

CRITICAL ROLE OF THE C-TERMINAL AMINO ACIDS OF THE HUMAN 5-HYDROXYTRYPTAMINE_{3A} RECEPTOR SUBUNIT

Amy S. Butler, Sarah A. Lindsay, Alice C. Dutton, Terri J. Dover, Anthony G. Hope and Nicholas M. Barnes. Cellular and Molecular Neuropharmacology Research Group, Department of Pharmacology, Division of Neuroscience, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT U.K.

The human (h) 5-HT_{3A} receptor forms a functional homomeric receptor (Barnes and Sharp, 1999). The homomeric nature of the receptor provides a useful model to study the cys-cys loop ligand-gated ion channel family, which includes the nicotinic acetylcholine receptor. Whilst the N-terminal domain, the putative second transmembrane domain and the large intracellular loop are recognised important components for function in this family (Karlin, 2002), the relatively short extracellular C-terminus has received little attention, although the present study indicates a significant role.

Specific mutations or stop codons were introduced into a myc-tagged h5-HT_{3A} subunit (Monk et al., 2004). Radioligand binding analysis ([³H]-granisetron; 1-5 nM; non-specific binding defined by ondansetron; see Monk et al., 2004) assessed the formation of a binding site in COS-7 cells transiently transfected with appropriate clones (see Monk et al., 2004). Cell membrane expression (COS-7 cells) for each h5-HT_{3A} construct was identified by immunocytochemistry (see Monk et al., 2004). The molecular weight of expressed h5-HT_{3A} constructs was assessed by SDS-PAGE/Western blotting (see Monk et al., 2004) and the molecular weight of the receptor complex was estimated by centrifugation (35,000 x g, 17 hrs) through a continuous sucrose density gradient (2.5-50%). 5-HT₃ receptor mediated [Ca²⁺]_i mobilisation was assessed, in HEK 293 cells, using a FlexStation with Fluo-4 as the Ca²⁺-sensitive dye.

Deleting the C-terminal 3 amino acids (-QYA; 4TM) of the h5-HT_{3A} subunit resulted in the loss of [³H]granisetron specific binding (wild type h5-HT_{3A}=1872 ± 330 fmol/mg, 4TM 0.8±0.3 % of wild-type levels, mean±SEM, n=9). Similarly a loss of cell membrane expression and [Ca²⁺]_i rise in response to 5-HT. Interestingly, mere removal of the C-terminal alanine also resulted in a considerable reduction in [³H]granisetron binding and cell membrane expression. The structural importance of the terminal alanine was emphasised by the consequences of mutation to glycine, valine and leucine; greater reductions in [³H]granisetron binding and cell membrane expression were evident with the substitution for the larger amino acid. The molecular weight of the h5-HT_{3A} receptor complex supported the formation of a pentamer (294±14.5 kDa, mean±SEM, n=11); similarly, evidence for a similar sized protein complex resulting from 4TM was apparent although this latter construct also gave rise to relatively greater proportions of protein likely expressed as subunit monomers.

These data demonstrate an important role for the C-terminus of the h5-HT_{3A} subunit.

Barnes NM & Sharp T (1999) *Neuropharmacology*, **38**:1083-1152. Karlin A (2002) *Nature Rev. Neurosci.*, **3**: 102-114. Monk SA, Williams JM, Hope AG & Barnes NM (2004) *Biochem. Pharmacol.*, **68**: 1787-1796.

Supported by The Wellcome Trust.