

Review

Sea anemone venom as a source of insecticidal peptides acting on voltage-gated Na⁺ channels

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Abstract

Sea anemones produce a myriad of toxic peptides and proteins of which a large group acts on voltage-gated Na⁺ channels. However, in comparison to other organisms, their venoms and toxins are poorly studied. Most of the known voltage-gated Na⁺ channel toxins isolated from sea anemone venoms act on neurotoxin receptor site 3 and inhibit the inactivation of these channels. Furthermore, it seems that most of these toxins have a distinct preference for crustaceans. Given the close evolutionary relationship between crustaceans and insects, it is not surprising that sea anemone toxins also profoundly affect insect voltage-gated Na⁺ channels, which constitutes the scope of this review. For this reason, these peptides can be considered as insecticidal lead compounds in the development of insecticides.

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Keywords: Sea anemone; Voltage-gated sodium channel; Insecticide; Toxin; Site 3; ATX-II

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1. Introduction

Sea anemones are ocean dwelling, solitary members of the phylum cnidaria and the class anthozoa invertebrates. The name Cnidaria (with a silent “c”)

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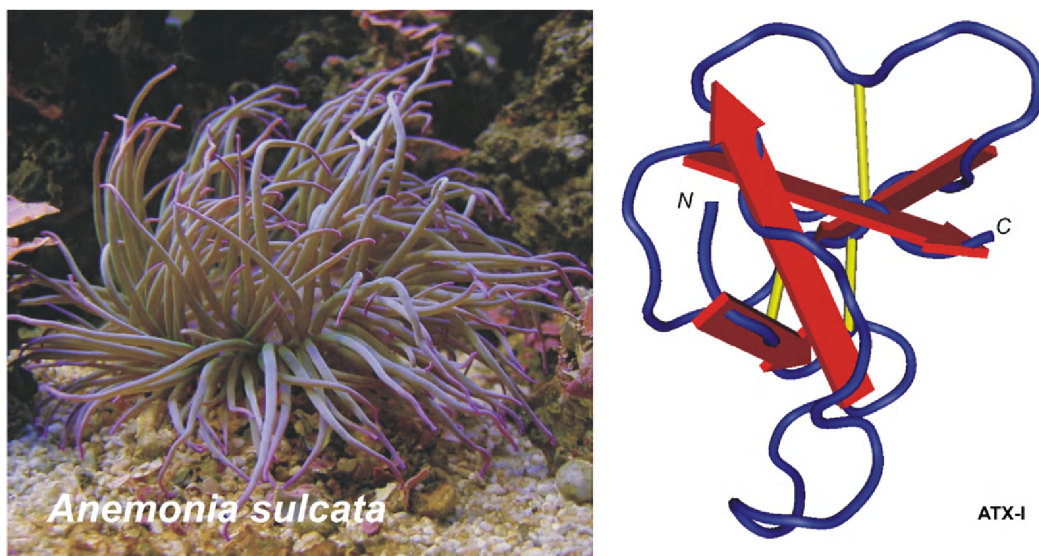


Fig. 1. Left panel: *Anemonia sulcata*; right panel: NMR structure of ATX-I (PDB accession code 1ATX).

refers to the cnidae, or nematocysts, which all Cnidarians possess. The phylum Cnidaria includes anemones, corals, jellyfish, and hydras. Sea anemones, named after a terrestrial flower, have a basic radial symmetry with tentacles that surround a central mouth opening (see Fig. 1). The tentacles are used to catch food and transfer it to their mouth. Each stinging capsule (nematocyst) in the tentacles, and other parts of the sea anemone, contains a coiled hollow filament, usually barbed, containing venom. This is used to immobilize smaller organisms, for defense against predators, and to fight territorial disputes. When triggered by mechanical or chemical stimulation, the capsule everts and drives the filament into its prey, discharging its venom.

This venom contains a variety of active compounds, including potent toxins affecting voltage-gated Na^+ and K^+ channels (including the hERG channel), acid-sensing ion channels, pore-forming toxins (actinoporins) and protease inhibitors (Belmonte et al., 1994; Béress et al., 1975; Bruhn and Béress, 1978; Diochot et al., 1998, 2003, 2004). The fact that sea anemone toxins affect voltage-gated ion channels seems logical since these targets are an important component of the action potential in the signal transduction process of both vertebrates and invertebrates. In fact, toxins from some species (e.g. *Anemonia sulcata*, *Phyllodiscus semoni*) can even be dangerous to humans. But, in most cases, a sting by the nematocysts will cause local inflammations, pain and sometimes edema. Actino-

porins have been reported as highly toxic to fish and crustaceans, which may be the natural prey of sea anemones. In addition to their role in predation, it has been suggested that actinoporins could act, when released in water, as efficient repellents against potential predators.

From an evolutionary point of view, the existence of crustacean-selective toxins in sea anemones is very interesting. It may help us to understand why we can encounter insect-selective toxins in sea anemones, even when insects and sea anemones in an everyday life will never “encounter one another”. Indeed, Zrzavy and Stys have proposed a taxon, the so-called ‘Pancrustacea’, comprising all crustaceans and hexapods (Zrzavy and Stys, 1997). It should hereby be explained that Hexapoda is a subphylum of the phylum Arthropoda and comprises the class Insecta, in addition to some wingless arthropods such as Collembola, Protura and Diplura. It is known that the taxonomy of Insecta is very extensive with the majority of invertebrates being classified as insects (approximately 1 million extant species) and 95% of the earth’s creatures being invertebrates. Furthermore, a monophyletic Pancrustacea taxon has been supported by several molecular studies (Giribet and Ribera, 2000; Nardi et al., 2003; Shultz and Regier, 2000), in which most of the subphylum Crustacea is paraphyletic with respect to insects. This means that insects are derived from crustacean ancestors and that by definition crustacean-selective toxins found in sea anemones may be considered as ‘lead

compounds' for molecules with an anti-insect profile, i.e. insecticides. On the basis of this peculiar evolutionary and pharmacological feature, this review will focus on sea anemone venom toxins as a source of insecticidal peptides acting on voltage-gated Na^+ (Na_v) channels (for a description of Na_v channels, see Section 3).

2. Sequences and structures

The characterization of sea anemone toxins began as early as 1968 when Shapiro purified a toxin which he called *Condylactis* toxin from the sea anemone *Condylactis gigantea* (Shapiro, 1968). This toxin appeared to cause an increase in action potential duration in lobster giant axons. Several years later, in 1975, Béress and co-workers isolated three toxins from *A. sulcata* venom (Béress et al., 1975) which became widely used tools to study Na_v channels.

Presently, some 50 toxins active on Na_v channels have been identified. Most of these proteins were first characterized as cardiac stimulants and neurotoxins (Barhanin et al., 1981; Sanchez-Rodriguez and Cruz-Vazquez, 2006; Schweitz et al., 1985). In 1991, Norton proposed a classification of sea anemone polypeptides dividing this group into three classes: two made up of molecules containing 46–49 amino acid residues including Type 1 (genera *Anthopleura* and *Anemonia* belonging to family Actiniidae) and Type 2 (genera *Radianthus* and *Stichodactyla* belonging to family Stichodactylidae) and one comprising shorter polypeptides containing 27–32 residues (Type 3; e.g. PaTX from *Entacmaea actinostoloides* and Da I and II from *Dofleinia armata*) (Honma et al., 2003; Nishida et al., 1985; Norton, 1991). Subsequently, other genera have been added to the Type 1 and Type 2 classes (see Fig. 2) and have therefore rendered this division

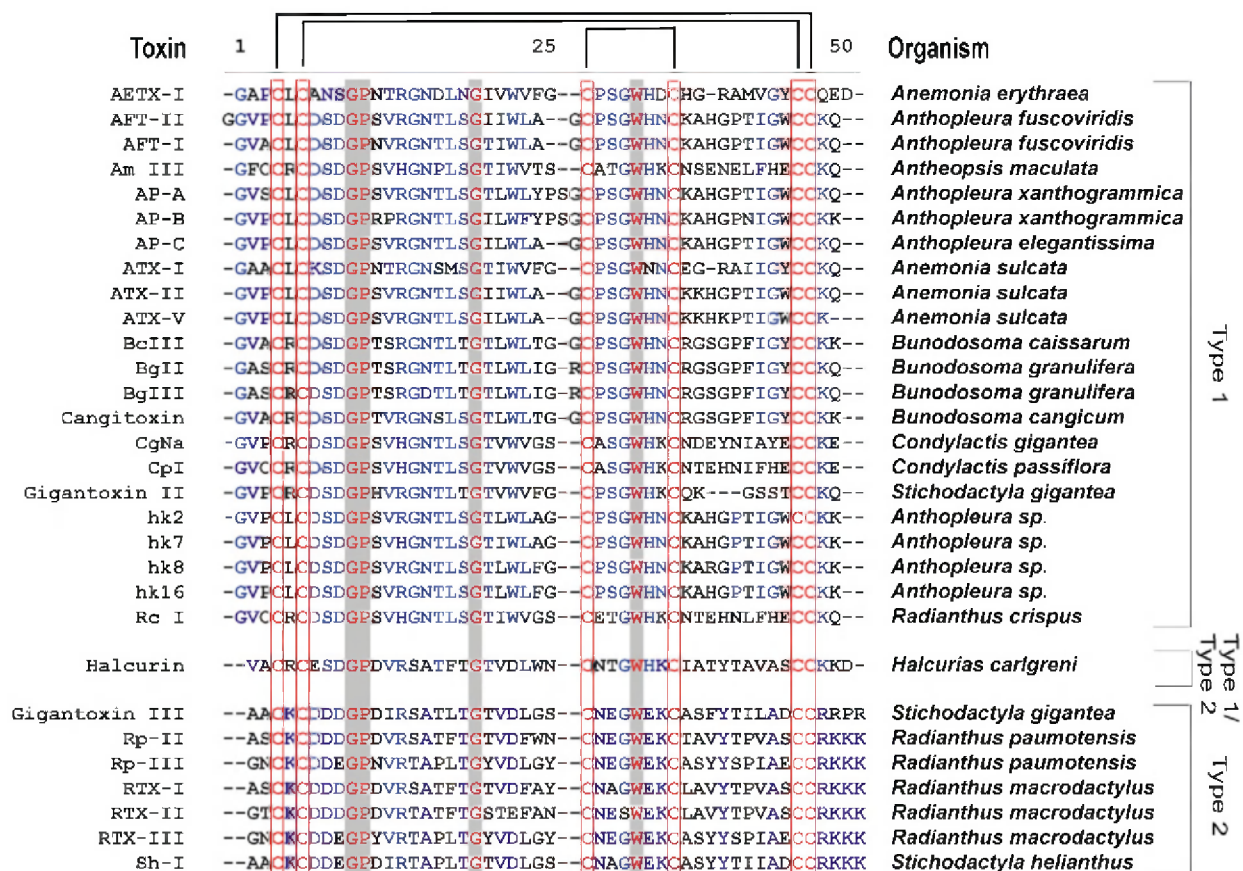


Fig. 2. Sequence alignment of Types 1 and 2 sea anemone toxins. The conserved cysteine spacing and bonding pattern is indicated by red squares and black bars above the sequence. Conserved residues over all sequences are indicated in red text with gray background. Highly conserved residues per Type are indicated in blue. Hydroxylated proline residues are indicated with the letter O. References for the toxins can be found throughout the text.

based on family classification obsolete. These two classes of polypeptides are similar with respect to the locations of the six half-cystines as well as several other residues thought to play a role in biological activity. Their molecular weight ranges between 3000 and 5000 Da. Due to the fact that the toxin halcurin possesses structural features of both Types 1 and 2 toxins and is present in the primitive *Halcurias* sp., both Types 1 and 2 toxins are considered to have evolved from the same ancestral gene (Ishida et al., 1997). Nevertheless, they are immunologically distinguishable from each other because there is no antigenic cross-reactivity between both types of toxins (Schweitz et al., 1985; Norton, 1991). Recently, three peptide toxins (Am I–III) with acute toxicity in crabs were isolated from the sea anemone *Anthopleura maculata* (Honma et al., 2005). Am I was weakly lethal to crabs (LD₅₀ 830 µg/kg) and Am III was potentially lethal (LD₅₀ 70 µg/kg), while Am II was only paralytic (ED₅₀ 420 µg/kg). The complete amino acid sequences of the three toxins were determined by cDNA cloning. Although Am III is an analogue of the Type 1 sea anemone Na_v channel toxins, both Am I (27 residues) and II (46 residues) are structurally novel peptide toxins (Honma et al., 2005).

The first three-dimensional structures of sea anemone toxins active on Na_v channels, determined by NMR spectroscopy, were AP-A in 1988 (Torda et al., 1988) and ATX-I in 1989 (Widmer et al., 1989). Both molecules contain a core of four strands of anti-parallel β -sheets connected by two loops (see Fig. 1). Meanwhile, the structures of Sh-I (Wilcox et al., 1993), ATX-III (Manoleras and Norton, 1994) and AP-B (Monks et al., 1995) have also been published. AP-B seems to exist in multiple conformations in solution as a result of *cis*–*trans* isomerization about the Gly⁴⁰–Pro⁴¹ peptide bond. Three loops connect the four β -sheets, the longest and least well defined being the first loop, extending from residues 8 to 17.

3. Na_v channels and site 3 toxins

Na_v channels are transmembrane protein complexes that form pores across the cell membrane through which specific ions can diffuse (Yu and Catterall, 2003). These channels are key elements in cellular function since they participate in the generation and propagation of action potentials in neurons and most electrically excitable cells present in different tissues from various organisms. Studies

have indicated that these channels are composed of a pore-forming α subunit (approximately 260 kDa) which can be associated with up to four different β subunits (30–40 kDa) (Yu et al., 2003). The α -subunit is sufficient for functional expression, but the kinetics, expression level and voltage dependence of channel gating can be modified by the β subunits. The α subunits are organized in four homologous domains (DI–IV), each containing six transmembrane α helices (S1–S6) and a pore lining loop located between the S5 and S6 segments. The S4 transmembrane segments in each domain contain positively charged amino acid residues at every third position and are thought to act as voltage sensors. The short intracellular loop connecting homologous domains III and IV, in particular a short sequence of hydrophobic residues (the IFM sequence), serves as the inactivation gate (Yu and Catterall, 2003). To date, nine mammalian channel isoforms have been identified (Goldin et al., 2000). The primary structure of insect Na_v channels is similar to that of mammals (Zlotkin, 1999). The first insect Na_v channel gene, *para*, was cloned from *Drosophila melanogaster* and heterologously expressed (Warmke et al., 1997). Since then, several other proteins, e.g. housefly Vssc1, cockroach Para^{CSMA} and cockroach BgNa_v1-1 have been functionally expressed in *Xenopus laevis* oocytes (Soderlund and Knipple, 2003; Tan et al., 2005). An auxiliary–regulatory β subunit for the insect Na_v channels, tipE, has also been identified (Feng et al., 1995).

As crucial components of the development of action potentials, Na_v channels are one of the foremost targets of venoms. Toxins from scorpion, sea anemone, cone snail, spider and insect venoms have been used to describe up to nine different receptor sites on the α -subunit of Na_v channels (Wang and Wang, 2003). All of them are linked to specific effects on channel function but only sites 1–5 are molecularly defined (Leipold et al., 2005). Sea anemone venoms contain toxins which are known to prolong action potential kinetics via their ability to bind to the extracellular site 3 (loop between S3 and S4 in DIV) on the Na_v channels in a membrane potential-dependent manner (see Fig. 3) (Bosmans et al., 2002; Cestèle et al., 1997; Gordon and Zlotkin, 1993; Leipold et al., 2004; Pauron et al., 1985; Rogers et al., 1996). Other ligands that bind to this site, although structurally unrelated, are scorpion α -toxins and some spider toxins (δ -atracotoxins) (Gordon et al., 1996; Nicholson et al., 2004; Possani et al., 1999).

	S3	Loop region	S4
rNav1.2	IVGMFLA	IIIEKYFVSPTLFRVIRL	
hNav1.5	IVGTVLS	IIIQKYFVSPTLFRVIRL	
Cockroach	ILGLVLS	IIIEKYFVSPTLLRVVRV	
Housefly	ILGLVLS	IIIEKYFVSPTLLRVVRV	
Drosophila	ILGLVLS	IIIEKYFVSPTLLRVVRV	
		DIV	

Fig. 3. Sequence comparison of the loop region between S3 and S4 in DIV in mammalian and insect Na_v channels. Residues indicated in red are reported to be important for scorpion α -toxin binding. Residues in yellow are reported to participate in sea anemone toxin binding. Residues in white text on a black background are reported to be crucial for binding of both groups.

In 1996, Rogers and co-workers converted extracellular acidic amino acids in domains I and IV of r $\text{Na}_v1.2a$ to neutral or basic amino acids using site-directed mutagenesis (Rogers et al., 1996). Conversion of individual residues in the DIV S3–S4 loop identified seven residues whose mutation caused significant effects on binding of scorpion α -toxin (LqTx) or sea anemone toxin (ATX-II). Moreover, chimeric Na_v channels in which amino acid residues at the extracellular end of S3 in DIV of $\text{Na}_v1.5$ were substituted into the r $\text{Na}_v1.2a$ sequence had reduced affinity for LqTx. Electrophysiological analysis showed that E1613R had 62 and 82-fold lower affinities for LqTx and ATX-II, respectively. These results indicated that non-identical amino acids of the DIV S3–S4 loop participate in scorpion α -toxin and sea anemone toxin binding to overlapping sites and that neighboring amino acid residues in the DIV S3 segment contribute to the difference in scorpion α -toxin binding affinity between cardiac and neuronal Na_v channels. Also, further experiments using AP-B on r $\text{Na}_v1.2a$ or $\text{Na}_v1.5$ mutants have suggested the involvement of this loop region and more specific Glu¹⁶¹³ and Glu¹⁶¹⁶ for $\text{Na}_v1.2$ and Asp¹⁶¹² for $\text{Na}_v1.5$ (see Fig. 3) (Benzinger et al., 1998; Blumenthal and Seibert, 2003; Honma and Shiomi, 2006). Other research groups, focusing on finding residues important for scorpion α -toxin binding, have also highlighted residues in this loop region in DIV (Leipold et al., 2004). Since all the aforementioned Na_v channel residues are similar or identical in their invertebrate counterparts, it could be hypothesized that changes in the selectivity of sea anemone toxins towards vertebrates or invertebrates could be caused by other channel residues in this vicinity. In concordance, Oliveira et al. suggested that nearby residues could also contribute to the interaction with sea anemone toxins (Oliveira et al., 2004).

4. Structure–function relationship of sea anemone toxins

Mutagenesis of AP-B resulted in the identification of a flexible loop in the region of residues 8–17 (Arg¹⁴ loop) as being important for binding to Na_v channels (Dias-Kadambi et al., 1996a, b). Furthermore, researchers in the lab of Dr. Blumenthal have shown that Leu¹⁸ is an absolute requirement for the binding of AP-B to its target (Blumenthal and Seibert, 2003; Honma and Shiomi, 2006). Neighboring residues are either less sensitive, or their sensitivity is dependent on the nature of the mutation, especially the introduction of negative charges at these positions that is poorly tolerated. In addition, Arg¹², Ser¹⁹ and Lys⁴⁹ are reported to be important for toxin affinity and channel isoform specificity of AP-B (Gallagher and Blumenthal, 1994; Seibert et al., 2004). It should be noted that AP-B has no selectivity between neuronal and cardiac Na_v channels, while AP-A is selective for the latter. Only two residues located outside the flexible loop region (Trp³³ and Lys³⁷) seem to be indispensable for pharmacological activity. It has been shown that Lys³⁷ can interact directly with Asp¹⁶¹² of r $\text{Na}_v1.5$ (Benzinger et al., 1998; Blumenthal and Seibert, 2003).

In comparison to scorpion α - and β -toxins (Cohen et al., 2005; Ye et al., 2005), not much work has been done on the insect- or mammalian-specificity of sea anemone toxins (Pelhate et al., 1984). Early work on ATX-I has indicated its preferential toxicity against crabs, rather than mice (see Table 1) (Norton, 1991; Schweitz et al., 1981). The proof of the link between insects and crustaceans has been described on a genetic level (Boore et al., 1998). Therefore, since both crabs (crustaceans) and insects belong to the same family (Arthropoda) one could hypothesize that ATX-I preferentially targets insects rather than mice. The same argument could be made for Sh-I and Rp-II. In fact, a study by Salgado and Kem in 1992 investigated the membrane actions of Sh-I, CgII and Cpl on action potentials and voltage-clamp membrane currents of the giant axon from the crayfish *Procambarus clarkii* (Salgado and Kem, 1992). Sh-I and CgII were also tested on the cockroach (*Periplaneta americana*) giant axon. Both toxins were particularly lethal to crustaceans, moderately toxic to an insect (cockroach), and essentially non-toxic to a mammal (mouse). Both toxins prolonged crayfish giant axon action potentials by selectively

Table 1
Activity of sea anemone toxins (based on Norton, 1991)

LD ₅₀ (μg/kg)				Rat brain synaptosomes (K _a in nM)	Cockroach central nervous system (IC ₅₀ in nM)	EC ₅₀ (nM) on rat heart muscle (Na _v 1.5)	EC ₅₀ (nM) on insect channel (para)
Toxin	Crab	Mice I.p.	Mice I.c.				
AFT-I	100–150	98				–2	
AFT-II	100–150	450				2.62 ^a	
ATX-I	2	> 4000	236	7000			
ATX-II	2	100	2.5	90–150	0.5–1.5 ^b	15–49 ^a	–10 ^a
ATX-V	5.2	19	1.6	50		2	
AP-A	11	66	5.3	120		3	
AP-B	39	8	0.2	35		2	
BgII			0.4 ^c	9		500 ^a	5.5 ^a
BgIII			21 ^c	72		5100 ^a	1300 ^a
Rp-I	36	145	1.5	900		3000	
Rp-II	15	4200	12	> 100 000		5000	
Sh-I	0.5–3 ^d	> 15 000	116	40 000 ^e		> 8000	

^aResults obtained from cloned channels expressed in cocytes and/or mammalian cells.

^bEC₅₀ in locust central nervous system is 6 nM. LD₅₀ in mice (s.c.) is 22 and 2.5 μmol/g in *B. Germanica*.

^ci.c.v. injections.

^dLD₅₀ varies with crab species.

^eK_D in crab is 14 nM.

slowing Na_v channel inactivation without affecting activation. However, more experiments on cloned Na_v channels of insects and mammals should be carried out. ATX-II is toxic to crabs but the activity on mice via i.c.v. injection is still pronounced (see Table 1) (Norton, 1991; Schweitz et al., 1981). However, ATX-II is also very effective on the *para* insect channel and binds with high affinity to cockroach neuronal membranes, while its binding affinity for rat brain synaptosomes is low (Gordon et al., 1996; Gordon and Zlotkin, 1993; Schweitz et al., 1981; Warmke et al., 1997). Mutagenesis of ATX-II has provided an insight into the pharmacologically important epitopes of this toxin: either acetylation or fluorescamine treatment of ATX-II that destroyed the positive charges of the three ε-amino groups of residues Lys³⁵, Lys³⁶ and Lys⁴⁶, and of the α-amino function of Gly¹, produced an almost complete loss of toxicity and a considerable decrease in binding activity (Barhanin et al., 1981). Furthermore, these authors showed that carbethoxylation of His³² and His³⁷ provoked an important decrease of both toxicity and binding activity, and it was also found that modification of the guanidine side chain of Arg¹⁴ could destroy both toxicity and binding of the toxin to Na_v channels. Last, but not least, Barhanin et al. concluded that modification of the carboxylate functions of Asp⁷,

Asp⁹ and Gln⁴⁷, with glycine ethyl ester in the presence of a soluble carbodimide completely abolished the toxicity but left the affinity for the sea anemone toxin receptor unchanged. Nevertheless, ATX-II displays a high potency when tested on the mammalian cardiac Na_v1.5 channel (Oliveira et al., 2004). Two toxins from the *Bunodosoma granulifera*, BgII and BgIII, have been thoroughly tested on mice, cloned channels, dorsal root ganglia and rat brain synaptosomes (Bosmans et al., 2002; Goudet et al., 2001; Loret et al., 1994; Salceda et al., 2002). Both toxins seem to have a preference for insects. BgII in particular has a 100-fold higher potency on the insect channel, *para*, as compared to other mammalian channels when expressed in *X. laevis* oocytes (see Table 1, Fig. 4). However, BgII does affect rat brain synaptosomes, with a K_D of 9 nM, but no experiments have yet been carried out on insect preparations. It should be stressed that BgII and BgIII only differ in one residue (N16D) which is situated in the aforementioned Arg¹⁴ loop. Yet, their potencies towards Na_v channels are remarkably different. Another trademark of BgII and BgIII is that they have a particularly devastating effect on the inactivation of the insect Na_v channel, *para*. This is in sharp contrast to vertebrate channels. The inactivation is extremely slowed such that the channel simply does not inactivate. This

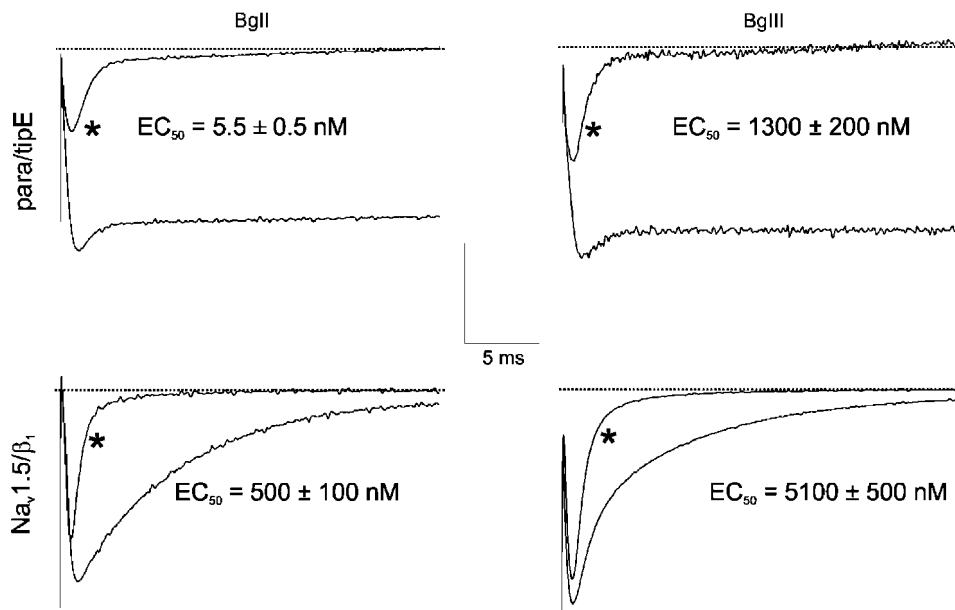


Fig. 4. Effects of sea anemone toxins on Na_v channels. The left column represents the effects of BgII, right column the effects of BgIII on the cloned Na_v channels expressed in *Xenopus laevis* oocytes. * represents control conditions where no toxin was added. Traces shown are before, and after, addition of: *Para*/BgII (20 nM); *Para*/BgIII (5 μM); $\text{Na}_v1.5$ /BgII (1 μM); $\text{Na}_v1.5$ /BgIII (60 μM). Current traces were evoked by depolarizations ranging from -20 to 10 mV depending on the Na_v channel, from a holding potential of -90 mV. Scale bar: y-axis scale for *para*/tipE is $0.5 \mu\text{A}$; $\text{Na}_v1.5/\beta_1$ is $1 \mu\text{A}$. EC_{50} values are shown in the figure.

removal of inactivation is also seen when ATX-II is applied on *para* (Gordon et al., 1996; Gordon and Zlotkin, 1993; Schweitz et al., 1981; Warmke et al., 1997).

Wang et al. (2004) have studied four 'naturally occurring mutant or isoform' toxins from an *Anthopleura* sp. that were expressed in *E. coli* and tested in contractile force studies. They suggest that residues at positions 14, 22, 25 and 37 (with an emphasis on Arg¹⁴) are important to explain the isoform-specific features of the toxins towards their pharmacological effects.

More recently, a new Type 1 peptide toxin with a strong paralytic activity on crustacea (LD_{50} approx. $1 \mu\text{g}/\text{kg}$) was isolated from the sea anemone *C. gigantea* (Standker et al., 2006). This new toxin, CgNa, increased action potential duration in dorsal root ganglia neurons under current clamp conditions. CgNa also prolonged the cardiac action potential duration and enhanced contractile force albeit at 100-fold higher concentrations than ATX-II. The action on Na_v channel inactivation and cardiac excitation–contraction coupling resembles previous results with compounds obtained from this and other sea anemones (see previous section).

In a very recent paper by Moran et al. (2006), the authors try to resolve the bioactive surface of ATX-II. To this end, they established an efficient expression system for this toxin and mutagenized it throughout. Six residues were found to constitute the anti-insect bioactive surface of ATX-II (Val², Leu⁵, Asn¹⁶, Leu¹⁸, and Ile⁴¹). Further analysis of nine ATX-II mutants on $\text{Na}_v1.5$ indicated that the bioactive surfaces interacting with insect and mammalian channels practically coincide but differ from the bioactive surface of AP-B. All residues important for activity excluding Arg¹² and Lys⁴⁹ appear in both ATX-II and AP-B. Yet, Ser¹⁹ seems to be important for the anti-mammalian activity but only has a small effect on the activity of ATX-II toward insects, suggesting that this residue is not a major contributor to the insecticidal activity of this toxin (as opposed to AP-B). The authors also investigated a major variation between the bioactive surfaces of AP-B and ATX-II that consists of a Trp³³ and Lys³⁷ in AP-B as compared to a Trp³¹ and a Lys³⁵ in ATX-II. Substitution of these residues in AP-B using their ATX-II equivalents had no effect on the insecticidal activity and only a slight effect on $\text{Na}_v1.5$. These conspicuous disparities in bioactive surfaces imply that despite

similarities in the Arg¹⁴ loop, the interaction site of ATX-II with site 3 is different from that of AP-B. Support for this conclusion can be found in the report that the binding sites of AP-A and AP-B on Na_v1.4 and Na_v1.5 are also slightly different (Benzinger et al., 1997).

5. Sea anemone toxins as insecticides?

Abundant use of one of the most commonly used insecticides in crop protection, pyrethroids, has led to the development of resistance in many insect species. One of the most important mechanisms is that of knockdown resistance (or *kdr*), caused by several mutations in the *para* gene (L1014F and M918T) which confers cross-resistance to the entire class of pyrethroids (Soderlund and Knipple, 2003; Zlotkin, 1999). Another problem is that most insecticides cause toxicity in organisms other than insects because of the general conservation of Na_v channel structure throughout the animal kingdom. Nevertheless, reports of toxins which show selectivity towards insects or mammals are being published (Bosmans et al., 2002). In fact, projects to replace classical chemical insecticides have already been undertaken (Zlotkin et al., 2000). Already in 1988, Bloomquist and Soderlund studied the effects of saturating concentrations of DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) and the pyrethroid insecticides cismethrin and deltamethrin on veratridine-dependent activation of Na_v channels using measurements of ²²Na⁺ uptake into mouse brain synaptosomes. They also conducted additional experiments to assess the interactions of insecticides and ATX-II as modifiers of alkaloid-dependent uptake. DDT and ATX-II acted synergistically to increase uptake stimulated by veratridine. Moreover, DDT shifted the potency of ATX-II for enhancing veratridine-dependent uptake to 5-fold lower concentrations. In contrast, DDT and sub-saturating concentrations of ATX-II acted independently in their enhancement of Na_v channels activation by batrachotoxin. Combining several insecticidal synergetic peptides like insect-selective sea anemone and scorpion toxins (e.g. Regev et al., 2003) in a baculovirus could also prove to be valuable. By itself this virus is already insect-selective (for a general overview on baculovirus biology, see Zlotkin et al., 2000; Inceoglu et al., 2001). However, its natural kill-rate is too slow and its host range is limited (which could also be interpreted as advantageous). In 1993, Hammock

and co-workers developed recombinant baculovirus insecticides using two approaches (Hammock et al., 1993). In one approach an insect-specific scorpion neurotoxin (AaHIT1) was expressed by the virus leading to a dramatic reduction in time to death (as compared to the native baculovirus). In the second approach an insect juvenile hormone esterase was expressed which lead to a significant reduction in feeding time indicating that an increase in lethality is not always necessary.

Despite this promising application, research on characterizing the differences in binding sites of insect- and mammalian-specific toxins towards Na_v channels is progressing slowly. The identification and comparison of the components that are involved in the interaction of sea anemone toxins with their targets is an absolute requirement to design novel insecticides. Beyond this, insect-selective toxins could be truncated (engineered) in order to become more stable when administering them. Importantly recent reports suggest that some insect-selective peptide toxins, such as ω -atracotoxin-Hv1a might be orally active in certain species (Mukherjee et al., 2006). Surprisingly, a ω -atracotoxin-Hv1a fusion protein was also topically effective (Khan et al., 2006).

Not only do arthropods destroy about 20–30% of the world's food supply (Oerke, 1994) but they are also responsible for the transmission of many human diseases. This should be an incentive to explore the sea anemone toxin world more carefully and by doing so it could mean the beginning of a new era in insect pest control.

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