ELSEVIER

α-Conotoxins as selective probes for nicotinic acetylcholine receptor subclasses

Robert W Janes

α-Conotoxins are selective antagonists of neuromuscular or neuronal nicotinic acetylcholine receptors. Individual family members are often highly selective towards distinct receptor subclasses, most notably within neuronal nicotinic acetylcholine receptors. As such they are being used as tools to probe for the type and diversity of receptor subclasses in distinct parts of the central and peripheral nervous systems. Many new α-conotoxins are being identified every year, broadening the available armoury because small variations in their sequences and structures often confer altered selectivity towards receptor subunits and subclasses. Many neurological diseases are being associated wholly or in part with functional changes within specific subclasses of nicotinic acetylcholine receptors. Significantly, with more structures of α-conotoxins also becoming available this enables ready comparison of their similarities and, more notably, of their subtle differences, which dictate subclass selectivity. As such, α-conotoxins offer the potential to become templates for the creation, through rational drug design strategies, of pharmaceuticals highly selective for specific subclasses of nicotinic acetylcholine receptors.

Addresses
School of Biological Sciences, Queen Mary, University of London, Mile End Road, London, E1 4NS, UK

Corresponding author: Janes, Robert W (r.w.janes@qmul.ac.uk)

Current Opinion in Pharmacology 2005, **5**:280–292

This review comes from a themed issue on
Musculoskeletal
Edited by Daniel Bertrand and Ronald Hogg

Available online 16th April 2005

1471-4892/$ – see front matter

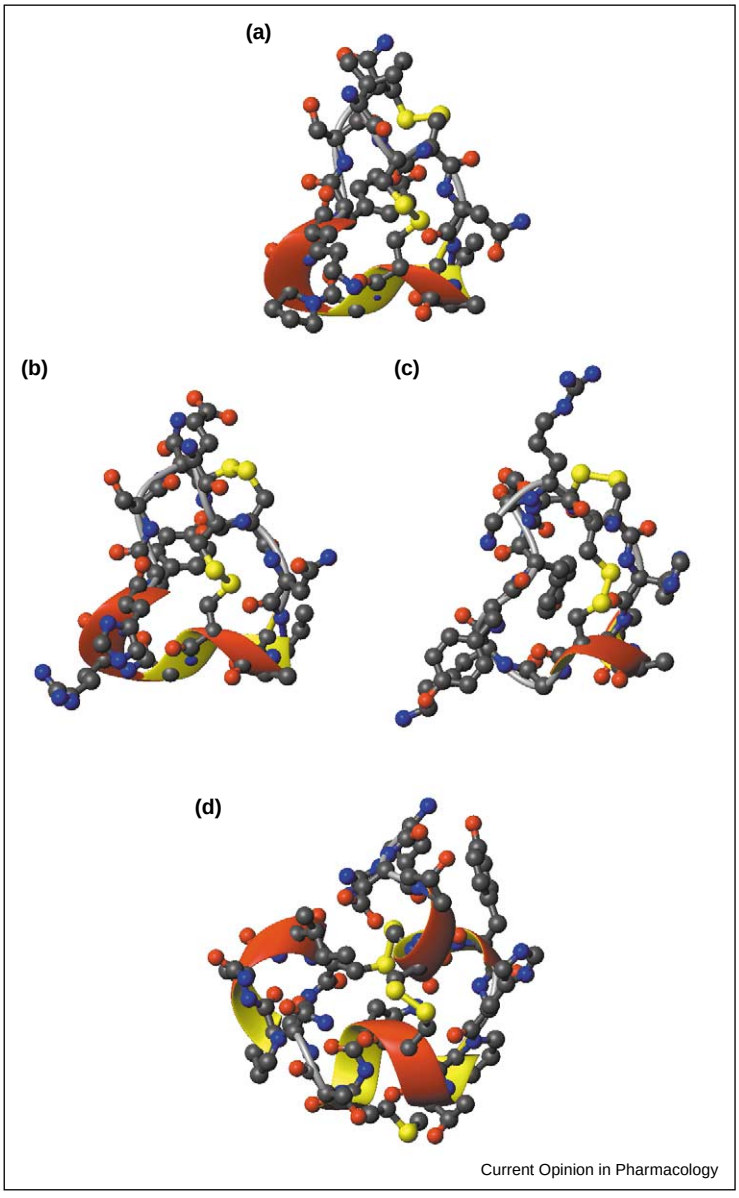
DOI 10.1016/j.coph.2005.01.013

Introduction

The venom of the *Conus* genus of marine snails contains a wealth of polypeptide toxins to enable them to capture and subdue their prey. One family of toxins found in cone snail venom is the α-conotoxins, which range in size between 12 and 19 amino acids and use key disulphide bonds to maintain their structure (Figures 1 and 2). These polypeptides are highly selective at blocking nicotinic acetylcholine receptors (nAChRs), ligand-gated channels that allow the passage of potassium, sodium or calcium ions across the synaptic membrane. Two classes of nAChRs exist – neuronal and neuromuscular – and each comprises five subunits, which can form heteropentameric or homopentameric membrane-bound channel structures. Two molecules of the neurotransmitter acetylcholine (ACh) are required to open the channel, stabilizing the receptor in the active open state for ion conductance. Many of the α-conotoxins are competitive antagonists at nAChRs, binding with high affinity at one or both of these ACh binding sites. Neuromuscular nAChRs (nm-nAChRs) are heteropentameric in character, and consist of two α_1 subunits, and one each of β_1, γ and δ subunits (with γ being found in the embryonic form, and replaced by ε during development). The structure and function of nAChRs are reviewed in [1••,2•]. Neuronal nAChRs (n-nAChRs) are more diverse in construction; they are formed of a variety of different α and β subunits, and none is identical to their neuromuscular cousins [3•]. The basic framework of these receptors takes the form $(\alpha)_2(\beta)_3$, but different α and β subunits can be involved in creating one receptor subclass [4••]. This variance in subunit construction creates a multitude of diverse subclasses of n-nAChR, which notably have temporal differences in their opening and closing characteristics. Moreover, they populate different areas of the central and peripheral nervous systems and perform discrete tasks, many of which are only now being identified. Significant diseases are being associated with changes in functional characteristics of nAChRs [5], and nicotine addiction is also clearly linked to neuronal receptors [6]. Alzheimer's disease [7•], Parkinson's disease [8•], genetic forms of schizophrenia [9•] and of frontal lobe epilepsy [10•], as well as myasthenia gravis [11•], all have strong connections with a specific class or subclass of nAChRs. As such, the nAChRs offer potential targets for drug intervention in these diseases [12].

The α-conotoxins are not only selective towards specific subclasses of receptor, but also towards ACh binding pockets between specific subunit pairs, which makes them ideal tools with which to probe the central and peripheral nervous systems for receptor distribution. Additionally, with a growing number of α-conotoxin structures becoming available, they have the potential to be used as templates from which to derive pharmaceutical agents. Several recent reviews report on conotoxins, and specifically on α-conotoxins [4••,13••–16••,17•,18,19], reflecting the current and developing interest in these potent polypeptides. This review considers the selectivity of different α-conotoxins, in conjunction with structural information where determined, with a focus on interactions with mammalian nAChRs. It covers the most recent developments concerning the more established neuromuscular family of α-conotoxins, then turns to the neuronal α-conotoxins. Emphasis is placed on new family members that have

Figure 1



Representative structures of neuromuscular α-conotoxins. The atoms of these conotoxins are illustrated in 'ball and stick' mode, and the disulphide bonds are shown joining the yellow sulphur side-chain atoms of the cysteine residues. The underlying secondary structure features are also indicated (helical regions in red/yellow banding, for example). Structures **(a)** to **(c)** are α3/5 neuromuscular. **(a)** Crystal structure of SI, (Protein Data Bank [pdb] code 1hje), **(b)** crystal structure of GI (pdb code 1not), **(c)** NMR solution structure of CnIA, (pdb code 1b45). **(d)** NMR solution structure of the α4/7 conotoxin EI, (pdb code 1k64). Not all known structures are shown. This figure was drawn using MOLMOL [81].

been recently identified. Restrictions in the length of this review mean that the few members of the larger α-A-conotoxins cannot be covered (see [4••,20] for reviews).

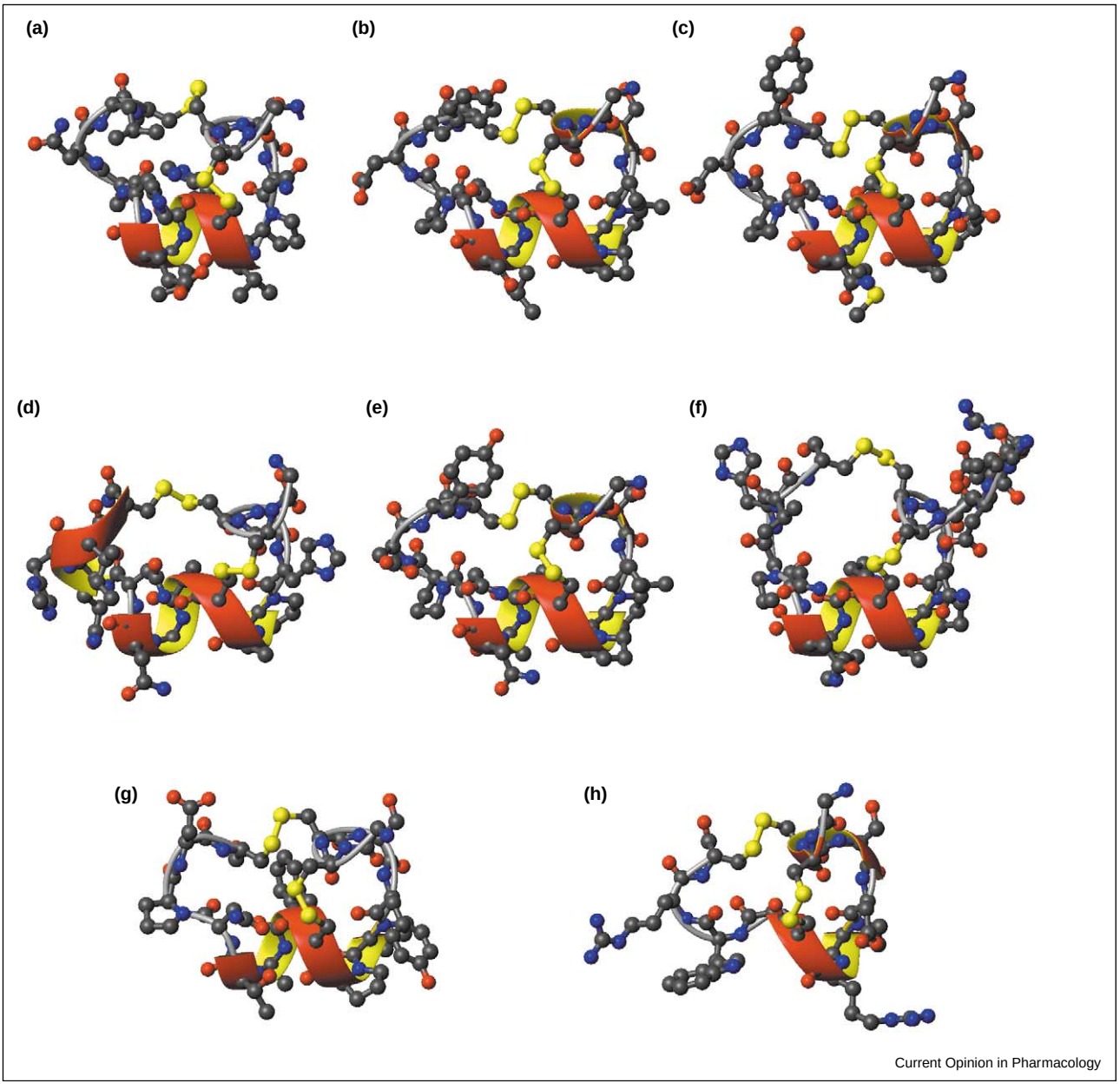
α-Conotoxin sequences and subunit interface preferences

Tables 1 and 2 summarise the sequences of known family members of the neuromuscular and neuronal α-conotoxins, respectively. Subunit interface preferences, where known, are summarised in Tables 3 and 4. For a full explanation of the naming of the conotoxins see [21]. Briefly, the initial letters associated with the conotoxins arise from the species of cone snail in which they were first identified. Structural features of the neuromuscular and neuronal α-conotoxins are show in Figures 1 and 2, respectively.

α-Conotoxin antagonists of neuromuscular nAChRs

With disulphides being a notable feature in their structural integrity, α-conotoxins have been compartmenta-

Figure 2



Representative structures of the neuronal α-conotoxins. The atoms of these conotoxins are illustrated in 'ball and stick' mode, and the disulphide bonds are shown joining the yellow sulphur side-chain atoms of the cysteine residues. The underlying secondary structure features are also indicated (helical regions in red/yellow banding, for example). Structures **(a)** to **(f)** are α4/7 conotoxins. **(a)** NMR solution structure of MII (pdb code 1mii), **(b)** crystal structure of PnIB (pdb code 1akg), **(c)** crystal structure of [Tyr15]EpI (pdb code 1a0m), **(d)** NMR solution structure of GIC (pdb code 1ul2), **(e)** crystal structure of PnIA (pdb code 1pen), and **(f)** solution structure of GID (pdb code 1mtq). **(g)** NMR solution structure of the α4/6 conotoxin AuIB (pdb code 1dg2), **(h)** NMR solution structure of the α4/3 conotoxin ImI (pdb code 1im1). Not all known structures are shown. This figure was drawn using MOLMOL [81].

lised into families based on the numbers of residues found in the 'loop' regions between cysteine (C) amino acids. Thus, as an example, for α-conotoxin GI [22] with the sequence ECCNPACGRHYSC(NH_2), the first loop region would be three (NPA) and the second would be five (GRHYS), and this conotoxin therefore belongs to the α3/5 family.

α3/5 α-Conotoxins

GI, MI, SI, SIA

α-Conotoxins GI from *Conus geographus* [22], MI from *Conus magus*, [23], and SI from *Conus striatus* [24] are considered the 'classical' α-conotoxins and have been studied for many years (reviewed in [20,21]). These neuromuscular α-conotoxins have highly species-

Table 1

Sequences of the neuromuscular α-conotoxins

Name	Sequence[a]	Reference
α3/5 α-Conotoxins		
GI	Glu Cys *Cys* Asn Pro Ala Cys Gly Arg His Tyr Ser *Cys* -NH_2	[22]
MI	Gly Arg Cys *Cys* His Pro Ala Cys Gly Lys Asn Tyr Ser *Cys* -NH_2	[23]
SI	Ile Cys *Cys* Asn Pro Ala Cys Gly Pro Lys Tyr Ser *Cys* -NH_2	[24]
SIA	Tyr Cys *Cys* His Pro Ala Cys Gly Lys Asn Phe Asp *Cys* -NH_2	[36]
SII[b]	Gly Cys Cys *Cys* Asn Pro Ala Cys Gly Pro Asn Tyr Gly *Cys* Gly Thr Ser Cys Ser	[37]
GIA	Glu Cys *Cys* Asn Pro Ala Cys Gly Arg His Tyr Ser *Cys* Gly Lys -NH_2	[22]
GII	Glu Cys *Cys* His Pro Ala Cys Gly Lys His Phe Ser *Cys* -NH_2	[22]
CnIA	Gly Arg Cys *Cys* His Pro Ala Cys Gly Lys Tyr Tyr Ser *Cys* -NH_2	[38]
α4/7 α-Conotoxins		
EI	Arg Asp Hyp[c] Cys *Cys* Tyr His Pro Thr Cys Asn Met Ser Asn Pro Gln Ile *Cys* -NH_2	[39]

[a] Disulphide bonds are linked as 'normal text' pairs and 'italicised text' pairs. In SII the third disulphide is linked as the underlined pair.
[b] This is an α3/5/3 loop α-conotoxin.
[c] Hydroxyproline.

dependent toxicity and binding properties, but usually have no or very little effect on n-nAChRs. Much work has involved the electric ray *Torpedo californica* because its electric organ is rich in nm-nAChRs, but these results differ fundamentally from those on mammalian receptors. Considering interactions with mammalian receptors, GI and MI preferentially block the α/δ interface of mouse receptors with high selectivity over the α/γ interface [25,26]. Incorporating an iodine on the Tyr12 of MI increases this selectivity such that the analogue retains the preference for the α/δ site but is now unable to bind at the α/γ interface [27]. SI by contrast, while retaining the preference for the α/δ site, shows greatly reduced toxicity towards mammalian nm-nAChRs, although its sequence is closer to GI than the sequence of MI is to GI [28]. Structural studies on GI [29,30], MI [31], and SI [32; Janes *et al.*, unpublished] have shown they all adopt a singular distinct fold: labelled the α-conotoxin fold. This is

Table 2

Sequences of the neuronal α-conotoxins.

Name	Sequence[a]	Reference
α4/7 α-Conotoxins		
MII	Gly Cys *Cys* Ser Asn Pro Val *Cys* His Leu Glu His Ser Asn Leu Cys -NH_2	[41]
AuIA	Gly Cys *Cys* Ser Tyr Pro Pro *Cys* Phe Ala Thr Asn Ser Asp Tyr Cys -NH_2	[51]
AuIC	Gly Cys *Cys* Ser Tyr Pro Pro *Cys* Phe Ala Thr Asn Ser Gly Tyr Cys -NH_2	[51]
PnIA	Gly Cys *Cys* Ser Leu Pro Pro *Cys* Ala Ala Asn Asn Pro Asp Tyr[b] Cys -NH_2	[52]
PnIB	Gly Cys *Cys* Ser Leu Pro Pro *Cys* Ala Leu Ser Asn Pro Asp Tyr[b] Cys -NH_2	[52]
EpI	Gly Cys *Cys* Ser Asp Pro Arg *Cys* Asn Met Asn Asn Pro Asp Tyr[b] Cys -NH_2	[60]
AnIA	Cys *Cys* Ser His Pro Ala *Cys* Ala Ala Asn Asn Gln Asp Tyr[b] Cys -NH_2	[63]
AnIB	Gly Gly Cys *Cys* Ser His Pro Ala *Cys* Ala Ala Asn Asn Gln Asp Tyr[b] Cys -NH_2	[63]
AnIC	Gly Gly Cys *Cys* Ser His Pro Ala *Cys* Phe Ala Ser Asn Pro Asp Tyr[b] Cys -NH_2	[63]
GIC	Gly Cys *Cys* Ser His Pro Ala *Cys* Ala Gly Asn Asn Gln His Ile Cys -NH_2	[64]
GID	Ile Arg Asp Gla[c] Cys *Cys* Ser Asn Pro Ala *Cys* Arg Val Asn Asn Hyp His Val Cys	[66]
Vc1.1[d]	Gly Cys *Cys* Ser Asp Pro Arg *Cys* Asn Tyr Asp His Pro Glu Ile Cys -NH_2	[68]
PIA	Arg Asp Pro Cys *Cys* Ser Asn Pro Val *Cys* Thr Val His Asn Pro Glu Ile Cys -NH_2	[46]
α4/6 α-Conotoxins		
AuIB	Gly Cys *Cys* Ser Tyr Pro Pro *Cys* Phe Ala Thr Asn Pro Asp Cys -NH_2	[51]
α4/3 α-Conotoxins		
ImI	Gly Cys *Cys* Ser Asp Pro Arg *Cys* Ala Trp Arg Cys -NH_2	[73]
ImII	Ala Cys *Cys* Ser Asp Arg Arg *Cys* Arg Trp Arg Cys -NH_2	[79]
ImIIA	Tyr Cys *Cys* His Arg Gly Pro *Cys* Met Val Trp Cys -NH_2	
α4/4 α-Conotoxins		
BuIA[e]	Gly Cys *Cys* Ser Thr Pro Pro *Cys* Ala Val Leu Tyr Cys -NH_2	[80•]

[a] Disulphide bonds are linked as 'normal text' pairs and 'italicised text' pairs.
[b] Sulphotyrosine. Note that data for the toxicity of PnIA and PnIB are for the non-sulphated form of the conotoxin.
[c] Carboxyglutamate.
[d] Vc1.1 is not the wild-type venom component, known as Vc1a, which contains post-translationally modified residues. See text for more details.
[e] No knowledge is currently available regarding PTMs present in the wild-type BuIA conotoxin, as discussed in the text.

Table 3

Subunit specificities of the neuromuscular α-conotoxins.

Name	Mammalian subunit interface selectivity		Reference
α3/5 α-Conotoxins			
	High affinity	Low affinity	
GI	α/δ	α/γ	[25]
MI	α/δ	α/γ	[26]
SI	α/δ	α/γ	[27]
SIA	α/δ	α/γ	[25]
SII	n.d.[a]	n.d.	[37]
GIA	α/δ	α/γ	[25]
GII	α/δ	α/γ	[25]
CnIA	n.d.	n.d.	[38]
α4/7 α-Conotoxins			
EI	α/δ	α/γ[b]	[39]

[a] n.d. No data available on the preferred site regarding the subunit interfaces.
[b] There is less difference in selectivity between the two sites and EI has high affinity for both interfaces.

surprising in the case of SI, where the Pro9 in the sequence does not distort the backbone despite constraints imposed by the cyclic nature of the side chain [32]. Differences in toxicity and binding profiles for the wild-type 'classical' α-conotoxins therefore stem from the topology and electrostatics of the side chains themselves, the backbone conformation acting as a scaffold from which to 'arrange' the side chains for their interactions. Several studies (for example [28,33]) have demonstrated that a key component associated with toxicity is having a potentially positively charged residue at position 9 (10 in MI). Thus, MI has a lysine and GI has an arginine, both being highly toxic, while SI has a neutral proline and is less toxic. This lack of toxicity could be exploited in that the basic framework of SI could act as a template for the controlled construction of a more potent agent while retaining selectivity towards nm-nAChRs. Identifying the differences between these neuromuscular conotoxins and the neuronal conotoxins, and maintaining those differences, would be essential for construction of such a focused pharmaceutical agent [34•].

Table 4

Subunit specificities of the neuronal α-conotoxins.

Name	Primary mammalian subunit selectivity[a]	References
α4/7 α-Conotoxins		
MII	α6β2 ≈ α3β2	[42,43]
AuIA	α3β4	[51]
AuIC	α3β4	[51]
PnIA	α3β2	[56]
PnIB	α7	[56]
EpI	α3β2, α3β4, α7[b]	[60,61]
AnIA	α3β2	[63]
AnIB	α3β2	[63]
AnIC	α3β2	[63]
GIC	α3β2 ≈ α6β2β3	[64,46]
GID	α3β2 ≈ α7	[66]
Vc1.1	α3β4	[68]
PIA	α6/α3β2β3[c]	[46]
α4/6 α-Conotoxins		
AuIB	α3β4	[51]
α4/3 α-Conotoxins		
ImI	α7	[74]
ImII	α7	[79]
ImIIA	n.d[d]	
α4/4 α-Conotoxins		
BuIA	α6/α3β2 ≈ α6/α3β4[c]	[80•]

[a] Only the subunit combination for which each neuronal α-conotoxin has the highest blocking potency is shown. The blocking potencies are comparable for the highest combinations where more than one is shown.
[b] Data from tissue receptor studies differ from Xenopus oocyte expression studies, as described in the text.
[c] α6/α3 refers to the chimera form of the α6 channel, as described in the text for PIA.
[d] Arises from mRNA sequenced data alone; no data on the toxin are known.

Replacing individual disulphides in SI by lactam bridges (putting glutamate and lysine in and joining their side chains to create a peptide bond) produced significant toxicity changes [35]. Although the Cys2–Cys7 lactam analogues were non-toxic, both Cys3–Cys13 lactam analogues showed toxicity: the Lys3–Glu13 was ~60-fold less toxic than wild-type SI, but the Glu3–Lys13 was ~70-fold more toxic than wild-type SI. This clearly shows that while the disulphides are important in these conotoxins, structural changes can be made that can enhance and/or alter the toxicity characteristics. SIA [36] from *C. striatus* preferentially blocks the α/δ subunit interface in mammalian smooth muscle cells over the α/γ interface [25]. It is more potent as a toxin than is SI, having a lysine at position 9. With a sequence not dissimilar to the other 'classical' family members, it might be expected SIA would retain the α-conotoxin fold. To avoid confusion with the growing number of structures from neuronal α-conotoxins, this should now strictly be termed 'the α3/5-conotoxin fold' to reflect the distinct arrangement of the loop residues in the sequence. The basic shape of these conotoxins can be described as a 'triangular wedge' with the N terminus and C terminus forming one corner and residues 5 and 9 (counting as for SI and GI) forming the other two. Specifically, this conformation enables presentation of the residue in the 9 position for interaction with the nm-nAChR.

SII, GIA, GII

SII from *C. striatus* [37] is thus far unique among neuromuscular α-conotoxins, having three disulphide bonds to support its structure, although its toxicity profile is similar to that of SI. Given this lack of toxicity in comparison to other neuromuscular α-conotoxins, and its complexity to synthesise (having three disulphide bonds), little further research has been conducted on this toxin to date. α-Conotoxins GIA and GII are two neuromuscular conotoxins from *C. geographus*, and were isolated at the same

time as GI [22]. However, like SII, almost no research work has been undertaken on them since their sequences were determined.

CnIA

CnIA, a neuromuscular α-conotoxin from the fish-hunting *Conus consors*, was isolated, characterised and had its structure solved in 1999 [38]. The sequence is very similar to MI, differing only at position 11 (10 in SI and GI), where it has a tyrosine instead of an asparagine, and this is also reflected in its toxicity profile towards nm-nAChRs. Likewise, its structure is similar to the other classical α-conotoxins (Figure 1); however, there is a residual toxicity towards neuronal receptors found in this conotoxin, when compared with the rest of the α3/5 family [38].

α4/7 α-Conotoxins

EI

First identified in 1995, EI is from the Atlantic fish-hunting marine snail *Conus ermineus* [39]. EI is a somewhat anomalous member of the neuromuscular conotoxins, in that it has an α4/7 loop motif more associated with neuronal conotoxins. Likewise, this conotoxin has high potency for both the α/δ and the α/γ subunit interfaces in mammalian nm-nAChRs, with only a slight preference for α/δ [39]. Comparing EI with α4/7 neuronal conotoxins, only two residues are found in its sequence that are unique and not found in any of the neuronal conotoxins (allowing for proline and hydroxyproline to be considered the same). These are a Tyr6, where neuronal conotoxins almost invariantly have a serine, and a Gln16, but even here some neuronal conotoxins have a glutamate in the comparable position. No data, however, have been reported regarding the binding of EI to neuronal nAChRs. Likewise, the determined structural conformation of EI is almost identical in backbone to the α4/7 neuronal α-conotoxins [40], which suggests that the major differences regarding the binding to their respective receptor subclasses lie in the surface charge, side-chain conformations and subtle differences in the overall topologies of these α-conotoxin peptides.

α-Conotoxin antagonists of neuronal nAChRs

Many of the neuronal α-conotoxins have been shown to be highly selective for specific subunit interfaces within subclasses of the n-nAChRs. Much of the work has been undertaken in *Xenopus* oocyte expression systems, and occasionally this has influenced the apparent selectivity of these conotoxins in a manner that is currently unclear, but due potentially to differences in the properties of the expressed receptors and those found in native source tissue. However, these differences might also result from the fact that the receptor subclass presumed to be abundant in the particular source tissue is not that actually present. Differences, where seen, are identified for each of the neuronal α-conotoxins discussed in this section.

α4/7 α-Conotoxins

MII

The neuronal α-conotoxin MII from *C. magus* was first purified and sequenced in 1996 [41] in a study specifically aimed at identifying blockers of receptors containing α3β2 subunits. Iodine-labelling of MII was made possible by the addition of a tyrosine residue to the N-terminal, with little change in toxicity. This addition, coupled with the slow dissociation rate from its receptor, allowed MII to be used to identify α3β2 n-nAChRs in many regions of the brain [42]. However, it is now well established that MII has a similar high affinity for receptors containing α6, a subunit that is highly homologous with α3 [43]. Indeed, an analogue of MII with alanine replacing His9 and Leu15 has been shown to be highly selective for α6-containing n-nAChRs over α3-containing receptors [44•]. Some recharacterisation of receptor subclass populations in the brain may be necessary. In addition, it is now suggested that MII may also favour receptors containing β2 and β4 combinations over α3 subunits [45,46]. Affinity for α3β2 is assured, however, and recent structure/function data suggest that residues Asn5, Pro6 and His12 contribute the most to the maintenance of this selectivity. This contribution is mediated either through direct interactions with residues in the receptor subunits, or through ensuring that the correct fold of the toxin is maintained, or elements of both [47•].

The structure of MII has been determined [48,49]. It follows the classical features associated with all α4/7 structures, resembling an omega ('ω') shape in its backbone conformation. As this has become a recognised structural framework for these conotoxins it should strictly be termed 'the α4/7-conotoxin fold' to reflect this fact. Indeed, it is probably appropriate when considering structures for conotoxins to add in the loop family designation to the fold description, as this would both emphasise the reproducibility of the given fold and enable differences, were they to be found, to be highlighted more effectively.

To demonstrate the ability to create a modified α-conotoxin more suited to crossing the blood-brain barrier, Blanchfield *et al.* [50] added a lipid molecule to the N-terminus of MII. This analogue with the 2-amino-D,L-dodecanoic acid attached proved far more able to permeate a test membrane system than did wild-type MII, thereby demonstrating one possible way of making α-conotoxins more bioavailable as drug agents in their own right [50].

AuIA, AuIC

AuIA and AuIC are two α4/7 conotoxins from *Conus aulicus*. They have some activity at blocking α3β4 mammalian n-nAChRs expressed in oocytes, but did not have high affinity for any of the other receptor subunit pairings tested in the experiment [51]. However, this experiment

was conducted before expression systems were available for α6-containing receptors, and no data are yet available on the affinity of conotoxins AuIA and AuIC for channels containing this subunit. AuIB, described later, is related to these two α-conotoxins.

PnIA, PnIB

First identified in 1994 from the venom of *Conus pennaceus* [52], PnIA and PnIB were assigned at that time as having a 'free tyrosine' in the 7-loop (residue Tyr15). However, a discrepancy was noted between the calculated and measured molecular weights for both their sequences; that is, a difference between the synthetically produced toxin and the purified fraction isolated from the venom itself. This discrepancy was resolved when it was determined that the tyrosine was actually sulphated in the wild-type conotoxins [53]. Despite this, seemingly all work involving these conotoxins has been undertaken on the non-sulphotyrosine form. The structures for PnIA [54] and PnIB [55], swiftly solved after their discovery, are not sulphated. These peptides share a high degree of structural similarity, not surprising, as they differ in sequence by only two residues (at positions 10 and 11). In addition, their structures are also comparable to most other α4/7 conformations, retaining the α4/7-conotoxin fold.

All studies of the subunit-selectivity of these conotoxins have been on the non-sulphated tyrosine form. These have established that PnIA is selective for α3β2-containing receptors and PnIB is selective for α7-containing receptors (rat subunits expressed in *Xenopus* oocytes [56]). With almost identical backbone conformations these selectivity differences result from the two different residues at positions 10 and 11, alanine and asparagine in PnIA, and leucine and serine in PnIB. Several studies switching just one of these residues [56–58] showed that the analogue [Ala10Leu]PnIA became highly selective for α7-containing receptors, more so than PnIB itself, and that [Asn11Ser]PnIA had reduced affinity for both subunit subclasses. A recent study with a mutated receptor suggests that PnIA and [Ala10Leu]PnIA block different functional states of the α7 receptor [59•], a feature that would be most useful were a pharmaceutical agent to be needed that could distinguish between the open and the closed state of the nAChR, for example.

EpI

First reported in 1998 [60], α-conotoxin EpI from *Conus episcopatus* was at the time considered to be unique in having a sulphated tyrosine residue, Tyr(SO_3H)15. Its subunit selectivity was determined initially in tissue thought to comprise specific n-nAChR subclasses. Thus EpI was highly potent at blocking n-nAChRs in bovine chromaffin cells, presumed rich in α3β4-containing receptors, and in rat intracardiac ganglia, where receptors containing α3β2 and α3β4 receptors were considered to predominate. No blocking of α7-containing receptors was reported [60]. However, when these same receptor subunit combinations, notably from rat, were expressed in *Xenopus* oocytes, EpI had at best limited blockade of α3β2 and α3β4 receptors and exhibited a significant selective block of α7-containing receptors, results diametrically opposite to those of the tissue studies [61]. Any of several factors could cause this significant difference: it is possible that as-yet unidentified or modified receptor subclasses may exist in intracardiac ganglia and possibly bovine chromaffin cells, there may be subtle differences in highly homologous subunits from different species, or differences in functional properties between receptor subclasses in expression systems and native tissue [4••,61]. There is evidence that receptor subclasses in intracardiac ganglia may be more complex than we currently understand, and it could well be that α-conotoxins will play a part in clarifying this further.

The structure of the non-sulphated form of EpI, [Tyr15]-EpI (which was reported to have similar toxicity levels to the sulphated wild-type [60]), has been solved [62]. Like other neuronal α-conotoxins of this family, it adopts the characteristic α4/7-conotoxin fold, although the solved structure illustrates that there are small elements of flexibility within this conformation that were not apparent from other crystal structures reported.

AnIA, AnIB, AnIC

Conus anemone is a worm-hunting cone snail from the southern Australian waters. Recent studies [63] have identified three α-conotoxins from its venom, AnIA, AnIB and AnIC, which share a high degree of sequence homology. AnIA and AnIB are essentially identical bar two extra glycine residues on the N terminus of the latter. AnIC retains these extra glycines, but differs from the others by two residues in the C-terminal region, Phe9 and Ser11 in place of the corresponding Ala9 and Asn11. All three conotoxins, like PnIA, PnIB and EpI, also from worm-hunting cone snails, contain a sulphotyrosine (Table 2). Selectivity studies against several different neuronal subunit pairings concluded that AnIB was the most active of these three, displaying a preference for α3β2 n-nAChRs. It also blocked α7-containing receptors, but with a potency two orders of magnitude below those of α3β2. Of interest, the non-sulphated tyrosine form of AnIB had lower affinity than wild-type AnIB at both these receptor subclasses, but showed even more distinction (selectivity) between them. Comparable differences in toxicity might well be seen if the sulphotyrosine forms of PnIA and PnIB were tested, as their sequences are highly homologous to AnIB, particularly in the C-terminal region.

GIC

C. geographus, a fish-hunting member of this marine snail genus, has not only potent nm-nAChR-selective components in its venom, but also n-nAChR-selective ones.

GIC, a toxin predicted from sequencing the genomic DNA of the snail, is one such neuronal toxin, being highly selective for neuronal nAChRs over neuromuscular receptors [64]. The toxin was characterised as most selective for α3β2-containing receptors by some two orders of magnitude over α3β4 and α4β2 receptors. Later studies have apparently established that this conotoxin is equally as potent towards α6β2β3 receptors ('unpublished observations' reported in [46]).

The structure of GIC has been reported recently [65]. The backbone fold is highly comparable to other members of the α4/7 family, so it is the side-chain composition and their charge and topology that results in the toxicity profile of GIC. Smaller hydrophobic residues at positions 7 and 10 in the sequence than those found in MII, for example (see Table 2), and the longer hydrophilic side chain of Gln13, may contribute to this profile [65].

GID

α-Conotoxin GID has also recently been isolated from crude *C. geographus* venom [66]. This polypeptide is currently unique among the α4/7 family, and indeed the rest of the conotoxins, in having four residues in its sequence prior to the first of the cysteine residues. Other unusual features are that one of these four residues has undergone post-translational modification (PTM) to a γ-carboxyglutamate; there is a hydroxyproline in the 7-loop; and the C terminus, amidated in the majority of conotoxins to date, is a free carboxylate in GID. Resultant from these many unusual features, GID has an interesting toxicity profile towards nAChRs. From data on mammalian subunits expressed in *Xenopus* oocytes, GID had no notable effect on blocking neuromuscular receptors, and only very limited effects on α3β4 and α4β4-containing neuronal receptors. By contrast, GID potently blocked α3β2 and α7 receptors, and had a significant block on the α4β2 subclass of receptors. Receptors containing α4β2 are implicated in dopamine release, a function believed associated with nicotine dependence [67], and have links with schizophrenia [9•]. Removing the N-terminal residues had little effect on the blocking potency of GID for the α3β2 and α7 receptors, although the duration of the block was significantly reduced in length. Removal of these four residues, however, greatly reduced the potency of GID for the α4β2 receptor subclass. This result suggests that addition of residues to the N termini of other n-nAChR-selective α-conotoxins might in turn alter their selectivity and toxicity profiles towards neuronal subclasses, which could be a notable avenue of exploration for rational drug design. Replacement of Arg12 with an alanine in GID (equivalent to position 9 in most other α4/7 conotoxins, see Table 2), resulted in a dramatic reduction in blocking potency for the α4β2 subclass. There was also significant and limited reduction in potency for receptors containing α7 and α3β2 subunits, respectively [66].

The overall backbone conformation of GID is again very similar to other α4/7 α-conotoxins, when considering the residues 4 to 19, the region corresponding to that of the more conventional α4/7 family members [66]. The four N-terminal residues appear disordered in the solution structure solved by nuclear magnetic resonance (NMR). This suggests that these residues only achieve some sort of structured conformation when GID binds into the receptor where specific interactions, such as hydrogen bonding or salt bridges, would take place to constrain these residues.

Vc1.1

Using the technique of polymerase chain reaction amplification to increase the small quantities of individual venom components, Sandall and co-workers isolated several conotoxins from *Conus victoriae* [68]. Eleven conotoxins were found and one, termed Vc1.1, a 16 residue polypeptide, was identified as a neuronally active nAChR antagonist, having no activity towards nm-nAChRs. The polypeptide synthesised using the sequence data available, with an amidated C terminus and disulphides identical in pairing to other α4/7 conotoxins, was tested on bovine chromaffin cells. The results suggested that it blocks α3β4-containing n-nAChRs. Of interest was that this polypeptide was also found to exhibit powerful analgesic properties *in vivo* which has led, thus far, to a patent and preclinical trials [13••]. However, Vc1.1 does not represent the wild-type toxin, labelled Vc1a, as apart from the C-terminal amidation and disulphide formation, two other PTMs to residues have been identified. These are a γ-carboxyglutamate at residue 14, and hydroxyproline at residue 6 [69]. This latter is indeed unusual when considering the strong similarity of the N-terminal region of Vc1.1 to other α-conotoxins (Table 2). Indeed, ImI is identical in the first eight, and EpI in the first nine, residues of their N termini, and yet neither exhibits a PTM of their equivalent proline. It might be, however, that hydroxyproline forms of these other conotoxins are present as venom components but in far smaller quantities than the unmodified forms. This demonstrates the diversity that can exist within the *Conus* species, and the conotoxins that they generate can be enriched by creation of post-translationally modified components. When the wild-type conotoxin Vc1a, with all PTMs in place, was subsequently synthesised and tested for toxicity, it exhibited none of the properties that its unmodified cousin had shown. It is unclear at this time why this is the case [69].

PIA

The α-conotoxin PIA from *Conus purpurasceus* is the first toxin reported as being able to distinguish between the highly homologous α3 and α6 subunits in n-nAChRs expressed in *Xenopus* oocytes [46]. α-Conotoxins with this distinguishing ability are important, as receptors that contain the α6 subunit are being considered as targets for

the treatment of Parkinson's disease [8•]. PIA exhibits a more potent blocking of channels containing either the full α6 subunit or a chimera comprised of the extracellular ACh-binding α6 region joined to the α3 membranous and intracellular region, than of channels containing the α3 subunit alone. The most potent block was of receptors containing α6/α3β2β3 (α6/α3 indicates the chimera subunit). Some tests were performed against channels comprising the mammalian α6β4 subunits, which PIA successfully blocked. However, this subclass of receptor has only been identified in chick at present [70]. Channels formed when β4 were included were blocked for longer periods than those where β2 were used [46].

α4/6 α-Conotoxins

AuIB

AuIB, from *C. aulicus*, is currently unique among the known neuronal α-conotoxins in that while having four residues in the first loop, there are only six in the second loop (see Figure 2 and Tables 2 and 4). Identified along with AuIA, and AuIC, this conotoxin was the most potent and selective of the three at blocking α3β4-containing receptors [51]. It was less potent, by two orders of magnitude, at blocking α7 receptors, and showed negligible effects on numerous other subunit pairings. Structurally, while retaining much of the characteristics of α4/7 conformations, the 'lack' of a tyrosine in the C-terminal loop renders this region of the polypeptide noticeably different from its larger cousins [71]. It is clear the differences in AuIB help promote its subclass selectivity. Many studies have investigated the disulphide bond arrangement of conotoxins, and almost invariably any alterations by changing the pairings leads to drastic to total loss of toxic characteristics. It was surprising, therefore, that a different arrangement of disulphides in AuIB, a so-called 'ribbon' structure, (wild-type being Cys2–Cys8, Cys3–Cys15, and the variant being Cys2–Cys15, Cys3–Cys8) resulted in 10-times higher potency towards α3β4 receptors than the wild-type bonding pattern [72]. However, data from oocyte-expressed α3β4 receptors showed that the ribbon structure conotoxin had substantially lower potency than the wild-type conotoxin at these receptors [61]. The NMR solution structure of this ribbon AuIB suggests there is a greater degree of flexibility between the N-terminal and C-terminal regions than is found in the native conformation, which might be one reason for these discrepancies in the results [61,72].

α4/3 α-Conotoxins

ImI, ImII, ImIIA

α-Conotoxin ImI, the first neuronally active toxin, was identified in 1994 and was shown to cause seizures in mice and rats [73]. It proved to be an exception in terms of its sequence, in that it is an α4/3 looped structure. ImI was reported to potently block nm-nAChRs from frogs but not mammalian or *T. californica* nm-nAChRs. Later work on its neuronal receptor selectivity showed that the block was against homopentameric α7 receptors, and that it had potency, although two orders less, towards α9 receptors [74]. Against all other subunit pairs ImI showed very little to no activity at all, making this a highly useful probe for studies on identifying tissue containing specifically α7 receptors. Analogues of ImI have shown residues in two key areas of the conotoxin to be important for activity: Asp5–Pro6–Arg7 in the 4-loop, and Trp10 in the 3-loop [75]. Structural studies were undertaken on ImI [76–78], and in comparisons with available α4/7 structures it can be seen that the conformations of the N-terminal 4-loop regions are very similar. Clearly the C-terminal loop regions are quite different, as would be expected given their different numbers of residues.

Recently α-conotoxin ImII has been reported, having the same α4/3 arrangement as ImI and also being similar in sequence, but with notable differences between the two [79]. In the 4-loop a proline conserved within other neuronal α-conotoxins is replaced by an arginine in ImII, and another arginine replaces an alanine found in ImI in the 3-loop. Not only does this introduce potentially charged side chains to these positions in the conotoxin, but also the loss of the structural constraints imposed by proline could result in conformational differences between ImII and ImI. It is interesting, therefore, that while both ImI and ImII block the α7 subclass of receptors with comparable potency, they seem to do so at different sites on these receptors [79]. Indeed, studies using analogues of ImI support the critical nature of Pro6 in giving rise to this difference between ImI and ImII. A structure determination of ImII might well resolve whether the conformation has changed resultant from the loss of constraint from Pro6 in the 4-loop.

ImIIA is only known from its mRNA sequence, which was isolated from the species itself, and no other data are available. It was submitted directly to the nucleotide database (Swiss-Prot database accession code: Q9U619). It is interesting that the proline residue, conserved in position in the 4-loop of all neuronal α-conotoxins to date, is in a different position in this toxin. What toxicity profile and what structure this produces will be of interest.

α4/4 α-Conotoxins

α-Conotoxin BuIA from *Conus bullatus*, a fish-hunting cone snail from the Indo-Pacific, is a highly novel polypeptide recently discovered [80•]. It was identified because the DNA genetic data located just before an α-conotoxin polypeptide is highly conserved across species, and this was used as an aid to hunt for new toxins. BuIA is the first α-conotoxin found to have four residues in both the first and second loops (Table 2). The BuIA α-conotoxin has not yet been isolated from the native venom of this species. For synthesis and characterisation, therefore, the disulphides were paired as commonly found in other conotoxins, and an amidated C terminus

was created, also a common feature. This was then tested against several neuronal receptors created using many different pairings of rat subunits expressed in *Xenopus* oocytes, and was found to be active against many of them, but to dramatically varying degrees and with interesting kinetic differences. BuIA was most potent at blocking α6-containing receptors (using the chimera receptor as described for PIA above), and next for those containing the highly homologous α3 subunit. In addition, blocking was observed against α2β4 and α7 receptors, but BuIA showed limited activity against α4β2 receptors. Significantly, this α-conotoxin dissociated at different rates from receptors with β2 or β4 as components where a common α subunit was used [80•]. Receptors containing the β2 subunit were relatively quick at releasing the conotoxin, whereas those containing the β4 subunit were substantially slower. Some receptors are believed to contain both β2 and β4 subunits, but no data are as yet available for BuIA at such receptors, although this would clearly be of interest [80•].

Conclusions

Nature has created a wealth of polypeptide agents within the venom of the *Conus* genus of marine snails to enable them to capture and subdue their prey. α-Conotoxins represent an important family group in that through subtle variations within their sequences, resulting in charge, side-chain conformation and topological differences, they possess the ability to be highly selective competitive antagonists at specific nicotinic acetylcholine receptor subclasses. Some of these individual subclasses may be involved in prominent diseases, and an approach to treatment might be through drug action on these receptors. For example, one α-conotoxin is already in preclinical trials as an analgesic agent. Many studies have identified structural features that focus the blocking abilities of the α-conotoxins towards their specific subclasses. A positive charge on one residue in the C-terminal region of the neuromuscular-selective α-conotoxins gives rise to greater potency than a neutral residue in that position. Subtle sequence variations within the C-terminal loop regions of the larger neuronal-selective α-conotoxins often correlate with selectivity for the specific β subunit found in the neuronal receptors studied. Likewise, differences in the N-terminal regions of these α-conotoxins seemingly have a bearing on the selective blocking of specific α-subunit-containing receptors. These results alone imply the interface preference in orientation of the larger neuronal α-conotoxins in their receptors, although many studies involving sequence changes in both receptor and conotoxin have gone a long way in identifying interactions, and hence binding orientation, between them.

Not only have wild-type α-conotoxins been employed but also analogues have been used as tools to further the knowledge about the topological requirements for specificity. Additionally, non-natural analogues (lactam derivatives, for example), have shown that changes can be made that result in an increase in potency over their wild-type conotoxin cousins. This then becomes the province of the synthetic chemist in creating analogues that cannot be found in nature, to assist the development of drug agents. There is every reason to suggest that with judicious substitutions of non-native side chains onto the backbone framework of the α-conotoxins, drugs could be developed that would single out, with very high selectivity, one receptor subclass over all others. The structures of the α-conotoxins determined thus far have offered considerable insight into the conformational requirements that could lead to the creation of such peptomimetic agents, and there is clear potential to use this structural knowledge in future rational drug design strategies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hogg RC, Raggenbass M, Bertrand D: **Nicotinic acetylcholine**
•• **receptors: from structure to brain function**. *Rev Physiol Biochem Pharmacol* 2003, **147**:1-46.

An extensive and detailed review of our current knowledge of nAChR biochemistry, structure, distribution, functions, and involvement in disease states.

2. Karlin A: **Emerging structure of the nicotinic acetylcholine**
• **receptors**. *Nat Rev Neurosci* 2002, **3**:102-114.

A clear and comprehensive review of our knowledge of the structure of the nAChR and of how this information was gained.

3. Dajas-Bailador F, Wonnacott S: **Nicotinic acetylcholine**
• **receptors and the regulation of neuronal signalling**. *Trends Pharmacol Sci* 2004, **25**:317-324.

A review giving insight into the functional role of neuronal nAChRs in their mediation of signalling resultant from Ca^{2+} permeability through these channels.

4. Nicke A, Wonnacott S, Lewis RJ: **α-Conotoxins as tools for the**
•• **elucidation of structure and function of neuronal nicotinic acetylcholine receptor subtypes**. *Eur J Biochem* 2004, **271**:2305-2319.

A detailed, comprehensive and data-filled review covering neuronal α-conotoxin toxicities against receptor sub-classes from diverse species, tissues and expression systems.

5. Rajendra W, Armugam A, Jeyaseelan K: **Neuroprotection and peptide toxins**. *Brain Res Brain Res Rev* 2004, **45**:125-141.

6. Hogg RC, Bertrand D: **What genes tell us about nicotine addiction**. *Science* 2004, **306**:983-984.

7. Tuppo EE, Arias HR: **The role of inflammation in Alzheimer's**
• **disease**. *Int J Biochem Cell Biol* 2005, **37**:289-305.

Within this review presentation is made of the possible link between n-nAChRs and neuroinflammation, which is integrally associated with Alzheimer's disease. As such it is suggested that nAChRs offer a therapeutic target for treatment of the disease.

8. Quik M, Kulak JM: **Nicotine and nicotinic receptors;**
• **relevance to Parkinson's disease**. *Neurotoxicology* 2002, **23**:581-594.

A review covering the link between nAChRs and Parkinson's disease, highlighting the importance of receptors containing the α6 subunit as potential targets for drug treatments.

9. Ripoll N, Bronnec M, Bourin M: **Nicotinic receptors and**
• **schizophrenia**. *Curr Med Res Opin* 2004, **20**:1057-1074.

A review reporting that dysfunction of specific subclasses of n-nAChR are linked to schizophrenia and how nicotine intake, shown to alleviate the symptoms, supports this role of n-nAChR involvement in the condition.

10. Combi R, Dalpra L, Tenchini ML, Ferini-Strambi L: **Autosomal**
• **dominant nocturnal frontal lobe epilepsy - A critical overview**. *J Neurol* 2004, **251**:923-934.
This overview identifies the known mutations found in neuronal nAChR subunits that have been implicated in frontal lobe epilepsy. It discusses the nature of the link between nAChRs and the development and progression of this disease, where there are still areas that need to be clarified.

11. Richman DP, Agius MA: **Treatment of autoimmune myasthenia**
• **gravis**. *Neurology* 2003, **61**:1652-1661.
This review discusses, amongst other issues, the current treatments available for myasthenia gravis and inherent problems associated with the non-specificity of drugs used.

12. Lloyd GK, Williams M: **Neuronal nicotinic acetylcholine receptors as novel drug targets**. *J Pharmacol Exp Ther* 2000, **292**:461-467.

13. Livett BG, Gayler KR, Khalil Z: **Drugs from the sea:**
•• **Conopeptides as potential therapeutics**. *Curr Med Chem* 2004, **11**:1715-1723.
A comprehensive review that identifies the characteristics of selected members of all conotoxin families, and how some of these are being developed as therapeutic agents to treat neurological conditions.

14. Loughnan ML, Alewood PF: **Physico-chemical characterization**
•• **and synthesis of neuronally active α-conotoxins**. *Eur J Biochem* 2004, **271**:2294-2304.
Identifying all the features present in an α-conotoxin sequence, specifically PTMs, is important as these may be critical components involved in the toxicity. This review describes means and methods for achieving this identification process, and shows that chemical synthesis has a central role, both now and in the future, for creation of α-conotoxins and of their analogues.

15. Millard EL, Daly NL, Craik DJ: **Structure-activity relationships of**
•• **α-conotoxins targeting neuronal nicotinic acetylcholine receptors**. *Eur J Biochem* 2004, **271**:2320-2326.
A review focussing on the structures of neuronal α-conotoxins, highlighting their differences, and how these and analogue structures are being used in mapping out the conformational characteristics important for receptor subclass selectivity and toxicity.

16. Dutertre S, Lewis RJ: **Computational approaches to understand**
•• **α-conotoxin interactions at neuronal nicotinic receptors**. *Eur J Biochem* 2004, **271**:2327-2334.
A review that highlights the use of computational protocols in gaining insight into the ways in which neuronal α-conotoxins might bind into their respective receptor sub-classes using molecular modelling and docking strategies. Insights gained in this way may well facilitate the development of novel peptomimetics through rational drug design.

17. Santos AD, McIntosh JM, Hillyard DR, Cruz LJ, Olivera BM:
• **The A-superfamily of conotoxins – Structural and functional divergence**. *J Biol Chem* 2004, **279**:17596-17606.
A paper identifying how the diversity of the α-conotoxins has arisen within the single *Conus* genus. By showing the similarities in the genetic data involved with creating these conotoxins, it illustrates one of the ways in which further toxin components could be identified.

18. Terlau H, Olivera BM: **Conus venoms: A rich source of novel ion channel-targeted peptides**. *Physiol Rev* 2004, **84**:41-68.

19. Tsetlin VI, Hucho F: **Snake and snail toxins acting on nicotinic acetylcholine receptors: fundamental aspects and medical applications**. *FEBS Lett* 2004, **557**:9-13.

20. Arias HR, Blanton MP: **α-Conotoxins**. *Int J Biochem Cell Biol* 2000, **32**:1017-1028.

21. McIntosh JM, Santos AD, Olivera BM: **Conus peptides targeted to specific nicotinic acetylcholine receptor subtypes**. *Annu Rev Biochem* 1999, **68**:59-88.

22. Gray WR, Luque A, Olivera BM, Barret J, Cruz LJ: **Peptide toxins from *Conus geographus* venom**. *J Biol Chem* 1981, **256**:4734-4740.

23. McIntosh M, Cruz LJ, Hunkapiller MW, Gray WR, Olivera BM: **Isolation and structure of a peptide from the marine snail *Conus-magus***. *Arch Biochem Biophys* 1982, **218**:329-334.

24. Zafaralla GC, Ramilo C, Gray WR, Karlstrom R, Olivera BM, Cruz LJ: **Phylogenetic specificity of cholinergic ligands: α-conotoxin SI**. *Biochemistry* 1988, **27**:7102-7105.

25. Groebe DR, Dumm JM, Levitan ES, Abramson SN: **α-Conotoxins selectively inhibit one of the 2 acetylcholine binding-sites of nicotinic receptors**. *Mol Pharmacol* 1995, **48**:105-111.

26. Kreienkamp HJ, Sine SM, Maeda RK, Taylor P: **Glycosylation sites selectively interfere with alpha-toxin binding to the nicotinic acetylcholine-receptor**. *J Biol Chem* 1994, **269**:8108-8114.

27. Luo S, McIntosh JM: **Iodo-α-conotoxin MI selectively binds the α/δ subunit interface of muscle nicotinic acetylcholine receptors**. *Biochemistry* 2004, **43**:6656-6662.

28. Groebe DR, Gray WR, Abramson SN: **Determinants involved in the affinity of α-conotoxins GI and SI for the muscle subtype of nicotinic acetylcholine receptors**. *Biochemistry* 1997, **36**:6469-6474.

29. Gehrmann J, Alewood PF, Craik DJ: **Structure determination of the three disulfide bond isomers of α-conotoxin GI: A model for the role of disulfide bonds in structural stability**. *J Mol Biol* 1998, **278**:401-415.

30. Guddat LW, Martin JA, Shan L, Edmundson AB, Gray WR: **Three-dimensional structure of the α-conotoxin GI at 1.2 Å resolution**. *Biochemistry* 1996, **35**:11329-11335.

31. Gouda H, Yamazaki K, Hasegawa J, Kobayashi Y, Nishiuchi Y, Sakakibara S, Hirono S: **Solution structure of α-conotoxin MI determined by H-1-NMR spectroscopy and molecular dynamics simulation with the explicit solvent water**. *Biochimica Biophysica Acta - Prot Struct Mol Enz* 1997, **1343**:327-334.

32. Benie AJ, Whitford D, Hargittai B, Barany G, Janes RW: **Solution structure of alpha-conotoxin SI**. *FEBS Lett* 2000, **476**:287-295.

33. Hann RM, Pagan OR, Gregory LM, Jacome T, Eterovic VA: **The 9-arginine residue of α-conotoxin GI is responsible for its selective high affinity for the αγ agonist site on the electric organ acetylcholine receptor**. *Biochemistry* 1997, **36**:9051-9056.

34. Janes RW: **Nicotinic acetylcholine receptors: α-conotoxins**
• **as templates for rational drug design**. *Biochem Soc Trans* 2003, **31**:634-636.
A paper illustrating how comparative topological studies between α-conotoxins can be used in rational drug design strategies.

35. Hargittai B, Sole NA, Groebe DR, Abramson SN, Barany G: **Chemical syntheses and biological activities of lactam analogues of α-conotoxin SI**. *J Med Chem* 2000, **43**:4787-4792.

36. Myers RA, Zafaralla GC, Gray WR, Abbott J, Cruz LJ, Olivera BM: **α-Conotoxins, small peptide probes of nicotinic acetylcholine-receptors**. *Biochemistry* 1991, **30**:9370-9377.

37. Ramilo CA, Zafaralla GC, Nadasdi L, Hammerland LG, Yoshikami D, Gray WR, Kristipati R, Ramachandran J, Miljanich G, Olivera BM, Cruz LJ: **Novel α-conotoxin and ω-conotoxin from *Conus striatus* venom**. *Biochemistry* 1992, **31**:9919-9926.

38. Favreau P, Krimm I, Le Gall F, Bobenrieth MJ, Lamthanh H, Bouet F, Servent D, Molgo J, Menez A, Letourneux Y, Lancelin JM: **Biochemical characterization and nuclear magnetic resonance structure of novel α-conotoxins isolated from the venom of *Conus consors***. *Biochemistry* 1999, **38**:6317-6326.

39. Martinez JS, Olivera BM, Gray WR, Craig AG, Groebe DR, Abramson SN, Mcintosh JM: **α-Conotoxin EI, a new nicotinic acetylcholine-receptor antagonist with novel selectivity**. *Biochemistry* 1995, **34**:14519-14526.

40. Park KH, Suk JE, Jacobsen R, Gray WR, McIntosh JM, Han KH: **Solution conformation of α-conotoxin EI, a neuromuscular toxin specific for the α_1/δ subunit interface of *Torpedo* nicotinic acetylcholine receptor**. *J Biol Chem* 2001, **276**:49028-49033.

41. Cartier GE, Yoshikami DJ, Gray WR, Luo SQ, Olivera BM, McIntosh JM: **A new α-conotoxin which targets α3β2 nicotinic acetylcholine receptors**. *J Biol Chem* 1996, **271**:7522-7528.

42. Whiteaker P, McIntosh JM, Luo SQ, Collins AC, Marks MJ: **^{125}I-α-Conotoxin MII identifies a novel nicotinic acetylcholine receptor population in mouse brain**. *Mol Pharmacol* 2000, **57**:913-925.

43. Kuryatov A, Olale F, Cooper J, Choi C, Lindstrom J: **Human α6 AChR subtypes: subunit composition, assembly, and pharmacological responses**. *Neuropharmacology* 2000, **39**:2570-2590.

44. • McIntosh JM, Azam L, Staheli S, Dowell C, Lindstrom JM, Kuryatov A, Garrett JE, Marks MJ, Whiteaker P: **Analogs of α-conotoxin MII are selective for α6-containing nicotinic acetylcholine receptors**. *Mol Pharmacol* 2004, **65**:944-952.

A paper highlighting that alterations to the basic sequence of α-conotoxins, to create analogues, can profoundly affect the subclass selectivity of the product compound. Specifically here, converting a parent α-conotoxin with limited preference between α3 and α6 subunits, into an analogue with high selectivity for α6 over α3.

45. Whiteaker P, Peterson CG, Xu W, McIntosh JM, Paylor R, Beaudet AL, Collins AC, Marks MJ: **Involvement of the α3 subunit in central nicotinic binding populations**. *J Neurosci* 2002, **22**:2522-2529.

46. Dowell C, Olivera BM, Garrett JE, Staheli ST, Watkins M, Kuryatov A, Yoshikami D, Lindstrom JM, McIntosh JM: **α-Conotoxin PIA is selective for α6 subunit-containing nicotinic acetylcholine receptors**. *J Neurosci* 2003, **23**:8445-8452.

47. • Everhart D, Cartier GE, Malhotra A, Gomes AV, McIntosh JM, Luetje CW: **Determinants of potency on α-conotoxin MII, a peptide antagonist of neuronal nicotinic receptors**. *Biochemistry* 2004, **43**:2732-2737.

A paper reporting the creation of analogues of α-conotoxin MII, which were used to determine the residues in the sequence critically important for potency. Of note is that each analogue was checked for structural integrity to ensure that those changes that were made in the sequence were not to the detriment of the overall conformation of the α4/7-conotoxin fold.

48. Shon KJ, Koerber SC, Rivier JE, Olivera BM, McIntosh JM: **Three-dimensional solution structure of α-conotoxin MII, an $\alpha_3\beta_2$ neuronal nicotinic acetylcholine receptor-targeted ligand**. *Biochemistry* 1997, **36**:15693-15700.

49. Hill JM, Oomen CJ, Miranda LP, Bingham JP, Alewood PF, Craik DJ: **Three-dimensional solution structure of alpha-conotoxin MII by NMR spectroscopy: Effects of solution environment on helicity**. *Biochemistry* 1998, **37**:15621-15630.

50. Blanchfield JT, Dutton JL, Hogg RC, Gallagher OP, Craik DJ, Jones A, Adams DJ, Lewis RJ, Alewood PF, Toth I: **Synthesis, structure elucidation, in vitro biological activity, toxicity, and Caco-2 cell permeability of lipophilic analogues of α-conotoxin MII**. *J Med Chem* 2003, **46**:1266-1272.

51. Luo SQ, Kulak JM, Cartier GE, Jacobsen RB, Yoshikami D, Olivera BM, McIntosh JM: **α-Conotoxin AuIB selectively blocks α3β4 nicotinic acetylcholine receptors and nicotine-evoked norepinephrine release**. *J Neurosci* 1998, **18**:8571-8579.

52. Fainzilber M, Hasson A, Oren R, Burlingame AL, Gordon D, Spira ME, Zlotkin E: **New mollusk-specific α-conotoxins block *aplysia* neuronal acetylcholine-receptors**. *Biochemistry* 1994, **33**:9523-9529.

53. Wolfender JL, Chu FX, Ball H, Wolfender F, Fainzilber M, Baldwin MA, Burlingame AL: **Identification of tyrosine sulfation in *Conus pennaceus* conotoxins α-PnIA and α-PnIB: Further investigation of labile sulfo- and phosphopeptides by electrospray, matrix-assisted laser desorption/ionization (MALDI) and atmospheric pressure MALDI mass spectrometry**. *J Mass Spectrom* 1999, **34**:447-454.

54. Hu SH, Gehrmann J, Guddat LW, Alewood PF, Craik DJ, Martin JL: **The 1.1 Å crystal structure of the neuronal acetylcholine receptor antagonist, α-conotoxin PnIA from *Conus pennaceus***. *Structure* 1996, **4**:417-423.

55. Hu SH, Gehrmann J, Alewood PF, Craik DJ, Martin JL: **Crystal structure at 1.1 Å resolution of α-conotoxin PnIB: comparison with α-conotoxins PnIA and GI**. *Biochemistry* 1997, **36**:11323-11330.

56. Luo S, Nguyen TA, Cartier GE, Olivera BM, Yoshikami D, McIntosh JM: **Single-residue alteration in alpha-conotoxin PnIA switches its nAChR subtype selectivity**. *Biochemistry* 1999, **38**:14542-14548.

57. Hogg RC, Miranda LP, Craik DJ, Lewis RJ, Alewood PF, Adams DJ: **Single amino acid substitutions in α-conotoxin PnIA shift selectivity for subtypes of the mammalian neuronal nicotinic acetylcholine receptor**. *J Biol Chem* 1999, **274**:36559-36564.

58. Broxton N, Miranda L, Gehrmann J, Down J, Alewood P, Livett B: **Leu^{10} of α-conotoxin PnIB confers potency for neuronal nicotinic responses in bovine chromaffin cells**. *Eur J Pharmacol* 2000, **390**:229-236.

59. • Hogg RC, Hopping G, Alewood PF, Adams DJ, Bertrand D: **α-conotoxins PnIA and [A10L] PnIA stabilize different states of the α7-L247T nicotinic acetylcholine receptor**. *J Biol Chem* 2003, **278**:26908-26914.

This paper demonstrates that with subtle sequence changes within these α-conotoxins, it is possible to create analogues that interact with nAChRs that are in different functional states. This is important in regard to the potential for developing pharmaceutical agents with comparable distinguishing characteristics.

60. Loughnan M, Bond T, Atkins A, Cuevas J, Adams DJ, Broxton NM, Livett BG, Down JG, Jones A, Alewood PF, Lewis RJ: **α-Conotoxin EpI, a novel sulfated peptide from *Conus episcopatus* that selectively targets neuronal nicotinic acetylcholine receptors**. *J Biol Chem* 1998, **273**:15667-15674.

61. Nicke A, Samochocki M, Loughnan ML, Bansal PS, Maelicke A, Lewis RJ: **α-conotoxins EpI and AuIB switch subtype selectivity and activity in native versus recombinant nicotinic acetylcholine receptors**. *FEBS Lett* 2003, **554**:219-223.

62. Hu SH, Loughnan M, Miller R, Weeks CM, Blessing RH, Alewood PF, Lewis RJ, Martin JL: **The 1.1 Å resolution crystal structure of [Tyr(15)]EpI, a novel α-conotoxin from *Conus episcopatus*, solved by direct methods**. *Biochemistry* 1998, **37**:11425-11433.

63. Loughnan ML, Nicke A, Jones A, Adams DJ, Alewood PF, Lewis RJ: **Chemical and functional identification and characterization of novel sulfated α-conotoxins from the cone snail *Conus anemone***. *J Med Chem* 2004, **47**:1234-1241.

64. McIntosh JM, Dowell C, Watkins M, Garrett JE, Yoshikami D, Olivera BM: **α-Conotoxin GIC from Conus geographus, a novel peptide antagonist of nicotinic acetylcholine receptors**. *J Biol Chem* 2002, **277**:33610-33615.

65. Chi SW, Kim DH, Olivera BM, Mcintosh JM, Han KH: **Solution conformation of α-conotoxin GIC, a novel potent antagonist of α3β2 nicotinic acetylcholine receptors**. *Biochem J* 2004, **380**:347-352.

66. Nicke A, Loughnan ML, Millard EL, Alewood PF, Adams DJ, Daly NL, Craik DJ, Lewis RJ: **Isolation, structure, and activity of GID, a novel α4/7-conotoxin with an extended N-terminal sequence**. *J Biol Chem* 2003, **278**:3137-3144.

67. Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, Marks MJ, Collins AC, Lester HA: **Nicotine activation of α4* receptors: sufficient for reward, tolerance, and sensitization**. *Science* 2004, **306**:1029-1032.

68. Sandall DW, Satkunanathan N, Keays DA, Polidano MA, Liping X, Pham V, Down JG, Khalil Z, Livett BG, Gayler KR: **A novel α-conotoxin identified by gene sequencing is active in suppressing the vascular response to selective stimulation of sensory nerves in vivo**. *Biochemistry* 2003, **42**:6904-6911.

69. Jakubowski JA, Keays DA, Kelley WP, Sandall DW, Bingham JP, Livett BG, Gayler KR, Sweedler JV: **Determining sequences and post-translational modifications of novel conotoxins in *Conus victoriae* using cDNA sequencing and mass spectrometry**. *J Mass Spectrom* 2004, **39**:548-557.

70. Vailati S, Hanke W, Bejan A, Barabino B, Longhi R, Balestra B, Moretti M, Clementi F, Gotti C: **Functional α6-containing nicotinic receptors are present in chick retina**. *Mol Pharmacol* 1999, **56**:11-19.

71. Cho JH, Mok KH, Olivera BM, McIntosh JM, Park KH, Han KH: **Nuclear magnetic resonance solution conformation of α-conotoxin AuIB, an $\alpha_3\beta_4$ subtype-selective neuronal nicotinic acetylcholine receptor antagonist**. *J Biol Chem* 2000, **275**:8680-8685.

72. Dutton JL, Bansal PS, Hogg RC, Adams DJ, Alewood PF, Craik DJ: **A new level of conotoxin diversity, a non-native disulfide bond connectivity in α-conotoxin AuIB reduces structural definition but increases biological activity**. *J Biol Chem* 2002, **277**:48849-48857.

73. McIntosh JM, Yoshikami D, Mahe E, Nielsen DB, Rivier JE, Gray WR, Olivera BM: **A nicotinic acetylcholine receptor ligand of unique specificity, α-conotoxin IMI**. *J Biol Chem* 1994, **269**:16733-16739.

74. Johnson DS, Martinez J, Elgoyhen AB, Heinemann SF, Mcintosh JM: **α-Conotoxin Im(I) exhibits subtype-specific nicotinic acetylcholine-receptor blockade - preferential inhibition of homomeric α7 and α9 receptors**. *Mol Pharmacol* 1995, **48**:194-199.

75. Quiram PA, Sine SM: **Identification of residues in the neuronal α7 acetylcholine receptor that confer selectivity for conotoxin ImI**. *J Biol Chem* 1998, **273**:11001-11006.

76. Maslennikov IV, Shenkarev ZO, Zhmak MN, Ivanov VT, Methfessel C, Tsetlin VI, Arseniev AS: **NMR spatial structure of α-conotoxin ImI reveals a common scaffold in snail and snake toxins recognizing neuronal nicotinic acetylcholine receptors**. *FEBS Lett* 1999, **444**:275-280.

77. Gehrmann J, Daly NL, Alewood PF, Craik DJ: **Solution structure of α-conotoxin ImI by H-1 nuclear magnetic resonance**. *J Med Chem* 1999, **42**:2364-2372.

78. Lamthanh H, Jegou-Matheron C, Servent D, Menez A, Lancelin JM: **Minimal conformation of the α-conotoxin ImI for the α7 neuronal nicotinic acetylcholine receptor recognition: correlated CD, NMR and binding studies**. *FEBS Lett* 1999, **454**:293-298.

79. Ellison M, McIntosh JM, Olivera BM: **α-Conotoxins ImI and ImII - similar α7 nicotinic receptor antagonists act at different sites**. *J Biol Chem* 2003, **278**:757-764.

80. • Azam L, Dowell C, Watkins M, Stitzel JA, Olivera BM, McIntosh JM: **α-Conotoxin BuIA, a novel peptide from *Conus bullatus* distiguishes among neuronal nicotinic acetylcholine receptors**. *J Biol Chem* 2005, **280**:80-87.

A significant paper that introduces a new arrival to the neuronal α-conotoxin family with a novel α4/4 loop arrangement. This offers a whole new avenue of exploration for investigating α-conotoxin interactions with specific subunits and provides yet more armoury for rational drug design strategies.

81. Koradi R, Billeter M, Wüthrich K: **MOLMOL: A program for display and analysis of macromolecular structures**. *J Mol Graph* 1996, **14**:51-55.