

Probing the function of residue 3.60 in peptide ligand-GPCRs

Richard Logan, Rachel Kendrick, Mark Wheatley. *University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK*

With the increasing availability of GPCR crystallographic data, identification of structural elements within loop regions is now possible. Secondary structure is present in both extracellular loops (reviewed in Wheatley *et al.*, 2012) and intracellular loop regions of GPCRs. Intracellular loop 2 (ICL2) has been revealed to contain a short helical segment in many GPCRs. This helix appears to be a dynamic feature, given that distinct ICL2 conformations are observed for the dopamine D3 receptor in each of the two receptor molecules of the crystallographic asymmetric unit (Chien *et al.*, 2010). It is noteworthy that in monoamine ligand-GPCRs, residue 3.60 located centrally within ICL2 is almost exclusively tyrosine. In contrast, tyrosine is excluded at this position in peptide ligand-GPCRs. The role of this residue was investigated in this study.

A QuikChange™ (Stratagene) site-directed mutagenesis strategy was used to generate point mutations at position 3.60 in N-terminal HA-tagged peptide-ligand GPCRs within mammalian expression vector pcDNA3.1(+) (Invitrogen). The human ghrelin receptor (ghrelin-R) and human vasopressin 1a receptor (V_{1a}R) are both G_{q/11} coupled peptide-ligand GPCRs with substantial constitutive activity (CA) (50-60% of E_{max}), and little/no constitutive activity, respectively. The contribution of the side chain properties at position 3.60 to receptor structure and function were assessed by competition binding assay (V_{1a}R constructs only) and inositol phosphates accumulation assay of transiently transfected HEK293T cells. Cell-surface expression was determined by ELISA. All data are expressed as mean ± s.e.m. of experiments performed in triplicate.

V_{1a}R receptor constructs T3.60A and T3.60S represent commonly observed residues at position 3.60 in peptide ligand-GPCRs and demonstrated wildtype-like function. T3.60F was also well tolerated while the substitution T3.60Y, typical of monoamine ligand-GPCRs, demonstrated increased cell-surface expression compared to wildtype (152 ± 3%). Ghrelin-R constructs A3.60F and A3.60Y possessed severe decreases in CA of 87 ± 14% and 71 ± 18% respectively, but exhibited wildtype cell-surface expression. Constructs were dose-responsive to ghrelin challenge but maximum signalling was impaired in A3.60Y (63 ± 11% of wildtype E_{max}) whereas A3.60F was wildtype-like.

In conclusion, introduction of Tyr^{3.60}, typical of monoamine ligand-GPCRs, apparently stabilised the V_{1a}R structure leading to increased cell-surface expression. In the ghrelin-R, an aromatic ring at position 3.60 (Phe) reduced constitutive signalling, whereas the additional presence of a hydroxyl (Tyr) also reduced maximal signalling.

Chien EY, Liu W, Zhao Q, Katrich V, Han GW, Hanson MA *et al.* (2010). Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science* 330: 1091–1095

Wheatley M, Wootten D, Conner MT, Simms J, Kendrick R, Logan R *et al.* (2012) Lifting the lid on GPCRs: the role of extracellular loops. *Br J Pharmacol* 165: 1688-1703