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**Identification of the first small molecule positive allosteric modulator of the Gastric Inhibitory Polypeptide Receptor**

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***Introduction:*** Peptide-based incretin mimetics are limited by poor oral bioavailability. Orally active small molecules that can modulate incretin receptor pharmacology are far more attractive therapeutics. Indeed, small molecule glucagon-like peptide-1 receptor (GLP-1R) positive allosteric modulators (PAMs) have been developed that modulate GLP-1R activity to promote insulin secretion (1). There are, as yet, no small molecule allosteric modulators of the gastric inhibitory polypeptide receptor (GIPR). In-silico docking identified 11 compounds predicted to bind the GIPR and an initial screen revealed that one compound (C9) had potential activity.

***Method:*** Mammalian HTRF-based second messenger assays for cAMP accumulation and intracellular calcium ($(Ca^{2+})_i$) mobilisation were utilised to measure the response of HEK-293 and CHO-K1 cells stably expressing the GIPR, and GLP-1R CRISPR KO INS-1 832/3 cells to GIP (1-42) or GIP (Pro3) in the presence and absence of C9. Glucose-stimulated insulin secretion (GSIS) assays were performed to investigate the effect of C9 on GIP (1-42) potentiation of insulin secretion from isolated human pancreatic islets. The operational model of allosterism was used to determine the allosteric cooperativity factor ($\log(\alpha\beta)$) for C9 at the GIPR (1). Data are given as mean±SEM (n individual repeats) and analysis was performed using Students t-test or one way ANOVA and Dunnetts post-test where appropriate.

***Results:*** The potency of GIP (1-42) or GIP (Pro3) to stimulate cAMP accumulation was significantly increased when cells were costimulated with C9 (Table 1) and resulted in an allosteric cooperativity factor for C9 greater than 1 ($\log(\alpha\beta)$; 1.09±0.11 and 1.28±0.28, respectively). This indicated that C9 was a PAM. 15 minutes pre-treatment of GIPR expressing HEK-293 cells with C9 produced a significant increase in $E_{max}$ (3.05 fold, $p<0.01$) and $pEC_{50}$ ($p<0.05$) for GIP (1-42) mediated ($(Ca^{2+})_i$) mobilisation. Pharmacological analysis of C9 was then extended to GLP-1R KO INS-1 cells where costimulation with C9 significantly increased the $pEC_{50}$ of GIP (1-42) to stimulate cAMP accumulation (Table 1 , $\log\alpha\beta$; 1.13±0.82). Furthermore, 1 hour pre-incubation of isolated human pancreatic islets with C9 resulted in a dramatic enhancement of GIP (1-42) potentiation of GSIS (Figure 1).

Table 1. $pEC_{50}$ values for cAMP accumulation in CHO-K1-GIPR, HEK 293-GIPR and GLP-1R KO INS-1 832/3 cells, stimulated with GIP (1-42) (CHO-K1-GIPR and GLP-1R KO INS-1 832/3) or GIP (Pro3) (HEK 293-GIPR) in the presence and absence of C9

| C9 | CHO-K1-GIPR | HEK 293-GIPR | GLP-1R KO INS-1 |
|---|---|---|---|
| 0 | 9.32±0.06 (19) | 7.30±0.06 (14) | 8.21±0.10 (6) |
| 10 µM | 9.61±0.07** (18) | 7.71±0.06*** (16) | 8.11±0.12 (6) |
| 31.6 µM | 9.71±0.10* (6) | N/A | 8.95±0.33* (3) |
| 100 µM | 10.04±0.06**** (25) | 8.29±0.07**** (17) | 8.79±0.13** (8) |

*, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001

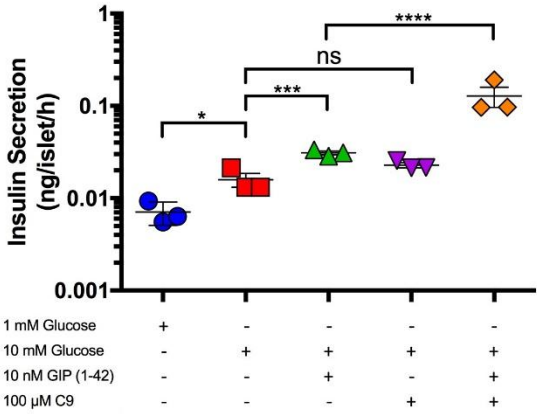


Figure 1. Glucose stimulated insulin secretion from isolated human pancreatic islets, in the presence and absence of 10 nM GIP (1-42) or 100 µM C9. *, p < 0.05; ***, p < 0.001; ****, p < 0.0001.

***Conclusions:*** We have identified the first small molecule PAM of the GIPR that can potently enhance cAMP accumulation, (($Ca^{2+}$)$_i$) mobilisation and importantly, insulin secretion. The roles that GIPR plays in blood glucose homeostasis and bone formation suggests that C9 may have therapeutic benefits for patients with type 1 or type 2 diabetes, or osteoporosis.

***References:***

(1). Wootten D et al. (2012). Mol Pharmacol 82(2): 281-290.