Activation and modulation of the GLP-2R by incretin hormones and RAMPs

Glucagon-like peptide 2 (GLP-2) is a 33 amino acid peptide derived from proglucagon and secreted from intestinal enteroendocrine L cells (1). Activation of the GLP-2 receptor (GLP-2R) causes crypt cell proliferation in the small intestine, and has been found to improve nutrient absorption in patients with short bowel syndrome (1, 2). The GLP-2R signals through Gαs leading to cAMP production. It has a 40% similarity to other proglucagon-derived peptides, GLP-1 and glucagon, which activate family B G-protein coupled receptors (GPCRs) to regulate blood glucose (1) and are currently targets for diabetes treatment. We therefore investigated the ability of these peptides and a therapeutic GLP-1 mimetic, lixisenatide, to activate the GLP-2R and stimulate cAMP production. The receptor was transiently transfected into HEK293 cells and cAMP production measured using a PerkinElmer LANCE assay kit (3). Each peptide bound and activated the receptor (Fig.1), with GLP-1 7-36 acting as a low potency full agonist (pEC_{50} = 5.81 ± 0.21, E_{max} = 86.15 ± 6.24), and glucagon (pEC_{50} = 5.76 ± 0.57, E_{max} = 63.69 ± 7.84) and lixisenatide (pEC_{50} = 9.1 ± 0.62, E_{max} = 46.37 ± 3.33) acting as partial agonists compared to full agonist GLP2 1-33 (pEC_{50} = 9.46 ± 0.37, E_{max} = 75.27 ± 3.86) (n = 9).

Receptor activity modifying proteins (RAMPs) are known to interact with some family B GPCRs where they modulate signal transduction and influence ligand specificity (3). However, a pharmacological analysis of these interactions for the GLP-2R has not been carried out. We investigated cAMP production of the GLP-2R by GLP2 1-33 in the presence of each of the RAMPs (Fig. 2). RAMP1 significantly increased basal signalling through Gαs ($P < 0.01$, one-way ANOVA and Dunnett's test), while RAMP2 significantly reduced basal signalling ($P < 0.001$) (n = 18). While there were no significant changes to pEC_{50}, RAMPs 2 and 3 significantly increased GLP-2R signalling range ($P < 0.005$ and $P < 0.01$, respectively). This data demonstrates that GLP-1, glucagon and lixisenatide can act at the GLP-2R, and signalling via this receptor is enhanced by RAMPs. This information should lead to the rational design of more targeted drugs with fewer side effects.

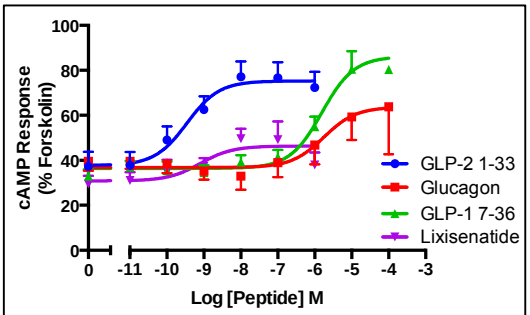

Figure 1: GLP2R activation by proglucagon-derived peptides and a GLP-1R agonist

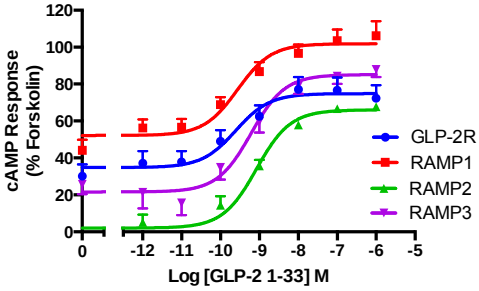

Figure 2: GLP-2R interaction with RAMPs alters basal signalling through Gs

References

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(2) Guan, X *et al* (2006) *Gastroenterology* **130***:* 1019 – 1021,
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