Investigating Histamine H_4 Receptor Trafficking In Response To β-Arrestin-2-Biased Agonists

We have recently demonstrated that the human histamine H_4 receptor (hH_4R) belongs to the growing number of GPCRs shown to exhibit signalling bias for $G_{i/o}$- or β-arrestin-mediated pathways. Specifically JNJ7777120 and JNJ10191584, known antagonists at the hH_4R, are capable of recruiting β-arrestin-2 independently of G protein activation[1,2]. One of the potential consequences of this type of biased agonism could be an increase in receptor internalization after prolonged 'antagonist' incubation, resulting in long-term down-regulation of the histamine-mediated hH_4R response. This may potentially provide better therapeutic potential than a traditional neutral antagonist. Here we have explored the kinetics of β-arrestin-2 recruitment to the hH_4R, and whether this translates into receptor internalization.

β-arrestin-2 recruitment was monitored in PathHunter™ U2OS-H_4/β-arrestin-2 (U2OS-arrestin-H_4) cells using the DiscoveRx enzyme-fragment complementation assay. Receptor internalization and recycling was monitored in U2OS cells stably expressing a venus-tagged hH_4R (U2OS-vH4), using immunofluorescence. Cells were treated with a range of hH_4R ligands for between 0 – 4 hr (arrestin) or 0 – 60 min (internalization). For receptor recycling experiments, cells were pre-incubated with ligands for 60 min, washed extensively and recycling of receptor to the cell surface monitored over 2 hr.

All ligands tested recruited β-arrestin-2 and caused hH_4R internalization in a concentration- and time-dependent manner (Table 1). VUF8430 was a slowly recruiting super agonist in the β-arrestin-2 recruitment assay compared to the endogenous ligand histamine, and the two arrestin-biased ligands JNJ7777120 and JNJ10191584 were partial agonists. In contrast to the β-arrestin-2 assay, VUF8430 was a partial agonist for receptor internalization. In this assay, clobenpropit was the slowest to cause receptor internalization, despite having higher intrinsic activity than either of the biased JNJ compounds.

	β-arrestin-2 recruitment			**hH_4R internalization**			**hH_4R Recycling**
Ligand	**pEC_{50}**	**E_{max} (% histamine)**	**Half-life (min)**	**pEC_{50}**	**E_{max} (% histamine)**	**Half-life (min)**	**Half-life (min)**
Histamine	7.06 ± 0.04	103 ± 1.6	47.7 ± 8.2	7.45 ± 0.05	100	10.7 ± 1.0	17.6 ± 0.5
4-methyl histamine	6.66 ± 0.04	96.6 ± 9.3	64.2 ± 22	6.79 ± 0.07	89.1 ± 3.6	11.6 ± 0.6	17.0 ± 1.0
VUF8430	6.21 ± 0.10	133 ± 7.8	64.3 ± 11	6.81 ± 0.15	80.9 ± 2.8	10.6 ± 0.5	14.3 ± 1.4
Clobenpropit	6.64 ± 0.11	57.8 ± 3.8	45.9 ± 8.7	6.99 ± 0.08	48.8 ± 3.8	14.9 ± 0.3	58.7 ± 13.7
JNJ7777120	7.63 ± 0.10	69.6 ± 1.9	155 ± 29	8.25 ± 0.21	37.1 ± 3.3	14.3 ± 1.7	22.7 ± 1.4
JNJ10191584	6.87 ± 0.11	76.8 ± 5.1	147 ± 34	7.66 ± 0.11	28.8 ± 2.0	11.3 ± 0.3	18.0 ± 4.1

Table 1. Potency, intrinsic activity and kinetics of hH_4R ligands.

Clobenpropit also appeared to cause prolonged receptor internalization when compared to the endogenous ligand histamine. In addition, only 38.4 ± 3.6 % of receptors were recycled to the membrane compared to 78.8 ± 3.6 % for histamine. There was no significant difference between the rate and degree of receptor recycling for all other ligands tested.

In conclusion, the biased agonists JNJ7777120 and JNJ10191584 are able to recruit β-arrestin-2 and internalize the hH_4R, independently of G protein activation. This additional receptor down-regulation may provide enhanced inhibition of hH_4R G protein signalling.

[1]Rosethorne EM & Charlton SJ (2011) *Mol Pharmacol* **79**:749.
[2]Nijmeijer, S *et al.* (2012) *Mol Pharmacol* **82(6)**:1174.