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Review

α-Conotoxins

Hugo R. Arias *, Michael P. Blanton

Departments of Pharmacology and Anesthesiology, School of Medicine, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA

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Abstract

α-Conotoxins (α-CgTxs) are a family of Cys-enriched peptides found in several marine snails from the genus *Conus*. These small peptides behave pharmacologically as competitive antagonists of the nicotinic acetylcholine receptor (AChR). The data indicate that (1) α-CgTxs are able to discriminate between muscle- and neuronal-type AChRs and even among distinct AChR subtypes; (2) the binding sites for α-CgTxs are located, like other cholinergic ligands, at the interface of α and non-α subunits (γ, δ, and ε for the muscle-type AChR, and β for several neuronal-type AChRs); (3) some α-CgTxs differentiate the high- from the low-affinity binding site found on either α/non-α subunit interface; and that (4) specific residues in the cholinergic binding site are energetically coupled with their corresponding pairs in the toxin stabilizing the α-CgTx-AChR complex. The α-CgTxs have proven to be excellent probes for studying the structure and function of the AChR family.

Keywords: α-Conotoxins; Competitive antagonists; Nicotinic acetylcholine receptors

Contents

Abbreviations: AChR, nicotinic acetylcholine receptor; 5-HT, 5-hydroxytryptamine; 5-HT_3R, 5-hydroxytryptamine type 3 receptor; NMDA, *N*-methyl-D-aspartate; α-BTx, α-bungarotoxin; α-CgTx, α-conotoxin; αA-CgTx, αA-conotoxin; μ-CgTx, μ-conotoxin; ω-CgTx, ω-conotoxin; κ-CgTx, κ-conotoxin; δ-CgTx, δ-conotoxin; μO-CgTx, μO-conotoxin; σ-CgTx, σ-conotoxin.

* Corresponding author. Tel.: +1-806-7432425, ext.: 244; fax: +1-806-7432744.

E-mail address: phrhra@ttuhsc.edu (H.R. Arias).

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1. Introduction

From an evolutionary point of view, snails from the genus *Conus* are among the most successful marine invertebrates (reviewed in [1]). The genus *Conus* is formed by over 500 venomous species. These marine mollusks prey on other marine species by means of a very large number of small peptides (10–50 amino acids in length) with specific pharmacological activities. Due to the remarkably fast interspecific divergence of peptide sequences, each *Conus* species has a repertoire of 50–200 different peptides (reviewed in [2]). These peptides, called conotoxins, affect the functioning of different voltage-gated ion channels and neurotransmitter-gated receptors (reviewed in [3,4]). Based on the molecular form, the approximately 50 000 conotoxins can be grouped into a minimum of seven superfamilies; the A- [e.g. α-conotoxins (α-CgTxs), αA-, and κA-CgTxs], M- (e.g. μ- and ψ-CgTxs), O- (e.g. ω-, κ-, δ-, and μO-CgTxs), P- (e.g. Spasmodic peptide; [5]), R-, S- (e.g. σ-CgTxs), and T-superfamilies (e.g. tx5a, p5a, au5a, and au5b; [6]) (reviewed in [4,7]). This review will focus on the structure and pharmacological activity of only the conotoxin family (e.g. α-CgTx and αA-CgTxs) which specifically bind to several nicotinic acetylcholine receptors (AChRs) (reviewed in [4,8]).

The AChR is the prototype of a superfamily of ion channel-coupled receptors which are gated by specific neurotransmitters. This superfamily also includes the type A γ-aminobutyric acid, glycine, and type 3 5-hydroxytryptamine (5-HT) receptors (reviewed in [8,9]). There are two main AChR types, the muscle- and neuronal-type. The muscle-type AChR is a pentamer comprised of two α1 subunits, one β1, one δ and, one γ or ε subunit, depending on whether the receptor is in an embryonic or adult stage, respectively. The α1 subunits contain two adjacent cysteines at position 192 and 193 (sequence number from *Torpedo* AChR), which are involved in the recognition and binding of cholinergic agonists and competitive antagonists. Based on the presence or the absence of these two cysteines, the neuronal-type AChR subunit classes are designated α (when they contain both cysteines) or β (when they do not). To date, eight α subunits (α2–α9) and three β subunits (β2–β4) have been identified. The α7, α8, and α9 polypeptides are the only subunits capable of forming homo-oligomeric ion channels. Interestingly, the two α subunits display non-equivalence for the binding of several cholinergic agonists and competitive antagonists in both muscle- and neuronal-type AChRs. In this regard, α-CgTxs have become one of the most powerful tools to support this non-equivalence (reviewed in [8,9]).

2. Structure

The initial purification and chemical characterization of an α-CgTx was performed by Olivera and co-workers in 1981 [10]. Since then, a great deal of structural information has been obtained. Most of them exhibit four Cys residues in the following conserved arrangements; CCX_3CX_5C (the $\alpha_{3/5}$ subfamily), CCX_4CX_3C (the $\alpha_{4/3}$ subfamily), and CCX_4CX_7C (the $\alpha_{4/7}$ subfamily). Each alternate Cys pair forms a disulfide loop (i.e. loops I and II, respectively). In each case, the X represents the number of amino acids between the Cys residues. The only representative of the $\alpha_{3/5/3}$ subfamily, which has three Cys bonds, is α-CgTx SII. The spacing between disulfide bonds is an important determinant of backbone structure. Additionally, a conserved Pro residue is found

between the second and the third Cys in almost all α-CgTxs that have been characterized to date. The exception is the αA-CgTx subfamily (e.g. αA-PIVA, αA-EIVA, and αA-EIVB), which has a core sequence that is very different than the other subfamilies [11–13]. Table 1 shows the primary and secondary structural features of some α- and αA-CgTxs as examples of each subfamily.

The three-dimensional structures of several α-CgTxs have been determined using either X-ray

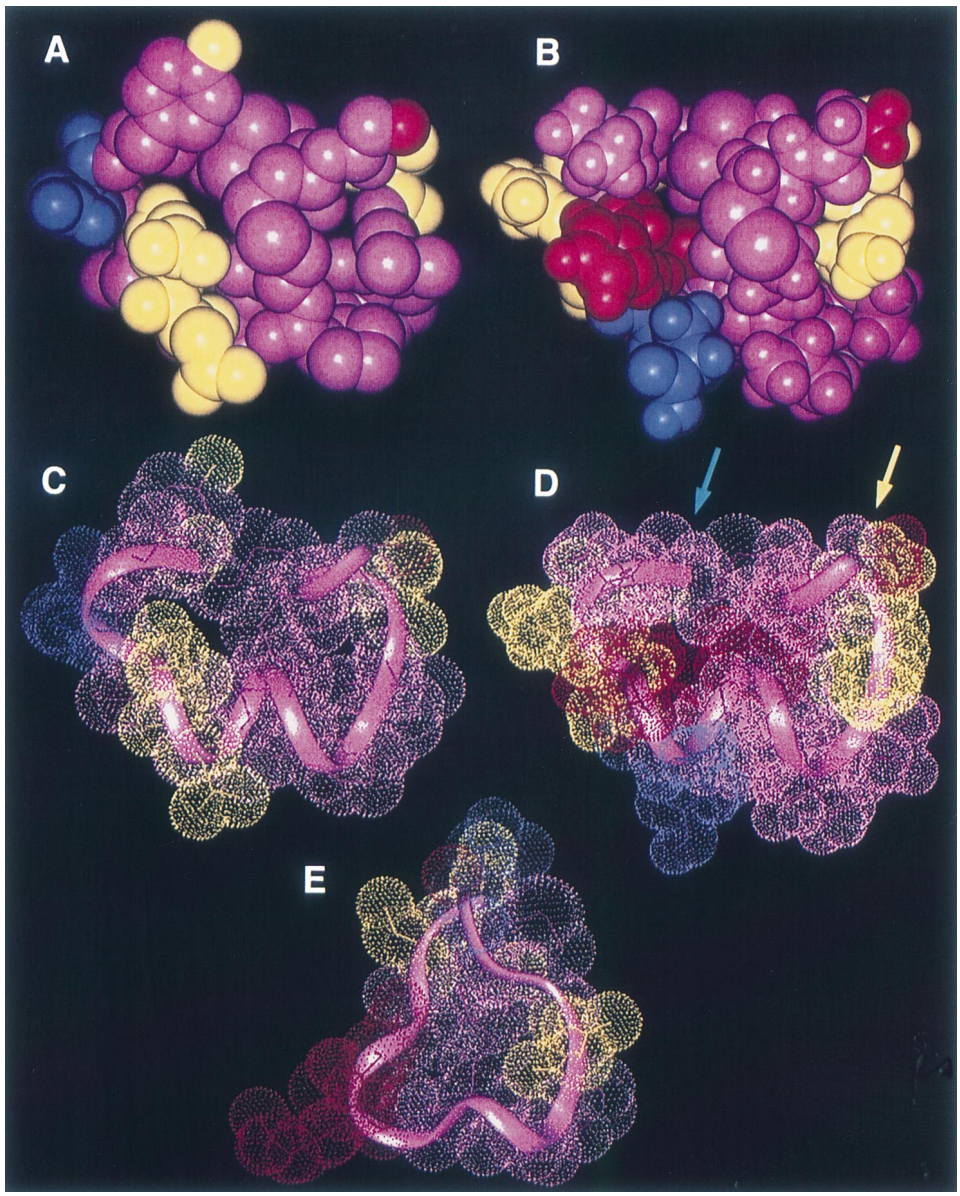

Fig. 1. Surface models of α-CgTxs PnIA (A) and MII (B) and backbone representations of α-CgTxs PnIA (C), MII (D), and GI (E) (taken from [16], with permission). Hydrophobic, polar, positive-charged, and negative-charged residues are displayed in purple, yellow, red, and blue, respectively. Backbone conformations of α-CgTxs PnIA (C), MII (D), and GI (E) are shown in ribbons. Surface distributions in dots with the same color codes. Arrows indicate carboxyl (blue) and amino (yellow) terminal residues.

crystallography or NMR spectroscopy [11,14–26]. These studies provide support for the idea that these small polypeptides achieve their conformational stability by means of disulfide bonding. Although α-CgTxs are apparently very rigid structures, two or more interconvertible conformers may exist in solution (reviewed in [4]). The overall shapes of the different α-CgTx subfamilies are as follow: the $\alpha_{4/7}$ subfamily is more rectangular, the $\alpha_{3/5}$ subfamily is more triangular, whereas the αA subfamily has been described as an iron. The structural comparison of several α-CgTxs specific for neuronal-type AChRs, such as α-CgTx ImI, PnIA, PnIB, MII, and EpI (Table 3), exhibit remarkable similarity in local backbone conformations and relative solvent-accessible surface areas [21]. A model of the tertiary structure of α-CgTxs PnIA, MII, and GI is shown in Fig. 1 (taken from [16]). The backbones of the α-CgTxs PnIA and MII structures (panels C and D, respectively) are very similar. Nevertheless, the surfaces of both the polypeptides are unique. The protruding Tyr residue on the surface of the α-CgTx PnIA structure (panel C, in purple) contrasts with the flat hydrophobic surface of α-CgTx MII (panel D, residues in purple). Furthermore, charged residues are exposed on the α-CgTx MII surface (panel D, negative residues in blue and positive residues in red), whereas they are not exposed on the α-CgTx PnIA surface (panel C). In addition, both the backbone and the surface of α-CgTx GI (panel E), which is specific for muscle-type AChRs (see Table 2), diverge significantly from the other PnIA and MII α-CgTxs.

3. Synthesis

The *Conus* venom is biosynthesized and stored in the venom duct of the venom apparatus (reviewed in [27]). When the snail is hunting prey, the venom, by contraction of the muscular venom bulb, is delivered through a harpoon-like radula tooth located in the proboscis.

Table 1
Primary and secondary structure of each conotoxin subfamily

α-Conotoxin	*Conus* species	Subfamily	Primary[a] and secondary[b] structure	References
MI	*Conus magus*	$\alpha_{3/5}$	GRCCHPACGKNYSC[c]	[30]
PnIA	*Conus pennaceus*	$\alpha_{4/7}$	GCCSLPPCAANNPDY[d]C[c]	[55]
ImI	*Conus imperialis*	$\alpha_{4/3}$	GCCSDPRCAWRC[c]	[56]
SII	*Conus striatus*	$\alpha_{3/5/3}$	GCCCNPACGPNYGCCGTSCS	[57]
αA-PIVA	*Conus purpurascens*	αA	GCCGSYONAACHOCSCKDROSYCG[c]	[13]

[a] The sequence alignment of the different α-CgTxs is shown in the standard one-letter amino acid code. The letter O represents *trans*-4-hydroxyproline.

[b] α-Conotoxin subfamilies $\alpha_{3/5}$, $\alpha_{4/7}$, and $\alpha_{4/3}$ are formed by two disulfide bonds, while the αA-CgTx subfamily and α-CgTx SII ($\alpha_{3/5/3}$ subfamily) have three disulfide bonds.

[c] Amidated COOH-terminus. Instead, α-CgTx SII presents a free COOH-terminus.

[d] Sulfated residue.

Table 2
α-Conotoxin specificity for different muscle-type nicotinic acetylcholine receptors

α-Conotoxin	AChR Source	IC_{50}[a] (subunit interface)		References
		High-affinity binding site NM	Low-affinity binding site μM	
MI	BC_3H-1	1.50 ± 0.24 (αδ)	22.0 ± 1.1 (αγ)	[30]
		0.42 ± 0.15 (αδ)	23.0 ± 4.1 (αγ)	[31]
		0.40 ± 0.01[b] ($\alpha_2\beta\gamma\delta$)		[12]
		0.40 ± 0.17[b] (αβδ)	18 ± 5 (αβγ)	[12]
		1.4 ± 0.1[b] (kinetic)		[12]
	Mouse	0.55 ± 0.06 ($\alpha_2\beta\gamma\delta$)	18.3 ± 0.93 ($\alpha_2\beta\gamma\delta$)	[42]
		1.34 (αβδ)	11.7 (αβγ)	[42]
		5.3 ($\alpha_2\beta\delta$)	13 ± 1.3 ($\alpha_2\beta\gamma\delta$)	[39]
		1.9 (αδ)	15 (αγ)	[32]
		12[b] (αδ)		[58]
		2.8 ± 1.3 (αδ)	7.8 ± 1.3 (αγ)	[33]
	Torpedo	2.6 ± 1.0 (αγ)	2.3 ± 0.7 (αδ)	[30]
		120 ± 10 (αγ)	21 ± 1 (αδ)	[45]
		4.50 ± 1.60 (αγ)	0.48 ± 0.10 (αδ)	[31]
		1.6 ± 1.4 ($\alpha_2\beta\gamma\delta$)	1.0 ± 0.3 ($\alpha_2\beta\gamma\delta$)	[40]
		32 ± 2 (αβγ)	1.2 ± 0.1 (αβδ)	[40]
	Human ($\alpha_2\beta\varepsilon\delta$)	9 ± 1 (αδ)	0.09 ± 0.01 (αε)	[48]
GI	BC_3H-1	4.9 ± 1.9 (αδ)	58 ± 12 (αγ)	[30]
		1.3 ± 0.3 (αδ)	60 ± 1 (αγ)	[43]
	Mouse	20[b] (αδ)		[58]
	Torpedo	360 ± 40 (αγ)	24 ± 2 (αδ)	[45]
		4.5 ± 1.3 (αγ)	0.09 ± 0.02 (αδ)	[43]
		41.3 ± 8.2[c] (αγ)		[59]
	Human ($\alpha_2\beta\varepsilon\delta$)	2 ± 1 (αδ)	0.14 ± 0.01 (αε)	[48]
	Frog		2-4[b]	[56]
	Fish (*Eigenmannia*)	50-100[b]		[56]
CnIA	*Torpedo*	190		[19]
SI	BC_3H-1	680 ± 160 (αδ)	220 ± 45 (αγ)	[30]
		1300 ± 430 (αδ)	290 ± 110 (αγ)	[43]
	Human ($\alpha_2\beta\varepsilon\delta$)	80 ± 14 (αε)	8.31 ± 1.2 (αδ)	[48]
	Torpedo		0.17 ± 0.04 (αγ or αδ)	[44]
			16 ± 1 (αγ or αδ)	[45]
			1	[57]
SIA	BC_3H-1	7.70 ± 0.14 (αδ)	200 ± 8.6 (αγ)	[30]
	Torpedo	590 ± 40 (αγ)	>260 (αδ)	[45]
		420		
EI	BC_3H-1	9.40 ± 1.20 (αδ)	0.28 ± 0.03 (αγ)	[31]
	Torpedo	0.41 ± 0.09 (αδ)	0.19 ± 0.02 (αγ)	[31]
SII	BC_3H-1		18 ± 6.6	[30]
	Torpedo		8	[57]
αA-EIVA	*Torpedo*	17[b]		[12]
	Mouse	11[b] ($\alpha_2\beta\gamma\delta$)		[12]
		11[b] (αβγ)		
		32[b] (kinetic)		
		15[b] (αβδ)		
		37[b] (kinetic)		
	BC_3H-1	150 ± 10		[12]
αA-EIVB	*Torpedo*	18[b]		[12]
αA-PIVA	*Torpedo*		~1	[13]

[a] These values were obtained by inhibition of [^{125}I]α-BTx binding except those determined by the following: [b] electrophysiology or [c] fluorescence spectroscopy.

Table 3
α-Conotoxin specificity for different neuronal-type nicotinic acetylcholine receptors

α-Conotoxin	AChR Subtype	IC_{50}[a] nM	References
CnIA	α7	14 800 [b]	[19]
MII	α3β2	3.5	[46]
		8.0 ± 1.1	[36]
		24.3 ± 2.9[c] (synaptosomes)	[36]
		17.3 ± 0.1[c] (slices)	[36]
	α4β2	~400	[46]
	α3β4	3000[d] (noradrenaline)	[62]
EpI	α3β4	84 ± 19[d] (adrenaline)	[54]
		210 ± 30[d] (noradrenaline)	[54]
	α3β2/α3β4	1.6	[54]
PnIA	α7/?	14	[50]
	α7	252	[51]
		176 (K_d)	[51]
	α7 (human)/5-HT_3R (rat)	$61\,200 \pm 1\,100$[b]	[53]
	α3β2	9.56	[51]
	α3β4	21 000–28 000[d] (noradrenaline)	[52]
AuIB	α3β4	500 ± 140 (K_d)	[37]
		750	[37]
		20 000[d] (noradrenaline)	[52]
	α7	>7000	[37]
PnIB	α7/?	33	[50]
	α7	61.3	[51]
		84.9 (K_d)	[51]
	α7 (human)/5-HT_3R (rat)	$29\,600 \pm 600$[b]	[53]
	α3β2	1970	[51]
	α3β4	700[b]	[30]
		700[d] (noradrenaline)	[52]
		1000[d] (adrenaline)	[52]
ImI	α7 (rat)	220	[58]
	α7	100	[60]
		300[d]	
	α7	86.2 ± 1.2	[61]
	α7 (human)	2450 ± 100[b]	[48]
	α9	1800	[58]
	α3β4/α3β4α5	2500 ± 1200[e]	[34]
	α3β4	>3000	[60]
	Aplysia	47 (desensitizing Cl^- response)	[35]
		>20 000 (sustained Cl^- response)	
		150 ± 22 (cationic response)	

[a] These values were obtained by electrophysiological techniques except those determined by the following: [b] inhibition of [^{125}I]α-BTx binding, inhibition of agonist–induced, [c] dopamine, [d] catecholamine (e.g. adrenaline or noradrenaline), [e] 5-HT release.

Cone snails generate novel polypeptide sequences by amino acid hypermutation (reviewed in [2,7]). The synthesis of conotoxins can be compared with a combinatorial library search strategy. From studies with the O-superfamily, conotoxins have been considered to be initially translated as larger prepropeptide precursors 70–120 amino acids in length with a single copy of the toxin present at the C-terminal end. Other neuropeptide precursors encode either multiple

copies of a specific peptide or several distinct toxins. The high number of polypeptide structures observed in different cone snails that inhibit a specific target are thought to have evolved by hypermutation of the amino acids located closer to the C-terminal. Exceptions are the Cys residues located in the toxin proper. The rest of the precursor sequence remains highly conserved. The signal sequence is the polypeptide region with the highest level of sequence conservation. Between the signal sequence and the mature toxin there exists an intervening pro-region ~ 40 amino acids in length which exhibits a low mutation rate.

4. Biological function

The pioneering studies done in Dr Baldomero Olivera's laboratory provide the initial methodology for determining the biological activity of α-CgTxs. Initial pharmacological data demonstrated that, in general, the family of α-CgTxs behave as competitive antagonists of the AChR (reviewed in [7,8]). The α-CgTxs compete for the binding sites of acetylcholine and cholinergic agonists. However, a distinct conotoxin from *Conus purpurascens*, called ψ-CgTx PIIIE, inhibits the functional activity of the AChR in a non-competitive manner [28]. The earliest studies showed that α-CgTxs inhibited muscle-type AChRs. Table 2 shows the α-CTx specificities for muscle-type AChRs from different species. Nonequivalent binding of some α-CgTxs at the two agonist/competitive antagonist binding domains in *Torpedo* AChR [29–31] is also summarized in Table 1. Although early studies focused on α-CgTxs action in muscle-type AChRs, more recent efforts have identified pharmacological effects of these compounds in neuronal-type AChRs. Table 3 shows the α-CgTx specificities for different neuronal-type AChRs.

In order to determine which subunits of the *Torpedo* AChR are involved in the α-CgTx binding site, purified α-CgTx MI was crosslinked to the AChR with bivalent succinimide reagents of different lengths [29]. With a 12-atom crosslinker, all the four subunits were labeled, whereas a 4-atom crosslinker labeled the β and γ subunits. In addition, two azidosalicylate α-CgTx GIA derivatives were used for photoaffinity labeling of the AChR [29]. These studies showed that depending on the α-CgTx derivative used, the specifically labeled AChR subunits were β and γ, or δ and γ. However, labeling of detergent-solubilized AChR was exclusive for residues 121 and 183 of the γ subunit. The *p*-benzoylphenylalanine derivative of α-CgTx GI also labeled the α subunits [38].

In order to identify the determinants of α-CgTx MI selectivity, Dr Steven Sine's laboratory used subunit chimeras and site-directed mutagenesis [33,39]. From these studies, it was found that the high affinity of subtype MI for the αδ subunit interface of mammalian AChRs is determined by amino acids S^{36}, Y^{113}, and I^{178} from the δ subunit, while the low affinity for the αγ interface is determined by residues K^{34}, S^{111}, Y^{117}, L^{119}, and F^{172} of the γ subunit. Since δY^{113} and γS^{111} are exchanged for Arg and Tyr in the *Torpedo* AChR, these two natural differences may account for the observed site-specificity between the two species. This idea is corroborated by the fact that the mutation δR113Y in the $\alpha_2\beta\delta_2$ or the mutation γY111R in $\alpha_2\beta\gamma_2$ *Torpedo* AChR results either in an enhancement of or a decrease in α-CgTx MI affinity, respectively [40]. The pairs $\gamma K^{34}/\delta S^{36}$ and $\gamma F^{172}/\delta I^{178}$, as primary determinants for α-CgTx MI selectivity in mouse AChR, coincide with that for the selectivity of carbamylcholine [41]. In contrast, neither the γS111Y nor δY113S mutation affected carbamylcholine affinity, suggesting that agonists and at least the α-CgTx MI subtype do not have identical selectivity determinants. Residues γK^{34}, γS^{111}, and γF^{172} contribute to loops D, F, and G, respectively, of the agonist/competitive antagonist binding site (reviewed in [8]). Since other residues from the α subunit are considered to be involved in the agonist/competitive antagonist binding sites (reviewed in [8,9]), Sugiyama et al. [42] examined the contribution of some of these residues to the binding of α-CgTx MI. Mutations αY190F and αY189F do not affect α-CgTx MI affinity, whereas removal of aromaticity by exchanging these residues for Thr has a marked influence on α-CgTx association. This suggests that aromaticity may be required to stabilize the cationic peptide. The effect elicited by substitutions of Y^{93} (loop A; see [8]) and D^{152} residue (loop B; see [8]) indicate that both peptide and nonpeptidic ligands bind to the same site in

the AChR, but unique though overlapping sets of amino acids contribute to the binding domain. In addition, substitution of Y12A on the MI toxin dramatically reduces its affinity for the high-affinity site (αδ) with little effect on toxin potency at the low-affinity site (αγ) [43]. This and additional data suggest that the orientation of residue Y^{12} is important in the formation of the α-CgTx MI-AChR complex.

In order to determine the existence of a linkage relationship between mutations in α and δ/γ subunits, a mutant cycle analysis was employed [42]. From this study, a high coupling energy between S^{36} and I^{178} of the δ subunit was demonstrated. In contrast, a relatively low linkage between residues $\alpha Y^{93}/V^{188}$ and pairs $\gamma K^{34}/\delta S^{36}$, $\gamma S^{111}/\delta Y^{113}$, and $\gamma F^{172}/\delta I^{178}$ is evident. Taking into account that the energetic contributions of amino acids in the α chain to α-CgTx MI association with the AChR seem to be independent from the ones at the δ/γ subunits, it is postulated that one of the surfaces of the neurotoxin molecule interacts with the α subunit, whereas the other surface interacts with the δ or the γ subunit. In this regard, recent conformational sudies using [H^1]-NMR spectroscopy suggest that both the faces of the α-CgTx GI are involved in the orientation of the molecule within the αδ subunit interface [25]. The binding face of α-CgTx GI, a toxin closely related to the MI subtype in structure, interacts by means of residues C^2, N^4, P^5, A^6, and C^7 (from loop I) with the α1 subunit, whereas the selectivity face comprising amino acids R^9 and H^{10} (from loop II) is oriented towards the δ subunit (the subunit forming the high-affinity α-CgTx GI locus in mammalian AChRs). Residues R^9 and H^{10} were found to be responsible for the high differential selectivity and affinity between both the cholinergic ligand binding sites [44,45]. The lack of effect of the mutation P^9 to the neutral residue Ala in the α-CgTx SI suggests that the cationic group of A^9 in the α-CgTx GI plays a major role in αγ selectivity in the *Torpedo* receptor. The critical difference between α-CgTx GI and SI has been ascribed to position 9 (reviewed in [24]). The importance of a cationic group for high selectivity is further substantiated by the fact that mutations on the α-CgTx MI at position K^{10}, the homologous residue of A^9, resulted in a loss of selectivity [45].

Taking into account that neuronal receptors containing the subunit composition α4β2, α2β2, or α3β4 are more than 200-fold less sensitive to α-CgTx MII than α3β2 AChRs, the determinants of α-CgTx selectivity were identified using chimeric subunits and subunits with single residue substitutions [46]. Residues $\beta 2T^{59}$, $\alpha 3K^{185}$, and $\alpha 3I^{188}$ were identified as specific determinants for α-CgTx MII sensitivity. The amino acid $\alpha 3K^{185}$ may electrostatically interact with E^{11} from α-CgTx MII.

Regarding α-CgTx ImI specificity, the pairs $\alpha 7W^{55}/\alpha 1R^{55}$, $\alpha 7S^{59}/\alpha 1Q^{59}$, and $\alpha 7T^{77}/\alpha 1K^{77}$ have been considered as components conferring high affinity binding to α7/5-HT_3R compared with α1/5-HT_3R homooligomeric chimeras [47] (reviewed in [8]). The third pair ($\alpha 7T^{77}/\alpha 1K^{77}$) may be considered as a new loop or an allosterically coupled loop. Experiments performed in parallel show that two regions in the α-CgTx ImI molecule are essential for binding to the α7/5-HT_3R chimera [48]: a region comprising residues D^5-P^6-R^7 in the first loop and a second region in loop II formed by W^{10}. The fact that D^5 functions as an N-terminal cap and P^6 as a helix-initiator suggests that the contribution of both the amino acids to binding may be due to their structural roles rather than due to direct interaction with the AChR [21]. The structural role of P^6 was recently corroborated by mutagenesis studies [49]. Subsequent thermodynamic mutant cycle analyses demonstrated the existence of a dominant pairwise interaction between α-CgTx ImI R^7 and $\alpha 7Y^{195}$ (located in loop C; reviewed in [8]), and multiple weak interactions between α-CgTx ImI D^5 and W^{149}, Y^{151}, and G^{153} of α7 (which are all located in loop B; reviewed in [8]), and between α-CgTx ImI W^{10} and $\alpha 7T^{77}$ (which is probably located in a new loop) and $\alpha 7N^{111}$ (located in loop F; reviewed in [8]) [49].

Although α-CgTxs PnIA and PnIB differ in only two amino acids (A10L and N11S, which are located on the helix face exposed to the solvent residues), the PnIA subtype preferentially inhibits the α3β2 AChR, whereas α-CgTx PnIB is selective for the α7 receptor [50] (Table 3). The fact that

the α-CgTx PnIA A10L mutant binds with higher affinity to the α7 receptor suggests that position 10 is important for the observed selectivity [50,51]. These studies also suggest that both A^{10} and N^{11} in α-CgTx PnIA independently interact with the α3β2 AChR whereas L^{10} in α-CgTx PnIB seems to be the only structural requirement for the binding to either α7 or α3β2 subtype [51], as well as for the inhibition of the nicotine-evoked catecholamine release [52]. Subsequent thermodynamic mutant cycle analyses demonstrated the existence of a dominant interaction between α-CgTx PnIB L^{10} and $\alpha7W^{149}$ (located in loop B; reviewed in [8]), and weaker interactions between α-CgTx PnIB P^6 and $\alpha7W^{149}$, and between both P^6 and P^7 of α-CgTx PnIB and $\alpha7Y^{93}$ (located in loop A; reviewed in [8]) [53]. The evidence from mutational experiments also suggests that the binding site for α-CgTx PnIA [50] or for α-CgTx PnIB [53] on the α7 receptor is different from the α-CgTx ImI site [49].

5. Possible medical applications

Nicotinic acetylcholine receptors appear to be important for a number of neurophysiological processes including cognition, learning, and memory. In addition, this receptor family has been implicated in the pathophysiology of several neuropsychiatric disorders including Alzheimer's and Parkinson's disease, schizophrenia, Tourette's syndrome, nocturnal frontal lobe epilepsy, as well as nicotine addiction, myasthenia gravis, and various congenital myasthenic syndromes (reviewed in [63]). Thus, the identification of a ligand with high specificity for certain AChR subtype will be of great importance in the development of new drugs with potential medical uses. In the future, α-CTxs might be used as therapeutic agents in the treatment of some of the above mentioned diseases. In this regard, additional *Conus* toxins not discussed in this review are being examined for possible clinical use. For example, conantokin-R which inhibits the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor might have use as an anticonvulsant agent [64]. The ω-CTx MVIIA, which is highly specific for the voltage-gated calcium channels containing the α_{1B} subunit, is being used in clinical trials for the treatment of certain chronic pain syndromes (e.g. intractable pain resulting from cancer, traumatic nerve demage, or amputation) and it has also proved to be useful as a neuroprotector of cerebral ischemia provoked by stroke, cardiac arrest, or head trauma (reviewed in [65]). Finally, an immunoprecipitation assay with [^{125}I]ω-CTx is used to diagnose the Lambert–Eaton myastenic syndrome (reviewed in [65]), an autoimmune disease in which antibodies recognize endogenous calcium channels.

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