Allosteric modulation of the activity of GLP-1 9-36 amide, a major glucagon-like peptide-1 metabolite, at the GLP-1 receptor

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Glucagon-like peptide-1 (GLP-1) released from intestinal L cells in response to nutrients has many physiological effects but particularly enhances glucose-dependent insulin release through the GLP-1 receptor (GLP-1R), a Family B GPCR. GLP-1 7-36 amide, the predominant circulating active form of GLP-1, has a short half life (1–2min) due to rapid truncation by dipeptidyl peptidase-4 to GLP-1 9-36 amide, which is generally considered inactive or even a low affinity GLP-1R antagonist. Given its role in glucose-dependent insulin release, the GLP-1R is targeted for treatment of type 2 diabetes, particularly by non-peptide, orally-active, small molecules. Recently, 'compound 2' has been described as an agonist and positive allosteric modulator of GLP-1 7-36 amide affinity at the GLP-1R (Knudsen *et al*., 2007). As GLP-1 9-36 amide is the major circulating form of GLP-1, here we examined functional interactions with compound 2 using previously characterized HEK293Flp-In cells with stable expression of the human GLP-1R (Coopman *et al*., 2010).

Challenge with GLP-1 9-36 amide (15min) in the presence of isobutylmethylxanthine (500μM) caused concentration-dependent increases in cellular cAMP levels (measured by radioreceptor assay) (pEC_{50} 6.51±0.04, E_{max} 457±19 pmol/mg protein compared to 10.21± 0.06 and 2635±32 pmol/mg protein for GLP-1 7-36 amide; all data mean±sem, $n \geq 3$). Pretreatment (10min) with GLP-1 9-36 amide (1μM) had little effect on potency of GLP-1 7-36 amide (pEC_{50} 9.87±0.06) but reduced the E_{max} to 2271±81 pmol/mg protein ($P<0.05$). We have previously shown a bell-shaped concentration-response for compound 2-mediated cAMP responses in these cells (E_{max} at 3μM ~70% of GLP-1 7-36 amide; Coopman *et al*., 2010). Here, GLP-1 9-36 amide (1μM, 10min pretreatment) significantly ($P<0.01$) increased potency of compound 2 (pEC_{50} 5.94± 0.04 to 6.55±0.07). Furthermore, cAMP responses to co-treatment were 48% higher than the numerical sum of responses to compound 2 (1μM) and GLP-1 9-36 (1μM) alone, suggesting increased efficacy. This effect was detectable at 5min and lasted for at least 60min. Furthermore, compound 2 (30nM to 3μM) concentration-dependently increased the potency of GLP-1 9-36 amide cAMP responses (pEC_{50} 6.51±0.02 and 8.41±0.22 at 0 and 3μM compound 2) and resulted in E_{max} values (based on 1μM GLP-1 9-36 amide) that were greater than the numerical addition of responses to compound 2 and GLP-1 9-36 alone. Indeed, concentrations of compound 2 ($\leq$0.1μM) that did not evoke cAMP responses, enhanced the potency and E_{max} of GLP-1 9-36 responses.

These data demonstrate low potency, partial agonism by both GLP-1 9-36 amide and the allosteric compound 2 at the GLP-1R. Further, compound 2 markedly enhances potency and efficacy of GLP-1 9-36 amide in contrast to similar studies with GLP-1 7-36 amide, in which E_{max} was unaffected and potency was either unaffected or reduced (Knudsen et al., 2007; Coopman *et al.*, 2010). It is unclear if such effects on GLP-1 9-36 amide could play a role in potential therapeutic effects of small molecule GLP-1R allosteric modulators or indeed whether this provides a therapeutic opportunity.

References: Knudsen *et al.* (2007) *Proc Natl Acad Sci USA,* 104: 937-942.

Coopman *et al.* (2010) *J Pharmacol Exp Ther,* 334: 795-808.