccessible to the native toxin (Zlotkin et al., 2000).

Several other insect-selective scorpion toxin have also been field-applicated in combination with baculoviruses. A comparative study was conducted in which the insecticidal efficacy of recombinant expressed depressant β-toxins was paralleled with this of excitatory β-toxins. This study revealed that the depressant toxin LqhIT2 displayed a higher insecticidal activity compared to its excitatory counterpart LqhIT1 (Gershburg et al., 1998). The observed difference in activity between these toxins, isolated from the scorpion Leiurus quinquestriatus hebraeus, was surprising as excitatory β-toxins generate an immediate paralyzing effect whereas depressant β-toxins generate a delayed effect. The difference might be explained by pharmacokinetic differences resulting from a higher stability and thus increased bioavailability of depressant compared to excitatory β-toxins (Gershburg et al., 1998).

Two insect-selective sea anemone toxins targeting Na\textsubscript{v} channels have been used in the recombinant baculovirus strategy. The site 3 toxins As II, from the sea anemone Anemonia sulcata and Sh I isolated from Stichodactyla helianthus, displayed an effective ability to reduce crop damage upon expression by AcMNPV (Prikhod’ko & Miller, 1996). Furthermore, the construction of AcMNPV expressing both the site 3 toxin As II and the site 4 toxin µ-Aga-IV, resulted in a synergistic enhanced insecticidal activity, underlining the potentiating of insecticidal activity by coproduction of toxins targeting allosterically coupled sites of Na\textsubscript{v} channels (Nicholson, 2007).

**7.2 Incorporation of insect-selective toxins in plants: transgenic plants**

Another strategy in the development of potent insect-selective insecticides without burden for the environment is the incorporation of neurotoxins in the genome of plants. It has been shown that the spider toxin ω-ACTX-Hv1a, from Hadronyche versuta, displayed remarkable insecticidal activity upon incorporation into the tobacco plant Nicotiana tabacum (Fletcher et al., 1997). Although ω-ACTX-Hv1a is an insect-selective Ca\textsubscript{v} and not a Na\textsubscript{v} channel toxin, the insecticidal efficacy of ω-ACTX-Hv1a fusion proteins against important pest species of transgenic plants expressing this insect-selective spider toxin is a strong argument evidencing the efficiency of the transgenic approach. Nevertheless, no insect-selective spider neurotoxins targeting Na\textsubscript{v} channels have been selected to be incorporated in
plants (Nicholson, 2007). However, the insect-selective spider toxin Magi 6, isolated from the Japanese spider *Macrothele gigas*, has been successfully expressed in transgenic tobacco. As described earlier is the exact target of this toxin still unknown, although its potent effects in vivo do suggest that Magi 6 is acting on a receptor within the nervous system (Corzo *et al.*, 2003). The incorporation of Magi 6 in transgenic plants conferred resistance to several insect pests and opens the possibility of employing this spider toxin to improve the resistance of diverse plants (Hernandez-Campuzano *et al.*, 2009).

Several scorpion toxins active on Na\textsubscript{V} channels are successfully used as insecticide by application of the transgenic approach. A gene encoding for the high insecticidal scorpion toxin BmKIT from *Buthus martensi Karsch* was, in combination with a gene encoding for an insect-specific chitinase, from *Manduca sexta*, introduced into *Brassica napus* plants rendering these plants resistance against agricultural important pests (Wang *et al.*, 2005). Chitin is a major component is of the cuticle and gut epidermis of many lepidoptera. The chitinase gene is normally not expressed in insects during feeding periods but only in a narrow period prior to molting (Kramer *et al.*, 1993). Continuously exposure to chitinase might lead to malfunction because of chitin degradation and loss of structural integrity of the gut epidermis (Regev *et al.*, 1996). The loss of this absorption barrier in the gut may also enhance the bioavailability of the co expressed neurotoxin as the access pathway for these toxins to reach their targets is strongly facilitated (Wang *et al.*, 2005).

### 7.3 Improved toxin stability and resistance equals improved activity?

A general criticism upon peptide-based insecticides might be the fact that these Na\textsubscript{V} channel targeting toxins despite their extraordinary insecticidal properties potentially suffer from generic problems encountered by all peptide-based drugs, such as short biological half-lives, proteolysis and poor absorption. However, in the last decade there has been an impressive number of studies focusing on the re-engineering of peptide toxins to address these biopharmaceutical problems (Halai & Craik, 2009). Even though the aim of application is different between clinical used drugs and insecticides, the properties of their general pharmacokinetic pathways are identical. As such can techniques used to improve pharmacological availability of peptide-based drugs be very useful to enhance the bioavailability of peptide-based insecticides.

One example of a very effective strategy to improve the drug-like properties of toxins is backbone cyclization, by either the addition of a linker to span the peptide termini or directly by head-to-tail linkage. The resulting cyclic peptides may have an improved binding efficacy but more over display an impressive decrease in susceptibility to proteolysis (Clark *et al.*, 2010). Many microorganisms are known to produce cyclic peptides, exemplified by the well known fungal product cyclosporine which has revolutionized organ transplant therapy because of its potent immunosuppressant activities (Clark *et al.*, 2010; Starzl, 1981). Furthermore, peptide cyclization is a widely used technique in the pharmaceutical industry in the development of a variety of drug design applications (Craik & Adams, 2007). For example, the conotoxin MII, isolated from the *Conus magus*, targets with high potency nicotine acetylcholine receptors (Shon *et al.*, 1997)). Cyclization of this toxin by adding a linker of seven amino acids resulted in maintenance of the structure of the native peptide and similar biological activity, but with an importantly enhanced resistance against proteolytic breakdown (Clark *et al.*, 2005). Cyclic peptides used in pharmaceutical applications are usual smaller than 12 amino acids which is noticeable shorter than most Na\textsubscript{V} channel targeting neurotoxins described in this review. However, a large number of
naturally occurring disulfide-rich cyclic peptides have been discovered in plants and animals and all of these natural cyclic peptides share the remarkable feature of displaying an exceptional stability and a compact structure (Clark et al., 2010). This suggests that cyclization of highly insecticidal neurotoxins from spiders, scorpions and sea anemone might be potentially useful to enhance their bioavailability by strongly increasing their stability and their resistance against proteolytic degradation. For peptide toxins and in general for all proteins, several amino acids are sensitive for chemical degradation. Therefore can potential stability problems with peptide-based insecticides be avoided by replacing these residues, provided of course that substituting these residues does not lead to a loss of biological activity (Craik & Adams, 2007). Examples of amino acids susceptible to chemical degradation are deamidation of asparagine, oxidation of methionine and isomerization or cleavage of asparagine-proline bonds (Wakankar & Borchardt, 2006).

A widely used technique in the peptide chemistry is the substitution of L-amino acids for D-amino acids to improve the resistance against proteases. The use of D-amino acids is particularly common in peptide-based drug design of cyclic peptides as described above (Schroeder et al., 2004). An interesting approach to improve the activity of the Conus striatus toxin SIIIA is the incorporation of non-natural backbone spacers. The use of flexible spacers such as amino-3-oxypenatnoic and 6-aminohexanoic acids to replace conformational constrained parts of the three disulfide bridges in SIIIA resulted in an enhanced activity (Green et al., 2007). Since SIIIA is a highly potent inhibitor of mammalian NaV channels and since it is folded accordingly the ICK motif which is shared by many insect-selective toxins, these observations of enhanced activity are of peculiar interest for the development of new insect-selective insecticides which act by inhibiting the sodium conductance such as HNTX-1.

Other approaches to improve bioavailability are the use of lipid tags on selected residues, terminal capping and peptide truncation. Capping involves C-terminal amidation because it is known that amidation leads to a reduced sensitivity to proteolytic breakdown by carboxypeptidases. Peptide truncation finds it application through the rationale that residues lying outside the defined cystine framework, display an increased susceptibility to proteolytic or chemical degradation due to the fact that these residues are likely to be more flexible (Craik & Adams, 2007).

All together it can be seen that the increasing knowledge on bypassing the generic barriers insures that the exquisite potency and selectivity of insecticidal peptide toxins derived from venomous animals can be exploited as promising lead compounds in the development of novel peptide-based pesticides.

8. Conclusions and further directions

Arthropods are the most widespread and diverse group of animals worldwide. Even though only a small percentage of all arthropods are considered as pest species, there impact on human society is of great importance. Vector-borne human and veterinary diseases, in which pest species function as vectors for transmission, are of increasing concern to the general population and more specific to the public health. They represent a significant threat to the productivity, health, the normal lifecycle of humans, livestock, companion animals and wild life, and by generalization, to the viability of life on earth (Nicholson, 2007).
Pest species are responsible for major devastations of crops, destroying up to 20% of the annual crop production worldwide (Nicholson, 2007). An increasing demand on crop production to feed the ever increasing world population, together with the fact that the majority of the world fertile land is being exploited raises a global insurmountable awareness for the urgent need of environmental compatible insecticides, capable of increasing crop production yields by decreasing the losses accountable by pest species. The increasing factors limiting the efficacy of conventional agrochemical pesticides are therefore worrying. Due to the wide usages and the narrow target range of classical agrichemicals, such as DDT and pyrethroids, arthropods have been submitted to a high degree of selection pressure, resulting in pest species with a far reaching resistance against these agents (Brogdon & McAllister, 1998). Consequently, the development of novel, potent and selective pesticides has become crucial for global welfare. Insect-selective neurotoxins might significantly contribute to the accomplishment of novel insecticides.

The range of organisms that is producing toxins for defense or for capturing their arthropod prey is remarkable diverse. Toxins isolated from the venoms of spiders, scorpions and sea anemones, as described in this review, are of particular interest because of their importantly high selectivity for insects over mammals, great potency and environmental friendly character. Spiders, scorpions and sea anemones have developed during their evolution a unique, complex pre-optimized combinatorial peptide library of neurotoxins which is now available as a valuable source of novel insecticides.

Voltage-gated sodium channels have gained great interest as target for future pesticides. This is mainly because of their important role in excitability, their pharmacological diversity exhibited in the large number of binding sites and their pharmacological distinctiveness as revealed by the ability of insect-selective neurotoxins to discriminate strongly between insect and mammalian Na\textsuperscript{V} channels (Zlotkin, 1999). A very important argument, favoring both neurotoxins and Na\textsuperscript{V} channels as key elements in the development of new insecticides is the existence of allosterically coupled sites as potentiating of the insecticidal activity. For instance, it has been shown that pyrethroids and scorpion toxins can operate synergistic with one another (Gilles et al., 2003). It was found that insects, which were first infected with a baculovirus expressing the scorpion toxin AahIT, were much more sensitive to the effects of pyrethroids then non-infected species (Sharkey et al., 1987). Interesting to note is the fact that pyrethroid-resistant strains do display an enhanced sensitivity towards AahIT and other site 4 toxins (Zlotkin, 1999). Furthermore it was also demonstrated that site 2, 3 and 5 toxins could enhance pyrethroid binding to locust neuronal membranes (Gilles et al., 2003). Using the toxin ATX II as a site 3 toxin representative, it was shown that sea anemone toxins could similarly enhance the potency of DDT and other pyrethriods (Bloomquist & Soderlund, 1988). All together these results do imply the interesting possibility to use the synergistic interaction of insect-selective Na\textsuperscript{V} channel toxins in order to not only overcome resistance but also to reduce the concentration of each toxin required to obtain an efficient control of pest species.

The synergistic action of insecticides should be seen beyond the boundaries of the Na\textsuperscript{V} channel toxins. Their exist many other interesting targets for insecticides such as Ca\textsubscript{V}, K\textsubscript{V}, GABA-gated Cl channels or other receptors such as nicotine acetylcholine receptors. Furthermore, baculoviruses, snowdrop lectine and chitinases have proven to be very useful in the battle against pest species whether this is because of their direct insecticidal properties or because they function as ideal transport vector for insect-selective toxins. It is recommendable to develop over the long term a strategy of applying synergistic mixtures
containing a variety of insecticidal substances acting on a broad range of different targets. The synergistic character of these mixtures will be the result of positive cooperativity between insecticidal agents acting on different targets and/or agents acting on allosterically coupled sites within the same target. Employment of such mixtures will significantly reduce the required doses of each insecticide and herewith not only their production cost but also their impact on the environment and public health.

Insect-selective Nav channel toxins from spiders, scorpion and sea anemones can contribute importantly to these synergistic mixtures. They deserve to be lead compounds in the development of new insecticides thanks to the exquisite selectivity, unseen affinity and high potency displayed towards their target, the insect voltage-gated sodium channel.

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10. References

Adams, M.E. (2004). Agatoxins: ion channel specific toxins from the American funnel web spider, Agelenopsis aperta. *Toxicon*, Vol.43, No.5, (Apr), pp. 509-25

Arnon, T.; Potikha, T.; Sher, D.; Elazar, M.; Mao, W.; Tal, T.; Bosmans, F.; Tytgat, J.; Ben-Arie, N. & Zlotkin, E. (2005). BjalphaIT: a novel scorpion alpha-toxin selective for insects--unique pharmacological tool. *Insect Biochem Mol Biol*, Vol.35, No.3, (Mar), pp. 187-95

Beress, L. & Beress, R. (1975). Purification of three polypeptides with neuro- and cardiotoxic activity from the sea anemone Anemonia sulcata. *Toxicon*, Vol.13, No.5, (Nov), pp. 359-67

Billen, B.; Bosmans, F. & Tytgat, J. (2008). Animal peptides targeting voltage-activated sodium channels. *Curr Pharm Des*, Vol.14, No.24, pp. 2492-502

Billen, B.; Vassilevski, A.; Nikolsky, A.; Debaveye, S.; Tytgat, J. & Grishin, E. (2010). Unique bell-shaped voltage-dependent modulation of Na+ channel gating by novel insect-selective toxins from the spider Agelena orientalis. *J Biol Chem*, Vol.285, No.24, (Jun 11), pp. 18545-54

Bloomquist, J.R.; Kinne, L.P.; Deutsch, V. & Simpson, S.F. (1996). Mode of action of an insecticidal peptide toxin from the venom of a weaving spider (Diguetia canities). *Toxicon*, Vol.34, No.9, (Sep), pp. 1072-5

Bloomquist, J.R. & Soderlund, D.M. (1988). Pyrethroid insecticides and DDT modify alkaloid-dependent sodium channel activation and its enhancement by sea anemone toxin. *Mol Pharmacol*, Vol.33, No.5, (May), pp. 543-50

Bonning, B.C. & Hammock, B.D. (1996). Development of recombinant baculoviruses for insect control. *Annu Rev Entomol*, Vol.41, pp. 191-210

Boore, J.L.; Lavrov, D.V. & Brown, W.M. (1998). Gene translocation links insects and crustaceans. *Nature*, Vol.392, No.6677, (Apr 16), pp. 667-8

Borges, M.H.; De Lima, M.E.; Stankiewicz, M.; Pelhate, M.; Cordeiro, M.N. & Beirão, P.S.L. (2009). Structural and functional diversity in the venom of spiders of the genus Phoneutria, In: *Animal toxins: state of the art*, De Lima, M.E., pp. 291-312, EDITORAufmg, 978-85-7041-735-0, Belo Horizonte
Bosmans, F.; Aneiros, A. & Tytgat, J. (2002). The sea anemone Bunodosoma granulifera contains surprisingly efficacious and potent insect-selective toxins. FEBS Lett, Vol.532, No.1-2, (Dec 4), pp. 131-4

Bosmans, F.; Martin-Eauclaire, M.F. & Tytgat, J. (2005). The depressant scorpion neurotoxin LqqIT2 selectively modulates the insect voltage-gated sodium channel. Toxicon, Vol.45, No.4, (Mar 15), pp. 501-7

Bosmans, F. & Tytgat, J. (2007a). Sea anemone venom as a source of insecticidal peptides acting on voltage-gated Na+ channels. Toxicon, Vol.49, No.4, (Mar 15), pp. 550-60

Bosmans, F. & Tytgat, J. (2007b). Voltage-gated sodium channel modulation by scorpion alpha-toxins. Toxicon, Vol.49, No.2, (Feb), pp. 142-58

Brogdon, W.G. & McAllister, J.C. (1998). Insecticide resistance and vector control. Emerg Infect Dis, Vol.4, No.4, (Oct-Dec), pp. 605-13

Bruce, C.; Fitches, E.C.; Chougule, N.; Bell, H.A. & Gatehouse, J.A. (2011). Recombinant conotoxin, TxVIA, produced in yeast has insecticidal activity. Toxicon, Vol.58, No.1, (Jul), pp. 93-100

Bulet, P. & Stocklin, R. (2005). Insect antimicrobial peptides: structures, properties and gene regulation. Protein Pept Lett, Vol.12, No.1, (Jan), pp. 3-11

Catterall, W.A. (2000). From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron, Vol.26, No.1, (Apr), pp. 13-25

Catterall, W.A.; Cestele, S.; Yarov-Yarovoy, V.; Yu, F.H.; Konoki, K. & Scheuer, T. (2007). Voltage-gated ion channels and gating modifier toxins. Toxicon, Vol.49, No.2, (Feb), pp. 124-41

Cestele, S.; Scheuer, T.; Mantegazza, M.; Rochat, H. & Catterall, W.A. (2001). Neutralization of gating charges in domain II of the sodium channel alpha subunit enhances voltage-sensor trapping by a beta-scorpion toxin. J Gen Physiol, Vol.118, No.3, (Sep), pp. 291-302

Cestele, S.; Yarov-Yarovoy, V.; Qu, Y.; Sampieri, F.; Scheuer, T. & Catterall, W.A. (2006). Structure and function of the voltage sensor of sodium channels probed by a beta-scorpion toxin. J Biol Chem, Vol.281, No.30, (Jul 28), pp. 21332-44

Chanda, B. & Bezanilla, F. (2002). Tracking voltage-dependent conformational changes in skeletal muscle sodium channel during activation. J Gen Physiol, Vol.120, No.5, (Nov), pp. 629-45

Chejanovsky, N.; Zilberberg, N.; Rivkin, H.; Zlotkin, E. & Gurevitz, M. (1995). Functional expression of an alpha anti-insect scorpion neurotoxin in insect cells and lepidopterous larvae. FEBS Lett, Vol.376, No.3, (Dec 4), pp. 181-4

Clark, R.J.; Akcan, M.; Kaas, Q.; Daly, N.L. & Craik, D.J. (2010). Cyclization of conotoxins to improve their biopharmaceutical properties. Toxicon, (Dec 10), pp.

Clark, R.J.; Fischer, H.; Dempster, L.; Daly, N.L.; Rosengren, K.J.; Nevin, S.T.; Meunier, F.A.; Adams, D.J. & Craik, D.J. (2005). Engineering stable peptide toxins by means of backbone cyclization: stabilization of the alpha-conotoxin MII. Proc Natl Acad Sci USA, Vol.102, No.39, (Sep 27), pp. 13767-72

Cohen, L.; Gilles, N.; Karbat, I.; Ilan, N.; Gordon, D. & Gurevitz, M. (2006). Direct evidence that receptor site-4 of sodium channel gating modifiers is not dipped in the phospholipid bilayer of neuronal membranes. J Biol Chem, Vol.281, No.30, (Jul 28), pp. 20673-9

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Cohen, L.; Troub, Y.; Turkov, M.; Gilles, N.; Ilan, N.; Benveniste, M.; Gordon, D. & Gurevitz, M. (2007). Mammalian skeletal muscle voltage-gated sodium channels are affected by scorpion depressant "insect-selective" toxins when preconditioned. *Mol Pharmacol*, Vol.72, No.5, (Nov), pp. 1220-7

Corzo, G.; Diego-Garcia, E.; Clement, H.; Peigneur, S.; Odell, G.; Tytgat, J.; Possani, L.D. & Alagon, A. (2008). An insecticidal peptide from the theraphosid Brachypelma smithi spider venom reveals common molecular features among spider species from different genera. *Peptides*, Vol.29, No.11, (Nov), pp. 1901-8

Corzo, G.; Escoubas, P.; Stankiewicz, M.; Pelhate, M.; Kristensen, C.P. & Nakajima, T. (2000). Isolation, synthesis and pharmacological characterization of delta-palutoxins IT, novel insecticidal toxins from the spider Paracoelotes luctuosus (Amaurobiidae). *Eur J Biochem*, Vol.267, No.18, (Sep), pp. 5783-95

Corzo, G.; Escoubas, P.; Villegas, E.; Karbat, I.; Gordon, D.; Gurevitz, M.; Nakajima, T. & Gilles, N. (2005). A spider toxin that induces a typical effect of scorpion alpha-toxins but competes with beta-toxins on binding to insect sodium channels. *Biochemistry*, Vol.44, No.5, (Feb 8), pp. 1542-9

Corzo, G.; Gilles, N.; Satake, H.; Villegas, E.; Dai, L.; Nakajima, T. & Haupt, J. (2003). Distinct primary structures of the major peptide toxins from the venom of the spider Macrothele gigas that bind to sites 3 and 4 in the sodium channel. *FEBS Lett*, Vol.547, No.1-3, (Jul 17), pp. 43-50

Craik, D.J. & Adams, D.J. (2007). Chemical modification of conotoxins to improve stability and activity. *ACS Chem Biol*, Vol.2, No.7, (Jul 20), pp. 457-68

Craik, D.J.; Daly, N.L. & Waine, C. (2001). The cystine knot motif in toxins and implications for drug design. *Toxicon*, Vol.39, No.1, (Jan), pp. 43-60

de Dianous, S.; Kopeyan, C.; Bahraoui, E.M. & Rochat, H. (1987). Purification of contracture-inducing insect toxins from Buthinae scorpion venoms by immunoaffinity and high pressure liquid chromatography. *Toxicon*, Vol.25, No.7, pp. 731-41

de la Vega, R.C. & Possani, L.D. (2007). Novel paradigms on scorpion toxins that affects the activating mechanism of sodium channels. *Toxicon*, Vol.49, No.2, (Feb), pp. 171-80

Defaix, A. & Lapied, B. (2005). Role of a novel maintained low-voltage-activated inward current permeable to sodium and calcium in pacemaking of insect neurosecretory neurons. *Invert Neurosci*, Vol.5, No.3-4, (Nov), pp. 135-46

Derst, C.; Walther, C.; Veh, R.W.; Wicher, D. & Heinemann, S.H. (2006). Four novel sequences in Drosophila melanogaster homologous to the auxiliary Para sodium channel subunit TipE. *Biochem Biophys Res Commun*, Vol.339, No.3, (Jan 20), pp. 939-48

Diochot, S.; Baron, A.; Rash, L.D.; Deval, E.; Escoubas, P.; Scarzello, S.; Salinas, M. & Lazzunski, M. (2004). A new sea anemone peptide, APETx2, inhibits ASIC3, a major acid-sensitive channel in sensory neurons. *Embo J*, Vol.23, No.7, (Apr 7), pp. 1516-25

Diochot, S.; Loret, E.; Bruhn, T.; Beress, L. & Lazzunski, M. (2003). APETx1, a new toxin from the sea anemone Anthopleura elegantissima, blocks voltage-gated human ether-a-go-go-related gene potassium channels. *Mol Pharmacol*, Vol.64, No.1, (Jul), pp. 59-69
Dong, K. (1997). A single amino acid change in the para sodium channel protein is associated with knockdown-resistance (kdr) to pyrethroid insecticides in German cockroach. Insect Biochem Mol Biol, Vol.27, No.2, (Feb), pp. 93-100

Dong, K. (2007). Insect sodium channels and insecticide resistance. Invert Neurosci, Vol.7, No.1, (Mar), pp. 17-30

Du, Y.; Nomura, Y.; Liu, Z.; Huang, Z.Y. & Dong, K. (2009). Functional expression of an arachnid sodium channel reveals residues responsible for tetrodotoxin resistance in invertebrate sodium channels. J Biol Chem, Vol.284, No.49, (Dec 4), pp. 33869-75

Feng, G.; Dvak, P.; Chopra, M. & Hall, L.M. (1995). Cloning and functional analysis of TipE, a novel membrane protein that enhances Drosophila para sodium channel function. Cell, Vol.82, No.6, (Sep 22), pp. 1001-11

Ferrat, G.; Bosmans, F.; Tytgat, J.; Pimentel, C.; Chagot, B.; Gilles, N.; Nakajima, T.; Darbon, H. & Corzo, G. (2005). Solution structure of two insect-specific spider toxins and their pharmacological interaction with the insect voltage-gated Na+ channel. Proteins, Vol.59, No.2, (May 1), pp. 368-79

Fletcher, J.I.; Smith, R.; O'Donoghue, S.I.; Nilges, M.; Connor, M.; Howden, M.E.; Christie, M.J. & King, G.F. (1997). The structure of a novel insecticidal neurotoxin, omega-atracotoxin-HV1, from the venom of an Australian funnel web spider. Nat Struct Biol, Vol.4, No.7, (Jul), pp. 559-66

Froy, O.; Zilberberg, N.; Gordon, D.; Turkov, M.; Gilles, N.; Stankiewicz, M.; Pelhate, M.; Lorez, E.; Oren, D.A.; Shaanan, B. & Gurevitz, M. (1999). The putative bioactive surface of insect-selective scorpion excitatory neurotoxins. J Biol Chem, Vol.274, No.9, (Feb 26), pp. 5769-76

Gershburg, E.; Stockholm, D.; Froy, O.; Rashi, S.; Gurevitz, M. & Chejanovsky, N. (1998). Baculovirus-mediated expression of a scorpion depressant toxin improves the insecticidal efficacy achieved with excitatory toxins. FEBS Lett, Vol.422, No.2, (Jan 30), pp. 132-6

Gilles, N.; Gurevitz, M. & Gordon, D. (2003). Allosteric interactions among pyrethroid, brevetoxin, and scorpion toxin receptors on insect sodium channels raise an alternative approach for insect control. FEBS Lett, Vol.540, No.1-3, (Apr 10), pp. 81-5

Gilles, N.; Harrison, G.; Karbat, I.; Gurevitz, M.; Nicholson, G.M. & Gordon, D. (2002). Variations in receptor site-3 on rat brain and insect sodium channels highlighted by binding of a funnel-web spider delta-atracotoxin. Eur J Biochem, Vol.269, No.5, (Mar), pp. 1500-10

Goldin, A.L. (1999). Diversity of mammalian voltage-gated sodium channels. Ann N Y Acad Sci, Vol.868, (Apr 30), pp. 38-50

Goldin, A.L. (2001). Resurgence of sodium channel research. Annu Rev Physiol, Vol.63, pp. 871-94

Goldin, A.L.; Barchi, R.L.; Caldwell, J.H.; Hofmann, F.; Howe, J.R.; Hunter, J.C.; Kallen, R.G.; Mandel, G.; Meisler, M.H.; Netter, Y.B.; Noda, M.; Tamkun, M.M.; Waxman, S.G.; Wood, J.N. & Catterall, W.A. (2000). Nomenclature of voltage-gated sodium channels. Neuron, Vol.28, No.2, (Nov), pp. 365-8

Gordon, D.; Karbat, I.; Iban, N.; Cohen, L.; Kahr, R.; Gilles, N.; Dong, K.; Stuhmer, W.; Tytgat, J. & Gurevitz, M. (2007). The differential preference of scorpion alpha-toxins
for insect or mammalian sodium channels: implications for improved insect control. *Toxicon*, Vol.49, No.4, (Mar 15), pp. 452-72

Gordon, D.; Martin-Eauclaire, M.F.; Cestele, S.; Kopeyan, C.; Carlier, E.; Khalifa, R.B.; Pelhate, M. & Rochat, H. (1996). Scorpion toxins affecting sodium current inactivation bind to distinct homologous receptor sites on rat brain and insect sodium channels. *J Biol Chem*, Vol.271, No.14, (Apr 5), pp. 8034-45

Goudet, C.; Ferrer, T.; Galan, L.; Artiles, A.; Batista, C.F.; Possani, L.D.; Alvarez, J.; Aneiros, A. & Tytgat, J. (2001). Characterization of two Bunodosoma granulifera toxins active on cardiac sodium channels. *Br J Pharmacol*, Vol.134, No.6, (Nov), pp. 195-206

Green, B.R.; Catlin, P.; Zhang, M.M.; Fiedler, B.; Bayudan, W.; Morrison, A.; Norton, R.S.; Smith, B.J.; Yoshikami, D.; Olivera, B.M. & Bulaj, G. (2007). Conotoxins containing nonnatural backbone spacers: cladistic-based design, chemical synthesis, and improved analgesic activity. *Chem Biol*, Vol.14, No.4, (Apr), pp. 399-407

Grolleau, F. & Lapied, B. (2000). Dorsal unpaired median neurones in the insect central nervous system: towards a better understanding of the ionic mechanisms underlying spontaneous electrical activity. *J Exp Biol*, Vol.203, No.Pt 11, (Jun), pp. 1633-48

Guan, R.J.; Xiang, Y.; He, X.L.; Wang, C.G.; Wang, M.; Zhang, Y.; Sundberg, E.J. & Wang, D.C. (2004). Structural mechanism governing cis and trans isomeric states and an intramolecular switch for cis/trans isomerization of a non-proline peptide bond observed in crystal structures of scorpion toxins. *J Mol Biol*, Vol.341, No.5, (Aug 27), pp. 1189-204

Gurevitz, M.; Froy, O.; Zilberberg, N.; Turkov, M.; Strugatsky, D.; Gershburg, E.; Lee, D.; Adams, M.E.; Tugarinov, V.; Anglister, J.; Shaanan, B.; Lore, E.; Stankiewicz, M.; Pelhate, M.; Gordon, D. & Chejanovsky, N. (1998). Sodium channel modifiers from scorpion venom: structure-activity relationship, mode of action and application. *Toxicon*, Vol.36, No.11, (Nov), pp. 1671-82

Gurevitz, M.; Karbat, I.; Cohen, L.; Ilan, N.; Kahn, R.; Turkov, M.; Stankiewicz, M.; Stuhmer, W.; Dong, K. & Gordon, D. (2007). The insecticidal potential of scorpion beta-toxins. *Toxicon*, Vol.49, No.4, (Mar 15), pp. 473-89

Halai, R. & Craik, D.J. (2009). Conotoxins: natural product drug leads. *Nat Prod Rep*, Vol.26, No.4, (Apr), pp. 526-36

Hassan, A.A.; el-Sewedy, S.M.; Minatogawa, Y. & Kido, R. (1990). Effects of some insecticides on several enzymes of tryptophan metabolism in rats. *J Environ Sci Health B*, Vol.25, No.3, (Jun), pp. 333-46

Hernandez-Campuzano, B.; Suarez, R.; Lina, L.; Hernandez, V.; Villegas, E.; Corzo, G. & Iturriaga, G. (2009). Expression of a spider venom peptide in transgenic tobacco confers insect resistance. *Toxicol*, Vol.53, No.1, (Jan), pp. 122-8

Hille, B. (2001). *Ion channels of excitable membranes* (3), Sinauer Associates, Inc., 978-0-87893-321-1, Sunderland, Massachusetts

Honma, T. & Shiomi, K. (2006). Peptide toxins in sea anemones: structural and functional aspects. *Mar Biotechnol (NY)*, Vol.8, No.1, (Jan-Feb), pp. 1-10

Hughes, P.R.; Wood, H.A.; Breen, J.P.; Simpson, S.F.; Duggan, A.J. & Dybas, J.A. (1997). Enhanced Bioactivity of Recombinant Baculoviruses Expressing Insect-Specific
The Sophisticated Peptide Chemistry of Venomous Animals as a Source of Novel Insecticides Acting on Voltage-Gated Sodium Channels

Spider Toxins in Lepidopteran Crop Pests. J Invertebr Pathol, Vol.69, No.2, (Mar), pp. 112-8
Hwang, D.F.; Tai, K.P.; Chueh, C.H.; Lin, L.C. & Jeng, S.S. (1991). Tetrodotoxin and derivatives in several species of the gastropod Naticidae. Toxicon, Vol.29, No.8, pp. 1019-24
Ingles, P.J.; Adams, P.M.; Knipple, D.C. & Soderlund, D.M. (1996). Characterization of voltage-sensitive sodium channel gene coding sequences from insecticide-susceptible and knockdown-resistant house fly strains. Insect Biochem Mol Biol, Vol.26, No.4, (Apr), pp. 319-26
Isbister, G.K. & White, J. (2004). Clinical consequences of spider bites: recent advances in our understanding. Toxicon, Vol.43, No.5, (Apr), pp. 477-92
Ishida, M.; Yokoyama, A.; Shimakura, K.; Nagashima, Y. & Shiomi, K. (1997). Halcurin, a polypeptide toxin from the sea anemone Halcurias sp., with a structural resemblance to type 1 and 2 toxins. Toxicon, Vol.35, No.4, (Apr), pp. 537-44
Jeziorski, M.C.; Greenberg, R.M. & Anderson, P.A. (2000). The molecular biology of invertebrate voltage-gated Ca(2+) channels. J Exp Biol, Vol.203, No.Pt 5, (Mar), pp. 841-56
Karbat, I.; Turkov, M.; Cohen, L.; Kahn, R.; Gordon, D.; Gurevitz, M. & Frolov, F. (2007). X-ray structure and mutagenesis of the scorpion depressant toxin LqhIT2 reveals key determinants crucial for activity and anti-insect selectivity. J Mol Biol, Vol.366, No.2, (Feb 16), pp. 586-601
King, G.F.; Escoubas, P. & Nicholson, G.M. (2008). Peptide toxins that selectively target insect Na(V) and Ca(V) channels. Channels (Austin), Vol.2, No.2, (Mar-Apr), pp. 100-16
Kramer, K.J.; Corpus, L.; Choi, H.K. & Muthukrishnan, S. (1993). Sequence of a cDNA and expression of the gene encoding epidermal and gut chitinases of Manduca sexta. Insect Biochem Mol Biol, Vol.23, No.6, (Sep), pp. 691-701
Krapcho, K.J.; Kral, R.M., Jr.; Vanwagenen, B.C.; Eppler, K.G. & Morgan, T.K. (1995). Characterization and cloning of insecticidal peptides from the primitive weaving spider Diguetia canities. Insect Biochem Mol Biol, Vol.25, No.9, (Oct), pp. 991-1000
Kuhn-Nentwig, L.; Schaller, J. & Nentwig, W. (2004). Biochemistry, toxicology and ecology of the venom of the spider Cupiennius salei (Ctenidae). Toxicon, Vol.43, No.5, (Apr), pp. 543-53
Lee, S.H.; Smith, T.J.; Ingles, P.J. & Soderlund, D.M. (2000). Cloning and functional characterization of a putative sodium channel auxiliary subunit gene from the house fly (Musca domestica). Insect Biochem Mol Biol, Vol.30, No.6, (Jun), pp. 479-87
Leipold, E.; Hansel, A.; Borges, A. & Heinemann, S.H. (2006). Subtype specificity of scorpion beta-toxin Tz1 interaction with voltage-gated sodium channels is determined by the pore loop of domain 3. Mol Pharmacol, Vol.70, No.1, (Jul), pp. 340-7
Li, D.; Xiao, Y.; Hu, W.; Xie, J.; Bosmans, F.; Tytgat, J. & Liang, S. (2003). Function and solution structure of hainantoxin-I, a novel insect sodium channel inhibitor from the Chinese bird spider Selenocosmia hainana. FEBS Lett, Vol.555, No.3, (Dec 18), pp. 616-22
Littleton, J.T. & Ganetzky, B. (2000). Ion channels and synaptic organization: analysis of the Drosophila genome. Neuron, Vol.26, No.1, (Apr), pp. 35-43
Loret, E.P.; del Valle, R.M.; Mansuelle, P.; Sampieri, F. & Rochat, H. (1994). Positively charged amino acid residues located similarly in sea anemone and scorpion toxins. *J Biol Chem*, Vol.269, No.24, (Jun 17), pp. 16785-8

Loughney, K.; Kreber, R. & Ganetzky, B. (1989). Molecular analysis of the para locus, a sodium channel gene in Drosophila. *Cell*, Vol.58, No.6, (Sep 22), pp. 1143-54

Maeda, S. (1989). Expression of foreign genes in insects using baculovirus vectors. *Annu Rev Entomol*, Vol.34, pp. 351-72

Martin-Eauclaire, M.F.; Cear, B.; Bosmans, F.; Rosso, J.P.; Tytgat, J. & Bougis, P.E. (2005). New "Birtoxin analogs" from Androctonus australis venom. *Biochem Biophys Res Commun*, Vol.333, No.2, (Jul 29), pp. 524-30

Martin, M.F.; Garcia y Perez, L.G.; el Ayeb, M.; Kopeyan, C.; Bechis, G.; Jover, E. & Rochat, H. (1987). Purification and chemical and biological characterizations of seven toxins from the Mexican scorpion, Centruroides suffusus suffusus. *J Biol Chem*, Vol.262, No.10, (Apr 5), pp. 4452-9

McCutchen, B.F.; Choudary, P.V.; Crenshaw, R.; Maddox, D.; Kamita, S.G.; Palekar, N.; Volrath, S.; Fowler, E.; Hammock, B.D. & Maeda, S. (1991). Development of a recombinant baculovirus expressing an insect-selective neurotoxin: potential for pest control. *Biotechnology (N Y)*, Vol.9, No.9, (Sep), pp. 848-52

Mebs, D. (2002). *Venomous and poisonous animals* (1), medpharm Scientific Publishers, 3-88763-093-9, Stuttgart

Moore, A.W.; Barbel, S.; Jan, L.Y. & Jan, Y.N. (2000). A genomewide survey of basic helix-loop-helix factors in Drosophila. *Proc Natl Acad Sci U S A*, Vol.97, No.19, (Sep 12), pp. 10436-41

Mouhat, S.; Jouirou, B.; Mosbah, A.; De Waard, M. & Sabatier, J.M. (2004). Diversity of folds in animal toxins acting on ion channels. *Biochem J*, Vol.378, No.Pt 3, (Mar 15), pp. 717-26

Narahashi, T. (2008). Tetrodotoxin: a brief history. *Proc Jpn Acad Ser B Phys Biol Sci*, Vol.84, No.5, pp. 147-54

Nardi, F.; Spinsanti, G.; Boore, J.L.; Carapelli, A.; Dallai, R. & Frati, F. (2003). Hexapod origins: monophyletic or paraphyletic? *Science*, Vol.299, No.5614, (Mar 21), pp. 1887-9

Nicholson, G.M. (2007). Insect-selective spider toxins targeting voltage-gated sodium channels. *Toxicon*, Vol.49, No.4, (Mar 15), pp. 490-512

Nicholson, G.M.; Little, M.J. & Birinyi-Strachan, L.C. (2004). Structure and function of delta-atractotoxins: lethal neurotoxins targeting the voltage-gated sodium channel. *Toxicon*, Vol.43, No.5, (Apr), pp. 587-99

Norton, R.S. (1991). Structure and structure-function relationships of sea anemone proteins that interact with the sodium channel. *Toxicon*, Vol.29, No.9, pp. 1051-84

Norton, R.S. & Pallaghy, P.K. (1998). The cystine knot structure of ion channel toxins and related polypeptides. *Toxicon*, Vol.36, No.11, (Nov), pp. 1573-83

Oliveira, J.S.; Fuentes-Silva, D. & Zaharenko, A.J. (2009). Sea anemone peptides, In: *Animal toxins: state of the art*, De Lima, M.E., pp. 49-72, EDITORAufmg, 978-85-7041-735-0, Belo Horizonte
Pimenta, A.M. & De Lima, M.E. (2005). Small peptides, big world: biotechnological potential in neglected bioactive peptides from arthropod venoms. J Pept Sci, Vol.11, No.11, (Nov), pp. 670-6

Platnick, N.I. (2000). The world spider catalog, version 12.0, In: The American Museum of Natural History, July 26th 2011, Available from: http://research.amnh.org/iz/spiders/catalog

Possani, L.D.; Becerril, B.; Delepierre, M. & Tytgat, J. (1999). Scorpion toxins specific for Na+ channels. Eur J Biochem, Vol.264, No.2, (Sep), pp. 287-300

Prikhod’ko, E.A. & Miller, L.K. (1996). Induction of apoptosis by baculovirus transactivator IE1. J Virol, Vol.70, No.10, (Oct), pp. 7116-24

Regev, A.; Keller, M.; Strizhov, N.; Sneh, B.; Prudovsky, E.; Chet, I.; Ginzberg, I.; Koncz-Kalman, Z.; Koncz, C.; Schell, J. & Zilberstein, A. (1996). Synergistic activity of a Bacillus thuringiensis delta-endotoxin and a bacterial endochitinase against Spodoptera littoralis larvae. Appl Environ Microbiol, Vol.62, No.10, (Oct), pp. 3581-6

Rodriguez de la Vega, R.C. & Possani, L.D. (2005). Overview of scorpion toxins specific for Na+ channels and related peptides: biodiversity, structure-function relationships and evolution. Toxicon, Vol.46, No.8, (Dec 15), pp. 831-44

Rosengren, K.J.; Wilson, D.; Daly, N.L.; Alewood, P.F. & Craik, D.J. (2002). Solution structures of the cis- and trans-Pro30 isomers of a novel 38-residue toxin from the venom of Hadronyche Infensa sp. that contains a cystine-knot motif within its four disulfide bonds. Biochemistry, Vol.41, No.10, (Mar 12), pp. 3294-301

Salceda, E.; Perez-Castells, J.; Lopez-Mendez, B.; Garateix, A.; Salazar, H.; Lopez, O.; Aneiros, A.; Standker, L.; Beress, L.; Forssmann, W.G.; Soto, E.; Jimenez-Barbero, J. & Gimenez-Gallego, G. (2007). CgNa, a type I toxin from the giant Caribbean sea anemone Condylactis gigantea shows structural similarities to both type I and II toxins, as well as distinctive structural and functional properties(1). Biochem J, Vol.406, No.1, (Aug 15), pp. 67-76

Salgado, V.L. & Kem, W.R. (1992). Actions of three structurally distinct sea anemone toxins on crustacean and insect sodium channels. Toxicon, Vol.30, No.11, (Nov), pp. 1365-81

Schroeder, C.I.; Smythe, M.L. & Lewis, R.J. (2004). Development of small molecules that mimic the binding of omega-conotoxins at the N-type voltage-gated calcium channel. Mol Divers, Vol.8, No.2, pp. 127-34

Schweitz, H. (1984). Lethal potency in mice of toxins from scorpion, sea anemone, snake and bee venoms following intraperitoneal and intracisternal injection. Toxicon, Vol.22, No.2, pp. 308-11

Sharkey, R.G.; Jover, E.; Couraud, F.; Baden, D.G. & Catterall, W.A. (1987). Allosteric modulation of neurotoxin binding to voltage-sensitive sodium channels by Ptychodiscus brevis toxin 2. Mol Pharmacol, Vol.31, No.3, (Mar), pp. 273-8

Shon, K.J.; Grilley, M.; Jacobsen, R.; Cartier, G.E.; Hopkins, C.; Gray, W.R.; Watkins, M.; Hillyard, D.R.; Rivier, J.; Torres, J.; Yoshikami, D. & Olivera, B.M. (1997). A noncompetitive peptide inhibitor of the nicotinic acetylcholine receptor from Conus purpurascens venom. Biochemistry, Vol.36, No.31, (Aug 5), pp. 9581-7
Shultz, J.W. & Regier, J.C. (2000). Phylogenetic analysis of arthropods using two nuclear protein-encoding genes supports a crustacean + hexapod clade. *Proc Biol Sci*, Vol.267, No.1447, (May 22), pp. 1011-9

Soderlund, D.M. & Knipple, D.C. (2003). The molecular biology of knockdown resistance to pyrethroid insecticides. *Insect Biochem Mol Biol*, Vol.33, No.6, (Jun), pp. 563-77

Song, W.; Du, Y.; Liu, Z.; Luo, N.; Turkov, M.; Gordon, D.; Gurevitz, M.; Goldin, A.L. & Dong, K. (2011). Substitutions in the domain III voltage-sensing module enhance the sensitivity of an insect sodium channel to a scorpion beta-toxin. *J Biol Chem*, Vol.286, No.18, (May 6), pp. 15781-8

Stapleton, A.; Blankenship, D.T.; Ackermann, B.L.; Chen, T.M.; Gorder, G.W.; Manley, G.D.; Palfreyman, M.G.; Coutant, J.E. & Cardin, A.D. (1990). Curtatoxins. Neurotoxic insecticidal polypeptides isolated from the funnel-web spider Hololena curta. *J Biol Chem*, Vol.265, No.4, (Feb 5), pp. 2054-9

Starzl, T.E. (1981). The succession from kidney to liver transplantation. *Transplant Proc*, Vol.13, No.1 Suppl 1, (Mar), pp. 50-4

Stephan, M.M.; Potts, J.F. & Agnew, W.S. (1994). The micr0 skeletal muscle sodium channel: mutation E403Q eliminates sensitivity to tetrodotoxin but not to mu-conotoxins GIIIA and GIIIB. *J Membr Biol*, Vol.137, No.1, (Jan), pp. 1-8

Stewart, L.M.; Hirst, M.; Lopez Ferber, M.; Merryweather, A.T.; Cayley, P.J. & Possee, R.D. (1991). Construction of an improved baculovirus insecticide containing an insect-specific toxin gene. *Nature*, Vol.352, No.6330, (Jul 4), pp. 85-8

Strugatsky, D.; Zilberberg, N.; Stankiewicz, M.; Ilan, N.; Turkov, M.; Cohen, L.; Pelhate, M.; Gilles, N.; Gordon, D. & Gurevitz, M. (2005). Genetic polymorphism and expression of a highly potent scorpion depressant toxin enable refinement of the effects on insect Na channels and illuminate the key role of Asn-58. *Biochemistry*, Vol.44, No.25, (Jun 28), pp. 9179-87

Tan, J.; Liu, Z.; Nomura, Y.; Goldin, A.L. & Dong, K. (2002a). Alternative splicing of an insect sodium channel gene generates pharmacologically distinct sodium channels. *J Neurosci*, Vol.22, No.13, (Jul 1), pp. 5300-9

Tan, J.; Liu, Z.; Tsai, T.D.; Valles, S.M.; Goldin, A.L. & Dong, K. (2002b). Novel sodium channel gene mutations in Blattella germanica reduce the sensitivity of expressed channels to deltamethrin. *Insect Biochem Mol Biol*, Vol.32, No.4, (Apr), pp. 445-54

Tomalski, M.D.; Kutney, R.; Bruce, W.A.; Brown, M.R.; Blum, M.S. & Travis, J. (1989). Purification and characterization of insect toxins derived from the mite, Pyemotes tritici. *Toxicon*, Vol.27, No.10, pp. 1151-67

Tugarinov, V.; Kustanovich, I.; Zilberberg, N.; Gurevitz, M. & Anglister, J. (1997). Solution structures of a highly insecticidal recombinant scorpion alpha-toxin and a mutant with increased activity. *Biochemistry*, Vol.36, No.9, (Mar 4), pp. 2414-24

Valdez-Cruz, N.A.; Batista, C.V.; Zamudio, F.Z.; Bosmans, F.; Tytgat, J. & Possani, L.D. (2004). Phaiodotoxin, a novel structural class of insect-toxin isolated from the venom of the Mexican scorpion Anuroctonus phaiodactylus. *Eur J Biochem*, Vol.271, No.23-24, (Dec), pp. 4753-61
Vassilevski, A.A.; Kozlov, S.A. & Grishin, E.V. (2009). Molecular diversity of spider venom. *Biochemistry (Mosc)*, Vol.74, No.13, (Dec), pp. 1505-34

Vijverberg, H.P.; Pauron, D. & Lazdunski, M. (1984). The effect of Tityus serrulatus scorpion toxin gamma on Na channels in neuroblastoma cells. *Pflugers Arch*, Vol.401, No.3, (Jul), pp. 297-303

Wakankar, A.A. & Borchardt, R.T. (2006). Formulation considerations for proteins susceptible to asparagine deamidation and aspartate isomerization. *J Pharm Sci*, Vol.95, No.11, (Nov), pp. 2321-36

Wang, J.; Chen, Z.; Du, J.; Sun, Y. & Liang, A. (2005). Novel insect resistance in Brassica napus developed by transformation of chitinase and scorpion toxin genes. *Plant Cell Rep*, Vol.24, No.9, (Nov), pp. 549-55

Wang, R.; Huang, Z.Y. & Dong, K. (2003). Molecular characterization of an arachnid sodium channel gene from the varroa mite (Varroa destructor). *Insect Biochem Mol Biol*, Vol.33, No.7, (Jul), pp. 733-9

Warmke, J.W.; Reenan, R.A.; Wang, P.; Qian, S.; Arena, J.P.; Wang, J.; Wunderler, D.; Liu, K.; Kaczorowski, G.J.; Van der Ploeg, L.H.; Ganetzky, B. & Cohen, C.J. (1997). Functional expression of Drosophila para sodium channels. Modulation by the membrane protein TipE and toxin pharmacology. *J Gen Physiol*, Vol.110, No.2, (Aug), pp. 119-33

Wilson, M.J.; Zhang, M.M.; Azam, L.; Olivera, B.M.; Bulaj, G. & Yoshikami, D. (2011). NaV[beta] Subunits Modulate the Inhibition of NaV1.8 by the Analgesic Gating Modifier {micro}O-Conotoxin MrVIB. *J Pharmacol Exp Ther*, Vol.338, No.2, (Aug), pp. 687-93

Xiao, Y.; Bingham, J.P.; Zhu, W.; Moczydlowski, E.; Liang, S. & Cummins, T.R. (2008). Tarantula huwentoxin-IV inhibits neuronal sodium channels by binding to receptor site 4 and trapping the domain ii voltage sensor in the closed configuration. *J Biol Chem*, Vol.283, No.40, (Oct 3), pp. 27300-13

Xiao, Y. & Liang, S. (2003). Inhibition of neuronal tetrodotoxin-sensitive Na+ channels by two spider toxins: hainantoxin-III and hainantoxin-IV. *Eur J Pharmacol*, Vol.477, No.1, (Sep 5), pp. 1-7

Yu, F.H. & Catterall, W.A. (2003). Overview of the voltage-gated sodium channel family. *Genome Biol*, Vol.4, No.3, pp. 207

Zamudio, F.Z.; Conde, R.; Arevalo, C.; Becerril, B.; Martin, B.M.; Valdivia, H.H. & Possani, L.D. (1997). The mechanism of inhibition of ryanodine receptor channels by imperatoxin I, a heterodimeric protein from the scorpion Pandinus imperator. *J Biol Chem*, Vol.272, No.18, (May 2), pp. 11886-94

Zhu, S.; Gao, B. & Tytgat, J. (2005). Phylogenetic distribution, functional epitopes and evolution of the CSalphabeta superfamily. *Cell Mol Life Sci*, Vol.62, No.19-20, (Oct), pp. 2257-69

Zlotkin, E. (1999). The insect voltage-gated sodium channel as target of insecticides. *Annu Rev Entomol*, Vol.44, pp. 429-55

Zlotkin, E.; Eitan, M.; Bindokas, V.P.; Adams, M.E.; Moyer, M.; Burkhart, W. & Fowler, E. (1991). Functional duality and structural uniqueness of depressant insect-selective neurotoxins. *Biochemistry*, Vol.30, No.19, (May 14), pp. 4814-21
Zlotkin, E.; Fishman, Y. & Elazar, M. (2000). AaIT: from neurotoxin to insecticide. *Biochimie*, Vol.82, No.9-10, (Sep-Oct), pp. 869-81

Zlotkin, E.; Gurevitz, M.; Fowler, E. & Adams, M.E. (1993). Depressant insect selective neurotoxins from scorpion venom: chemistry, action, and gene cloning. *Arch Insect Biochem Physiol*, Vol.22, No.1-2, pp. 55-73

Zlotkin, E.; Kadouri, D.; Gordon, D.; Pelhate, M.; Martin, M.F. & Rochat, H. (1985). An excitatory and a depressant insect toxin from scorpion venom both affect sodium conductance and possess a common binding site. *Arch Biochem Biophys*, Vol.240, No.2, (Aug 1), pp. 877-87
This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through Bacillus sphaericus and B. thuringiensis, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.
