Identifying key amino acid residues for α-conotoxin AuIB inhibition of α3β4 nicotinic acetylcholine receptors

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Neuronal nicotinic acetylcholine receptors (nAChR) are a family of ligand-gated cation channels expressed in the central and peripheral nervous systems. They are involved in numerous physiological functions and are activated by the endogenous neurotransmitter acetylcholine (ACh). While all nAChRs have a similar pentameric structure, each subtype has specific pharmacological and functional properties due to the different subunit combinations that can be formed.

α-Conotoxin AuIB, isolated from the marine snail *Conus aulicus*, is a peptide consisting of 15 amino acid residues with a 4/6 intercysteine spacing. AuIB has been characterized on oocyte-expressed nAChRs and was revealed as a selective antagonist primarily for the nAChR subtype α3β4 (Luo *et al.*, 1998). As well as its activity on the α3β4 nAChR, AuIB recently was shown to inhibit voltage-gated N-type calcium channels by activating G protein-coupled $GABA_B$ receptors (Klimis *et al.*, 2011; Cuny *et al.*, 2012). Interestingly, AuIB is one of a few α-conotoxins with analgesic activity *in vivo*, so it is a potential drug lead for the development of treatments for neuropathic pain. To develop better drugs based on AuIB but without side effects, we must understand how AuIB binds to the $GABA_B$ receptor and α3β4 nAChR.

This study aimed to identify the amino acid residues responsible and critical for interaction between AuIB and the α3β4 nAChR. Alanine scanning mutagenesis of AuIB and pharmacological testing on *Xenopus* oocytes expressing α3β4 nAChRs indicated that the phenylalanine at position 9 (Phe9) is critical for AuIB inhibition of ACh-evoked currents mediated by α3β4 nAChRs. Homology modelling and molecular dynamics (MD) simulations suggested that interaction between the Phe9 of AuIB and a binding pocket formed by Trp79 and Lys81 on the β4 subunit of α3β4 nAChR may be pivotal for this inhibition. Site-directed mutagenesis of Trp79 and Lys81 on the β4 subunit confirmed they are key residues for AuIB interaction with the receptor. It also suggested that a hydrophobic interaction between AuIB and Trp79 is important.

We substituted Phe9 on AuIB with various residues to confirm its hydrophobic interaction with the binding pocket on α3β4 nAChR. Analysis using these AuIB-analogues revealed that the size of the hydrophobic residue at position 9 in AuIB strongly affects its interaction with the binding pocket. We also analysed the molecular interaction of AuIB with the α3 subunit using homology modelling and MD simulations. Experiments to validate the model included testing AuIB analogues on other nAChR subtypes. This study revealed that α-conotoxin AuIB binds to a different domain from the agonist (ACh) binding domain on the α3β4 nAChR. We propose a mechanism for α3β4 nAChR inhibition by identifying the key interacting residues of AuIB and the α3β4 nAChR.

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