

P004 Differential orexin receptor internalisation and recycling kinetics attributed to distinct beta-arrestin interactions
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The orexin receptors (OxRs) 1 and 2 are Family A GPCRs that have similar high affinity and inositol phosphate signalling efficacy when binding the endogenous agonist orexin A (OxA). Consequently, the aim of this study was to establish subtype-specific differences in receptor function upon activation by this ligand. We have demonstrated that OxR1 internalises and recycles significantly more rapidly than OxR2 using ELISA. Bioluminescence resonance energy transfer (BRET) kinetic and dose-response data show that OxR1 interacts with beta-arrestin 1 and 2 more transiently and with lower affinity compared to OxR2. Further evidence for OxR1 rapid recycling was provided using the SB334867 OxR1 competitive antagonist, which was shown to rapidly inhibit OxA-induced OxR1/beta-arrestin 1 and 2 interactions in real time extended BRET (eBRET) assays. OxR1 contains fewer putative GRK phosphorylation sites in the C-terminus compared to OxR2 indicating that the extent of receptor phosphorylation may be a determinant of subtype-specific differences in internalisation and recycling. Mutation of beta-arrestins to produce phosphorylation-independent receptor binding increased affinity for OxR1, but not OxR2, a finding consistent with this hypothesis.