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Review article

α-Conotoxins to explore the molecular, physiological and pathophysiological functions of neuronal nicotinic acetylcholine receptors

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ABSTRACT

The vast diversity of neuronal nicotinic acetylcholine subunits expressed in the central and peripheral nervous systems, as well as in non-neuronal tissues, constitutes a formidable challenge for researchers and clinicians to decipher the role of particular subtypes, including complex subunit associations, in physiological and pathophysiological functions. Many natural products target the nAChRs, but there is no richer source of nicotinic ligands than the venom of predatory gastropods known as cone snails. Indeed, every single species of cone snail was shown to produce at least one type of such α-conotoxins. These tiny peptides (10–25 amino acids), constrained by disulfide bridges, proved to be unvaluable tools to investigate the structure and function of nAChRs, some of them having also therapeutic potential. In this review, we provide a recent update on the pharmacology and subtype specificity of several major α-conotoxins.

1. General introduction about α-conotoxins

Cone snails belong to the large Conoidean group of venomous marine gastropods, comprising Turridae, Terebridae and Conidae mollusks. These predatory snails display complex and specific feeding behavior, whether they prey on worms (vermivorous), mollusks (molluscivorous) or fish (piscivorous). They use fast-acting and often paralyzing venoms that is usually injected into their prey or predator through a hypodermic needle-like modified radula tooth [1]. Cone snail venoms contain a mixture of many diverse compounds, including small molecules, peptides and enzymes, yet peptidic toxins called conotoxins largely dominate these cocktails [2]. Typically, each cone snail venom contains 50–200 major conotoxins, which are mostly small disulfide rich peptides (between 10 and 40 residues and 2–4 disulfilde bonds) that synergize together to induce the rapid immobilization of preys [3]. Disulfide bridges provide exceptional structural stability, enabling a tight interaction with their molecular targets, mainly ion channels, transporters and GPCRs [4]. Conotoxins are classified after i) the signal sequence of their precursors, which defines the gene superfamily (A-Q, S, T, V, Y) ii) their cysteine frameworks, which describes the cysteine pattern (I–XXVI) iii) the pharmacology, which is determined by the pharmacological target (α, γ, δ, ε, ι, κ, μ, ρ, σ, τ, χ, ω) [4]. Among all the different classes of conotoxins, α-conotoxins are the most studied and the most pharmacologically characterized [5,6]. α-Conotoxins target the nicotinic acetylcholine receptors (nAChRs), which are well known targets for other potent animal toxins, such as snake neurotoxins [7]. Whereas a toxin that blocks the muscle type nAChR is an obvious weapon to neutralize preys, there are surprisingly more α-conotoxins that target neuronal nAChRs identified, suggesting that cone snail venoms work in a much more subtle way than simply muscle paralysis. Most of these α-conotoxins act as competitive antagonists of nAChRs, however there are few examples where α-conotoxins show allosteric modulation or even agonist action in certain circumstances. Historically, the bioassays were performed on rat or human nAChRs, although it is expected that conotoxins show a greater potency for their prey's membrane receptors. In this review, we will focus on recent advances on α-conotoxins targeting neuronal nAChRs and their use as pharmacological tools to decipher the function of these receptors in normal and pathological states.

2. Classification and primary structure of α-conotoxins

α-conotoxins usually belong to the A superfamily and present a type I cysteine framework (CC-C-C, ≈75%) or eventually a type IV (CC-C-C-C-C, ≈10%). However conotoxins targeting nAChRs were found outside the A superfamily (≈20%), including nine other superfamilies (B, D, J, L, M, O1, S, T, and a yet unnamed family) with at least seven more cysteine frameworks. The rules established for naming conotoxins are based on what suggested McIntosh et al. Olivera et al. and Gray et al. [8–11]. The first greek letter specify the conotoxin's pharmacological target, a majuscule letter can be added to indicate the superfamily. The next one or two letters stand for the species from which the toxin was

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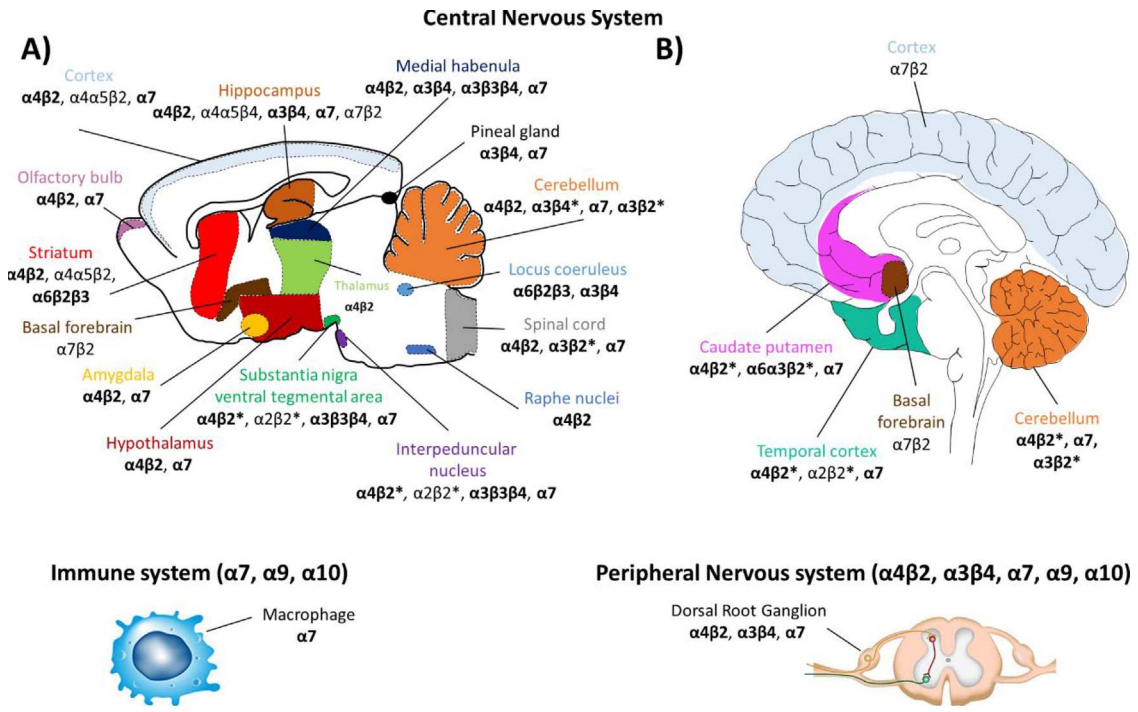

Fig. 1. Distribution of neuronal nAChR subunits in CNS (top panel): A) subtypes identified to date in rodent brain B) subtypes identified to date in human brain, Immune system (left panel) and PNS (right panel). Conotoxin-targeted subunit combinations are shown in bold. Top panel A) was adapted from Gotti et al. [66] and B) from Zoli et al.[147].

discovered. This letter is followed by a roman number that determines the cysteine framework and finally, a capital letter designates the order of the discovery within that category [11]. However, this nomenclature is not always respected for every conotoxins, mainly for historical reasons. In addition, for the conotoxins discovered by sequencing techniques (e.g. cloning or RNAseq), the cysteine framework and order of the discovery of the clone is noted by Arabic numbers separated by a dot [12]. The classic α-conotoxin contains 12–20 residues with four cysteines connected to form the "globular fold" (Cys_1-Cys_3/Cys_2-Cys_4). They can be further classified based on the number of amino acids in between the cysteine residues (loops). Interestingly, in most case the size and the nature of these cysteine loops seem to predispose the specificity of the α-conotoxin for α-homomeric (3/4), muscle-type (3/5), or neuronal (4/4, 4/6, and 4/7) nAChR subtypes [13].

3. α-conotoxins as probes for α7 nAChRs

Homomeric α7 neuronal nAChR has been intensely studied since its original discovery [14]. In the central nervous system, this subtype is mainly distributed in the hippocampus and the cerebral cortex, in regions associated with learning and memory mechanisms [15] (Fig. 1). Major human pathologies such as epilepsy, myasthenic syndromes, schizophrenia, Parkinson's and Alzheimer's diseases result from a dysfunction of the α7 nAChRs [16,17]. The α-conotoxin's binding sites are localized at the interfaces between identical α7 subunits. Like the snake neurotoxin α-bungarotoxin, which has been used to determine the precise location of α7 nAChRs at cellular and subcellular levels [18], the high selectivity of some α-conotoxins for α7 nAChRs can provide unique opportunities for deciphering the complex pharmacology of these receptors. One unusual example is the [A10L]PnIA which has been described by Hogg et al. as acting as an antagonist on the wt-α7 but as an agonist on the α7-L247T mutant [19]. One plausible hypothesis is that [A10L]PnIA mutant has the capacity to stabilize the desensitized form of the receptor, which is not conducting for the wt-α7 but conducting for the α7-L247T mutant. Since many channelopathies are due to a mutation that modifies the biophysical properties of the channel, such α-conotoxins that can act differently on mutated neuronal nAChRs (agonist versus antagonist) may have potential as therapeutics [20]. Ellison et al. reported α-conotoxin ImII [21] to be structurally closely related to previously described α-conotoxin ImI [22] although they both block α7 with similar affinity (respectively 571 nM and 595 nM). However, in contrary to α-conotoxin ImI, α-conotoxin ImII does not compete with radiolabeled α-bungarotoxin (a classical competitive inhibitor of the α7 nAChR). This suggests that the binding site of ImII in α7 is different from that of α-bungarotoxin (at the interfaces between identical α7 subunits) and ImI. Indeed, two mutations (Q117S and N111S) within the orthosteric ligand binding site of the α7 nAChR were shown to strongly affect antagonism potency of α-ImI but not α-ImII [23]. Therefore, α-conotoxin ImII represents a unique probe for targeting a novel antagonist-binding site, or microsite, on the α7 nAChR. α-conotoxin ImI has been used as a template molecule for the design of synthetic analogues with a significant higher binding affinity and antagonist activity at the α7 nAChR [24]. For instance, [P6/5-(R)-Phenyl]ImI exhibited a K_i value of 0.19 μM at the α7/5-HT_{3A} (*vs.* 1.2 μM for the wild type) and an IC_{50} value of 0.7 μM (*vs.* 2.6 μM for the wild type) at the α7. The authors demonstrated, by studying the binding site with the AChBP, that there were additional hydrophobic interactions at proline-modified residue in position 6. Armishaw et al. used combinatorial chemistry to generate many synthetic variants of α-conotoxin ImI and three analogs containing the Nva9–Dmt10–His11 (Dmt: 2,6-dimethyltyrosine), Leu9–Aph10–Abu11 (Abu: α-aminobutyric acid), and Nva9–Dmt10–Trp11 combinations exhibited ~12-, 14- and 10-fold increases in α7 nAChR inhibition respectively, when compared to wild type ImI [25]. α-conotoxin ArIB was synthesized directly from a cDNA library of *Conus arenatus* and its synthetic variant [V11L,V16D]ArIB was 800- to > 1000-fold more potent against α7 than all other nAChR [26] (Table 1). This synthetic mutant has been found to reduce both the lactate dehydrogenase level in tumor cells and the inflammatory leukocyte infiltration in tumor tissue in mice [27]. In addition, ^{125}I-α-conotoxin [V11L,V16D]ArIB was proposed and validated as an alternative to radiolabeled α-bungarotoxin as pharmacological tool [28].^{125}I-α-conotoxin [V11L,V16D]ArIB is a highly α7-selective conotoxin confirmed by autoradiography using α7-null mutant tissue. Because the ^{125}I-α-conotoxin [V11L,V16D]ArIB