BRET imaging of ligand interactions with the human β2 adrenoceptor using novel ICI 118,551 based fluorescent antagonists

J. Goulding[1,2], S. J. Mistry[3,2], S. J. Briddon[1,2], J. Woolard[1,2], B. Kellam[3,2], S. J. Hill[1,2]. [1]Division of Physiology, Pharmacology and Neuroscience, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom, [2]Compare, University of Nottingham, Nottingham, United Kingdom, [3]School of Pharmacy, University of Nottingham, Nottingham, United Kingdom.

Introduction The β2 adrenoceptor (β2AR) is a G protein-coupled receptor implicated in the pathology of asthma and heart disease. Fluorescent antagonists allow both visualisation and study of real-time ligand-receptor interactions in living cells. However few highly selective β2AR fluorescent antagonists are available. Therefore, we have synthesised three new fluorescent analogues of the selective β2AR antagonist ICI-118,551 (1, β2AR pK_D 9.3) with varying linkers. We show that these ligands retain β2AR/β1AR selectivity and can be used for single cell ligand binding using Bioluminescence Resonance Energy Transfer (BRET) imaging.

Methods Three BODIPY630/650-X conjugates of the 3-((3-hydroxy-4-((7-methyl-2,3-dihydro-1*H*-inden-4-yl)oxy)butan-2-yl)amino core of ICI-118,551 were synthesised with either a 3-(2-(2-(2-aminoethoxy)ethoxy)ethyl- or dipeptidyl (Gly-Ala/β-Ala-β-Ala) linkers. Confocal imaging was performed with a Zeiss LSM880 microscope using HEK293 cells stably expressing an N-terminal SNAP-tagged β2AR (SNAP_β2AR) labelled with 0.5μM SNAPSurface488. Whole cell saturation NanoBRET ligand binding assays were performed on HEK293 cells stably expressing N-terminal nanoluciferase- (Nluc) tagged human β2AR or β1AR as previously reported (2). BRET imaging was performed on Nluc_β2AR cells on an Olympus LV200 bioluminescence microscope using furimazine (4μM) and fluorescent antagonist (100nM). Emission was collected at 438/24nm bandpass filter (20s) and 647nm Long Pass (4min) and image analysis performed using Fiji plugin *Time Series Analyzer V3*.

Results Clear saturable specific binding was detected at the β2AR using NanoBRET with all three fluorescent ICI,118551 analogues (Table 1). The polyether linked ICI118,551-BODIPY630/650-X conjugate (PEG3) demonstrated the highest affinity and selectivity for the β2AR (Table 1). Confocal imaging of all three antagonists displayed membrane binding, co-localising with that of the SNAP_ β2AR. Displaceable binding (10μM propranolol) was observed for the Gly-Ala-ICI118,551-BY650/650X conjugate by confocal imaging and by BRET imaging for the β-Ala-β-Ala-ICI118,551-BY650/650X and PEG3-ICI118,551-BY650/650X conjugates.

Linker	β2AR pK_D	β1AR pK_D
PEG3	7.74 ± 0.05 (6)	<5 (5)
Gly-Ala	7.06 ± 0.10 (5)	<5 (4)
β-Ala-β-Ala	7.36 ± 0.15 (5)	6.32 ± 0.25 (5)

Table 1. Affinity of ICI118,551 derivatives at the human β1 and β2AR in HEK293 cells using NanoBRET saturation binding. Data represent mean ± S.E.M of n repeat experiments performed in duplicate. 10μM propranolol was used to define non-specific binding.

Conclusion We have developed novel selective β2AR fluorescent antagonists that can be used for confocal and BRET imaging. We thank the MRC for financial support.

References

1 Baker (2005). *Br J Pharmacol* **144(3)**: 317-22
2 Stoddart *et al.* (2015). *Nat Methods* **12(7)**: 661-663