Investigation of Ligand-Binding Sites of the Acetylcholine Receptor Using Photoactivatable Derivatives of Neurotoxin II from *Naja naja oxiana*†

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ABSTRACT: Several photoaffinity derivatives of neurotoxin II from the venom of the central Asian cobra *Naja naja oxiana* have been prepared. After reaction of the ^{125}I-labeled derivatives with the nicotinic acetylcholine receptor from electric organ, the α-subunit of the nAChR is almost exclusively labeled by the derivative carrying the photoactivatable group in position Lys46. In contrast to this, a reactive group at Lys26 predominantly labels the γ- and δ-subunits, while the α- and β-subunits incorporate much less radioactivity. Competition experiments with *d*-tubocurarine show that the γ-subunit is labeled when this derivative occupies the high affinity *d*-tubocurarine-binding site, while the δ-subunit is labeled by the toxin bound at the low-affinity *d*-tubocurarine site. A model is discussed for the orientation of different loops of the toxin molecules in the binding site for agonists and competitive antagonists.

The nicotinic acetylcholine receptor (nAChR)[1] is the neurotransmitter receptor involved in chemical transmission at the vertebrate neuromuscular junction. Binding of two molecules of acetylcholine leads to the opening of an ion channel which is an integral part of the receptor. The structure of this protein has been the subject of numerous investigations [reviewed in Stroud et al. and (1990) Karlin (1991)]. The nAChR consists of five subunits with the stoichiometry $\alpha_2\beta\gamma\delta$. The two binding sites for agonists and competitive antagonists have been localized on the two α-subunits. Several affinity-labeling studies performed on the nAChR from Torpedo electric organ have further defined the region of the α-subunit which interacts with cholinergic ligands. Residues Cys192/Cys193 (which form a vicinal disulfide), as well as Tyr190 and Tyr198, have been shown to contribute to the binding region (Kao et al., 1984; Abramson et al., 1989; Dennis et al., 1988; Middleton & Cohen; 1991). In the desensitized state, which is characterized by an increased affinity for agonists, Tyr93 and Trp149 are also labeled by the photoaffinity reagent [^{3}H]DDF (Galzi et al., 1991). The importance of the α-180–200 region for the formation of the binding site has been supported by a series of binding studies with α-neurotoxins from snake venom (Wilson et al., 1984, 1985). α-Neurotoxins are a group of basic polypeptides (7–8 kDa) which bind to the nAChR with very high affinity (K_D 10^{-9}–10^{-11} M) and thus block neuromuscular transmission. In model experiments with synthetic or proteolytic fragments of the Torpedo receptor α-subunit, the sequence α-185–196 has been identified as the minimum required to detect binding of α-BgTx (Neumann et al., 1986).

The two binding sites of the nAChR molecule for agonists and competitive antagonists are not equivalent. Several ligands discriminate between these sites; the competitive antagonist *d*-tubocurarine shows a difference in K_D values of 2 orders of magnitude (Neubig & Cohen, 1979). The molecular basis for this discrepancy is, presumably, the different environment of the two α-subunits in a likely circular $\alpha\gamma\alpha\beta\delta$ subunit arrangement (Karlin, 1991). Blount and Merlie showed, by expression of different pairs of subunits of the mouse nAChR, that the high-affinity site for *d*-tubocurarine is formed by coexpression of α and γ, while the low-affinity *d*-tubocurarine site is observed with α and δ (Blount & Merlie, 1989). Photolabeling with *d*-tubocurarine yielded a similar result (Pedersen & Cohen, 1990): α and γ were labeled when *d*-tubocurarine was bound in its high-affinity site, while in the low-affinity site, the label was incorporated into α and δ.

α-Neurotoxins from snake venom are suitable tools for the detection of non-α-subunits in the binding site or in its vicinity. Because of their size they are expected to cover a larger area of the receptor surface than low molecular weight ligands. This has been exploited in several chemical and photochemical cross-linking studies (Hucho, 1979; Witzemann et al., 1979; Oswald & Changeux, 1982; Hamilton et al., 1985). Depending on the type of activation, the size of the cross-linker, and the type of neurotoxin, labeling of β, γ, and δ has been reported in addition to labeling of the α-subunit. A preliminary report on the preparation of neurotoxin derivatives with photoactivatable groups in defined sequence positions of the neurotoxin molecule and their binding to solubilized nAChR has been published by Tsetlin et al. (1983). Employing similar derivatives, Chatrenet et al. (1990) showed that, for the neurotoxin from *Naja naja nigricollis*, Lys47 (which is in the binding region of the toxin) cross-links to the α-subunit, Lys51 and Lys15, which do not contribute to high-affinity binding, make contacts to β and δ and to α and γ, respectively. We intended to probe the relationship between the toxin-binding sites and the above-mentioned low- and high-affinity sites for low molecular weight agonists/antagonists. For this purpose, we used derivatives of the neurotoxin II (NT-II) from the venom of the Central Asian cobra *Naja naja oxiana* with the photoactivatable groups in positions Lys26 and Lys46. These two residues are located on the concave side of the toxin

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[1] Abbreviations: AChR, acetylcholine receptor; α-BgTx, α-bungarotoxin; [^{3}H]DDF, *p*-[^{3}H](dimethylamino)benzenediazonium fluoroborate; NT-II, neurotoxin II from the venom of *Naja naja oxiana*; nAChR, nicotinic acetylcholine receptor.

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molecule which presumably interacts with the nAChR (Endo & Tamiya, 1987).

MATERIALS AND METHODS

Materials. Neurotoxin II from the venom of the Central Asian cobra *Naja naja oxiana* was obtained from Kemotex (Tallinn, Estonia). α-Bungarotoxin, *d*-tubocurarine, chloramine T, and *N*-hydroxysuccinimidyl azidobenzoate were purchased from Sigma (Munich, Germany). $Na^{125}I$ was purchased from Amersham-Buchler (Braunschweig, Germany); [^{125}I]BgTx was prepared by lactoperoxidase-catalyzed iodination of α-BgTx (Phillips & Morrison, 1970). Liquid N_2-frozen electric tissue form *Torpedo californica* was obtained from C. Winkler, San Pedro, CA.

Preparation of Photoactivatable Neurotoxin II Derivatives. The preparation of NT-II photoderivatives followed essentially a procedure preliminarly described by Tsetlin et al. (1983). All steps were carried out under dim light conditions or in the dark. A 10-mg sample of neurotoxin II (1.42 μmol) was treated with 1.42 μmol of *N*-hydroxysuccinimidyl azidobenzoate in 6 M guanidinium hydrochloride, 0.2 M $NaPO_4$, pH 8.0, for 16 h at room temperature. Reagents were removed by gel filtration on a Sephadex G-25 column (2.5 × 38 cm) in 0.1 M CH_3COOH. After lyophilization of the protein fraction, the poly- and monomodified NT-II derivatives were separated by cation-exchange chromatography on a Bio-Rex70 (1 × 20 cm) column with a linear gradient from 10 to 100 mM ammonium acetate, pH 7.5. Unmodified NT-II was recovered by elution with 200 mM ammonium acetate. Ion-exchange HPLC on a TSK-CM-3SW column was used to check the purity of the obtained derivatives and, if necessary, as a means of additional purification of those peaks poorly resolved on the Bio-Rex70 column. Quantitation was achieved by measuring the UV absorption, assuming an extinction coefficient of E_{270} = 20 000 L mol^{-1} cm^{-1}. This coefficient was determined as the sum of E_{270} = 14 000 for the native toxin and E_{270} = 6000 for the label.

Iodination of Photoactivatable Derivatives. $Na^{125}I$ was isotopically diluted with KI (2 mg/mL) to a specific radioactivity of 560 000 cpm/nmol. NT-II derivative (80 nmol) in 300 μL of $NaPO_4$ buffer (50 mM, pH 7.4) was incubated with 30 μL of $K^{125}I$ and 60 μL of chloramine T (1 mg/mL) for 5 min at room temperature. The reaction was stopped by addition of 120 μg of $Na_2S_2O_5$. The reaction products were separated by reverse-phase HPLC (VydacC-18, 4.6 × 250 mm) employing a binary gradient HPLC system (Knauer) with 0.1% TFA as the aqueous phase and 0.1% TFA in acetonitrile as the organic phase. Free ^{125}I was eluted at 15% organic phase. Several protein fractions were obtained with a linear gradient from 15 to 65% organic phase in 30 min. The peak with the lowest specific radioactivity eluted at 54% acetonitrile. It contained more than 50% of the injected protein; only this peak was used for further experiments in order to avoid complications introduced by multiple iodination.

Preparation of nAChR-Rich Membranes. Preparation of nAChR-rich membranes from Torpedo electric tissue was performed as described previously (Schiebler et al., 1977). Specific [^{125}I]BgTx-binding activity was determined according to Hartig and Raftery (1979) after dilution in 0.1% Triton X-100. Typically, the membrane preparation had a specific activity of 4 nmol of BgTx-binding sites/mg of protein.

Photolabeling Experiments. AChR-rich membranes (40 pmol of BgTx-binding sites) were incubated with 20 pmol of [^{125}I]NT-II photoactivatable derivatives for 30 min in the dark in a total volume of 200 μL of 50 mM $NaPO_4$ buffer, pH 7.4. This ratio was chosen because not all of the BgTx-binding sites (determined in detergent) are available for toxin binding in membranes. (Part of the binding sites may be oriented toward the internal side of the membrane vesicles, and some are not accessible due to the high receptor density in the membrane.) In competition experiments with unmodified NT-II or *d*-tubocurarine, the nAChR was preincubated with a 10-fold excess of NT-II over BgTx-binding sites (or the indicated amount of *d*-tubocurarine) for 30 min. The mixture was then irradiated for 4 min with a UV lamp (Type 5241, Quarzlampen GmbH Hanau) from a distance of 8 cm. The samples were centrifuged at 12000*g*, and the pellets were dissolved in 80 μL of sample buffer for SDS–PAGE. Experiments in 1% Triton X-100 were performed with the same amounts of AChR and NT-II derivative, but in a total volume of 50 μL. SDS sample buffer was added directly after irradiation to a total volume of 80 μL.

SDS–Polyacrylamide Gel Electrophoresis. Receptor subunits were separated on polyacrylamide slab gels (3% stacking gel, 10% separating gel) according to Laemmli (1970). The gels were stained with 0.1% Serva Blue R in 25% 2-propanol and 10% acetic acid and destained in the same solvent without dye. The dried gels were examined by autoradiography with an Kodak X-Omat XAR-2 film. Radioactivity incorporated into the nAChR subunits was quantitated in two ways: in the experiments with *d*-tubocurarine, radioactive bands were cut out of the dried gels and counted in the γ-counter. In the other experiments, electrophoresis was performed in a preparative tube gel system (BRL, Bethesda, MD) with continuous elution at the lower end of the gel (Oberthür et al., 1986). The eluting subunits were fractionated, and radioactivity was determined in the γ-counter.

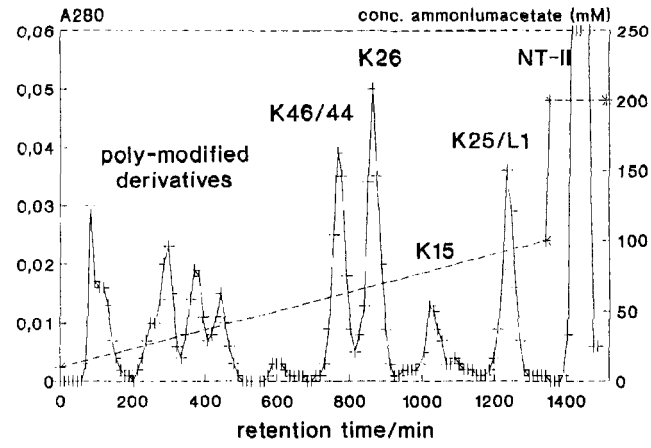

FIGURE 1: Separation of NT-II photoactivatable derivatives on a BioRex 70 cation-exchange column. The peaks designated K26, K15, etc., represent monomodified neurotoxin molecules carrying the photoactivatable group at sequence position Lys26, Lys15, etc. (The labeling site was determined by protein sequencing).

RESULTS

Preparation of Photolabile Derivatives of Neurotoxin II. The separation of the photoactivatable derivatives of neurotoxin II by ion-exchange chromatography is shown in Figure 1. Several polymodified derivatives, which were not further characterized, eluted at low salt concentrations. They were followed by four peaks which contained monolabeled derivatives. Toxins derivatized in positions Lys46 and Lys44 as well as Lys25 and Leu1 were not separated under these conditions. For the Lys44/Lys46 mixture, a ratio of 1:3 could be observed in a subsequent chromatography on a TSK-CM-3SW (HPLC) ion-exchange column (data not shown). The

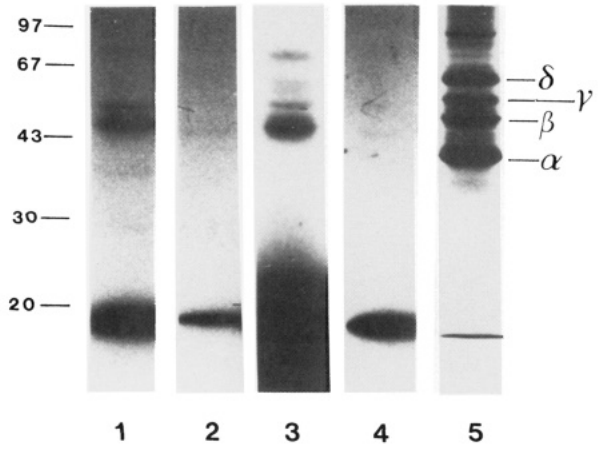

FIGURE 2: Autoradiography of nAChR subunits after reaction with [^{125}I]NT-II/Lys46 derivative: (1) reaction with the membrane-bound nAChR; (2) reaction in the presence of an excess of unmodified NT-II; (3) reaction with the receptor solubilized in 1% Triton X-100; (4) reaction with solubilized receptor in the presence of unmodified NT-II; (5) Coomassie Blue stain of AChR subunits.

separation of Lys44 and Lys46 was usually omitted since it had been shown previously that the Lys44 derivative does not react covalently with the nAChR (Tsetlin et al., 1983). For the Lys15, Lys26, Lys44, and Lys46 derivatives, the modified amino acids were confirmed by proteolytic cleavage and peptide sequencing (data not shown). For the Lys25 and Leu1 derivative, we rely on the determination of the sequence positions of the photoactivatable groups in a previous report (Tsetlin et al., 1983).

The yield of photoderivatives was 980 μg (140 nmol) for Lys26 and 700 μg (100 nmol) for the Lys44/Lys46 mixture starting from 10 mg (1.42 μmol) of toxin. The purity of the toxin derivatives is clearly an important aspect. From the different nonoverlapping receptor-labeling patterns, it is evident that there is no contamination of the Lys26 derivative with the Lys46 derivative.

Reaction of the nAChR with the Lys46 Derivative. Binding assays in the dark with ^{125}I-photoactivatable Lys26 and Lys46 derivatives of neurotoxin II yielded practically the same number of binding sites per receptor molecule as with unmodified [^{125}I]NT II or [^{125}I]BgTx. This means that the two photoactivatable derivatives are still capable of binding to both neurotoxin binding sites of the nAChR.

The results of electrophoretic analysis of photoinduced cross-links between the nAChR and the [^{125}I]Lys46 derivative are shown in Figure 2. There is only one major labeled band with an apparent molecular mass of 47 kDa. This corresponds to the molecular mass of the α-subunit (40 kDa) plus 7 kDa of the neurotoxin. A very weak band corresponding to the molecular weight of the β-subunit–neurotoxin–cross-link represents less than 10% of the radioactivity in the α-subunit–toxin product. In some experiments, very weak labeling of the δ-subunits was also observed, possibly due to traces of Lys26-derivatized toxin in the Lys46 derivative fraction.

The specificity of the reaction was shown by preincubation of the nAChR-containing membranes with an excess of unmodified NT-II, which precluded labeling of the nAChR subunits. We did not find differences in the labeling pattern when Lys46 derivative purified by HPLC was used for cross-linking (data not shown). This is in agreement with earlier spectroscopic and photolabeling data showing that Lys44 is not involved in receptor binding (Tsetlin et al., 1979, 1983).

When photolabeling was performed with the nAChR solubilized in 1% Triton X-100, two additional weak bands were

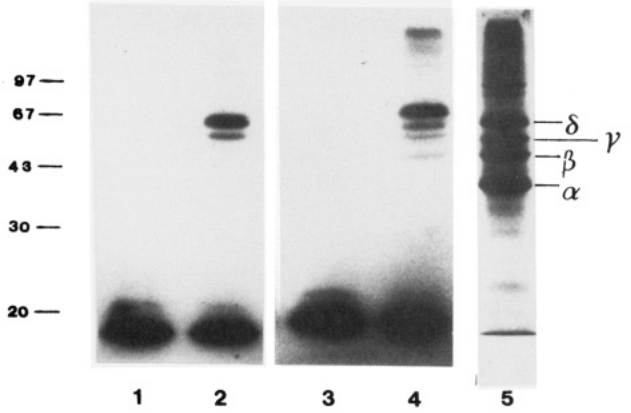

FIGURE 3: Autoradiography of nAChR subunits after reaction with [^{125}I]NT-II/Lys26 derivative: (1 and 3) control experiments in the presence of excess NT-II; (2) reaction with the membrane-bound receptor; (4) reaction with the receptor solubilized in 1% Triton X-100; (5) Coomassie Blue stain of AChR subunits.

observed in the autoradiography at 53 and at 70 kDa. This corresponds to labeling of the β- and δ-subunits. Again there was no incorporation in the presence of an excess of NT-II.

The cross-linking yield was determined for the experiment with membrane-bound AChR; 7 ± 1% of the radioactivity applied to the gel was incorporated into the α-subunit.

Reaction of the nAChR with the Lys26 Derivative. The autoradiography of [^{125}I]Lys26-labeled subunits is shown in Figure 3. There are two major labeled bands at molecular masses of 61 and 70 kDa. This indicates labeling of the γ- and δ-subunits. At longer exposition times of the autoradiography film, weak labeling of α and β was also observed.

In membranes solubilized in 1% Triton there was no qualitative difference compared with the experiment performed in native membranes. Labeling of α and β was slightly enhanced. The incorporation of radioactivity was specific in all cases, as shown by competition with an excess of unmodified NT-II. The yield of the photoreaction was again quantified for the experiment with the membrane-bound nAChR. The cross-linking yields were 3.0 ± 0.2% for δ and 1.6 ± 0.1% for γ. α and β were not separately quantified because of bad separation of these subunits in the preparative gel system. The combined yield for these two subunits was 1.0 ± 0.2%. The relative yield of γ- and δ-cross-linking was not the same in each experiment. On the average (calculated from 12 preparative gel runs), the amount of radioactivity incorporated into the γ-subunit was 45% when compared to the δ-subunit.

Discrimination between the Two Binding Sites for Agonists and Competitive Antagonists. Since the labeling pattern with the Lys26 derivative is complex and involves non-α-subunits, this label may allow discrimination between the two agonist/competitive antagonist-binding sites of the receptor. A suitable ligand for this purpose is *d*-tubocurarine, because the difference in K_D values for the two sites is approximately 2 orders of magnitude (Neubig & Cohen, 1979). When the irradiation experiment with the Lys26 derivative and nAChR-rich membranes was performed in the presence of increasing amounts of *d*-tubocurarine, at first the labeling of the γ-subunit was abolished at low concentrations of *d*-tubocurarine (Figure 4A).

Incorporation of radioactivity into the δ-subunit increased in parallel to the decrease of γ-labeling (Figure 4b), possibly because more neurotoxin was available for occupation of the second site. At higher *d*-tubocurarine concentrations, labeling of the δ-subunit was also blocked (Figure 4a). The IC_{50} values

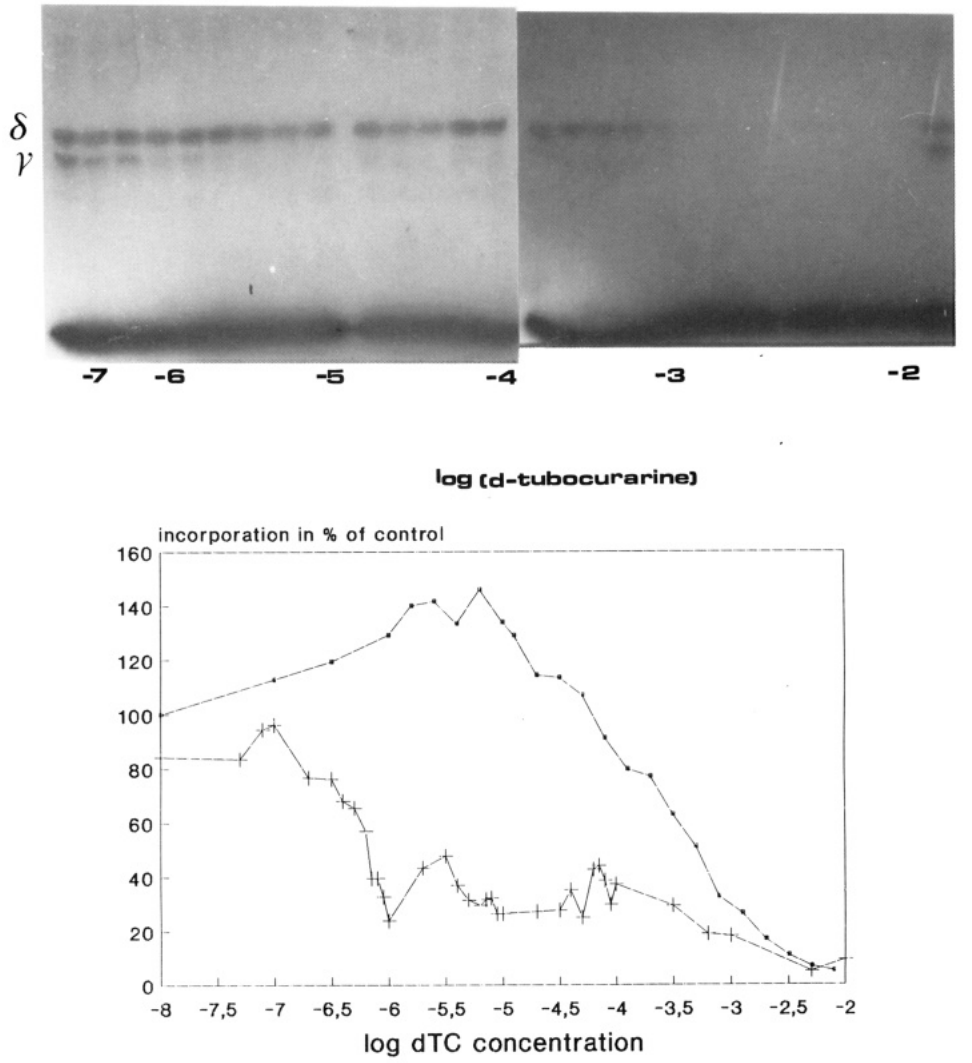

FIGURE 4: (a, top) Autoradiography of receptor subunits after reaction with [^{125}I]NT-II/Lys26 derivative in the presence of increasing concentrations of *d*-tubocurarine. The tracks on the right and on the left ends of the photo show controls representing labeling in the absence of *d*-tubocurarine. (b, bottom) Incorporation of [^{125}I]Lys26 derivative into the γ-(+) and δ-subunits (■) of the receptor as a function of the *d*-tubocurarine concentration. (The radioactive protein bands were excised from the gel and counted.)

for the inhibition of cross-linking were 636 ± 74 nM for the γ-subunit and 412 ± 138 μM for the δ-subunit.

DISCUSSION

In this paper we analyze the contribution of different subunits of the nAChR to the formation of the neurotoxin-binding sites. The aim of the investigation is to find out which parts of the AChR molecule are in contact with which parts of the toxin. To this end, photoactivatable derivatives have been prepared which carry the photolabile group in well-defined positions: in the central loop (Lys26) and in the third loop (Lys46) of the neurotoxin molecule. On the basis of EPR and fluorescence spectroscopy, Tsetlin et al. (1979) predicted that both residues are in contact with the nAChR.

The Lys46 derivative predominantly labels the α-subunit. This result is in agreement with the findings of Chatrenet et al. (1990), who showed that a photolabile group at the analogous residue (Lys47) in the α-toxin from *Naja naja nigricollis* also mainly labels the α-subunit. In the presence of the nonionic detergent Triton X-100, which has frequently been utilized for solubilization and affinity purification of the nAChR, slight changes in the labeling pattern are observed. This indicates that the photoaffinity labeling technique described here is sensitive to subtle conformational changes of the receptor molecule. Our data [and the fact that modification of Lys46 reduces the affinity of the toxin (Tsetlin et al., 1979; Chatrenet et al., 1990)] thus confirm that Lys46 forms part of the binding region of the toxin.

The Lys26 derivative shows a more complex labeling pattern; most of the radioactivity is incorporated into the γ- and δ-subunits while the α- and β-subunits are labeled only slightly. This proves that the Lys26 neurotoxin derivative is virtually free of Lys46 derivative.

The sequence of a large number of neurotoxins is known [for review see Endo and Tamiya (1987)]. The numerous amino acid residues conserved within this protein family (among others, the lysine residues modified in our experiments, Lys27 and Lys53 in the aligned sequences) all represent candidates for the formation of the high-affinity AChR-binding site common to postsynaptically acting neurotoxins. The chemical modification of invariant amino acids was widely used for deducing the structure/function relationship of α-neurotoxins. The outcome of these studies, however, is not always clear-cut; e.g., the contribution of lysine residues to receptor binding has been controversial for a long time. As a general rule, there seems to be no single lysine residue essential for toxicity (Karlsson et al., 1972), and even toxin molecules fully modified at all lysines retain significant toxicity

(Martin et al., 1983). For Lys26, a contribution to the binding surface of the toxin molecule had been questioned by Johnson et al. (1990). The authors showed that a fluorescent group introduced at the corresponding position in α-toxin from *Naja naja siamensis* (Lys23) reduced the binding affinity only to a small degree and was still accessible to fluorescence-quenching agents in the medium. The second observation, which applies to all lysine derivatives investigated by the authors, again casts doubt on the contribution of lysine residues to receptor binding.

In other cases, however, the modification of specific lysine residues is shown to have a strong influence on receptor binding. The biotinylation of Lys23 and Lys49 in the *Naja naja siamensis* toxin 3 (Lobel et al., 1985) and, similarly in *Naja nigricollis* neurotoxin, acetylation of the two corresponding residues (Faure et al., 1983) sharply decrease the affinity for nAChR. For the toxin investigated here (*Naja naja oxiana* NT-II), our earlier reports show that dansylation or acetylation of Lys26 and Lys46 results in lower receptor affinity and in reduced toxicity (Tsetlin et al., 1979).

The reasons for these discrepancies are not fully understood. Some authors suggest that they are a function of the labeling moiety (Johnson et al., 1990), but they may also arise from subtle differences in the binding areas of different species or reflect differences between long and short neurotoxins. For example, upon oxidation of the invariant tryptophan, the toxicity of *Naja naja atra* toxin (a short toxin) is reduced whereas it is retained in α-bungarotoxin (a long toxin) (Chang et al., 1990). We think that the observation that toxin molecules differentially labeled at lysine residues specifically and covalently interact with different parts of their target receptor [Chatrenet et al. (1990) and this paper] makes a strong point for the participation of these residues in specific high-affinity receptor binding. The fact that Lys26 and Lys46, which are rather far apart in the toxin structure, recognize different receptor subunits supports the concept of multipoint attachment and of a rather extended contact area between the two molecules.

By fluorescence energy transfer measurements, Johnson et al. (1984) found that in AChR-rich membranes derived from Torpedo electric organ the toxin molecules bound on neighboring AChR molecules are closer to each other than the toxin molecules bound to one receptor. One could therefore argue that our labeling with Lys26 represents an intermolecular cross-link between a toxin bound to one receptor and a labeling site on another receptor. However, we think that we can exclude this possibility, as dilution of the receptor-rich membranes in Triton X-100 does not significantly alter the cross-linking pattern.

The nonequivalence of the two binding sites has been explained by the presence of different non-α-subunits surrounding the binding regions on the (identical) α-subunits (Karlin, 1980). This hypothesis was substantiated by the experiments of Pedersen and Cohen (1990) and Blount and Merlie (1989), who showed that the high-affinity binding site for *d*-tubocurarine is located at the interface between the α- and γ-subunits, while the low-affinity site is formed by the α- and δ-subunits. For a better understanding of the orientation of the toxin molecule in its binding site, we investigated whether the labeling of γ and δ by the Lys26 derivative occurs when the toxin is bound at one binding site or whether γ and δ are labeled from different sites. The competition experiment with *d*-tubocurarine clearly shows that the γ-subunit is labeled by the toxin bound in the high-affinity dTC site, while δ is labeled by the toxin bound in the low-affinity dTC site. The difference in the IC_{50} values is larger than 2 orders of magnitude, in agreement with the data of Neubig and Cohen (1979) in which K_D values for dTC of 33 nM and 7.7 μM were observed.

It should be noted that the nonequivalence of the two neurotoxin-binding sites in terms of covalent cross-linking sites has been observed earlier. Tsetlin et al. (1983) were able to displace the Lys15 azidobenzoyl derivative from half of the binding sites of the Triton X-100-solubilized receptor with NT-II trifluoroacetylated at all six amino groups, thereby abolishing labeling of the γ- and δ-subunits, but not of the α-subunit. The Lys51 derivative of the α-toxin from *Naja naja nigricollis* labels δ when it is bound to the site with higher affinity for this toxin and δ and γ when it is bound to both sites (Chatrenet et al., 1990). As it is unclear which of these sites has the higher affinity for dTC, it is difficult to compare the results directly. Because of the different effects on the labeling of γ and δ, one can speculate that the high-affinity *d*-tubocurarine site is the low-affinity α-neurotoxin site mentioned by Chatrenet et al. (1990). This is further supported by a report by Dowding and Hall (1987), who showed that the high-affinity *d*-tubocurarine site shows the lower binding rate constant for α-BgTX. Hamilton et al., (1985) investigated the pattern of cross-links formed by the toxin from *Naja naja siamensis* and a bifunctional reagent via nonidentified positions of the toxin. Incorporation of toxin into the γ- and β-subunits was affected more strongly by low concentrations of dTC than incorporation into the α- and δ-subunits.

Our studies with the Lys46 and the Lys26 derivative of NT-II give additional support to the concept that the concave side of loops 2 (containing Lys26) and 3 (Lys46) of the neurotoxin molecule contributes to binding to the AChR. Taking into account that the binding sites for agonists and small competitive antagonists are at the interfaces of the α- and γ-chains and the α- and δ-chains, respectively, we propose the following model for the orientation of the α-neurotoxin in these sites: the central loop of the toxin structure binds directly onto the agonist-binding site at the subunit interfaces. Thus some residues, like Lys26, make contact with the respective non-α-subunits (γ or δ). The third loop of the toxin makes additional contacts to the α-subunit; these additional contacts account for the extremely high affinity of α-neurotoxins for the nAChR.

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