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**Interacting Domains of Anti-Insect Scorpion Toxins
and their Sodium Channel Binding Sites: Structure,
Cooperative Interactions with Agrochemicals and
Application**

**M. Gurevitz, M.E. Adams, B. Shaanan, O. Froy, D. Gordon, D. Lee,
Y. Zhao**

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Investigators

Michael Gurevitz (PI)

Michael E. Adams (PI)

Boaz Shaanan (CI)

Oren Froy (Ph.D. student)

Dalia Gordon (CI)

Daewoo Lee (Ph.D. student)

Yong Zhao (Ph.D. student)

Institutions

Tel-Aviv University

UC Riverside

Hebrew University

Tel-Aviv University

Tel-Aviv University

UC Riverside

UC Riverside

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Abbreviations: Lqh α IT: *Leiurus quinquestriatus hebraeus* alpha insect toxin; Aah2: *Androctonus australis hector* toxin 2; Lqh2: *Leiurus quinquestriatus hebraeus* toxin 2; Bj-xtrIT: *Buthotus judaicus* excitatory insect toxin; LqhIT2: *Leiurus quinquestriatus hebraeus* insect toxin 2.

PI Signatures:

M. Gurevitz *M. Gurevitz*

M.E. Adams

Institution's Authorizing Official:

Abstract

Integrated pest management in modern crop protection may combine chemical and biological insecticides, particularly due to the risks to the environment and livestock arising from the massive use of non-selective chemicals. Thus, there is a need for safer alternatives, which target insects more specifically. Scorpions produce anti-insect selective polypeptide toxins that are biodegradable and non-toxic to warm-blooded animals. Therefore, integration of these substances into insect pest control strategies is of major importance. Moreover, clarification of the molecular basis of this selectivity may provide valuable information pertinent to their receptor sites and to the future design of peptidomimetic anti-insect specific substances. These toxins may also be important for reducing the current overuse of chemical insecticides if they produce a synergistic effect with conventional pesticides.

Based on these considerations, our major objectives were:

- 1) To elucidate the three-dimensional structure and toxic-site of scorpion excitatory, depressant, and anti-insect alpha toxins.
- 2) To obtain an initial view to the sodium channel recognition sites of the above toxins by generating peptide decoys through a phage display system.
- 3) To investigate the synergism between toxins and chemical insecticides.

Our approach was to develop a suitable expression system for toxin production in a recombinant form and for elucidation of toxin bioactive sites via mutagenesis. In parallel, the mode of action and synergistic effects of scorpion insecticidal toxins with pyrethroids were studied at the sodium channel level using electrophysiological methods.

Objective 1 was achieved for the alpha toxin, Lqh α IT (Zilberberg *et al.*, 1996, 1997; Tugarinov *et al.*, 1997; Froy *et al.*, 2002), and the excitatory toxin, Bj-xtrIT (Oren *et al.*, 1998; Froy *et al.*, 1999; unpublished data). The bioactive surface of the depressant toxin, LqhIT2, has been clarified and a crystal of the toxin is now being analyzed (unpublished). **Objective 2** was not successful thus far as no phages that recognize the toxins were obtained. We therefore initiated recently an alternative approach, which is introduction of mutations into recombinant channels and creation of channel chimeras. **Objective 3** was undertaken at Riverside and the results demonstrated synergism between Lqh α IT or AaIT and pyrethroids (Lee *et al.*, 2002). Furthermore, negative cross-resistance between pyrethroids and scorpion toxins (Lqh α IT and AaIT) was demonstrated at the molecular level.

Although our study did not yield a product, it paves the way for future design of selective pesticides by capitalizing on the natural competence of scorpion toxins to distinguish between sodium channels of insects and vertebrates. We also show that future application of anti-insect toxins may enable to decrease the amounts of chemical pesticides due to their synergism.

Achievements

Tel Aviv University

Several approaches were employed for elucidating the bioactive surface of toxin representatives of three pharmacologically distinct groups. 1) Massive point mutagenesis, where each toxin variant was produced using our established bacterial expression and *in vitro* folding protocols (Zilberberg *et al.*, 1996; Turkov *et al.*, 1997; Gurevitz *et al.*, 1998; Froy *et al.*, 1999; Gurevitz *et al.*, 2000; Gurevitz *et al.*, 2002); 2) Determination of three-dimensional structure either by X-ray crystallography or 2D-NMR (Tugarinov *et al.*, 1997; Oren *et al.*, 1998); 3) Generation of toxin chimeras to verify the bioactive site and identify structural elements involved in selectivity (Froy *et al.*, 2002).

The alpha anti-insect toxin Lqh α IT: The putative toxic surface of Lqh α IT for insect sodium channels was highlighted by substituting more than 60 residues that are exposed to the solvent. The mutagenesis was based on the 3-D structure of Lqh α IT (Tugarinov *et al.*, 1997) and focused on the molecule hemisphere shown previously to bear bioactive residues (Zilberberg *et al.*, 1997). Seven residues that form two adjacent clusters were found to define the bioactive surface of Lqh α IT (Gurevitz *et al.*, 2001; Froy *et al.*, 2002): Arg58 and Lys62 of the C-tail with Tyr10 of the 5-residue turn (residues 8-12) form the 'C-tail cluster', to which Ile57 and Val59 may also be associated due to their involvement in bioactivity. The ' α/β -cluster' is composed of Phe17 and Arg18, derived from the loop preceding the α -helix, and Trp38 and Asn44, which reside on the boundaries of the second and third β -strands (Tugarinov *et al.*, 1997).

Since the carbon backbone of Lqh α IT is very similar to that of the anti-mammalian toxins, Aah2 (from *Androctonus australis hector*), and Lqh2 (from the Israeli scorpion *Leiurus quinquestriatus hebraeus*), we verified the identity of the bioactive surface of Lqh α IT, by mobilizing it onto the scaffold of Lqh2. The toxin chimera, Lqh2^{Lqh α IT(face)}, acquired the characteristics of Lqh α IT as demonstrated by toxicity assays, binding affinity, and electrophysiological effect on an insect sodium channel expressed in *Xenopus* oocytes. Construction of the reciprocal chimera, Lqh α IT^{Lqh2(face)} yielded a toxin capable of binding rat brain sodium channels similarly to Lqh2, whereas Lqh α IT is unable to recognize this channel subtype. These experiments validated unequivocally the identity of the bioactive surface of Lqh α IT and highlighted that of Lqh2 (Froy *et al.*, 2002). They also supported our hypothesis that all 'long chain' scorpion toxins diversified from a common progenitor (Froy *et al.*, 1999; Gurevitz and Froy, 1998); and demonstrated that engineering of scorpion toxins to obtain selectivity for sodium channel subtypes is feasible.

The excitatory anti-insect toxin Bj-xtrIT: Functional expression of the excitatory anti-insect selective toxin, Bj-xtrIT, enabled mutagenic dissection as well as determination of the crystal structure (Oren *et al.*, 1998). More than 50 mutations were introduced at the putative bioactive surface focusing on the toxin hemisphere, which according to previous studies was similar to models of other excitatory toxins (AahIT, LqhIT1), and shown to bear the bioactive C-tail (Froy *et*

al., 1999 and unpublished). We extended the mutagenic dissection into the molecule surface surrounding the α_0 module that precedes the main α -helix (α_1). From the analysis, the bioactive surface of excitatory toxins seems to include seven residues, some of which correspond to positions that also are important in α -toxins, e.g. residues in the region preceding the α -helix, and other residues that surround the C-tail region (Gurevitz *et al.*, 2001; and unpublished results).

Mutagenesis at α_0 that distinguishes Bj-xtrIT from the excitatory toxin AahIT suggests that this region is likely to be involved as well in bioactivity and may be responsible for the pharmacological differences between Bj-xtrIT and the heralded excitatory toxin, AahIT. This study is now being pursued by determination of electrophysiological effects on the *para* sodium channel from *Drosophila*, which is available to us in a recombinant form in *Xenopus* oocytes.

The depressant toxin LqhIT2: The depressant group of toxins is the most promising for design of insecticides as implicated from its baculovirus (AcMNPV) mediated expression in Noctuid larvae (Gershburg *et al.*, 1998; Froy *et al.*, 2000). We studied the structure/function relationship of the anti-insect selective toxin, LqhIT2, for several years (unpublished). The toxin was expressed in a functional form (Turkov *et al.*, 1997) and more than 45 mutations unraveled its bioactive surface, which surrounds the toxin exterior onto which the C-tail is anchored (Gurevitz *et al.*, 2001; and unpublished results). Despite continuous difficulties to determine its structure in solution by 2D-NMR, we succeeded recently to crystallize it and the structure is now under analysis (in collaboration with Prof. F. Frolow, Institute of Biotechnology, Tel Aviv University; unpublished).

UC Riverside

Molecular Physiology of Scorpion Toxin Action: We examined how the alpha-scorpion toxin, Lqh α IT modifies synaptic transmission in a model physiological preparation using the fly, *Musca domestica* (Lee *et al.*, 2000), and characterized sodium channels of an insect pest and their modification by a scorpion toxin (Lee and Adams, 2000). We found that the toxin, Lqh α IT, generates two main effects on insect nerve terminals: increased neurotransmitter release and initiation of repetitive activity. The molecular basis of these physiological effects was further examined by voltage-clamp analysis of sodium currents in neurons dissociated from the fly CNS. Elevated transmitter release results from suppression of sodium current inactivation. Repetitive activity is a consequence of incomplete steady state activation (Lee *et al.*, 2000).

We characterized sodium channels in central neurons of the tobacco budworm, *Heliothis virescens*. We found that these channels are sensitive to low concentrations of the alpha-scorpion toxin Lqh α IT and the insect-specific excitatory toxin AaIT. The latter toxin shifts the voltage-dependent activation to more negative potentials, providing an explanation for the repetitive action potential activity observed in insects injected with the toxin.

Synergism between scorpion toxins and pyrethroid insecticides: We investigated possible joint actions of insect-selective scorpion toxins and the pyrethroid, permethrin. The dose-dependent elevation of tail current by permethrin was measured in the absence and presence of the toxin,

Lqh α IT. We chose 200 pM Lqh α IT because this concentration caused negligible elevation (<2%) of steady-state sodium current (Lee *et al.*, 1999). Modification by permethrin was greatly enhanced in the presence of 200 pM Lqh α IT (Fig. 1). The action of 0.3 μ M permethrin was enhanced ~8-fold in the presence of Lqh α IT.

The second interaction examined was the effect of a low permethrin concentration on AaIT action. The major effect of AaIT on the *H. virescens* sodium channel is to shift voltage-dependent activation, resulting in repetitive firing of action potentials (Lee and Adams, 2000). In the presence of 10 nM permethrin, which alone did not show any significant effect, the action of AaIT (100 nM) was potentiated (Fig. 2A). Normalized currents, before and after permethrin, showed a clear shift of voltage-dependent activation in the hyperpolarizing direction (Fig. 2B,C). This potentiation by permethrin was observed only at relatively negative test potentials (<-30 mV), which was the effective range for AaIT alone.

Pyrethroid resistant insects show enhanced sensitivity to scorpion toxins: We analyzed a pyrethroid-resistant strain of *Heliothis virescens* carrying a single mutation, V421M, in the I-S6 transmembrane segment. While this strain is ~20-fold resistant to permethrin, it showed a 2-fold increased sensitivity to Lqh α IT. This enhanced sensitivity to the scorpion toxin represents a rare case of negative cross-resistance (Lee *et al.*, 1999). Similar negative cross resistance was observed for the excitatory scorpion toxin, AaIT (Fig. 3; Lee and Adams, 2002).

Heterologous expression of cloned sodium channels in oocytes: In parallel to the above analyses, we developed the *Xenopus* oocyte heterologous expression system for analysis of voltage-activated sodium channel pharmacology and function in order to examine the synergistic interactions between the scorpion toxins and chemical insecticides. We co-expressed the *Drosophila para* channel with the accessory protein, Tip-E. Fig. 4 shows suppression of sodium channel inactivation by Lqh α IT, which is consistent with our previous data on sodium channels in central neurons of *M. domestica* (Lee *et al.*, 2000) and *H. virescens* (Lee and Adams, 2000). However, we observed differences in the way the excitatory toxin, Bj-xtrIT modified neuronal sodium channels as compared with *para* expressed in oocytes. Fig. 5 shows modification of housefly central neurons by Bj-xtrIT (100 nM), in which the toxin shifts activation to more negative potentials and partially inhibits inactivation (Zhao and Adams, in preparation). In contrast, nanomolar concentrations of this toxin had no effect on the *para* sodium channel. At higher concentrations (>1 μ M), the toxin suppresses sodium currents and shifts voltage-dependent activation to more positive potentials (Fig. 6). The discrepancy in Bj-xtrIT action in these two systems could be due to the particular splice variant of *para* used in this study. Further work is needed with additional splice variants of *para* to clarify these results. However, these experiments clearly show that *para* currents assayed in a heterologous expression system are modified by recombinant Lqh α IT and Bj-xtrIT (recombinant toxins produced in Tel Aviv).

The oocyte system enables us to analyze whether mutations in the channel causing pyrethroid resistance in insects change the sensitivity of the channel to scorpion toxins. We confirmed that two

channel mutations associated with pyrethroid resistance in *H. virescens*, V421M and L1029H, confer decreased sensitivity to permethrin (Zhao *et al.*, 2000). Surprisingly, neither mutation increased the sensitivity to Lqh α IT (Fig. 7; Zhao and Adams, 2002), as had been observed in *Heliothis* central neurons (Lee *et al.*, 1999). This is likely due to differences in the structures of the *Drosophila* and *Heliothis* sodium channels. Further work with the oocyte expression system is underway to provide a more complete analysis of negative cross-resistance and synergism between pyrethroids and scorpion toxins.

Analysis of *H. virescens* point mutations at positions 421 and 1029, which confer moderate insensitivity (~20x) to pyrethroids, show that they confer absolute insensitivity to the Site 2 toxins aconitine, veratridine and batrachotoxin (Zhao and Adams, 2002). It is noteworthy that position 421 lies within the proposed area of transmembrane segment I-S6, which is thought to include part of the batrachotoxin binding site.

During this project, we observed pronounced allosteric coupling between binding Sites 3 (alpha-scorpion toxins; e.g., Lqh α IT) and 4 (beta-scorpion toxins; e.g., AaIT, Bj-xtrIT).

Future impact

Our results raise future possibilities for developing alternative means in insect pest control as well as ways for reducing the current overuse of chemical insecticides. This is still a **long term** objective but it may be highly rewarding in view of the massive use of chemicals in insect control and the risks they pose on the environment and humans.

Cooperation

Both laboratories contributed unique expertise to this project. The Tel Aviv laboratory cloned, expressed, mutagenized, and analyzed the various toxins. The binding assays and electrophysiological characterization of the recombinant toxins were performed in collaboration with Drs. Dalia Gordon and Nicolas Gilles, Saclay, France, and Prof. Marcel Pelhate, Anger, France. The Adams laboratory at UC Riverside analyzed the recombinant toxins Lqh α IT and Bj-xtrIT produced in Tel Aviv by using the patch clamp technique and on the recombinant *para* channel expressed in *Xenopus* oocytes. These analyses revealed the marked synergism between Lqh α IT and the pyrethroid, permethrin as well as the synergism between AaIT and permethrin. These results indicate that the pyrethroid binding site is allosterically coupled to both Sites 3 and 4 on sodium channels. In light of our past results showing increase in insecticidal efficacy by baculovirus mediated expression of Lqh α IT (Chejanovsky *et al.*, 1995), the synergism of this toxin with pyrethroids is highly promising for future development of means to control lepidopterous pests. It is noteworthy that it is still required to eliminate the anti-mammalian activity of Lqh α IT and develop an appropriate way for its administration in the field, a task now under investigation in the Tel Aviv laboratory.

BARD supported publications (IS-2901-97C designated by asterisk)

- Zilberberg, N., Gordon, D., Pelhate, M., Adams, M.E., Norris, T., Zlotkin, E. & Gurevitz, M. (1996) Functional expression and genetic alteration of an alpha scorpion neurotoxin. *Biochemistry* 35:10215-10222.
- Tugarinov, V., Kustanovich, I., Zilberberg, N., Gurevitz, M. & Anglister, J. (1997) Solution structure of a highly insecticidal recombinant scorpion α -toxin and a mutant with increased activity. *Biochemistry* 36:2414-2424.
- Zilberberg, N., Froy, O., Cestele, S., Loret, E., Arad, D., Gordon, D. & Gurevitz, M. (1997) Identification of structural elements of a scorpion alpha neurotoxin important for receptor-site recognition. *J. Biol. Chem.* 272:14810-14816.
- Turkov, M., Rashi, S., Zilberberg, N., Gordon, D., Benkhalifa, R., Pelhate, M. & Gurevitz, M. (1997) Expression in *Escherichia coli* and reconstitution of a functional recombinant scorpion depressant neurotoxin specific against insects. *Prot. Express. Purific.* 10:123-131.
- Benkhalifa, R., Stankiewicz, M., Lapied, B., Turkov, M., Zilberberg, N., Gurevitz, M. & Pelhate, M. (1997) Refined electrophysiological analysis suggests that scorpion depressant neurotoxins are sodium channel openers rather than blockers. *Life Sci.* 61:819-830.
- *Oren, D., Froy, O., Amit, E., Kleinberger-Doron, N., Gurevitz, M., & Shaanan, B. (1998) An excitatory scorpion toxin with a distinctive feature: an additional α -helix at the C-terminus and its implications for interaction with insect sodium channels. *Structure* 6:1095-1103.
- *Gershburg, E., Stockholm, D., Froy, O., Rashi, S., Gurevitz, M. & Chejanovsky, N. (1998) Baculovirus-mediated expression of a scorpion depressant toxin improves significantly the insecticidal efficacy achieved with excitatory toxins. *FEBS Lett.* 422:132-136.
- Gordon, D., Savarin, P., Gurevitz, M. & Zinn-Justin, S. (1998) Functional anatomy of scorpion toxins affecting sodium channels. *J. Toxicol. Toxin Rev.* 17:131-158.
- *Froy, O. and Gurevitz, M. (1998) Membrane potential modulators: a thread of scarlet from plants to humans. *FASEB J.* 12:1793-1796.
- *Gurevitz, M., Froy, O., Zilberberg, N., Turkov, M., Strugatsky, D., Gershburg, E., Lee, D., Adams, M. E., Tugarinov, V., Anglister, J., Shaanan, B., Loret, E., Stankiewicz, M., Pelhate, M., Gordon, D. & Chejanovsky, N. (1998) Sodium channel modifiers from Scorpion venom: Structure-activity relationship, mode of action, and application. *Toxicon* 36:1671-1682.
- *Froy, O., Zilberberg, N., Gordon, D., Turkov, M., Gilles, N., Stankiewicz, M., Pelhate, M., Loret, E., Oren, D., Shaanan, B. & Gurevitz, M. (1999) The putative bioactive surface of insect-selective scorpion excitatory neurotoxins. *J. Biol. Chem.* 274:5769-5776.
- *Froy, O., Sagiv, T., Poreh, M., Urbach, D., Zilberberg, N. and Gurevitz, M. (1999) Dynamic diversification from a putative common progenitor of scorpion toxins affecting sodium, potassium and chloride channels. *J. Mol. Evol.* 48:187-196.
- *Froy, O., Zilberberg, N., Chejanovsky, N., Anglister, J., Loret, E., Shaanan, B., Gordon, D. & Gurevitz, M. (2000) Scorpion neurotoxins: Structure/function relationships and application in agriculture. *Pest. Manag. Sci.* 56:472-474.
- *Gurevitz, M., Froy, O., Zilberberg, N., Shaanan, B., Adams, M. E., Anglister, J., Pelhate, M., Chejanovsky, N. & Gordon, D. (1999) Elucidation of the active site and potential applications of sodium channel modifiers from scorpion venom. *Progress in Neuropharmacology and Neurotoxicology of Pesticides and Drugs* (D. J. Beadle, Ed.) Oxford, UK, pp. 67-80.

- *Lee, D., Gurevitz, M. & Adams, M. E. (2000) Modification of synaptic transmission and sodium channel inactivation by the insect-selective scorpion toxin Lqh α IT. *J. Neurophysiol.* 83:1181-1187.
- *Lee, D. and Adams, M.E. (2000) Sodium channels in central neurons of the tobacco budworm, *Heliothis virescens*: basic properties and modification by scorpion toxins. *J. Insect Physiol.* 46(4):499-508.
- *Gilles, N., Krimm, I., Bouet, F., Froy, O., Gurevitz, M., Lancelin, J-M. & Gordon, D. (2000) Structural implications on the interaction of scorpion α -like toxins with the sodium channel receptor site inferred from toxin iodination and pH-dependent binding. *J. Neurochem.* 75:1735-1745.
- *Zhao, Y., Park, Y. & Adams, M. E. (2000) Functional and evolutionary consequences of pyrethroid resistance mutations in S6 transmembrane segments of a voltage-gated sodium channel. *Biochem. Biophys. Res. Commun.* 278(3):516-521.
- *Gurevitz, M., Gordon, D., Ben-Natan, S., Turkov, M. & Froy, O. (2001) Diversification of neurotoxins by C-tail 'wiggling' - a scorpion recipe for survival. *FASEB J.* 15:1201-1205.
- *Gurevitz, M., Zilberberg, N., Froy, O., Turkov, M., Wilunsky, R., Karbat, I., Anglister, J., Shaanan, B., Pelhate, M., Adams, M. R., Gilles, N. & Gordon, D. (2002) Diversification of toxic sites on a conserved protein scaffold - a scorpion recipe for survival. In *Perspectives in Molecular Toxinology* (Menez, A., Ed.), Wiley and Sons, Chichester, UK. In press.
- *Gilles, N., Harrison, G., Karbat, I., Gurevitz, M., Nicholson, G. M. & Gordon, D. (2002) Variations in site-3 receptors on rat brain and insect sodium channels highlighted by binding of a funnel-web spider δ -Atracotoxin. *Eur. J. Biochem.* Accepted.
- *Regev, A., Rivkin, H., Inceoglu, B., Gershburg, E., Hammock, B. D., Gurevitz, M. & Chejanovsky, N. (2002) co-expression of scorpion toxins that act cooperatively on sodium channels improves the insecticidal efficacy of recombinant baculoviruses. *Biocontrol*, Accepted.
- *Froy, O., Gilles, N., Turkov, M., Karbat, I., Ilan, N., Benveniste, M., Gordon, D. & Gurevitz, M. Inversion of scorpion alpha- toxin selectivity for insect or mammalian neuronal sodium channels. Submitted.
- *Zhao, Y. & Adams, M. E. (2002) The influence of pyrethroid resistance mutations on allosteric Site 2 of the voltage-dependent sodium channel. Submitted.
- *Lee, D., Gurevitz, M. & Adams, M. E. (2002) Modulation of sodium channels by permethrin: Enhancement by joint actions with scorpion toxins. Submitted.

Appendix

Fig. 1. Potentiation of permethrin action by 200 pM Lqh α IT. (A) Concentration-dependent modification of sodium channels by permethrin alone. (B) Concentration-dependent modification by permethrin in the presence of 200 pM Lqh α IT. (C) Percent modified channels as a function of permethrin concentration. As can be seen in (B), Lqh α IT dramatically synergized permethrin action by increasing the percent of modified channels. Each point in the presence of 200 pM Lqh α IT is an average of at least 3 experiments.

Fig. 2. Potentiation of AaIT action by permethrin. (A) Superimposed sodium currents evoked by V_T of -43 mV (refer to a down-arrow in C) before and after exposure to the toxins (AaIT and permethrin). The increased sodium conductance caused by 100 nM AaIT is enhanced in the presence of 10 nM permethrin. The effect of 10 nM permethrin is shown in (B) and (C) as a shift of voltage-dependent activation in the hyperpolarizing direction.

Fig. 3. Differential effect of an excitatory scorpion toxin AaIT on sodium currents in pyrethroid-susceptible and -resistant *H. virescens* neurons. I/V relationship before and after AaIT application was converted to gNa/V curves. (A) and (B) show representative gNa/V curves in the absence and presence of AaIT with various concentrations in pyrethroid-susceptible (A) and -resistant (B) *H. virescens* neurons. Since AaIT shifts voltage dependent activation to more negative potentials, the shift of V_T to activate 50% of channels was plotted as a function of AaIT concentration. (C) Concentration-response curve of AaIT in both strains. Sodium channels in resistant *H. virescens* showed 4-fold higher sensitivity in 50 nM AaIT.

Fig. 4. Dose response curve of Lqh α IT on wild-type channel. A, sodium currents recorded under 0, 0.3, 1, 3 and 10 nM Lqh α IT. B. Dose response curve plotted by normalized inactivation currents (inactivation current/peak current) vs Lqh α IT concentration (n=5). Inactivation currents were collected at 15 msec after testing potentials. Lqh α IT began to effect the Para sodium channel at concentration of 0.3 nM and reached maximum effect at 30 nM., (n=5). The normalized inactivation currents were calculated by dividing inactivation currents with peak current.

Fig. 5. Effects of the excitatory scorpion toxin Bj-xtrIT (100 nM) on sodium channel currents recorded in housefly central neurons. Control currents (upper left) show slowing of inactivation (upper right) and a shift of voltage-dependent activation to more hyperpolarizing potentials (lower).

Fig. 6. Effects of Bj-xtrIT (10 μM) on *para* sodium channel currents recorded in *Xenopus* oocytes. The toxin has effects only at relatively higher concentrations, and the effect is to suppress currents and shift voltage-dependent activation to more positive potentials.

Fig. 7. V421M and L1029H mutations do not change channel sensitivity to LqhαIT. A, representative currents recorded from wild-type, V421M and L1029H sodium channels before (control) and after application 10 nM of LqhαIT. B, *kdr* mutations do not change channel sensitivity to LqhαIT. The inactivation currents induced by 10 nM LqhαIT were normalized with maximum peak currents. The normalized inactivation currents after 10 nM LqhαIT were: 75.5 ± 10.2 , 72.2 ± 10.0 and 71.3 ± 9.1 for wild-type ($n=6$), V421M ($n=6$) and L1029H ($n=6$), respectively. The differences are not significant ($P < 0.05$).