Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue2abst046P.pdf

THE EFFECT OF NOVEL GHRELIN RECEPTOR AGONISTS ON HEK-293 CELL GROWTH

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Ghrelin receptor 1a (GHSR1a) mediates the effects of ghrelin, a major hunger-inducing peptide that is produced mainly in the stomach. GHSR1a has high constitutive activity, being able to increase IP_3 levels and activate both serum and CREB response-element reporter assays in cells in the absence of ghrelin. Ghrelin treatment increases activation of these signaling pathways and novel agonists, such as wFw-Isn-NH_2 (a partial agonist) and KwFwLL (an inverse agonist) have been used to further study GHSR1a signaling, with an aim to develop anti-obesity therapies without side effects (Sivertsen *et al.* 2011).

Ghrelin can increase the growth of different types of cancer cell lines, via mitogen activated protein kinase (MAPK) activation (Chopin *et al.* 2011). Therefore, we aimed to use two different cell lines, 1) HEK-293 (which have endogenous levels of GHSR1a and ghrelin) and 2) HEK-GHSR1a (HEK-293 cells stably transfected with GHSR1a) in a sulphorhodamine B growth assay (Vichai & Kirtikara, 2006) to study the effects of novel GHSR1a agonists and an antagonist on growth.

HEK-GHSR1a cells showed significantly increased growth when treated with serum (ANOVA, $P < 0.001$), compared to HEK-293 cells (but not when cells were grown in the absence of serum). We found that ghrelin increased growth in a concentration-dependent manner in HEK-293 and HEK-GHSR1a cells (EC_{50} 0.25 (0.04 – 1.58) and 0.03 (0.004 – 25.84) nM respectively) and this was MAPK mediated as the MAPK inhibitor, PD98059, inhibited 1 nM ghrelin-induced growth significantly ($P < 0.01$) at 1µM (and with IC_{50} values of 0.34 and 0.46 µM, respectively).

Surprisingly, we found that the inverse agonist KwFwLL increased growth in both cell lines (EC_{50} 0.1 (0.02 – 0.59) nM and 7.76 (0.99 – 60.66) pM respectively), with similar efficacies to that of ghrelin. However, the partial agonist wFw-Isn-NH_2 only increased growth significantly in HEK-293 cells (EC_{50} <0.01 (0.002 – 0.02) pM) and exhibited parital agonism (at 10^{-14} - 10^{-12} M) in stably transfected cells. Furthermore, a novel GHSR1a antagonist *per se* also increased growth in HEK-293 cells (EC_{50} 6.4 (1.2 – 34.0) pM) and in HEK-GHSR1a cells (EC_{50} 1.73 (0.6 – 5.02) pM). Taken together this data demonstrates a very different ligand pharmacology in terms of growth response, from their original classification as determined by IP_3 accumulation.

Supported by: EU 7th Framework Programme, Grant Agreement: no.223057 (GIPIO).

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