Constrained NPY Y1 / Y4 receptor BiFC heterodimers show pharmacology consistent with their orthosteric binding sites acting independently.

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The functional relevance of Class A GPCR heterodimerisation remains controversial, but may offer opportunities for new pharmacological targets[1]. This altered pharmacology could be due to cooperativity between the two ligand binding sites of the dimer or by modified coupling to effector proteins such as β-arrestins. However the stoichiometry of binding of β-arrestins, in particular for proposed GPCR dimers, remains unknown[2,3].

Here we have used bimolecular fluorescence complementation (BiFC) to constrain Neuropeptide Y (NPY) Y1 and Y4 receptor subtypes as discrete heterodimers of known composition. The NPY receptor subtypes Y1 and Y4, were chosen as they have been shown to internalise selectively in response to distinct agonists (NPY, pancreatic polypeptide PP respectively) in a β-arrestin dependent manner[4]. Quantitative platereader imaging (recomplemented YFP) allowed the measurement of BiFC dimer internalisation as an indirect readout of β-arrestin recruitment and dimer function[5]. N terminal SNAP tagging of the Y1 population (identified by SNAP AF647 labelling) allowed the simultaneous measurement of endocytosis of the overall Y1 receptor population. Granularity analysis was performed on images of both populations, to allow the quantification of receptor internalisation on a per cell basis. All data is expressed as mean ± s.e.m. Using these techniques, we investigated whether NPY Y1/Y4 BiFC dimer internalisation exhibited novel pharmacology compared to Y1 or Y4 receptors expressed alone.

For Y1/Y4 BiFC dimers, NPY induced internalisation of both the BiFC dimer and SNAP-tagged Y1 receptor populations, with similar agonist potencies (pEC_{50} values: BiFC dimer 7.8 ± 0.1; SNAP population 7.7 ± 0.2; n=4). These potencies were also comparable to NPY induced endocytosis of SNAP-tagged Y1 receptors when expressed alone (pEC_{50} 8.4 ± 0.1; n=4). Pre-treatment of Y1/Y4 BiFC dimers with the Y1 selective antagonist BIBO3304 (30nM; 30 minutes), which has an affinity for the Y4 receptor Ki > 1μM ([6]), produced a 10 fold rightward parallel shift of NPY concentration response curves (estimated pKB BiFC dimer 8.5 ± 0.2; SNAP population 8.4 ± 0.2, n=4), expected from its affinity at the Y1 receptor[5]. In contrast PP was able to induce internalisation of the BiFC dimer population (% NPY response at 1μM PP 84.2% ± 7.0 n=4) but not of the SNAP Y1 receptor population. BIBO3304 treatment had no effect on this (pEC_{50} values:± 30nM BIBO 8.0 ± 0.1-0.2, n=4). These PP induced responses were comparable to internalisation of Y4 receptors when expressed alone (Y4-GFP), with internalisation seen in response to PP treatment but only with high concentrations of NPY (% PP response at 1μM NPY 56.8% ± 2.8; n=4).

Thus constrained NPY Y1/Y4 receptor BiFC dimers are capable of undergoing agonist induced internalisation in response to selective Y1 or Y4 receptor ligands. However based on the selective antagonism of BIBO3304 on NPY but not PP responses, we obtained no evidence for co-operation between the Y1 and Y4 orthosteric binding sites. This suggests that single promoter occupancy of the Y1 / Y4 dimer is sufficient for its internalisation, and that constrained heterodimers do not give rise to novel antagonist pharmacology.

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