strong evidence that non-competitive binding sites exist at non-α interfaces of heteromeric nAChRs. Furthermore, non-competitive antagonist binding of natural alkaloids has been shown to occur at the channel pore. In previous studies using Analogue 1, the ion channel pore appears to be the main binding site for this compound. In contrast Analogue 2 does not bind to this site. Here, we report studies of the non-competitive binding site within the N-terminal domain on the α4β2 nAChR. Since this β(−) α(+) subunit interface of the N-terminal domain is not well studied, water accessibility of the residues was first examined using the Substituted Cysteine Accessibility Method (SCAM). The residues on Loop D (N88, V89, W90, V91, K92, Q93 and E94) of the α4 subunit and Loop A (V116, V117, L118, Y119, N120, N121, A122, D123 and G124) of the β2 subunit were individually mutated to cysteine, expressed in *Xenopus* oocytes and analysed using two-electrode voltage clamp recordings. Surface accessibility was tested by evaluating the reaction of sulfhydryl reagent ethylammonium-methanethiosulfonate (MTSEA) in the opened (in the presence of ACh) and closed channel states (in the absence of ACh). The site was then evaluated using two methods: (1) The antagonists were competed with the sulfhydryl reagents where protection from irreversible inhibition infers the binding site. (2) Analogue 1 and 2 were synthesized into a thiol reactive probe capable of reacting with cysteine directly. Irreversible inhibition infers the binding site. All mutants generated functional receptors and most were accessible to MTSEA. Both competition and reactive probe experiments showed that neither of these analogues bind within the N-terminal domain. Other loops within the non-competitive β(−) α(+) interface and the competitive α(+) β(−) interface will be studied in the future.

doi:10.1016/j.bcp.2009.06.045

1.17

Mutation of proline enables subtype selectivity of α-conotoxin BuIA

Yelena Filchakova*, Amy B. Godoy, Sean Christensen, J. Michael McIntosh

University of Utah, Department of Biology, 257 South 1400 East, Salt Lake City, UT, United States

α-Conotoxins are neuroactive peptides, isolated from the venom of carnivorous snails that act as competitive antagonists of nicotinic acetylcholine receptors (nAChRs). α-Conotoxins are small peptides that have two cysteine loops and a highly conserved proline (Pro) in the first loop. Crystal structures of α-conotoxins in complex with the acetylcholine binding protein (AChBP) show that the α-conotoxin Pro side chain is positioned to potentially interact with the ACh binding pocket. BuIA is a 13 amino acid α–conotoxin that kinetically discriminates between β2- and β4-containing nAChRs; the off-rate of BuIA is slow for all β4- vs. β2- containing nAChRs. Three residues on the β subunit at positions 59, 111 and 119 are critical for binding of some α-conotoxins. These residues line the putative acetylcholine-binding pocket and differ between β2 and β4 nAChR subunits. Site-directed mutagenesis has demonstrated that Thr59 is an important determinant of sensitivity for α-conotoxins as well as other competitive antagonists. Homology modeling studies with the AChBP identified Val111 and Phe119 as likely residues interacting with α-conotoxins MII and PnIA. BuIA contains two Pro residues. In the present study we explored the role of the BuIA Pro residues in the ability of BuIA to discriminate between β2- and β4-containing nAChRs. We hypothesized that Pro6 and/or Pro7 interacts with non-conserved residues on the nAChR β subunit and through this interaction influences the subtype selectivity of BuIA. BuIA as well as BuIA analogs were synthesized using Fmoc chemistry. Pro6 or Pro7 was substituted with 4-*trans* hydroxyproline or 3-*trans* hydroxyproline. nAChR residues present in the β4 subunit were introduced as point mutations in the homologous positions in the β2 subunit. These mutations included β2T59K, β2V111I and β2F119Q. In addition, one mutant β4 subunit was made, β4K59T. Two-electrode voltage clamp of oocytes injected with cRNA encoding wild type and mutant nAChRs was used to assess the activity of the conotoxin analogs. The interaction between the α-conotoxin BuIA analogs and the β subunit of the nAChR was assessed by double-mutant cycle analysis; pair-wise interaction energies of Pro6 and Pro7 with nAChR residues (at positions 59, 111 and 119) were determined. Pro6 interacts with Thr59 (on the β2 subunit) with a coupling energy of 2.4 kcal/mol and Pro6 interacts with Lys59 (on the β4 subunit) with a coupling energy of 2.6 kcal/mol (energies are absolute values). The introduction of 4-*trans* hydroxyproline in the 6th position selectively decreased binding of BuIA to α3β2 nAChRs thus enabling BuIA to selectively block α3β4 vs. α3β2 nAChRs (Fig. 1). Pro6 thus represents an amino acid that may be mutated to create α-conotoxins with improved subtype selectivity.

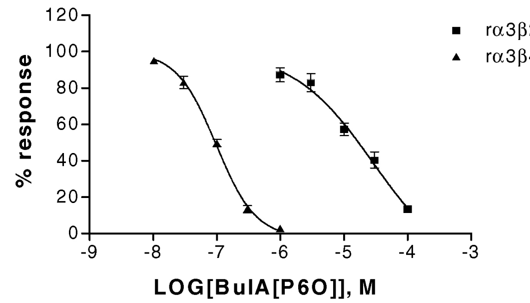

Fig. 1. Presence of hydroxyproline enables α-conotoxin BuIA to discriminate between rα3β2 and rα3β4 nAChRs.

Acknowledgements: This work was funded by NIH grants MH53631 and GM48677.

doi:10.1016/j.bcp.2009.06.046

1.18

Isoanatabine, a naturally occurring α4β2 nicotinic receptor agonist

H. Xing*, A. Rouchaud, S. Keshwah, W.R. Kem

Dept Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, FL32610-0267, United States

Anabaseine was the first nemertine alkaloid to be isolated and pharmacologically characterized; benzylidene-substituted anabaseines including DMXBA (GTS-21) are being investigated as potential therapeutic agents for treating cognitive dysfunction (Kem, 2000; Freedman et al., 2008). Here we examine the pharmacological properties of isoanatabine [2-(3-pyridyl)-1,2,5,6-tetrahydropyridine], an anabaseine isomer, that was isolated from a different species of nemertine. Enantiomers of synthetic isoanatabine and anatabine were obtained by chiral HPLC. Functional properties (EC_{50} and I_{max}) were assessed on *Xenopus* oocytes ($n \geq 4$)) using 100 μM (alpha4beta2) or 1000 μM (alpha7) ACh as standards; α4β2 nAChR binding was measured by displacement of [3H]-cytisine using rat brain membranes. Data were fitted with Prism software, to yield calculated properties shown below: