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The short-neurotoxin-binding regions on the α-chain of human and *Torpedo californica* acetylcholine receptors

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The continuous regions for short-neurotoxin binding on the α-chains of *Torpedo californica* (electric ray) and human acetylcholine receptors (AChR) were localized by reaction of ^{125}I-labelled cobrotoxin (Cot) and erabutoxin b (Eb) with synthetic overlapping peptides spanning the entire extracellular part of the respective α-chains. On *Torpedo* AChR, five Cot-binding regions were found to reside within peptides α1–16, α23–38/α34–49 overlap, α100–115, α122–138 and α194–210. The Eb-binding regions were localized within peptides α23–38/α34–49/α45–60 overlap, α100–115 and α122–138. The main binding activity for both toxins resided within region α122–138. In previous studies we had shown that the binding of long α-neurotoxins [α-bungarotoxin (Bgt) and cobratoxin (Cbt)] involved the same regions on *Torpedo* AChR as well as an additional region within residues α182–198. Thus region α182–198, which is the strongest binding region for long neurotoxins on *Torpedo* AChR, was not a binding region for short neurotoxins. On human AChR, peptide α122–138 possessed the highest activity with both toxins, and lower activity was found in the overlap α23–38/α34–49/α45–60 and in peptide α194–210. In addition, peptides α100–115 and α56–71 showed strong and medium binding activities to Eb, but low activity to Cot, whereas peptide α1–16 exhibited low binding to Cot and no binding to Eb. Comparison with previous studies indicated that, for human AChR, the binding regions of short and long neurotoxins were essentially the same. The finding that the region within residues α122–138 of both human and *Torpedo* AChR possessed the highest binding activity with short neurotoxins indicated that this region constitutes a universal binding site for long and short neurotoxins on AChR from various species.

INTRODUCTION

The nicotinic acetylcholine receptor (AChR) plays a central role in postsynaptic neuromuscular transmission by mediating ion flux across the cell membrane in response to binding of acetylcholine (Karlin *et al.*, 1980; Conti-Tronconi & Raftery, 1982; Changeux *et al.*, 1984; McCarthy *et al.*, 1986). The binding of an α-neurotoxin to AChR blocks postsynaptic neuromuscular transmission by inhibiting the channel-opening activity of acetylcholine (Popot & Changeux, 1984; Stroud & Finer-Moore, 1985; Hucho, 1986). Snake venom postsynaptic neurotoxins form a large family of related proteins, of which two subgroups, the long and short neurotoxins, are major constituents (see the Discussion section). Both long and short neurotoxins are known to bind specifically to the α-chain of AChR in a competitive manner with cholinergic ligands (Meunier *et al.*, 1974; Maelicke *et al.*, 1977; Haggerty & Froehner, 1981), but display differences in their association and dissociation kinetics. Identification of the binding sites on the toxins and the receptor should provide a molecular explanation for the observed differences between the two toxin groups in their actions on AChR.

By application of a comprehensive synthetic-peptide strategy (Kazim & Atassi, 1980), we have recently reported the localization of the full profile of the continuous binding regions for long α-neurotoxin on the extracellular part (residues α1–210) of the α-chains of *Torpedo* (electric ray) (Mulac-Jericevic & Atassi, 1987*a*,*b*) and human AChRs (Mulac-Jericevic *et al.*, 1988; Ruan *et al.*, 1990). In *Torpedo* AChR, the binding regions reside within (but may not include all of) residues α1–10, α32–49, α100–115, α122–138 and α182–198. In human AChR, long neurotoxins bind to regions α32–49, α100–115, α122–138 and α194–210. In the present paper the two panels of *Torpedo* and human AChR synthetic overlapping peptides were employed to localize short-neurotoxin-binding regions on the extracellular part of α-chains of the two respective species of AChR. Comparison between the short- and long-neurotoxin-binding regions on AChR revealed important differences in the AChR-toxin contacts, particularly with *Torpedo* AChR.

MATERIALS AND METHODS

Neurotoxins

Cobrotoxin (Cot) and erabutoxin b were prepared by Dr. Bruce Meade (Fort Detrick address). Cobrotoxin was isolated from the venom of the Formosan Cobra (*Naja naja atra*) as described by Lee *et al.* (1968) and Yang *et al.* (1969). Erabutoxin b (Eb) was prepared from the venom of the banded sea krait (*Laticauda semifasciata* by the procedure of Tamiya & Arai (1966). Crude venoms were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The preparations were monitored by SDS/15%-(w/v)-PAGE. The authenticity of the pure toxin preparations was confirmed by their amino acid compositions and partial *N*-terminal-sequence analysis. The amino acid composition of each neurotoxin and the sequence of its first 15 amino acid residues were in excellent agreement with those expected from its respective reported covalent structure (Yang *et al.*, 1969; Nishida *et al.*, 1985). The LD_{50} values (3 μg/28–30 g mouse) were very similar to the reported values (Tamiya & Arai, 1966). α-Bungarotoxin (Bgt) and cobratoxin (Cbt) were obtained from Miami Serpentarium Laboratories (Salt Lake City, UT, U.S.A.).

Abbreviations used: AChR, acetylcholine receptor; Bgt, α-bungarotoxin; Cbt, cobratoxin; Cot, cobrotoxin; Eb, erabutoxin b; PBS, phosphate-buffered saline (0.15 M-NaCl in 0.01 M-sodium phosphate buffer, pH 7.2).

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Peptide position	Species	Structure
α1–16	H	S E H E T R L V A K L F K D Y S
	T	- - - - - - - - - N - L E N - N
α12–27	H	F K D Y S S V V R P V E D H R Q
	T	L E N - N K - I - - - - H - T H
α23–38	H	E D H R Q V V E V T V G L Q L I
	T	- H - T H F - D I - - - - - - -
α34–49	H	G L Q L I Q L I N V D E V N Q I
	T	- - - - - - - - S - - - - - - -
α45–60	H	E V N Q I V T T N V R L K Q Q W
	T	- - - - - - - E - - - - - R - - -
α56–71	H	L K Q Q W V D Y N L K W N P D D
	T	- R - - - I - V R - R - - - A -
α67–82	H	W N P D D Y G G V K K I H I P S
	T	- - - A - - - - I - - - R L - -
α78–93	H	I H I P S E K I W R P D L V L Y
	T	- R L - - D D V - L - - - - - -
α89–104	H	D L V L Y N N A D G D F A I V K
	T	- - - - - - - - - - - - - - - H
α100–115	H	F A I V K F T K V L L Q Y T G H
	T	- - - - - H M - - L - - D - - - K
α111–126	H	Q Y T G H I T W T P P A I F K S
	T	D - - - K - M - - - - - - - - - -
α122–138	H	A I F K S Y G E I I V T H F P F D
	T	- - - - - - - - - - - - - - - - -
α134–150	H	H F P F D E Q N G S M K L G T W T
	T	- - - - - Q- - - -T - - - - I - -
α146–162	H	L G T W T Y D G S V V A I N P E S
	T	- - I - - - - - T K - S - S - - -
α158–174	H	I N P E S D Q P D L S N F M E S G
	T	- S - - - - R - - - - T - - - - -
α170–186	H	F M E S G E W V I K E S R G W K H
	T	- - - - - - - - M - D Y - - - - -
α182–198	H	R G W K H S V T Y S G G P D T P Y
	T	- - - - - W - Y - T - - - - - - -
α194–210	H	P D T P Y L D I T Y H F V M Q R L
	T	- - - - - - - - - - - I - - - I
α262–276	H	E L I P S T S S A V P L I G K
	T	- - - - - - - - - - - - - - -

Fig. 1. Covalent structures of the synthetic overlapping peptides representing the extracellular part of each of the α-chains of human (H) and *Torpedo californica* (T) AChRs

The upper sequences of each pair of peptides give the full primary structures of the human AChR peptides and, under these, only the residues that are different in the corresponding *Torpedo* peptides are given. Segments in **bold type** represent the five-residue overlaps between consecutive peptides.

AChR and receptor peptides

The preparation of AChR from the electric-organ tissue of *Torpedo californica* (Pacific Bio-Marine Laboratories, Venice, CA, U.S.A.) was carried out as described elsewhere (Mulac-Jericevic *et al.*, 1987). The four-subunit composition ($\alpha_2\beta\gamma\delta$) of pure AChR and the binding activity of its α-chain were confirmed by SDS/PAGE (Laemmli, 1970) and Western blotting (Towbin *et al.*, 1979). Freshly prepared AChR had a Bgt-binding activity of 8.7–9.1 nmol/mg of AChR. The peptides (Fig. 1), which corresponded to the extracellular part (residues 1–210) of the α-chains of *Torpedo* and human AChR (Noda *et al.*, 1982, 1983) were synthesized, purified and characterized as previously described (Mulac-Jericevic & Atassi, 1987*a*; Mulac-Jericevic *et al.*, 1988).

Binding of toxins to the synthetic AChR peptides

The toxins were labelled with ^{125}I by using the chloramine-T method (Hunter & Greenwood, 1962). Radioiodinated materials were used immediately after labelling. The specific radioactivities of the labelled toxins were: Cot, 2.5×10^3 c.p.m./pmol; Eb, 3.3×10^3 c.p.m./pmol. The coupling of proteins and peptides to CNBr-activated Sepharose CL-4B was carried out under optimum conditions as described by Twining & Atassi (1979). At least three preparations of each adsorbent were studied. Protein and peptide contents of the adsorbents were determined by duplicate amino acid analysis of acid hydrolysates. The adsorbents contained 0.8 ± 0.1 mg/ml and 0.43 ± 0.04 mg/ml of packed volume respectively.

Quantitative adsorbent titrations were performed in phosphate-buffered saline (PBS; 0.15 M-NaCl in 0.01 M-sodium phosphate buffer, pH 7,2) containing 0.1% BSA with fixed amounts of ^{125}I-labelled toxin and various amounts of protein or peptide adsorbents. Titrations were also performed using fixed amounts (25 μl of a 1:1, v/v, suspension) of each adsorbent with increasing amounts of ^{125}I-labelled toxin. Binding studies were done at room temperature for 16 h with gentle rocking, after which the tubes were washed four times by centrifugation with PBS and then counted for radioactivity in a γ-radiation counter. The studies on each panel of peptides were done three times, each in triplicate. Non-specific binding was determined by titrating, under identical conditions, equivalent volumes of uncoupled Sepharose CL-4B and Sepharose adsorbents of unrelated proteins (BSA, horse myoglobin) and synthetic peptides of similar size [sperm-whale myoglobin synthetic peptides corresponding to residues 1–17, 25–41 and 121–137 (Bixler & Atassi, 1983)]. The specificity of binding ^{125}I-labelled toxin to fixed amounts (5 μl, packed volume) of peptide adsorbents was confirmed by inhibition studies using various amounts of unlabelled toxin (Mulac-Jericevic & Atassi, 1987*b*) as inhibitor. Unrelated proteins [BSA, myoglobin and the aforementioned myoglobin synthetic peptides (Bixler & Atassi, 1983)] were used as control inhibitors.

RESULTS

Binding of short neurotoxins to *Torpedo* AChR peptides

The binding profiles of ^{125}I-labelled short neurotoxins to *Torpedo* AChR peptides are summarized in Fig. 2. The results showed that the main binding activity for both neurotoxins, Cot and Eb, resided within region α122–138. A lower binding activity was exhibited by the peptides α23–38/α34–49 overlap and α100–115. Peptides α1–16 and α194–210 had low binding activity only with Cot, and little or no activity with Eb. On the other hand, peptide α45–60 showed low, but significant, binding to Eb, whereas its binding to Cot was considerably lower. Finally, ^{125}I-short neurotoxins bound, as expected, to *Torpedo* AChR (positive control), but not to any unrelated proteins and peptides (negative controls).

The finding here that the region α182–198 of *Torpedo* AChR did not show any significant binding to short neurotoxins was unexpected, in view of the fact that this constitutes a major binding region to long neurotoxins (Mulac-Jericevic & Atassi, 1986, 1987*a*,*b*). To confirm further this major difference in the binding site for long and short neurotoxins on *Torpedo* AChR, quantitative radiometric titrations were carried out using a constant amount of peptide adsorbents [25 μl; 1:1 (v/v) suspension in PBS] and increasing amounts of ^{125}I-labelled short (Cot and Eb) or long (Bgt) neurotoxins. The peptide α122–138, which binds to both long and short neurotoxins equally well, was used as a positive control. The results (Fig. 3) showed that the long neurotoxin, Bgt, bound to both peptides α122–138 and α182–198, clearly confirming that the binding activity of the

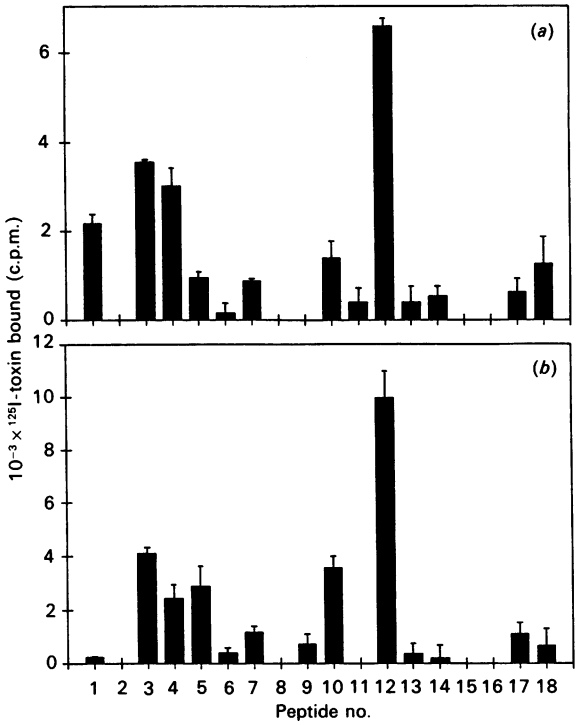

Fig. 2. Summary of the profiles of (*a*) Cot and (*b*) Eb binding to the synthetic overlapping peptides of the extracellular part of the α-chain of *Torpedo* AChR

The bars represent the binding values of 25 μl of a 1:1 suspension (v/v) of each peptide adsorbent. Titrations were carried out in triplicates in PBS containing 0.1% BSA. The reaction volume was 60 μl and the amount of ^{125}I-labelled toxin added was 350000 c.p.m./tube. After the reaction, the adsorbents were washed by centrifugation four times with PBS and their radioactivity was counted. *Torpedo* AChR was used as a positive control. The results, which represent averages for three experiments, each in triplicate, have been corrected for non-specific binding to unrelated proteins and peptides. The peptides were: 1, α1–16; 2, α12–27; 3, α23–38; 4, α34–49; 5, α45–60; 6, α56–71; 7, α67–82; 8, α78–93; 9, α89–104; 10, α100–115; 11, α111–126; 12, α122–138; 13, α134–150; 14, α146–162; 15, α158–174; 16, α170–186; 17, α182–198; 18, α194–210 (Mulac-Jericevic & Atassi, 1987*a*; Mulac-Jericevic *et al.*, 1988). The binding values of ^{125}I-labelled Cot and Eb to *T. californica* AChR were 55140±1350 and 68550±1520 c.p.m. respectively. Binding to unrelated proteins (BSA, horse myoglobin) and peptides [sperm-whale myoglobin synthetic peptides 1–17, 25–41 and 121–137 (Bixler & Atassi, 1983)] (negative controls) was 650±220 c.p.m.

peptide α182–198 had not been destroyed in the present adsorbent preparation. This same preparation did not bind the short neurotoxins Cot and Eb (Fig. 3), whereas peptide α122–138 was fully capable of binding these two toxins. Finally, adsorbents of unrelated proteins and peptides did not bind any of these toxins, thus confirming the specificity of the aforementioned binding results.

Binding of short neurotoxins to human AChR peptides

The results for short neurotoxin binding to human AChR peptides are summarized in Fig. 4. The main binding activity for both toxins resided within peptide α122–138. A lower binding activity was present in the overlap α23–38/α34–49/α45–60 and in peptide α194–210. Peptides α100–115 and α56–71 showed strong and medium binding respectively to Eb, but low binding to Cot. Peptide α1–16 had a low binding activity only with Cot and showed negligible binding to Eb. Finally, these two toxins did not bind to unrelated proteins and peptide controls.

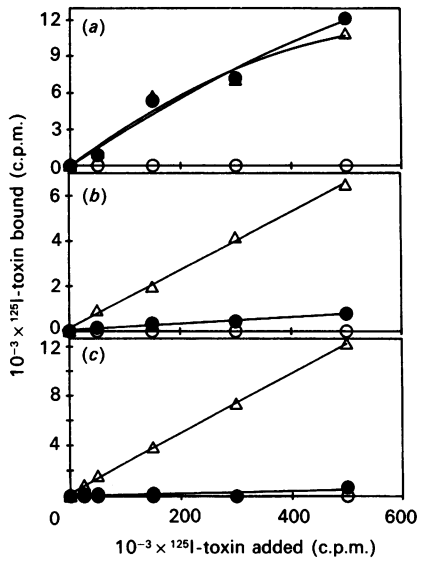

Fig. 3. Comparison of the extents of binding of ^{125}I-labelled (*a*) Bgt, (*b*) Cot and (*c*) Eb to peptides (△) α122–138 and (●) α182–198, of the α-chain of *Torpedo* AChR and to the unrelated synthetic peptides given in Fig. 2 (○)

Increasing amounts of the ^{125}I-labelled toxins were added to a fixed volume [25 μl; 1:1 (v/v) suspension in PBS/0.1% BSA] of each peptide adsorbents. The experiments were carried out as described in Fig. 2 and the text. Each experimental point represents the average result for six replicate analyses which varied by ±2.4% or less.

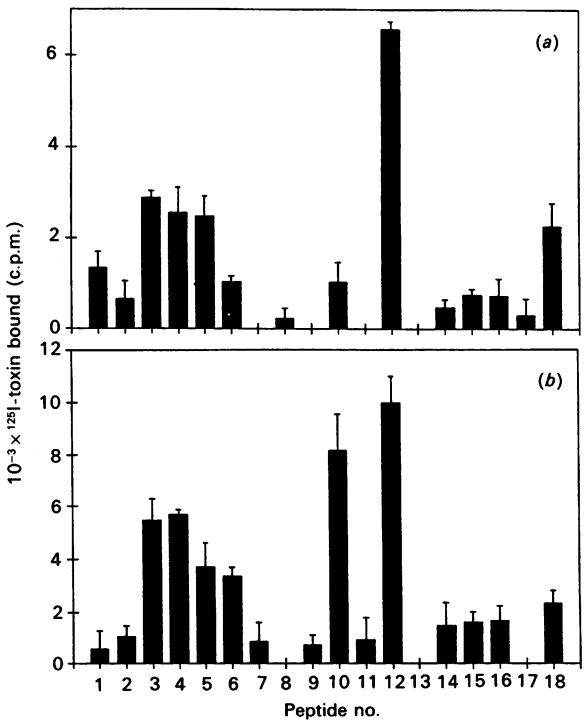

Fig. 4. Summary of the binding profiles of (*a*) Cot and (*b*) Eb to the synthetic overlapping peptides of the extracellular part of the α-chain of human AChR

The sequence positions of the 18 synthetic overlapping peptides of the α-chain of human AChR and the assay conditions were as described in Fig. 1.

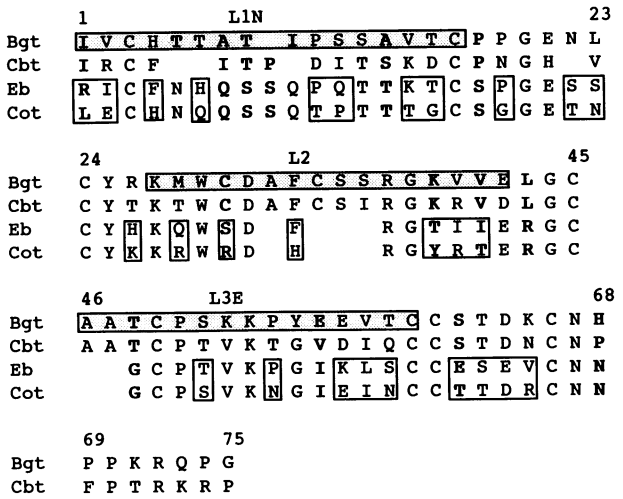

Fig. 5. Comparison of the consensus sequences of the short neurotoxins, Cot and Eb, and the long neurotoxins, Bgt and Cbt

Letters in **bold type** denote differences between consensus sequences of long and short neurotoxins. The boxed regions in Cot and Eb denote sequence differences between the two short neurotoxins. The shaded boxed parts in the Bgt structure indicate the regions of the AChR-binding loops which were localized and confirmed by the use of synthetic peptides (Atassi *et al.*, 1988).

DISCUSSION

Venoms of snakes from Elapidae and Hydrophiidae families possess proteins having very pronounced pharmacological activities (Dufton & Hider, 1983; Endo & Tamiya, 1987). Some members of this family are potent cytotoxins, whereas others are presynaptic or postsynaptic neurotoxins. The postsynaptic neurotoxins are divided into short and long neurotoxins. Both classes of toxins are known to bind specifically (Meunier *et al.*, 1974; Maelicke *et al.*, 1977; Haggerty & Froehner, 1981; Mishina *et al.*, 1984) and tightly (Weber & Changeux, 1974) to AChR. This binding is, in a competitive manner, linked to the binding of cholinergic ligands (one of which is the physiological native channel-opening molecule acetylcholine). However, unlike the cholinergic ligands, binding of neurotoxins to AChR does not lead to opening, but rather to relatively permanent closure, of the channel. The extremely tight, non-covalent, association between receptor and neurotoxins (dissociation constant range 10^{-9} M–10^{-11} M) in comparison with that of acetylcholine (10^{-6} M) makes them useful tools with which to investigate the function of the neuromuscular synapse and its receptors.

Short and long neurotoxins have very similar dissociation constants with AChR (Lee *et al.*, 1972; Chicheportiche *et al.*, 1975) (10^{-10}–10^{-11} M) and LD_{50} values (typically, for mice, between 50 and 150 μg/kg). They differ chiefly in their rates of association and dissociation from the receptor. Long neurotoxins generally associate and dissociate much more slowly (Weber & Changeux, 1974; Chicheportiche *et al.*, 1975). These differing rates are reflections of major sequence differences between the two types of toxins.

The primary structure of short neurotoxins is composed of 60, 61 or 62 amino acid residues, all of which are intramolecularly cross-linked by four disulphide bridges. One short neurotoxin, Eb, has been crystallized and its X-ray structure has been determined (Low *et al.*, 1976, 1984; Tsernoglou & Petsko, 1976, 1977; Kimball *et al.*, 1979). The disulphide bonds of this short neurotoxin are localized at one end of the molecule and, accordingly, produce a knotted structure with a globular head and three protruding loops. The predominant secondary structural characteristic is β-sheet with β-turns located at the chain reversals. Most of the invariant residues either are localized in the immediate vicinity of the disulphide bridges in the globular head or are found toward the distal ends of the three major loops. In contrast, the least conserved residues tend to be grouped across the top of the globular head.

Long neurotoxins also have the four disulphide bridges of short neurotoxins, but possess an additional disulphide bond in the central loop of the molecule. In addition, apart from insertions and deletions within the main chain itself, long neurotoxins have a long polypeptide chain (between 65 and 74 residues) giving rise to a characteristic *C*-terminal tail. The three-dimensional structures of two long neurotoxins (Cbt and Bgt) have been determined, and the overall structure is highly similar to that of Eb (Walkinshaw *et al.*, 1980). Apparently, where there are differences in sequence or chain length, these alterations do not disrupt the clustering of the disulphide bridges or the major loops. There are proportionately fewer conserved or invariant residues in the long neurotoxins. However, there are marked similarities in and around the disulphide bridges and in the loops. In long neurotoxins, the least-conserved regions tend to be found in the *C*-terminal tail and the first loop.

The application of a comprehensive synthetic approach, previously introduced in this laboratory (Kazim & Atassi, 1980, 1982), enabled the mapping of the full profile of binding regions for long neurotoxins on the extracellular part of the α-chains of *Torpedo californica* (Mulac-Jericevic & Atassi, 1987*a*,*b*) and human (Mulac-Jericevic *et al.*, 1988) AChR. Determination of the binding regions for short neurotoxins on *Torpedo* and human AChR should, therefore, permit the comparison of the binding regions for the two classes of toxin on a given AChR and provide a rationale for the differences in their binding kinetics.

The AChR of *T. californica* has five regions on its α-subunit which are involved in the binding to long neurotoxins (Bgt and Cbt) (Mulac-Jericevic & Atassi, 1986, 1987*a*,*b*). These regions reside within, but may not include all of, residues α1–16, the overlap α23–38/α34–49, α100–115, the overlap α122–138/α134–150, α182–198. In the human receptor, the binding activities of peptides α1–16 and α182–198 are lost because of adverse amino acid replacements (Fig. 1) (Mulac-Jericevic *et al.*, 1988). A low binding activity is retained by the human peptide α194–210. The main difference, however, in the binding of long neurotoxins to the overlapping peptides of *T. californica* and human AChR, is the great decrease in the contribution of peptide α182–198 to the binding of the human receptor. It has been found (Sine, 1988) that Bgt binds to human and *T. californica* AChR with the same forward rate constant (1.8×10^5 M$^{-1}\cdot$s^{-1}). But there were remarkable differences in the dissociation of the toxin from human and *T. californica* receptors. The dissociation time constant was 6 h for the human receptor from intact TE671 human medulloblastoma cells and 24 h for membrane-associated *Torpedo* receptor. The differences in the reversibility of long neurotoxins binding to *Torpedo* AChR must be due to the contribution of region α182–198 to binding in *Torpedo* AChR and the absence of this contribution in human AChR.

Binding studies with whole human AChR and short neurotoxins have not been performed. Reversibility studies of neuromuscular blockade by long and short neurotoxins were done with species other than human and *Torpedo* (Lee *et al.*, 1972). With rat phrenic-nerve preparations, binding of the short neurotoxins Eb and Cot was slowly reversible, whereas that of Bgt was not. Binding of Cot to the sciatic-nerve sartorius-muscle preparation of the frog (*Rana tigrina*) was reversible, whereas that of Bgt was irreversible. This clearly indicates that the sequence differences between short and long neurotoxins are reflected in their AChR-binding properties. In the present work

the main difference in the binding of long and short neurotoxins to the overlapping peptides of *T. californica* AChR lies in the behaviour of peptide α182–198. This peptide possessed the highest binding activity of all the *T. californica* peptides for long neurotoxins, but showed little or no binding to short neurotoxins. The inability of the region α182–198 in both human and *Torpedo* AChR to bind short neurotoxins and in human AChR to bind long neurotoxins confirms the previous conclusions (Mulac-Jericevic *et al.*, 1988) that the region α182–198 may not play a significant role in neuromuscular blockage. It may be concluded that the differences in reversibility between long and short neurotoxins are due to the inability of short neurotoxins to bind to the contact region within residues α182–198 of AChR. Thus the participation or otherwise of region α182–198 in the neurotoxin binding may explain the differences in the association and dissociation rates between long and short neurotoxins.

The AChR-binding regions on Bgt were recently mapped using synthetic peptides corresponding to the various loops and most of the surface areas of the toxin (Atassi *et al.*, 1988). It was found that Bgt has three AChR-binding regions within the loop peptides L1N, L2 and L3E (Fig. 5). Comparison of the loop sequences in the short neurotoxins (Cot and Eb) with the corresponding regions in the long neurotoxins (Bgt and Cbt) (Fig. 5), will help to explain some of the differences in their binding behaviours towards the region α182–198 of *Torpedo* AChR. The consensus sequences exhibited several amino acid replacements within the three loops, but loop L1N exhibited a much higher number of amino acid replacements than did loops L2 and L3E. This could indicate that loop L1N of the long neurotoxins is their main contact with region α182–198 of the *Torpedo* AChR. Short neurotoxins are unable to bind to peptide α182–198 of *Torpedo* AChR, probably because of several adverse amino acid replacements in loop L1N (Fig. 5).

With the peptide panel of a given AChR, there were differences in the binding profiles of Cot and Eb (Figs. 2 and 4). The main difference was that peptide α1–16 in both receptors bound to Cot, but not to Eb. Sequence comparison of Cot and Eb showed several amino acid differences within the AChR-binding loops (Fig. 5). These replacements may explain the slight differences in the binding profiles of the two short neurotoxins.

It should also be noted that some quantitative differences were observed between human and *Torpedo* AChR peptides in the binding to a given short neurotoxin. For example, Cot showed considerably lower binding to *Torpedo* peptide α45–60 than to the corresponding human peptide (Figs. 2 and 4). This region has two amino acid replacements (Thr-51→Glu and Lys-57→Arg) in *Torpedo* AChR relative to the human receptor (Fig. 1). The decrease in binding is most likely due to the adverse effect resulting from the creation of a negatively charged side chain at position 51. On the other hand, Eb showed lower binding to *Torpedo* peptides α34–49, α56–71, α100–115 and α194–210 than to the corresponding human peptides (Figs. 2 and 4). These differences are caused by the amino acid substitutions in these regions (Fig. 1). The quantitative differences in the effects of these substitutions on the binding of the two short neurotoxins should also be influenced by sequence differences between Eb and Cot (Fig. 5).

The Bgt-binding cavity on human AChR was recently derived from peptide-to-peptide binding studies of the human receptor peptides and the aforementioned Bgt synthetic loops, followed by modelling (Ruan *et al.*, 1990). The region α122–138, which is a main universal long-neurotoxin-binding region on AChR α-chain from various species (Mulac-Jericevic *et al.*, 1988), forms a face (subsite) within the toxin-binding cavity. This region also carries essential contact residues of the acetylcholine-binding site (McCormick & Atassi, 1984). The present studies have shown that this region possessed the highest activity of binding to the short neurotoxins. Clearly, this region is the main universal binding site for both long and short neurotoxins. It is noteworthy that the affinity of neurotoxins to the receptor is several orders of magnitude higher than that of acetylcholine. Therefore the binding of toxin will be expected to prevent that of acetylcholine (and thus disrupt receptor function) completely, even in the presence of a large excess of the latter.

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