Biochemical Pharmacology 78 (2009) 720–731

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

Polypeptide and peptide toxins, magnifying lenses for binding sites in nicotinic acetylcholine receptors

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ARTICLE INFO

Article history:
Received 1 April 2009
Accepted 21 May 2009

Keywords:
Acetylcholine-binding proteins
Nicotinic acetylcholine receptors
Three-fingered toxins
α-Conotoxins
α-Neurotoxins

ABSTRACT

At present the cryo-electron microscopy structure at 4 Å resolution is known for the *Torpedo marmorata* nicotinic acetylcholine receptor (nAChR), and high-resolution X-ray structures have been recently determined for bacterial ligand-gated ion channels which have the same type of spatial organization. Together all these structures provide the basis for better understanding functioning of muscle-type and neuronal nAChRs, as well as of other Cys-loop receptors: 5HT3-, glycine-, GABA-A and some other. Detailed information about the ligand-binding sites in nAChRs, necessary both for understanding the receptor functioning and for rational drug design, became available when the X-ray structures were solved for the acetylcholine-binding proteins (AChBP), excellent models for the ligand-binding domains of all Cys-loop receptors. Of special value in this respect are the X-ray structures of AChBP complexes with agonists and antagonists. Among the latter are the complexes with polypeptide and peptide antagonists, that is with protein neurotoxins from snake venoms and peptide neurotoxins (α-conotoxins) from poisonous marine snails of *Conus* genus. The role of a bridge between the AChBP and nAChRs is played by the X-ray structure of the ligand-binding domain of α1 subunit of nAChR in the complex with α-bungarotoxin.

The purpose of this review is to show the role of well-known and new polypeptide and peptide neurotoxins, from the earlier days of nAChRs research until present time, in identification of different nAChR subtypes and mapping their binding sites.

Contents

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doi:10.1016/j.bcp.2009.05.032

1. Introduction

Investigation of the spatial structure of nicotinic acetylcholine receptor (nAChR) has a long history and the first studies were carried out in eighties of the last century (for review see [1]). Now nAChR is one of the best studied neuroreceptors. The progress in nAChR research was backed up by two favorable factors: the availability of high quantities of the receptor protein from electric organ of *Torpedo* rays as well as the discovery of very selective and efficient receptor ligands—polypeptide α-neurotoxins from snake venoms. Although large amount of information about the nAChR structure has been accumulated to the present moment, only a few structures of the whole receptor are available. Thus, the three-dimensional structure of the *Torpedo marmorata* nAChR was determined by cryo-electron microscopy at 4 Å resolution [2]. Recently, high-resolution X-ray structures have been solved for bacterial ligand-gated ion channels [3–5]. In combination, these EM and X-ray structures shed light on the three-dimensional organization of muscle-type and neuronal nAChRs, as well as of other Cys-loop receptors: 5HT3-, glycine-, GABA-A and some other. The X-ray structure of a water-soluble acetylcholine-binding protein (AChBP) [6] provided an excellent model for the ligand-binding domains of nAChRs and other members of the Cys-loop family. The most accurate information about the topography of ligand-binding sites was obtained from the X-ray structures of different AChBPs in complexes with diverse agonists and antagonists [7–12]. For better understanding the differences in the specificity of distinct nAChR subtypes and for design of potential drugs, an important role may play the X-ray structures of the AChBP complexes with a snake venom α-neurotoxin [9] and α-conotoxins [8,10–12], neurotoxic peptides from poisonous marine snails of *Conus* genus. The EM structure of the *Torpedo* nAChR [2] corresponds to the receptor containing no ligand. However, the X-ray structure of ligand-binding domain of α1 subunit of muscle nAChR in the complex with α-bungarotoxin has been recently solved [13]. In fact, this structure gave one more confirmation for correctness of using the structures of AChBPs and their complexes for modeling ligand-binding sites of nAChRs. Thus, it is quite clear that neurotoxins from snake venoms, including both well-known and new structural types, in combination with a growing number of α-conotoxins from numerous *Conus* species continue to play important role in nAChR research by helping to identify and quantify different nAChR subtypes, probe their function and map their ligand-binding sites.

2. Protein and peptide neurotoxins utilized in research on nAChRs

2.1. "Classical" snake venom three-fingered α-neurotoxins

At the very beginning of studies on nAChRs as proteins, snake toxins played a very important role. The first toxin acting on nAChR was identified in 1963 in *Bungarus multicinctus* venom [14]. Later on this type of toxins rapidly expanded and they were called α-neurotoxins. (For more details see review [15]). There are two structural types of α-neurotoxins: short-chain α-neurotoxins (60–62 amino acid residues, 4 disulfide bridges) (Fig. 1A) and long-chain ones (66–75 amino acid residues, 5 disulfide bonds, the fifth bond being present in the central polypeptide loop) (Fig. 1B). α-Neurotoxins possessing high affinity to nAChRs served as efficient tools for receptor identification and isolation. Their importance in nAChR studies is comparable to that of electric organs from the electric eels and rays, which served as rich sources of the receptor protein. α-Neurotoxins were widely used for the functional detection of nAChR at the neuro-muscular junction causing block of its function. Nowadays these receptors are called muscle-type

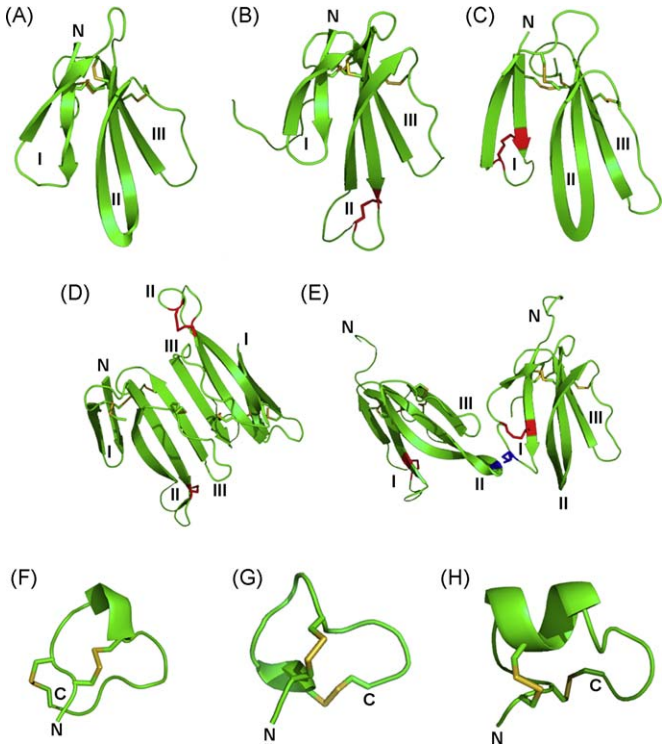

Fig. 1. Spatial structures of toxins interacting with nAChR. A—erabutoxin a (PDB ID: 5EBX); B—neurotoxin I *Naja oxiana* (1NTN); C—candoxin (1JGK); D—kappa-bungarotoxin (1KBA); E—irditoxin (2H7Z); F—α-conotoxin GI (1NOT); G—α-conotoxin ImI (1IM1); H—α-conotoxin MII (1MII). Fifth disulfide bonds in snake toxins are shown in red; disulfide bond between the monomers in irditoxin is shown in blue.

nAChRs. The receptors from the *Torpedo* electric organ also belong to this type. α-Neurotoxins were used for preparation of the affinity resin for purification of the muscle-type nAChR. This allowed researches to characterize the nAChR as an oligomeric protein with molecular mass of about 220 kDa consisting of five subunits (two α, β, γ and δ). In 1976 the X-ray structure of α-neurotoxin erabutoxin b was determined [16]. However, this structure per se did not give the answer to the question what are the interacting surfaces of the α-neurotoxin and nAChR.

The first direct data revealing the toxin amino acid residues interacting with nAChR were obtained using reporter groups attached to defined positions in the α-neurotoxin amino acid sequence. Thus, the use of fluorescence and spin-labeled derivatives of short type α-neurotoxin II from *Naja oxiana* showed that all three loops of this toxin take part in the interaction with the receptor [17]. Later on, extensive mutations of another short-chain neurotoxin (erabutoxin b) [18] confirmed these data and gave more detailed information about amino acid residues involved in the interaction with nAChR. (As will be discussed below, the X-ray data for complexes of long α-neurotoxins confirmed the involvement of loops I and II in the interaction, but for short toxins such data are still lacking.) Data on the binding surfaces at the receptor started to appear later. Thus, the results of photoaffinity labeling and site-directed mutagenesis (see reviews [1,19]) showed that the toxin-binding sites in the *Torpedo* nAChR are localized at the α/γ and α/δ interfaces. Moreover, it has been shown that the toxin-binding sites are not at the very top of the receptor molecule, as it was suggested earlier (similarly to MIR region, a binding site for autoimmune antibodies), but should be embedded into the nAChR extracellular domain.

Both short- and long-chain α-neurotoxins interact with *Torpedo* nAChR. In addition to this interaction, the long-chain toxins (in

particular α-bungarotoxin) were able to bind to specific sites in brain (e.g., [20]). Later on these binding sites were identified as homooligomeric α7 nAChR. During further studies several nAChR subtypes were characterized in nervous system. They are either homooligomeric nAChRs (e.g., α7) consisting of five subunits of one type, or heterooligomeric ones, comprising five subunits of different types (e.g., α3β2, α4β2). At present nine α-subunits (α2–α10) and three β-subunits (β2–β4) are characterized in nervous system. Only long-chain α-neurotoxins are able to interact with homooligomeric neuronal nAChR (α7, α8, α9). It worth to mention, that so-called κ-bungarotoxins were isolated from krait (*Bungarus* genus) venom. These toxins specifically interact with α3β2 nAChR subtype. More detailed information about these toxins will be given below.

Although most of the studies on neurotoxin–nAChR interactions have been done on the whole receptor (either membrane-bound or solubilized), it was also shown that the isolated α-subunit was able to bind α-neurotoxin on the blots [21]. It was found that even relatively short fragments of the α-subunit could bind α-neurotoxins, although with a lower affinity as compared to the native receptor. These fragments were shown later to contain the amino acid residues which are essential for the interaction of nAChR with α-neurotoxins. In fact, some information about the receptor ligand-binding site could be obtained from these studies, but the conclusions about the topography of these sites made from NMR or X-ray analysis of such model complexes are of only limited value. Interestingly enough, binding of α-neurotoxins is not limited to nAChRs. Recently one subtype of the GABA-A receptor was shown to interact with α-bungarotoxin [22], which blocked the receptors containing the adjacent β3 subunits.

2.2. Weak (non-conventional) three-fingered neurotoxins

Weak or non-conventional neurotoxins is the group of three-fingered toxins consisting of 62–68 amino acid residues with five disulfide bridges (Fig. 1C). This is a fairly divergent group, the main structural feature of which is the presence of fifth disulfide bridge in the first polypeptide loop [23]. The toxicities for the most of group members are very low (5–80 mg/kg) in contrast to classical α-neurotoxins with toxicities in the range from 0.04 to 0.3 mg/kg. However, some very potent toxins (e.g., γ-bungarotoxin with LD_{50} of 0.15 mg/kg and candoxin—0.83 mg/kg) are also included in this group. Although a first weak toxin was isolated more than 30 years ago, biological targets of these proteins remained unknown until recently. The interest to this toxins group was revived after discovery of their unusual interaction with the α7 and muscle-type nAChRs [24]. It was found that weak toxin WTX from *Naja kaouthia* venom interacted with micromolar affinity with the two nAChR subtypes, the binding being practically irreversible [24]. Later it was found [25] that candoxin from this group interacted both with α7 and muscle-type nAChRs with high affinity, however its interaction with the muscle-type receptor was easily reversible. Another member of this group denmotoxin isolated from *Colubrid* snake *Boiga dendrophila* (Mangrove Catsnake) displays a remarkable species specificity, being able to interact irreversibly and with high affinity with chick muscle nAChR, but only with low affinity with mouse nAChR [26].

The only structural data available for weak toxin–nAChR interaction are those obtained by computational modeling [27]. Docking and molecular dynamics simulations predicted fast kinetics of candoxin association with nAChR and no gross changes in the toxin conformation (with smaller toxin flexibility on α7 nAChR). A slow binding of WTX to nAChR was predicted, associated with slow irreversible rearrangements both of the tip of the toxin loop II and of the nAChR binding pocket residues locking finally the toxin molecule.

2.3. Non-covalently and covalently bound dimers of three-fingered toxins

Oligomerization may be regarded as an intrinsic property of three-fingered toxins. Thus, three-fingered cytotoxins interact with lipid membranes as dimers [28]. κ-Bungarotoxins shortly mentioned above also confirm this suggestion. These toxins consist of 66 amino acid residues and contain 5 disulfide bridges, the fifth disulfide being located in the central polypeptide loop II. According to X-ray and other data [29,30] these toxins exist as non-covalently bound dimers (Fig. 1D). κ-Bungarotoxins specifically bind with high affinity to neuronal α3β2 nAChR. Toxin mutagenesis studies indicated the involvement of certain amino acids in the interaction with nAChR. Thus, it was shown that Arg34 and position 36 within the loop II are essential for the high affinity binding to nAChR. Also it was suggested that dimerization is necessary for toxin association with nAChR. However, direct experimental data confirming this suggestion were absent until recent discovery of disulfide-bound dimers of the three-fingered toxins. During proteomic studies of *N. kaouthia* cobra venom in our laboratory, several dimers of three-fingered toxins, covalently bound by disulfide bridges, have been isolated [31]. These are homodimer of α-cobratoxin (a long-chain α-neurotoxin) and heterodimers of α-cobratoxin with cytotoxins 1, 2 and 3 from the same venom. α-Cobratoxin within dimers preserved to some extent its capacity to interact with the α7 and muscle-type nAChRs, but the cytotoxin moiety completely lost its cytotoxicity. Interestingly, the α-cobratoxin homodimer, in contrast to monomeric α-cobratoxin, acquired the capacity to interact with α3β2 nAChR [31]. Although the affinity of homodimer to the α3β2 nAChR was lower than that of κ-bungarotoxin, these data indicate that dimerization is necessary for binding of a three-finger toxin to neuronal nAChR. However, the high K_D value suggests that some other factors are also very important for binding.

One more disulfide-bound three-fingered toxin dimer – irditoxin – was isolated from the Colubrid snake *Boiga irregularis* [32]. In contrast to cobra dimers which are present in venom in minor amounts, irditoxin is the main component of boiga venom. Monomers forming this toxin belong to non-conventional toxin type and each monomer contains an extra sulfhydryl group which forms a disulfide bridge between the monomers (Fig. 1E). Irditoxin, similarly to denmotoxin, is taxon-specific: it interacts with high affinity with avian muscle-type nAChR, but only with low affinity with mammalian nAChR. Taken together, the above data suggest that dimerization may represent a new way of biological activity diversification for three-fingered toxins.

2.4. Waglerins, neurotoxic peptides from Tropidolaemus wagleri

Polypeptide waglerins isolated from the venom of South Asian snake *Tropidolaemus wagleri* consist of 22–24 amino acids and contain one disulfide bridge [33,34]. At present four waglerins are known. These toxins with high affinity interact with muscle-type nAChR [35]. An interesting property of waglerins is their capacity to distinguish embryonic ($\alpha1_2\beta1\gamma\delta$) and "mature" ($\alpha1_2\beta1\varepsilon\delta$) muscle-type nAChRs [36]. Thus, waglerin-1 efficiently blocks the epsilon form, but not the gamma form of the muscle-type nAChR. It was found also that waglerin-1 binds 2100-fold more tightly to the α-ε than to the α-δ binding site interface of the mouse nAChR [37]. Several amino acid residues in nAChR subunits participating in waglerin binding were identified by site directed mutagenesis [38], Asp59 and Asp173 being important for waglerin binding at both sites. It should be noted that waglerin 1 modulated currents induced by GABA in murine hypothalamic neurons [39] and inhibited GABA-A current of neurons in the nucleus accumbens of

Table 1
Most studied members of α-conotoxin family.

Name	*Conus* species	Sequence	nAChR subtypes selectivity
3/5 α-conotoxins			
GI	*C. geographus*	ECCNPACGRHYSC*	α1β1γ/εδ [41,54,56,86]
MI	*C. magus*	GRCCHPACGKNYSC*	α1β1γ/εδ [54,56,59,85,86]
SI	*C. striatus*	ICCNPACGPKYSC*	α1β1γ/εδ [56–58,86]
SIA	*C. striatus*	YCCHPACGKNFDC*	α1β1γ/εδ [54,58,76,86]
4/3 α-conotoxins			
ImI	*C. imperialis*	GCCSDPRCAWRC*	α7 [92,93,107]; α9α10 [92,108];α3β2 [94]; α3β4 [95]
ImII	*C. imperialis*	ACCSDRRCRWRC*	α7 [94,107]; α1β1εδ [94]
RgIA	*C. regius*	GCCSDPRCRYR CR	α9α10 [60,108]
4/4 α-conotoxins			
BuIA	*C. bullatus*	GCCSTPPCAVLYC*	α3(α6)β2, α3(α6)β4 [109]
4/6 α-conotoxins			
AuIB	*C. aulicus*	GCCSYPPCFATNPDC*	α3β4 [102]
4/7 α-conotoxins			
PnIA	*C. pennaceus*	GCCSLPP CAANNPDY^{S}C*	α3β2 [63,64,70,71]
PnIB	*C. pennaceus*	GCCSLPP CALSNPDY^S C*	α7 [63,64]
MII	*C. magus*	GCCSNPV CHLEHSNL C*	α3β2, α3β2β3 [69,71,96,98];α6-containing [97,99,100,101]
GID	*C. geographus*	IRDγCCSNPACRVNNOHV C	α3β2, α7, α4β2 [71,105]
ArIB	*C. arenatus*	DECCSNPACRVNNPHV CRRR	α7, α6α3β2β3, α3β2 [104]
TxIA	*C. textile*	GCCSRPP CIANNPDL C*	α3β2 [12]
EI	*C. ermineus*	RDOCCYHPTCNMSNPQI C*	α1β1γ/εδ [106]; α3β4, α4β2 [106]
SrIB	*C. spurius*	RTCCSROTCRMEYPγL CG*	α4β2, α1β1γδ [106]

In all cases the shaded Cys residues are closed in C1–C3 and C2–C4 disulfides.
* Indicates an amidated C-terminus; O—4-*trans* hydroxyproline; γ—carboxyglutamate; Y^S—sulfated tyrosin.

neonatal rats [40]. This data may indicate the interaction of waglerins with GABA-A receptors present in brain.

2.5. α-*Conotoxins, neurotoxic peptides from Conus snails*

In the early 80^s, peptide toxins from marine snail *Conus geographus* venom which caused postsynaptic inhibition at the neuromuscular junction in frog were discovered and named as conotoxins [41]. During past quarter of the last century a few dozen similar toxic peptides from different members of *Conus* genera (including more than 500 species) were purified and characterized (most examined are listed in Table 1) (Fig. 1F–H). Functionally all of them are the competitive antagonists of nAChRs and form the group of α-conotoxins by common structural features: the cysteine-framework CC-C-C (that distinguishes them from ψ-, αA-, αA$_S$-, αC-, αS- and αD-families acting on nAChRs as well [42–49]) bridged in C1–C3 and C2–C4 disulfides (in contrast to ρ-conotoxin TIA with C1–C4 and C2–C3 closing, α$_1$-adrenoreceptor ligand [50]). Two exceptions are known at present: α-conotoxin SII from *C. striatus* with the additional N- and C-terminal cysteines closed in a third disulfide [51] and peptide lt14a with C-C-C-C framework identified in *C. litteratus* [52] which is an inhibitor of neuronal-type nAChRs. α-Conotoxins are structurally subdivided into subgroups by numbering the amino acid residues between the second and third cysteines and the third and the forth cysteines (Table 1). These fragments form the first and second loops, respectively, and determine in essential the α-conotoxin specificity to different nAChR subtypes. All known at present 3/5 α-conotoxins, for example, are potent blockers of muscle-type nAChRs ('muscle' conotoxins). The members of other subgroups (4/3, 4/4, 4/6, 4/7) act on various neuronal nAChR subtypes ('neuronal' conotoxins) and do not reveal any obvious correlation between the affinity to the receptor subtype and their subgroup membership. Moreover, usually neuronal α-conotoxins interact with two or more nAChR subtypes (see Table 1).

A characteristic feature of the majority of 3/5 α-conotoxins is their ability to discriminate two binding sites on the muscle or *Torpedo* nAChRs. This capacity can be species specific. A striking example of this effect is the affinity preference by 10,000 times of α-conotoxins MI, GI, SIA for α1/δ—over α1/γ site in muscle nAChR [53,54], in contrast to more effective binding of these peptides to α1/γ site in the *Torpedo* species nAChRs [55,56].

With the aid of synthetic α-conotoxin analogs ("mutagenesis" of α-conotoxins), it was possible to pinpoint the amino acid residues governing the potency and specificity for definite nAChRs including the above-mentioned selectivity for the two binding sites on the muscle-type nAChRs. This way the crucial role of Arg9 of α-conotoxin GI [57,58] and Pro6 and Tyr12 of α-conotoxin MI [59] for discriminating the α1/γ- and α1/δ-sites was revealed. The importance of Arg9 in neuronal 4/3 α-conotoxin RgIA for its α9α10 nAChR specificity was recently discovered [60]. Another set of amino acid residues was found to define the selectivity for homomeric α7 nAChR of α-conotoxin ImI (triad Asp5-Pro6-Arg7 and Trp10) [61,62]. Interestingly, a single [Ala10Leu] mutation in α-conotoxin PnIA shifted its preference from α3β2 to α7 nAChR type [63,64]. On the other hand, α-conotoxin MII high affinity for α3β2 nAChR is determined by Asn5, Pro6 and His12 [65].

Mutation studies of various muscle and neuronal nAChR subunits had the goal to explain the differences in specificity and reveal the loci of α-conotoxins' binding sites. From numerous publications it followed that six fragments of nAChR (so-called A, B and C from the principal and D, E and F from the complementary sides whose nomenclature was given in [66]) were participating in binding of peptides (see, for example [67–71]). These data were also confirmed in studies of pairwise analysis (as earlier mentioned for α-neurotoxins and waglerins) where the sets of mutated nAChR subunits and conotoxins were applied in different biochemical tests that suggest the amino acid contacts between the ligand and its target. Particularly, the dominant interaction of α-conotoxin ImI Arg7 with Tyr195 of α7 nAChR [72] or α-conotoxin PnIB Leu10 with Trp149 of the same receptor were supposed [73]. A few definite contacts between muscle-type nAChRs and α-conotoxin MI were also suggested [74,75].

Detection of the direct contacts between the ligand and its target is possible also by photolabeling approach. This method was tested only for muscle α-conotoxins and native *Torpedo* nAChR. In earlier works the preparation and characterization of the peptide photoactivatable analogs in labeling of the whole subunits was

Table 2
Preparation and characterization of some α-conotoxin analogs.

Conotoxin	Sequence	Affinities (nM) to	References
		α7 nAChR/α9α10 nAChR	
RgIA:	GCCSDPRCRYRCR	3300/8.5	[60]
RgIA(A^4):	GCCADPRCRYRCR	5700/15	[60]
RgIA(E^5):	GCCSEPRCRYRCR	27,000/6600	[60]
RgIA(V^6):	GCCSDVRCRYRCR	19,000/4100	[60]
RgIA(K^7):	GCCSDPKCRYRCR	47,000/13,000	[60]
RgIA(A^9):	GCCSDPRCAYRCR	570/13,000	[60]
RgIA(W^{10}):	GCCSDPRCRWRCR	2900/11	[60]
RgIA(desR^{13})*:	GCCSDPRCRYRC*	1150/7.8	[60]
		α7 nAChR/α3β2 nAChR/α6α3β2β3 nAChR	
MII:	GCCSNPVCHLEHSNLC*	~200/2.2/0.39	[96,99]
MII(A^{11}):	GCCSNPVCHLAHSNLC*	1050/8.7/0.16	[99]
MII(A^9,A^{15}):	GCCSNPVCALEHSNAC*	>10,000/4850/2.4	[99]
MII(A^4,A^{11},A^{15}):	GCCANPVCHLAHSNAC*	~10,000/1400/1.2	[103]
ArIB:	DECCSNPACRVNNPHVCRRR	1.8/60/6.5	[104]
ArIB(L^{11}):	DECCSNPACRLNNPHVCRRR	0.54/39/-	[104]
ArIB(L^{11},A^{16}):	DECCSNPACRLNNPHACRRR	0.36/75/120	[104]
ArIB(L^{11},D^{16}):	DECCSNPACRLNNPHDCRRR	1.1/>10,000/830	[104]
		Ls-AChBP/α7 nAChR/α3β2 nAChR	
TxIA:	GCCSRPPCIANNPDLC*	1.7/390/3.6	[12]
TxIA(L^{10}):	GCCSRPPCILNNPDLC*	1.1/39/2.0	[12]
PnIA(L^{10}):	GCCSLPPCALNNPDYC*	80/13/99	[12,64]
PnIA(R^5,L^{10}):	GCCSRPPCALNNPDYC*	6.2/10/4.6	[12]

Substituted amino acid residues in analogs are shaded.

achieved that first revealed the position of α-conotoxin-binding sites at the interfaces of the adjacent α1- and non-α1 nAChR subunits, as well as the involvement of the peptide N- and C-termini in the formation of complex with nAChR [76,77]. Later the employment of the phoactivatable analogs of α-conotoxins GI and MI resulted in detection of the crosslinked fragments of α1-, γ- and δ-subunits and partial mapping of the α1/γ [78] and α1/δ [79] sites from two *Torpedo* nAChRs, respectively.

It is worth to mention, that goals of α-conotoxin analogs preparation are not limited to structural studies of nAChRs, but embrace numerous tasks (a fairly full list of synthesized α-conotoxin variants and derivatives is given in recent review [80]). On the basis of some α-conotoxins (MII, GI, MI, ImI, ArIB), their radioactive derivatives are prepared and utilized in radioligand assays (see, for example, [77,78,81–88]). The investigations directed to generation of more stable at physiological conditions (selenoconotoxins [89] or cyclizated peptides [90]) analogs or peptides with the ability to penetrate through the blood–brain barrier (α-conotoxin MII modified with lipoamino acid [91]) are of great interest in view of possible use of α-conotoxins in medicinal practice. The real progress was achieved also in synthesis of α-conotoxin analogs possessing more potency and/or selectivity than the respective naturally occurring peptides. This task is of great importance because of lack of native α-conotoxins exclusively targeting species—specifically the definite neuronal nAChR subtype (see Table 1). For instance, α-conotoxin ImI was earlier described as a blocker of rodent homomeric α7 or α9 nAChRs [92,93], then its higher affinity for the recombinant human α3β2 type [94] and possible interaction with α3β4 nAChR from bovine adrenal chromaffin cells [95] were discovered. In a similar way, α-conotoxin MII originally was characterized as α3β2 nAChR ligand [69,96], but later it was found to target with a higher efficacy the receptors containing the α6 and β3 subunits [97–101]. In addition, the evidence of the difference in the activity and selectivity of some α-conotoxins for the same recombinant or native nAChR subtype was published [102].

As a result of purposeful design and synthesis of potent and specific α-conotoxin analogs, a series of peptides on the base of various 'muscle' (GI, SI, SIA) and 'neuronal' (PnIA, MII, ArIB, TxIA) α-conotoxins were described. Most of these analogs were built either on the base of similarities with amino acid sequences of other known α-conotoxins or for the Ala-scanning mutagenesis studies or with purposeful introducing the hydrophobic or charged residues. For example, introduction of positively charged amino acid residues (Lys or Arg) in the C-terminal part of the 'muscle' α-conotoxins or PnIA(L^{10}) analog resulted in gaining the potency to *Torpedo californica* [86] or chicken α7 nAChRs [8], respectively. The importance of definite amino acid residues in α-conotoxins for the nAChR subtype specificity (in addition to the above-mentioned [Ala10Leu] substitution in α-conotoxin PnIA) was demonstrated recently also for α-conotoxin RgIA [60]. The substitution of Ala9 for Arg resulted in a crucial decrease of the peptide affinity for α9α10 nAChR (see above), but with a considerable increase in potency for α7 nAChR subtype (Table 2). Multiple substitutions of alanine residues in α-conotoxin MII gave a few effective analogs with the affinity for α6α3β2β3 nAChR 1000-fold higher than for relative α3β2 subtype (see data from [99,103] given in Table 2). At present, the most potent and specific antagonists of the α7 nAChRs are synthetic variants of α-conotoxin ArIB (see data from [104] in Table 2). Design and preparation of α-conotoxin analogs targeting other neuronal nAChR subtypes are in progress now and stimulated by ongoing discovery of new members of the α-conotoxin family directly purified from *Conus* venoms [12,105,106], derived from the respective DNA libraries [104,107–109] or by computer modeling (see later).

3. Acetylcholine-binding proteins, models of nAChR ligand-binding domains

3.1. X-ray structures of AChBPs

In 2001 a water-soluble acetylcholine-binding protein that modulated synaptic transmission was found in glia of *Lymnaea stagnalis* snail [110]. AChBP was pulled down with the use of α-bungarotoxin, similarly as it was with the *Torpedo* nAChR more than 30 years ago. Successful heterologous expression of this protein resulted in crystal structure at 2.7 Å resolution [6]. Pharmacological profile of AChBP, close relationship to the α-subunits of nAChRs in sequence alignment, as well as the comparison of the AChBP X-ray structure with the available

low-resolution cryo-electron microscopy structure of *Torpedo* nAChR [2] allowed the researchers to conclude that this protein is an excellent structural model of the N-terminal ligand-binding domains of all nAChRs. In active form AChBP is a homopentamer. In spite of low total sequence homology with the extracellular domains of any nAChR subunits (not more than 25%), AChBP contains all amino acid residues principal for binding of true cholinergic ligands. The crystal structure provided the coordinates of these residues situated at the interfaces between the subunits (AChBP protomers) and for the first time outlined the spatial arrangement of the whole ligand-binding site.

At present, the X-ray structures of two other molluscan AChBPs from *Aplysia californica* and *Bulinus truncatus* are known [10,111]. All three proteins are characterized with low total sequence homology, but the same type of spatial structure. There are essential differences in the sensitivity of these AChBPs to various α-neurotoxins and α-conotoxins that are resembling functional differences between the nAChR subtypes. Indeed, AChBP from *L. stagnalis* has a 60–140-fold higher affinity for α-bungarotoxin as compared to AChBP from *A. californica* and a 120–16,000-fold weaker potency for α-conotoxin ImI [8,112].

3.2. X-ray structures of AChBP complexes with agonists and antagonists (including α-neurotoxins and α-conotoxins)

The first crystal structure of *L. stagnalis* AChBP revealed the occupation of all five ligand-binding sites with 5 HEPES molecules [7]. This buffer molecule contains positively charged quaternary ammonium, resembling classical nAChR agonists, and therefore could be considered as a putative ligand, like acetylcholine. Soon the crystal structures of *L. stagnalis* and *A. californica* AChBPs bound with true cholinergic agonists carbamoylcholine and nicotine [8], as well as α-lobeline and epibatidine [10] were solved. In all cases ligand molecules were plunged in the same pocket formed (as was predicted from photoaffinity labeling and mutagenesis studies) by loops A, B and C of the principal side and loops D, F and E of the complementary side. This ligand-binding pocket is a peculiar aromatic box that is formed by side chains of *L. stagnalis*/*A. californica* AChBP amino acids: Tyr89/93 (A), Trp143/147 (B), Tyr185/188, vicinal Cys187-Cys188/190-191 and Tyr192/195 (C), Trp53/Tyr55 and Gln55/57 (D), Arg104/Val108, Leu112/Met116 and Met114/Ile118 (E), Tyr164/168 (F) with numbering according to [6,112]. All listed amino acids of the principal side are present also in α-subunits of different nAChR subtypes in contrast to amino acids of the complementary side varying in different receptors.

A comparison of these structures with the X-ray structure of AChBP occupied with buffer molecules [6], and especially with *A. californica* AChBP which has a free ligand-binding site [10] shows that on binding an agonist, the loop C moves to the center of the molecule and embraces the bound agonist molecule.

The first complex of AChBP with an antagonist was that with α-cobratoxin, although it was done not at very high (4.2 Å) resolution [9]. In general, it showed the correctness of earlier ideas about the location of the toxin-binding site. It also demonstrated the expected multi-point mode of toxin binding where the central loop II (including the additional disulfide characteristic only for long-chain α- and κ-neurotoxins) and loop I are involved. However, there were no obvious interactions with loop III as could be expected from pair-wise mutagenesis studies of α-cobratoxin binding to α7 nAChR. It should be stressed here that AChBP seems to correspond best of all to homooligomeric α7 neuronal nAChRs. There might be expected some differences in the mode of interaction of other types of snake neurotoxins with distinct nAChR subtypes, for example of short-chain α-neurotoxins binding to muscle-type nAChR. In particular, in modeling, on the basis of cryo-electron microscopy structure of *Torpedo* nAChR [2] and X-ray structures of AChBP complexes, of the *Torpedo* nAChR complex with neurotoxin II from *N. oxiana*, a short-chain α-neurotoxin, some differences in the approach of the tip of the central loop to the binding sites between the long and short α-neurotoxin were suggested [113].

The X-ray structure of the AChBP complex with α-cobratoxin could not answer the question what is the type of interactions between the neuronal heteromeric nAChRs and κ-bungarotoxins. Bourne et al. [9] proposed that the tip of the central loop of κ-bungarotoxins (where additional disulfide, like in long-chain α-neurotoxins is located) is the most important for interaction, and the selectivity to α3β2 nAChR is provided by a positive charge of Lys29 which is lacking in long-chain α-neurotoxins. To check this hypothesis, such a positive charge was introduced into a chimeric long-chain α-neurotoxin: the [Ala29Lys] substitution considerably decreased the affinity to α7 nAChR, but did not bring about a shift in favor of α3β2 nAChR subtype [114]. On the other hand, we have recently shown that dimerization is a prerequisite for the long-chain α-neurotoxin capacity to recognize a heteromeric nAChR: a naturally occurring disulfide-bound dimer of α-cobratoxin has a 10-fold decreased affinity for the α7-, but inhibits α3β2 nAChR with IC_{50} 150 nM (about 50-fold less potently than κ-bungarotoxin) [31].

In the above paragraph we were focused on what is the binding surface of snake α-neurotoxins in the complex with AChBP and what conclusions can be done about the mode of snake neurotoxins binding to distinct nAChR subtypes. However, this X-ray structure revealed one feature concerning the organization and functioning of the binding site in the nAChR itself. As already mentioned, on agonist binding, loop C changes its position, embracing the agonist and moving closer to the central axis of the AChBP pentameric complex. However, in the complex with α-cobratoxin, loop C occupies an outward position, the difference from its location in the complex with an agonist being about 7 Å. In fact such a position is similar to that in the *Torpedo* nAChR which has been analyzed in apparently closed state [2]. So, at first approximation the X-ray structures of the AChBP complexes with the agonists might correspond to conformation of the nAChR in the desensitized state, while the complexes with antagonists might have common features characteristic for the resting and closed states. However, interpretation of the nAChR states in terms of the X-ray structures of AChBP complexes might be not so straightforward because such antagonists as α-conotoxins ImI and PnIA(L^{10}, K^{14}) behaved as agonists towards [L247T] mutant of α7 nAChR [11,115], and the agonist activity was also observed for α-conotoxins EI, SrIA and SrIB [106].

Soon after elucidation of the X-ray structure of the *L. stagnalis* AChBP complex with α-cobratoxin, a high-resolution (2.4 Å) structure was established for α-conotoxin complex with *A. californica* AChBP [8]. In fact, it was the first X-ray structure for a representative of a large family of various conotoxins in complex with a biological target. The crystals were obtained for α-conotoxin PnIA variant having two substitutions (namely, PnIA(L^{10},K^{14}) analog) which revealed high affinity both for *L. stagnalis* and *A. californica* AChBPs and potently inhibited the acetylcholine-induced currents in α7 nAChRs expressed in oocytes [8].

Interaction of PnIA(L^{10},K^{14}) with *A. californica* AChBP is dominated by hydrophobic contacts (Fig. 2A). At the principal side, these contacts are formed basically by highly conserved aromatic residues—Trp145, Tyr186, Tyr193, as well as by vicinal disulfide Cys188-Cys189. Aliphatic residues are forming binding pocket from the complementary side (Val106, Met114, Ile116). A few additional AChBP amino acids are completing the α-conotoxin-binding cavity: Val146, Ser148, Glu151 and Glu191 of the principal and Gln55, Arg57, Asp162, Ser164 and Thr34 of the complementary sides.

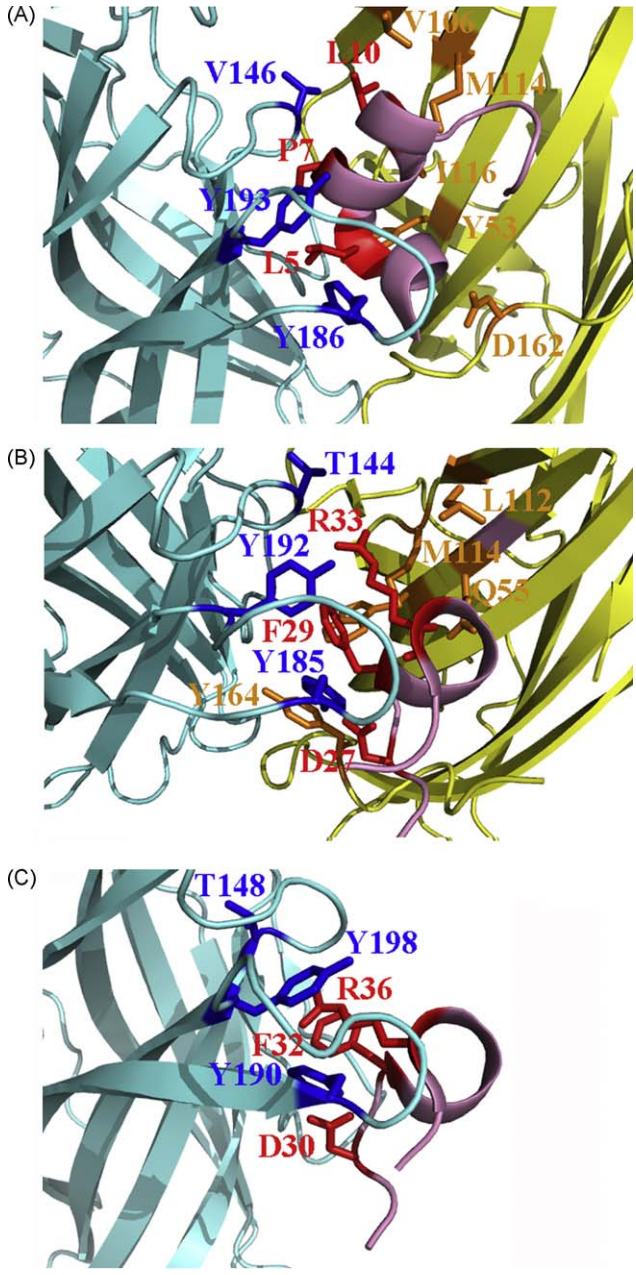

Fig. 2. Ligand-binding sites of the AChBP/nAChR domain-toxin complexes from their crystal structures. (A) α-Conotoxin PnIA(L^{10},K^{14}) analog bound to *A. californica* AChBP (PDB ID: 2BR8); (B) α-Cobratoxin bound to *L. stagnalis* AChBP (PDB ID: 1YI5); (C) α-Bungarotoxin bound to the N-terminal domain of nAChR α1 subunit (PDB ID: 2QC1). α-Neurotoxins are represented by the tips of the central loop II. All toxins are in magenta; the principal and complementary sides of AChBPs are in blue and yellow, respectively. Side chains of some important amino acid residues of toxins and targets are shown and marked.

Among the listed *A. californica* AChBP amino acid residues contacting with α-conotoxin PnIA(L^{10},K^{14}) an essential part (respective residues of *L. stagnalis* AChBP) participated also in binding of α-cobratoxin [9]. It is absolutely true for residues from the principal side, while most differences in the interactions were observed on the complementary side. It might be the reason of lower (in respect to bound α-conotoxin) disposition of α-cobratoxin central loop tip in the binding pocket (compare Fig. 2A and B). However, despite these differences, the crucial common feature for both antagonists is their similar influence on the outward conformation of the loop C.

3.3. Computer modeling of nAChR complexes with agonists and antagonists based on the X-ray structures of AChBP complexes, design of antagonists

As soon as the X-ray structure of AChBPs have appeared, computer modeling has been started to rationalize a whole body of protein chemistry, molecular biology data and the results of the X-ray and NMR analysis on model systems in order to explain the experimental results and to have a more realistic picture of ligand interactions with different nAChR subtypes. At first, only the structure of *L. stagnalis* AChBP served as a starting model, and the agonist- or antagonist-induced conformational changes, which became known later, could not be taken into account. However, it should be noted that the model of α-bungarotoxin complex with AChBP suggested on the basis of the X-ray structure of α-bungarotoxin in complex with just a short peptide homologous to the loop C [116] proved to be quite close in terms of general shape to what was later found for the *L. stagnalis* AChBP complex with α-cobratoxin [9]. The possibilities and accuracy of modeling considerably increased when the coordinates of the *Torpedo* nAChR became available [2] and the high-resolution structures of AChBP complexes with distinct agonists and antagonists were solved [7–11,112].

As already mentioned, modeling (docking and molecular dynamics) is needed to rationalize the experimentally observed varying affinities of different ligands for particular nAChR subtypes, to shed light on the molecular mechanisms of ligand–receptor interactions and to get the basis for design of more potent and more selective analogs which would allow targeting more selectively distinct receptor subtypes. The latter task seems especially important because recent publications from several leading laboratories on a series of knock-out mice demonstrated nonselectivity of practically all available antibodies for rigorous detection of a particular nAChR subtype, at least in histochemistry and Western blot experiments (see, for example, [117,118]). In this respect, α-conotoxins appear especially promising, due to a wide range of naturally occurring compounds characterized by different degrees of selectivity in respect to muscle and distinct neuronal nAChRs, the possibility of their chemical synthesis and a wide choice of further chemical modifications of their structures.

Below are several examples of modeling complexes with bound α-conotoxins. When the X-ray structure of the *A. californica* AChBP in complex with α-conotoxin PnIA(L^{10},K^{14}) was solved, modeling of the complex with *L. stagnalis* AChBP was straightforward since the X-ray structure of this protein has been earlier known. Additional [D14K] substitution increased more than 10-fold the affinity of PnIA(L^{10}) for *L. stagnalis* AChBP, but had no effect on the affinity for *A. californica* AChBP [8]. The X-ray structure disclosed no interactions between the introduced positive charge of Lys14 and its microenvironment in *A. californica* AChBP, whereas modeling predicted a salt bridge with Glu110 in *L. stagnalis* AChBP [8]. To explain a gain in the affinity to *Torpedo* nAChR contributed by the introduction of positive charge at 12th position in 'muscle' α-conotoxins GI, SI and SIA (with the strongest registered increase for the SIA(K^{12})), modeling was also applied and suggested an ionic bond between Lys12 and Glu57 of the γ-subunit as the most probable explanation of the increased affinity [86].

Modeling helps to design α-conotoxins with a higher specificity to a particular nAChR subtype. We checked the possibilities of such an approach by designing, as a control, α-conotoxins which would show a greater difference in affinity between the *L. stagnalis* and *A. californica* AChBPs. The substitutions proposed on the basis of modeling resulted in the analog PnIA(D^{5},R^{7},L^{10}) which had a 760-fold difference in the affinity between the two proteins (Kasheverov et al., unpublished). Interestingly, design on the basis of the same α-conotoxin PnIA(L^{10}) analog and α-conotoxin ImI resulted in the compound (ImI(5-(*R*)-phenyl-L-Pro^{6})) which had a higher

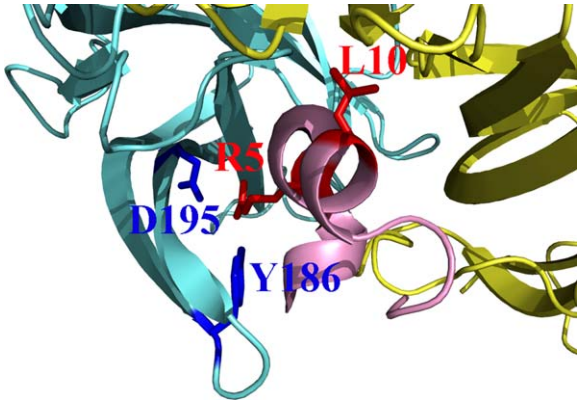

Fig. 3. Crystal structure of ligand-binding site in *A. californica* AChBP with bound α-conotoxin TxIA(L^{10}) (PDB ID: 2UZ6), top view. The principal and complementary faces of AChBP are in cyan and yellow, respectively; α-conotoxin is in magenta with red colored marked Arg5 and Leu10 residues. The amino acid residues of AChBP that interact with peptide Arg5 are in blue.

affinity for α7 nAChR [119]. The most impressive example in modeling the effective α-conotoxin analogs is the preparation of PnIA(R^5,L^{10}) [12]. The resolved X-ray structure of the related α-conotoxin TxIA(L^{10}) analog (acting on α3β2 nAChR and *L. stagnalis* AChBP) complexed with *A. californica* AChBP [12] revealed the interaction of the Arg5 in peptide with Tyr186 and Asp195 in AChBP (Fig. 3). A similar mutation in α-conotoxin PnIA(L^{10}) generated PnIA(R^5,L^{10}) analog that acquired the increased potencies both for α3β2 nAChR and *L. stagnalis* AChBP (see data from [12] presented in Table 2).

In recent years there were considerable achievements in increasing the potency and affinity of α-conotoxins basing both on the analysis of available X-ray structures and thorough comparisons of the sequences for α-conotoxins distinct from each other in having a higher selectivity to one or another nAChR subtype. In Section 2.5 we already mentioned the design and synthesis of a series of potent α-conotoxin MII analogs capable to discriminate between the α6- and α3-subunit-containing nAChR subtypes, as well as the variants of α-conotoxin ArIB which are the most effective antagonists of α7 nAChR at present. The latter had the advantage that their dissociation rates from the α7 nAChR were apparently slow thus making possible staining of α7 nAChR in tissues by radioiodinated derivative of one of these α-conotoxins [88].

It should be emphasized that in general α-conotoxins have high association and dissociation rates hampering their application for staining procedures. In this respect, virtual irreversibility of α-bungarotoxin binding is very valuable for reliable detection of α7 nAChR with the aid of radioactive or fluorescent derivatives. High specificity of such interactions has been demonstrated in the experiments with α7 knock-out mouse [120]. However, since α-bungarotoxin or α-cobratoxin can also bind to α8 and α9/α10 nAChRs, their presence should be checked by independent means. It should be also mentioned that on detecting α7 nAChR in non-neuronal tissues, possible presence of the α1 subunits, which also can bind α-neurotoxins, should be taken into account. It was recently shown that in histochemical experiments staining of muscle nAChR with fluorescent toxin (Alexa-α-bungarotoxin) can be prevented both by short- and long-chain α-neurotoxins, while the interaction with α7 nAChR can be inhibited only by long-chain α-neurotoxins [120].

3.4. Interaction of AChBPs with agonists and antagonists analyzed by spectroscopic approaches

AChBPs are very convenient objects because they are water-soluble, can be prepared in large amounts, and the static pictures obtained by X-ray crystallography of AChBP themselves and of their complexes can be supplemented by studies in solution. It is not only determination of the dissociation and inhibition constants by radioligand analysis and other techniques (for example, isothermal calorimetry or fluorescence spectroscopy), but also detection of conformational changes in the process of AChBP-ligand recognition affecting one or both of the interacting partners and identification of residues in each of them essential for binding. These studies embracing a whole range of agonists and antagonists were done mostly by P. Taylor and his collaborators and were covered in a review [121] in the previous volume of Biochemical Pharmacology devoted to nAChRs. That is why this research will not be discussed here. We will mention here only a few publications. Using the hydrodynamic and fluorescence analysis, it was concluded that α-cobratoxin in the complex with *L. stagnalis* AChBP is not rigidly oriented and possesses a certain degree of flexibility [122]. This result is interesting to compare with molecular modeling of α-neurotoxin interactions with AChBPs and α7 nAChRs [123,124]. In a free form, without adding α-neurotoxins, both the α7 subunits and AChBP protomers manifest a capacity to some movements (slight rotations) which were surmised to be essential for realization of agonist-induced conformational changes and subsequent opening of the channel. Binding of α-neurotoxins fixed the structure of the AChBP or α7 nAChR and prevented their mobility. At the moment it is not clear, how in such a case a toxin itself would demonstrate the above-mentioned mobility. NMR spectroscopy was also applied for studying the AChBP solution conformations. That allowed to assume the multiple conformations of the loop C without acetylcholine. Upon agonist binding the conformation of the loop C becomes restricted [125].

4. X-ray structure of the extracellular domain of muscle nAChR α1 subunit in complex with α-bungarotoxin

Until now we were considering the X-ray and EM analyses of closely related but independent objects of studies: acetylcholine-binding proteins and *Torpedo* nAChR.

The close inter-relationships between AChBPs and all muscle-type and neuronal nAChRs (as well as with other Cys-loop receptors) are beyond any doubts because the chimera of AChBP and transmembrane domains of Cys-loop receptors after minor structural changes manifested a functional receptor activity [126]. However, the structures of complexes of nAChR agonists and antagonists until recently were available only for the AChBPs. That is why when researchers wished to analyze in three dimensions the interactions of ligands (and toxins) with the muscle- or neuronal-type nAChRs, they had to rely on complexes built by computer modeling. Fortunately, one of the bridges between the AChBPs and nAChRs spatial structures has been recently open: the X-ray structure has been determined for the α-bungarotoxin complex with heterologously expressed α1 subunit of mouse muscle nAChR [13].

A number of laboratories wanted to get information about the spatial structure of ligand-binding sites by heterologously expressing the N-terminal ligand-binding domains of α1 subunit of muscle-type nAChRs or of α7 subunit of neuronal nAChRs. In both cases binding of α-neurotoxins, although not with a very high affinity, was the indication that these domains apparently possess a part of functional activities [127–129]. Interactions with toxins could be analyzed not only by radioligand analysis, but also using ^{19}F NMR to detect binding of trifluoroacetylated α-cobratoxin with a monomeric form of α1 *Torpedo* domain [130]. This domain was also obtained in *E. coli* harvested on a medium where all Trp residues were exchanged for 5-F-Trp; unfortunately, the signals in the ^{19}F NMR spectra were too broad to get accurate information

about the spatial structure [130]. Concerning the latter, the only achievement was the demonstration of predominance of β-structure revealed by circular dichroism spectroscopy (see, for example [128]).

In the case of heterologously expressed ligand-binding domain of α7 subunit, the main problem was the formation of high-molecular weight aggregates, rather than of pentamers or monomers. Aggregation could be diminished by [C116S] mutation [131], and usage of this mutation in combination with the substitution of the hydrophobic Cys-loop for a more hydrophilic one from AChBP allowed Tzartos and his colleagues to get as a result of expression in yeast a protein in predominantly pentameric form [132]. Moreover, this protein in electron microscopy showed the rosettes resembling those of the *Torpedo* nAChR. The rosettes were also observed for the protein assembled from the domains of all muscle-type nAChR subunits [133], but none of the described examples ended with a successful crystallization.

In view of the above-said, the work of Dellisanti et al. [13] is clearly a breakthrough. Using random mutagenesis, they have chosen a protein with a low tendency to aggregation. Although it happened to have the mutation of Trp149 (known to be important for binding agonists and antagonists) for Arg, the protein could bind α-bungarotoxin. It was namely the complex of α-bungarotoxin rather than the free domain which the authors managed to crystallize. (Thus, in addition to helping isolate the *Torpedo* nAChR and *L. stagnalis* AChBP, α-neurotoxins played again an important role in crystallization of the nAChR subunit ligand-binding domain.) The structure of the complex has been solved at very high resolution (1.94 Å).

Although this domain is a monomer, its spatial structure is very similar to an AChBP protomer in a pentameric complex. A molecule of bound α-bungarotoxin occupies the position similar to that of α-cobratoxin in complex with *L. stagnalis* AChBP (compare Fig. 2B and C). All the same aromatic amino acids of nAChR (tyrosines 93, 190 and 198) as well as Thr148 and Arg149 interact with Asp30, Phe32 and Arg36 of α-neurotoxin (Fig. 2C). It should be emphasized that in the complex with α1 domain, α-bungarotoxin utilized for interaction only the principal side [13], while α-cobratoxin in complex with the pentameric AChBP has contacts with both principal and complementary sides at the subunit interface. However, instead of this, α-bungarotoxin forms contacts with the sugar moiety present in the nAChR but lacking in AChBPs.

5. X-ray structure of bacterial ligand-gated ion channels: new data on the ligand-binding sites?

The X-ray structures of ligand-gated ion channels from bacteria *Erwinia chrysantemi* and *Gloebacter violareus* (the channels being abbreviated as ELIC and GLIC, respectively) have been recently determined [3–5,134]. The channel in ELIC is in apparently closed state, while it is open in GLIC which can be activated by low pH and does not desensitize. The observed changes between the two structures were interpreted as a result of conformational changes induced by ligand binding and leading to opening of the channel. However, the two proteins are not highly homologous and the respective conclusion would have been more rigorous if the structure of GLIC in the closed state were known.

With the exception of the proton-activated GLIC, ligands of bacterial ligand-gated channels are not known, and at the moment it is difficult to say what new information, useful for design of new agonists and antagonists of different nAChRs, can be extracted from the X-ray structures of bacterial receptors. Although the N-terminal α-helical fragment and two Cys residues (which in other receptors fix the Cys-loop giving the name to the whole family), are lacking, the structure of the ligand-binding domain in ELIC and GLIC is very similar (including the "C-loop") to the structure of AChBPs and to the ligand-binding domain of the *Torpedo* nAChR. The advantage of these structures is that they provide a high-resolution not for the isolated ligand-binding domains (like AChBPs or domain of α1 subunit in complex with α-bungarotoxin), but for the domain within the whole receptor complex. Therefore, when new compounds are found (probably, even peptide and polypeptide neurotoxins) which interact with the bacterial ligand-gated ion channels and the structures of respective complexes are solved, new ideas might appear concerning the design of drugs acting on nAChRs and other true Cys-loop receptors.

6. Summary

As can be seen from this review, peptide and polypeptide neurotoxins played and continue to play an important role in research on nAChRs. Historically, neurotoxins were used for discovery, isolation and characterization of these receptors. Later their interaction with nAChRs and AChBPs provided valuable information about the respective binding sites. Discoveries of new types of polypeptide toxins (non-conventional three-finger toxins, covalently bound α-neurotoxin dimers etc.) widen an array of tools for nAChR studies. Naturally occurring α-conotoxins, their derivatives and analogs are of special interest as they distinguish different subtypes of nAChRs. Moreover, some α-conotoxins also distinguish the two binding sites within one muscle-type nAChR. In summary, one can state that the more neurotoxins are available, the more detailed information about nAChR structure and function can be obtained. There are all reasons to expect that better understanding of the toxin-receptor interactions will lead to development of new drugs having better selectivity to a particular nAChR subtype.

Acknowledgements

The work on this review was supported by FP7 program Neurocypres, by grants of Russian Foundation of Basic Research ##06-04-89400 (NWO), 08-04-00801, 09-04-01061, 09-04-01476, and by RAS grant "Molecular and Cellular Biology".

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