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# Evaluation of the insulinotropic and glucose-lowering actions of zebrafish GIP in mammalian systems: Evidence for involvement of the GLP-1 receptor

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ABSTRACT

The insulinotropic properties of zebrafish GIP (zfGIP) were assessed *in vitro* using clonal pancreatic β-cell lines and isolated mouse islets and acute effects on glucose tolerance and insulin release *in vivo* were evaluated in mice. The peptide produced a dose-dependent increase in the rate of insulin release from BRIN-BD11 rat clonal β-cells at concentrations ≥30 nM. Insulin release from 1.1 B4 human clonal β-cells and mouse islets was significantly increased by zfGIP (10 nM and 1 μM). The *in vitro* insulinotropic activity of zfGIP was decreased after incubating BRIN-BD11 cells with the GLP-1 receptor antagonist, exendin-4(9-39) ($p < 0.001$) and the GIP receptor antagonist, GIP (6-30) Cex-K$^{40}$[Pal] ($p < 0.05$) but the glucagon receptor antagonist [des-His$^{1}$,Pro$^{4}$,Glu$^{9}$]glucagon amide was without effect. zfGIP (10 nM and 1 μM) produced significant increases in cAMP concentration in CHL cells transfected with the human GLP-1 receptor but was without effect on HEK293 cells transfected with the human glucagon receptor. Conversely, zfGIP, but not human GIP, significantly stimulated insulin release from CRISPR/Cas9-engineered INS-1 clonal β-cells from which the GIP receptor had been deleted. Intraperitoneal administration of zfGIP (25 and 75 nmol/kg body weight) to mice together with an intraperitoneal glucose load (18 mmol/kg body weight) produced a significant decrease in plasma glucose concentrations concomitant with an increase in insulin concentrations. The study provides evidence that the insulinotropic action of zfGIP in mammalian systems involves activation of both the GLP-1 and the GIP receptors but not the glucagon receptor.

## 1. Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is a member of an extended family of structurally related regulatory peptides that includes glucagon, glucagon-like peptide-1(GLP-1), glucagon-like peptide-2, secretin, vasoactive intestinal polypeptide, peptide histidine methionine, pituitary adenylate cyclase-activating peptide, and growth hormone-releasing hormone [1]. In addition, a gene encoding glucagon-related peptide with structural similarity to the Gila monster venom peptides, exendin-3 and -4 is present in the genomes of a range of tetrapod taxa but is absent from zebrafish and mammals [2,3]. The peptides are related evolutionarily having arisen from an ancestral gene either by tandem duplications or as a result of ancient whole genome duplications (2R hypothesis) [4,5]. The physiological effects of these peptides are mediated largely by interaction with a family of structurally and evolutionarily related class B (secretin–like) G-protein coupled receptors [6].

GIP was first isolated from a porcine intestinal extract as a 42-amino-acid peptide on the basis of its ability to inhibit gastric acid production at supraphysiological concentrations [7]. Subsequently, the gene encoding the peptide has been identified in a range of mammalian species (reviewed in [1]), chicken [8], the frogs *Xenopus tropicalis* and *Xenopus laevis* [8], and in a teleost, the zebrafish *Danio rerio* [8]. A gene encoding a GIP-like sequence is present in the genome of the sea lamprey *Petromyzon marinus* but transcripts were not detected [9]. Similarly, a GIP-like molecule has not yet been identified in an elasmobranch but it is of interest that a mammalian GIP is a potent stimulant of cAMP production and chloride secretion in the rectal gland of the skate *Leucoraja erinacea* [10]. The predicted primary structure of zebrafish GIP (zfGIP) is only 31 amino acid residues and it has been speculated that the peptide is more similar to the ancestral gene product from which the glucagon family of peptides has arisen than are mammalian GIP peptides [9].

GIP is described as an incretin hormone in mammals owing to its ability to promote insulin release from pancreatic β-cells at physiologically relevant concentrations. GIP is released into the circulation from intestinal enteroendocrine K- cells in response to ingestion of nutrients and, along with GLP-1, is the major physiologic incretin in the human

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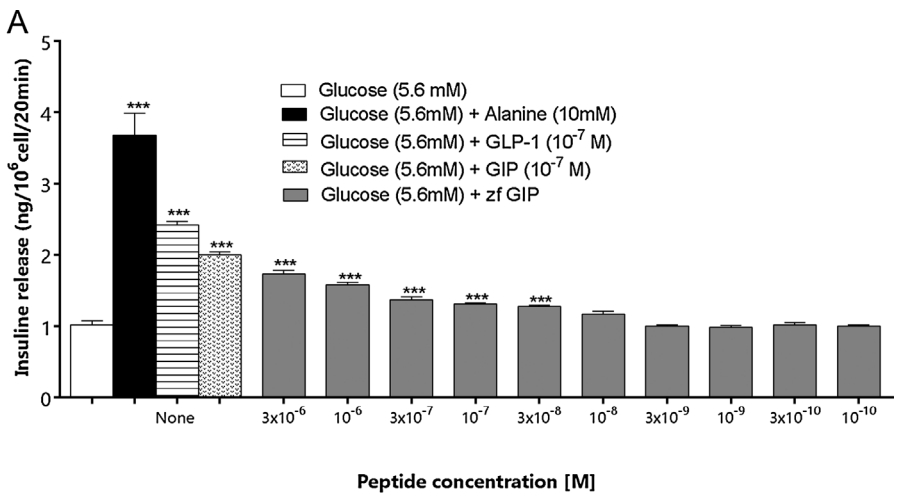


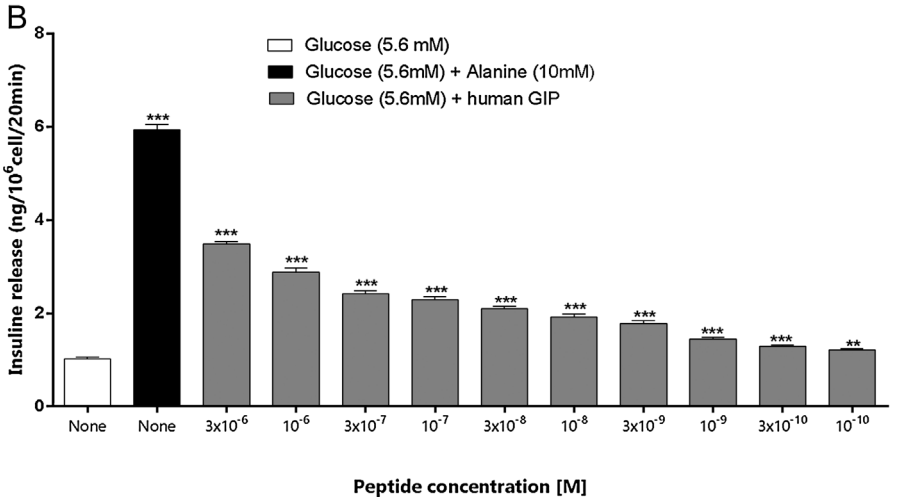


**Fig. 1.** Concentration-dependent effects of (A) zfGIP and (B) human GIP on insulin release from BRIN-BD11 rat clonal β-cells. Values are mean ± S.E.M., n = 8. **P < 0.01, ***P < 0.001 compared to 5.6 mM glucose alone.

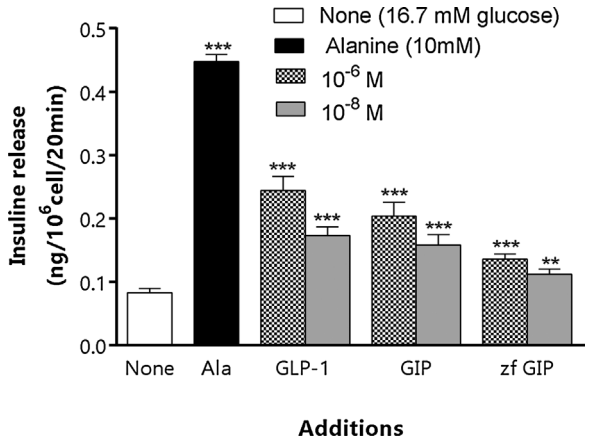


**Fig. 2.** Effects of zfGIP on insulin release from human 1.1 B4 cells. Values are mean ± S.E.M.,$n$ = 8 **$P$ < 0.01, ***$P$ < 0.001 compared to 16.7 mM glucose alone.

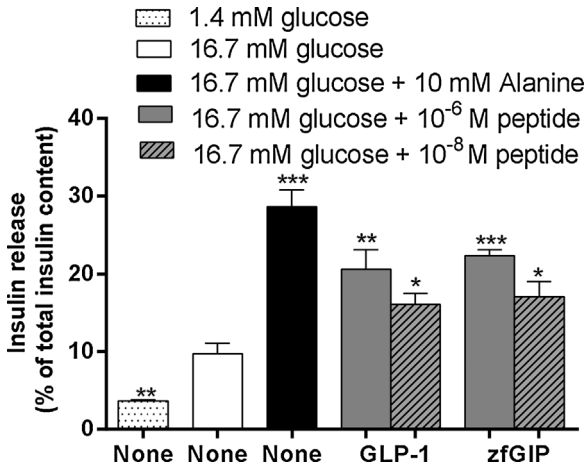


**Fig. 3.** Effects of zfGIP on insulin release from pancreatic islets isolated from NIH Swiss mice. The values are mean ± SEM for n = 4; *P < 0.5, **P < 0.01, ***P < 0.001 compared to 16.7 mM glucose alone.

[11]. Recent research has indicated that functionally relevant GIP is also present in the pancreatic islet cells of mice [12]. In the zebrafish, RT-PCR and immunohistocemical studies have demonstrated that GIP expression in the intestine is restricted to cells located near the base of the vill and the peptide is located principally in endocrine cells of the pancreas [13]. This has led investigators to speculate that GIP may not function as an incretin in this species [13]. No gene that closely resembles the human GLP-1R gene has been found in the genomes of the zebrafish or other bony fish [6] and the fact that GLP-1 does not function as an incretin hormone in teleosts, rather as a glucagon-like stimulant of glycogenolysis and gluconeogenesis, is well established [14].