Sea Anemone Toxin and Scorpion Toxin Share a Common Receptor Site Associated with the Action Potential Sodium Ionophore*

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Toxin II isolated from the sea anemone Anemonia sulcata enhances activation of the action potential sodium ionophore of electrically excitable neuroblastoma cells by veratridine and batrachotoxin. This heterotropic cooperative effect is identical to that observed previously with scorpion toxin but occurs at a 110-fold higher concentration. Depolarization of the neuroblastoma cells inhibits the effect of sea anemone toxin as observed previously for scorpion toxin. Specific scorpion toxin binding is inhibited by sea anemone toxin with $K_D = 90$ nm. These results show that the polypeptides scorpion toxin and sea anemone toxin II share a common receptor site associated with action potential sodium ionophores.

Extracts of sea anemone nematocysts cause repetitive action potentials in crustacean giant axons (1) by inhibiting the inactivation process of the action potential sodium ionophore (2). Recently, three polypeptide toxins have been purified from the sea anemone Anemonia sulcata (3, 4). The most abundant of these (designated sea anemone toxin II) inhibits inactivation of sodium ionophores in crustacean giant axons and in frog myelinated nerve (5–7).

Venom of the scorpions Leiurus quinquestriatus and Buthus tamulus and a polypeptide toxin purified from L. quinquestriatus venom also inhibit inactivation of sodium ionophores in nerve axons and muscle (8, 9). Scorpion toxin binds to a specific receptor site associated with action potential sodium ionophores of electrically excitable neuroblastoma cells (10, 11). Binding to this site is highly dependent upon membrane potential (10, 11). Occupancy of this receptor site by scorpion toxin causes a striking enhancement of activation of sodium ionophores by veratridine, batrachotoxin, aconitine, and greyanotoxin (12, 13). This heterotropic cooperative interaction can be described by a simple allosteric model, which assumes that the activating toxins bind with high affinity to the active state of the sodium ionophore and that scorpion toxin reduces the energy required for the transition from the inactive to the active state (13).

Since both sea anemone and scorpion toxins are polypeptides and inhibit inactivation of sodium ionophores, it seemed likely that these two toxins might act by a common mechanism. In these experiments, we have compared the interaction of sea anemone and scorpion toxins with specific receptor sites associated with sodium channels in electrically excitable neuroblastoma cells.

EXPERIMENTAL PROCEDURES

Materials—Sea anemone toxin II was purified from A. sulcata as described previously (3, 4). Scorpion toxin was purified from the venom of L. quinquestriatus (13) and iodinated (11) as described previously. Batrachotoxin was kindly provided by Drs. J. Daly and B. Witkop (Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health). Other materials were purchased commercially as previously described (11).

Cell Cultures—Clone N18 of mouse neuroblastoma C1300 was used for all studies. Cell cultures were prepared as described previously (12).

Sodium Permeability Measurements—Sodium permeability was determined by measuring the initial rate of $^{22}\text{Na}^+$ influx from medium of low sodium concentration (10 mm) after inhibition of (Na$^+$,K$^+$)-ATPase by ouabain. The details of these procedures have been described previously (13, 14). Control experiments have shown that $^{22}\text{Na}^+$ influx ($J_{\text{Na}}$) is linearly related to sodium permeability ($P_{\text{Na}}$) under these conditions (14).

Scorpion Toxin Binding Measurements—Scorpion toxin binding was measured exactly as described previously using pure scorpion monocrotophos as the binding ligand (11).

RESULTS

Cooperative Activation of Sodium Ionophores by Sea Anemone Toxin and Veratridine or Batrachotoxin—Sea anemone toxin has no effect on sodium permeability when added to neuroblastoma cells alone (Fig. 1, 0). When electrically excitable neuroblastoma cells are incubated with increasing concentrations of sea anemone toxin for 30 min and rinsed, and then sodium permeability is measured in the presence of 200 $\mu$M veratridine, sea anemone toxin causes a large increase in veratridine-dependent sodium permeability (Fig. 1, $\Delta$). Both the increase in sodium permeability caused by veratridine alone (Fig. 1, points on ordinate) and the increase caused by veratridine plus sea anemone toxin are completely inhibited by 1 $\mu$M tetrodotoxin, a specific inhibitor of action potential sodium ionophores (Fig. 1, C). These results are similar to those described previously for scorpion toxin (13) and suggest a heterotropic cooperative interaction between veratridine and sea anemone toxin in activating the action potential sodium ionophore.

In order to study this interaction in more detail, complete concentration-effect curves were determined for veratridine...
and batrachotoxin in the presence of different fixed sea anemone toxin concentrations (Fig. 2). Under the conditions of these experiments, veratridine is a partial agonist activating approximately 8% of the sodium ionophores (14). Sea anemone toxin increases the fraction of sodium ionophores activated at saturating concentrations of veratridine and reduces the concentration of veratridine required to give 50% maximum activation (Fig. 2, left). Batrachotoxin is a full agonist activating greater than 95% of the sodium ionophores (14). Sea anemone toxin reduces \( K_{D,5} \) for batrachotoxin (Fig. 2, right) but has little effect on \( P_{s} \) since nearly all the sodium ionophores are activated by batrachotoxin alone. The curves in Fig. 2 are computed fits to a simple hyperbolic saturation function of the form \( P(A) = P_{a}A/(K_{D,5} + A) \) where \( A \) is the concentration of veratridine or batrachotoxin. The data points conform closely to these theoretical curves, indicating that no heterotropic cooperativity is involved in activation of sodium ionophores by veratridine and batrachotoxin in the presence or absence of sea anemone toxin.

An allosteric model has been described previously which successfully fits the data on the heterotropic cooperative interaction between scorpion toxin and the activating toxin (14). In this model, the sodium ionophore is assumed to exist in (at least) two states, active (R) and inactive (T). There are reversible transitions between the two states characterized by an equilibrium constant \( (M_{R,T}) \). Veratridine and batrachotoxin cause activation by binding more tightly to the active (R) state; that is, \( K_{R} < K_{T} \) where \( K_{R} \) and \( K_{T} \) are equilibrium dissociation constants for binding of veratridine and batrachotoxin to the R and T states. Scorpion toxin and sea anemone toxin reduce the value of \( M_{R,T} \) and thereby enhance the activation by veratridine and batrachotoxin. The fraction of ionophores activated \( (P_{a}) \) as a function of activator concentration \( (A) \) is given by:

\[
P_{a}(A) = \frac{1}{1 + M_{R,T} + A/K_{R} + A/K_{T}}
\]

The values of these parameters derived from least squares fits the data are presented in Table I. As found previously for scorpion toxin (14), the assumption that sea anemone toxin reduces the allosteric equilibrium constant \( M_{R,T} \) allows an adequate fit of the data. Thus, the effects of both sea anemone toxin and scorpion toxin can be accommodated by this allosteric model.

**Competition between Sea Anemone and Scorpion Toxins**—Since the binding of scorpion toxin to its receptor site on the action potential sodium ionophore can be measured directly, it is possible to test whether sea anemone toxin competes with scorpion toxin for that receptor site. In these experiments, cells were incubated with 0.5 nM \(^{125}\)I-labeled scorpion toxin and increasing concentrations of either unlabeled scorpion toxin or sea anemone toxin and specifically bound \(^{125}\)I-labeled scorpion toxin was measured. Bound scorpion toxin was displaced by unlabeled scorpion toxin with a \( K_{D,5} \) of approximately 1.4 nM (Fig. 3). Bound scorpion toxin was also displaced by sea anemone toxin but with a \( K_{D,5} \) of approximately 150 nM. Assuming simple competitive inhibition, the \( K_{D,5} \) for sea anemone toxin at this receptor site is 90 nM. This concentration of sea anemone toxin is similar to the \( K_{D,5} \) observed for enhancement of veratridine activation of sodium ionophores (Fig. 1). These results indicate that sea anemone toxin binds to the same receptor site as scorpion toxin and has similar effects on the sodium ionophore.

Data from a more extensive experiment like that in Fig. 3 are replotted in Fig. 4 using coordinates analogous to those of a Scatchard plot. A straight line is obtained consistent with the presence of a single class of independent binding sites for sea anemone toxin with \( K_{D} = 78 \) nM.

Tetrodotoxin and saxitoxin inhibit sodium ionophores in neuroblastoma cells by binding to a different receptor site from scorpion toxin (11, 17). Tetrodotoxin \((1 \mu M)\) has no effect

**Table I**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>( K_{T} ) (M)</th>
<th>( K_{R} ) (M)</th>
<th>( K_{T}/K_{R} ) (M)</th>
</tr>
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<tbody>
<tr>
<td>Veratridine</td>
<td>( 3.3 \times 10^{-5} )</td>
<td>( 5.3 \times 10^{-8} )</td>
<td>( 6.2 \times 10^{2} )</td>
</tr>
<tr>
<td>Batrachotoxin</td>
<td>( 1.5 \times 10^{-5} )</td>
<td>( 1.9 \times 10^{-19} )</td>
<td>( 1.4 \times 10^{15} )</td>
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<table>
<thead>
<tr>
<th>Toxin</th>
<th>( K_{D} ) (M)</th>
<th>( K_{D}/K_{R} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea anemone toxin</td>
<td>( 7.2 \times 10^{4} )</td>
<td>( 6.9 \times 10^{4} )</td>
</tr>
<tr>
<td>Scorpion toxin</td>
<td>( 5.5 \times 10^{4} )</td>
<td>( 5.5 \times 10^{4} )</td>
</tr>
<tr>
<td>Veratridine</td>
<td>( 2.2 \times 10^{4} )</td>
<td>( 2.4 \times 10^{4} )</td>
</tr>
<tr>
<td>Batrachotoxin</td>
<td>( 1.5 \times 10^{5} )</td>
<td>( 6.7 \times 10^{5} )</td>
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</table>

The abbreviations used are: \( K_{D} \), the concentration of toxin causing 50% maximal effect on \(^{22}\)Na\(^{+}\) uptake or scorpion \(^{125}\)I toxin binding; \( P_{s} \), the maximum Na\(^{+}\) permeability at saturating concentrations of toxin.
on displacement of bound scorpion toxin by sea anemone toxin (Fig. 3, A), indicating that tetrodotoxin and sea anemone toxin also bind to separate receptor sites.

**Effect of Membrane Potential on Sea Anemone Toxin Action**—The heterotropic cooperative effect of scorpion toxin on activation of sodium ionophores by veratridine and batrachotoxin is dependent on membrane potential (10). Depolarization of the neuroblastoma cells by K+ or other agents causes a large increase in $K_{o,5}$ for scorpion toxin action and in $K_D$ measured in direct binding experiments (11). Similar experiments were carried out to test the effect of membrane potential on $K_{o,5}$ for sea anemone toxin. In these experiments, cells were incubated with increasing concentrations of sea anemone toxin for 30 min in 5 mM K+ (membrane potential = $-41 \text{ mV}$ (10)) or in 135 mM K+ (membrane potential = 0 mV (10)). The cells were then briefly rinsed and sodium permeability was measured in 5 mM K+ and 200 µM veratridine. The results of these experiments show that depolarization increases $K_{o,5}$ for sea anemone toxin approximately 5-fold (Fig. 5, left). These data, when plotted on a double reciprocal plot, do not yield straight lines (Fig. 5, right). The curves obtained are most consistent with a common ordinate intercept, indicating no effect of membrane potential on $P_{\text{in}}$ and different slopes, indicating an increase in $K_{o,5}$ due to depolarization.

The effect of membrane potential on scorpion toxin binding is due mainly to an increase in the rate of dissociation of the scorpion toxin-receptor complex (10, 11). In order to test the effect of membrane potential on the rate of reversal of sea anemone toxin action, cells were incubated for 30 min with 0.3 µM sea anemone toxin in 5 mM K+, rinsed, and incubated

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**Fig. 3.** Inhibition of scorpion toxin binding by sea anemone toxin. N18 cells were incubated for 60 min at 36°C in sodium-free choline-substituted medium (11) containing 0.5 nM scorpion mono-¹¹Ijodotoxin (SCTX) and the indicated concentrations of unlabeled scorpion toxin (O) or sea anemone toxin (O, A). Tetrodotoxin (1 µM) was added to one group of sea anemone toxin samples (A). Specifically bound scorpion toxin was measured as described previously (11). The amount of scorpion mono-¹¹Ijodotoxin bound in the presence of a saturating concentration (200 nM) of unlabeled scorpion toxin was considered nonspecific and was subtracted from the results.

**Fig. 4.** Analysis of sea anemone toxin binding. Binding competition data from a more extensive experiment carried out as in Fig. 3 is plotted in a form analogous to the Scatchard chart. Per cent inhibition of specific scorpion toxin binding by sea anemone toxin (ordinate and abscissa) is proportional to fractional saturation of the receptor sites by sea anemone toxin. Therefore, plot of per cent inhibition divided by free sea anemone toxin (ATX II) concentration versus per cent inhibition is formally analogous to a Scatchard or Eadie-Hofstee plot where the slope is $-1/K_{o,5}$.

**Fig. 5.** Effect of depolarization on the action of sea anemone toxin. N18 cells were incubated for 30 min at 36°C in medium containing 5 mM K+ (O) or 135 mM K+ (C). $^2$Na+ uptake was then measured in medium containing 5 mM K+ and 200 µM veratridine as described previously (10). The data are presented as a velocity ($v$) versus concentration plot (left) and a double-reciprocal plot (right).
for a variable period of time in the absence of toxin in either
5 mm K+ or 105 mm K+, and then sodium permeability was
measured in the presence of 200 μM veratridine and 5 mm K+
In experiments of this kind, the rate of reversal of sea anemone
toxin action is substantially increased by depolarization (Fig.
6). The semilogarithmic plots are consistently nonlinear pre-
venting precise determination of rate constants. The initial
rate of reversal is increased almost 5-fold by depolarization
suggesting that most of the observed increase in Kd is due to
an increase in the rate constant for dissociation of the
toxin receptor complex.

### Discussion

The effects of sea anemone toxin II and scorpion toxin on
sodium ionophores in neuroblastoma cells are similar in sev-
eral respects. Neither toxin has a measurable effect on sodium
permeability when tested alone. Each toxin enhances the
activation of action potential sodium ionophores by veratri-
dine and batrachotoxin. In both cases, this heterotropic co-
operative effect is inhibited by depolarization of the cells. The
effect of membrane depolarization is primarily to increase the
rate of dissociation of the toxin receptor complex. These
results are consistent with the conclusion that sea anemone
toxin and scorpion toxin alter the properties of the action
potential sodium ionophore by a common mechanism. In
direct binding assays using 35S-labeled scorpion toxin, sea
anemone toxin II inhibits specific binding of scorpion toxin to
its receptor site associated with action potential sodium ion-
ophores. Considered together, these results provide strong
evidence that scorpion toxin and sea anemone toxin share a
common receptor site. Scorpion toxin (Kd = 0.8 nM) has 110-
fold greater affinity for this site than sea anemone toxin (Kd
= 90 nM).

Although our results strongly support the conclusion that
the site and mechanism of action of scorpion and sea anemone
toxins are identical, some differences between the action of
the two toxins have been observed. Depolarization of the cells
causes a 70-fold increase in Kd for scorpion toxin action (10)
but only a 5-fold increase in Kd for sea anemone toxin action.
Since it is likely that the change in Kd represents a difference
in toxin binding to two different conformational states of the
receptor site (11), these results imply that sea anemone toxin
discriminates less effectively between these two receptor site
conformations. In addition, concentration effect curves for
scorpion toxin are consistently hyperbolic (10, 13) whereas
curves for sea anemone toxin deviate from simple hyperbolae
as illustrated by the curved double-reciprocal plots of Fig. 5.
In contrast, binding curves for sea anemone toxin were con-
sistently hyperbolic as illustrated by the linear Scatchard plot
in Fig. 4. Comparison of the data from these two different
types of experiments suggests that the deviation from hyperbo-
lar behavior observed in the ion flux experiments resulted
from dissociation of sea anemone toxin during the ion flux
measurements which must be made in the absence of toxin
and did not reflect complexity in the interaction between toxin
and receptor site. To test this possibility, flux experiments
were carried out in two ways. In the first, cells were incubated
with anemone toxin for 30 min and then uptake was measured
in the presence of 200 μM veratridine but no anemone toxin.
This experimental design is required for the experiment of
Fig. 5. In the second experimental design, sea anemone toxin
was present in both preincubation and assay. Results from
experiments of this second type showed smaller deviation from
hyperbolic behavior. Thus, it seems likely that at least
part of the deviation from hyperbolic behavior is due to the
manipulations required to test for membrane potential
dependence as in Fig. 5. The more rapid rate of dissociation of

### Table II

<table>
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<th>Table II Properties of neurotoxin receptor sites</th>
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### References

1. Shapiro, B. I. (1968) Comp. Biochem. Physiol. 27, 519-531
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