Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol15Issue1abst009.pdf

A potential role for ligand kinetics in the recruitment of β-arrestin to the human histamine H4R

D. A. Sykes, C. T. Long, X. Y. Ooi, K. C. Ting, Y. L. Wong, N. Holliday, E. M. Rosethorne, S. J. Charlton. School of Life Sciences, The University of Nottingham, Nottingham, United Kingdom

Introduction: Histamine (HA) is an endogenous biogenic amine which binds the human histamine H4 receptor (hH4R) on hematopoietic cells eg. mast cells causing G protein-mediated intracellular Ca^{2+} mobilization, resulting in recruitment of effector cells leading to chronic inflammation. JNJ7777120 and JNJ10191584 were developed as selective hH4R antagonists and display anti-inflammatory effects *in-vivo*. More recently, these ligands were demonstrated to display signal bias through selective activation of the β-arrestin 2 signalling pathway (Rosethorne *et al*., 2011). A role for kinetics in β-arrestin-biased signalling has been suggested previously (Sykes et al., 2014) and the aim of this study was to explore whether kinetics contributes to hH4R β-arrestin signalling bias.

Methods: Kinetic parameters were assessed using a time-resolved fluorescence resonance energy transfer (TR-FRET) based competition binding assay. This approach involves simultaneous addition of both fluorescent ligand (F-Clobenpropit) and competitor to SNAP-tagged hH4R expressed on the HEK293 cell membranes. The degree of competition between F- Clobenpropit and unlabelled H4R ligand was assessed at multiple time points by homogeneous time-resolved fluorescence (HTRF) detection on a Pherastar FS (BMG Labtech). The *k*on and *k*off of unlabelled H4R ligands at uncoupled and β-arrestin-2 pre-bound hH4R preparations was determined using the Motulsky-Mahan competition association binding approach. Bimolecular fluorescence complementation (BiFC) technique was used to pre-couple β-arrestin-2 to the H4R (see Kilpatrick et al., 2010).

Results: The fluorescent tracer F- Clobenpropit showed a significant reduction in *k*on and *k*off at the β-arrestin 2 pre-coupled receptor form with no overall change in equilibrium affinity. All agonists including the biased ligands JNJ7777120 and JNJ10191584, displayed lower affinity for the β-arrestin 2 pre-coupled receptor form, with an apparent reduction in measured on and off-rates, suggesting that β-arrestin 2 pre-coupling specifically alters H4R conformation.

Discussion & Conclusions: An overall trend was observed for slower association and dissociation parameters for all H4R agonists bound to the β-arrestin-2 coupled receptor. A positive relationship between agonist receptor residency time (1/*k*off) and β-arrestin-2 levels was uncovered, suggesting that kinetics may play a role in the recruitment of β-arrestin-2 to the H4R following receptor activation. This work suggests that ligand-receptor kinetics is one possible contributing factor which should be routinely studied when characterising biased molecules during lead optimisation phases, but does not by itself explain bias.

References

Kilpatrick et al., (2010) Br J Pharmacol. 160, 892–906.

Rosethorne and Charlton SJ (2011) Mol Pharmacol. 79:749.

Sykes et al., (2014) Br J Pharmacol. 171(21):4797-807.