Investigating the Activation Mechanism of the Oxytocin Receptor

Oxytocin (OT) signals through the oxytocin receptor (OTR), a member of the neurohypophysial peptide hormone receptor subfamily of family A G-protein-coupled receptors (GPCRs). The aim of this work was to understand the molecular basis of OTR activation by defining exact contacts between agonist (OT) and receptor (OTR). A glutamyl residue (E1.35) at the top of transmembrane helix 1 is conserved between all four human receptors in the neurohypophysial hormone receptor subfamily and has previously been shown to be required for high-affinity agonist binding and signalling at the OTR (1).

Interactions between OT and the human OTR were probed using a combination of site-directed mutagenesis at position E1.35 of the OTR and modification of OT at specific points. Positions 4 and 5 of OT were independently changed from glutamine and asparagine respectively to glutamic acid, producing the modified ligands [Glu^4]OT and [Glu^5]OT. The glutamyl residue at position 1.35 of the OTR was independently mutated to glutamine or asparagine, producing mutant receptors [E1.35Q]OTR and [E1.35N]OTR, thus effectively swapping residues between the receptor and ligand at specific loci to probe for direct interactions between agonist and receptor using double reciprocal mutation. The wild-type (WT) and mutant receptors were expressed in HEK 293T cells and inositol phosphate signalling was assayed following stimulation with either OT, [Glu^4]OT or [Glu^5]OT. Cell-surface expression of receptors was determined by enzyme-linked immunosorbent assays (ELISAs). Site-directed mutagenesis, cell culture, transfection and inositol phosphate assay protocols were as previously described (1). The ELISA method was based on that previously described (2), but using antibody dilutions of 1:3500 plus *o*-phenylenediamine dihydrochloride (OPD) as the substrate in the final step. After the addition of OPD, reactions were left to develop in the dark for 1-5 min and then terminated by mixing a 100 µL aliquot with an equal volume of 1 M sulphuric acid before measuring absorbance at 492 nm.

Mutant receptors exhibited similar cell-surface expression to WT OTR. At the WT OTR_5 the E_{max} of [Glu^5]OT signalling was only 24 ± 5 % (*n*=5) of that stimulated by OT. This [Glu^5]OT signalling was abolished when E1.35 of the OTR was mutated to asparagine (*n*=5). However, when E1.35 of the OTR was mutated to glutamine, [Glu^5]OT became an effective agonist with a pEC_{50} = 6.47 ± 0.08 (*n*=5) and an E_{max} = 89 ± 32 % (*n*=5) compared to the E_{max} of OT at the WT OTR. In contrast, [Glu^4]OT did not signal at either the [E1.35Q]OTR or [E1.35N]OTR mutant receptors (*n*=4). These data demonstrate the importance of both E1.35 of the OTR and Asn^5 of OT in receptor activation and provide evidence for a direct interaction between receptor and agonist which is dependent on the very specific side-chain geometry at these respective loci. These findings help to define the agonist binding site and improve our understanding of the molecular basis of receptor activation, which may facilitate future rational drug design.

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(1) Wootten DL *et al.* (2011). *Mol Cell Endocrinol* **333:** 20–27.
(2) Hawtin SR *et al.* (2006). *J Biol Chem* **281:** 38478–38488.