Substitutions in the Domain III Voltage-sensing Module Enhance the Sensitivity of an Insect Sodium Channel to a Scorpion β-Toxin*

Weizhong Song1, Yuzhe Du1, Zhiqi Liu2, Ningguang Luo3, Michael Turkov4, Michael Gordon5, Michael Gurevitz5, Alan L. Goldin6, and Ke Dong1†‡¶

From the 1Department of Entomology and the Genetics and Neuroscience Programs, Michigan State University, East Lansing, Michigan 48824, the 2Department of Plant Molecular Biology and Ecology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel, and the 3Department of Microbiology and Molecular Genetics, University of California, Irvine, California 92697

Scorpion β-toxins bind to the extracellular regions of the voltage-sensing module of domain II and to the pore module of domain III in voltage-gated sodium channels and enhance channel activation by trapping and stabilizing the voltage sensor of domain II in its activated state. We investigated the interaction of a highly potent insect-selective scorpion depressant β-toxin, Lqh-dprIT3, from Leiurus quinquestriatus hebraeus with insect sodium channels from Blattella germanica (BggNa). Like other scorpion β-toxins, Lqh-dprIT3, shifts the voltage dependence of activation of BggNa channels expressed in Xenopus oocytes to more negative membrane potentials but only after strong depolarizing prepulses. Notably, among 10 BggNa splice variants tested for their sensitivity to the toxin, only BggNa1-1 was hypersensitive due to an L1285P substitution in IIIS1 resulting from a U-to-C RNA-editing event. Furthermore, charge reversal of a negatively charged residue (E1290K) at the extracellular end of IIIS1 and the two innermost positively charged residues (R4E and R5E) in IIIS4 also increased the channel sensitivity to Lqh-dprIT3. Besides enhancement of toxin sensitivity, the R4E substitution caused an additional 20-mV negative shift in the voltage dependence of activation of toxin-modified channels, inducing a unique toxin-modified state. Our findings provide the first direct evidence for the involvement of the domain III voltage-sensing module in the action of scorpion β-toxins. This hypersensitivity most likely reflects an increase in IIIS4 trapping via allosteric mechanisms, suggesting coupling between the voltage sensors in neighboring domains during channel activation.

Voltage-gated sodium (Na+) channels are essential for the initiation and propagation of action potentials in most excitable cells. They consist of a large pore-forming α-subunit that is associated with a variable number of smaller subunits in different excitable tissues (1). The α-subunit comprises four repeat homologous domains (I–IV), each having six membrane-spanning segments (S1–S6). The S1–S4 segments constitute the voltage-sensing module. S4 in each domain contains four to seven positively charged residues and moves outward in response to membrane depolarization, which initiates the channel activation process (1). The S5 and S6 segments and their connecting P loops compose the pore-forming module. Each reentrant P loop contains two short segments (SS1 and SS2) that span the membrane as a hairpin and form the lining of the pore.

Mammals produce functionally and pharmacologically diverse Naαα-subunits by selective expression of distinct sodium channel genes (at least nine in rat and human) in different tissues (2, 3). In insects such as Drosophila melanogaster and Blattella germanica, extensive alternative splicing and RNA editing of a single gene generate a broad array of variants that are diverse in gating and pharmacological properties (4–6).

Because of their pivotal role in excitability, Naαα channels are targeted by a variety of toxins derived from plants and animals as part of their defense or preying strategies (1, 7–10). Among venomous animals, scorpions produce a rich repertoire of 61–76-residue-long peptide toxins that modify sodium channel gating upon binding to distinct extracellular receptor sites in the α-subunit (7, 8, 11–13). The toxins that affect Naαα channels are divided into α- and β-classes according to their mode of action and binding properties (14, 15). α-Toxins inhibit channel fast inactivation in a voltage-dependent manner upon binding at receptor site 3, assigned mainly to domains I and IV. β-Toxins interact with receptor site 4, assigned to the voltage-sensing module of domain II and the pore module of domain III, and shift the voltage dependence of activation to more negative membrane potentials (11, 16–19). The sensitivity of mammalian Naαα subtypes to scorpion β-toxins differs greatly. Rat brain (rNaα,1.2)2 and skeletal muscle (rNaα,1.4) channels are often more sensitive than the cardiac channel (rNaα,1.5) to β-toxins of South American scorpions such as Css4 from Centruroides suffusus suffusus, Ts1 from Tityus serrulatus, and TdVIII from Tityus discrepans (16–20). Moreover, Tz1 from Tityus zulius is especially active at rNaα,1.4 compared with rNaα,1.2 and human Naα,1.5 (18).

* This work was supported, in whole or in part, by National Institutes of Health Grant GM057440 (to K. D.). This work was also supported by United States-Israel Binational Agricultural Research and Development Grant IS-4066-07 (to D. G., M. G., and K. D.), National Science Foundation Grant IBN 9808156 (to K. D.), and Israeli Science Foundation Grant 107/08 (to M. G. and D. G.).

1 To whom correspondence should be addressed. Tel.: 517-432-2034; Fax: 517-353-4354; E-mail: dongk@msu.edu.

2 The abbreviations used are: rNaα, rat Naαα; BggNaαα, B. germanica Naαα.
Domain III Voltage Sensor Is Critical for β-Toxin Action

The prominent activity of Css4 on rNa,1.2 and rNa,1.4 compared with its very weak effect on rNa,1.5 motivated Catterall and co-workers (16) to analyze each of the 16 extracellular loops of rNa,1.5 in the background of rNa,1.2. Their analysis revealed that S5S-S1I, S1S2-S2, I1S3-S4, and IIIS2-S6, particularly a G845N substitution in IIIS3-S4, play a role in determining toxin preference, suggesting that scorpion β-toxins bind to the S3-S4 loop (16). In a similar fashion, differences in potency of the toxin Tz1 on rat muscle, cardiac, and neuronal Na channels motivated Heinemann and co-workers (18) to swap rNa,1.2a sequences in the background of rNa,1.4. Their analysis revealed that three amino acid residues in the C-terminal pore loop (SS2-S6) of domain III determine the Tz1 preference for the skeletal muscle Na channel (18).

To account for enhanced sodium channel activation by Css4, a voltage sensor-trapping model was proposed in which the S4 voltage sensor of domain II (IIS4) is trapped and stabilized in its outward activated position by the scorpion β-toxin (16, 21). Neutralization or reversal of gating charges in the voltage sensor of domain II enhances the action of scorpion β-toxins. According to the classical models of sodium channel gating, the voltage sensors of the sodium channel activate independently, and at least three of them have to be in an activated position for the channel to open (22, 23). For a scorpion β-toxin to shift the threshold of activation, more than one voltage sensor could be affected by the toxins. Bezanilla and co-workers (24) combined electrophysiological and spectroscopic measurements to determine the structural rearrangements induced by the scorpion β-toxin Ts1 on the rNa,1.4 channel. Consistent with studies using Css4, Ts1 binding to the channel has been shown to be restricted to a single binding site in the voltage sensor of domain II, where it traps IIS4 in the activated state. Interestingly, Ts1 binding to S4 of domain II allosterically potentiates activation of the other three voltage sensors at more hyperpolarized potentials. However, it is unknown how voltage-sensing modules in other domains contribute to the voltage sensor trapping by β-toxins.

In this study, we analyzed the sensitivity of 10 B. germanica Na, (BgNa,) variants (25) to a potent insect-selective Lqh-dprIT, depressant toxin (26). The discovery of one variant that is hypersensitive to Lqh-dprIT, led to the identification of the critical effect of Leu-1285 and Glu-1290 in IIS1 on channel sensitivity to the toxin. We further show that substitutions of the two innermost positively charged residues (R4E and R5E) in IIS4 also increase the effect of Lqh-dprIT, variants produced by the scorpion Leiarius quinques-triatris hebraeus, the highly potent variant c, hereafter referred to as Lqh-dprIT, was produced in recombinant form and analyzed as described previously (26).

The voltage dependence of sodium channel conductance (G, measured as peak conductance) was calculated by measuring the peak current at test potentials ranging from −80 to +65 mV in 5-mV increments and divided by (V − Vrev), where V is the test potential and Vrev is the reversal potential for sodium ions. Peak conductance values were normalized to the maximal peak conductance (Gmax) and fit with a two-state Boltzmann equation of the form G/Gmax = (1 + exp(V − V1/2)/k)−1 or with the sum of two such expressions, where V is the potential of the voltage pulse, V1/2 is the voltage for half-maximal activation, and k is the slope factor.

The percentage of channel modification by Lqh-dprIT, was determined by the percentage of channels with the voltage dependence of activation shifted to negative membrane potentials, which was derived from double Boltzmann fits of the conductance-voltage relationships. Data are presented as means ± S.D. Statistical significance was determined by one-way analysis of variance (p < 0.05).

RESULTS

Effects of Lqh-dprIT, on Sodium Channel Splice Variant BgNa,1-1—We first examined a well characterized cockroach sodium channel, BgNa,1-1 (27), for the effect of Lqh-dprIT, Previous studies showed that, for most of scorpion β-toxins, a strong depolarization prepulse is required for the toxins to induce a negative shift in the voltage dependence of activation (11, 16–19). For example, a 1-ms depolarization prepulse to 50 mV followed by a depolarizing test pulse to −65 mV activates Css4-modified Na,1.2 channels but not unmodified channels (16). We initially used a similar protocol to examine the effect of Lqh-dprIT, on BgNa,1-1 channels and found that a brief depolarization pulse was not sufficient to detect Lqh-dprIT, action (data not shown). We then applied a 20-Hz train of 50 5-ms depolarizing prepulses to 50 mV as the conditional pulses, followed by a 20-ms depolarizing test pulse between −80 and −65 mV from a holding potential of −120 mV. As expected, in the absence of the toxin, no sodium currents were detected under any of the test pulses (Fig. 1A). However, in the presence of 300 nm Lqh-dprIT, substantial sodium currents were detected at −75 to −65 mV (Fig. 1A), indicating that Lqh-dprIT, modified the gating of BgNa,1-1 channels.
The toxin effect was also evident in analyses of current-voltage relation and conductance curves exhibiting a negative shift in the voltage dependence of channel activation after conditional prepulses in the presence of toxin (Fig. 1, B and D). In addition, a significant increase in peak current was observed at 300 nM but not at a lower concentration (100 nM) (Fig. 1B). Neither of these effects was observed without the prepulses (Fig. 1C). The voltage dependence of channel inactivation was not affected by the toxin (data not shown). At both 100 and 300 nM, the current-voltage and conductance-voltage relations determined from Lqh-dprIT3 effects were biphasic (Fig. 1D). Fitting the conductance curves with the sum of two Boltzmann relations revealed that the voltage dependence of activation of 40% (with 100 nM Lqh-dprIT3) and 80% (with 300 nM Lqh-dprIT3) of BgNav1-1 channels shifted 40 mV to a more hyperpolarizing membrane potential. The extent of the toxin-induced hyperpolarizing shift in the voltage dependence of channel activation ranged from −23 to −42 mV (Table 1).

Because the type 1 splice variants BgNa1-1-2 and BgNa1-1-3 were less sensitive to Lqh-dprIT3 compared with BgNa1-1, we reasoned that the alternative exons common to these three variants were not responsible for the hypersensitivity to Lqh-dprIT3. Compared with BgNa1-1-2 and BgNa1-1-3, four amino acid residues are different in BgNa1-1: R502G, L1285P, V1685A, and I1806L (Fig. 3). Of these four differences, L1285P and V1685A have been shown to result from U-to-C RNA-editing events (25). We examined the effect of Lqh-dprIT3 on a recombinant channel, BgNa1-1a, in which these four unique residues were replaced with those of BgNa1-1-2 and BgNa1-1-3 (25). We found that the sensitivity of BgNa1-1a to Lqh-dprIT3 decreased prominently, with only 18% of the channels modified by 300 nM toxin (Fig. 2 and Table 2). This result suggested that one or more of these four amino acids were responsible for the hypersensitivity of BgNa1-1 to Lqh-dprIT3.
**Domain III Voltage Sensor Is Critical for β-Toxin Action**

**FIGURE 2. Effect of Lqh-dprIT3 on 10 BgNav variants.** A, conductance-voltage relations in the absence (○) and presence (●) of 300 nM Lqh-dprIT3, for eight BgNav variants and the recombinant BgNav1-1a channel, originating from BgNav1-1. The conductance-voltage relation was measured using the protocol described under "Experimental Procedures." B, dose-response curve of BgNav1-3 in comparison with BgNav1-1. C, conductance-voltage relation in the absence (○) and presence (●) of 300 nM Lqh-dprIT3, for the recombinant BgNav1-1a channel. Four amino acid changes in BgNav1-1 were reversed by site-directed mutagenesis generating BgNav1-1a (25).

**TABLE 1**

Voltage dependence of activation of BgNav splices variants before and after application of 300 nM Lqh-dprIT3

| Toxin-free | Lqh-dprIT3 (300 nM) |
|------------|---------------------|
|            | V_{0.5} (mV) | k | % | V_{0.5} (mV) | k | % | A1 | V_{0.5} (mV) | k | % | A2 |
| BgNav1-1   | -23.2 ± 1.0 | 6.3 ± 0.3 | -21.0 ± 0.4 | 5.3 ± 0.3 | 20 ± 5* | -62.8 ± 2.6 | 7.9 ± 0.5 | 80 ± 5* |
| BgNav1-1a  | -28.4 ± 2.1 | 4.1 ± 0.6 | -28.1 ± 1.5 | 5.0 ± 0.3 | 82 ± 5 | -60.0 ± 3.3 | 10.4 ± 1.6 | 18 ± 5 |
| BgNav1-2   | -55.2 ± 2.8 | 5.1 ± 1.1 | -55.2 ± 2.1 | 6.1 ± 1.4 | 81 ± 3 | -58.1 ± 1.2 | 8.6 ± 0.8 | 19 ± 3 |
| BgNav1-3   | -30.4 ± 2.1 | 4.3 ± 0.6 | -30.6 ± 1.5 | 5.5 ± 0.4 | 86 ± 4 | -68.7 ± 0.8 | 5.4 ± 0.3 | 14 ± 4 |
| BgNav5     | -35.9 ± 2.6 | 3.7 ± 0.5 | -35.7 ± 0.7 | 4.0 ± 0.4 | 77 ± 1 | -59.3 ± 3.0 | 11.5 ± 0.9 | 23 ± 1 |
| BgNav6     | -27.9 ± 2.4 | 4.1 ± 0.9 | -29.2 ± 2.6 | 5.2 ± 0.9 | 79 ± 5 | -59.0 ± 5.8 | 10.9 ± 2.3 | 21 ± 3 |
| BgNav7     | -31.7 ± 1.9 | 4.8 ± 0.4 | -32.5 ± 2.3 | 3.1 ± 0.6 | 79 ± 6 | -56.7 ± 2.8 | 11.8 ± 2.1 | 21 ± 5 |
| BgNav8     | -26.5 ± 1.0 | 4.8 ± 0.2 | -26.2 ± 0.9 | 5.5 ± 0.2 | 86 ± 3 | -60.0 ± 0.5 | 8.2 ± 0.2 | 14 ± 3 |
| BgNav9     | -30.7 ± 3.1 | 6.4 ± 1.2 | -31.0 ± 2.9 | 7.5 ± 1.4 | 82 ± 5 | -60.0 ± 2.6 | 10.1 ± 1.5 | 18 ± 5 |
| BgNav10    | -34.1 ± 2.4 | 3.5 ± 0.5 | -35.8 ± 0.7 | 3.9 ± 0.7 | 75 ± 6 | -58.6 ± 2.0 | 14.3 ± 2.6 | 25 ± 6 |
| BgNav11    | -49.3 ± 2.0 | -3.2 ± 0.5 | -43.2 ± 2.7 | 5.6 ± 0.7 | 73 ± 3 | -73.7 ± 3.0 | 2.9 ± 1.1 | 21 ± 3 |

* Significant differences from other splice variants (p < 0.05).

**L1285P in III1 Is Responsible for the Hypersensitivity of BgNav1-1 to Lqh-dprIT3.**—To determine which of the four amino acid substitutions in BgNav1-1 is responsible for the hypersensitivity to Lqh-dprIT3, we substituted each of the four amino acids in the background of BgNav1-1a with those found in BgNav1-1 (i.e. R502G, L1285P, V1685A, and I1806L) and determined the effect of Lqh-dprIT3. Whereas none of the substitutions had an effect on the voltage dependence of channel activity, L1285P conferred the effect of hypersensitivity to Lqh-dprIT3 to BgNav1-1.
activation in the absence of toxin (Table 2), 300 nM Lqh-dprIT₃ caused a shift in all four channel mutants (Fig. 3). The fraction of toxin-modified channels was 71% for the L1285P mutant compared with BgNa₁-1. In contrast, the fraction of toxin-modified channels for the other three mutants was 13–14%, similar to that for BgNa₁-1a (Fig. 3 and Table 2). These results indicated that the L1285P substitution was responsible for the hypersensitivity of BgNa₁-1 to Lqh-dprIT₃.

To further determine the impact of the side chain of the residue at position 1258 on Lqh-dprIT₃ action, we substituted Leu-1285 with an aromatic residue (Phe), two neutral residues (Gly and Cys), and a positively charged Lys residue in the background of BgNa₁-1a. In contrast to L1285P, none of these substitutions increased the channel sensitivity to Lqh-dprIT₃ (Fig. 4 and Table 3). In addition, none of these substitutions significantly altered the voltage dependence of channel activation. However, the L1285G substitution caused a 13-mV positive shift in the voltage dependence of steady-state inactivation (data not shown).

**FIGURE 3.** L1285P is responsible for the hypersensitivity of BgNa₁-1 to Lqh-dprIT₃. A, the four amino acid changes in BgNa₁-1 are indicated in the topology of the BgNa₁ sodium channel protein. B, conductance curves in the absence (○) and presence (○) of 300 nM Lqh-dprIT₃ for four BgNa₁-1a channel mutants. Each of the four mutations was introduced into the BgNa₁-1a channel. The conductance-voltage relation was measured using the protocol described under "Experimental Procedures."

**TABLE 2**

Voltage dependence of activation of BgNa₁-1a and mutants before and after the application of 300 nM Lqh-dprIT₃

| Channel               | V₀.5 (mV) | kₐ | k₉ | A₁ | V₀.5 (mV) | k₉ | A₂ |
|-----------------------|-----------|----|----|----|-----------|----|----|
| BgNa₁-1a              | −28.4 ± 2.1 | 4.1 ± 0.6 | −28.1 ± 1.5 | 5.0 ± 0.3 | 82 ± 5 | −60.0 ± 3.3 | 10.4 ± 1.6 | 18 ± 5 |
| R502G                 | −28.3 ± 0.6 | 4.0 ± 0.2 | −27.5 ± 1.3 | 5.1 ± 0.1 | 86 ± 2 | −60.0 ± 3.5 | 11.6 ± 1.5 | 14 ± 2 |
| L1285P                | −25.0 ± 1.0 | 5.4 ± 0.2 | −25.1 ± 1.6 | 5.5 ± 0.5 | 29 ± 4* | −64.3 ± 1.5 | 8.6 ± 0.9 | 71 ± 4* |
| V1685A                | −27.5 ± 0.7 | 3.9 ± 0.2 | −26.0 ± 1.5 | 4.7 ± 0.3 | 86 ± 4 | −61.3 ± 2.0 | 7.4 ± 1.0 | 14 ± 4 |
| I1806L                | −26.2 ± 0.5 | 4.4 ± 0.3 | −24.6 ± 1.2 | 5.1 ± 0.2 | 87 ± 3 | −63.5 ± 2.0 | 6.6 ± 0.8 | 13 ± 3 |

* Significant differences from BgNa₁-1a (p < 0.05).
Domain III Voltage Sensor Is Critical for β-Toxin Action

Voltage dependence of activation for the modified R4E channels, which started to activate at membrane potentials as negative as −100 mV.

DISCUSSION

The differential sensitivities of BgNa1 sodium channel splice variants from B. germanica have been valuable in elucidating the channel regions involved in channel function and resistance to pyrethroid insecticides (4, 5). Here, we examined the sensitivity of 10 BgNa1 channel variants to the insect-selective scorpion depressant β-toxin Lqh-dprIT3. This screening unexpectedly revealed that substitutions at the voltage-sensing module of domain III influenced the action of the β-toxin. The binding site of the β-toxin Css4 has been shown to involve the voltage-sensing module of domain II and the pore module of domain III (16, 19, 31, 32). We found that a Leu-to-Pro substitution at position 1285 in IIIS1, resulting from an RNA-editing event, was responsible for the hypersensitivity of the sodium channel variant BgNa1-1 to Lqh-dprIT3. Further site-directed mutagenesis identified additional residues whose substitution affected the action of Lqh-dprIT3, including a negatively charged residue at the extracellular end of IIIS1 (Glu-1290) and the two innermost positively charged gating residues in IIIS4. Charge reversal of either Glu-1290 or each of the two gating charged residues in IIIS4 did not hinder but rather increased the channel sensitivity to the toxin.

A preliminary three-dimensional model of Css4 docking at rNa1.2a, constructed using as template the crystal structure of the bacterial voltage-gated potassium channel K_A1 (21), suggested that the toxin binds at a crevice between S1–S2 and S3–S4 in domain II, thus controlling IIIS4 movement during activation. When the two outermost positively charged residues in IIIS4 of rNa1.2 were neutralized or charge-reversed, Css4 activity increased, most likely due to their increased mobility during activation, thus enhancing the trapping by the toxin (31).

The voltage-sensing module of domain III has not previously been implicated in the binding and/or action of scorpion β-toxins. The increased sensitivity to Lqh-dprIT3 by the L1285P and E1290A/K substitutions in IIIS1 might indicate that the Lqh-dprIT3 toxin binds at the crevice juxtaposed to S1 of domain III in the channel mutants. Alternatively, alterations in domain III may indirectly facilitate trapping of the voltage sensor in domain II by the toxin. The enhanced channel sensitivity to Lqh-dprIT3 when Leu-1285 was substituted specifically with proline (Table 2) seems more consistent with the latter explanation. Proline inserts a kink in the distal part of the S1 α-helix, which would disrupt the local arrangement of IIIS1, including Glu-1290. Because these alterations increased rather than decreased the effect of Lqh-dprIT3, we speculate that the unmodified IIIS1 in BgNa1 imposes some structural constraint that limits the full effect of the toxin in binding to the domain II voltage sensor and/or in the voltage sensor trapping, and this constraint is lifted by the above substitutions.

The most dramatic effect inspected was when the two innermost gating charges in domain III were substituted with negative charges. This result is quite different from the effects of substituting negative charges in the S4 voltage sensor of domain II. In domain II, neutralization of the two outermost charged residues markedly enhanced β-scorpion toxin activation, and neutralization of the three innermost positively charged residues had no effect (31). In contrast, we observed that reversal of the three outermost positively charged residues in IIIS4 had no effects on toxin activity, and reversal of the two innermost charges greatly enhanced the effect of the toxin. It is unlikely that the two innermost charges directly interact with the toxin because these residues are embedded in the membrane even when the channel is in the activated state. The effects on toxin sensitivity are also unlikely due to effects on the voltage dependence of activation because the effect of the R4E substi-

**TABLE 3**
Voltage dependence of activation of BgNa1-1a and L1285 substitutions before and after the application of 300 nM Lqh-dprIT3

Each value represents the mean ± S.D. for at least five oocytes.

| Toxin-free | Lqh-dprIT3 (300 nM) |
|------------|---------------------|
| **V_{0.5}** | **k** | **k** | **A** | **A** |
| BgNa1-1a   | −28.4 ± 2.1 | 4.1 ± 0.6 | −28.1 ± 1.5 | 5.0 ± 0.3 | 82 ± 5 | −60.0 ± 3.3 | 10.4 ± 1.6 | 18 ± 5 |
| L1285P     | −25.0 ± 1.0 | 5.4 ± 0.2 | −25.1 ± 1.6 | 5.5 ± 0.5 | 29 ± 4* | −64.3 ± 1.5 | 8.6 ± 0.9 | 71 ± 4* |
| L1285F     | −28.6 ± 1.1 | 4.2 ± 0.2 | −25.0 ± 1.0 | 4.8 ± 0.2 | 87 ± 4 | −61.6 ± 3.1 | 8.3 ± 0.2 | 13 ± 4 |
| L1285G     | −28.7 ± 0.8 | 3.9 ± 0.3 | −27.8 ± 1.2 | 4.0 ± 0.6 | 85 ± 3 | −50.6 ± 6 | 10.4 ± 2.0 | 15 ± 3 |
| L1285K     | −30.5 ± 2.1 | 4.6 ± 0.4 | −27.5 ± 1.5 | 5.9 ± 0.6 | 86 ± 2 | −67.2 ± 1.9 | 8.5 ± 1.2 | 14 ± 2 |
| L1285C     | −28.7 ± 0.3 | 4.1 ± 0.3 | −25.9 ± 1.6 | 4.6 ± 0.2 | 85 ± 2 | −59.6 ± 3.1 | 11.0 ± 1.6 | 15 ± 2 |

* Significant differences from BgNa1-1a (p < 0.05).
Domain III Voltage Sensor Is Critical for β-Toxin Action

Each value represents the mean ± S.D. for at least five oocytes.

TABLE 4
Voltage dependence of activation of BgNa1-1a and mutants before and after the application of 300 nM Lqh-dprIT3

| Toxin-free | Lqh-dprIT3 (300 nM) |
|------------|---------------------|
|            | $V_{0.5}$ | $k$         | $V_{0.5}$ | $k$             | $A_1$ | $V_{0.5}$ | $k$ | $A_2$ |
| BgNa1-1a   | -28.4 ± 2.1 | 4.1 ± 0.6 | -28.1 ± 1.5 | 5.0 ± 0.3 | 82 ± 5 | -60.0 ± 3.3 | 10.4 ± 1.6 | 118 ± 5 |
| E1290A     | -24.5 ± 1.8 | 4.9 ± 0.3 | -24.0 ± 3.5 | 6.9 ± 0.2 | 48 ± 12* | -60.0 ± 2.4 | 6.1 ± 0.5 | 52 ± 12* |
| E1290K     | -24.2 ± 1.2 | 6.6 ± 0.4 | -68.1 ± 2.8 | 7.5 ± 0.4 |          |          |          |      |

* Significant differences from BgNa1-1a ($p < 0.05$).

TABLE 5
Voltage dependence of activation of BgNa1-1a and mutants before and after the application of 300 nM Lqh-dprIT3

| Toxin-free | Lqh-dprIT3 (300 nM) |
|------------|---------------------|
|            | $V_{0.5}$ | $k$         | $V_{0.5}$ | $k$ | $A_1$ | $V_{0.5}$ | $k$ | $A_2$ |
| BgNa1-1a   | -28.4 ± 2.1 | 4.1 ± 0.6 | -28.1 ± 1.5 | 5.0 ± 0.3 | 82 ± 5 | -60.0 ± 3.3 | 10.4 ± 1.6 | 18 ± 5 |
| K1E        | -35.5 ± 2.5 | 4.7 ± 0.4 | -35.8 ± 3.9 | 4.8 ± 0.3 | 82 ± 4 | -70.0 ± 6.1 | 10.7 ± 2.1 | 18 ± 4 |
| R2E        | -34.3 ± 2.8 | 5.1 ± 0.3 | -32.9 ± 2.1 | 5.5 ± 0.4 | 81 ± 4 | -75.5 ± 4.5 | 7.4 ± 0.8 | 19 ± 4 |
| R3E        | -34.2 ± 2.5 | 5.0 ± 0.2 | -34.7 ± 2.7 | 6.8 ± 0.3 | 75 ± 5 | -76.1 ± 8.7 | 7.0 ± 0.6 | 25 ± 5 |
| R4E        | -33.7 ± 3.8 | 5.7 ± 0.5 | -26.9 ± 3.6 | 8.1 ± 0.6 | 43 ± 8* | -87.9 ± 3.6 | 5.1 ± 0.5 | 57 ± 8* |
| R5E        | -24.0 ± 1.9 | 7.2 ± 0.4 | -25.0 ± 3.9 | 8.6 ± 0.8 | 35 ± 7* | -63.6 ± 5.0 | 7.9 ± 0.6 | 65 ± 7* |

* Significant differences from BgNa1-1a ($p < 0.05$).
Domain III Voltage Sensor Is Critical for β-Toxin Action

differential effects are more consistent with an allosteric modulation of channel gating than a direct interaction between the substituted residues and the toxin. Bezanilla and co-workers (33) proposed that the voltage sensors of the sodium channel are intrinsically tightly coupled. They have shown that binding of the scorpion β-toxin Ts1 to S4 of domain II allosterically activates the movement of all the other voltage sensors in the sodium channel, with the maximal effect on the neighboring voltage sensors of domains I and III rather than on the distant S4 of domain IV. Our results suggest that alterations in the voltage-sensing module of domain III are likely to enhance the effect of the toxins on IIS4. The role of the voltage-sensing module of domain III in the action of Lqh-dprIT3 on insect sodium channels, as revealed in this study, may be conserved in mammalian sodium channel interaction with other scorpion β-toxins. Future research should test this possibility.

REFERENCES
1. Catterall, W. A. (2000) Neuron 26, 13–25
2. Goldin, A. L. (1999) Ann. N.Y. Acad. Sci. 868, 38–50
3. Goldin, A. L., Barchi, R. L., Caldwell, J. H., Hofmann, F., Howe, J. R., Hunter, J. C., Kallen, R. G., Mandel, G., Meisler, M. H., Netter, Y. B., Noda, M., Tamkun, M. M., Waxman, S. G., Wood, J. N., and Catterall, W. A. (2000) Neuron 28, 365–368
4. Dong, K. (2007) Invert. Neurosci. 7, 17–30
5. Dong, K. (2010) in Insect Pharmacology (Gilbert, L. I., and Gill, S. S., eds) pp. 25–27, Elsevier B.V., Oxford, United Kingdom
6. Soderlund, D. M. (2010) Sodium Channels, Elsevier B. V., Oxford, United Kingdom
7. Catterall, W. A. (1992) Physiol. Rev. 72, S15–S48
8. Gordon, D. (1997) Invert. Neurosci. 3, 103–116
9. Terlau, H., and Olivera, B. M. (2004) Physiol. Rev. 84, 41–68
10. Ceste`le, S., and Catterall, W. A. (2000) Biochimie 82, 883–892
11. Gordon, D. (1997) in Toxins and Signal Transduction (Gutman, Y., and Lazarovici, P., eds) pp. 119–149, Harwood, Amsterdam, The Netherlands
12. Martin-Eauclaire, M. F., and Couraud, F. (1995) in Handbook Neurotoxicology (Chang, L. W., and Dyer, R. S., eds) Marcel Dekker, New York
13. Martin-Eauclaire, M. F., and Couraud, F. (1995) in Handbook Neurotoxicology (Chang, L. W., and Dyer, R. S., eds) Marcel Dekker, New York
14. Gordon, D., Karbat, I., Ilan, N., Cohen, L., Kahn, R., Gilles, N., Dong, K., Stühmer, W., Tytgat, J., and Gurevitz, M. (2007) Toxicon 49, 452–472
15. Gurevitz, M., Karbat, I., Cohen, L., Ilan, N., Kahn, R., Turkov, M., Stankiewicz, M., Stühmer, W., Dong, K., and Gordon, D. (2007) Toxicon 49, 473–489
16. Ceste`le, S., Qu, Y., Rogers, J. C., Rochat, H., Scheuer, T., and Catterall, W. A. (1998) Neuron 21, 919–931
17. Cohen, L., Ilan, N., Gur, M., Stühmer, W., Gordon, D., and Gurevitz, M. (2007) J. Biol. Chem. 282, 29242–29240
18. Leipold, E., Hansel, A., Borges, A., and Heinemann, S. H. (2006) Mol. Pharmacol. 70, 340–347
19. Marcotte, P., Chen, L. Q., Kallen, R. G., and Chahine, M. (1997) Circ. Res. 80, 363–369
20. Tsushima, R. G., Borges, A., and Backx, P. H. (1999) Pflugers Arch. 437, 661–668
21. Ceste`le, S., Yarov-Yarovoy, V., Qu, Y., Sampieri, F., Scheuer, T., and Catterall, W. A. (2006) J. Biol. Chem. 281, 21332–21344
22. Armstrong, C. M., and Bezanilla, F. (1977) J. Gen. Physiol. 70, 567–590
23. Hodgkin, A. L., and Huxley, A. F. (1952) Cold Spring Harb. Symp. Quant. Biol. 17, 43–52
24. Campos, F. V., Chanda, B., Beirão, P. S., and Bezanilla, F. (2007) J. Gen. Physiol. 130, 257–268
25. Song, W., Liu, Z., Tan, J., Nomura, Y., and Dong, K. (2004) J. Biol. Chem. 279, 32554–32561
26. Strugatsky, D., Zilberberg, N., Stankiewicz, M., Ilan, N., Turkov, M., Cohen, L., Pelhate, M., Gilles, N., Gordon, D., and Gurevitz, M. (2005) Biochemistry 44, 9179–9187
27. Tan, J., Liu, Z., Nomura, Y., Goldin, A. L., and Dong, K. (2002) J. Neurosci. 22, 5300–5309
28. Feng, G., Deik, P., Chopra, M., and Hall, L. M. (1995) Cell 82, 1001–1011
29. Warmke, J. W., Reenan, R. A., Wang, P., Qian, S., Arena, J. P., Wang, J., Wunderler, D., Liu, K., Kaczorowski, G. J., Van der Ploeg, L. H., Ganetzky, B., and Cohen, C. J. (1997) J. Gen. Physiol. 110, 119–133
30. Tan, J., Liu, Z., Wang, R., Huang, Z. Y., Chen, A. C., Gurevitz, M., and Dong, K. (2005) Mol. Pharmacol. 67, 513–522
31. Ceste`le, S., Scheuer, T., Mantegazza, M., Rochat, H., and Catterall, W. A. (2001) J. Gen. Physiol. 118, 291–302
32. Cohen, L., Karbat, I., Gilles, N., Ilan, N., Benveniste, M., Gordon, D., and Gurevitz, M. (2005) J. Biol. Chem. 280, 5045–5053
33. Chanda, B., Asamoah, O. K., and Bezanilla, F. (2004) J. Gen. Physiol. 123, 217–230