Published in final edited form as:

Toxicol. 2007 February ; 49(2): 142–158.

Voltage-gated sodium channel modulation by scorpion α -toxins

Frank Bosmans* and Jan Tytgat

Laboratory of Toxicology, University of Leuven, O&N 2, Postbus 922, Herestraat 49, 3000 Leuven, Belgium

Abstract

Voltage-gated Na^+ channels are integral membrane proteins that function as a gateway for a selective permeation of sodium ions across biological membranes. In this way, they are crucial players for the generation of action potentials in excitable cells. Voltage-gated Na^+ channels are encoded by at least nine genes in mammals. The different isoforms have remarkably similar functional properties, but small changes in function and pharmacology are biologically well-defined, as underscored by mutations that cause several diseases and by modulation of a myriad of compounds respectively. This review will stress on the modulation of voltage-gated Na^+ channels by scorpion alpha-toxins. Nature has designed these two classes of molecules as if they were predestined to each other: an inevitable 'encounter' between a voltage-gated Na^+ channel isoform and an alpha-toxin from scorpion venom indeed results in a dramatically changed Na^+ current phenotype with clear-cut consequences on electrical excitability and sometimes life or death. This fascinating aspect justifies an overview on scorpion venoms, their alpha-toxins and the Na^+ channel targets they are built for, as well as on the molecular determinants that govern the selectivity and affinity of this 'inseparable duo'.

1. Introduction

Voltage-gated Na^+ channels (VGSCs) 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 (Denac et al., 2000; Goldin, 2001). Studies have indicated that VGSCs 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) (Catterall et al., 2005; Yu and Catterall, 2003; Yu et al., 2003). 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 S5 and S6. The S4 segment in each domain contains positively charged amino acid residues at every third position and is believed to play a key role in channel activation (Guy and Seetharamulu, 1986). The short intracellular loop connecting homologous DIII and DIV, in particular a short sequence of hydrophobic residues (the IFM sequence), serves as the inactivation gate (Yu and Catterall, 2003).

To whom correspondence should be addressed: J. Tytgat, Laboratory of Toxicology, University of Leuven, O&N 2, Postbus 922, Herestraat 49, 3000 Leuven, Belgium. Fax: +3216323405, Tel.: +3216323403. E-mail: Jan.Tytgat@pharm.kuleuven.be; <http://www.toxicology.be>.

*Current address: Molecular Physiology and Biophysics Section, Porter Neuroscience Research Center, Building 35, 3B 211, NINDS, NIH, Bethesda, MD 20892, USA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The β -subunits of the VGSC appear to have dual functions: modulation of channel gating and cell-cell interaction (Stevens et al., 2001; Yu et al., 2003). They consist of a large, glycosylated extracellular domain, a single transmembrane segment, and a small intracellular domain. Expression of the α -subunits of brain or skeletal muscle VGSCs alone in *Xenopus laevis* oocytes yields sodium currents that activate and inactivate more slowly than expected from recordings of dissociated neurons or myocytes. Co-expression with the β -subunits accelerates channel gating to normal rates. Up till now, four β -subunits have been identified and functionally expressed. The structural similarity of the β -subunits to cell adhesion molecules suggests that they perform similar functions, and direct experimental support for this idea has come from recent experiments on interaction of the β subunits with extracellular proteins such as tenascin-C and tenascin-R (Yu and Catterall, 2003).

A variety of different VGSCs has been identified by electrophysiological recording, biochemical purification, and cloning (SCNA genes). To eliminate confusion resulting from the multiplicity of names, a standardized nomenclature (e.g., $\text{Na}_v1.1a$) was developed (Catterall et al., 2005; Goldin et al., 2000). The nine mammalian VGSC isoforms that have been identified so far are all more than 50% identical in amino acid sequence in the transmembrane and extracellular domains. There is a tenth isoform, termed Na_x , which has approximately 50% sequence identity to the other mammalian VGSCs. Because the Na_x channel has not been functionally expressed, it is possible that this gene does not encode a VGSC, which is why it has not been assigned a number yet. Phylogenetic analysis has been previously applied to SCNA genes in order to determine the evolutionary history of the vertebrate SCNA gene family. The phylogenetic estimates of previous studies differ significantly. An state-of-the-art overview combined with new data can be found in a recent article by Novak et al. (2006) (but see also Catterall et al., 2005).

The primary structure of insect VGSCs is similar to that of mammalian VGSCs. The first insect VGSC gene, *para*, was cloned from *Drosophila melanogaster* (Warmke et al., 1997). Since then, several insect VGSC proteins, e.g. *Drosophila Para*, 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 arachnid VGSC gene from the Varroa mite (*VmNa*) was also identified but has not yet been functionally expressed (Wang et al., 2003). Recently, two new VGSCs were cloned from arachnids; the scorpion *Buthus martensii* (*BmNa_v1*) and from the spider *Ornithoconus huwena* (*OhNa_v1*) (Zuo et al., 2006).

An auxiliary-regulatory β subunit for the insect VGSC has also been identified. It was found that the tipE protein, an acidic membrane protein of 50 kDa with two putative membrane-spanning domains, stimulates functional expression of *para* VGSCs (Feng et al., 1995).

In addition to the differences in cellular and tissue expression, mammalian VGSCs also have differential expression profiles during development and different subcellular localizations, consistent with a distinct role for each channel in mammalian physiology (Goldin, 2001). Four isoforms, $\text{Na}_v1.1$, $\text{Na}_v1.2$, $\text{Na}_v1.3$ and $\text{Na}_v1.6$, are expressed at high levels in the central nervous system (CNS). Two isoforms are abundant in muscle: $\text{Na}_v1.4$ in adult skeletal muscle and $\text{Na}_v1.5$ in embryonic and denervated skeletal muscle and heart muscle. Three isoforms, $\text{Na}_v1.7$, $\text{Na}_v1.8$ and $\text{Na}_v1.9$, are expressed primarily in the peripheral nervous system. A number of human diseases have been identified that result from aberrant VGSC activity. Mutations in $\text{Na}_v1.4$ have been shown to cause three neuromuscular diseases: hyperkalemic periodic paralysis, paramyotonia congenita, and the potassium-aggravated myotonias (Cannon, 2001; Goldin, 2001). Mutations in $\text{Na}_v1.5$ have been shown to cause a cardiac pathology called the long QT syndrome. Several types of general epilepsy with febrile seizures plus (GEFS+) have been demonstrated to result from mutations in human CNS VGSC-related genes (Hains et al., 2003; Spanpanato et al., 2001). GEFS+ type 1 results from a mutation in

the β_1 subunit gene and GEFS+ type 2 results from mutations in the gene encoding $\text{Na}_v1.1$. In the literature there is ample evidence that several VGSCs are also major players in pain sensation (Baker and Wood, 2001; Julius and Basbaum, 2001). $\text{Na}_v1.8$ seems to play first fiddle in nociception. This channel is expressed not only in the central nervous system, but also in peripheral neurons such as the small diameter nociceptor neurons of dorsal root ganglia (DRG) that originate in afferent nociceptive fibers such as the C-fibers and A δ fibers (Akopian et al., 1996; Akopian et al., 1999).

Also in insects, VGSCs play an important role. Pyrethroids, which affect the insect VGSC, are commonly used as insecticides in crop protection, animal health, and the control of insects that endanger human health. Pyrethroids paralyze flying insects and, as such, are known as knockdown insecticides. The intensive use of these insecticides has led to the development of resistance in many insect species. One of the most important mechanisms of resistance in insects is the 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 (Inceoglu et al., 2001; Soderlund and Knipple, 2003; Vais et al., 2001).

Venoms are produced in specialized tissues or glands, which often are connected with delivery structures (stings, teeth, etc.). *Envenomation* takes place after parenteral application or upon contact with venoms. Most often, venoms consist of complex mixtures of different chemical substances. *Toxins* are toxic substances occurring in venoms and poisons with in many cases very specific actions on biological systems. As crucial components of the development of action potentials, VGSCs are one of the foremost targets of animal venoms and toxins. Toxins from scorpion, sea anemone, cone snail, spider and insect venoms have been used to describe nine different receptor sites on the α -subunit of VGSCs. All of them are linked to specific effects on channel function (Wang and Wang, 2003) but only sites 1–5 are molecularly defined (Leipold et al., 2005). Site 1 binds toxins that interfere with the ion transport by physically occluding the pore: these toxins include tetrodotoxin (TTX) from the puffer fish, saxitoxin from dinoflagellates, and the μ -conotoxins from cone snails. TTX was discovered nearly 40 years ago and enabled scientists to purify and study more closely the outer pore of the VGSCs (Agnew, 1984; Catterall, 2000; Miller et al., 1983). All VGSC isoforms can be divided into two groups according to their sensitivity to TTX. $\text{Na}_v1.5$, $\text{Na}_v1.8$ and $\text{Na}_v1.9$ are TTX-resistant (TTX-r) and the other isoforms are TTX-sensitive (TTX-s). A wide variety of polypeptides isolated from the venom of marine and terrestrial invertebrates are known to prolong action potential kinetics via their ability to bind to the extracellular Site 3 on the VGSC in a membrane potential-dependent manner. Among these ligands are the scorpion α -toxins, sea anemone and some spider toxins (δ -atracotoxins) (Alsen et al., 1981; Benzinger et al., 1997; Gordon et al., 1996; Grolleau et al., 2001; Pelhate et al., 1984). In fact, Site 3 was identified as a result of studying insect VGSCs by binding of radiolabeled toxins such as ^{125}I -Lqh α IT, ^{125}I -ATX II, and ^{125}I - δ -atracotoxin Hv1a (Cestele et al., 1997; Gilles et al., 2002; Gordon et al., 1996; Gordon and Zlotkin, 1993; Pauron et al., 1985). In addition to the large number of scorpion toxins that delay inactivation by binding to Site 3, there is a second class of scorpion toxins that shift the membrane potential dependence of channel activation by binding to Site 4. These scorpion β -toxins were first identified more than 20 years ago and were quickly shown not to compete with α -toxins in binding to VGSCs (Couraud et al., 1982; Couraud et al., 1986; Fontecilla-Camps et al., 1980; Zlotkin et al., 1985). Recently, a study by Corzo et al. (Corzo et al., 2005) indicated that this classification into receptor sites and the consequent physiological effects might not be so exclusive. δ -Palutoxins from the spider *Paracoelotes luctuosus* slow VGSC inactivation in a fashion similar to that of receptor Site 3 modifiers but they actually bind with high affinity to the topologically distinct receptor Site 4 linked to scorpion β -toxins.

2. Scorpion venoms and toxins

2.1. Venom composition

Scorpions (class of Arachnida, Order Scorpiones) have been roaming this planet for more than 400 million years which makes them one of the most ancient groups of animals. Approximately 1500 different species exist, which have conserved their morphology almost unaltered (Fet et al., 2000). Out of all these species, 50 are dangerous to humans. Scorpion stings cause a wide range of conditions, from severe local skin reactions to neurologic, respiratory, and cardiovascular collapse. Almost all of these lethal scorpions, except the *Hemiscorpius* species, belong to the family of the *Buthidae*. This family is characterized by a triangular-shaped sternum, in contrast to the pentagonal-shaped sternum found in the other five scorpion families. Dangerously venomous scorpions tend to have weak-looking pincers, thin bodies, and thick tails, as opposed to the strong heavy pincers, thick bodies, and thin tails present in non lethal scorpions. The lethal members of the *Buthidae* family include the genera of *Androctonus* (Northern Africa to Southeast Asia), *Buthus* (Mediterranean), *Centruroides* (Southwest USA, Mexico, Central America), *Leiurus* (Northern Africa and Middle East), *Mesobuthus* (Asia), *Parabuthus* (Western and Southern Africa), and *Tityus* (Central and South America, Caribbean). However, these scorpions may be found outside their habitat range of distribution when inadvertently transported with luggage and cargo.

Scorpion venoms are highly complex mixtures of enzymes, peptides, nucleotides, lipids, mucoproteins, biogenic amines and other unknown substances. Neurotoxins present in scorpion venoms have evolved towards a specific bioactivity aimed at attacking and immobilizing prey and as a defense against predators. In addition, multiple toxins can be present in the venom of a single species of scorpion, which pretty much guarantees that the venom will be capable of producing potent synergistic effects when injected into another party. The bioactivity of the neurotoxins present in scorpion venoms also exhibits a high level of specificity: the venom from a single species of scorpion may contain a toxin preferentially targeting mostly invertebrates, other only vertebrates. More information about the phylogeny of scorpion toxins can be found in a recent comprehensive paper by de la Vega et al. (2005).

The best studied groups of components from scorpion venoms are the polypeptides that recognize ion channels and receptors in excitable membranes. In fact, the overall toxicity of scorpion venom to humans has mainly been attributed to the activity of long chain toxins affecting VGSCs (Martin-Eauclaire and Couraud, 1995). This group can be divided into two classes: α -toxins which bind to Site 3 and β -toxins which bind to Site 4. According to their different pharmacological preferences, the scorpion α -toxins can be divided into three subgroups: classical α , α -like, and insect α -toxins. Classical α -toxins are very toxic to mammals (e.g., AaH II from *Androctonus australis* Hector, Lqh II from *Leiurus quinquestriatus hebraeus*). They are highly active in mammalian brain (reported LD₅₀'s by intracerebroventricular (*i.c.v.*) injection into mice range between 0.5 to 20 ng per 20g bodyweight) and bind with high affinity to rat brain synaptosomes ($K_D = 0.2\text{--}5$ nM) (Jover et al., 1978; Legros et al., 2005). Furthermore, the binding of AaH II is increased by veratridine binding to Site 2 (Gordon and Zlotkin, 1993). Site 5 and Site 7 toxins like brevetoxin and several pyrethroids have also been shown to allosterically alter the binding of scorpion α -toxins to Site 3 (Cestele et al., 1995; Gilles et al., 2003; Sharkey et al., 1987). Insect α -toxins are especially toxic for insects (e.g., Lqh α IT from *Leiurus quinquestriatus hebraeus*) (Housset et al., 1994; Li et al., 1996; Tugarinov et al., 1997). Their toxicity towards mice is very low (LD₅₀ >1 μ g/20 g mouse by *i.c.v.* injection), but their binding affinity to insect neuronal preparations is high ($K_D = 0.06\text{--}1$ nM). In contrast to classical α -toxins, the binding of Lqh α IT to insect neuronal membranes is reported to be independent of membrane potential (Gordon and Zlotkin, 1993). The α -like toxins act on both mammals and insects but do not bind to rat brain synaptosomes (mostly Na_v1.2) despite a high toxicity by intravenous injection

(e.g., BmK M1 from *Buthus martensii* Karsch, Lqh III from *Leiurus quinquestriatus hebraeus*) (Bosmans et al., 2005; Brone et al., 2003; Goudet et al., 2001; He et al., 1999; Krimm et al., 1999). These toxins are active on mice by *i.c.v.* injection ($LD_{50} = 23\text{--}50\text{ ng}/20\text{ g mouse}$), but barely compete for ^{125}I -AaHII binding to rat brain synaptosomes (Gordon et al., 1996; Krimm et al., 1999; Sautiere et al., 1998; Vargas et al., 1987). Scorpion β -toxins bind to receptor Site 4 of the VGSC, located in the extracellular linkers of DII, thereby modifying the activation process of the channel (Cestele and Catterall, 2000; Cestele et al., 2001; Mejri et al., 2003). This group of toxins includes both mammalian and insect-selective toxins. Although α -toxins and β -toxins bind at two different sites on the VGSC, synergic effects were reported recently when both were co-injected into insects. Using radio-ligand binding assays, Cohen and co-workers found positive allosteric interactions between the excitatory toxin Bj-xtrIT, and the depressant toxins LqhIT2 and Lqh α IT (Cohen et al., 2006). Even a nontoxic mutant Bj-xtrIT-E15R was able to enhance Lqh α IT binding and toxicity similarly to the native Bj-xtrIT. The authors conclude that mere binding of a nontoxic ligand to receptor Site 4 induces a conformational change that does not alter channel gating, but influences toxin binding at receptor Site 3 leading to enhanced toxicity. Their finding suggests a new functional role for weakly toxic polypeptides in enhancing the effect of other active neurotoxins in arthropod venom.

2.2. Importance of scorpion toxins

Scorpion toxins have already proven to be important pharmacological tools for probing the structure of the VGSC and studying the activation and inactivation processes that are a fundamental trademark of VGSCs (e.g. Cestele et al., 2001). Another important application of scorpion toxins is based on their ability to discriminate between vertebrate and invertebrate channels. Insect-selective scorpion toxins could serve as templates for further development of novel insecticides (Arnon et al., 2005; Gordon, 1997; Zlotkin, 1999; Zlotkin et al., 2000). Since the intensive use of classical insecticides has led to the development of resistance in many insect species, projects to replace these chemical substances have already been undertaken. The lack of toxicity of AaHIT in mammals, for example, and its highly potent activity on insects has been used to reinforce the natural insecticidal ability of baculoviruses (Zlotkin et al., 2000). The insertion of the gene coding for AaHIT causes a higher killing speed of insects by the baculovirus (particularly against lepidopterans). More recent research in this field focused on the enhancement of the baculovirus' insecticidal efficacy with scorpion toxins that interact cooperatively (Regev et al., 2003). However, more work is needed before recombinant baculoviruses can be considered as an alternative or complement for the chemical insecticides that are used nowadays.

3. Sequences and structures of scorpion α -toxins

Up to now, hundreds of distinct peptides have been purified from ± 30 different species of scorpions (see Figure 1) (Possani et al., 1999; Possani et al., 2000). The purification process generally starts with chromatographic columns that separate venoms into peptide fractions based on molecular mass (size-exclusion gel filtration), followed by ion-exchange resins and finally reverse-phase columns. Currently, direct separation of peptides by HPLC (in combination with mass spectrometry), or use of HPLC directly after a molecular mass separation of soluble venoms is more common.

Predictions suggest that close to 100 000 distinct polypeptides are present in all known scorpion species (Possani et al., 1999). From this total, only 1% is currently known. Furthermore, for several of these peptides, a function has yet to be assigned. Lately, the amino acid sequence of VGSC toxins have been obtained by sequencing cDNA clones. These new sequences are not included in Figure 1, because of the uncertainty about the C-terminal sequence of mature peptides.

Structure-function studies only became possible after the purification of toxins on a large scale. Yet, scorpion venoms are not easy to collect and their venom quantity is generally low (as compared to snakes, for instance). Consequently, two techniques have contributed to overcome the scarcity and cost of natural toxins: recombinant expression and chemical synthesis. The insecticidal toxin Lqh α IT (Zilberberg et al., 1997; Zilberberg et al., 1996) and several mutants were produced by recombinant expression and used for identifying residues important for activity. The α -like toxin BmKM1 was expressed in yeast (Shao et al., 1999). Recombinant variants of this toxin were used in several extensive studies (Liu et al., 2005; Sun et al., 2003; Ye et al., 2005).

Chemical synthesis may be considered as an alternative to recombinant expression of more difficult toxins. This technique offers the possibility of making mutants with unusual residues. Successful chemical syntheses of short scorpion neurotoxins affecting voltage-gated potassium channels have been reported (Giangiacomo et al., 1993; Kharrat et al., 1996; Sabatier et al., 1994; Torres et al., 2003). However, chemical synthesis of long-chain toxins has proven to be more difficult. Only recently, the first successful chemical synthesis using a Fmoc strategy of Aah I, by M'Barek et al. (2004), was reported (M'Barek et al., 2004). The synthetic Aah I showed physical-chemical, biochemical, immunochemical and pharmacological properties similar to those of the natural toxin. In voltage-clamp experiments, Na_v1.2 inactivation was prevented by both the application of the synthetic toxin or of the natural toxin in a similar way.

In general, scorpion neurotoxins targeting VGSCs are single chain polypeptides composed of 60–76 amino acids cross-linked by four disulfide bridges which possess a highly conserved, dense core formed by an α -helix and two to three strands of β -sheet structural motifs, stabilized by the disulfide bridges. The scorpion toxin fold families are based on their β -sheet and α -helical structures and include the $\beta\alpha\beta\beta$ family (Housset et al., 1994) and the $\beta\alpha\alpha\beta\alpha$ family (Oren et al., 1998) which are all stabilized by the four disulfide bridges (Ali et al., 2006; Mouhat et al., 2004). To better understand the role of these disulfide bridges, Sun et al. (Sun et al., 2002) performed a mutagenesis analysis of BmK M1, from the scorpion *Buthus martensii* Karsch for the four disulfide bonds, 12–63, 16–36, 22–46 and 26–48. All cysteines were replaced by serines. Recombinant variants (C22S, C46S) and (C26S, C48S) were not expressed at all; (C16S, C36S) could be expressed at trace levels but was extremely unstable. (C12S, C63S) could be expressed in an amount comparable with that of unmodified BmK M1, but had no detectable bioactivities. The results indicated that among the four disulfide bonds, loss of either bridge C22 C46 or C26 C48 is critical for the general folding of the molecule. Bridge C16 C36 mainly contributes to the global stability of the folded scaffold, and bridge C12 C63 plays an essential role in the functional performance of scorpion toxins.

4. Electrophysiological aspects of scorpion α -toxins

The foremost effects of scorpion α -toxins are a remarkable slowing of fast inactivation of VGSCs and minor modifications of the voltage dependence of channel activation (Chen et al., 2002; Chen et al., 2005). Since these Site 3 toxins prevent a component of outward gating charge movement associated with channel inactivation (Sheets et al., 1999), it is likely that they are able to slow inactivation by preventing the outward movement of the DIV S4 segment, a conformational change necessary for fast inactivation (Cestele and Catterall, 2000). In this sense, scorpion α -toxins can be considered gating-modifier toxins like graminotoxin; (McDonough et al., 1997) and hanatoxin (Li-Smerin and Swartz, 1998; Swartz and MacKinnon, 1997; Winterfield and Swartz, 2000). A consequence of scorpion α -toxins in vivo is that they prolong the action potentials of excitable cells. As a consequence, these toxins can kill organisms by inducing paralysis and arrhythmia. However, the binding affinity of scorpion α -toxins to mammalian VGSCs is reduced by membrane depolarization and increased by alkaloid binding at Site 2 (Catterall, 1979; Catterall et al., 1992; Chen et al., 2000; Conti et al.,

1976; Strichartz et al., 1987). In 2001, Chen and co-workers studied the interaction of scorpion α -toxins Lqh II, Lqh III, and Lqh α IT with Na_v1.5 (Chen and Heinemann, 2001). As seen before, the toxins removed fast inactivation. However, association and dissociation rates of Lqh III were much slower than those of Lqh II and Lqh α IT, to the extent that Lqh III would not dissociate from the channel during a cardiac activation potential. Toxin dissociation remained voltage dependent even at high voltages. Slow inactivation of Na_v1.5 was significantly enhanced by Lqh II and Lqh III. The half-maximal voltage of steady-state slow inactivation was shifted to negative values, the voltage dependence was increased and slow inactivation at high voltages became more complete indicating that VGSC slow inactivation is directly modulated by scorpion α -toxins.

5. Molecular determinants of scorpion α -toxin selectivity

5.1. Focus on the toxin

The molecular dissection of the interaction between scorpion α -toxins and VGSCs has been a broadly studied theme in toxinology. Since the pioneering work of several researchers in the 60's and 70's, the availability of producing recombinant and/or synthetic toxins and the cloning and heterologous expression of almost all subtypes of VGSCs has caused major advancements in this field. As reader you will notice that a recurring theme in scorpion α -toxin research is AaH II, the mammalian-specific toxin from *Androctonus australis* Hector, on which most studies are based.

In 1976, Habersetzer-Rochat and Sampieri studied the chemical modification of some trifunctional amino acid residues in AaH I, II, and III of the scorpion *Androctonus australis* Hector (Habersetzer-Rochat and Sampieri, 1976). The results indicated that: (1) reduction and methylation of one disulfide bridge destroyed the toxic activity of AaH II; (2) the only tryptophan residue of AaH II (at position 38) is not included in the active site of the molecule; (3) modification of five carboxylates out of the seven contained in AaH II suppresses the toxic activity; (4) acetylation of the lysine and tyrosine residues leads to the loss of both toxic and antigenic activity; (5) citraconylation of AaH II and AaH III leads to complete loss of toxicity; decitraconylation restores full activity; (6) alkylation of AaH II by iodoacetic acid affects both amino groups and histidine residues. The loss of toxicity was mainly due to the modification of the lysine residues.

In 1986, five antibody populations selected for their specificity toward various regions of AaH II were used to probe the interaction of this toxin with its receptor site on the VGSC (el Ayeb et al., 1986). These studies indicated that two antigenic sites, one located around the disulfide bridge including residues 12–63 and one encompassing residues 50–59, are involved in the molecular mechanisms of toxicity neutralization. Fab fragments specific to the region around disulfide bridge 12–63 inhibited binding of the ¹²⁵I-labeled toxin to its receptor site. Furthermore, these two antigenic regions were inaccessible to their antibodies when the toxin is bound to its receptor site.

When the 3D structures of AaH II (Figure 2) and CsE V3 were elucidated about twenty years ago, the conserved hydrophobic surface (CHS) region, mainly including Tyr5, Tyr35 and Trp/Tyr47, was proposed to be responsible for the pharmacological effect of these toxins (Fontecilla-Camps et al., 1982; Fontecilla-Camps et al., 1980; Fontecilla-Camps et al., 1988). Though the CHS was found in all scorpion toxin structures known today, this assumption required experimental identification. The individual residues in this cluster (e.g. Trp38 in AaH II, Tyr49 in Lqh α IT) have been assessed by chemical modification (Kharrat et al., 1989) or mutagenesis analysis (Zilberberg et al., 1997; Zilberberg et al., 1996) and showed to play an important role in bioactivity. In 1989, Kharrat and co-workers suggested that aromatic residues belonging to the CHS, to the C-terminal and to the loop region 37–44 were involved in the

molecular mechanisms by which scorpion α -toxins act (Kharrat et al., 1989). Charged residues in the N-terminal and C-terminal also contribute to the high efficacy of the binding process (Figure 2). They stated that all important residues are clustered on one face of the toxin, suggesting a multipoint interaction with the VGSC. In 1990, they studied the contribution of arginine residues to the general toxicity of scorpion α -toxins by modifying these amino acids in AaH I and AaH II by phenylglyoxal or p-hydroxyphenylglyoxal (Kharrat et al., 1990). Their results furnished a more precise picture of those residues involved in the "toxic region", which appeared to be composed of residues belonging to the CHS and to the C-terminal and N-terminal sequences. Recently, the importance of these conserved aromatic residues in BmK M1 was re-investigated using site-directed mutagenesis in combination with tests on mice and cloned VGSCs expressed in *Xenopus laevis* oocytes (Sun et al., 2003). In this study, it was shown that Trp38 and Tyr42 are mostly involved in the functional performance of the toxin and that the side chains of residues Trp47, Tyr14 and Tyr35 are important for the structural stability of BmK M1. Tyr5 appears to be vital for the correct folding of the toxin. Tyr21, residing in the α -helix of the toxin, is putatively not a crucial determinant for the structure and pharmacological function of the toxin.

In 2004, Benkhadir et al. (Benkhadir et al., 2004), cloned and expressed the scorpion α -toxin BotIII and confirmed the critical role of the C-terminal region for its interaction with VGSCs. The importance of this region is reported in other studies as well (Gurevitz et al., 2001). In the study of Benkhadir et al. (2004), BotIII was studied together with AaH II which was expressed before. They came to the following conclusions: (a) the affinity of recombinant BotIII-OH and recombinant AaH II-OH (rBotIII-OH with R10V, V51L, N64H) for the VGSCs is reduced compared to the native toxins; (b) the single mutation N64H is responsible for the difference of toxicity and affinity between rBotIII-OH and rAaHII-OH and (c) the addition of the sequence GR to rBotIII-OH leads to the loss of biological activity. Remarkably, in this study it was reported that C-terminal carboxylation of AaH II decreases its potency on rat brain synaptosomes. However, in 2005, Legros et al. (Legros et al., 2005) expressed AaH II and some of its mutants in order to identify putative key bioactive elements. There it was stated that the lack of amidation on the C-terminal residue His64 does not affect the pharmacological activity of AaH II. On the contrary, the replacement of Lys58 by a hydrophobic (Val and Ile) or acidic (Glu) amino acid residue immediately led to an inactive analogue leading to the conclusion that this position is imperative for the pharmacological function of this toxin family.

The importance of the C-terminal region for the target specificity of BmK M1 was also reported in another study in 2005 (Liu et al., 2005). Here, it was stated that there are three epitopes that determine the VGSC specificity of this toxin: 1) the first three N-terminal residues, 2) the five-residue-turn (residues 8–12) in combination with the C-tail (residues 57–61), and 3) the loop between the β_2 and β_3 sheet including the adjacent Gly in the β_3 sheet (residues 40–43). These three domains were in fact a fine-tuning of a previously reported hypothesis of Karbat et al. (Karbat et al., 2004). Here, the molecular basis of the high insecticidal potency of scorpion α -toxins was investigated by extensive mutagenesis and X-ray crystallography (see also (Zilberberg et al., 1996)) of the insect scorpion α -toxin Lqh α IT (importance of Tyr10, Phe17, Lys8, Arg18, Lys62 Arg64 and the structural reconfiguration of the C-tail). Consequently, two distinct domains of this toxin were transferred to AaH II (mammal-specific) resulting in insect activity of this toxin. These two domains consist of a conserved 'Core domain' formed by four to five residues located on the short loops connecting the conserved secondary structure elements of the molecule core, and the five-residue-turn with the C-terminal segment (residues 56–64) which forms the 'NC-domain'. Furthermore, the high insecticidal potency of Lqh α IT was suggested to be associated with the protruding conformation of the 'NC domain', which is observed in most known scorpion α -toxins active on insects (Guan et al., 2004; Karbat et al., 2004). This geometry is correlated with a non-proline cis peptide bond between residues 9 and 10 in the five-residue turn of these toxins. A different, flat geometry of the 'NC-domain' was

seen in the crystal structures of the classical α -toxins, AaH II and BmKM8, in which the peptide bond between residues 9 and 10 is in trans conformation (Fontecilla-Camps et al., 1988; He et al., 1999). In agreement, the crystal structure of a toxin chimera, AaH II^{Lqh α IT(face)}, in which the 'NC-domain' of Lqh α IT was placed on the AaH II scaffold, revealed a protruding conformation of the 'NC-domain'. This chimera was weakly active on Na_v1.2a but was much more active on insect VGSCs (Karbat et al., 2004). Bearing the importance of this domain in mind, the role of six critical amino acids located in the five-residue reverse turn (RT) and C-tail (CT) of the scorpion α -like toxin BmK M1 were further dissected by Ye et al. (2005). The residues were individually substituted and subjected to a bioassay on mice, an electrophysiological characterization on three cloned voltage-gated Na⁺ channels (Na_v1.2a, Na_v1.5 and para), and an X-ray crystallographic investigation. The results revealed two molecular sites, a couple of residues (8–9) in the RT and a hydrophobic surface consisting of residues 57 and 59–61 in the CT, where substitution with specific residues could redirect the α -like characteristics of BmK M1 to either total insect or much higher mammal specificity. Crystal structures revealed that the pharmacological ramification of these mutants is accompanied by the reshaping of the 3D structure surrounding position 8. Furthermore, this study also revealed that residues 57 and 59–61, located at the CT, enclose the critical residue 58 in order to form a hydrophobic 'gasket'. Mutants of BmK M1 that interrupt this hydrophobic surface significantly gain insect-selectivity.

Another molecular determinant for scorpion α -toxin selectivity between VGSC subtypes was reported in 2003 by Alami and co-workers (Alami et al., 2003). They screened the venom of the scorpion *Androctonus mauretanicus* mauretanicus by use of a specific serum directed against AaH II, with the aim of identifying new analogues. This led to the isolation of Amm VIII (7382.57 Da), but this toxin was totally devoid of toxicity when injected subcutaneously into mice. Voltage-clamp experiments revealed a preference for Na_v1.2a over Na_v1.4. Amm VIII shows 87% sequence identity with AaH II, but carries an unusual extension at its C-terminal end, consisting of an additional Asp due to a point mutation in the cDNA penultimate codon. The authors hypothesized that this extra amino acid residue could induce steric hindrance and dramatically reduce recognition of the target by Amm VIII. Molecular modelling showed that this C-terminal extension does not lead to an overall conformational change in Amm VIII, but drastically modifies the charge repartition and, consequently, the electrostatic dipole moment of the molecule which could explain the discrimination between different VGSC subtypes.

5.2. Focus on Site 3

Already in 1978, Catterall indicated that scorpion α -toxins and sea anemone toxins share a common receptor site associated with action potential sodium ionophores (Catterall and Beress, 1978) (Figure 3). In the same year, Couraud and co-workers labeled AaH II with ¹²⁵I and showed that this neurotoxin binds specifically to a single class of non interacting binding sites of electrically excitable neuroblastoma cells (Couraud et al., 1978). The sea anemone toxin ATX II was shown to modify ¹²⁵I-labeled AaH II binding to neuroblastoma cells by increasing the apparent K_D for labeled scorpion toxin without modification of the number of binding sites. Therefore, the authors concluded that both AaH II and ATX II interact competitively with a regulatory component of the VGSC.

Several years later, Thomson and Catterall suggested that the extracellular loops between segments S5 and S6 of DI and DIV comprised at least part of the scorpion α -toxin receptor site (also studied in (Tejedor and Catterall, 1988)) by evaluating the effect of antibodies on voltage-dependent binding of radiolabeled toxin isolated from *Leiurus quinquestriatus* to both reconstituted Na_v1.2a and rat brain synaptosomes (Thomsen and Catterall, 1989). They also suggested a VGSC membrane topology model in which DI and DIV are adjacent in the tertiary

structure of the channel protein and six transmembrane sequences are contained in each of the four homologous domains.

In 1996, Rogers and co-workers converted extracellular acidic amino acids in DI and DIV of 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 (ATXII) indicating their common binding site. In addition, chimeric VGSCs in which amino acid residues at the extracellular end of S3 in DIV of Na_v1.5 were substituted into the Na_v1.2 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 VGSCs. Other research groups, focusing on finding residues important for scorpion α -toxin binding, have highlighted residues in this loop region in DIV too (Leipold et al., 2004). Chen and co-workers tested Lqh2 and Lqh3 on rat(r) Na_v1.2 and human(h) Na_v1.7 (Chen et al., 2000). At Site 3, rNa_v1.2 and hNa_v1.7 channels differ only in two residues (E/D and K/T). To examine the importance of these substitutions for toxin binding, they constructed a mutant of rNa_v1.2, E1613D/K1617T, in which receptor Site 3 became identical to that of hNa_v1.7. This double-site mutation conferred sensitivity of rNa_v1.2 channels towards Lqh3 whilst the sensitivity to Lqh2 was not modified and did follow that of hNa_v1.7. The authors hypothesize that this result may indicate that other parts of the channel protein, such as the S5-6 linker of domain 1 (Rogers et al., 1996) could be particularly important for Lqh2 binding.

In 2000, Gilles and co-workers also studied Lqh3 but from a different perspective, namely that this toxin binds with higher affinity to receptor Site 3 on the insect VGSCs but does not bind to rat brain synaptosomes (Na_v1.2) (Gilles et al., 2000). Radioiodination of Tyr14 in Lqh3 abolished its binding to locust but not cockroach VGSCs. In contrast, radioiodination of Tyr10 or Tyr21 in the structurally similar α -toxin Lqh α IT, as well as their substitution by phenylalanine, had only minor effects on binding to cockroach neuronal membranes. However, substitution of Tyr21 but not Tyr14 by leucine decreased the binding affinity of Lqh α IT severely. These results highlight subtle architectural variations between locust and cockroach receptor Site 3, in addition to previous results demonstrating the competence of Lqh3 to differentiate between insect and mammalian VGSC subtypes.

In 2004, Leipold and co-workers found that rNa_v1.4 was equally sensitive to Lqh2, Lqh α IT, and Lqh3 (Leipold et al., 2004). For this reason, they used this VGSC for studying the effects of substitutions based on the sequences of other channel subtypes. By transferring the linker of DIV S3–S4 from Na_v1.2 and Na_v1.3 to Na_v1.4, they abolished the sensitivity to Lqh3 and to Lqh α IT, but not to Lqh2. Substitution with the DIV S3–S4 linker from Na_v1.6 had only a minor effect on the activity of all toxins. Remarkably, the same linker from hNa_v1.7 significantly reduced the Lqh α IT effect and to a lesser extent that of Lqh2. Surprisingly, this substitution had no effect on Lqh3 activity. When individually studying the residues of these loops, the authors found that Asp1428 in Na_v1.4 (equivalent to Glu1613 in rNa_v1.2a), was critical. A conserved substitution, D1428E, greatly decreased the effects of Lqh3 and Lqh α IT, while the effect of Lqh2 persisted. Furthermore, the potency of Lqh α IT on Na_v1.4 strongly decreased when both Asp1428 and Lys1432 in Na_v1.4 were substituted, whereas the affinity of Lqh2 was only moderately affected. Recent work by the same authors also implicated the importance of the conserved hydrophobic residues Tyr1433, Phe1434, and Val1435 in DIV S3–S4 of rNa_v1.4 for the activity of Lqh2 but not Lqh3 (Leipold et al., 2005).

Scorpion α -like toxins target both mammalian VGSCs and insect VGSCs but do not bind to rat brain synaptosomes (mostly $\text{Na}_v1.2$). Zuo and Ji (2004) looked deeper into this interesting feature by comparing a large amount of structural and functional data (Zuo and Ji, 2004). By comparing sequences they noticed that an important anionic residue (Asp) for α -toxins is present in the insect VGSC and $\text{rNa}_v1.4$, 1.5 and 1.6 (Figure 3). Consequently, this residue could not be the only determinant of the binding of insect α -toxins. Further inspection of this region revealed that the consecutive residues in insect VGSCs are Leu-Val-Leu-Ser, Leu-Ala-Leu-Ser in the sensitive mammalian isoform $\text{rNa}_v1.4$, and Thr-Val-Leu-Ser in $\text{rNa}_v1.5$. However, these residues correspond to Met-Phe-Leu-Ala in the insensitive isoforms $\text{rNa}_v1.1$ –1.3 and $\text{rNa}_v1.6$. Consequently, the authors conclude that receptor sites for scorpion α -like toxins and insect α -toxins are not identical but do overlap. More evidence for this statement was found in the sequence comparison of $\text{rNa}_v1.7$ and $\text{hNa}_v1.7$ which reveals that these channels are almost identical in DIV S3–S4, with the exception of a Glu that is critical for scorpion α -toxin binding (Asp in $\text{hNa}_v1.7$). Yet, according to mutagenesis experiments on $\text{rNa}_v1.2$, this distinction does not determine its sensitivity to mammalian α -toxins. Therefore, the authors conclude that the sequence variation in DIV S3–S4 alone is not adequate to explain selective recognition of mammal-selective α -toxins. The molecular basis of the selectivity of mammal α -toxins possibly involves additional external loops like S5–S6 loops of DI and DIV, as suggested previously (Catterall and Beress, 1978).

In a league of its own, $\text{Na}_v1.8$ has puzzled researchers for years. This intriguing VGSC seems to be insensitive to all tested scorpion peptide toxins. However, some spider toxins have been reported to act on this channel (Bosmans et al., 2006; Middleton et al., 2002). In order to find out more about this phenomenon, Saab and co-workers tested the effects of the scorpion venom from *Leiurus quinquestriatus* (LqTx) on the TTX-resistant VGSC $\text{Na}_v1.8$ in sensory neurons (Saab et al., 2002). As expected, $\text{Na}_v1.8$ current was found to be resistant whereas LqTx slowed inactivation of the current generated by $\text{Na}_v1.4$ and induced a persistent current. Sequence analysis reveals that the DIV S3–S4 linker is longer in $\text{Na}_v1.8$ than in $\text{Na}_v1.4$ by four amino acids: Ser, Leu, Glu, and Asp (SLEN) (Figure 3). $\text{Na}_v1.4$ -SLEN, a chimera construct carrying SLEN at the analogous position in the DIV S3–S4 linker, was also found to be resistant to LqTx. Therefore, the authors concluded that the tetrapeptide SLEN at the DIV S3–S4 linker region is sufficient to make $\text{Na}_v1.8$ resistant to LqTx. A reverse chimera, however, was not constructed.

Finally, it has been shown recently that toxins which modulate the gating properties of voltage-gated ion channels are able to bind to phospholipids membranes, leading to the suggestion that these toxins are able to access a channel binding site that remains membrane restricted. Based on this information, Smith et al. (Smith et al., 2005) examined the ability of AP-B, a sea anemone toxin that binds to Site 3, to bind to liposomes. The authors also looked into the activity of the structurally unrelated Site 3 toxins LqqV. They concluded that toxins that modify inactivation kinetics via binding to Site 3 lack the ability to bind phospholipids.

In summary, these results all support the view that residues in the S3–S4 loop of DIV are involved in the differential preference of scorpion α -toxins towards VGSCs. However, they also imply that diverse classes of α -toxins interact in a different manner with this channel region, depending on the bioactive surface of each toxin (Leipold et al., 2005; Leipold et al., 2004). Moreover, other parts of the channel may also be involved. This assumption is supported by the low affinity of Lqh2 for insect VGSCs in which DIV S3–S4 is almost identical in its mammalian counterpart, $\text{Na}_v1.6$, which is a high affinity target of Lqh2. Furthermore, structural flexibility issues of scorpion α -toxins are also likely to contribute to VGSC subtype selectivity (Krimm et al., 1999; Tugarinov et al., 1997). Critical structural changes in the receptors caused by individual residue replacement (e.g., (Leipold et al., 2004)) could evoke

various sorts of steric hindrance for toxin binding. Depending on the flexibility of the toxin to adapt to the changed receptor, differences in selectivity could be explained.

Acknowledgements

This work was supported in part by grants G.0330.06 (F.W.O.-Vlaanderen) and OT-05-64 (K.U.Leuven). F.B. is a postdoctoral fellow in the National Institutes of Health (NIH) – Research Foundation – Flanders (FWO) Research Career Transition Award Program and is an honorary Fellow of the Belgian American Educational Foundation.

References

- Agnew WS. Voltage-regulated sodium channel molecules. *Annu Rev Physiol* 1984;46:517–530. [PubMed: 6324661]
- Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature* 1996;379:257–262. [PubMed: 8538791]
- Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, et al. The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. *Nat Neurosci* 1999;2:541–548. [PubMed: 10448219]
- Alami M, Vacher H, Bosmans F, Devaux C, Rosso JP, Bougis PE, Tytgat J, Darbon H, Martin-Eauclaire MF. Characterization of Amm VIII from *Androctonus mauretanicus mauretanicus*: a new scorpion toxin that discriminates between neuronal and skeletal sodium channels. *Biochem J* 2003;375:551–560. [PubMed: 12911331]
- Ali SA, Wang B, Alam M, Beck A, Stoeva S, Voelter W, Abbasi A, Duszenko M. Structure-activity relationship of an alpha-toxin Bs-Tx28 from scorpion (*Buthus indicus*) venom suggests a new alpha-toxin subfamily. *Arch Biochem Biophys* 2006;445:81–94. [PubMed: 16309623]
- Alsen C, Harris JB, Tesseraux I. Mechanical and electrophysiological effects of sea anemone (*Anemonia sulcata*) toxins on rat innervated and denervated skeletal muscle. *Br J Pharmacol* 1981;74:61–71. [PubMed: 6115695]
- Arnon T, Potikha T, Sher D, Elazar M, Mao W, Tal T, Bosmans F, Tytgat J, Ben-Arie N, Zlotkin E. BjalphIT: a novel scorpion alpha-toxin selective for insects--unique pharmacological tool. *Insect Biochem Mol Biol* 2005;35:187–195. [PubMed: 15705498]
- Baker MD, Wood JN. Involvement of Na⁺ channels in pain pathways. *Trends Pharmacol Sci* 2001;22:27–31. [PubMed: 11165669]
- Benkhadir K, Kharrat R, Cestele S, Mosbah A, Rochat H, El Ayeb M, Karoui H. Molecular cloning and functional expression of the alpha-scorpion toxin BotIII: pivotal role of the C-terminal region for its interaction with voltage-dependent sodium channels. *Peptides* 2004;25:151–161. [PubMed: 15062995]
- Benzinger GR, Drum CL, Chen LQ, Kallen RG, Hanck DA, Hanck D. Differences in the binding sites of two site-3 sodium channel toxins. *Pflugers Arch* 1997;434:742–749. [PubMed: 9306007]
- Bosmans F, Brone B, Sun YM, Zhu RH, Xiong YM, Wang DC, Van Kerkhove E, Tytgat J. Pharmacological comparison of two different insect models using the scorpion alpha-like toxin BmK M1 from *Buthus martensii* Karsch. *Protein Pept Lett* 2005;12:363–367. [PubMed: 15907182]
- Bosmans F, Rash L, Zhu S, Diochot S, Lazdunski M, Escoubas P, Tytgat J. Four novel tarantula toxins as selective modulators of voltage-gated sodium channel subtypes. *Mol Pharmacol* 2006;69:419–429. [PubMed: 16267209]
- Brone B, Tytgat J, Wang DC, Van Kerkhove E. Characterization of Na(+) currents in isolated dorsal unpaired median neurons of *Locusta migratoria* and effect of the alpha-like scorpion toxin BmK M1. *J Insect Physiol* 2003;49:171–182. [PubMed: 12770010]
- Cannon SC. Voltage-gated ion channelopathies of the nervous system. *Clin Neurosci Res* 2001;1:104–117.
- Catterall WA. Binding of scorpion toxin to receptor sites associated with sodium channels in frog muscle. Correlation of voltage-dependent binding with activation. *J Gen Physiol* 1979;74:375–391. [PubMed: 479827]
- Catterall WA, Beress L. Sea anemone toxin and scorpion toxin share a common receptor site associated with the action potential sodium ionophore. *J Biol Chem* 1978;253:7393–7396. [PubMed: 29897]

- Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev* 2005;57:397–409. [PubMed: 16382098]
- Catterall WA, Trainer V, Baden DG. Molecular properties of the sodium channel: a receptor for multiple neurotoxins. *Bull Soc Pathol Exot* 1992;85:481–485. [PubMed: 1340350]
- Cestele S, Ben Khalifa RB, Pelhate M, Rochat H, Gordon D. Alpha-scorpion toxins binding on rat brain and insect sodium channels reveal divergent allosteric modulations by brevetoxin and veratridine. *J Biol Chem* 1995;270:15153–15161. [PubMed: 7797499]
- Cestele S, Catterall WA. Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. *Biochimie* 2000;82:883–892. [PubMed: 11086218]
- Cestele S, Gordon D, Kopeyan C, Rochat H. Toxin III from *Leiurus quinquestriatus quinquestriatus*: a specific probe for receptor site 3 on insect sodium channels. *Insect Biochem Mol Biol* 1997;27:523–528. [PubMed: 9304794]
- Cestele S, Scheuer T, Mantegazza M, Rochat H, Catterall WA. 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* 2001;118:291–302. [PubMed: 11524459]
- Chai Z, Zhu M, Bai Z, Liu T, Tan M, Pang X, Ji Y. BmK alphaIV, a novel modulator of sodium channels: from genomic organization to functional analysis. *Biochem J*. 2006(epub ahead of print)
- Chen H, Gordon D, Heinemann SH. Modulation of cloned skeletal muscle sodium channels by the scorpion toxins Lqh II, Lqh III, and Lqh alphaIT. *Pflugers Arch* 2000;439:423–432. [PubMed: 10678738]
- Chen H, Heinemann SH. Interaction of scorpion alpha-toxins with cardiac sodium channels: binding properties and enhancement of slow inactivation. *J Gen Physiol* 2001;117:505–518. [PubMed: 11382802]
- Chen H, Lu S, Leipold E, Gordon D, Hansel A, Heinemann SH. Differential sensitivity of sodium channels from the central and peripheral nervous system to the scorpion toxins Lqh-2 and Lqh-3. *Eur J Neurosci* 2002;16:767–770. [PubMed: 12270053]
- Chen J, Tan ZY, Zhao R, Feng XH, Shi J, Ji YH. The modulation effects of BmK I, an alpha-like scorpion neurotoxin, on voltage-gated Na(+) currents in rat dorsal root ganglion neurons. *Neurosci Lett* 2005;390:66–71. [PubMed: 16129561]
- Chen T, Folan R, Kwok H, O'Kane EJ, Bjourson AJ, Shaw C. Isolation of scorpion (*Androctonus amoreuxi*) putative alpha neurotoxins and parallel cloning of their respective cDNAs from a single sample of venom. *Regul Pept* 2003;115:115–121. [PubMed: 12972326]
- Cohen L, Lipstein N, Gordon D. Allosteric interactions between scorpion toxin receptor sites on voltage-gated Na channels imply a novel role for weakly active components in arthropod venom. *FASEB J*. 2006(epub ahead of print)
- Conti F, Hille B, Neumcke B, Nonner W, Stampfli R. Conductance of the sodium channel in myelinated nerve fibres with modified sodium inactivation. *J Physiol* 1976;262:729–742. [PubMed: 1087644]
- Corzo G, Escoubas P, Villegas E, Karbat I, Gordon D, Gurevitz M, Nakajima T, Gilles N. A Spider Toxin That Induces a Typical Effect of Scorpion alpha-Toxins but Competes with beta-Toxins on Binding to Insect Sodium Channels. *Biochemistry* 2005;44:1542–1549. [PubMed: 15683238]
- Corzo G, Villegas E, Nakajima T. Isolation and structural characterization of a peptide from the venom of scorpion with toxicity towards invertebrates and vertebrates. *protein and peptide letters* 2001;8:385–393.
- Couraud F, Jover E, Dubois JM, Rochat H. Two types of scorpion receptor sites, one related to the activation, the other to the inactivation of the action potential sodium channel. *Toxicon* 1982;20:9–16. [PubMed: 6281941]
- Couraud F, Jover E, Dubois JM, Rochat H. Two types of scorpion toxin receptor sites, one related to the activation, the other to the inactivation of the action potential sodium channel. *Toxicon* 1986;20:9–13. [PubMed: 6281941]
- Couraud F, Rochat H, Lissitzky S. Binding of scorpion and sea anemone neurotoxins to a common site related to the action potential Na+ ionophore in neuroblastoma cells. *Biochem Biophys Res Commun* 1978;83:1525–1530. [PubMed: 29635]

- del Rio-Portilla F, Hernandez-Marin E, Pimienta G, Coronas FV, Zamudio FZ, Rodriguez de la Vega RC, Wanke E, Possani LD. NMR solution structure of Cn12, a novel peptide from the Mexican scorpion *Centruroides noxius* with a typical beta-toxin sequence but with alpha-like physiological activity. *Eur J Biochem* 2004;271:2504–2516. [PubMed: 15182366]
- Denac H, Mevissen M, Scholtysik G. Structure, function and pharmacology of voltage-gated sodium channels. *Naunyn Schmiedebergs Arch Pharmacol* 2000;362:453–479. [PubMed: 11138838]
- el Ayeb M, Bahraoui EM, Granier C, Rochat H. Use of antibodies specific to defined regions of scorpion alpha-toxin to study its interaction with its receptor site on the sodium channel. *Biochemistry* 1986;25:6671–6678. [PubMed: 2431711]
- Feng G, Deak P, Chopra M, Hall LM. Cloning and functional analysis of TipE, a novel membrane protein that enhances *Drosophila* para sodium channel function. *Cell* 1995;82:1001–1011. [PubMed: 7553842]
- Fet, V.; Sissom, DW.; Lowe, G.; Braunwalder, ME. Catalog of the scorpions of the world (1758–1998). The New York Entomological Society; 2000.
- Fontecilla-Camps JC, Almassy RJ, Suddath FL, Bugg CE. The three-dimensional structure of scorpion neurotoxins. *Toxicon* 1982;20:1–7. [PubMed: 7080025]
- Fontecilla-Camps JC, Almassy RJ, Suddath FL, Watt DD, Bugg CE. Three-dimensional structure of a protein from scorpion venom: a new structural class of neurotoxins. *Proc Natl Acad Sci U S A* 1980;77:6496–6500. [PubMed: 6256740]
- Fontecilla-Camps JC, Habersetzer-Rochat C, Rochat H. Orthorhombic crystals and three-dimensional structure of the potent toxin II from the scorpion *Androctonus australis* Hector. *Proc Natl Acad Sci U S A* 1988;85:7443–7447. [PubMed: 3174645]
- Giangiacomo KM, Sugg EE, Garcia-Calvo M, Leonard RJ, McManus OB, Kaczorowski GJ, Garcia ML. Synthetic charybdotoxin-iberiotoxin chimeric peptides define toxin binding sites on calcium-activated and voltage-dependent potassium channels. *Biochemistry* 1993;32:2363–2370. [PubMed: 7680230]
- Gilles N, Gurevitz M, Gordon D. Allosteric interactions among pyrethroid, brevetoxin, and scorpion toxin receptors on insect sodium channels raise an alternative approach for insect control. *FEBS Lett* 2003;540:81–85. [PubMed: 12681487]
- Gilles N, Harrison G, Karbat I, Gurevitz M, Nicholson GM, Gordon D. 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* 2002;269:1500–1510. [PubMed: 11874465]
- Gilles N, Krimm I, Bouet F, Froy O, Gurevitz M, Lancelin JM, Gordon D. Structural implications on the interaction of scorpion alpha-like toxins with the sodium channel receptor site inferred from toxin iodination and pH-dependent binding. *J Neurochem* 2000;75:1735–1745. [PubMed: 10987857]
- Goldin AL. Resurgence of sodium channel research. *Annu Rev Physiol* 2001;63:871–894. [PubMed: 11181979]
- Goldin AL. Mechanisms of sodium channel inactivation. *Curr Opin Neurobiol* 2003;13:284–290. [PubMed: 12850212]
- Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, et al. Nomenclature of voltage-gated sodium channels. *Neuron* 2000;28:365–368. [PubMed: 11144347]
- Gong J, Kini RM, Gwee MC, Gopalakrishnakone P, Chung MC. Makatoxin I, a novel toxin isolated from the venom of the scorpion *Buthus martensi* Karsch, exhibits nitrenergic actions. *J Biol Chem* 1997;272:8320–8324. [PubMed: 9079654]
- Gordon D. A new approach to insect-pest control—combination of neurotoxins interacting with voltage sensitive sodium channels to increase selectivity and specificity. *Invert Neurosci* 1997;3:103–116. [PubMed: 9783437]
- Gordon D, Martin-Eauclaire MF, Cestele S, Kopeyan C, Carlier E, Khalifa RB, Pelhate M, Rochat H. Scorpion toxins affecting sodium current inactivation bind to distinct homologous receptor sites on rat brain and insect sodium channels. *J Biol Chem* 1996;271:8034–8045. [PubMed: 8626486]
- Gordon D, Zlotkin E. Binding of an alpha scorpion toxin to insect sodium channels is not dependent on membrane potential. *FEBS Lett* 1993;315:125–128. [PubMed: 8380269]

- Goudet C, Chi CW, Tytgat J. An overview of toxins and genes from the venom of the Asian scorpion *Buthus martensi* Karsch. *Toxicon* 2002;40:1239–1258. [PubMed: 12220709]
- Goudet C, Huys I, Clynen E, Schoofs L, Wang DC, Waelkens E, Tytgat J. Electrophysiological characterization of BmK M1, an alpha-like toxin from *Buthus martensi* Karsch venom. *FEBS Lett* 2001;495:61–65. [PubMed: 11322948]
- Grolleau F, Stankiewicz M, Birinyi-Strachan L, Wang XH, Nicholson GM, Pelhate M, Lapied B. Electrophysiological analysis of the neurotoxic action of a funnel-web spider toxin, delta-atracotoxin-HV1a, on insect voltage-gated Na⁺ channels. *J Exp Biol* 2001;204:711–721. [PubMed: 11171353]
- Guan RJ, Xiang Y, He XL, Wang CG, Wang M, Zhang Y, Sundberg EJ, Wang DC. 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* 2004;341:1189–1204. [PubMed: 15321715]
- Gurevitz M, Gordon D, Ben-Natan S, Turkov M, Froy O. Diversification of neurotoxins by C-tail 'wiggling': a scorpion recipe for survival. *Faseb J* 2001;15:1201–1205. [PubMed: 11344088]
- Guy HR, Seetharamulu P. Molecular model of the action potential sodium channel. *Proc Natl Acad Sci U S A* 1986;83:508–512. [PubMed: 2417247]
- Habersetzer-Rochat C, Sampieri F. Structure-function relationships of scorpion neurotoxins. *Biochemistry* 1976;15:2254–2261. [PubMed: 819030]
- Hains BC, Klein JP, Saab CY, Craner MJ, Black JA, Waxman SG. Upregulation of sodium channel Nav1.3 and functional involvement in neuronal hyperexcitability associated with central neuropathic pain after spinal cord injury. *J Neurosci* 2003;23:8881–8892. [PubMed: 14523090]
- Hamon A, Gilles N, Sautiere P, Martinage A, Kopeyan C, Ulens C, Tytgat J, Lancelin JM, Gordon D. Characterization of scorpion alpha-like toxin group using two new toxins from the scorpion *Leiurus quinquestriatus hebraeus*. *Eur J Biochem* 2002;269:3920–3933. [PubMed: 12180969]
- He XL, Li HM, Zeng ZH, Liu XQ, Wang M, Wang DC. Crystal structures of two alpha-like scorpion toxins: non-proline cis peptide bonds and implications for new binding site selectivity on the sodium channel. *J Mol Biol* 1999;292:125–135. [PubMed: 10493862]
- Hodgkin AL, Huxley AF. Propagation of electrical signals along giant nerve fibers. *Proc R Soc Lond B Biol Sci* 1952;140:177–183. [PubMed: 13003922]
- Housset D, Habersetzer-Rochat C, Astier JP, Fontecilla-Camps JC. Crystal structure of toxin II from the scorpion *Androctonus australis Hector* refined at 1.3 Å resolution. *J Mol Biol* 1994;238:88–103. [PubMed: 8145259]
- Inceoglu AB, Kamita SG, Hinton AC, Huang Q, Severson TF, Kang K, Hammock BD. Recombinant baculoviruses for insect control. *Pest Manag Sci* 2001;57:981–987. [PubMed: 11695193]
- Jalali A, Bosmans F, Amininasab M, Clynen E, Cuypers E, Zaremirakabadi A, Sarbolouki MN, Schoofs L, Vatanpour H, Tytgat J. OD1, the first toxin isolated from the venom of the scorpion *Odonthobuthus doriae* active on voltage-gated Na⁺ channels. *FEBS Lett* 2005;579:4181–4186. [PubMed: 16038905]
- Jover E, Martin-Moutot N, Couraud F, Rochat H. Scorpion toxin: specific binding to rat synaptosomes. *Biochem Biophys Res Commun* 1978;85:377–382. [PubMed: 33674]
- Julius D, Basbaum AI. Molecular mechanisms of nociception. *Nature* 2001;413:203–210. [PubMed: 11557989]
- Karbat I, Frolow F, Froy O, Gilles N, Cohen L, Turkov M, Gordon D, Gurevitz M. Molecular basis of the high insecticidal potency of scorpion alpha-toxins. *J Biol Chem* 2004;279:31679–31686. [PubMed: 15133045]
- Kharrat R, Darbon H, Granier C, Rochat H. Structure-activity relationships of scorpion alpha-neurotoxins: contribution of arginine residues. *Toxicon* 1990;28:509–523. [PubMed: 2389252]
- Kharrat R, Darbon H, Rochat H, Granier C. Structure/activity relationships of scorpion alpha-toxins. Multiple residues contribute to the interaction with receptors. *Eur J Biochem* 1989;181:381–390. [PubMed: 2714290]
- Kharrat R, Mabrouk K, Crest M, Darbon H, Oughideni R, Martin-Eauclaire MF, Jacquet G, el Ayeb M, Van Rietschoten J, Rochat H, Sabatier JM. Chemical synthesis and characterization of maurotoxin, a short scorpion toxin with four disulfide bridges that acts on K⁺ channels. *Eur J Biochem* 1996;242:491–498. [PubMed: 9022673]

- Kontis KJ, Goldin AL. Sodium channel inactivation is altered by substitution of voltage sensor positive charges. *J Gen Physiol* 1997;110:403–413. [PubMed: 9379172]
- Krimm I, Gilles N, Sautiere P, Stankiewicz M, Pelhate M, Gordon D, Lancelin JM. NMR structures and activity of a novel alpha-like toxin from the scorpion *Leiurus quinquestriatus hebraeus*. *J Mol Biol* 1999;285:1749–1763. [PubMed: 9917409]
- Legros C, Ceard B, Vacher H, Marchot P, Bougis PE, Martin-Eauclaire MF. Expression of the standard scorpion alpha-toxin AaH II and AaH II mutants leading to the identification of some key bioactive elements. *Biochim Biophys Acta*. 2005
- Leipold E, Hansel A, Olivera BM, Terlau H, Heinemann SH. Molecular interaction of delta-conotoxins with voltage-gated sodium channels. *FEBS Lett* 2005;579:3881–3884. [PubMed: 15990094]
- Leipold E, Lu S, Gordon D, Hansel A, Heinemann SH. Combinatorial interaction of scorpion toxins Lqh-2, Lqh-3, and LqhalphaIT with sodium channel receptor sites-3. *Mol Pharmacol* 2004;65:685–691. [PubMed: 14978247]
- Li-Smerin Y, Swartz KJ. Gating modifier toxins reveal a conserved structural motif in voltage-gated Ca²⁺ and K⁺ channels. *Proc Natl Acad Sci U S A* 1998;95:8585–8589. [PubMed: 9671721]
- Li HM, Wang DC, Zeng ZH, Jin L, Hu RQ. Crystal structure of an acidic neurotoxin from scorpion *Buthus martensii* Karsch at 1.85 Å resolution. *J Mol Biol* 1996;261:415–431. [PubMed: 8780783]
- Liu LH, Bosmans F, Maertens C, Zhu RH, Wang DC, Tytgat J. Molecular basis of the mammalian potency of the scorpion alpha-like toxin, BmK MI. *Faseb J* 2005;19:594–596. [PubMed: 15677695]
- M'Barek S, Fajloun Z, Cestele S, Devaux C, Mansuelle P, Mosbah A, Jouirou B, Mantegazza M, Van Rietschoten J, El Ayeb M, et al. First chemical synthesis of a scorpion alpha-toxin affecting sodium channels: the Aah I toxin of *Androctonus australis hector*. *J Pept Sci* 2004;10:666–677. [PubMed: 15568681]
- Martin-Eauclaire, MF.; Couraud, F. Scorpion neurotoxins: Effects and mechanisms. In: Chang, LW.; Dyer, RS., editors. *Handbook of Neurotoxicology*. Marcel Dekker; NY, NY: 1995. p. 683-716.
- McDonough SI, Lampe RA, Keith RA, Bean BP. Voltage-dependent inhibition of N- and P-type calcium channels by the peptide toxin omega-grammotoxin-SIA. *Mol Pharmacol* 1997;52:1095–1104. [PubMed: 9415720]
- Mejri T, Borchani L, Srairi-Abid N, Benkhalifa R, Cestele S, Regaya I, Karoui H, Pelhate M, Rochat H, El Ayeb M. BotIT6: a potent depressant insect toxin from *Buthus occitanus tunetanus* venom. *Toxicon* 2003;41:163–171. [PubMed: 12565735]
- Middleton RE, Warren VA, Kraus RL, Hwang JC, Liu CJ, Dai G, Brochu RM, Kohler MG, Gao YD, Garsky VM, et al. Two tarantula peptides inhibit activation of multiple sodium channels. *Biochemistry* 2002;41:14734–14747. [PubMed: 12475222]
- Miller JA, Agnew WS, Levinson SR. Principal glycopeptide of the tetrodotoxin/saxitoxin binding protein from *Electrophorus electricus*: isolation and partial chemical and physical characterization. *Biochemistry* 1983;22:462–470. [PubMed: 6186277]
- Mouhat S, Jouirou B, Mosbah A, De Waard M, Sabatier JM. Diversity of folds in animal toxins acting on ion channels. *Biochem J* 2004;378:717–726. [PubMed: 14674883]
- Noda M, Shimizu S, Tanabe T, Takai T, Kayano T, Ikeda T, Takahashi H, Nakayama H, Kanaoka Y, Minamino N. Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence. *Nature* 1984;312:121–127. [PubMed: 6209577]
- Novak AE, Jost MC, Lu Y, Taylor AD, Zakon HH, Ribera AB. Gene duplications and evolution of vertebrate voltage-gated sodium channels. *J Mol Evol* 2006 Aug;63(2):208–21. [PubMed: 16830092]
- Oren DA, Froy O, Amit E, Kleinberger-Doron N, Gurevitz M, Shaanan B. An excitatory scorpion toxin with a distinctive feature: an additional alpha helix at the C terminus and its implications for interaction with insect sodium channels. *Structure* 1998;6:1095–1103. [PubMed: 9753689]
- Pauron D, Barhanin J, Lazdunski M. The voltage-dependent Na⁺ channel of insect nervous system identified by receptor sites for tetrodotoxin, and scorpion and sea anemone toxins. *Biochem Biophys Res Commun* 1985;131:1226–1233. [PubMed: 2413857]
- Pelhate M, Laufer J, Pichon Y, Zlotkin E. Effects of several sea anemone and scorpion toxins on excitability and ionic currents in the giant axon of the cockroach. *J Physiol (Paris)* 1984;79:309–317. [PubMed: 6152295]

- Possani LD, Becerril B, Delepierre M, Tytgat J. Scorpion toxins specific for Na⁺-channels. *Eur J Biochem* 1999;264:287–300. [PubMed: 10491073]
- Possani LD, Merino E, Corona M, Bolivar F, Becerril B. Peptides and genes coding for scorpion toxins that affect ion-channels. *Biochimie* 2000;82:861–868. [PubMed: 11086216]
- Regev A, Rivkin H, Inceoglu B, Gershburg E, Hammock BD, Gurevitz M, Chejanovsky N. Further enhancement of baculovirus insecticidal efficacy with scorpion toxins that interact cooperatively. *FEBS Lett* 2003;537:106–110. [PubMed: 12606040]
- Rodriguez de la Vega RC, Possani LD. Overview of scorpion toxins specific for Na⁺ channels and related peptides: biodiversity, structure-function relationships and evolution. *Toxicon* 2005;46:831–844. [PubMed: 16274721]
- Rogers JC, Qu Y, Tanada TN, Scheuer T, Catterall WA. Molecular determinants of high affinity binding of alpha-scorpion toxin and sea anemone toxin in the S3–S4 extracellular loop in domain IV of the Na⁺ channel alpha subunit. *J Biol Chem* 1996;271:15950–15962. [PubMed: 8663157]
- Saab CY, Cummins TR, Dib-Hajj SD, Waxman SG. Molecular determinant of Na(v)1.8 sodium channel resistance to the venom from the scorpion *Leiurus quinquestriatus hebraeus*. *Neurosci Lett* 2002;331:79–82. [PubMed: 12361845]
- Sabatier JM, Fremont V, Mabrouk K, Crest M, Darbon H, Rochat H, Van Rietschoten J, Martin-Eauclaire MF. Leiurotoxin I, a scorpion toxin specific for Ca(2+)-activated K⁺ channels. Structure-activity analysis using synthetic analogs. *Int J Pept Protein Res* 1994;43:486–495. [PubMed: 8070973]
- Sampaio SV, Coutinho-Netto J, Arantes EC, Marangoni S, Oliveira B, Giglio JR. Isolation of toxin TsTX-VI from *Tityus serrulatus* scorpion venom. Effects on the release of neurotransmitters from synaptosomes. *Biochem Mol Biol Int* 1996;39:729–740. [PubMed: 8843341]
- Sautiere P, Cestele S, Kopeyan C, Martinage A, Drobecq H, Doljansky Y, Gordon D. New toxins acting on sodium channels from the scorpion *Leiurus quinquestriatus hebraeus* suggest a clue to mammalian vs insect selectivity. *Toxicon* 1998;36:1141–1154. [PubMed: 9690781]
- Shao F, Xiong YM, Zhu RH, Ling MH, Chi CW, Wang DC. Expression and purification of the BmK M1 neurotoxin from the scorpion *Buthus martensii* Karsch. *Protein Expr Purif* 1999;17:358–365. [PubMed: 10600453]
- Sharkey RG, Jover E, Couraud F, Baden DG, Catterall WA. Allosteric modulation of neurotoxin binding to voltage-sensitive sodium channels by *Ptychodiscus brevis* toxin 2. *Mol Pharmacol* 1987;31:273–278. [PubMed: 2436034]
- Sheets MF, Kyle JW, Kallen RG, Hanck DA. The Na channel voltage sensor associated with inactivation is localized to the external charged residues of domain IV, S4. *Biophys J* 1999;77:747–757. [PubMed: 10423423]
- Smith JJ, Alphy S, Seibert AL, Blumenthal KM. Differential phospholipid binding by site 3 and site 4 toxins. Implications for structural variability between voltage-sensitive sodium channel domains. *J Biol Chem* 2005;280:11127–11133. [PubMed: 15632158]
- Soderlund DM, Knipple DC. The molecular biology of knockdown resistance to pyrethroid insecticides. *Insect Biochem Mol Biol* 2003;33:563–577. [PubMed: 12770575]
- Spampanato J, Escayg A, Meisler MH, Goldin AL. Functional effects of two voltage-gated sodium channel mutations that cause generalized epilepsy with febrile seizures plus type 2. *J Neurosci* 2001;21:7481–7490. [PubMed: 11567038]
- Srinivasan KN, Nirthan S, Sasaki T, Sato K, Cheng B, Gwee MC, Kini RM, Gopalakrishnakone P. Functional site of bukatoxin, an alpha-type sodium channel neurotoxin from the Chinese scorpion (*Buthus martensii* Karsch) venom: probable role of the (52)PDKVP(56) loop. *FEBS Lett* 2001;494:145–149. [PubMed: 11311230]
- Stevens EB, Cox PJ, Shah BS, Dixon AK, Richardson PJ, Pinnock RD, Lee K. Tissue distribution and functional expression of the human voltage-gated sodium channel beta3 subunit. *Pflugers Arch* 2001;441:481–488. [PubMed: 11212211]
- Strichartz G, Rando T, Wang GK. An integrated view of the molecular toxinology of sodium channel gating in excitable cells. *Annu Rev Neurosci* 1987;10:237–267. [PubMed: 2436544]
- Sun YM, Bosmans F, Zhu RH, Goudet C, Xiong YM, Tytgat J, Wang DC. Importance of the conserved aromatic residues in the scorpion alpha-like toxin BmK M1: the hydrophobic surface region revisited. *J Biol Chem* 2003;278:24125–24131. [PubMed: 12692120]

- Sun YM, Liu W, Goudet C, Tytgat J, Wang DC. Role of disulfide bridges in scorpion toxin BmK M1 analyzed by mutagenesis. *J Peptide Res* 2002;60:247–256. [PubMed: 12383115]
- Swartz KJ, MacKinnon R. Hanatoxin modifies the gating of a voltage- dependent K⁺ channel through multiple binding sites. *Neuron* 1997;18:665–673. [PubMed: 9136774]
- Tan J, Liu Z, Wang R, Huang ZY, Chen AC, Gurevitz M, Dong K. Identification of amino acid residues in the insect sodium channel critical for pyrethroid binding. *Mol Pharmacol* 2005;67:513–522. [PubMed: 15525757]
- Tejedor FJ, Catterall WA. Site of covalent attachment of alpha-scorpion toxin derivatives in domain I of the sodium channel alpha subunit. *Proc Natl Acad Sci U S A* 1988;85:8742–8746. [PubMed: 2847174]
- Thomsen WJ, Catterall WA. Localization of the receptor site for alpha-scorpion toxins by antibody mapping: implications for sodium channel topology. *Proc Natl Acad Sci U S A* 1989;86:10161–10165. [PubMed: 2557622]
- Torres AM, Bansal P, Alewood PF, Bursill JA, Kuchel PW, Vandenberg JI. Solution structure of CnErg1 (Ergtoxin), a HERG specific scorpion toxin. *FEBS Lett* 2003;539:138–142. [PubMed: 12650941]
- Tugarinov V, Kustanovich I, Zilberberg N, Gurevitz M, Anglister J. Solution structures of a highly insecticidal recombinant scorpion alpha-toxin and a mutant with increased activity. *Biochemistry* 1997;36:2414–2424. [PubMed: 9054546]
- Vais H, Williamson MS, Devonshire AL, Usherwood PN. The molecular interactions of pyrethroid insecticides with insect and mammalian sodium channels. *Pest Manag Sci* 2001;57:877–888. [PubMed: 11695180]
- Vargas O, Martin MF, Rochat H. Characterization of six toxins from the venom of the Moroccan scorpion *Buthus occitanus mardochei*. *Eur J Biochem* 1987;162:589–599. [PubMed: 3104036]
- Vedantham V, Cannon SC. Slow inactivation does not affect movement of the fast inactivation gate in voltage-gated Na⁺ channels. *J Gen Physiol* 1998;111:83–93. [PubMed: 9417137]
- Wang R, Huang ZY, Dong K. Molecular characterization of an arachnid sodium channel gene from the varroa mite (*Varroa destructor*). *Insect Biochem Mol Biol* 2003;33:733–739. [PubMed: 12826100]
- Wang SY, Wang GK. Voltage-gated sodium channels as primary targets of diverse lipid-soluble neurotoxins. *Cell Signal* 2003;15:151–159. [PubMed: 12464386]
- Warmke JW, Reenan RA, Wang P, Qian S, Arena JP, Wang J, Wunderler D, Liu K, Kaczorowski GJ, Van der Ploeg LH, et al. Functional expression of *Drosophila* para sodium channels. Modulation by the membrane protein TipE and toxin pharmacology. *J Gen Physiol* 1997;110:119–133. [PubMed: 9236205]
- Winterfield JR, Swartz KJ. A hot spot for the interaction of gating modifier toxins with voltage-dependent ion channels. *J Gen Physiol* 2000;116:637–644. [PubMed: 11055992]
- Ye X, Bosmans F, Li C, Zhang Y, Wang DC, Tytgat J. Structural basis for the voltage-gated Na⁺ channel selectivity of the scorpion alpha-like toxin BmK M1. *J Mol Biol* 2005;353:788–803. [PubMed: 16209876]
- Yu FH, Catterall WA. Overview of the voltage-gated sodium channel family. *Genome Biol* 2003;4:207. [PubMed: 12620097]
- Yu FH, Westenbroek RE, Silos-Santiago I, McCormick KA, Lawson D, Ge P, Ferriera H, Lilly J, DiStefano PS, Catterall WA, et al. Sodium channel beta4, a new disulfide-linked auxiliary subunit with similarity to beta2. *J Neurosci* 2003;23:7577–7585. [PubMed: 12930796]
- Zilberberg N, Froy O, Loret E, Cestele S, Arad D, Gordon D, Gurevitz M. Identification of structural elements of a scorpion alpha-neurotoxin important for receptor site recognition. *J Biol Chem* 1997;272:14810–14816. [PubMed: 9169449]
- Zilberberg N, Gordon D, Pelhate M, Adams ME, Norris TM, Zlotkin E, Gurevitz M. Functional expression and genetic alteration of an alpha scorpion neurotoxin. *Biochemistry* 1996;35:10215–10222. [PubMed: 8756487]
- Zlotkin E. The insect voltage-gated sodium channel as target of insecticides. *Annu Rev Entomol* 1999;44:429–455. [PubMed: 9990721]
- Zlotkin E, Fishman Y, Elazar M. AaIT: from neurotoxin to insecticide. *Biochimie* 2000;82:869–881. [PubMed: 11086217]

- Zlotkin E, Kadouri D, Gordon D, Pelhate M, Martin MF, Rochat H. An excitatory and a depressant insect toxin from scorpion venom both affect sodium conductance and possess a common binding site. *Arch Biochem Biophys* 1985;240:877–887. [PubMed: 2411224]
- Zuo XP, He HQ, He M, Liu ZR, Xu Q, Ye JG, Ji YH. Comparative pharmacology and cloning of two novel arachnid sodium channels: Exploring the adaptive insensitivity of scorpion to its toxins. *FEBS Lett* 2006;580:4508–4514. [PubMed: 16870180]
- Zuo XP, Ji YH. Molecular mechanism of scorpion neurotoxins acting on sodium channels: insight into their diverse selectivity. *Mol Neurobiol* 2004;30:265–278. [PubMed: 15655252]

et al., 2003; Ali *et al.*, 2006; Arnon *et al.*, 2005; Chai *et al.*, 2006; Chen *et al.*, 2003; Corzo *et al.*, 2001; del Rio-Portilla *et al.*, 2004; Gong *et al.*, 1997; Goudet *et al.*, 2002; Hamon *et al.*, 2002; Jalali *et al.*, 2005; Sampaio *et al.*, 1996; Srinivasan *et al.*, 2001).

Figure 2. Structures of representative scorpion α -toxins

AaHII: classical scorpion α -toxin (mammalian-selective). Indicated are important residues for structure/function.

BmKM1: scorpion α -like toxin (active on both mammals and insects). Indicated are three important regions for function as defined by Liu *et al.* (Liu *et al.*, 2005).

Lqh α IT: scorpion insect α -toxin (insect-selective). Indicated are three important regions for function as defined by Karbat *et al.* (Karbat *et al.*, 2004).

	S3	Loop region	S4	
(r) hNa _v 1.1	SIVGMFLAELIEK--YFVSPTLFRVIRL		(NP110502)	P35498
(r) hNa _v 1.2 (a)	SIVGMFLAELIEK--YFVSPTLFRVIRL		(P04775)	Q99250
rNa _v 1.3	SIVGMFLAELIEK--YFVSPTLFRVIRL		P08104	
hNa _v 1.3	SIVGMFLAEMIEK--YFVSPTLFRVIRL			Q9NY46
(r) hNa _v 1.4	SIVGLALS SD LIQK--YFVSPTLFRVIRL		(P15390)	M81758
(r) hNa _v 1.5	SIVGTVLS DI IQK--YFVSPTLFRVIRL		(P15389)	Q14524
(r) hNa _v 1.6	SIVGMFLADIIEK--YFVSPTLFRVIRL		(AF049239)	Q9NYX2
rNa _v 1.7	SIVGMFLAEMIEK--YFVSPTLFRVIRL		O08562	
hNa _v 1.7	SIVGMFLADLIET--YFVSPTLFRVIRL			Q15858
rNa _v 1.8	SIGSLL FS SAILK SL ENYFSPPTLFRVIRL		Q62968	
hNa _v 1.8	SIASLIFSAILKSLQSYFSPPTLFRVIRL			Q9Y5Y9
rNa _v 1.9	SIISTLVSRLEDSDISFPPTLFRVVRL		Q9UI33	
hNa _v 1.9	SIVSTMISTLENQEHIFPPTLFRIVRL			Q9UI33
Cockroach	SILGLVLS DI IEK--YFVSPTLLRVVVRV		U73583	
Fruitfly	SILGLVLS DI IEK--YFVSPTLLRVVVRV		M32078	
Housefly	SILGLVLS DI IEK--YFVSPTLLRVVVRV		CAA65448	
Mite	SILGTVLKD LI AA--YFVSPTLLRVVVRV		AAP13992	
Scorpion	SILGIAIKDLIAS--YFVSPTLLRVVVRV		AAQ84279	
Spider	SILGIVLKD LI AK--YFVSPTLLRVVVRV		DQ839489	
		DIV		

Figure 3. Voltage-gated Na⁺ channel Site 3 comparisons

Alignment of Site 3 of vertebrate VGSCs and invertebrate VGSCs. Accession numbers are given on the right (left column: rat; right column: human). If the rat (r) and human (h) channel are different in Site 3, both of them are displayed beneath one another. Functionally important residues for scorpion α -toxins and sea anemone toxins are indicated in white with a black background. Residues shown to be important for scorpion α -toxins are indicated with a red background. The yellow box indicates residues studied by Saab *et al.* 2002.