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# BINDING OF [$^3$H]PERHYDROHISTRIONICOTOXIN AND [$^3$H] PHENCYCLIDINE TO THE NICOTINIC RECEPTOR–ION CHANNEL COMPLEX OF *TORPEDO* ELECTROPLAX

## INHIBITION BY HISTRIONICOTOXINS AND DERIVATIVES

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**Abstract**—Histrionicotoxin, a spiropiperidine alkaloid, and twenty-two analogs inhibited binding of [$^3$H] perhydrohistrionicotoxin ([$^3$H]$H_{12}$-HTX) and of [$^3$H]phencyclidine ([$^3$H]PCP) to sites on the acetylcholine receptor–ion complex of *Torpedo* electroplax membranes. Structural alterations to the nitrogen (secondary amine) or oxygen (alcohol) functions or to the five carbon and four carbon side chain of histrionicotoxin altered the potency versus [$^3$H]$H_{12}$-HTX and [$^3$H]PCP binding measured in the presence or absence of a receptor agonist, carbamylcholine. Histrionicotoxin itself was 3-fold more potent versus [$^3$H]PCP binding than versus [$^3$H]$H_{12}$-HTX binding. N-Methylation or O-acetylation increased this difference, while alterations to the side chains either slightly decreased or markedly increased this difference. Histrionicotoxin was some 3.5-fold more potent versus [$^3$H]$H_{12}$-HTX binding in the presence of carbamylcholine than in its absence. O-Acetylation increased this selectivity for the carbamylcholine-activated state of the receptor channel complex, while alterations in the side chains either reduced or increased the selectivity. Histrionicotoxin was some 2.2-fold more potent versus [$^3$H] PCP binding in the presence of carbamylcholine than in its absence. N-Methylation of *O*-acetylhistrionicotoxin greatly increased this selectivity, while alterations in the side chains either reduced or had no effect on selectivity.

Histrionicotoxins are unique spiropiperidine alkaloids, originally obtained from the skins of the poison frog *Dendrobates histrionicus* [1]. A variety of natural and synthetic histrionicotoxins have been structurally defined (see Ref. 2 for a review). The first pharmacological studies of these alkaloids on the neuromuscular junction led to identification through electrophysiology and biochemistry of a target site on the nicotinic receptor–ionic channel complex [3–5]. Specifically, histrionicotoxins are potent blockers of acetylcholine-evoked ionic conductance at the neuromuscular junction [6–8]. The histrionicotoxins block neuromuscular transmission, not through blockade of the acetylcholine receptor site, but through interaction with at least two sites associated with the ionic channel [3, 5, 6, 8]. The interaction of histrionicotoxin with the acetylcholine receptor–ionic channel complex increases the affinity of acetylcholine for its binding site causing the appearance of desensitized species (see Ref. 9 for a review of desensitization) and also produces a blockade of the ionic channel in open and closed conformations [3, 6, 7]. Histrionicotoxins are noncompetitive antagonists at the nicotinic receptor channel complex and produce a use-dependent effect on the endplate current. Histrionicotoxins depress the peak amplitude of the endplate current and depress the depolarizing response induced by microiontophoretically applied acetylcholine in a frequency-dependent manner. In addition, histrionicotoxins shorten channel life time and cause a depression of peak amplitude which is accompanied by time and voltage dependence seen as nonlinearity and hysteresis in the peak current relationship [4, 6, 10]. The ability of histrionicotoxin to affect function of the nicotinic receptor-channel complex has now been demonstrated at the level of single channels using a patch clamp technique.¶

Compounds which, like histrionicotoxins, produce these effects are frequently referred to as "channel blockers", although the relationship of the site at which the ion channel blockers act to modify channel conductance to the ion channel mechanism is not generally known [6, 8, 10]. Biochemical analysis of nicotinic receptors, including purification and functional reconstitution, leaves little doubt that the ion translocation mechanism resides in the five poly-

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¶ Y. Aracava, J. W. Daly and E. X. Albuquerque, *IUPHAR Ninth International Congress of Pharmacology*, 601P (1984).

peptides and associated lipids which constitute the nicotinic receptor complex. Binding studies with radioactive ligands provide evidence for distinct but functionally linked sites on the nicotinic receptor–ion channel complex. One of these ligands is [$^3$H] perhydrohistrionicotoxin ([$^3$H]$H_{12}$-HTX) which has been used extensively as a direct probe of "ion channels" associated with the nicotinic acetylcholine receptor in *Torpedo* electric organs [5, 11–15]. [$^3$H] $H_{12}$-HTX binds to a discrete population of sites on *Torpedo* electrocytes with a dissociation constant of about 100 nM. This binding is displaced by most electrophysiologically-identified ion channel blockers, but not by classical receptor antagonists (see Ref. 16 for a review). The kinetics of [$^3$H]$H_{12}$-HTX binding are greatly affected by the functional state of the receptor-channel complex [11, 17]. For example, the rate of binding is greatly stimulated in the presence of receptor agonists and this stimulation is mitigated under desensitizing conditions. Thus, [$^3$H] $H_{12}$-HTX has proved particularly useful in the characterization of drug interactions with the end-plate receptor complex. In the present study, the interactions of twenty-three natural, synthetic and derivatized histrionicotoxins (Fig. 1) with the nicotonic receptor ion channel complex from *Torpedo* electric organ were characterized using radiolabeled probes for both receptor and channel binding sites.

## MATERIALS AND METHODS

*Tissue preparation.* Electric organs from *Torpedo californica* were purchased from Pacific Biomarine (Venice, CA) and stored for up to 3 months at −70°. A crude particulate fraction was prepared by disrupting the tissue in a blender in 5 vol. of 50 mM Tris–Cl, pH 7.4, containing 1 mM phenylmethylsulfonyl fluoride to inhibit proteolysis. This homogenate was filtered through three layers of cheesecloth to remove collagenous and other undisrupted material and was centrifuged at 30,000 *g* for 20 min. The pellets were resuspended in buffer at a protein concentration of 1–2 mg/ml, and kept at 0° for up to 2 days before use. Protein content was determined by a modification of the method of Lowry *et al.* [18].

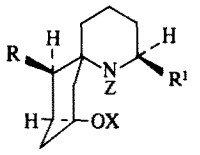
*Binding measurements.* Nicotinic receptor binding was measured using tritiated acetylcholine ([$^3$H] ACh; 80 Ci/mmole, New England Nuclear) in an equilibrium dialysis procedure. An aliquot of *Torpedo* membranes was exposed to 100 μM diisopropylfluorophosphate for 20 min at room temperature to inhibit acetylcholinesterase activity, placed inside Spectra/Por 2 dialysis tubing (Fisher), and incubated in a medium containing 100 mM Tris–Cl, pH 7.4, 10 nM [$^3$H]ACh and the competing histrionicotoxin, as required by the experiment. After incubation on a rotary shaker for 4 hr at room temperature, the radioactivity content inside and outside the dialysis tubing was determined by liquid scintillation counting. Non-specific binding, which was less than 10% of total binding, was determined in the presence of 100 μM *d*-tubocurarine.

Ion channel interactions were measured using two radiolabeled probes, [$^3$H]$H_{12}$-HTX and [$^3$H]phencyclidine ([$^3$H]PCP; 48 Ci/mmole, New England Nuclear). [$^3$H]$H_{12}$-HTX was prepared by reduction of 6.6 mg of *dl*-octahydrohistrionicotoxin with 25 Ci of tritium gas by New England Nuclear and had a specific activity of 54.5 Ci/mmole. An aliquot of *Torpedo* membranes (25–50 μg protein) was incubated with 3 nM [$^3$H]PCP or 2 nM [$^3$H]$H_{12}$-HTX in 50 mM Tris–Cl, pH 7.4, for 45 min at room temperature. The suspension was then filtered through Whatman GF/B glass fiber filters which had been wetted with a 1% organo-saline solution (Sigmacote, Sigma Chemical Co.) to eliminate [$^3$H]$H_{12}$-HTX binding to the filters. The radioactivity content of the tissue trapped on the filters was determined by liquid scintillation counting. Non-specific binding was measured in the presence of 100 μM amantadine [19]. Ion channel binding was routinely measured in the presence and absence of 1 μM carbamylcholine, since channel binding is sensitive to the state (resting, activated, blocked or desensitized) of the receptor-ion channel complex [19].

*Histrionicotoxin analogs and derivatives.* Histrionicotoxin, dihydrohistrionicotoxin, isodihydrohistrionicotoxin, neodihydrohistrionicotoxin, allodihydrohistrionicotoxin, tetrahydrohistrionicotoxin, isotetrahydrohistrionicotoxin, octahydrohistrionicotoxin, $\Delta^{17,18}$-*trans*-histrionicotoxin, and histrionicotoxin 259 were isolated from skin extracts of *D. histrionicus* [1, 20–22]. All of these natural histrionicotoxins are the levorotatory enantiomers [2]. *l*-Perhydrohistrionicotoxin was prepared from isodihydrohistrionicotoxin and was converted to 7-deoxy-$\Delta^{7,8}$-perhydrohistrionicotoxin and 7-deoxyperhydrohistrionicotoxin as described [22]. The latter compounds were provided by Dr. T. Tokuyama (Osaka City University) *dl*-Octahydrohistrionicotoxin, *dl*-$H_{10}$-(l-pentenyl)histrionicotoxin and *dl*-$H_{10}$-(l-butenyl)histrionicotoxin were provided by Dr. Y. Kishi (Harvard University). *d*-Perhydrohistrionicotoxin, *dl*-desbutylperhydrohistrionicotoxin, *dl*-desamylperhydrohistrionicotoxin, and *dl*-desamylperhydrohistrionicotoxin methiodide were provided by Drs. K. Takahashi and A. Brossi (National Institutes of Health).

N-*Methylhistrionicotoxin.* Histrionicotoxin was converted to *N*-methylhistrionicotoxin by reductive formylation [23] as follows. Histrionicotoxin (22.5 mg, 0.080 mmole) and 150 μl of 37% aqueous formaldehyde (1.8 mmoles) were dissolved in acetonitrile. Sodium cyanoborohydride (10.0 mg, 0.16 mmole) was then added to the stirred solution. The pH of the solution was monitored, and a total of 5 μl glacial acetic acid was added to maintain a pH near neutrality. After 3 hr at room temperature, the mixture was diluted with 5 ml of 5% aqueous $NaHCO_3$ and extracted three times with diethyl ether. The ether layers were dried over anhydrous $MgSO_4$, filtered, and evaporated at reduced pressure to give 22.8 mg of a colorless glass which crystallized on standing. Column chromatography of 14.6 mg of the crude product on silica gel 60 using 4:1 $CH_2Cl_2$:$CH_3OH$ gave 11.6 mg *N*-methylhistrionicotoxin, a single spot on thin-layer chromatography (TLC), m.p. 125–126°.

N-*Methyl*-O-*acetylhistrionicotoxin.* *N*-Methyl histrionicotoxin (11.6 mg, 0.039 mmole) was dissolved in 1 ml acetone, and to this solution was added 50 μl acetic anhydride (0.53 mmole) and 5 mg sodium



Natural I - Enantiomer
unless otherwise specified X and Z = H

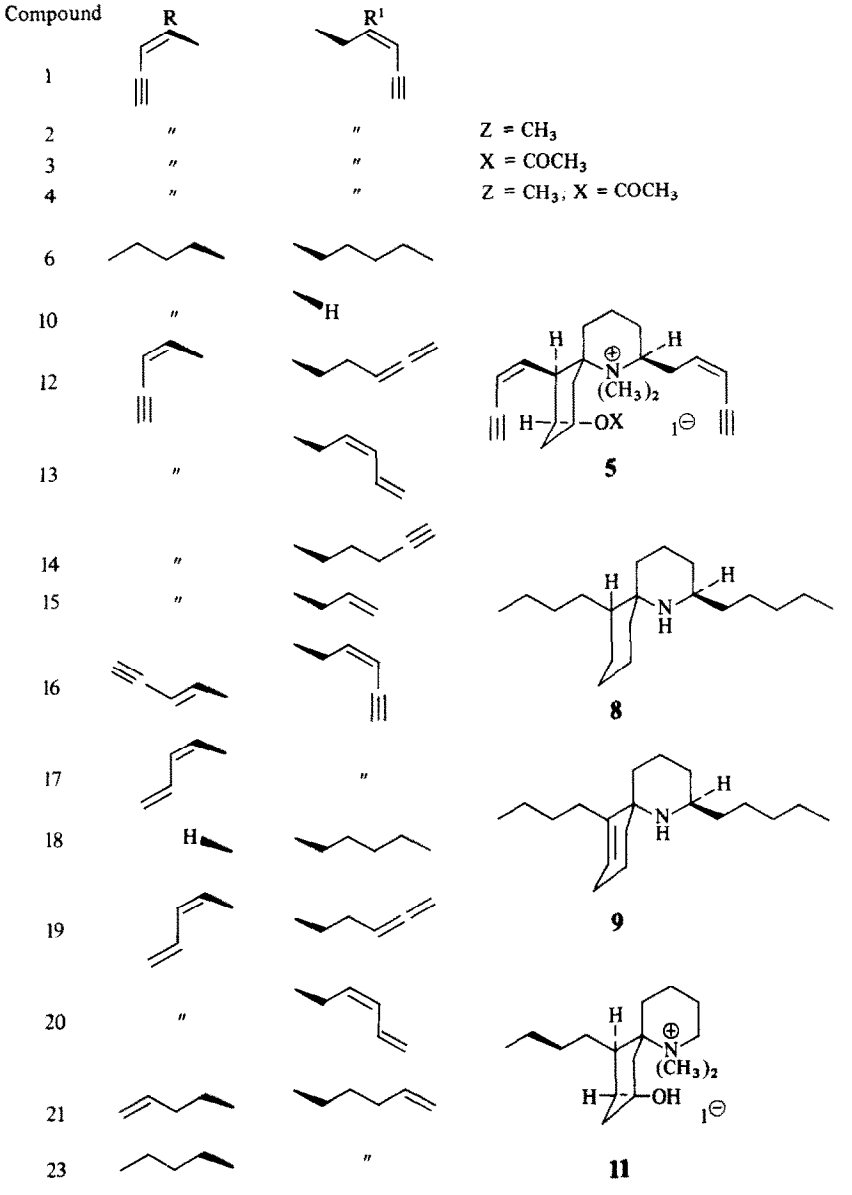



Fig. 1. Structures of natural and synthetic histrionicotoxins.

acetate trihydrate (0.037 mmole). The mixture was stirred at room temperature overnight. Methanol (250 $\mu$l) was then added and stirring was continued for 15 min. After dilution with 7 ml water, the solution was extracted with methylene chloride. The methylene chloride layers were dried over anhydrous sodium sulfate, filtered, and evaporated at reduced pressure to give 11.8 mg (89%) of a pale yellow–orange oil, consisting of a single component by TLC and high pressure liquid chromatography. Column chromatography of this crude material on silica gel 60 with eluting solvents ranging from $CH_2Cl_2$ to

10:1 $CH_2Cl_2$:$CH_3OH$ gave 10.5 mg *N*-methyl-*O*-acetylhistrionicotoxin, a pale green oil, a single spot on TLC. *N*-Methyl-*O*-acetylhistrionicotoxin was also prepared using the reverse order of reactions, i.e. acetylation of histrionicotoxin followed by methylation of *O*-acetylhistrionicotoxin.

*O-Acetylhistrionicotoxin.* A solution of 11.0 mg histrionicotoxin (0.039 mmole), 50 μl acetic anhydride (0.53 mmole), and 3.8 mg potassium acetate (0.039 mmole) was stirred at room temperature for 7 hr. Methanol (100 μl) was added to the solution, which was stirred for an additional 15 min. Dilution of the reaction mixture with 5 ml of 5% aqueous $NaHCO_3$ was followed by repeated extraction with diethyl ether. Ether layers were combined and dried over anhydrous $MgSO_4$, filtered, and evaporated at reduced pressure to give a quantitative yield of crude product, a yellow–orange glass. Column chromatography (silica gel 60, 9:1 $CCl_4$:$CH_3OH$) of this residue provided 10.1 mg (80%) of *O*-acetylhistrionicotoxin, a single component by TLC.

*O-Acetylhistrionicotoxin methiodide.* To a solution of *N*-methyl-*O*-acetylhistrionicotoxin (36.3 mg, 0.1 mmole) in 1 ml acetonitrile was added 300 μl $CH_3I$ in three portions at intervals of 7 days. After 25 days, the reaction mixture was evaporated *in vacuo* to yield 50.1 mg crude product. Extraction with methylene chloride yielded 21.8 mg of methiodide, pure by TLC analysis. The methylene chloride extract was partitioned with water to yield an additional 12.7 mg of methiodide. Mass spectral analysis using a thermal spray inlet indicated a single component with a positively charged molecular ion at 354. No significant conversion of *N*-methylhistrionicotoxin to the methiodide was possible, in agreement with results of Takahashi *et al.* [24], which indicate that only the O-acetyl derivatives of various synthetic analogs of histrionicotoxin can be converted to methiodides. Hydrolysis of *O*-acetylhistrionicotoxin methiodide to histrionicotoxin methiodide was unsuccessful under a variety of conditions using $K_2CO_3$.

The carbon-13 magnetic resonance spectra of *N*-methylhistrionicotoxin, *O*-acetylhistrionicotoxin and *N*-methyl-*O*-acetylhistrionicotoxin were consistent with the structures.

## RESULTS

*Interactions of histrionicotoxin and analogues with the nicotonic ACh receptor.* All of the histrionicotoxins had little affinity for the nicotinic receptor (the ACh binding site). Specific [$^3$H]ACh binding was depressed by less than 15% by each of the histrionicotoxins at 10 μM (data not shown; see Refs. 25–29).

*Competitive interactions between phencyclidine and perhydrohistrionicotoxin.* [$^3$H]PCP binding to ion channel sites was measured at seven concentrations from 0.3 to 100 nM in the absence or presence of five concentrations of unlabeled $H_{12}$-HTX from 0.03 to 3 μM. Double-reciprocal plots of these binding data were consistent with a competitive interaction between the two compounds: the number of [$^3$H]PCP binding sites was the same in the presence of $H_{12}$-HTX and a plot of the slope of the reciprocal

Table 1. Effects of alterations of oxygen and nitrogen functions on the interactions of histrionicotoxins with ion channel sites associated with nicotinic acetylcholine receptors in *Torpedo california* electric organ*

| No. | Compound | $K_i$ (μM) | | | |
|---|---|---|---|---|---|
| | | [$^3$H]$H_{12}$-HTX | [$^3$H]$H_{12}$-HTX + Carb | [$^3$H]PCP | [$^3$H]PCP + Carb |
| 1 | Histrionicotoxin | 0.63 ± 0.05 | 0.18 ± 0.03 | 0.21 ± 0.04 | 0.095 ± 0.008 |
| 3 | *N*-Methylhistrionicotoxin | 0.60 ± 0.06 | 0.20 ± 0.08 | 0.10 ± 0.02 | 0.028 ± 0.004 |
| 3 | *O*-Acetylhistrionicotoxin | 1.1 ± 0.3 | 0.10 ± 0.02 | 0.13 ± 0.02 | 0.066 ± 0.007 |
| 4 | *N*-Methyl-*O*-acetylhistrionicotoxin | 1.5 ± 0.4 | 0.19 ± 0.03 | 0.22 ± 0.04 | 0.010 ± 0.003 |
| 5 | *O*-Acetylhistrionicotoxin methiodide | 100 ± 9 | 15 ± 4 | 23 ± 5 | 8.3 ± 0.3 |
| 6 | *l*-Perhydrohistrionicotoxin | 0.14 ± 0.04 | 0.090 ± 0.006 | 0.025 ± 0.003 | 0.022 ± 0.004 |
| 7 | *d*-Perhydrohistrionicotoxin | 0.17 ± 0.02 | 0.089 ± 0.005 | 0.085 ± 0.004 | 0.040 ± 0.002 |
| 8 | 7-Deoxyperhydrohistrionicotoxin | 0.20 ± 0.03 | 0.16 ± 0.03 | 0.11 ± 0.02 | 0.09 ± 0.02 |
| 9 | 7-Deoxy-$\Delta^{7,8}$-perhydrohistrionicotoxin | 0.21 ± 0.03 | 0.14 ± 0.02 | 0.075 ± 0.007 | 0.063 ± 0.009 |
| 10 | Desamylperhydrohistrionicotoxin | 3.5 ± 0.7 | 2.3 ± 0.8 | 0.63 ± 0.07 | 0.40 ± 0.06 |
| 11 | Desamylperhydrohistrionicotoxin methiodide | 6.3 ± 0.9 | 7.9 ± 0.9 | 2.2 ± 0.6 | 1.4 ± 0.4 |

* Inhibition constant (μM) is for inhibition of 2 nM [$^3$H]$H_{12}$-HTX and 3 nM [$^3$H]PCP binding to ion channel sites in *Torpedo* electrocyte membranes. Binding was measured in the presence or absence of 1 μM carbamylcholine. The means and standard deviations from three independent determinations are listed.

plot as a function of $H_{12}$-HTX concentration was linear and indicated a $K_i$ for $H_{12}$-HTX of 0.1 $\mu$M (data not shown). Similar findings were obtained when measuring [$^3$H]$H_{12}$-HTX binding (0.3 to 180 nM) in the presence of unlabeled PCP (0.1 to 3 $\mu$M). These findings indicate that PCP and $H_{12}$-HTX binding to ion channel sites in *Torpedo* electric tissue is mutually exclusive.

*Interactions of histrionicotoxin analogues with the nicotinic receptor ion channel.* Interactions of the histrionicotoxins with sites associated with the nicotinic ion channel were determined from their ability to inhibit the specific binding of 2 nM [$^3$H]$H_{12}$-HTX and 3 nM [$^3$H]PCP in the presence and absence of 1 $\mu$M carbamylcholine. Inhibition constants were calculated from the $IC_{50}$ concentrations according to the relationship:

$$K_i = IC_{50}/(1 + D/K_D)$$

where $D$ and $K_D$ are the concentration and dissociation constants, respectively, of the radiolabeled probes ([$^3$H]$H_{12}$-HTX or [$^3$H]PCP). The $K_D$ values used for [$^3$H]$H_{12}$-HTX were 0.089 and 0.135 $\mu$M in the presence and absence of 1 $\mu$M carbamylcholine. The corresponding values for [$^3$H]PCP were 0.097 and 0.54 $\mu$M. Insofar as the concentrations of [$^3$H]$H_{12}$-HTX and [$^3$H]PCP used (2 and 3 nM respectively) were small fractions of their dissociation constants, only small corrections (less than 3%) of the $IC_{50}$ values were necessary to obtain the inhibition constants.

Twenty-three histrionicotoxins were evaluated as antagonists of binding of [$^3$H]$H_{12}$-HTX and of [$^3$H]PCP to sites apparently associated with the acetylcholine receptor–ion channel complex. The $K_i$ values are given in Tables 1 and 2. Binding isotherms for certain histrionicotoxins are illustrated in Figs. 2 and 3. Such curves were consonant with interaction with a single class of binding sites for either [$^3$H]$H_{12}$-HTX or [$^3$H]PCP. Structural variations to the "parent" histrionicotoxin include (i) alterations at the nitrogen and oxygen functions; and (ii) alterations to the five carbon and four carbon side chains. Tables 2 and 3 are organized to facilitate discussion with respect to such alterations. In addition, the effect of the absolute stereochemistry was probed for *l*- and *d*-perhydrohistrionicotoxin (Table 1, compounds *6* and *7*). No significant stereoselectivity versus [$^3$H]$H_{12}$-HTX binding was observed, in confirmation of earlier electrophysiological data [24, 29]. However, the natural *l*-enantiomer was somewhat more potent than the *d*-enantiomer versus [$^3$H]PCP binding. A similar tendency was manifest for natural *l*-octahydrohistrionicotoxin (Table 2, compound *21*) compared to synthetic *dl*-octahydrohistrionicotoxin (compound *22*).

Alterations in the nitrogen and oxygen functions did not have remarkable effects on activity in the

Table 2. Effects of alterations of side chains on the interactions of histrionicotoxins with ion channel sites associated with nicotinic acetylcholine receptors in *Torpedo california* electric organ*

| No. | Compound | $K_i$ ($\mu$M) | | | |
|---|---|---|---|---|---|
| | | [$^3$H]$H_{12}$-HTX | [$^3$H]$H_{12}$-HTX + Carb | [$^3$H]PCP | [$^3$H]PCP + Carb |
| | Alterations of five carbon side chain | | | | |
| 1 | Histrionicotoxin | 0.63 ± 0.05 | 0.18 ± 0.03 | 0.21 ± 0.04 | 0.095 ± 0.008 |
| 12 | Isodihydrohistrionicotoxin | 0.10 ± 0.03 | 0.07 ± 0.01 | 0.047 ± 0.008 | 0.032 ± 0.005 |
| 13 | Dihydrohistrionicotoxin | 0.13 ± 0.02 | 0.11 ± 0.02 | 0.060 ± 0.02 | 0.026 ± 0.004 |
| 14 | Allodihydrohistrionicotoxin | 0.19 ± 0.02 | 0.093 ± 0.007 | 0.063 ± 0.008 | 0.050 ± 0.006 |
| 15 | Histrionicotoxin 259 | 3.4 ± 0.4 | 0.48 ± 0.05 | 0.29 ± 0.03 | 0.13 ± 0.03 |
| 17 | Neodihydrohistrionicotoxin | 0.07 ± 0.01 | 0.056 ± 0.005 | 0.030 ± 0.007 | 0.026 ± 0.004 |
| 20 | Tetrahydrohistrionicotoxin | 0.48 ± 0.06 | 0.073 ± 0.007 | 0.045 ± 0.009 | 0.034 ± 0.006 |
| 6 | *l*-Perhydrohistrionicotoxin | 0.14 ± 0.04 | 0.090 ± 0.006 | 0.025 ± 0.003 | 0.022 ± 0.004 |
| 10 | Desamylperhydrohistrionicotoxin | 3.5 ± 0.7 | 2.3 ± 0.8 | 0.63 ± 0.07 | 0.40 ± 0.06 |
| | Alterations of four carbon side chain | | | | |
| 1 | Histrionicotoxin | 0.63 ± 0.05 | 0.18 ± 0.03 | 0.21 ± 0.04 | 0.095 ± 0.008 |
| 16 | $\Delta^{17,18}$-*trans*-Histrionicotoxin | 3.4 ± 0.5 | 0.48 ± 0.07 | 0.25 ± 0.04 | 0.15 ± 0.04 |
| 17 | Neodihydrohistrionicotoxin | 0.07 ± 0.01 | 0.056 ± 0.005 | 0.030 ± 0.007 | 0.026 ± 0.004 |
| 12 | Isodihydrohistrionicotoxin | 0.10 ± 0.03 | 0.07 ± 0.01 | 0.047 ± 0.008 | 0.032 ± 0.005 |
| 19 | Isotetrahydrohistrionicotoxin | 0.035 ± 0.007 | 0.050 ± 0.006 | 0.016 ± 0.002 | 0.020 ± 0.003 |
| 13 | Dihydrohistrionicotoxin | 0.13 ± 0.02 | 0.11 ± 0.02 | 0.060 ± 0.02 | 0.026 ± 0.004 |
| 20 | Tetrahydrohistrionicotoxin | 0.48 ± 0.06 | 0.073 ± 0.007 | 0.045 ± 0.009 | 0.034 ± 0.006 |
| 21 | Octahydrohistrionicotoxin | 0.14 ± 0.03 | 0.043 ± 0.005 | 0.021 ± 0.003 | 0.022 ± 0.004 |
| 22 | *dl*-Octahydrohistrionicotoxin | 0.16 ± 0.02 | 0.05 ± 0.01 | 0.066 ± 0.008 | 0.027 ± 0.005 |
| 23 | *dl*-$H_{10}$-(pentenyl)histrionicotoxin | 0.21 ± 0.04 | 0.13 ± 0.03 | 0.050 ± 0.007 | 0.028 ± 0.007 |
| 6 | *l*-Perhydrohistrionicotoxin | 0.14 ± 0.04 | 0.090 ± 0.006 | 0.025 ± 0.003 | 0.022 ± 0.004 |
| 18 | Desbutylperhydrohistrionicotoxin | 5.0 ± 0.6 | 2.2 ± 0.3 | 1.7 ± 0.4 | 1.3 ± 0.2 |

* Inhibition constant ($\mu$M) is for inhibition of 2 nM [$^3$H]$H_{12}$-HTX and 3 nM [$^3$H]PCP binding to ion channel sites in *Torpedo* electrolyte membranes. Binding was measured in the presence or absence of 1 $\mu$M carbamylcholine. The mean and standard deviations from three independent determinations are listed.

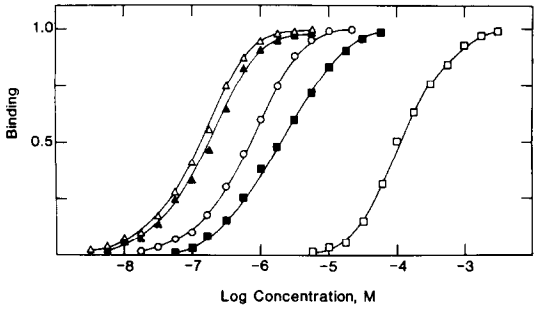



Fig. 2. Binding of histrionicotoxin analogs to the nicotinic receptor–channel complex from *Torpedo* electric organ. Fractional receptor occupancy of histrionicotoxin (○), *O*-acetylhistrionicotoxin (■), *O*-acetylhistrionicotoxin methiodide (□), octahydrohistrionicotoxin (Δ), and 7-deoxy-$H_{10}$-histrionicotoxin (▲) was inferred from their inhibition of 2 nM [$^3$H]$H_{12}$-HTX binding measured in the absence of carbamylcholine. Each point represents the mean from four determinations.

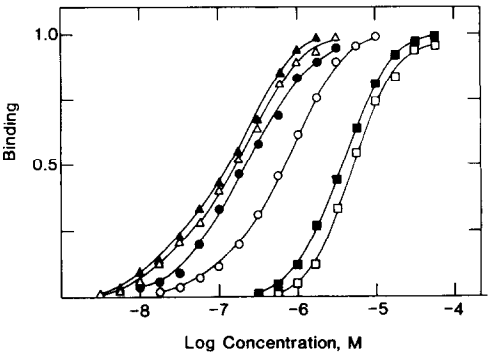



Fig. 3. Binding of histrionicotoxin and perhydrohistrionicotoxin analogs to the nicotinic receptor–channel complex from *Torpedo* electric organ. Fractional receptor occupancy of histrionicotoxin (○), *l*-perhydrohistrionicotoxin (▲), *d*-perhydrohistrionicotoxin (△), 7-deoxyperhydrohistrionicotoxin (●), desamylperhydrohistrionicotoxin (■), and desamylperhydrohistrionocotoxin methiodide (□) was inferred from their inhibition of specific 2 nM [$^3$H]$H_{12}$-HTX binding measured in the absence of carbamylcholine. Each point represents the mean from four determinations.

histrionicotoxins with a few exceptions. Thus, N-methylation of histrionicotoxin had little effect on activity as an antagonist of [$^3$H]$H_{12}$-HTX binding, while increasing potency versus [$^3$H]PCP 2- to 3-fold. It is noteworthy that [$^3$H]$H_{12}$-HTX and histrionicotoxin (compound *1*) are secondary amines, while [$^3$H]PCP and *N*-methylhistrionicotoxin (compound *2*) are tertiary amines. O-Acetylation of the hydroxy function of histrionicotoxin had only slight effects, decreasing potency (compound *3*) somewhat for inhibition of [$^3$H]$H_{12}$-HTX binding in the absence of carbamylcholine and increasing potency some-

Table 3. Comparison of relative potencies of histrionicotoxins in the absence of carbamylcholine, the ratio of potencies versus [$^3$H]$H_{12}$-HTX and [$^3$H]PCP binding in the absence of carbamylcholine, and the ratios of potencies in the absence and presence of carbamylcholine for [$^3$H]$H_{12}$-HTX and [$^3$H]PCP binding sites*

| No. | Compound | Relative potency [$^3$H]$H_{12}$-HTX/[$^3$H]PCP −Carbamylcholine | | Ratio potency [$^3$H]$H_{12}$-HTX/[$^3$H]PCP −Carbamylcholine | Ratio potency [$^3$H]$H_{12}$-HTX/[$^3$H]PCP −/+Carbamylcholine | |
|---|---|---|---|---|---|---|
| 1 | Histrionicotoxin | 1 | 1 | 3.0 | 3.5 | 2.2 |
| 2 | *N*-Methylhistrionicotoxin | 1.05 | 2.1 | 6.0 | 3.0 | 3.6 |
| 3 | *O*-Acetylhistrionicotoxin | 0.57 | 1.6 | 8.4 | 11.0 | 2.0 |
| 4 | *N*-Methyl-*O*-acetylhistrionicotoxin | 0.42 | 0.95 | 6.8 | 7.9 | 22 |
| 5 | *O*-Acetylhistrionicotoxin methiodide | 0.006 | 0.009 | 4.3 | 6.7 | 2.8 |
| 6 | *l*-Perhydrohistrionicotoxin | 4.5 | 8.4 | 5.6 | 1.6 | 2.1 |
| 7 | *d*-Perhydrohistrionicotoxin | 3.7 | 2.5 | 2.0 | 1.9 | 2.1 |
| 8 | 7-Deoxyperhydrohistrionicotoxin | 3.2 | 1.9 | 1.8 | 1.3 | 1.2 |
| 9 | 7-Deoxy-$\Delta^{7,8}$-perhydrohistrionicotoxin | 3.0 | 2.8 | 2.8 | 1.5 | 1.2 |
| 10 | Desamylperhydrohistrionicotoxin | 0.18 | 0.33 | 5.6 | 1.5 | 1.6 |
| 11 | Desamylperhydrohistrionicotoxin methiodide | 0.10 | 0.09 | 2.9 | 0.80 | 1.6 |
| 12 | Isodihydrohistrionicotoxin | 6.3 | 4.5 | 2.1 | 1.4 | 1.3 |
| 13 | Dihydrohistrionicotoxin | 4.8 | 3.5 | 2.2 | 1.2 | 2.3 |
| 14 | Allodihydrohistrionicotoxin | 3.3 | 3.5 | 3.0 | 2.0 | 1.3 |
| 15 | Histrionicotoxin 259 | 0.19 | 0.72 | 11.7 | 7.1 | 2.2 |
| 16 | $\Delta^{17,18}$-*trans*-Histrionicotoxin | 0.19 | 0.84 | 13.6 | 7.1 | 1.7 |
| 17 | Neodihydrohistrionicotoxin | 9.0 | 7.0 | 2.3 | 1.3 | 1.2 |
| 18 | Desbutylperhydrohistrionicotoxin | 0.13 | 0.12 | 2.9 | 2.3 | 1.3 |
| 19 | Isotetrahydrohistrionicotoxin | 18 | 13 | 2.2 | 0.70 | 0.80 |
| 20 | Tetrahydrohistrionicotoxin | 1.3 | 4.7 | 10.7 | 6.6 | 1.3 |
| 21 | Octahydrohistrionicotoxin | 4.5 | 10 | 6.7 | 3.3 | 0.95 |
| 22 | *dl*-Octahydrohistrionicotoxin | 3.9 | 3.2 | 2.4 | 3.2 | 2.4 |
| 23 | *dl*-$H_{10}$-(pentenyl)histrionicotoxin | 2.9 | 4.2 | 4.4 | 1.7 | 1.8 |

* Histrionicotoxin was assigned a potency of one versus binding of radioactive ligand.

what in the presence of carbamylcholine. Potency was increased slightly by O-acetylation for inhibition of [$^3$H]PCP binding in both the presence and the absence of carbamycholine. The O-acetyl-N-methyl derivative (compound *4*) of histrionicotoxin was somewhat less potent than histrionicotoxin itself for [$^3$H]$H_{12}$-HTX binding in the absence of carbamylcholine while being equipotent to histrionicotoxin in the presence of carbamycholine. The converse was true with respect to potency versus [$^3$H]PCP binding. Thus, the derivative was equipotent to histrionicotoxin in the absence of carbamylcholine but was nearly 10-fold more potent than histrionicotoxin in the presence of carbamylcholine. Quarternization of *O*-acetyl-*N*-methylhistrionicotoxin to yield the methiodide (compound *5*) had a very pronounced effect on activity, resulting in about a 100-fold reduction in potency versus either [$^3$H]$H_{12}$-HTX or [$^3$H]PCP. In contrast, the methiodide (compound *11*) of desamylperhydrohistrionicotoxin was only about 2- to 3-fold less potent than the parent secondary amine (compound *10*). Lack of the hydroxyl function of perhydrohistrionicotoxin as in the 7-deoxy compounds *8* and *9* had no marked effect on potency versus [$^3$H]$H_{12}$-HTX binding, while decreasing potency 3- to 4-fold versus [$^3$H]PCP binding.

Alterations in the nature and length of the five carbon and four carbon sides chains of histrionicotoxin had marked effects on potency versus [$^3$H]$H_{12}$-HTX and [$^3$H]PCP binding (Table 2). Both steric (conformational) and electronic effects are probably important. In many cases, more than one change relative to the parent histrionicotoxin has been made. Only the effect of single changes will be presently considered, although it appears that changes in one side chain can significantly affect the importance or nature of interactions of the other side chain with the site.

A single alteration to the five carbon side chain of histrionicotoxin pertains in four compounds. In isodihydrohistrionicotoxin (compound *12*), the *cis*-ene-yne bonds of histrionicotoxin have been replaced with a terminal allene grouping. This changes both electronic and steric factors. The two methylenes instead of one methylene group yield greater conformational freedom. Indeed, the five carbon side chain of isodihydrohistrionicotoxin is positioned quite differently than that of histrionicotoxin in the crystal lattice [1]. Either conformational or electronic factors might be responsible for the 4- to 6-fold greater potency of isodihydrohistrionicotoxin than histrionicotoxin as an antagonist of binding of [$^3$H]$H_{12}$-HTX and [$^3$H]PCP in the absence of carbamylcholine and the 2- to 3-fold greater potency in the presence of carbamylcholine. Reduction of the terminal acetylenic moiety in the five carbon side chain of histrionicotoxin to a double bond yields dihydrohistrionicotoxin (compound *13*) which was also more potent than histrionicotoxin. Reduction of the *cis*-double bond in the five carbon side chain yields allodihydrohistrionicotoxin (compound *14*), which was also more potent than histrionicotoxin. For both compounds *13* and *14*, as for compound *12*, the increase in potency relative to histrionicotoxin was greater in the absence than in the presence of carbamylcholine. The results indicate that the side chain of histrionicotoxin is far from optimal for activity; increases in conformational freedom of the five carbon side chain (compounds *12* and *14*) and replacement of the terminal acetylenic moiety with a double bond (compound *13*) conferred higher activity. However, reduction of the acetylenic moiety of the five carbon chain to a double bond in neodihydrohistrionicotoxin (compound *17*) to yield tetrahydrohistrionicotoxin (compound *20*) resulted in a 7-fold *decrease* in potency versus binding of [$^3$H]$H_{12}$-HTX and a 2-fold decrease versus [$^3$H]PCP, both in the absence of carbamylcholine, while having little or no effect on potency in the presence of carbamylcholine. When the five carbon side chain of histrionicotoxin was shortened by removal of the terminal 2-carbon (acetylene) moiety, the resulting histrionicotoxin 259 (compound *15*) had much lower activity (3- to 5-fold) versus [$^3$H]$H_{12}$-HTX binding, while having potency similar to that of histrionicotoxin versus [$^3$H]PCP binding. The last two carbons of the five carbon side chain thus appear to be more important for antagonism of [$^3$H]$H_{12}$-HTX binding than for antagonism of [$^3$H]PCP binding. Removal of the five carbon side chain of perhydrohistrionicotoxin (compound *6*) yields desamylperhydrohistrionicotoxin (compound *10*) with a resultant 20-fold or more decrease in potency versus [$^3$H]$H_{12}$-HTX and [$^3$H]PCP binding.

A single alteration to the four carbon side chain of histrionocotoxin pertains in only two compounds. In one, the $\Delta^{17,18}$-*cis*-double bond of histrionicotoxin is replaced with a $\Delta^{17,18}$-*trans*-double bond (compound *16*) with a resultant marked change in conformation. This alteration decreased potency some 5-fold versus binding of [$^3$H]$H_{12}$-HTX in the absence of carbamylcholine and 3-fold in the presence of carbamylcholine. Potency versus [$^3$H]PCP binding, on the other hand, was not affected by this alteration. Reduction of the terminal acetylenic moiety of the four carbon side chain of histrionicotoxin to a double bond yields neodihydrohistrionicotoxin (compound *17*) which was 9-fold more potent than histrionicotoxin versus [$^3$H]$H_{12}$-HTX binding in the absence of carbamylcholine and 3-fold more potent than histrionicotoxin in the presence of carbamylcholine. Neodihydrohistrionicotoxin was 7- and 4-fold more potent than histrionicotoxin versus [$^3$H]PCP binding in the absence and presence of carbamylcholine respectively. Reduction of the same terminal acetylenic moiety to a double bond in dihydrohistrionicotoxin (compound *13*) to yield tetrahydrohistrionicotoxin (compound *20*) caused a *decrease* in potency versus [$^3$H]$H_{12}$-HTX in the absence of carbamylcholine, while having no significant effect on potency in the presence of carbamylcholine nor on potency versus [$^3$H]PCP binding. Clearly, the nature of the five carbon side chain influenced the effects of changes in the four carbon side chain and vice versa (*vide supra*). No compound was available to assess the effect of increases in conformational freedom of the four carbon side chain on activity. Octahydrohistrionicotoxin (compound *21*), with marked conformational freedom in both the five and four carbon side chains, was a very active compound. Reduction of the terminal

double bond of the four carbon side chain of octahydrohistrionicotoxin yields $H_{10}$-(pentenyl)histrionicotoxin (compound *23*) with little alteration in potency versus [$^3$H]$H_{12}$-HTX or [$^3$H]PCP. Reduction of both terminal double bonds yields perhydrohistrionicotoxin (compound *6*), again with little alteration in potency. It would appear that the electronic nature of the terminal two carbons is relatively unimportant when there is a high degree of conformational freedom (compound *6* vs *21*), but it is important where there is a low degree of conformational freedom, as, for example, in histrionicotoxin (compound *1*) with terminal triple bonds compared to tetrahydrohistrionicotoxin (compound *20*) with terminal double bonds. Removal of the four carbon side chain to yield desbutylperhydrohistrionicotoxin (compound *18*) resulted in a 25- to 35-fold reduction in potency versus [$^3$H]$H_{12}$-HTX binding and a 60- to 70-fold reduction in potency versus [$^3$H]PCP binding relative to perhydrohistrionicotoxin. It would appear that the presence of a four carbon side chain is more important to potency versus [$^3$H]PCP binding than for potency versus [$^3$H]$H_{12}$-HTX binding.

## DISCUSSION

A variety of structurally modified analogs of histrionicotoxin have been evaluated with respect to their binding versus two compounds that affect the function of the acetylcholine receptor–ion channel complex by binding to ill-defined sites, presumably on the ion channel. These two ligands were [$^3$H]$H_{12}$-HTX, which is, of course, closely related structurally to the various histrionicotoxin analogs, and [$^3$H]PCP, which is quite different structurally. Antagonism of binding of the ligands by histrionicotoxin analogs could occur by direct competition for a single site, as would be expected for [$^3$H]$H_{12}$-HTX because of close structural similarities, but could also ccur by binding to other sites with resultant allosteric alteration of the channel proteins and, thereby, prevention or reduction of binding at the ligand site. Both mechanisms would result in competitive interactions between PCP and $H_{12}$-HTX, as were observed in the present studies. It is likely that PCP and the histrionicotoxins bind to the same site, but with different affinities depending on the conformation of the receptor complex (resting, activated, desensitized, etc.). Thus, the equilibrium binding constants for [$^3$H]PCP and [$^3$H]$H_{12}$-HTX would reflect both the microscopic dissociation constants that define ligand binding to each conformation of the site *and* the isomerization constants that define the distribution of the receptor between the various conformational states. In such a situation, the various histrionicotoxin analogues could differ in their abilities to inhibit [$^3$H]PCP versus [$^3$H]$H_{12}$-binding, even though [$^3$H]PCP and [$^3$H]$H_{12}$-HTX bind to the same ion channel site. This scheme is supported by the observations that: (1) $H_{12}$-HTX and PCP binding interactions were competitive, (2) the nicotinic receptor complex assumed several conformations, each associated with specific patterns of ion channel binding (see discussion below), and (3) closely related histrionicotoxin analogues had considerably different abilities to inhibit [$^3$H]$H_{12}$-HTX compared to [$^3$H]PCP binding to ion channel sites. Allosteric mechanisms could result in changes in off rates of binding of the radioligand but such data are not yet available.

Noncompetitive blockers of neuromuscular transmission bind to several categories of sites associated with the nicotinic acetylcholine receptor–ion channel complex [14, 15, *]. Phencyclidine and histrionicotoxin bind selectively to the "high affinity" sites that are believed to be located within the central hydrophilic depression of the receptor complex which is probably the site of ion translocation gated by cholinergic agonists [30]. The evidence for this, as summarized by Oswald *et al.* [31], is that (1) high-affinity noncompetitive blockers act at least partially on the $\delta$-subunit, but the sites are in close proximity to all of the polypeptides that constitute the receptor, (2) high-affinity binding sites are present with a stoichiometry of one site per receptor complex, (3) high-affinity binding sites are located one-half to three-quarters of the way across the membrane, and (4) access to the high-affinity sites is controlled by cholinergic ligands. As a consequence of this binding, a desensitized conformation of the receptor complex characterized by a high affinity for receptor agonists is stabilized [14, 28]. In the presence of receptor agonists, histrionicotoxin and phencyclidine may sterically block ion fluxes as well as affect conformational shifts [31]. Conversely, occupancy of receptor binding sites alters [$^3$H]$H_{12}$-HTX and [$^3$H]PCP binding. Carbamylcholine increased the affinity of [$^3$H]$H_{12}$-HTX and [$^3$H]PCP for their binding sites 1.5- and 5.4-fold, respectively, without altering the number of binding sites (unpublished results). In the absence of cholinergic ligands, [$^3$H]$H_{12}$-HTX binding approaches equilibrium quite slowly. This has been interpreted as indicating severe steric restrictions for access of [$^3$H]$H_{12}$-HTX to its binding sites [14, 17]. Apparently, these steric hindrances are eliminated upon assumption of activated and/or desensitized conformations by the receptor.

The present results on twenty-three histrionicotoxins are most readily discussed in terms of a single, high-affinity ion channel binding site which may exist in a variety of low energy conformations. The distribution among these conformations is strongly influenced by receptor agonists and by the ion channel probes themselves. Differences in the ability of histrionicotoxins to inhibit [$^3$H]$H_{12}$-HTX versus [$^3$H]PCP binding are assumed to reflect the different dissociation constants of both radiolabeled probe and unlabeled histrionicotoxin for the binding site in its various conformations.

*[$^3$H]$H_{12}$-HTX binding.* Many of the histrionicotoxins (compounds *1–7, 14–16, 18, 20–23*) were more potent as antagonists of [$^3$H]$H_{12}$-HTX binding in the presence of carbamylcholine, suggesting a higher affinity for activated forms of the ion channel binding site, while some analogs (compounds *8–13, 17, 19*) were nearly equipotent in the absence or presence

* Y. Aracava, J. W. Daly and E. X. Albuquerque, *IUPHAR Ninth International Congress of Pharmacology*, 601P (1984).

of carbamylcholine. It is unclear what structural features confer selectivity for the [$^3$H]$H_{12}$-HTX binding site in the "activated" form of the channel complex. Histrionicotoxin itself and its O- and N-modified derivatives were selective. Indeed, the O-acetyl moiety appeared to enhance selectivity, resulting in compounds with potencies 7- to 11-fold greater at the activated form, compared to only a 3-fold selectivity for histrionicotoxin and *N*-methylhistrionicotoxin. Perhydrohistrionicotoxin was somewhat selective but its 7-deoxy derivatives (compounds *8* and *9*) were not. The desbutyl-derivative (compound *18*) was moderately selective, while the desamyl-derivatives (compounds *10* and *11*) were not. However, minor structural modifications in either the four or five carbon side chains altered binding potency or selectivity for the activated form. Modification of the five carbon side chain of histrionicotoxin, a selective blocker of the activated form, yielded isodihydrohistrionicotoxin (compound *12*) and dihydrohistrionicotoxin (compound *13*) which were very potent nonselective blockers. Both analogs with allene groups in the five carbon side chain (compounds *12* and *19*) had high potency versus [$^3$H]$H_{12}$-HTX binding, but little selectivity for the activated form. Other modifications of the five carbon side chain of histrionicotoxin yielded allodihydrohistrionicotoxin (compound *14*) and histrionicotoxin 259 (compound *15*), both of which retained selectivity for sites on the activated form. Similarly, modification of the four carbon chain of histrionicotoxin yielded compounds with either greater or lesser potency versus [$^3$H]$H_{12}$-HTX binding, and with either greater or lesser selectivity for the activated form of the ion channel: $\Delta^{17,18}$-*trans*-histrionicotoxin (compound *16*) was much less potent, but more selective, than histrionicotoxin, while neodihydrohistrionicotoxin (compound *17*) was more potent, but less selective, than histrionicotoxin. The reduction in potency of $\Delta^{17,18}$-*trans*-histrionicotoxin was remarkable and suggests that the binding site will not readily accommodate a rigid and extended *trans*-ene-yne side chain. Remarkably, tetrahydrohistrionicotoxin (compound *20*) was a very selective (7-fold) compound. Thus, single structural changes that rendered neodihydrohistrionicotoxin (compound *17*) and dihydrohistrionicotoxin (compound *13*) nonselective, resulted, when combined in tetrahydrohistrionicotoxin, in a highly selective compound. Conversion of tetrahydrohistrionicotoxin to the O-acetyl derivative, based on the histrionicotoxin series, would be expected to result in even greater selectivity.

There appears to be some correlation between potency and selectivity. The four most potent compounds (compounds *19*, *17*, *12*, and *13*) were nonselective. The most potent analog with high selectivity was octahydrohistrionicotoxin (compounds *21* and *22*). Perhydrohistrionicotoxin (compound *6*), allodihydrohistrionicotoxin (compound *14*), and $H_{10}$-(pentenyl)histrionicotoxin (compound *23*) were also quite potent, but their selectivity was only about 2-fold.

*[$^3$H]PCP-binding.* Potencies of the histrionicotoxins versus [$^3$H]PCP binding did not always parallel the data on [$^3$H]$H_{12}$-HTX binding. In all cases, the various histrionicotoxins were more potent versus [$^3$H]PCP binding than versus [$^3$H]$H_{12}$-HTX binding. The difference ranged from about 2-fold for many compounds to as much as 50-fold for *N*-methyl-*O*-acetylhistrionicotoxin (compound *4*) in the presence of carbamylcholine. The selectivity of the histrionicotoxins versus [$^3$H]PCP binding in the absence or presence of carbamylcholine was often less than was the case versus [$^3$H]$H_{12}$-HTX in the absence or presence of carbamylcholine. This was the case for histrionicotoxin, *O*-acetylhistrionicotoxin, *O*-acetylhistrionicotoxin methiodide, histrionicotoxin 259, $\Delta^{17,18}$-*trans*-histrionicotoxin, allodihydrohistrionicotoxin, tetrahydrohistrionicotoxin, octahydrohistrionicotoxin, and desbutylperhydrohistrionicotoxin (compounds *1*, *3*, *5*, *15*, *16*, *14*, *20*, *21*, and *18*). Indeed, the last four compounds, in the case of [$^3$H]PCP binding, became nearly nonselective. Conversely, dihydrohistrionicotoxin (compound *13*), which was nonselective for [$^3$H]$H_{12}$-HTX binding, was selective for [$^3$H]PCP binding. *N*-Methylhistrionicotoxin (compound *2*) and *dl*-octahydrohistrionicotoxin (compound *22*) had a selectivity of about 3-fold in both cases, while *N*-methyl-*O*-acetylhistrionicotoxin (compound *4*) was more selective for the case of [$^3$H]PCP binding with a selectivity of about 20-fold compared to 8-fold for [$^3$H]$H_{12}$-HTX binding.

*Correlations with electrophysiological effects.* Blockade of acetylcholine–receptor channel complexes by certain histrionicotoxins has been studied in detail in mammalian and amphibian neuromuscular preparations [4, 6–8, 10, 16, 24, 29]. Comparisons of such data with the present binding data are complicated by a number of factors, including the use and time dependence of electrophysiological blockade. With regard to endplate currents, histrionicotoxins appear to have two probably independent effects. The first is a relatively rapid decrease in the decay time constant and the second a use- and time-dependent decrease in peak amplitude. Limitations of supplies of naturally occurring histrionicotoxins have prevented a detailed study of many of the compounds of the present study. There are, however, some correlations between the binding and electrophysiological data. Thus, there is little difference in potencies for the *d*- and *l*-enantiomers of perhydrohistrionicotoxin with regard to either electrophysiology [24, 29] or binding (Table 1). The lack of the five carbon side chain results in a marked decrease in potency with regard to neuromuscular transmission [24, 29] and with regard to binding (Table 1). N-Methylation or O-acetylation appears to have little effect on potency of histrionicotoxin analogs with regard to blockage of neuromuscular transmission [8, 29], nor did these alterations greatly affect potency for binding sites (Table 1). There are, however, many aspects of such structure–activity relationships that require further delineation. For example, the time to onset of neuromuscular blockade for a series of histrionicotoxins each at 70 $\mu$M is as follows: histrionicotoxin < isodihydrohistrionicotoxin, dihydrohistrionicotoxin, isotetrahydrohistrionicotoxin, tetrahydrohistrionicotoxin < neodihydrohistrionicotoxin < octahydrohistrionicotoxin < perhydrohistrionicotoxin [8]. Such a

rank order does not correlate well with any of the binding data but may, in large part, reflect kinetic effects related to lipid solubility in the case of the nerve-muscle preparation. Similarly, a rank order of potencies with respect to decreases in decay constant of endplate currents is as follows: isotetrahydrohistrionicotoxin > histrionicotoxin, perhydrohistrionicotoxin > octahydrohistrionicotoxin [4]. This rank order does not correlate well with any of the binding data. Since the binding data reflect composite interaction of the alkaloids with various sites on open, closed, and desensitized states of the nicotinic receptor–channel complex and since electrophysiological measurements occur under conditions in which the states will differ considerably from those under which the binding data were obtained, it is perhaps not unexpected that the rank order of potencies for a set of derivatives will not exactly correlate for the two paradigms.

The present binding data do delineate sets of histrionicotoxins which, if available synthetically, would be useful probes for the acetylcholine receptor–channel complex (Table 3). Thus, of all the histrionicotoxins, the isotetrahydrohistrionicotoxin (compound *19*) was the most potent in binding assays versus both [$^3$H]$H_{12}$-HTX and [$^3$H]PCP binding (Table 3). It was also virtually unselective for unactivated and carbamylcholine-activated complexes. Both N-methylation and O-acetylation, based on the present data, might be expected to increase selectivity of isotetrahydrohistrionicotoxin for the carbamylcholine-activated state without affecting its potency. Isodihydrohistrionicotoxin (compound *12*), the only other analog with an allene in the five carbon side chain, was also quite potent and relatively nonselective. The most selective natural analog, at least with respect to [$^3$H]$H_{12}$-HTX binding in the presence and absence of carbamylcholine was tetrahydrohistrionicotoxin (compound *20*). None of the histrionicotoxins were particularly selective with respect to [$^3$H]PCP binding in the presence and absence of carbamylcholine except for *N*-methyl-*O*-acetylhistrionicotoxin (compound *4*). The alkaloids showing the greatest differences in this regard were $\Delta^{17,18}$-*trans*-histrionicotoxin, tetrahydrohistrionicotoxin, histrionicotoxin 259, *O*-acetylhistrionicotoxin, and octahydrohistrionicotoxin (compounds *16*, *20*, *15*, *3*, and *21*) which were 7- to 14-fold more potent versus [$^3$H]PCP binding than versus [$^3$H]$H_{12}$-HTX binding. N-Methylation and/or O-acetylation would be expected, based on the results with histrionicotoxin, to increase further this difference in potency versus [$^3$H]PCP and [$^3$H]$H_{12}$-HTX. Unfortunately, except for histrionicotoxin, isodihydrohistrionicotoxin and allohistrionicotoxin, none of the other natural histrionicotoxins are available in significant amounts from natural sources and further studies on these must await synthetic material, in particular histrionicotoxin 259, isotetrahydrohistrionocotoxin, tetrahydrohistrionicotoxin and octahydrohistrionicotoxin. In view of enantiomeric selectivity at the [$^3$H]PCP sites, such compounds would be most desirable as the *l*- and/or *d*-enantiomers.

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