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The binding orientation of epibatidine at α7 nACh receptors

Andrew J. Thompson [b, *], Simon Metzger [a], Martin Lochner [a, c], Marc-David Ruepp [a, **]

[a] Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland
[b] Department of Pharmacology, University of Cambridge, Cambridge, UK
[c] Institute of Biochemistry and Molecular Medicine, University of Bern, Bern, Switzerland

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ABSTRACT

Epibatidine is an alkaloid toxin that binds with high affinity to nicotinic and muscarinic acetylcholine receptors, and has been extensively used as a research tool. To examine binding interactions at the nicotinic receptor, it has been co-crystallised with the structural homologue acetylcholine binding protein (AChBP; PDB ID 2BYQ), and with an AChBP chimaera (3SQ6) that shares 64% sequence identity with the α7 nACh receptor. However, the binding orientations revealed by AChBP co-crystal structures may not precisely represent their receptor homologues and experimental evidence is needed to verify the ligand poses. Here we identify potential binding site interactions between epibatidine and AChBP residues, and substitute equivalent positions in the α7 nACh receptor. The effects of these are probed by [^{3}H]epibatidine binding following the expression α7 nACh receptor cysteine mutants in HEK 293 cells. Of the sixteen mutants created, the affinity of epibatidine was unaffected by the substitutions Q55C, L106C, L116C, T146C, D160C and S162C, reduced by C186A and C187A, increased by Q114C and S144C, and abolished by W53C, Y91C, N104C, W145C, Y184C and Y191C. These results are consistent with the predicted orientations in AChBP and suggest that epibatidine is likely to occupy a similar location at α7 nACh receptors. We speculate that steric constraints placed upon the C-5 position of the pyridine ring in 3SQ6 may account for the relatively poor affinities of epibatidine derivatives that are substituted at this position.

1. Introduction

α7 nACh receptors are considered to be valuable drug targets as they modulate many physiological responses and are associated with neurological disorders and pathological conditions such as Alzheimer's, Parkinson's and schizophrenia (Hernandez and Dineley, 2012; Mazurov et al., 2011; Thomsen et al., 2010). Consequently there have been continued efforts to develop new ligands that target these receptors, both as drug candidates and as pharmacological research tools. Knowledge of how the ligands orientate in the receptor binding site can provide insights into where modifications might be tolerated. Such changes can be used to improve their pharmacological properties and aid the coupling of functional groups such as fluorescent labels, thiol-reactive moieties and photo-affinity probes (Lochner and Thompson, 2015).

α7 nACh receptors belong to the Cys-loop family of transmembrane ligand-gated ion-channels that are responsible for fast synaptic neurotransmission in the central and peripheral nervous systems. All members of this family are composed of five subunits, each of which contains an extracellular, a transmembrane and an intracellular domain (Thompson et al., 2010; Unwin, 2005). Competitive ligands such as acetylcholine or epibatidine bind at an extracellular, orthosteric binding site that is located at the interface of two adjacent subunits (Fig. 1). This site is a hydrophobic cavity created by the convergence of loops A – C from the principal subunit and loops D – F from the complementary subunit (Fig. 1). However, owing to the difficulty of crystallising membrane proteins, a soluble structural homologue (acetylcholine binding protein; AChBP), has been commonly used to study the binding orientations of α7 nACh receptor ligands. Examples include the

Abbreviations: 5-HT, 5-hydroxytryptamine; nACh, nicotinic acetylcholine; GABA, gamma-aminobutyric acid; HEK, human embryonic kidney; AChBP, acetylcholine binding protein; 5HTBP, an AChBP mutant modified to resemble the 5-HT$_3$R binding site.

* Corresponding author. Department of Pharmacology, Tennis Court Road, Cambridge CB2 1PD, UK.
** Corresponding author. Departement für Chemie und Biochemie, Universität Bern, Freiestrasse 3, Bern CH-3012, Switzerland.
E-mail addresses: ajt44@cam.ac.uk (A.J. Thompson), marc.ruepp@dcb.unibe.ch (M.-D. Ruepp).

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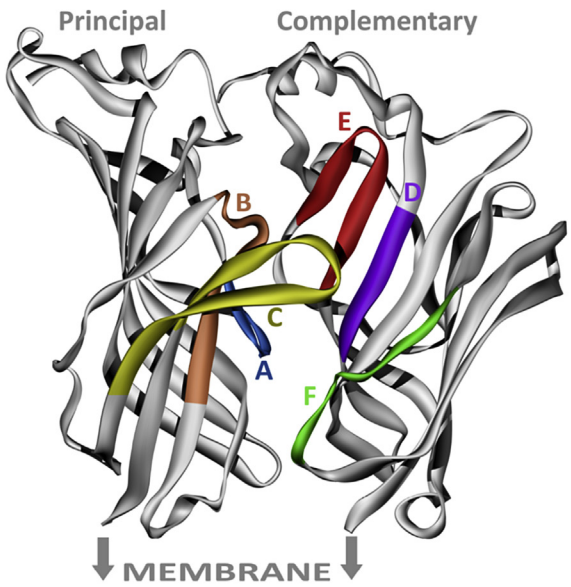
A



B

```
                                                  Loop D
                        30        40        50        60        70        80
α7m5HT3          SQPLTVYFTLSLMQIMDVDEKNQVLTTNIWLQMYWTDHYLQWNVSEYPGVKNVRFPDGLI
3SQ6             R-PVTVYFSLSLLQIMDVDEKNQVVDVVFWLQMSWTDHYLQWNVSEYPGVKQVSVPISSL
2BYQ             --PLTVTLGFTLQDIVKADSSTNEVDLVYYEQQRWKLNSLMWDPNEYGNITDFRTSAADI

                 Loop A                Loop E
                        90       100       110       120       130       140
α7m5HT3          WKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRWFPFDVQKCNLK
3SQ6              WVPDLAAYNAISK-PEVLTPQLALVNSSGHVQYLPSIRQRFSCDVSGVDTESGAT-CKLK
2BYQ              WTPDITAYSSTRP-VQVLSPQIAVVTHDGSVMFIPAQRLSFMCDPTGVDSEEGAT-CAVK

                 Loop B                Loop F                Loop C
                       150       160       170       180       190
α7m5HT3          FGSWTYGGWSLDLQMQEADISG------YISNGEWDLVGIPGKRTESFYECCKEPYPDIT
3SQ6             FGSWTHHSRELDLQMQEADISG------YIPYSRFELVGVTQKRSERFYECCKEPYPDVT
2BYQ             FGSWVYSGFEIDLKTDTDQVDLSS----YYASSKYEILSATQTRQVQHYSCCPEPYIDVN
```

C

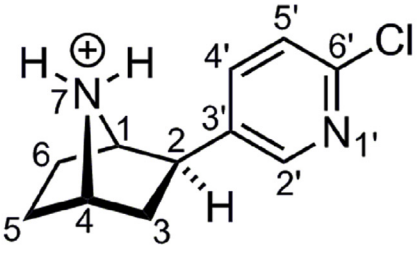


Fig. 1. Locations of residues mutated in this study. **A**. A cartoon showing the binding site of AChBP with binding loops A − F highlighted in colour. The binding site is found at the interface of two adjacent subunits, and for clarity only two of the five subunits of the native receptor are shown. The structure is 3SQ6, but the location of the membrane has been indicated to show where it would be located in the α7 nACh receptor. **B**. An amino acid sequence alignment of the α7 nACh receptor and sequences from the two crystal structures (2BYQ & 3SQ6) in which epibatidine has been co-crystallised. The binding loops are shown in the same colours as in panel A, and amino acids that are within 5 Å of epibatidine are highlighted as white text in grey boxes on the AChBP structures. The equivalent α7 nACh residues substituted in this study are shown as white text in black boxes. α7m5HT3 = chick α7nACh chimaera; 3SQ6 = *Ls*-AChBP-α7 nACh chimaera; 2BYQ = *Ac*-AChBP. The residue numbering used in this manuscript corresponds to residues in the crystal structure 3SQ6. 2BYQ and 3SQ6 share 31.8% identity and 49.8% similarity in their sequences (EMBOSS Needle; Rice et al., 2000). **C**. The structure and atom numbering of protonated epibatidine is shown below the alignment.