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Binding of an α scorpion toxin to insect sodium channels is not dependent on membrane potential

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The insect-specific LqhαIT toxin resembles α scorpion toxins affecting mammals by its amino acid sequence and effects on sodium conductance. The present study reveals that LqhαIT does not bind to rat brain membranes and possesses in locust neuronal membranes a single class of high affinity (K_d = 1.06 ± 0.15 nM) and low capacity (B_{max} = 0.7 ± 0.19 pmol/mg protein) binding sites. The latter are: (1) distinct from binding sites of other sodium channel neurotoxins; (2) inhibited by sea anemone toxin II; (3) cooperatively interacting with veratridine; (4) not dependent on membrane potential, in contrast to the binding sites of α toxins in vertebrate systems. These data suggest the occurrence of (a) conformational–structural differences between insect and mammal sodium channels and (b) the animal group specificity and pharmacological importance of the α scorpion toxins.

Sodium channel; Neurotoxin; Scorpion toxin; *Locusta*

1. INTRODUCTION

The various neurotoxins affecting voltage-sensitive sodium channels were shown to occupy at least four different receptor sites on the vertebrate sodium channels [1,2]. Receptor binding site 3 is recognized by polypeptide α scorpion and sea anemone toxins, which induce an extreme prolongation of the action potential due to a slowing or blocking of the sodium channel inactivation, and their binding is voltage-dependent and enhanced by lipid-soluble toxins such as veratridine [1–6].

Insect sodium channels were shown to resemble their vertebrate counterparts by their primary structure [7], organization [7–9] and basic biochemical [8–11] and pharmacological [12] properties.

On the other hand, a possible uniqueness of the insect sodium channels, when compared to their vertebrate counterparts, was suggested by the tolerance of insects to certain α scorpion toxins [13] and the very existence of the excitatory and depressant insect-selective polypeptide neurotoxins derived from scorpion venom, which specifically affect sodium conductance exclusively in insect neuronal preparations [14]. It was recently demonstrated that the two groups of insect-selective toxins bind to distinct receptor sites in insect sodium channels ([9] and Moskowitz et al., unpublished).

Recently, a new insect-specific scorpion toxin LqhαIT, was isolated and classified as an α insect toxin [15] due to its (a) effects on sodium conductance, namely an extreme prolongation of the action potential and slowing of sodium current inactivation, in an insect isolated axon and (b) resemblance in its primary structure to other α scorpion toxins [15].

The present study examines the interaction of the LqhαIT with insect neuronal membranes on the background of the well established binding properties of α toxins in vertebrates.

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Abbreviations: AaIT and AaH II, an excitatory insect selective toxin and an α mammal toxin, respectively, from the venom of the scorpion *Androctonus australis*; ATX II, toxin 2 from the sea anemone *Anemonia sulcata*; $LqhIT_2$, a depressant insect selective toxin from the venom of the scorpion *Leiurus quinquestriatus hebraeus*; Ts VII, a β toxin from the venom of the scorpion *Tityus serrulatus*; TTX, tetrodotoxin.

2. MATERIALS AND METHODS

2.1. *Materials*

The insect selective neurotoxins LqhαIT, $LqhIT_2$ and AaIT were purified according to [15], [16] and [13], respectively. The α toxin AaH II and the β toxin Ts VII were generous gifts of Dr. H. Rochat (School of Medicine, Biochemistry, Marseille, France) and Dr. L.D. Possani (UNAM, Cuernavaca 62271, Morelos, Mexico), respectively. Tetrodotoxin, ATX II, veratridine and gramicidin A were purchased from Sigma (USA).

2.2. *Radioiodination*

The LqhαIT toxin was iodinated by Iodogen (Pierce Chem. Co. Rockland, USA) according to the method described by [17], using 0.5 mCi carrier free [^{125}I]Na (Nuclear Research Center, Negev, Israel) and 5 μg of LqhαIT. The monoiodotoxin was purified as described [9] by a LiChrospher 100 RP-8 (5 μm) column (Merck). The concentration of the radiolabeled toxin was determined according to the specific radioactivity of the ^{125}I corresponding to 2,424 dpm/fmol monoiodotoxin.

2.3. *Neuronal membranes*

Locus (*Locusta migratoria*) synaptosomes (P_2L fraction) and their derived membrane vesicles (mvP_2L, obtained by an osmotic shock of synaptosomes) were prepared from the CNS of adult locusts according to established methods [18,19]. In the experiments presented in Table I, the mvP_2L were loaded by 0.1 M potassium phosphate, pH 6.8, 1 mM $MgSO_4$, for 5 min at 36°C according to [19,20]. It has been previously established [19,20] that membrane polarization (negative inside) is induced upon dilution of the loaded membrane vesicles by potassium-free medium [19]. Rat brain synaptosomes were prepared according to [21].

2.4. *Binding assays*

These were performed in the form of equilibrium saturation assays using increasing concentrations of the unlabeled toxin in the presence of a constant low concentration of the labeled toxin (0.11–0.2 nM), as described [9]. Analysis of the binding assays was performed using the iterative computer program LIGAND (P.J. Munson and D. Rodbard, modified by G.A. McPherson, 1985). With the exception of the binding assays presented in Table I, the membrane vesicles (mvP_2L) used were not polarized. Early kinetic experiments have shown that saturation of [^{125}I]LqhαIT binding is achieved after 30 min of incubation, regardless of membrane polarization (data not shown), thus all binding assays were performed after 40–50 min of incubation at 22°C.

Insect neuronal membranes (mvP_2L) were incubated in 0.3 ml of standard binding medium containing 0.15 M choline chloride, 1 mM $CaCl_2$, 0.8 mM $MgSO_4$, 10 mM HEPES/Tris, pH 7.4 and 1 mg/ml BSA. Termination, filtration (GF/F filters, Whatman, UK) and washing was according to [9]. Non-specific binding was determined in the presence of 1 μM unlabeled toxin and corresponded to 15–25% of the total binding.

2.5. *Protein determination*

Membrane protein was determined according to [22] using BSA as a standard.

Table I

Effect of depolarization on LqhαIT toxin binding

Conditions	LqhαIT bound (%)
Membrane vesicles (mvP_2L)	
Choline medium	100
Potassium medium	111 ± 4
Synaptosomes (P_2L)	
Choline medium	100
Potassium medium	95 ± 2
Sodium medium	100
Sodium medium, 10 μg/ml of gramicidin A	107 ± 1
Sodium medium, 10 μg/ml of gramicidin A, veratridine 100 μM	161 ± 6

Locust synaptosomal membrane vesicles (mvP_2L) loaded in 0.1 M potassium phosphate buffer or synaptosomes (P_2L, see section 2) were incubated with 0.18 nM [^{125}I]LqhαIT for 40 min at 22°C after 20-fold dilution in the following media: Choline medium (standard binding medium for mvP_2L, see section 2) or, for P_2L, 120 mM choline chloride, 0.8 mM $MgCl_2$, 20 mM HEPES/Tris, pH 7.4, 0.1% BSA. In other media, the choline was replaced with potassium or sodium, respectively. Gramicidin A (10 μg/ml) alone or with veratridine (100 μM) were added to some incubation mixtures. The results are reported as the percent of the binding measured in choline medium (100%) for each membrane preparation and represent mean ± S.E.M. of three separate experiments.

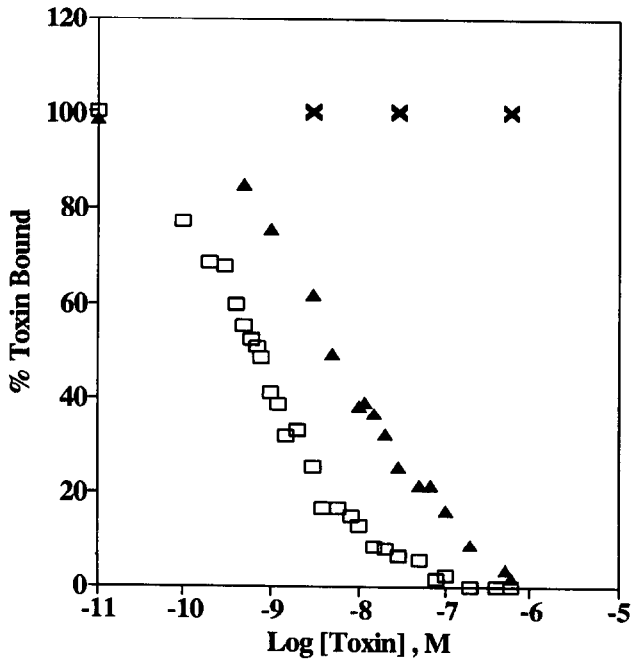

Fig. 1. Displacement of [^{125}I]LqhαIT binding by sodium channel neurotoxins. Locust neuronal membranes (mvP_2L, 54 μg membrane protein) were incubated in the presence of 0.18 nM [^{125}I]LqhαIT and increasing concentrations of each of the following toxins: LqhαIT (□); ATX II (▲); AaIT, $LqhIT_2$, Ts VII, AaH II, and TTX, which did not inhibit binding, are represented for clarity, by a single symbol (×). Non-specific binding, determined in the presence of 1 μM LqhαIT, was subtracted. The binding was measured as described in section 2 and analyzed by the computer program LIGAND. The IC_{50} for LqhαIT is 0.7 ± 0.2 nM and the IC_{50} value for ATX II equals 5.9 ± 1.21 nM (mean ± S.E. of three experiments). The calculated K_i for ATX II is 5.27 nM.

3. RESULTS AND DISCUSSION

3.1. *Inhibition of LqhαIT binding by sodium channel toxins*

In order to classify the receptor binding sites of the LqhαIT, we examined the inhibition of its specific binding by several known sodium channel neurotoxins. The data presented in Fig. 1 reveal that the binding of LqhαIT to locust neuronal membranes was not affected by: (a) the excitatory and depressant insect selective scorpion toxins, shown to bind with high affinity to insect sodium channels [9,19,23]; (b) the β toxin Ts VII, shown to compete with other β toxins on binding to receptor site 4 in vertebrate sodium channels [24,25] as well as with the above insect-selective toxins on binding to insect sodium channels [19,17,26]; (c) TTX, the universal sodium blocker, binding to receptor site 1 in vertebrate [1,2] and insect [11,23] sodium channels and (d) the α scorpion toxin AaH II, extremely toxic to mammals, that binds to receptor site 3 in vertebrate sodium channels [1,2]. The latter is in accordance with previous results indicating that AaH II was not toxic to insects [13] and devoid of specific binding to insect neuronal membranes [19].

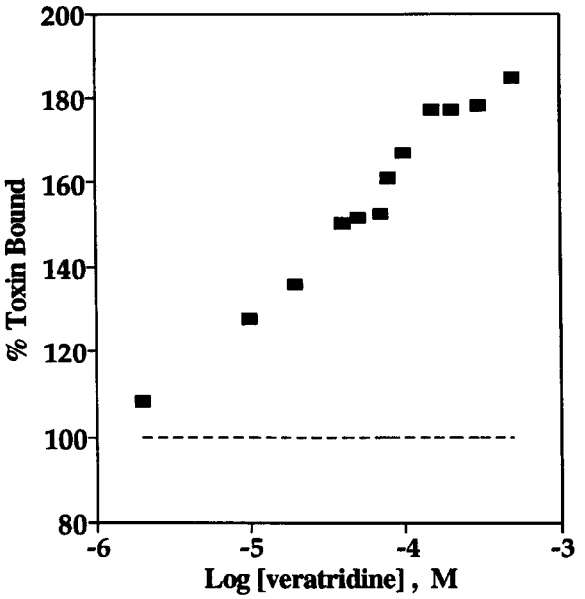

Fig. 2. Enhancement of [^{125}I]LqhαIT binding by veratridine. Locust neuronal membranes (mvP$_2$L, 28 μg membrane protein) were incubated in the presence of 0.18 nM [^{125}I]LqhαIT and the binding was determined as described in section 2. Specific binding of [^{125}I]LqhαIT was measured in the presence of 1 μM LqhαIT (100 %) and the indicated concentrations of veratridine.

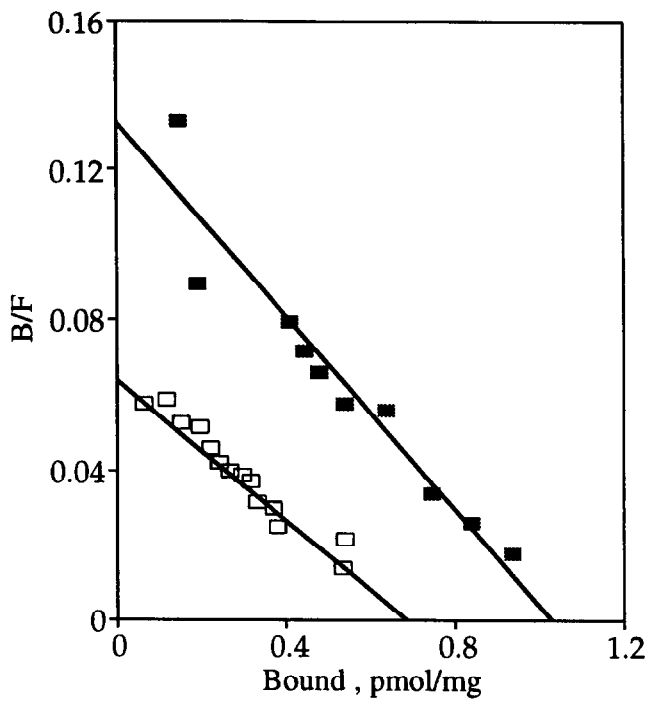

Fig. 3. Effect of veratridine on [^{125}I]LqhαIT binding. Locust neuronal membranes (mvP$_2$L, 28 μg membrane protein) were incubated in the presence of 0.18 nM [^{125}I]LqhαIT and increasing concentrations of LqhαIT in the presence (■) or absence (□) of 100 μM veratridine, under conditions as described in section 2. Scatchard analysis of LqhαIT specific binding was performed by the computer program LIGAND, yielding the presence of a single class of binding sites with the following constants: K_d = 1.06 ± 0.15 nM and B_{max} = 0.7 ± 0.19 pmol/mg protein (□); K_d = 0.88 ± 0.17 nM and B_{max} = 1.03 ± 0.06 pmol/mg protein in the presence of veratridine (■). The calculated binding constants represent mean ± S.E. of three experiments.

On the other hand, sea anemone toxin ATX II, shown to competitively inhibit the binding of α scorpion toxin to receptor site 3 in vertebrate sodium channel [2], completely inhibits, with high affinity, [^{125}I]LqhαIT binding to insect neuronal membranes (Fig. 1). Thus, sea anemone toxin displaces LqhαIT from its receptor site in locust neuronal membranes, which is distinct from the receptor sites of the other neurotoxins. It is noteworthy that ATX II was previously shown to bind to fly head neuronal membranes [27].

3.2. *Enhancement of LqhαIT binding by veratridine*

The data presented in Fig. 2 indicate that veratridine causes 1.8-fold increase in LqhαIT binding in a dose-dependent manner (Fig. 2). As shown (Fig. 3), veratridine (100 μM) increases both the binding affinity and capacity of LqhαIT. Thus, the LqhαIT and veratridine receptor sites interact cooperatively in locust neuronal membranes, as previously shown for α scorpion toxin binding in rat brain membranes [6]. The LqhαIT, however, either in the presence or absence of veratridine, did not reveal any significant specific binding to rat brain synaptosomes (data not shown).

3.3. *Binding of LqhαIT is not dependent on membrane potential*

As previously established [19,20], locust synaptosomes and their derived membrane vesicles after loading with potassium phosphate (see section 2) maintain ion gradients and the membrane potential in a modifiable manner. In order to test the membrane potential dependence of [^{125}I]LqhαIT toxin binding, depolarization of the locust neuronal membranes was induced by two treatments (Table I): (a) incubations of LqhαIT toxin in 150 mM potassium, shown to completely depolarize synaptosomal membranes [19,20], or (b) incubation in sodium medium in the presence of gramicidin A. Gramicidin A forms cation-selective ion channels and depolarizes excitable cells and synaptosomes completely [6,28]. As shown in Table I, the saturable binding of LqhαIT toxin was not affected by any of these treatments, indicating that the binding is not dependent on the membrane potential. The enhancement of LqhαIT binding by veratridine is also membrane potential-independent (Figs. 2, 3 and Table I).

3.4. *Concluding remarks*

When comparing the effects of LqhαIT in insect neuronal preparation to those of the α scorpion toxins in vertebrate preparations it may be concluded that:

(1) The LqhαIT reveals the typical pharmacology of α scorpion toxins by its (a) electrophysiological effects on

sodium conductance [15]; (b) cooperativity with veratridine; (c) displacement by the sea anemone ATX II toxin, and (d) absence of any effect on its binding by the various non-α sodium channel toxins.

(2) LqhαIT, however, differs from the α scorpion toxins by possessing voltage-independent binding sites. The binding of ATX II to the insect neuronal preparation is equally membrane potential-independent, in contrast to its binding to mammalian neuronal membranes. The latter may suggest that the receptor binding site of LqhαIT on insect sodium channels, is structurally different from the homologous (or, perhaps, analogous) receptor site 3 on vertebrate sodium channels.

(3) Clarification, on the molecular level, of LqhαIT receptor binding sites in the insect sodium channel may reveal the (a) unique properties of insect sodium channel related to its inactivation; (b) structural features responsible for animal group specificity of scorpion toxins and (c) provision of a new target for future selective insecticides.

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