A structural model for the ligand-binding sites at the nicotinic acetylcholine receptor

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The structural properties of a selected segment of the 4 polypeptide chains of the acetylcholine receptor from *Torpedo californica* have been compared by model building studies. The particular segment (residues 135–142) is identical for the β, γ and δ subunits but differs in one position from the otherwise identical α-peptide. We conclude that the exchange of a tryptophanyl by a glutaminyl residue may produce a sufficiently different folding and charge pattern to provide for the specific binding of cholinergic ligands to the α-peptide.

Nicotinic acetylcholine receptor *Ligand binding* *α-Cobratoxin* *Peptide sequence*

1. INTRODUCTION

Based on the available amino acid sequences of nicotinic acetylcholine receptors [1–6], several structural models for this integral membrane protein have been developed [3–5,7–10]. These focus almost exclusively on the prediction of transmembrane α-helices and on the topological arrangement of these within the overall structure of the receptor. In contrast, the large extracellular portions of the receptor's polypeptides are believed to contain mainly β-structural elements and, thus, are much less accessible to prediction [11]. As a further limitation, the disulphide bridges of these areas have not yet been unequivocally assigned to particular cysteines. Accordingly, the existing structural predictions for the location of the ligand binding sites on the receptor have of necessity been rather vague [1,7,10,12].

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Abbreviations: ACh, acetylcholine; AChR, nicotinic acetylcholine receptor; BAC, bromoacetylcholine; MBTA, 4-(*N*-maleimidobenzyl)trimethylammonium; CTX, α-cobratoxin; DTT, dithiothreitol

Prompted by the detailed knowledge available on the interaction with specific ligands, we have adopted a new approach to pinpoint regions of the receptor possibly involved in ligand binding: Initially, we searched for the longest stretch of amino acids in the extracellular portions of the receptor's polypeptides identical in the 3 larger subunits but differing from the α-subunit. Secondly, we employed secondary structure prediction methods to identify the structural elements likely to govern these selected sequence segments. Finally, if different structural predictions were obtained for the α-peptide as compared to the β,γ and δ-peptides, detailed model building studies involving several representative cholinergic ligands were initiated. These studies showed that the exchange of a tryptophanyl (β,γ,δ-peptides) by a glutaminyl residue (α-peptide) in an otherwise identical octapeptide (residues 135–142) can suffice to provide the α-subunit with the structural properties required for the specific binding of cholinergic ligands.

2. METHODS

2.1. *Secondary structure prediction*

Predictions of the secondary structure of the

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peptides 129–147 of the α and he β,γ,δ-subunits of the AChR were carried out according to the algorithms of [13–16] using computer programs written by authors in [17] for a DEC 10 computer.

2.2. *Model building*

Model building was done using the FRODO software [18] run on an Evans & Sutherland PS 300 Graphics System connected to a VAX 11/750 computer. The peptide conformations were constructed according to the results of secondary structure predictions with their geometry being refined by applying the method of [19]. Model building and refinement for the peptide CTX complex was performed by the same methods, keeping the coordinates for CTX as determined by X-ray crystallography [20].

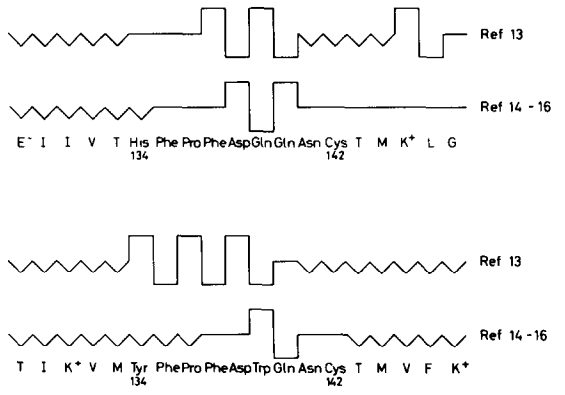

Fig.1. Secondary structure prediction [13] for the peptides 129–147 of the α-subunit (upper top trace) and the β-subunit (upper lower trace) from *T. californica*. The resulting average of the 4 different predictive methods mentioned in the text [13–16] (i.e., top lower and bottom lower traces) is also presented. $\wedge\wedge$, β-strand; $\sqcap\sqcap$, β-turn; ——, random coil.

3. RESULTS AND DISCUSSION

The maximal stretch of amino acids completely identical in the extracellular portions of the β,γ and δ-subunits but differing from the α-subunit, is the nonapeptide Tyr-Phe-Pro-Phe-Asp-Trp-Gln-Asn-Cys (residues 134–142). The related α-peptide has the sequence His-Phe-Pro-Phe-Asp-Gln-Gln-Asn-Cys. The latter is conserved in all receptor species sequenced so far [5,6].

As shown by X-ray crystallographic analysis of another protein [21], the exchange of a single amino acid in an otherwise completely homologous primary structure is sufficient to produce an entirely different folding of the respective peptide chain. The same seems to apply for the exchange of Gln for Trp in the above two nonapeptide sequences. As shown in fig.1, the algorithm developed by authors in [13] predicts a β-turn for the segment Phe-Asp-Gln-Gln of the α-peptide whereas this turn is shifted by one amino acid to the segment Pro-Phe-Asp-Trp in the β,γ,δ-peptides. Alternative prediction methods [14–16], while failing to exactly reproduce the β-turns outlined above, all predict β-turns in this region. Moreover, the average of these predictions closely matched the result obtained with the algorithm of [13] (fig.1).

At first view both the α-peptide and the β,γ,δ-peptides seem to meet the requirements for an ACh binding site: (i) Since both appear to form β-turns and also contain an N-glycosylation site (Asn-141), they probably reside at the surface of the receptor. (ii) They both contain a negatively charged residue (Asp-138) required as an acceptor site for the quaternary nitrogen moiety of ACh. (iii) They both contain a cysteine (Cys-142) which is known to exist in close vicinity of the ACh binding site: In the presence of cholinergic ligands a particular disulfide bridge on the α-subunit of the receptor is protected against reduction [22–24]. After reduction in the absence of protecting ligands, it is specifically affinity alkylated by the agonist BAC and the blocking agent MBTA [23,24].

However, if the two peptides are inspected more closely by model building (fig.2,3), it is readily seen that only the α-peptide exhibits the structural properties of an ACh binding site: (i) Only in this peptide are Asp-138 and Cys-142 positioned on the same side of the β-structure (fig.2a), thereby providing the proposed distance of $\approx$10 Å between the alkylation site and a centre of negative charge [22]. In contrast, in the predicted structure for the β,γ,δ-peptides (fig.2b) this distance exceeds this limiting value. (ii) The only N-glycosylation site in the entire α-subunit (Asn-141) [1] is positioned 'out of the way' of the presumed ACh binding site, thereby allowing free access of the ligand to this region. Such a distinction does not exist for the β,γ,δ-peptides. In addition and although posi-

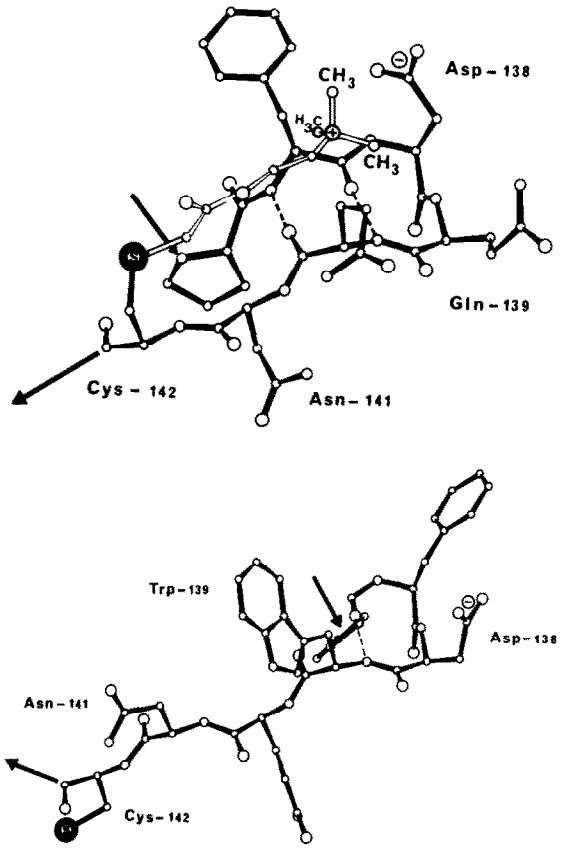

Fig.2. (a) Predicted conformation of the putative acetylcholine binding site on the α-subunit (solid lines Pro-136 to Cys-142). Open lines represent the ACh moiety covalently attached at Cys-142 as a result of affinity alkylation by BAC of DTT-treated tissue (see text). Note the ionic interaction between the onium group of the ligand and the carboxylate of Asp-138 of the peptide sequence. (b) Predicted conformation of the peptide sequence Pro-136 to Cys-142 in the β,γ and δ-subunits of the AChR. Since the side-chains of Cys-142 and Asp-138 orient to different sides of the β-strand, affinity alkylation with BAC (or MBTA) as shown in the α-subunit is not possible. ⟶ Indicates the direction of the polypeptide chain.

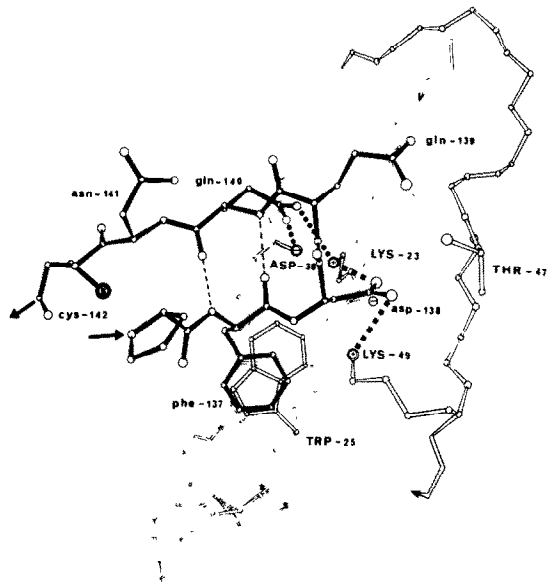

Fig.3. Suggested binding of the α-subunit peptide (utilising residues 136–142; solid lines) to CTX (open lines). For CTX the backbone of residues 19–50 is shown. These residues make up the central long loop that is believed to bind to the AChR [20]. Only those toxin side-chains interacting with the receptor in our model are shown. For clarity the large lettering corresponds to amino acids belonging to CTX and the small to amino acids of the α-peptide sequence. The amino acid side-chains of CTX involved in binding to the α-subunit are kept in the same conformation as found in the crystal structure [20] with the exception of Lys-49 which is 'flipped over' [35]. The proposed interactions between the α-peptide and CTX are (numbering corresponds to notation in the figure): (i) Stack between peptide Phe-137 and CTX TRP-25; (ii) ionic interactions between peptide Asp-138 and CTX LYS-23 and CTX LYS-49 (■ ■); (iii) H-bonding between peptide Gln-139 and CTX THR-47 (– –); (iv) dipole enhanced H-bonding between Gln-140 of the peptide and ASP-38 and LYS-23 of CTX (●●●). All the toxin residues utilised here in binding to the α-subunit sequence are highly conserved in a large series of snake toxin sequences [36]. ⟶ Indicates the direction of the polypeptide chain.

tioned 'out of the way', different levels of glycosylation of the α-peptide Asn-141 could nevertheless result in differential binding properties at this site [25]. (iii) The predicted structure for the α-peptide provides an excellent fit to the known structure of the central, long loop of CTX (fig.3). The residues of CTX shown to specifically interact with the α-peptide in the proposed model (fig.3) have been suggested by chemical modification studies [26,27] to form essential components of the multipoint attachment pattern between receptor and toxin. Comparable fits with the predicted structure of the β,γ,δ-peptides were not possible.

The above outlined differences in the predicted structures of the α-peptide as compared to the

β,γ,δ-peptides provide the basic rationale for a functional distinction between two closely homologous but not identical sequence segments of the receptor's polypeptides. Assuming that the α-peptide indeed contains the structural elements of the ACh binding site, several more observations can be accommodated: (i) While the β,γ,δ-peptides do not exhibit the established properties of an acetylcholine binding site, they remain closely homologous to this site and also may provide low affinity sites for cholinergic ligands [28] and be involved in recognition of different receptor ligands. Since the replacement of Gln by Trp in position 139 makes this region more hydrophobic, the more hydrophobic class of receptor ligands, i.e., the local anaesthetics come into mind. Assuming the β,γ and δ-peptides form a circular arrangement at the mouth of the receptor channel that is biased to the reception of local anaesthetics, the combination of local anaesthetic sites (β,γ,δ-peptides) and ACh sites (α-peptides) could form an allosteric network of sites with overlapping specificities. This agrees with the established pharmacology of these classes of receptor ligands [29], the variation in subunit labeling patterns with irreversible noncompetitive blockers [30,31], and the complex competition patterns of receptor ligands observed with monoclonal antibodies to the AChR [32]. (ii) The stretch of closely homologous amino acids in the 4 polypeptides of the receptor suggests a common ancestry with functional specialisation during evolution. From this viewpoint it is intriguing to note the existence in *Aplysia* of a 'nicotinic type' receptor that is depolarised by procaine (a hydrophilic local anaesthetic) in a manner almost indistinguishable from ACh [33]. Furthermore, a recent study on *Locust* nicotinic receptors suggests that these consist of only a single type of polypeptide chain [34]. Finally, it should be noted that the genetic codes for Trp and Gln differ in the first two base positions, thereby conferring considerable evolutionary stability.

The proposed location of the ACh binding site can be tested by several biochemical methods. In addition to chemical modification and site-direction mutagenesis, enzymatic modification (e.g., glutaminase) and antigenic site analysis [32] appear particularly suited for this purpose.

Note added in proof: While this manuscript was being reviewed, some direct experimental evidence for an MBTA binding site at the proposed position of Cys-142 was published [37].

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