Investigation of YIL781 Partial Agonism through Different Ghrelin Receptor Signalling Pathways and its Dependence on Constitutive Activity

S Tavakol, RL Thomas, ND Holliday. University of Nottingham, Nottingham, UK

Ghrelin is a stomach hormone which stimulates appetite through the GhrelinR, a 7TM receptor with high constitutive activity (Holliday *et al.*, 2007; Holst *et al.*, 2010). Although GhrelinR inverse agonists have been considered as obesity treatments, their action on regulatory as well as signalling pathways may limit future clinical efficacy because of compensatory increases in GhrelinR expression (Holliday *et al*., 2007; Pedersen *et al.*, 2009). Potentially this might be avoided by functionally selective ligands which influence a subset of signalling pathways. Here we investigate YIL781 (6-(4-fluorophenoxy)-2-methyl-3-[[(3*S*)-1-(1-methylethyl)-3-piperidinyl]methyl]-4(3*H*)-quinazolinone), reported as a GhrelinR antagonist (Esler *et al*., 2007), in calcium mobilisation and internalisation assays. We also compare wild type receptor responses with a PheV:13A mutant (F221A) exhibiting reduced constitutive activity (Holst *et al.*, 2010).

Calcium mobilisation was assessed in HEK293TR cells stably transfected with SNAP-tagged human GhrelinR cDNAs (Sivertsen *et al*., 2011), by measuring Fluo-4 fluorescence increases on an MDC FlexStation. Receptor internalisation was via IX Ultra platereader imaging (MDC) and automated analysis (Sivertsen *et al*., 2011). Individual triplicate responses were expressed as fold increases over basal level, and pooled concentration response curves were fitted using GraphPad Prism. A ligand bias factor (β) represented the logarithmic ratio of relative activities between YIL781 and ghrelin for each pathway, calculated as Rajagopal *et al*. (2011). Dissociation constant estimates from YIL781 displacement of ghrelin concentration-response profiles (pK_P) were corrected for partial agonism by equiactive comparison based on the operational model (Leff *et al*., 1993).

YIL781 was a partial agonist in both assays using HEK293TR GhrelinR cells, but was surprisingly more potent in stimulating low level internalisation (Table 1) – with a bias factor β=-2.63±0.99 ($p<0.01$ Dunnett's compared to ghrelin, β=0). In the internalisation assay, 30nM YIL781 pretreatment also right-shifted ghrelin concentration response curves, leading to a pK_P estimate of 8.43±0.33 (*n*=3). In the GhrelinR F221A mutant, the YIL781 R_{max} increased relative to ghrelin in calcium and internalisation assays (Table 1), without significant bias (β=0.76±1.63). The YIL781 pK_P in internalisation assays was also lower, 7.61±0.22 (*n*=3). Thus this study (i) demonstrates partial agonism, rather than antagonism, of YIL781 at the GhrelinR, (ii) suggests YIL781 may exhibit selectivity for ghrelinR conformations engaging internalisation compared to calcium signalling pathways, and (iii) implies that such selectivity depends on constitutive activity, and /or integrity of the GhrelinR PheV:13: TrpVI:13 activation microswitch (Holst *et al*., 2010).

Table 1. Summary of pEC_{50} and R_{max} values for wild-type (wt) and F221A GhrelinR.

		Calcium mobilisation			GhrelinR internalisation		
		pEC_{50}	R_{max}	n	pEC_{50}	R_{max}	n
WT	Ghrelin	8.46±0.29	5.27±0.58	5	8.01±0.29	0.25±0.03	5
	YIL781	7.37±0.27	1.43±0.19*	4	9.45±0.62†	0.08±0.02*	3

F221A	Ghrelin	7.95±0.13	24.60±1.48	2	7.87±0.22	0.37±0.04	9
	YIL781	7.87±0.16	15.33±1.13	4	7.28±0.69‡	0.17±0.07*	3

R_{max} expressed as fold over basal. *p<0.05 (1 way ANOVA and Dunnett's post-test cf. ghrelin); †$F_{2,96}$ = 4.53, p = 0.013; ‡F1,58 = 0.004; p = 0.95 (cf. calcium pEC_{50})

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