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Stoichiometry dependent inhibition of rat α3β4 nicotinic acetylcholine receptor by the ribbon isomer of α-conotoxin AuIB

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ABSTRACT

The ribbon isomer of α-conotoxin AuIB has 10-fold greater potency than the wild-type globular isomer at inhibiting nicotinic acetylcholine receptors (nAChRs) in rat parasympathetic neurons, and unlike its globular isoform, ribbon AuIB only targets a specific stoichiometry of the α3β4 nAChR subtype. Previous electrophysiological recordings of AuIB indicated that ribbon AuIB binds to the α3(+)α3(−) interface within the nAChR extracellular domain, which is displayed by the $(\alpha3)_3(\beta4)_2$ stoichiometry but not by $(\alpha3)_2(\beta4)_3$. This specificity for a particular stoichiometry is remarkable and suggests that ribbon isoforms of α-conotoxins might have great potential in drug design. In this study, we investigated the binding mode and structure-activity relationships of ribbon AuIB using a combination of molecular modeling and electrophysiology recording to determine the features that underpin its selectivity. An alanine scan showed that positions 4 and 9 of ribbon AuIB are the main determinants of the interaction with $(\alpha3)_3(\beta4)_2$ nAChR. Our computational models indicate that the first loop of ribbon AuIB binds in the "aromatic box" of the acetylcholine orthosteric binding site, similar to that of globular AuIB. In contrast, the second loop and the termini of the ribbon isomer have different orientations and interactions in the binding sites to those of the globular isomer. The structure-activity relationships reported herein should be useful to design peptides displaying a ribbon α-conotoxin scaffold for inhibition of nAChR subtypes that have hitherto been difficult to selectively target.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated cation-selective ion channels that belong to the Cys-loop family of receptors [1]. Neuronal nAChRs are homo- or heteropentamers of α2–α10 and β2–β4 subunits, and the different pentameric isoforms are differentially expressed in different parts of the brain [2,3]. Neuronal nAChR isoforms are involved in a range of diseases and conditions, including Alzheimer's disease, Parkinson's disease, schizophrenia, epilepsy, nicotine addiction, anxiety, depression and pain [3].

Heteropentameric nAChRs can assemble into different stoichiometries, with some displaying diverse pharmacological and biophysical properties [3]. For example, the α4β2 nAChR subtype, which is the most abundant nAChR subtype in the human brain, exists in two main stoichiometries: $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$ [4]. These two stoichiometries have contrasting pharmacological properties: the $(\alpha4)_2(\beta2)_3$ has a long open lifetime and is insensitive to the agonist NS-9283, whereas the $(\alpha4)_3(\beta2)_2$ has a short open lifetime and is potentiated by NS-9283 [4]. Subunit stoichiometries of other nAChR subtypes such as the α3β4, α7β2 and α9α10 nAChR have also been reported [5–7]. Although the various subunit stoichiometries of these nAChR subtypes have disparate functions, the relationship between the different functions and pathophysiology states remains unknown, mostly because detecting nAChR subtype stoichiometries is challenging. In this study we established some groundwork on the structure-activity relationship (SAR) of the ribbon isoform of α-conotoxin AuIB (rAuIB) [8,9], which specifically inhibits one of the two major stoichiometries of the α3β4 nAChR [10].

Structurally, nAChRs are composed of three domains: an extracellular domain (ECD), a transmembrane domain (TMD), and an intracellular domain (ICD), as shown in Fig. 1 [1,11]. Acetylcholine (ACh) binds at the interface between two subunits in the ECD, triggering the opening of the channel [1]. Each subunit in the ECD is composed of one α-helix, which lines the pore, and two β-sheets made of 10 β-strands (Fig. 1A and B). The ACh orthosteric binding site is

Abbreviations: ACh, acetylcholine; AChBP, acetylcholine-binding protein; ECD, extracellular domain; gAuIB, globular AuIB; ICD, intracellular domain; nAChR, nicotinic acetylcholine receptor; rAuIB, ribbon AuIB; SAR, structure-activity relationship; TMD, transmembrane domain

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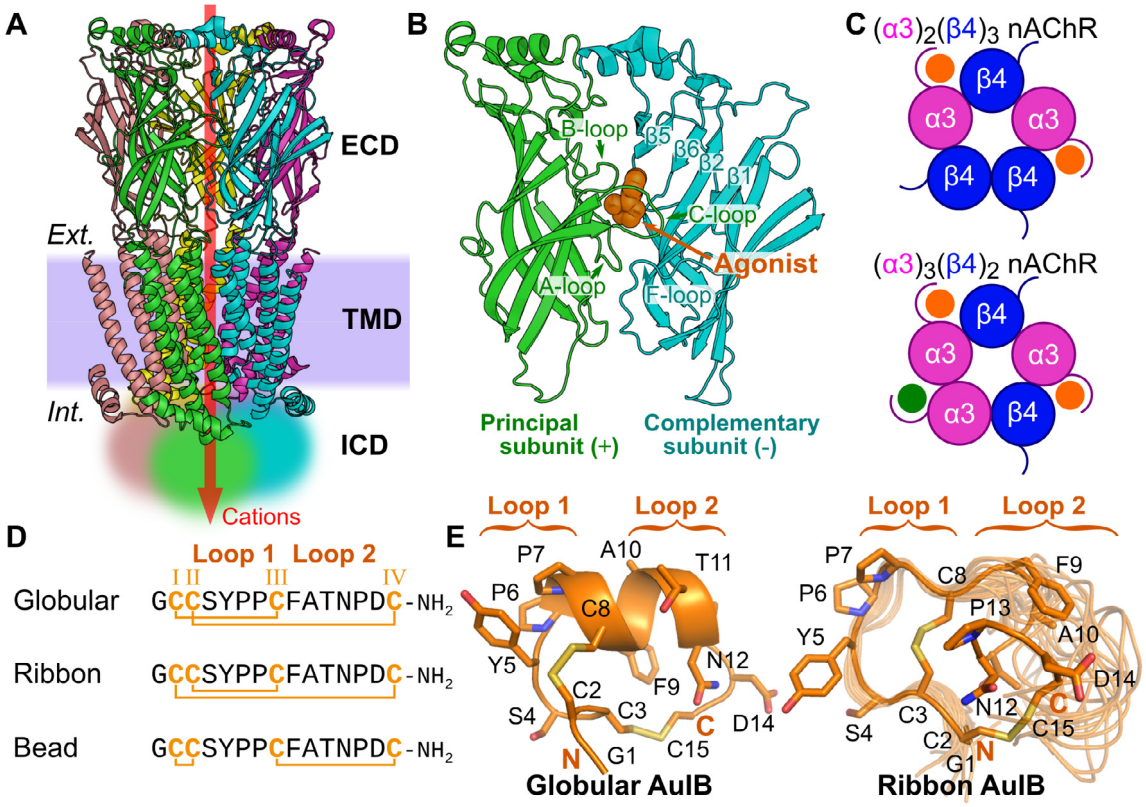

Fig. 1. Overview of the three-dimensional structure of nAChRs, the two main stoichiometries of α3β4 nAChRs, the three disulfide isomers of AuIB and the NMR spectroscopy solution structures of ribbon AuIB (rAuIB) and globular AuIB (gAuIB). A: The structure of nAChRs comprises three domains: an extracellular domain (ECD), a transmembrane domain (TMD), and an intracellular domain (ICD; ICD has not been resolved in the crystal structure). An arrow indicates the direction of cation movement through the central pore. The ribbon representation of the nAChR used the coordinates from the X-ray crystallography structure of the α4β2 nAChR (PDB: 5KXI). The membrane is represented by a purple rectangle and the extracellular (*Ext.*) and intracellular (*Int.*) sides of the membrane are indicated. B: Structure of the agonist binding site in the ECD, which is at the interface between a "principal" subunit (green) and a "complementary" subunit (cyan). The binding site is occupied by nicotine (orange). The ECD of each subunit displays one α-helix and 10 β-strands (β1 to β10), and the A-, B- , C- and F-loops are indicated. C: Illustration of the two main stoichiometries of homopentameric α3β4 nAChRs and identification of the functional binding sites. The illustration represents the nAChR as seen from the extracellular side and parallel to the membrane; the pore is in the center of the pentamer. The C-loop of each subunit is indicated by a curved line. The α3(+)β4(−) and α3(+)α3(−) agonist binding sites are indicated by orange and green discs, respectively. D: Amino acid sequence of the disulfide isomers of AuIB. Cys residues are numbered with Roman numerals (I–IV) and the disulfide bonds are shown as orange sticks. E: Three-dimensional NMR solution structures of gAuIB (PDB: 1MXN) and rAuIB (PDB: 1MXP). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

located at the interface between two subunits in the ECD; the "principal" subunit, denoted (+), contacts the agonist with the A-, B- and C-loops, and the "complementary" subunit, denoted (−), contributes to the ACh binding site through the β-strands 2, 5 and 6 (Fig. 1B). Functional binding sites cannot be formed with β subunits as the principal subunit; therefore the number of functional binding sites displayed by heteropentameric nAChRs depend on their stoichiometries. As illustrated in Fig. 1C, the $(\alpha3)_2(\beta4)_3$ nAChR subtype has two α3(+)β4(−) functional binding sites, and the $(\alpha3)_3(\beta4)_2$ subtype has an additional functional binding site between two α3 subunits, i.e. α3(+)α3(−) [6]. Concatemeric assemblies of α3 and β4 subunits with the arrangements described in Fig. 1C were compared for their pharmacological properties to subtypes assembled freely from subunits expressed in oocytes [12]. We note that in the current study we focused on engineered α3-containing nAChRs of these compositions but in native tissue the ganglionic nAChRs are more complex and could include accessory subunits of α3, α5, α6, β2 or β4 [13].

Conotoxins are a large family of disulfide-rich peptides isolated from the venom of marine cone snails [14,15]. Due to their potency and exquisite selectivity for ion channels and transporters of the nervous system, these peptides are considered as valuable pharmacological tools and drug leads [16]. The largest characterized pharmacological class of conotoxins are the α-conotoxins, which inhibit nAChRs and typically have 12–20 amino acid residues [14,16]. Because of their small size, α-conotoxins can readily be chemically synthesized, and their selectivity and potency against important nAChR subtypes make them interesting candidate drug leads [16–18]. Most α-conotoxins have the cysteine pattern CC-C-C, corresponding to the conotoxin cysteine Framework I. These conotoxins are further classified into *m/n* groups according to the length of the two loops, which are defined as the segments between Cys II and Cys III (loop 1, *m* residues) and between Cys III and Cys IV (loop 2, *n* residues) [19,20]. The four Cys residues can theoretically form three disulfide connectivities, resulting in the globular isomer (Cys I-III, Cys II-IV), ribbon isomer (Cys I-IV, Cys II-III) or bead isomer (Cys I-II, Cys III-IV) (Fig. 1D). The globular isomer is typically displayed by wild-type α-conotoxins isolated from venoms, apart from one instance where the ribbon connectivity of an α-conotoxin was detected in the venom of *Conus imperialis* [21]. Some ribbon isomers are as active as or more potent than the globular isomer at inhibiting nAChRs, and consequently display altered pharmacological properties [8,22].

α-Conotoxin AuIB (Fig. 1D and E) belongs to the 4/6 class of conotoxins [9], and the globular form (gAuIB) inhibits the rat α3β4 nAChR with an IC_{50} of 1–3 μM [10]. rAuIB was the first ribbon isomer reported to have higher potency than the globular isomer, with rAuIB displaying a 10-fold improved inhibition of Ach-evoked current in rat parasympathetic neurons compared to gAuIB, with IC_{50}s of 0.1 nM and 1.2 nM, respectively [8]. However, another study reported that gAuIB was more potent than rAuIB at inhibiting rat α3β4 nAChR expressed in