

Binding of Scorpion Toxin to Receptor Sites Associated with Sodium Channels in Frog Muscle

Correlation of Voltage-Dependent Binding with Activation

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ABSTRACT Purified scorpion toxin (*Leiurus quinquestriatus*) slows inactivation of sodium channels in frog muscle at concentrations in the range of 17–170 nM. Mono[¹²⁵I]iodo scorpion toxin binds to a single class of sites in frog sartorius muscle with a dissociation constant of 14 nM and a binding capacity of 13 fmol/mg wet weight. Specific binding is inhibited more than 90% by 3 μM sea anemone toxin II and by depolarization with 165 mM K⁺. Half-maximal inhibition of binding is observed on depolarization to -41 mV. The voltage dependence of scorpion toxin binding is correlated with the voltage dependence of activation of sodium channels. Removal of calcium from the bathing medium shifts both activation and inhibition of scorpion toxin binding to more negative membrane potentials. The results are considered in terms of the hypothesis that activation of sodium channels causes a conformational change in the scorpion toxin receptor site resulting in reduced affinity for scorpion toxin.

INTRODUCTION

Venoms of North African scorpions and polypeptide toxins purified from scorpion venoms inhibit inactivation of sodium channels in voltage clamp experiments on frog myelinated nerve (Koppenhöfer and Schmidt, 1968), invertebrate giant axons (Narahashi et al., 1972; Romey et al., 1976), and tunicate eggs (Okamoto et al., 1977). These toxins also enhance persistent activation of sodium channels by grayanotoxin and the alkaloids veratridine, batrachotoxin, and aconitine in ion flux experiments with electrically excitable neuroblastoma cells (Catterall, 1975, 1976, 1977 *b*). This effect of scorpion toxin is highly dependent on membrane potential (Catterall et al., 1976), and experiments with [¹²⁵I]-labelled scorpion toxin have shown that binding of scorpion toxin to receptor sites associated with sodium channels in neuroblastoma cells is inhibited by depolarization (Catterall et al., 1976; Catterall, 1977 *a*; Ray and Catterall, 1978). The experiments described in this report were

undertaken to determine the voltage dependence of binding of scorpion toxin to receptor sites in frog muscle and to compare it to the voltage dependence of activation and inactivation of sodium channels measured in voltage clamp studies of frog muscle (Adrian et al., 1970; Ildefonse and Rougier, 1972; Campbell and Hille, 1976).

METHODS

Scorpion toxin was purified from the venom of *Leiurus quinquestriatus* as described previously (Catterall, 1976). Mono[¹²⁵I]iodo scorpion toxin was prepared by lactoperoxidase-catalyzed iodination and purified as described previously (Catterall, 1977 a). Sea anemone toxin II was purified as described by Beress et al. (1975) and was a generous gift of Dr. Laszlo Beress, University of Kiel, West Germany.

Scorpion toxin binding experiments were carried out on intact sartorius muscles of small *Rana pipiens* (1.5 to 2.5 inches, Northern variety). Muscles were dissected, drained, and wet weight was determined. Each muscle was incubated for 8 h at room

TABLE I
COMPOSITION OF SOLUTIONS USED FOR DEPOLARIZATION

| Component | Concentration | | | | | |
|--|---------------|-----|------|-----|-----|-----|
| | Solution | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | <i>mM</i> | | | | | |
| K ⁺ | 2.5 | 10 | 20 | 30 | 75 | 165 |
| Cl ⁻ | 120 | 30 | 15 | 10 | 4 | 2 |
| Na ⁺ | 120 | 83 | 64.5 | 55 | 6 | 3 |
| Ca ²⁺ | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| HPO ₄ ²⁻ + H ₂ PO ₄ ⁻ | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| SO ₄ ²⁻ | - | 36 | 42 | 44 | 46 | 101 |
| Sucrose | - | 85 | 99 | 103 | 109 | - |
| Membrane potential, <i>mV</i> | -94 | -68 | -52 | -37 | -16 | +2 |

Solution composition and membrane potentials are from Hodgkin and Horowicz (1959).

temperature in multiwell plates containing 0.5 ml frog Ringer. The samples were gently shaken on a rotary shaker. At the end of the incubation period, the muscles were rinsed three times for 1 min each in 3-ml volumes of frog Ringer and bound radioactivity was determined in a gamma scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.).

The frog Ringer solution consisted of 115 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 1 mM MgSO₄, 10 mM HEPES buffer (adjusted to pH 7.4 with NaOH), 10 mM glucose, and 1 mg/ml bovine serum albumin. In all experiments reported here, 1 μM tetrodotoxin and 2 μM α-bungarotoxin were included in the solutions. For experiments in which the muscles were depolarized, solutions with increasing K⁺ concentrations, constant [K⁺] [Cl⁻] product, and constant osmolarity were used as described by Hodgkin and Horowicz (1959). The composition of these solutions is presented in Table I together with the membrane potentials reported by Hodgkin and Horowicz. Measurement of membrane potentials after incubation at room temperature for 30 min or 8 h in solutions 1 and 4 verified that these potentials applied in the present

work. Bovine serum albumin (1 mg/ml), tetrodotoxin (1 μ M), and α -bungarotoxin (2 μ M) were also added to these solutions.

RESULTS

Voltage Clamp Analysis of Scorpion Toxin Action in Frog Muscle

The toxin used in these studies was purified from venom of the North African scorpion *Leiurus quinquestriatus* using the cooperative enhancement of veratridine activation of sodium channels in neuroblastoma cells as a specific assay system (Catterall, 1976). Voltage clamp experiments were carried out on semitendinosus fibers of *Rana pipiens* in order to determine the physiological effect of this toxin and to estimate its effective concentration in frog muscle.

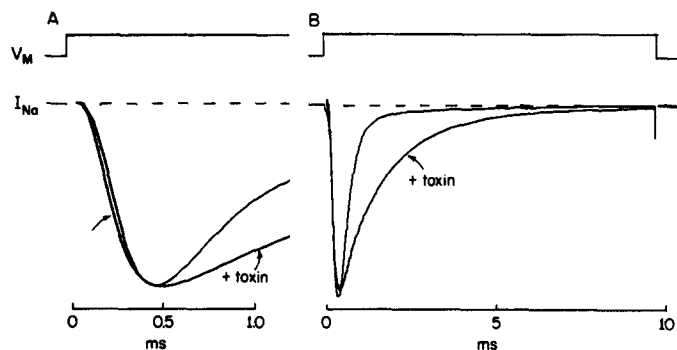


FIGURE 1. Effect of scorpion toxin on activation and inactivation of sodium channels in frog muscle fibers under voltage clamp. The traces, drawn by computer, are the membrane current minus leakage and capacity current in fibers bathed in a standard Ringer's solution containing 2 mM Ca^{++} and 1 mg/ml bovine serum albumin at 12°C. Ends of the fibers are cut in 120 mM CsF. (A) Sodium currents in a step depolarization from a holding potential of -90 to -24 mV, before and 150 s after adding 170 nM *Leiurus* toxin. The current in toxin (arrows) has been scaled $\times 1.14$ to make it about the same size as the control. (B) Sodium currents in a different fiber depolarized for 10 ms to -10 mV, before and 200 s after adding 170 nM *Leiurus* toxin (arrow).

These experiments were performed by Dr. Wolfgang Schwarz and Dr. Philip Palade in Dr. Bertil Hille's laboratory (University of Washington) using the method of Hille and Campbell (1976).

In four single muscle fibers the major effect of toxin on sodium currents was to slow the inactivation of the sodium conductance with little effect on the activation process. Fig. 1 A shows the time-course of Na currents in a step depolarization to -24 mV before and in the presence of 170 nM toxin. The rate of turn-on of the currents is changed little, while the turn-off is slowed appreciably. Fig. 1 B shows on a slower time base with a different fiber that inactivation is slowed about threefold, but not made incomplete in toxin-treated muscle fibers. No unusual sodium currents are seen upon repolarization from the 10-ms test pulse. Experiments with even longer test pulses show that

inactivation is at least 90% complete in the presence of purified toxin. Crude *Leiurus* venom (Sigma Chemical Co., St. Louis, Mo.) was also tested on four other muscle fibers yielding a similar slowed, but nearly complete, inactivation. Although not studied in detail, the voltage dependence of steady-state activation and inactivation seemed to remain within a few millivolts of the normal position on the voltage axis. The peak sodium current was also reduced 5–30% after toxin or venom treatment. To permit easier comparison of time-courses, the current in the presence of toxin in Fig. 1 A was scaled up by 14%, making it appear the same size as the control. The currents in Fig. 1 B are not scaled up.

Experiments were carried out with 17 nM and 170 nM scorpion toxin solutions. At 17 nM the effect seemed incomplete, as if only 60–75% of the channels were slowly inactivating and the remainder still rapidly inactivating; whereas at 170 nM, all channels seemed to be slowly inactivating. The maximal effect was observed within less than 3 min of addition of toxin, and the effect was never appreciably reversed by washing out the toxin solution with Ringer's solution several times in a 5–10 min period. Taken together, the results of the electrophysiologic experiments indicate that scorpion toxin receptor sites having a dissociation constant (K_D) of less than 17 nM are associated with sodium channels in frog muscle, and that binding of scorpion toxin to these receptor sites results in a marked slowing of inactivation.

Binding of [¹²⁵I]-Labelled Scorpion Toxin to Receptor Sites in Frog Muscle

Purified mono[¹²⁵I]iodo scorpion toxin prepared as previously described (Catterall, 1977 *a*) was used in these experiments. This toxin derivative retains full biologic activity in experiments with neuroblastoma cells.

The rate of toxin uptake by intact sartorius muscles bathed in frog Ringer at room temperature was determined in preliminary experiments. Uptake reached a plateau after 8 h incubation. Since the toxin was effective in a few minutes when incubated with individual fibers under voltage clamp, it is likely that the long incubation is required for toxin penetration throughout the intact muscle. All subsequent incubations were carried out for 8 h.

In neuroblastoma cells, binding of scorpion toxin is inhibited by depolarization (Catterall et al., 1976; Catterall, 1977 *a*). In order to prevent depolarization of the muscles due to release of acetylcholine from the cut nerve or due to repetitive action potentials induced by scorpion toxin, incubations were carried out in frog Ringer containing 2 μ M α -bungarotoxin and 1 μ M tetrodotoxin. Neither of these toxins affects scorpion toxin binding in neuroblastoma cells or in frog muscle.

In all binding experiments, a fraction of the binding ligand associated with the tissue is bound nonspecifically to sites other than the receptor site of interest. It is therefore essential to develop criteria to identify the specific and nonspecific components of binding. Earlier studies of binding to neuroblastoma cells and the electrophysiologic experiments described above suggest three criteria by which to define specific binding to receptors associated with

sodium channels. (a) The binding should be saturable with a K_D of less than 17 nM. (b) The binding should be blocked by sea anemone toxin since these two polypeptides compete for a common receptor site in neuroblastoma cells (Catterall and Beress, 1978). (c) The binding should be blocked by depolarization since depolarization inhibits both the binding and the physiologic effect of the toxin in neuroblastoma cells. Experiments were therefore designed to measure a saturable component of binding with these characteristics.

In order to minimize variation among individual frogs, measurements of total binding and nonspecific binding were carried out on paired muscles from the same frog. One muscle was incubated with 2 nM [125 I]-labelled scorpion toxin in modified frog Ringer to measure total binding. An average of 2.6 fmol of toxin per milligram wet weight was bound under these conditions. The second muscle was incubated with the same solution containing 500 nM unlabelled toxin in addition to labelled toxin. This sample provided a measure

TABLE II
EFFECT OF GRAMICIDIN, K^+ , AND SEA ANEMONE TOXIN ON
SCORPION TOXIN BINDING

| Conditions | Specific [125 I]ScTX bound <i>fmol/mg</i> |
|--------------------------------|--|
| Control (frog Ringer) | 1.57 |
| 3 μ M Sea anemone toxin II | 0.13 |
| Control (solution 1) | 1.68 |
| 165 mM K^+ (solution 6) | 0.16 |
| Control (frog Ringer) | 1.75 |
| 10 μ g/ml Gramicidin A | 0.77 |
| 100 μ g/ml Gramicidin A | 0.57 |

Paired sartorius muscles were incubated 8 h in frog Ringer, solution 1, or solution 6 (Table I) containing 2 nM monoiodo scorpion toxin or 2 nM monoiodo scorpion toxin plus 500 nM unlabelled scorpion toxin. Sea anemone toxin II and gramicidin A were added to the incubation media where indicated. Bound scorpion toxin was measured and specific binding was determined from the difference between binding by the paired muscles. The results are the mean of six pairs of muscles in each case.

of nonsaturable (and therefore nonspecific) binding, since 500 nM unlabelled toxin is sufficient to saturate all of the physiologically relevant scorpion toxin receptor sites. An average of 1.0 fmol of toxin per milligram wet weight was bound nonspecifically. The difference between these two values represents the saturable component of binding. All data presented represent the difference between muscles treated in this manner.

The results of typical experiments are illustrated in Table II. The saturable component of binding ranged from 1.57 to 1.75 fmol/mg in the three experiments presented. Saturable binding is inhibited 92% by 3 μ M sea anemone toxin and 90% by depolarization to +2 mV with solution 6 containing 165 mM K^+ (see Methods). These agents have no effect on the nonsaturable component of binding. Saturable binding is also inhibited 67%

by depolarization with gramicidin A. These results support the conclusion that the effect of K^+ is due to depolarization rather than to a chemical effect of K^+ per se. The data with gramicidin A are not as reliable as those with sea anemone toxin and K^+ , however, because gramicidin A affects both saturable and nonsaturable binding. The results of Table II indicate that the saturable component of binding represents binding to receptor sites associated with sodium channels since it is nearly completely inhibited by sea anemone toxin and by depolarization.

The affinity of these receptor sites for unlabelled scorpion toxin was determined by measuring the competition of unlabelled scorpion toxin with binding of mono[125 I]iodo scorpion toxin (Fig. 2). Half-maximal displacement

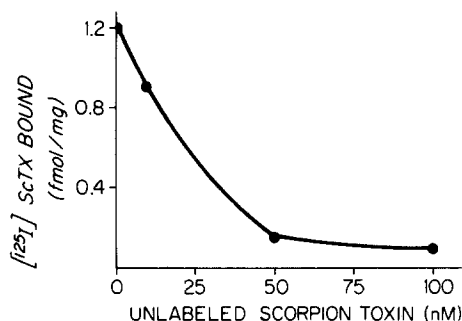


FIGURE 2. Concentration dependence of binding of unlabelled scorpion toxin ([ScTX]). Paired sartorius muscles were incubated for 8 h with 2 nM monoiodo scorpion toxin and the concentrations of unlabelled scorpion toxin indicated on the abscissa or with 500 nM unlabelled scorpion toxin in frog Ringer. Bound scorpion toxin was measured and specific binding was determined from the difference between binding by the paired samples. Specific binding is plotted as a function of unlabelled scorpion toxin concentration.

of specifically bound [125 I]-labelled scorpion toxin was observed at 15–20 nM unlabelled toxin. This concentration of unlabelled toxin is similar to the concentration required for half-maximal effect on the rate of sodium channel inactivation. Therefore, all of the criteria for definition of specific binding have been fulfilled. On the basis of the results in this section, I conclude that the saturable component of scorpion toxin binding represents binding to receptor sites associated with sodium channels in frog muscle, that sea anemone toxin competes with scorpion toxin for binding to these sites, and that depolarization inhibits toxin binding. Similar conclusions were reached in more extensive studies with neuroblastoma cells (Catterall et al., 1976; Catterall, 1977 a; Ray and Catterall, 1978; Catterall and Beress, 1978) and synaptosomes (Ray et al., 1978).

Density and Binding Affinity of Scorpion Toxin Receptor Sites in Frog Muscle

Specific and nonspecific binding of mono[125 I]iodo scorpion toxin were measured as a function of toxin concentration. Nonspecific binding measured in the presence of 500 nM unlabelled scorpion toxin varied linearly with toxin

concentration (Fig. 3, ●). The dependence of total binding on concentration fit a curve for the sum of a linear nonsaturable binding component and a hyperbolic binding component of the form $B = k[\text{ScTX}] + B_{\text{max}} [\text{ScTX}] / (K_D + [\text{ScTX}])$ (Fig. 3, ○). The specific binding component determined from the difference between total binding and the fitted line for nonspecific binding (Fig. 3) is presented as a Scatchard plot in Fig. 4. The data are consistent with the presence of a single class of binding sites with a K_D of 14 nM and a binding capacity of 13 fmol/mg wet weight. Taking a value of 0.45 cm² of muscle surface membrane per wet weight (Ritchie and Rogart, 1977), the data lead to an estimate of 170 sites/μm² of surface membrane. This density estimate

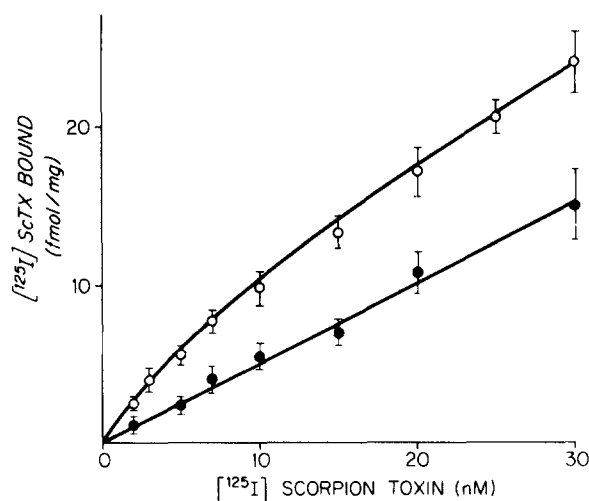


FIGURE 3. Concentration dependence of specific and nonspecific binding of monoiodo scorpion toxin. Paired sartorius muscles were incubated 8 h in frog Ringer containing the indicated concentrations of monoiodo scorpion toxin with (●) or without (○) 500 nM unlabelled scorpion toxin. Bound toxin was measured as described in Methods. The curves drawn through the points correspond to $B = 0.49 [\text{ScTX}]$ for (●) and $B = 0.49 [\text{ScTX}] + 12.7 [\text{ScTX}] / (14.3 + [\text{ScTX}])$ for (○). Error bars correspond to the standard error of the mean for 24 muscle pairs.

assumes that only surface sites are reached by the labelled toxin. Since it is possible that a significant fraction of the sodium channels in frog muscle is in the transverse (T) tubule system (Jaimovich et al., 1976), the calculated surface density would be lower if scorpion toxin reaches the T system. The density and properties of scorpion toxin receptor sites are compared to those of saxitoxin receptor sites in the Discussion.

Dependence of Scorpion Toxin Binding on Membrane Potential

Since scorpion toxin binding was markedly inhibited by depolarization (Table II), it was of interest to determine the form of the voltage dependence and to compare it to the voltage dependence of activation and inactivation of sodium

channels measured in voltage clamp experiments. In these experiments, the membrane potential was varied using the approach of Hodgkin and Horowicz (1959) by increasing extracellular K^+ while maintaining constant $[K^+][Cl^-]$ product and constant osmolarity with substitution of SO_4^{2-} and sucrose for Cl^- . The composition of the solutions used is presented in Table I. The membrane potentials presented are those measured by Hodgkin and Horowicz (1959).

Fig. 5 illustrates the dependence of scorpion toxin binding on membrane potential between -94 mV and $+2$ mV. Binding is inhibited 90% by depolarization. Half-maximal inhibition is obtained at -41 mV. The reduction in scorpion toxin binding at a low scorpion toxin concentration (2 nM) could result from an increase in K_D or a reduction in the number of available

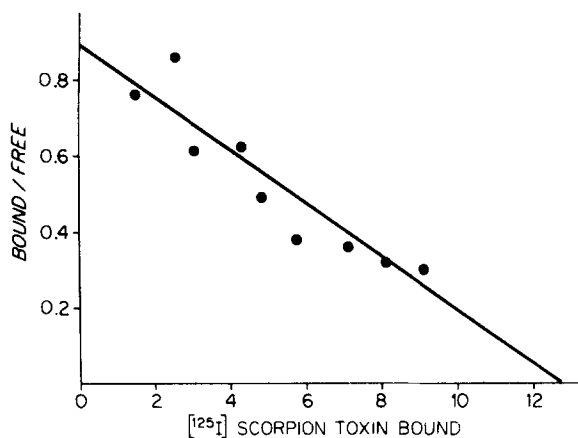


FIGURE 4. Scatchard analysis of specific scorpion toxin binding. Specific binding was calculated by subtracting the best fit value for nonspecific binding (0.49 [ScTX], Fig. 3) from measured total binding at each concentration (Figure 3). The data are presented as a Scatchard plot with $\text{fmol} \cdot \text{mg}^{-1} \cdot \text{nM}^{-1}$ as the ordinate and $\text{fmol} \cdot \text{mg}^{-1}$ as the abscissa. The regression line drawn corresponds to $B_{\text{max}} = 12.7 \text{ fmol/mg}$ and $K_D = 14.3 \text{ nM}$.

receptor sites. Because the ratio of specific to nonspecific binding is poor at high scorpion toxin concentrations (Fig. 3), it is not possible to obtain a complete binding curve under depolarized conditions in frog muscle. Therefore, this question cannot be resolved directly. However, in neuroblastoma cells (Catterall, 1977 *a*) and synaptosomes (Ray et al., 1978), depolarization causes an increase in K_D for scorpion toxin binding with no change in the number of binding sites. On the basis of those results, it is likely that the reduction in scorpion toxin binding observed in frog muscle also is due to an increase in K_D for scorpion toxin. Since K_D would then be inversely proportional to the amount of bound toxin at low toxin concentrations, the 90% reduction in binding suggests a 10-fold increase in K_D on depolarization.

The voltage dependence of activation and inactivation of sodium channels in frog skeletal muscle has been studied by voltage clamp methods in three

laboratories (Adrian et al., 1970; Ildefonse and Rougier, 1972; Campbell and Hille, 1976). Values of m_{∞} , the activation parameter of Hodgkin and Huxley (1952), are plotted as a function of membrane potential in Fig. 6 (smooth curves). The midpoints for these m_{∞} vs. V curves range from -50 to -41 mV. For comparison, the data of Fig. 5 are replotted in Fig. 6 as % inhibition of specific scorpion toxin binding (Δ). The voltage dependence of inhibition of scorpion toxin binding follows the m_{∞} curves closely. In contrast, h_{∞} vs. V curves describing the voltage dependence of inactivation of sodium channels in frog muscle have midpoints near -75 mV where scorpion toxin binding is independent of membrane potential (Adrian et al., 1970; Ildefonse and Rougier, 1972; Campbell and Hille, 1976). These results suggest that the

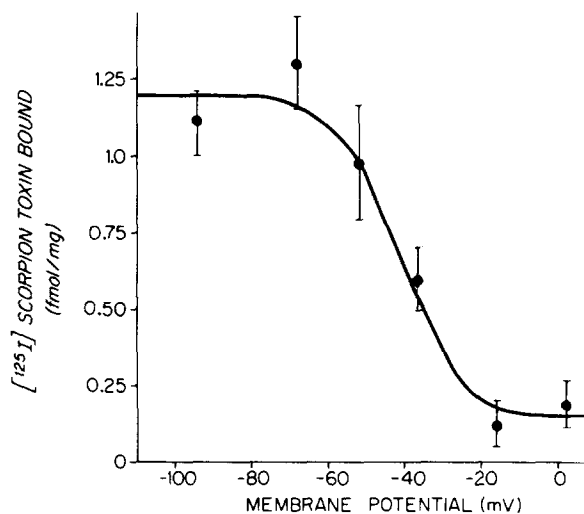


FIGURE 5. Voltage dependence of scorpion toxin binding. Paired sartorius muscles were incubated with 2 nM monoiodo scorpion toxin with and without 500 nM unlabelled scorpion toxin in solutions 1-6 (see Methods). Specifically bound toxin was calculated from the difference between the paired muscles and plotted as a function of membrane potential (see Methods). The error bars correspond to standard error of the mean. The smooth curves were drawn by eye.

voltage dependence of scorpion toxin binding arises from the voltage dependent activation of sodium channels. This result is unexpected since scorpion toxin slows inactivation markedly but has little effect on activation (Fig. 1).

Campbell and Hille (1976) showed that omission of Ca^{2+} from the frog Ringer caused a 15 mV shift in P_{Na} vs. V curves to more negative membrane potentials. In order to test the correlation between the voltage dependence of scorpion toxin binding and that of m_{∞} , parallel binding measurements were carried out in normal and Ca^{2+} -deficient Ringer. Omission of Ca^{2+} from the Ringer solution caused a marked shift in the voltage curve to more negative membrane potentials (Fig. 7). The curves drawn by eye in Fig. 7 give a 19 mV shift at their midpoints. Considering the uncertainty in the results, the

agreement between the binding data and physiological data is satisfactory. On the basis of the results of Figs. 6 and 7, I suggest the hypothesis that the voltage dependence of scorpion toxin binding results from the voltage-dependent change of state which leads to activation. This hypothesis is considered in more detail in the Discussion.

DISCUSSION

Scorpion Toxin Action in Frog Muscle

North African scorpion venoms and toxins purified from those venoms have been shown to inhibit sodium channel inactivation in frog myelinated nerve,

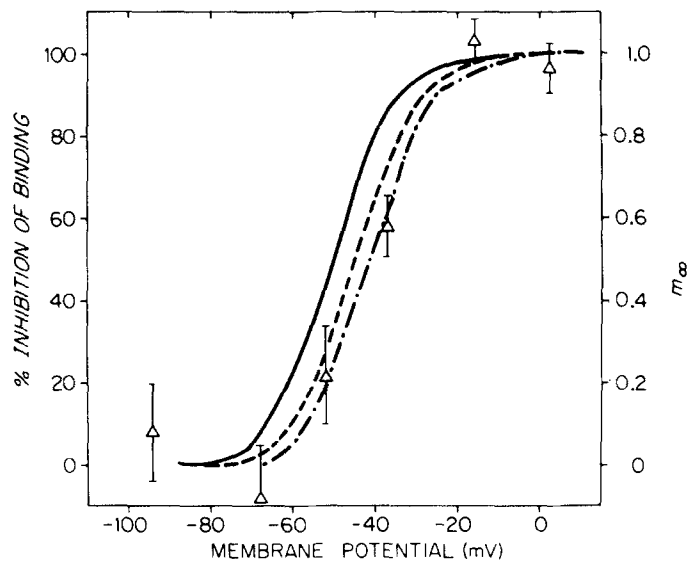


FIGURE 6. Correlation between the voltage dependence of scorpion toxin binding and m_{∞} . Percent inhibition of scorpion toxin binding (Δ) is compared to m_{∞} from the data of Adrian et al., 1970 (---), Ildefonse and Rougier, 1972 (—), and Campbell and Hille, 1976 (- - -).

invertebrate giant axons, and tunicate eggs (Koppenhöfer and Schmidt, 1968; Narahashi et al., 1972; Romey et al., 1976; Okamoto et al., 1977). Voltage clamp studies (Fig. 1 and unpublished data) show that the principal effect of both *Leiurus quinquestriatus* venom and a purified *Leiurus* toxin is to slow inactivation of sodium channels in frog muscle also. Thus, the action of *Leiurus* toxin in frog muscle is similar to its action in frog myelinated nerve. These results suggest that the pharmacological similarity of sodium channels in frog nerve and muscle described by Campbell and Hille (1976) can be extended to include interaction with scorpion toxin. Although the action of *Leiurus* toxin on frog muscle and frog nerve is generally similar, one difference was noted. In frog muscle, inactivation was greatly slowed but was always complete, whereas in frog nerve scorpion venom caused incomplete inactivation (Kop-

penhöfer and Schmidt, 1968). This difference may indicate that there are minor differences in the interaction of scorpion toxin with sodium channels in nerve and muscle.

Specificity of Scorpion Toxin Binding

The specificity of binding of scorpion toxin has been most thoroughly studied in neuroblastoma cells (Catterall, 1977 *a*). In those experiments, several lines of evidence indicated that the saturable component of scorpion toxin binding represented binding to voltage-dependent sodium channels. (*a*) The concentration curves for saturable binding of mono[¹²⁵I]iodo scorpion toxin and for enhancement of veratridine activation of sodium channels by mono[¹²⁵I]iodo scorpion toxin are superimposable. (*b*) Both binding and physiologic effect of

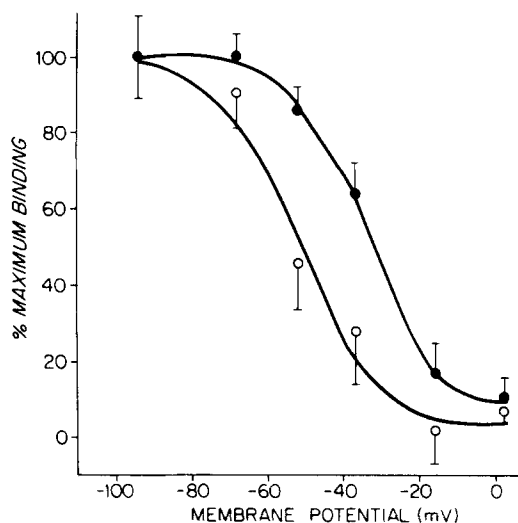


FIGURE 7. Effect of Ca^{2+} on voltage dependence of scorpion toxin binding. The voltage dependence of scorpion toxin binding was measured as described in the legend to Fig. 5 in medium with 2.0 mM Ca^{2+} (●) or in nominally Ca^{++} -free medium (○). The smooth curves were drawn by eye.

the toxin have identical voltage sensitivity. (*c*) Specific binding is absent in variant clones of neuroblastoma cells that lack sodium-dependent action potentials. (*d*) Specific binding is completely inhibited by sea anemone toxin with a concentration dependence similar to the concentration dependence of the physiologic effect of the toxin (Catterall and Beress, 1978). These data leave little doubt that scorpion toxin binds specifically to receptor sites associated with sodium channels in neuroblastoma cells.

In these experiments on frog muscle, three of the criteria of specificity developed in the experiments on neuroblastoma cells have been fulfilled. The saturable component of scorpion toxin binding has a K_D similar to the concentration giving a half-maximal physiologic effect, and saturable binding is specifically inhibited by depolarization and by sea anemone toxin. These

results support the conclusion that scorpion toxin binds specifically to receptor sites associated with voltage-sensitive sodium channels in frog muscle.

Sea anemone toxin II, like scorpion toxin, is a basic polypeptide which slows sodium channel inactivation (Romey et al., 1976; Bergman et al., 1976; Conti et al., 1976) and enhances persistent activation of sodium channels by veratridine and batrachotoxin (Catterall and Beress, 1978). The action of sea anemone toxin is inhibited by depolarization, although the effect is much smaller than observed with scorpion toxin (Catterall and Beress, 1978). Sea anemone toxin blocks scorpion toxin binding in neuroblastoma cells (Catterall and Beress, 1978), in brain synaptosomes (Ray et al., 1978), and in frog muscle (Table II). Therefore, it is likely that sea anemone toxin and scorpion toxin share a common receptor site associated with sodium channels in each of these excitable membranes.

Comparison of Binding of Saxitoxin, Tetrodotoxin, and Scorpion Toxin

Experiments in frog muscle (Almers and Levinson, 1975; Ritchie and Rogart, 1977; Jaimovich et al., 1976) and in other excitable tissues (Reed and Raftery, 1976; Ritchie et al., 1976; Catterall and Morrow, 1978) have demonstrated that tetrodotoxin and saxitoxin bind to a common receptor site associated with sodium channels. The receptor sites for saxitoxin/tetrodotoxin are distinct from the receptor sites for scorpion toxin (Catterall, 1976; Catterall and Morrow, 1978; Ray et al., 1978). Tetrodotoxin had no effect on scorpion toxin binding in frog muscle confirming that the receptor sites for these two toxins are separate and noninteracting in frog muscle as well. The receptor sites for tetrodotoxin/saxitoxin also differ from those for scorpion toxin in their voltage sensitivity. Complete depolarization of frog muscle had no effect on tetrodotoxin binding (Almers and Levinson, 1975) whereas depolarization causes a 90% inhibition of scorpion toxin binding. These results are consistent with the view that the receptor sites for scorpion toxin and tetrodotoxin are located on functionally separate components of the sodium channel.

The density of saxitoxin/tetrodotoxin binding sites in frog muscle ranges from 195 sites per μm^2 (Ritchie and Rogart, 1977) to 380 sites per μm^2 (Jaimovich et al., 1976; Almers and Levinson, 1975), referred in each case to the area of the surface membrane. The density of scorpion toxin sites (170 per μm^2 , Fig. 3) appears to be lower, although the variation among different estimates of saxitoxin/tetrodotoxin site density does not allow a quantitative comparison at present. Parallel measurements of saxitoxin (or tetrodotoxin) binding and scorpion toxin binding under identical conditions on the same muscles are required. Such measurements have been carried out on neuroblastoma cells where the ratio of specific to nonspecific binding is more favorable (Catterall and Morrow, 1978). Those results indicated that there are 2.8 saxitoxin receptor sites for each scorpion toxin receptor site. Measurements of this toxin binding ratio in frog muscle and other excitable membranes are now in progress.

Correlation of Voltage-Dependent Scorpion Toxin Binding with Activation of Sodium Channels

The voltage dependence of scorpion toxin binding is strongly correlated with the voltage dependence of m_{∞} , the Hodgkin-Huxley activation parameter (Figs. 6 and 7). In the model of Hodgkin and Huxley as applied to frog muscle, the peak conductance during voltage clamp steps to different mem-

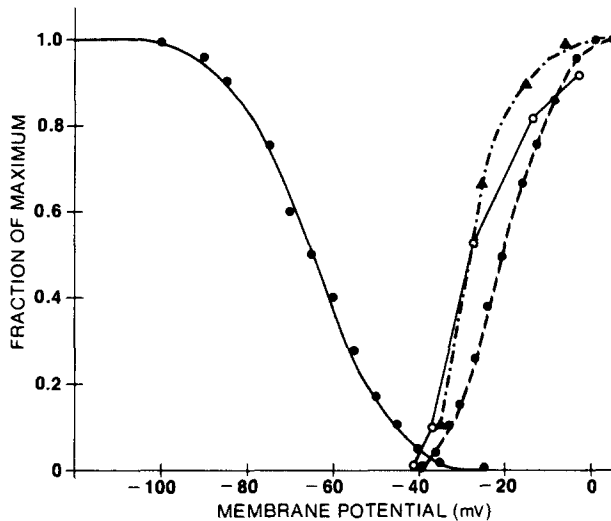


FIGURE 8. Comparison of the voltage dependence of scorpion toxin binding and sodium conductance activation and inactivation in neuroblastoma cells. Scorpion toxin binding was measured at five different membrane potentials as described (Catterall, 1977 *a*). Fractional inhibition of specific scorpion toxin binding is plotted vs. membrane potential (O). Values of sodium conductance calculated from data in the literature are presented as the fraction of the maximum conductance observed in each experiment. (●—●) Inactivation of the peak sodium conductance during voltage steps from the potentials indicated on the abscissa to -10 mV from the data of Moolenaar and Spector (1978) on clone N1E-115; (●---●) activation of the peak sodium conductance during voltage steps from -85 mV to the potentials indicated on the abscissa from the data of Moolenaar and Spector (1978) on clone N1E-115; (▲---▲) activation of the peak sodium conductance during voltage steps from -100 mV to the potentials indicated on the abscissa from the data of Veselovsky et al. (1977) on clone N18.

brane potentials is proportional to m_{∞}^3 . Curves of m_{∞}^3 vs. V for the three available sets of voltage clamp data have midpoints ranging from -41 to -32 mV. Considering the uncertainties in the binding and voltage clamp data, the correlation with m_{∞}^3 is nearly as good as with m_{∞} . Therefore, the results demonstrate a correlation between the voltage dependence of toxin binding and the voltage dependence of activation of sodium channels but are not sufficiently precise to distinguish between a correlation with m_{∞} or m_{∞}^3 .

The voltage dependence of scorpion toxin binding was first described in experiments with neuroblastoma cells (Catterall et al., 1976; Catterall, 1977 *a*). At the time those results were published, no voltage clamp data on neuroblastoma cells were available. Recently, the first voltage clamp studies of neuroblastoma cells have been described (Veselovsky et al., 1977; Moolenaar and Spector, 1978). The voltage dependence of activation and inactivation of sodium channels observed in those experiments is compared to the voltage dependence of scorpion toxin binding from my earlier work (Catterall, 1977 *a*) in Fig. 8. The inhibition of toxin binding in clone N18 cells closely parallels the activation of the peak sodium conductance in both clone N1E-115 and clone N18. Inactivation occurs at more negative membrane potentials. Preliminary estimates of m_{∞} from the data of Moolenaar and Spector (1978) also agree well with the scorpion toxin binding data. Thus, the voltage dependence of scorpion toxin binding in neuroblastoma cells also correlates closely with the voltage dependence of activation.

Although the correlation with activation is good in both these experimental systems, some caution is required in drawing conclusions. The voltage clamp measurements are made after short voltage pulses whereas the toxin binding experiments require an 8-h incubation at various membrane potentials in the frog muscle experiments or a 1-h incubation with the neuroblastoma cells. The physiologic properties of sodium channels after long-term depolarization are not well understood, and there is evidence for multiple voltage-dependent slow inactivation processes (cf. Adelman and Palti, 1969; Khodorov et al., 1976). Although none of the slow events described to date has a voltage dependence similar to that of scorpion toxin binding, it remains possible that the voltage sensitivity of scorpion toxin binding results from a slow voltage-dependent process and not from the activation process described by the voltage clamp data. This problem cannot be resolved using currently available methods since the binding and physiologic experiments must be carried out on different time scales. In view of these uncertainties, the interpretation of the results which follows is presented as a hypothesis.

The correlation of the voltage dependence of scorpion toxin binding with the voltage dependence of activation suggests that the change of state leading to activation results in a conformational change at the scorpion toxin receptor site leading to reduced affinity for scorpion toxin. Scorpion toxin may therefore bind to a component of the sodium channel which senses the membrane potential and undergoes a voltage-dependent conformation change leading ultimately to activation of the ion channel. If this interpretation is correct, measurements of scorpion toxin binding provide information on the functional state of a component of the sodium channel without measurement of the resulting changes in sodium permeability. The results provide direct chemical evidence for a voltage-dependent conformational change which is initiated by depolarization and which is maintained throughout the period of depolarization. This conformational change has some of the properties expected of the "activation gate" or m process of Hodgkin and Huxley (1952). Therefore, these results provide direct chemical support for one of the main features of the Hodgkin-Huxley model, the existence of a regulatory component control-

ling activation of sodium channels which undergoes a voltage-dependent change of state that is maintained during depolarization as the ion channel activates and then inactivates.

Gating current experiments have also provided direct evidence for a voltage-dependent change of state associated with activation of sodium channels that is maintained during depolarization as the ion channel activates and then inactivates (Bezanilla and Armstrong, 1974; Armstrong and Bezanilla, 1977). Although gating currents of sodium channels in frog muscle have not been studied, the close correspondence between the voltage dependence of gating currents and the voltage dependence of activation in squid giant axon (Keynes and Rojas, 1974; Armstrong and Bezanilla, 1977) and in frog myelinated nerve (Nonner et al., 1975) suggests that gating current and scorpion toxin binding may have similar voltage dependence in frog muscle. These two different experimental approaches may therefore detect the same or closely related voltage-dependent changes of state.

It is initially surprising that scorpion toxin blocks inactivation whereas the voltage dependence of toxin binding correlates with activation. However, gating current experiments show that inactivation is coupled to activation and may derive much of its voltage dependence from the activation process (Armstrong and Bezanilla, 1977). If this is correct, scorpion toxin may bind to a region of the sodium channel involved in voltage-dependent activation and interfere with the coupling of activation to inactivation causing a slowing of the rate of inactivation. These considerations also suggest an explanation for the marked difference in action of the North African scorpion venoms and toxins, which block inactivation, and the venom of the North American scorpion *Centruroides sculpturatus*, which alters activation (Cahalan, 1974). These different scorpion toxins may bind to a common receptor site on a component of the sodium channel involved in activation but, due to structural differences, exert their major effect either on the process of activation itself or on the coupling of activation to inactivation. Further experiments are required to test these ideas.

Our results show that scorpion toxin can be used as a specific molecular probe of functional properties of sodium channels. The toxin should be a valuable tool in studying the functional properties of sodium channels in membrane preparations and in solubilized and purified preparations of sodium channels.

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