Specific binding of \([^{3}H]\)batrachotoxinin A 20-\(\alpha\)-benzoate to sodium channels in synaptosomes has been studied. Specific binding is increased 10- to 20-fold by the polypeptide scorpion toxin which binds at a different receptor site on the sodium channel. The \(K_d\) for enhancement of \([^{3}H]\)batrachotoxinin A 20-\(\alpha\)-benzoate binding by scorpion toxin is 35 nM in medium containing 5 mM K\(^+\). Depolarization of synaptosomes with 135 mM K\(^+\) increases \(K_d\) to 300 nM. The \(K_d\) for scorpion toxin binding to its receptor site is closely correlated with \(K_d\) for enhancement of binding of \([^{3}H]\)batrachotoxinin A 20-\(\alpha\)-benzoate at both membrane potentials. Sea anemone toxin II has the same effect as scorpion toxin at 200-fold higher concentration. In the presence of scorpion toxin, the alkaloids batrachotoxin, veratridine, and aconitine block binding of \([^{3}H]\)batrachotoxinin A 20-\(\alpha\)-benzoate with \(K_d\) values of 0.05 \(\mu\)M, 7 \(\mu\)M, and 1.2 \(\mu\)M, respectively. These \(K_d\) values are closely correlated with values of \(K_d\) for activation of sodium channels by these alkaloid toxins. Scatchard analysis of binding of \([^{3}H]\)batrachotoxinin A 20-\(\alpha\)-benzoate binding in the presence of 1 \(\mu\)M scorpion toxin indicates a single class of sites with a \(K_d\) of 82 nM and a binding capacity of 2.1 \(\pm\) 0.2 pmol/mg of protein. At lower scorpion toxin concentrations, \(K_d\) is increased with no change in binding capacity. The results demonstrate the value of \([^{3}H]\)batrachotoxinin A 20-\(\alpha\)-benzoate as a binding probe of the polypeptide neurotoxin receptor site on the sodium channel and provide direct confirmation of the allosteric mechanism of toxin binding and action at this site proposed previously.

Voltage-sensitive sodium channels in nerve and neuroblastoma cells have three separate receptor sites for neurotoxins associated with protein components of the channels (for reviews see Refs. 1-3). The inhibitors tetrodotoxin and saxitoxin bind at receptor site 1 and inhibit ion flux through the sodium channel (2). Grayanotoxin and the alkaloids veratridine, batrachotoxin, and aconitine act at receptor site 2 to cause persistent activation of sodium channels (1, 3). The polypeptides scorpion toxin and sea anemone toxin bind at receptor site 3 (3). These toxins slow sodium channel inactivation (reviewed in Ref. 3) and enhance persistent activation of sodium channels by toxins acting at site 2 through an allosteric mechanism (4, 5).

Neurotoxins acting at these three receptor sites have been valuable probes of sodium channel structure and function. Radioactive derivatives of saxitoxin, tetrodotoxin, scorpion toxin, and sea anemone toxin have been prepared and used in direct binding studies to examine the properties of receptor sites 1 and 3 (2, 6-9). Direct binding studies of receptor site 2 have proven difficult because the ligands acting at that site are hydrophobic and have relatively low affinity. Recently, Brown et al. have described the preparation of a radiolabeled congener of batrachotoxin, batrachotoxinin A 20-\(\alpha\)-benzoate, shown that this derivative retains full biological activity, and studied its binding to lysed and washed membrane preparations from mouse brain (10). Specific binding of \([^{3}H]\)BATX-B\(^{1}\) was observed but, because of the low affinity and high lipid solubility of the toxin, specific binding was less than 10% of total binding (10). One approach to circumvent these difficulties is to take advantage of the positively cooperative interaction between batrachotoxin and scorpion toxin (4, 5) to enhance the affinity of \([^{3}H]\)BATX-B binding and thereby increase the ratio of specific to nonspecific binding.

The binding and action of scorpion toxin are highly voltage- and temperature-dependent (5, 7). Synaptic nerve ending particles (synaptosomes) prepared from rat brain retain a membrane potential (11, 12) and bind scorpion toxin with high affinity (13, 14). Scorpion toxin binding is blocked by depolarization or lysis of the synaptosomes (13, 14) and is markedly enhanced by batrachotoxin (13, 15). The enhancement by batrachotoxin reflects a direct allosteric interaction between receptor sites 2 and 3 (12-16). Ion flux studies show that sodium channels in synaptosomes have the same functional properties as those in intact nerve cells (15-17). Synaptosomes therefore provide a useful experimental system in which to study the effect of scorpion toxin on \([^{3}H]\)BATX-B binding. In the experiments described here, we show that scorpion toxin enhances \([^{3}H]\)BATX-B binding to synaptosomes 10- to 20-fold, allowing convenient measurement and investigation of binding at neurotoxin receptor site 2.

**EXPERIMENTAL PROCEDURES**

**Materials**—Chemicals were obtained from the following sources: veratridine from Aldrich; tetrodotoxin from Calbiochem; and scorpion venom (Leiurus quinquestriatus) and aconitine from Sigma. Batrachotoxin was purified as described by Tokuyama et al. (18). BTX-B and \([^{3}H]\)BTX-B were prepared and purified as described by Brown et al. (10). \([^{3}H]\)BTX-B is stable in storage for up to 1 year and is stable at 36 °C for the duration of these experiments. Scorpion toxin was

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1. The abbreviations used are: BTX-B, batrachotoxinin A 20-\(\alpha\)-benzoate; \([^{3}H]\)ScTX, scorpion monol\(^{3}\)Iodo toxin.
purified, labeled with $^{131}$T by lactoperoxidase-catalyzed iodination, and repurified by ion exchange chromatography as described by Catterall (7). Scorpion mono-$^{125}$Iodo toxin was used in the experiments presented. Anemone sulcata toxin II was purchased from Ferring GmbH, Kiel, West Germany.

Preparation of Synaptosomes—Synaptosomes were prepared by a combination of differential and density gradient centrifugation by a modification of the method of Gray and Whittaker (19) as described previously (13).

Measurement of $[^{3}H]$BTX-B Binding—$[^{3}H]$BTX-B binding was measured using a modification of the procedure previously described for measuring scorpion toxin binding. The standard binding medium contained 130 mm choline chloride, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (adjusted to pH 7.4 at 36°C with Tris base), 5.5 mm glucose, 0.8 mm MgSO$_4$, 5.4 mm KCl, 1 mg/ml of bovine serum albumin, and 1 $\mu$m tetrodotoxin. Tetrodotoxin blocks sodium channels noncompetitively with respect to both polypeptide toxins and alkaloid toxins (reviewed in Ref. 3). It therefore has no effect on binding of $[^{3}H]$BTX-B (10). It was included in all incubation solutions in order to prevent ion movements through sodium channels activated by scorpion toxin plus BTX-B. Binding reactions were initiated by addition of synaptosomes (approximately 200 $\mu$g of protein) suspended in 100 $\mu$l of standard binding medium to a reaction mixture containing 10 nM $[^{3}H]$BTX-B and other effectors as noted in the figure legends in 150 $\mu$l of standard binding medium. Samples were mixed and incubated for 30 min at 36°C. The reactions were stopped by addition of 3 ml of ice-cold wash medium consisting of 163 mm choline chloride, 5 $\text{mM}$ 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (adjusted to pH 7.4 at 36°C with Tris base), 1.8 $\text{mM}$ CaCl$_2$, 0.8 $\text{mM}$ MgSO$_4$, and 1 $\mu$g/ml of bovine serum albumin. The synaptosomes were immediately collected on glass fiber filters (Whatman GF/C) under vacuum and washed 3 times with 3 ml of wash medium. The filters were suspended in liquid scintillation fluid and $[^{3}H]$cpm determined in a liquid scintillation spectrometer. Synaptosomal protein was determined by the method of Peterson (20) using bovine serum albumin as standard and data recorded as fmol $[^{3}H]$BTX-B bound per mg of protein.

In preliminary experiments, the dependence of bound $[^{3}H]$BTX-B on total synaptosomal protein in the assay was determined. Bound $[^{3}H]$BTX-B increased linearly with synaptosomal protein up to 250 $\mu$g/assay. A protein concentration of 300 $\mu$g/assay was chosen for subsequent work.

Measurement of $[^{3}H]$ScTX Binding—Scorpion toxin binding was measured exactly as described for $[^{3}H]$BTX-B except that 10 nM BTX-B or batrachotoxin was substituted for $[^{3}H]$BTX-B in the assay and 0.1 nM $[^{125}]$ScTX was added. The washes of the GF/C filters were performed at 36°C, since nonspecifically bound scorpion toxin is more efficiently removed at that temperature. Nonspecific binding of $[^{125}]$ScTX was determined in the presence of 200 nM unlabeled scorpion toxin in experiments in standard binding medium, or in the presence of 20 $\mu$m unlabeled scorpion toxin in experiments with depolarized synaptosomes in standard binding medium with 130 $\mu$m KCl replacing the choline chloride. $[^{125}]$ScTX bound to filters was determined in a gamma scintillation spectrometer.

Data Analysis—Except where specifically mentioned, the data presented are the results of a single experiment that are representative of four or more similar experiments. All data points are the average of 2 or 3 replicate determinations. Unless specifically illustrated, standard errors were less than $\pm$10% of the maximum specific binding observed. Smooth curves describing the data points were drawn by eye. Straight lines on Scatchard and Hill plots were computed by linear regression.

RESULTS

Enhancement of $[^{3}H]$BTX-B Binding by Polypeptide Neurotoxins—In ion flux studies with synaptosomes (15), veratridine, aconitine, and batrachotoxin were shown to compete for a common receptor site as in neuroblastoma cells (5). Veratridine activation of sodium channels in synaptosomes was half-maximal at 15 $\mu$m (15, 16). In previous binding studies (10), veratridine competed with $[^{3}H]$BTX-B with a $K_d$ of approximately 2 $\mu$m. Therefore, in this study we have determined nonspecific binding by measuring $[^{3}H]$BTX-B bound in the presence of 300 $\mu$m veratridine. The experiment in Fig. 1 illustrates the effect of scorpion toxin on specific and nonspecific binding of 10 nM $[^{3}H]$BTX-B to sodium channels in synaptosomes. Nonspecific binding (○) is unaffected by scorpion toxin. Total binding (●) is markedly enhanced by scorpion toxin. In the absence of scorpion toxin, specific binding (the difference between total and nonspecific) was 8 fmol. In the presence of 1 $\mu$m scorpion toxin, specific binding was increased to 158 fmol. Under these conditions, 83% (range, 70 to 85%) of total binding was specific. Thus, scorpion toxin causes a 20-fold increase in specific binding of $[^{3}H]$BTX-B and improves the ratio of specific to nonspecific binding dramatically.

Fig. 1 also illustrates the saturation of the scorpion toxin receptor by scorpion toxin (△). Synaptosomes were incubated with 0.1 nM scorpion mono-$^{125}$Iodo toxin and increasing concentrations of unlabeled scorpion toxin under the conditions of the $[^{3}H]$BTX-binding assay and bound $[^{125}]$labeled scorpion toxin was measured by filtration on glass fiber filters as for $[^{3}H]$BTX-B. The results show that saturation of the scorpion toxin receptor site with unlabeled scorpion toxin occurs over the same concentration range as enhancement of $[^{3}H]$BTX-B binding by scorpion toxin. Thus, binding of scorpion toxin to receptor site 3 leads to a marked increase in affinity for batrachotoxin at receptor site 2.

The correlation between the $K_d$ for scorpion toxin binding and the $K_{d,5}$ for enhancement of $[^{3}H]$BTX-B binding is not exact. In Fig. 1, 36 nM scorpion toxin was required for a half-maximal increase in $[^{3}H]$BTX-B binding, whereas only 15 nM scorpion toxin was required to saturate half the sites labeled by $[^{125}]$ScTX. In several experiments, there was a consistent 2- to 4-fold difference between $K_{d,5}$ and $K_{d}$. This difference is probably due to the voltage-dependence of scorpion toxin binding (7, 13, 14). Only synaptosomes having a substantial resting membrane potential will bind scorpion toxin with high affinity. Thus, the titration curve for block of $[^{125}]$ScTX binding reflects mainly the contribution of synaptosomes with high affinity, whereas the titration curve for enhancement of $[^{3}H]$BTX-B binding represents the contribution of all synaptosomes equally. The exact correlation observed between $K_{d}$ and $K_{d,5}$ in depolarized synaptosomes (see below) supports the conclusion that scorpion toxin binding and enhancement of $[^{3}H]$BTX-B binding result from scorpion toxin binding at a single receptor site.

The time course of formation and dissociation of the $[^{3}H]$
Scorpion toxin is required to give a half-maximal enhancement of synaptosomal resting membrane potential (Fig. 1). In other experiments (not shown) binding at 45 and 60 min was the same or slightly lower than at 30 min. All subsequent experiments were incubated at 36 °C for 30 min.

In order to measure the rate of dissociation of the toxin-receptor complex, synaptosomes were incubated with 10 nM [³H]BTX-B and 1 μM scorpion toxin for 30 min at 36 °C, veratridine was added to a final concentration of 200 μM to stop the binding reaction, and samples were filtered at increasing times after addition of veratridine to measure bound [³H]BTX-B. At 36 °C, the toxin receptor complex dissociates with a half-time of approximately 60 min (Fig. 2B, C). At 0 °C, dissociation is greatly slowed. In fact, no loss of specifically bound [³H]BTX-B is observed during a 50-min experiment (Fig. 2B, O). The slow rate of toxin dissociation correlates well with the slow rate of reversal of batrachotoxin activation of sodium channels (t½ = 30 to 60 min at 36 °C in neuroblastoma cells (11)). With this slow dissociation rate, loss of bound [³H]BTX-B during filtration can be ignored.

Scorpion toxin binding and action are voltage-dependent (6, 7, 13). In synaptosomes, depolarization inhibits high affinity scorpion toxin binding (13) and increases the concentration of scorpion toxin required to enhance ion flux activation. Fig. 3 illustrates scorpion toxin enhancement of [³H]BTX-B binding in the presence of 135 mM K⁺ which completely depolarizes synaptosomes (11, 12). In depolarized synaptosomes, 300 nM scorpion toxin is required to give a half-maximal enhancement of [³H]BTX-B binding (Fig. 3, O) compared to 35 nM at the resting membrane potential (Fig. 1). We have also estimated the Kd for scorpion toxin directly in scorpion toxin binding experiments carried out in 135 mM K⁺. Since the Kd is greatly increased under these conditions, the ratio of specific to non-specific binding is poor and the binding data have large standard errors. In the experiments illustrated in Fig. 3, total binding was 1.20 fmol and nonspecific binding 0.87 fmol. Increasing concentrations of unlabeled scorpion toxin displaced specifically bound [³H]ScTX with a Kd of approximately 300 nM (Fig. 3, O). Therefore, there is a close correlation between Kd for scorpion toxin binding and Kd for the scorpion toxin enhancement of [³H]BTX-B binding with depolarized synaptosomes in 135 mM K⁺.

Sea anemone toxin II from A. sulcata competes with scorpion toxin for binding at receptor site 3 and has similar physiologic effects (8, 22). In depolarized synaptosomes, a sea anemone toxin II has 100-fold lower affinity than scorpion toxin (13, 14). Fig. 4 illustrates the effect of sea anemone toxin II on [³H]BTX-B binding (C). Like scorpion toxin, sea anemone toxin II enhances [³H]BTX-B binding. The effect of sea anemone toxin II does not occur at concentrations over the range studied. It is clear, however, that a 100- to 200-fold higher concentration of sea anemone toxin II is required to give an enhancement to scorpion toxin.

The results presented so far show that the enhancement of [³H]BTX-B binding by scorpion toxin has the characteristics expected for binding and action of scorpion toxin at receptor site 3 as previously described (7, 8, 22). The observed enhancement occurs over approximately the same concentration range as scorpion toxin binding, Fig. 1 is voltage-dependent (Fig. 3), and requires 200-fold greater concentrations of sea anemone toxin II for an equivalent effect (Fig. 4). Our results therefore provide direct evidence for allosteric coupling between neurotoxin receptor sites 2 and 3 that results in enhancement of alkalioid toxin binding at receptor site 2 in response to polypeptide toxin binding at receptor site 3. This allosteric interaction has been inferred previously from ion flux studies (5, 15, 16).

**Competitive Interactions among Alkaloid Neurotoxins**—In ion flux studies, batrachotoxin is a full agonist activating all the sodium channels in neuroblastoma cells or synaptosomes (4, 5, 15, 16), whereas aconitine, grayanotoxin, and veratridine are partial agonists activating only a fraction of sodium channels at saturation (4, 5, 15, 16). Veratridine and aconitine act as competitive antagonists of activation of sodium channels by the full agonist batrachotoxin (4, 5, 15). These results led to the conclusion that these three alkaloid toxins and grayanotoxin act at a common receptor site on the sodium channel (4, 5, 15). Brown et al. (10) have found that batrachotoxin, veratridine, and grayanotoxin inhibit the specific component of binding of [³H]BTX-B in agreement with interaction of these three toxins at a common receptor site.
The results of Fig. 5 show that batrachotoxin (●), aconitine (△), and veratridine (○) all completely inhibit specific [3H] BTX-B binding in the presence of scorpion toxin. The displacement curves for the three toxins are not precisely parallel (Fig. 5). Hill plots of the same data give straight lines with apparent Hill numbers of 1.0 for veratridine, 0.88 for aconitine, and 0.85 for batrachotoxin. While Hill coefficients less than 1.0 are indicative of negative cooperativity or heterogeneity of binding affinity, we believe these small deviations from a value of 1.0 are not significant for considerations of the mechanism of toxin action. Thus, we consider the linearity of the Hill plots and the values of the Hill coefficients to be consistent with the presence of a single class of noninteracting receptor sites for these toxins.

The $K_d$ values for the three alkaloid toxins are in close agreement with values of $K_{a,b}$ for activation of sodium channels in synaptosomes and neuroblastoma cells as measured in ion flux studies (Table I). Although there is some tendency for $K_d$ values to be less than $K_{a,b}$ values measured in synaptosomes, this probably results from slight underestimation of $K_{a,b}$ in ion flux studies due to the difficulties in measuring true initial rates of $^{22} \text{Na}^+$ uptake after full activation of sodium channels in synaptosomes because of their small internal volume. Values of $K_{a,b}$ for neuroblastoma cells, in which initial rates of influx are more easily determined, agree somewhat more closely with the $K_d$ values (Table I). In either case, the agreement is close enough to provide strong support for the conclusion that the three alkaloid toxins bind at a common receptor site in causing persistent activation of sodium channels. [3H]BTX-B provides the first labeled derivative of these toxins for which specific binding can be measured.

**Table I**

**Comparison of $K_d$ and $K_{a,b}$ values for alkaloid toxins**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Preparation</th>
<th>Toxin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$ (μM)</td>
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<td>Batrachotoxin</td>
<td>0.05</td>
</tr>
<tr>
<td>$K_{a,b}$ (μM)</td>
<td>Synaptosomes</td>
<td>Veratridine</td>
<td>0.03</td>
</tr>
<tr>
<td>$K_{a,b}$ (μM)</td>
<td>Synaptosomes</td>
<td>Aconitine</td>
<td>0.03</td>
</tr>
<tr>
<td>$K_{a,b}$ (μM)</td>
<td>Neuroblastoma</td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>

![Fig. 4. Enhancement of [3H]BTX-B binding by scorpion toxin and sea anemone toxin.](image)

[3H]BTX-B binding was measured as described under "Experimental Procedures" in standard binding medium containing 10 nm [3H]BTX-B, 1 μm tetrodotoxin, and increasing concentrations of scorpion toxin (●) or sea anemone toxin II (○). Nonspecific binding in the presence of 300 μm veratridine has been subtracted from the data.

![Fig. 5. Competitive displacement of bound [3H]BTX-B by batrachotoxin, veratridine, and aconitine.](image)

Synaptosomes were incubated for 10 min at 36 °C with the indicated concentrations of batrachotoxin (●), aconitine (△), or veratridine (○) in standard binding medium containing 1 μm scorpion toxin and 1 μm tetrodotoxin. [3H]BTX-B was then added in a small volume to a final concentration of 10 nm and the samples were incubated 30 min at 36 °C. Bound [3H]BTX-B was determined as described under "Experimental Procedures." Nonspecific binding in the presence of 300 μm veratridine has been subtracted from the data.

![Fig. 6. Scatchard analysis of [3H]BTX-B binding.](image)

**A.** Binding of 10 nm [3H]BTX-B was measured in standard binding medium containing 1 μm scorpion toxin, 1 μm tetrodotoxin, and increasing concentrations of unlabeled BTX-B (●). Nonspecific binding in the presence of 300 μm veratridine is illustrated by the dotted line. **B.** Specific binding calculated from the data in A is illustrated as a Scatchard plot. At each concentration of BTX-B from the data in A, the cpm of specifically bound [3H]BTX-B was determined and converted to total specifically bound BTX-B (labeled plus unlabeled) using the specific radioactivity appropriate for the concentration of unlabeled BTX-B added. Free BTX-B (labeled plus unlabeled) was calculated as the difference between total added BTX-B and bound BTX-B. Bound/free was then plotted versus bound in the form of a Scatchard plot. This method is identical with saturating the receptors with [3H]BTX-B since the labeled and unlabeled compounds are chemically identical.
**Batrachotoxin Receptor Site on the Sodium Channel**

![Scatchard analysis of the effect of scorpion toxin on \([^3H]BTX-B binding.\)](image)

**DISCUSSION**

[^3H]BTX-B is the first ligand at neurotoxin receptor site 2 of the sodium channel which has shown promise as a probe in receptor binding studies (10). Previous work with[^3H]veratridine (23), and [^3H]grayanotoxin (24) detected only nonspecific binding. The results described here greatly improve prospects for use of[^3H]BTX-B as a receptor probe. In the presence of scorpion toxin, binding of[^3H]BTX-B is enhanced 10- to 20-fold so that approximately 75% of the toxin binding enhances activation of sodium channels by the alkaloid toxins (4,5,15,16,22). Concentration-effect curves for the full agonist batrachotoxin are shifted to lower concentrations with no change in the maximum sodium permeability (4,5,15,16,22). Concentration-effect curves for the partial agonists are shifted to lower concentrations and the maximum sodium permeability is increased (4,5,15,16,22). The allosteric model presented previously accurately describes this heterotropic cooperative interaction (5). The activation of sodium channels by alkaloid toxins is assumed to result from preferential binding to active (A) states of sodium channels with respect to inactive (T) states. Scorpion toxin enhances alkaloid toxin action by reducing the value of the allosteric equilibrium constant, \(K_{	ext{ER}}\), for the transition from inactive (nonconducting) states to active states. In this case, the concentration of alkald which occupies 50% of the receptor sites (\(K_D\)) depends on \(M_{	ext{ER}}\) and the equilibrium dissociation constants for the inactive states (\(K_T\)) and the active states (\(K_A\)) according to Equation 1 as derived in References 5 and 7.

\[
K_D = \frac{M_{	ext{ER}} + 1}{K_T + \frac{1}{K_A}}
\]

Since, for the sodium channel, \(M_{	ext{ER}} \gg 1\) (5), \(K_D\) should vary linearly with \(M_{	ext{ER}}\). It is expected therefore, that scorpion toxin and sea anemone toxin II will reduce the \(K_D\) for[^3H]BTX-B and enhance[^3H]BTX-B binding. The results of Figs. 1, 3, 4, and 7 show that this expectation is fulfilled.[^3H]BTX-B binding is increased at least 10-fold by scorpion toxin. The \(K_A\) for[^3H]BTX-B in the presence of scorpion toxin (80 nm) is 9-fold less than that reported in the absence of scorpion toxin (700 nm, 10). Thus, the change in \(K_D\) can account for both the increased binding observed in the presence of scorpion toxin and the 10-fold reduction in \(K_{	ext{ER}}\) for batrachotoxin action observed in neuroblastoma cells (4,5,22) and synaptosomes.
(15, 16) in the presence of scorpion toxin. These results provide quantitative confirmation of the allosteric model used to account for enhancement of alkaloid toxin action by polypeptide toxins.

Our results also provide an estimate of the batrachotoxin receptor site density in synaptosomes. The binding capacity for [3H]BTX-B is 2.1 ± 0.2 pmol/mg of protein. This compares to average values of 1.4 and 1.7 pmol/mg of protein for scorpion toxin receptor sites determined in two previous investigations using the methods employed in the present work (13, 29). Since only synaptosomes which retain a membrane potential have high affinity receptor sites for scorpion toxin (13, 14), scorpion toxin binding underestimates the total density of scorpion toxin receptors by approximately 15% (13). In view of this, there are approximately equal densities of scorpion toxin receptor sites and batrachotoxin receptor sites in synaptosomes. In contrast, synaptosomes prepared as in this work have 4.4 pmol saxitoxin receptor sites/mg of protein (29). Thus, there are 2 to 3 times as many saxitoxin receptor sites as scorpion toxin or batrachotoxin receptor sites in synaptosomes prepared as in this work. This result may reflect the presence of sodium channels having an intact saxitoxin receptor site and missing or low affinity scorpion toxin and/or batrachotoxin receptor sites, or it may reflect a 2:1 or 3:1 stoichiometry of protein components bearing these receptor sites in the sodium channel structure.

Other workers have also studied neurotoxin binding in synaptosome preparations. Since varying methods have been used for synaptosome preparation, labeled toxin preparation and characterization, toxin binding assay, and protein assay, it is instructive to review the range of results obtained. Estimates for saxitoxin binding capacity range from 1.4 pmol/mg of protein to 4.4 pmol/mg of protein (29, 32, 33). Estimates of scorpion toxin binding capacity range from 0.3 pmol/mg of protein to 1.7 pmol/mg of protein (9, 13, 14). This broad range of results emphasizes the difficulties in determining binding capacities and stoichiometries when these values depend critically on the purity, integrity, and membrane potential of the synaptosome preparation, the purity and experimentally determined specific radioactivity of the labeled toxins used, and extent of proteolysis and other losses of labile binding activities that occur during isolation and assay. We consider the binding capacities and stoichiometries derived from this work to be working estimates that may require revision as our understanding and control of critical experimental factors improves.

REFERENCES


