Nicotinic acetylcholine receptors: α-conotoxins as templates for rational drug design

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Abstract

Nicotinic acetylcholine receptors (nAChRs) mediate the passage of potassium and sodium ions across synaptic membranes. Two classes of receptors exist: the neuromuscular nAChRs, which mediate signals between nerve and muscle cells, and the neuronal nAChRs, which are found throughout the nervous system. For treatment of diseases involving nAChRs, drugs must be designed with a high level of selectivity towards only one of these classes or subclasses (in the case of neuronal receptors). α-Conotoxins, small polypeptides isolated from the venoms of marine snails, represent molecules with just this type of selectivity, with specificity even towards certain subclasses of nAChRs. The availability of high-resolution crystal structures of α-conotoxins provides the opportunity to examine the structural features that orchestrate their preferential blocking action. In the present study of a neuromuscular- and a neuronal-specific α-conotoxin, SI and EpI respectively, important and significant differences can be seen in the shapes of the molecules, which must reflect topological features of the different types of target receptor subunits. These then provide a template for computational docking studies with the homologous acetylcholine-binding protein, whose structure is known, so drug analogues of the naturally occurring toxins can be developed with the desired specificities.

Introduction

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated transmembrane channels, which enable the passage of potassium and sodium ions across synaptic membranes. There are two classes of these receptors: neuronal, found in the central and peripheral nervous system, and neuromuscular, found between nerves and muscles. These receptors comprise five homologous subunits, arranged either as a homo-pentameric, or a pseudo-pentameric, structure, depending on the types of subunits present (reviewed in [1]). The small molecule acetylcholine (ACh), the activating ligand of these receptors, binds to two sites in a receptor, inducing a conformational change that is transmitted allosterically from the binding site through the subunits. The channels open as a result, permitting potassium and/or sodium ion passage across the membrane.

α-Conotoxins are small disulphide-rich polypeptide components of the venoms of marine snails of the species *Conus*, and are specific inhibitors of nAChRs. The α-conotoxins are antagonists of ACh binding, and, being competitive inhibitors, they bind in the same region as ACh and prevent the channel from opening. In parallel with the two classes of nAChR, there are also two families of α-conotoxins, each favouring binding to one class over the other with a high degree of specificity.

Key words: computational docking study, conotoxin, molecular topology, X-ray crystallography.
Abbreviations used: ACh, acetylcholine, nAChR, nicotinic ACh receptor, PDB, Protein Data Bank.
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Diseases involving nAChRs

nAChRs have been identified as being causally involved in a number of diseases [2–4]. For neuromuscular receptors, these include slow-channel syndrome (where the nAChR is slow to close, and hence muscles get easily fatigued), the converse fast-channel syndrome (where channels are slow to open, yet fast to close, which also leads to fatigue), and arthrogryposis multiplex congenita (a defect affecting the foetal γ-subunit, resulting in severe problems of movement from birth). Other diseases are associated with the neuronal receptors. These include the genetic disease autosomal dominant frontal lobe epilepsy [a mutation involving the $(\alpha 4)_2(\beta 2)_3$ subclass], schizophrenia (where, if of genetic-linked origin, it involves mutations in α7 subunits), and possibly Alzheimer's and Parkinson's diseases.

Treatments for some of these diseases are designed to maintain increased ACh concentrations. The neurotransmitter is therefore left in elevated levels throughout all types of tissues, leading to unwanted side-effects through actions on channels not affected by disease. Clearly, methods of treatment for these diseases, if they are to be of benefit, should be focused preferentially towards the class or subclass of receptor that is affected. Since α-conotoxins are highly specific towards the different classes, these polypeptide toxins should make ideal template structures from which to devise novel and highly focused therapeutics. A key question that should be addressed is: what critical structural features make the α-conotoxins so receptor-class-specific? In the present study, the similarities and differences in the overall topologies of the ACh-binding sites within the two classes of receptor were examined by comparing the crystal structure of the

Figure 1 | Ball-and-stick models of the polypeptide backbones of α-conotoxin SI (shown in black and with black lettering), and α-conotoxin EpI (shown in grey and with grey lettering)
The two views are at 90° to each other.

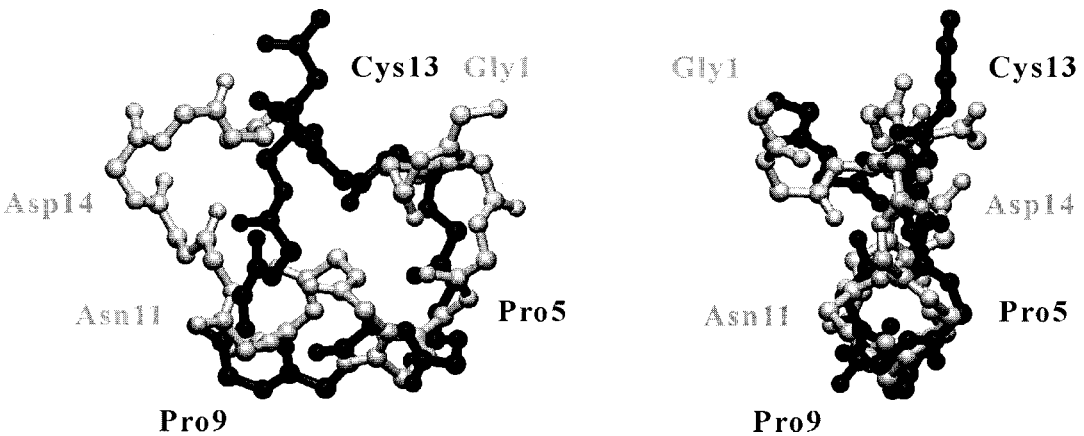
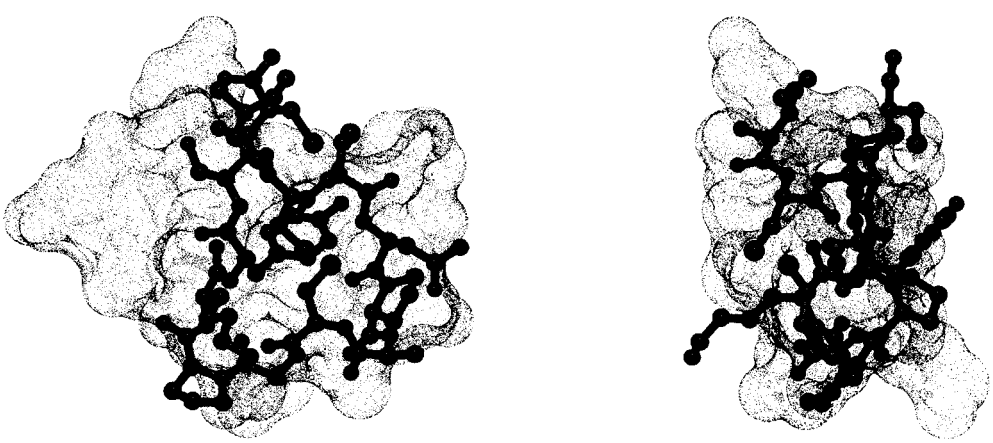


Figure 2 | Ball-and-stick structure (including side chains) of α-conotoxin SI superimposed on to the dotted surface structure representation of EpI
The two views are as in Figure 1.



neuromuscular α-conotoxin SI [R.W. Janes, B.J. Carrington, B. Hargittai, G. Barany, E.J. MacLean and S.J. Teat, unpublished work; Protein Data Bank (PDB) code 1hje], and the neuronal α-conotoxin [Tyr15]EpI crystal structure ([5]; PDB code 1a0m). These toxins were chosen because SI exhibits a low toxicity towards mammals, and is the α-conotoxin that is least selective between the ACh-binding sites of *Torpedo californica* neuromuscular receptors [6], whereas EpI, although highly potent, is nonetheless the least specific of the neuronals, binding to both the $(\alpha 3)_2(\beta 2)_3$ and $(\alpha 3)_2(\beta 4)_3$ subclasses of receptor [5].

Structural comparisons between α-conotoxins SI and EpI

This study focused on similarities and differences in structure and topology of these two conotoxins, rather than electrostatic interactions that must also play a role in binding of these toxins to their respective sites. The primary aim was to explore the overall complementary topologies of the ACh-binding sites of the two classes of receptors to see what distinctive features in the conotoxins might give rise to their specificity. The backbones of these conotoxins were superimposed, matching residues 2–12 of SI (with the end residues being neglected, since they were less well defined), with similar length stretches of residues in EpI using the programme MOLMOL [7]. By changing incrementally the alignment by one residue in each trial, the best fit between the backbones was found to be for residues 2–12 of SI and residues 3–13 of EpI, with a root-mean-square deviation of 2.924 Å (1 Å = 0.1 nm). In comparing these backbone conformations (Figure 1), there is clearly a considerable difference in overall shape between the C-terminal regions of the molecules. This

difference is a reflection of the larger number of residues in the neuronal versus the neuromuscular conotoxin between the third and fourth cysteine residues. However, when comparing the 'profile view' of the two conotoxins (90° rotation), there is a notable degree of similarity between the two backbones, which are presented to the different ACh-binding sites. Figure 2 compares surface feature differences between the two conotoxins. Overall α-conotoxin SI is significantly smaller than α-conotoxin EpI. A comparison of solvent-accessible surface area (calculated in MOLMOL) gives values of 1126.1 Å^2 and 1427.1 Å^2 for α-conotoxins SI and EpI respectively. The notable difference here is that the neuronal conotoxin is substantially longer, and slightly narrower, than the neuromuscular conotoxin. From a purely volume-and-dimensions viewpoint, a neuromuscular conotoxin might be able to be accommodated in a neuronal ACh-binding site far easier than vice versa. The difference in the 'profile' dimension appears somewhat small, but might account for the selectivity if this were on purely topological grounds. Furthermore, the crystal structure of the Ach-binding protein [8], which is considered to have a resemblance to neuronal nAChRs, has provided a test system for these templates by computational docking studies (R.W. Janes and B.J. Carrington, unpublished work).

Conclusions

The various α-conotoxins exhibit different specificities towards the two classes of nAChRs. Some similarities and differences in their structures can be related to differences in the shapes of the ACh-binding site pockets between the classes of nAChRs. The studies suggest that the neuronal ACh-binding site might be longer and narrower than its neuromuscular counterpart, a feature which could be exploited in drug design.

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