Diversity of *Conus* peptides that target the nicotinic acetylcholine receptors

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The cone snail (genus *Conus*) peptides known as conopeptides or conotoxins typically consist of about 10-50 amino acid (AA) residues. The peptides are processed from larger precursors with many more AA residues. Different regions of the peptide precursors (signal sequence, propeptide, mature peptide) have significantly diverged and are practically used as basis for the classification of *Conus* peptides into superfamilies. Peptides of a superfamily have highly homologous signal sequences and few variable AA residues in propeptide regions. Generally, the mature peptides (conopeptides) have relatively more variable AA residues. Peptide diversity also occurs in members of the same superfamily that differ in their molecular targets or members of different superfamilies that have the same molecular target. Conotoxins are highly selective antagonists of ligand-gated and voltage-gated ion channels. Consequently, they are used to characterize specific subtypes of receptors or ion channels. This overview describes the structural and functional diversity of conotoxins that influence neurotransmission through their action on nicotinic acetylcholine receptors (nAChRs), to demonstrate molecular diversity in various classes of *Conus* peptides. The primary structures, cysteine patterns, disulfide bonding frameworks, physiological effects and target specificities are described. The occurrence of these peptides in *Conus* venoms gives insights into the evolutionary tactics of cone snails. The conotoxins are useful as tools for investigating the structure and physiological roles of nAChRs. As the structural and functional diversity of conotoxins continues to be explored, these peptides can possibly reveal important neuropharmacological applications and therapeutic potential.

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INTRODUCTION

Nearly 700 extant cone snail (*Conus*) species express in their venoms diverse collections of peptide toxins interchangeably referred to as conotoxins or conopeptides (Kaas et al 2010, Kaas et al 2012). Each species of cone snails produces a distinct suite of about a hundred or more conotoxins, aimed against its prey, predators or competitors. This strategy enables the cone snails to dwell in diverse habitats in both tropical and subtropical marine waters. Based on their prey, cone snails are classified as fish-hunting, worm-hunting or mollusk-hunting species.

The immense diversity of conopeptides can be attributed to the different gene superfamilies into which they are classified

(Puillandre et al 2012). Precursor peptides under a superfamily share homologous signal sequences and propeptide regions, but often with high variability in the sequences of mature peptide regions that generally retain a unique cysteine pattern (Olivera et al 1999, Woodward et al 1990). Most conopeptides are rich in disulfide bonds that allow them to fold in three-dimensional structures necessary in binding to receptors or ion channels with high affinity and notable specificity (Olivera 1997, Terlau and Olivera 2004). Although conopeptides of a superfamily are genetically related, they attain molecular diversity by fast divergence in AA sequences. The structural diversity has resulted in different molecular targets for conotoxins belonging to the same superfamily or the same molecular target for those under different superfamilies (Terlau and Olivera 2004, Teichert et al 2009). Such strategy owing to the remarkable structural and functional diversity of conotoxins has seemingly helped the cone snails to survive under severe environmental pressure.

It has been established that *Conus* peptides are selective antagonists of receptor or ion channel subtypes. Hence, conopeptides have shown a diversity of pharmacological functions (Olivera 1997, Terlau and Olivera 2004, Olivera and Teichert 2007, Teichert et al 2009). Specifically, those that bind to the nicotinic acetylcholine receptors (nAChRs) have been employed in the studies of the functional roles of these molecular targets. Conotoxins targeted to the nAChRs that have been identified are characterized by high affinity and specificity of interactions with various receptor subunits and/or subtypes. Many of these are the well studied α-conotoxins that belong to the A-superfamily (McIntosh et al 1999, Santos et al 2004). Several α-conotoxins were reported to have significant therapeutic potential (Livett et al 2006, Quik and McIntosh 2006, Satkunanathan et al 2005).

An important application of conotoxin research is defining other binding sites (such as specific subtypes/subunits) on already known molecular targets. In addition to the numerous members of the α-conotoxin family, there are other families and subfamilies of conotoxins that target the nAChRs. Characterized members of these divergent families and subfamilies are reviewed in this paper with focus on their primary structures, cysteine patterns, disulfide bonding frameworks, physiological effects and target specificities. More recently, individual conotoxins with multiple targets including nAChRs have been discovered; these are also reviewed. The nAChRs have been particularly implicated in several neurological disorders, such as Parkinson's disease, Alzheimer's disease and schizophrenia (Haydar and Dunlop 2010, Ishikawa and Hashimoto 2011, Quik and McIntosh 2006, Olincy and Stevens 2007). Thus, conotoxins that affect neurotransmission through their action on the nAChRs have impact on the physiological and neurological conditions.

THE MOLECULAR TARGET: NICOTINIC ACETYLCHOLINE RECEPTOR

The nAChR forms ligand-gated ion channel in cell's plasma membrane. The channel is permeable to cations, such as Ca^{2+}, whose entry through the receptor channel leads to the regulation of Ca^{2+}-dependent cellular processes, including neurotransmitter release and synaptic plasticity (Fucile 2004). Based on their primary sites of expression, the nAChRs are broadly classified into muscle type and neuronal type. The muscle type nAChR is located in the neuromuscular junction; stimulation of this receptor leads to muscle contraction. The neuronal type nAChR is found in the nervous system and is involved in neurotransmission and regulation of neuronal activity.

The nAChR (MW~290 kDa) has a pentameric structure composed of five transmembrane subunits (Kalamida et al 2007). Seventeen nAChR subunits, such as α1 to α10, β1 to β4,

Figure 1. Shells of *Conus* species from which some nicotinic acetylcholine receptor-targeted conotoxins were identified. Left, *Conus radiatus*, the radial cone; right, *Conus purpurascens*, the purple cone. Bar = 1 cm.

γ, δ and ε, have been found in vertebrates; these can assemble to produce a diverse family of nAChR subtypes (Millar and Gotti 2009). The multiple subunits provide the basis for the heterogeneity of structures and functions of the nAChR subtypes (Kalamida et al 2007). Two to four different types of subunits often comprise native functional heteromeric nAChR complexes. Most vertebrate nAChR subtypes consist of two α-subunits and three other non-α subunits. The muscle nAChR subtypes consist of two α, one β, one δ, and either one γ or one ε that distinguishes the fetal and adult muscle types (Mishina et al 1986), respectively, for e.g., $(\alpha1)_2\beta1\gamma\delta$ for fetal type and $(\alpha1)_2\beta1\varepsilon\delta$ for adult type. The neuronal nAChR subtypes are generally made up of only of two α and three β subunits or exclusively α subunits (Itier and Bertrand 2001), for e.g., $(\alpha3)_2(\beta4)_3$, $(\alpha4)_2(\beta2)_3$) and $(\alpha7)_5$.

The acetylcholine binding sites of the nAChR are located on the outer surface of the α subunits close to the N termini. Binding of two acetylcholine molecules triggers a conformational change resulting in the opening of a pore (Grosman et al 2000). Opening of the ion channel allows positively charged ions, such as Ca^{+2}, to enter the cell.

DIVERGENT CONOTOXINS THAT TARGET THE NICOTINIC ACETYLCHOLINE RECEPTORS

Conotoxins that bind to the nAChRs exhibit remarkable specificity. Many of these are members of the α-conotoxin family that belongs to the A-superfamily (McIntosh et al 1999, Santos et al 2004). Conotoxins belonging to a superfamily are classified into families on the basis of common cysteine patterns and molecular targets. Divergent families and subfamilies of conotoxins targeted to nAChRs have been identified and grouped according to their cysteine patterns and disulfide-bonding frameworks, and are compared with the broadly distributed and more studied α-conotoxins (Table 1). Based on the classification, different families and subfamilies of nAChR-targeted conotoxins identified in venoms of various *Conus* species are reviewed in this report.

In the conventional nomenclature that is commonly used, the peptide families are designated with the Greek letter α to indicate nAChR as the molecular target, followed by a capital letter indicating the superfamily, except in α-conotoxin and α1-conotoxin which belong to the A-superfamily, and ψ-conotoxin which is under the M-superfamily. The Roman numeral following the one-letter or two-letter symbol for *Conus* species stands for the class assigned to the peptide.

The αA-conotoxin family: αA_L- and αA_S-conotoxin subfamilies

Members of the αA-conotoxin family fall under the A-superfamily, and are competitive antagonists of the muscle type nAChRs. The αA-conotoxin family shares the same cysteine pattern (–CC–C–C–C–C–) (Table 1) with the κA-conotoxin

Table 1. Cysteine patterns and disulfide-bonding frameworks of divergent conotoxin families/subfamilies targeted to nicotinic acetylcholine receptors, compared to those of α-conotoxin.

Family/ Subfamily	Super-family	Class	Cysteine pattern	Disulfide bonding framework	Reference
α-conotoxin	A	I, II	–CC–C–C–	[Cys1–Cys3, Cys2–Cys4]	Gray et al 1984, Cartier et al 1996
αA_L-conotoxin	A	IV	–CC–C–C–C–C–	[Cys1–Cys5, Cys2–Cys3, Cys4–Cys6]	Hopkins et al 1995
αA_S-conotoxin	A	IV	–CC–C–C–C–C–	[Cys1–Cys3, Cys2–Cys5, Cys4–Cys6]	Teichert et al 2004
ψ-conotoxin	M	III	–CC–C–C–CC–	[Cys1–Cys4, Cys2–Cys5, Cys3–Cys6]	Shon et al 1997
αS-conotoxin	S	VIII	–C–C–C–C–C–C–C–C–C–C–	Unknown	Teichert et al 2005a
αC-conotoxin	C	X	–C–C–	[Cys1–Cys2]	Jimenez et al 2007
α1-conotoxin	A	XIV	–C–C–C–C–	[Cys1–Cys3, Cys2–Cys4]	Peng et al 2010
αD-conotoxin	D	XII	–C–CC–C–CC–C–C–C–C–	Unknown	Loughnan et al 2006
καJ-conotoxin*	J	XIV	–C–C–C–C–	[Cys1–Cys3, Cys2–Cys4]	Imperial et al 2006
μαM-conotoxin*	M	III	–CC–C–C–CC–	[Cys1–Cys4, Cys2–Cys5, Cys3–Cys6]	Favreau et al 2012

*Tentative designation of families.

family. The αA-conotoxins are paralytic conotoxins that inhibit nAChRs similar to α-conotoxins (Hopkins et al 1995), while the κA-conotoxins are excitatory toxins that block potassium channels (Craig et al 1998). The αA-conotoxins can be subdivided into two subfamilies: the αA_L-conotoxin (subscript L stands for long chain) and the αA_S-conotoxin (subscript S stands for short chain). Both groups have the class IV cysteine pattern, but they differ in their disulfide-bonding frameworks: [Cys1–Cys5, Cys2–Cys3, Cys4–Cys6] for the αA_L-conotoxin (Hopkins et al 1995), and [Cys1–Cys3, Cys2–Cys5, Cys4–Cys6] for the αA_S-conotoxin (Teichert et al 2004).

The αA_L-conotoxin subfamily includes αA_L-PIVA which was isolated from venom of *C. purpurascens*, and αA_L-EIVA and αA_L-EIVB from venom of *C. ermineus*. Whereas αA_L-PIVA consists of 25 amino acid (AA) residues, both αA_L-EIVA and αA_L-EIVB have 30 AA residues. The three peptides are amidated at the C-terminus, and are notable in the presence of multiple residues of post-translationally modified AA, 4-hydroxyproline (Table 2). In competition binding assay using α-bungarotoxin, αA_L-PIVA blocked muscle type nAChR at α/γ, α/δ interfaces (Hopkins et al 1995). Both αA_L-EIVA and αA_L-EIVB inhibited torpedo and mouse α1-containing muscle type nAChRs expressed in *Xenopus* oocytes; αA-EIVA inhibited α1/γ

Table 2. Amino acid sequences and receptor subtype specificity of divergent conotoxins that target the nicotinic acetylcholine receptors.

Peptide	*Conus* species	Amino acid sequence	Specificity (nAChR type/subunits)	Reference
αA_L-PIVA	*C. purpurascens*	GCCGSYONAACHOCSCKDROSYCGQ*	muscle α/γ, α/δ interfaces	Hopkins et al 1995
αA_L-EIVA	*C. ermineus*	GCCGPYONAACHOCGCKVGROOYCDROSGG*	muscle α1/γ, α1/δ interfaces	Jacobsen et al 1997
αA_L-EIVB	*C. ermineus*	GCCGKYONAACHOCGCTVGROOYCDROSGG*	muscle α1/γ, α1/δ interfaces	Jacobsen et al 1997
αA_S-OIVA	*C. obscurus*	CCGVONAACHOCVCKNTC*	muscle α1β1γδ, α/γ interface	Teichert et al 2004, 2006
αA_S-OIVB	*C. obscurus*	CCGVONAACPOCVCNKTCG*	muscle α1β1γδ, α/γ interface	Teichert et al 2005b, 2006
αA_S-PeIVA	*C. pergrandis*	CCGVONAACHOCVCTGKC	muscleα1β1γδ, α/γ interface	Teichert et al 2006
αA_S-PeIVB	*C. pergrandis*	CCGIONAACHOCVCTGKC	muscleα1β1γδ, α/γ interface	Teichert et al 2006
ψ-PIIIE	*C. purpurascens*	HOOCCLYGKCRRYOGCSSASCCQR*	muscle type	Shon et al 1997
ψ-PIIIF	*C. purpurascens*	GOOCCLYGSCROFOGCYNALCCRK*	muscle type	Van Wagoner et al 2003
ψ-PrIIIA	*C. parius*	AARCCTYHGSCLKEKCRRKYCC*	muscle α1β1εδ > α1β1γδ	Lluisma et al 2008
αS-RVIIIA	*C. radiatus*	KCNFDKCKGTGVYNCGγSCSCγGLHSCRCT YNIGSMKSGCACICTYY	muscle α1β1εδ, α1β1γδ neuronal α7	Teichert et al 2005a
αC-PrXA	*C. parius*	TYGIYDAKPOFSCAGLRGGCVLPONLROKFKE*	muscle α/δ > α/γ interface	Jimenez et al 2007
α1-pu14a	*C. pulicarius*	DCPPHPVPGMHKCVCLKTC	muscle α1β1γδ, α6α3β2 neuronal α3β2	Peng et al 2010
α1-ts14a	*C. tessulatus*	DGCPPHPVPGMHPCMCTNTC	no data	Peng et al 2010
lt14a	*C. litteratus*	MCPPLCKPSCTNC*	unknown subtype	Sun et al 2011
αD-VxXIIA	*C. vexillum*	DVQDCQVSTOGSKWGRCCLNRVCGPMCCPA SHCYCVYHRGRGHGCSC	neuronal α7, β2-containing subtype	Loughnan et al 2006
αD-VxXIIB	*C. vexillum*	DDγSγCIINTRDSPWGRCCRTRMCGSMCCP RNGCTCVYHWRRGHGCSCPG	neuronal α7, β2-containing subtype	Loughnan et al 2006
αD-VxXIIC	*C. vexillum*	DLRQCTRNAPDSTWGRCCLNPMCGNFCCPR SGCTCAYNWRRGIYCSC	neuronal α7, β2-containing subtype	Loughnan et al 2006
αD-Ms	*C. mustelinus*	DVRECQVNTPGSKWGKCCMTRMCGTMCCAR SGCTCVYHWRRGHGCSCPG	neuronal α7, α3β2, α4β2	Kauferstein et al 2009
αD-Cp	*C. capitaneus*	EVQECQVDTPGSSWGKCCMTRMCGTMCCSR SVCTCVYHWRRGHGCSCPG	neuronal α7, α3β2, α4β2	Kauferstein et al 2009

nAChR, nicotinic acetylcholine receptor; *amidated C-terminus; O, 4-hydroxyproline; γ, γ-carboxyglutamate. Cysteine residues are underlined.

and α1/δ interfaces with equally high affinity although with different kinetics of inhibition (Jacobsen et al 1997).

Members of the αA_S-conotoxin subfamily that were characterized are αA_S-OIVA and αAs-OIVB from *C. obscurus*, and αAs-PeIVA and αAs-PeIVA from *C. pergrandis*. The αA_S-OIVA and αAs-OIVB contain 18 and 19 AA residues, respectively, and both are amidated at the C-termini. On the other hand, both αAs-PeIVA and αAs-PeIVB have 18 AA residues with free C-termini; they differ only in a single amino acid at the 4th position (Table 2). Similar to αA_L-PIVA (Hopkins et al 1995), both αA_S-OIVA and αA_S-OIVB caused paralysis in fish (Teichert et al 2004, 2005b). The αA_S-conotoxins are selective antagonists of the fetal muscle type nAChR and compete with acetylcholine for binding only at the α/γ subunit interface. However, they showed different kinetics of inhibition of the receptor, particularly in the dissociation rates (Teichert et al 2005b, 2006).

The ψ-conotoxin family

The ψ-conotoxins under the M superfamily have the cysteine pattern –CC–C–C–CC– and disulfide framework [Cys1–Cys4, Cys2–Cys5, Cys3–Cys6] (Table 1). Structurally, the ψ-conotoxins are most closely related to the class III M-superfamily conotoxins, namely μ-conotoxins that inhibit Na^+ channels (Cruz et al 1985) and κM-conotoxins that inhibit K^+ channels (Ferber et al 2003). Unlike most other nAChR antagonists, the ψ-conotoxins are noncompetitive antagonists of the muscle type nAChRs (Shon et al 1997).

The ψ-conotoxin family includes ψ-PIIIE and ψ-PIIIF which were isolated from venom of *C. purpurascens*. Both peptides have 24 AA residues with amidated C-termini, and are rich in post-translationally modified AA, 4-hydroxyprolines (Table 2). The ψ-PIIIE did not inhibit the binding of α-bungarotoxin, a competitive antagonist of nAChR, indicating that it blocked the receptor at a site other than the acetylcholine binding site (Shon et al 1997). Both ψ-PIIIE and ψ-PIIIF caused flaccid paralysis in goldfish; they inhibited mouse and torpedo nAChRs, although ψ-PIIIF was much less potent (Van Wagoner et al 2003).

The amino acid sequence of ψ-PrIIIE was inferred from cDNA clone from fish-hunting *C. parius*. The ψ-PrIIIE consists of 22 AA residues, about one-fourth of which are charged AA (Table 2). The peptide caused flaccid paralysis in fish. Electrophysiological assays using mouse nAChRs expressed in *Xenopus* oocytes showed that ψ-PrIIIE was about 30-fold more potent than ψ-PIIIE, and was more potent in the adult muscle type (α1β1εδ) than the fetal muscle type (α1β1γδ) nAChR (Lluisma et al 2008).

The αS-conotoxin family

The αS-conotoxin family under the S-superfamily has the class VIII cysteine pattern –C–C–C–C–C–C–C–C–C–C– (Table 1). Earlier, only one member of the S-superfamily conotoxin was reported; this is the σ-conotoxin GVIIIA which is a specific competitive antagonist of 5-HT_3 serotonin receptor (England et al 1998).

The αS-RVIIIA purified from venom of fish-hunting *C. radiatus* has 47 AA, with two residues of the post-translationally modified AA, γ-carboxyglutamate (Table 2). The conotoxin caused paralysis in fish and mice, and also seizure in mice. The αS-RVIIIA blocked both muscle α1β1εδ and α1β1γδ nAChRs, as well as neuronal α7 nAChR (Teichert et al 2005a). In venom of *C. radiatus*, only the αS-RVIIIA has so far been found to inhibit the nAChRs, despite the presence of α-conotoxin like peptides. Thus, the αS-conotoxin may be used by *C. radiatus* as its major antagonist of nAChRs (Teichert et al 2005a).

The αC-conotoxin family

The αC-conotoxin that belongs to the C-superfamily is in class X with the cysteine pattern –C–C– forming a single disulfide bond (Table 1). The αC-PrXA purified from venom of fish-hunting *C. parius* contains 32 AA residues (Table 2). It is very similar in its biochemical features to waglerin-I which was isolated from venom of Wagler's pit viper (*Trimeresurus wagleri*) (Weinstein et al 1991). The conotoxin caused paralysis

Table 3. Amino acid sequences and multiple molecular targets of divergent conotoxins.

Peptide	*Conus* species	Amino acid sequence	Molecular targets	Reference
pl14a	*C. planorbis*	FPRPRICNLACRAGIGHKYPFCHCR*	neuromuscular nAChR α1β1εδ, neuronal nAChR α3β4, potassium channel Kv1.6	Imperial et al 2006
μ-CnIIIC	*C. consors*	ZGCCNGPKGCSSKWCRDHARCC*	neuronal nAChR α3β2 > α7, α4β2, sodium channels Nav1.2, NaV1.4	Favreau et al 2012

nAChR, nicotinic acetylcholine receptor; *amidated C-terminus; O, 4-hydroxyproline; Z, pyroglutamate.
Cysteine residues are underlined.

in mice and fish. It blocked the muscle type nAChR and competed with α-bungarotoxin for binding at the α/γ and α/δ subunit interfaces, with higher affinity for the α/δ interface (Jimenez et al 2007).

The α1-conotoxin family

The α1-conotoxin family with the –C–C–C–C– cysteine pattern and disulfide framework [C1–C3, C2–C4], belongs to class XIV (Table 1). Conotoxin pu14a was identified by cDNA cloning from worm-hunting *C. pulicarius*, while Ts14a was purified from venom of worm-hunting *C. tessulatus* (Peng et al 2010). The signal sequence of Pu14.1 is homologous to that of α-conotoxins, and the inferred mature peptide, α1-pu14a, has high sequence similarity to α1-ts14a. The α1-pu14a and α1-ts14a have 19 and 20 residues, respectively, without any post-translationally modified AA residue aside from disulfide-bonded cysteines (Table 2). The α1-pu14a induced sleep in mice and caused seizure and paralysis in fish. With the *Xenopus* oocyte heterologous expression system, α1-pu14a blocked the rat neuronal α3β2 and the mouse muscle α1β1γδ nAChR, with fast dissociation rate (Peng et al 2010).

Conotoxin α1-lt14a isolated from venom of *C. litteratus* has 13 AA residues with an amidated C-terminus (Table 2). This peptide could inhibit nAChR and control pain (Sun et al 2011).

The αD-conotoxin family

The αD-conotoxins of the D-superfamily have the cysteine pattern –C–CC–C–CC–C–C–C–C– and are in class XII (Table 1). The αD-VxXIIA, αD-VxXIIB and αD-VxXIIC were purified from venom of *C. vexillum*. These conotoxins are dimers of paired peptides with 47 to 50 AA residues (about 11kDa) (Table 2). In binding assays and two-electrode voltage clamp analyses, the conotoxins potently and selectively inhibited the neuronal nAChR subtypes containing α7 and β2 subunits (Loughnan et al 2006).

The αD-Ms and αD-Cp isolated from the venom of *C. mustelinus* and *C. capitaneus*, respectively, have high sequence homology to αD-VxXIIA, αD-VxXII-B and αD-VxXII-C, and are also dimers of similar or identical peptides consisting of 49 AA residues (Table 2). These conotoxins specifically block neuronal nAChRs with α7, α3β2 and α4β2 subtypes (Kauferstein et al 2009).

Conotoxin families with multiple target receptors including nAChRs

A family of conotoxins belonging to J-superfamily, class XIV, has the cysteine pattern –C–C–C–C– and disulfide-bonding framework [C1–C3, C2–C4] (Table 1). Conotoxin pl14a from *C. planorbis* contains 25 AA residues with elongated six-residue N-terminal tail and amidated C-terminus (Table 2). The peptide elicited in mice excitatory symptoms leading to seizure. With the oocyte heterologous expression system, pl14a blocked the potassium channel Kv1.6 subtype, as well as the neuronal α3β4 and muscle α1β1εδ nAChR subtypes (Imperial et al 2006). The family of this conotoxin is tentatively referred to as καJ, provided that more members with the same activities will be identified. Three other peptides from *C. planorbis* and two peptides from *C. ferrugineus* under the J superfamily were identified by cDNA cloning (Imperial et al 2006), but whether they have similar targets as pl14a remains to be tested.

The μ-CnIIIC isolated from *C. consors* has class III cysteine pattern –CC–C–C–CC– and disulfide framework [Cys1–Cys4, Cys2–Cys5, Cys3–Cys6] (Table 1) similar to the M-conotoxin superfamily (Corpuz et al 2005) and the ψ-conotoxin family (Shon et al 1997). The μ-CnIIIC consists of 22 AA residues with post-translationally modified AA, pyroglutamate, at the N-terminus and amidated C-terminus (Table 3). This conotoxin inhibited both the sodium channel Nav1.2 and Nav1.4 subtypes, as well as the neuronal type α3β2 more than the α7 and α4β2 nAChRs (Favreau et al 2012). The family of this conotoxin is tentatively referred to as μαM, provided that more members with the same activities will be identified later.

The occurrence of conotoxins with multiple molecular targets is consistent with the evolutionary tactics of cone snails to be able to readily paralyze their prey, predators or competitors. However, from the point of view of drug development, this strategy would be a deviation from high specificity that cone snail peptides are known with respect to the molecular target subtypes.

THE nAChRs AS TARGETS AND SUBTYPE-SPECIFIC CONOTOXINS AS POTENTIAL THERAPEUTIC AGENTS

In addition to their use as tools for probing nAChRs, several conopeptides have been shown to have important therapeutic potential (Olivera 2006). The localization of distinct nAChR subtypes in specific regions of the central nervous system indicates the possibility of developing drugs for neurological disorders involving the affected areas. For instance, the therapeutic applications of α-conotoxins that target nAChR subtypes containing the α6 and α9 subunits are being developed.

The α-MII is used as ligand for the identification of α6-containing nAChR subtypes that are expressed in dopaminergic nerve terminals (Grady et al 2007). The α6-containing nAChRs are localized in the nigrostriatal area; thus, they represent targets for certain neurological disorders, such as Parkinson's disease, which is characterized by progressive degeneration of the nigrostriatal system (Olivera et al 2008, Quik and McIntosh 2006).

The discovery of α-RgIA from venom of *C. regius* led to the identification of a function for α9-containing nAChRs (Ellison et al 2006). The α-conotoxins that target α9-containing nAChR subtypes have potential use as analgesics for the treatment of neuropathic pain caused by nerve injury. Inhibition of α9α10 nAChR can relieve chronic pain resulting from peripheral nerve injury or inflammation and speed up the recovery of injured neurons (Vincler et al 2006). For instance, the α9α10 nAChR antagonist Vc1.1 showed potent analgesic effect in preclinical animal models of human neuropathic pain, and seemed to hasten the recovery of damaged neurons (Satkunanathan et al 2005). Conotoxin Vc1.1 (ACV-1) has reached Phase II clinical trials as treatment for neuropathic pain and is intended as a cure for diabetic neuropathy, sciatica and shingles (Livett et al 2006).

The nAChRs play a role in neurotransmission and neurotransmitter release, but in neurological conditions they are often involved in the pathology of certain diseases. The diversity and distribution of nAChR subtypes that are the targets of numerous conotoxins present an opportunity for the discovery of new drugs. Specifically, studies have suggested that the neuronal α7 and α4β2 nAChR subtypes are implicated in the pathology of schizophrenia and Alzheimer's disease (Haydar and Dunlop 2010, Ishikawa and Hashimoto 2011, Olincy and Stevens 2007). Thus, these nAChR subtypes could also be investigated as potential therapeutic targets. Inasmuch as the neuropharmacological applications of several α-conotoxins have been documented, the divergent conotoxins that target other nAChR subunits/subtypes could also be explored for their therapeutic potential

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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