Breakthroughs and Views

Glucose-dependent insulinotropic polypeptide analogues and their therapeutic potential for the treatment of obesity-diabetes

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Abstract

Glucose-dependent insulinotropic polypeptide (GIP) is a key incretin hormone, released postprandially into the circulation in response to feeding, producing a glucose-dependent stimulation of insulin secretion. It is this glucose-dependency that has attracted attention towards GIP as a potential therapeutic agent for the treatment of type 2 diabetes. A major drawback to achieving this goal has been the rapid degradation of circulating GIP by the ubiquitous enzyme, dipeptidylpeptidase IV (DPP IV). However, recent studies have described a number of novel structurally modified analogues of GIP with enhanced plasma stability, insulinotropic and antihyperglycaemic activity. The purpose of this article was to provide an overview of the biological effects of several GIP modifications and to highlight the potential of such analogues in the treatment of type 2 diabetes and obesity.
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Keywords: Dipeptidylpeptidase IV; Glucagon-like peptide-1; Glucose-dependent insulinotropic polypeptide; GIP analogues; Insulin secretion; Obesity; Type 2 diabetes

Glucose-dependent insulinotropic polypeptide (GIP) is a 42 amino acid gastrointestinal hormone secreted from enteroendocrine K-cells in response to food and nutrient absorption [1]. Although initially characterized for its ability to inhibit histamine-induced gastric acid secretion, the primary role of GIP as an incretin hormone moderating glucose-induced insulin secretion is widely recognized [2]. In addition to its actions on the pancreatic beta cell, GIP is also known to exert various extrapancreatic effects, which further enhance its glucose lowering ability. In particular, GIP has been shown to augment insulin-dependent inhibition of glycogenolysis in the liver [3] and to exert stimulatory effects on glucose uptake and metabolism in muscle [4]. Furthermore, functional GIP receptors have been identified on adipocytes [5] and have been shown to stimulate glucose transport [6], increase fatty acid synthesis [7], and stimulate lipoprotein lipase activity [8]. More recent studies have shown GIP to stimulate beta cell mitogenesis and inhibit apoptosis [9,10]. Consequently, this wide spectrum of biological activities has sparked the recent interest in GIP as a novel therapeutic candidate for the treatment of type 2 diabetes [11].

Solutions to difficulties posed by GIP as a therapeutic agent

Currently there are two main concerns in attempting to utilize GIP as a potential therapeutic agent. First, the native peptide has a short biological half-life in the circulation (approximately 3–5 min), due primarily to degradation by the ubiquitous enzyme, dipeptidylpeptidase IV (DPP IV; EC 3.4.14.5). After release into the circulation, the native peptide (GIP 1-42) is rapidly hydrolysed at the amino terminus removing Tyr^1–Ala^2 to produce the truncated metabolite GIP(3-42) [12]. This major degradation product was initially thought to be inactive [13], however, recent observations have shown it to function as a GIP receptor antagonist in vivo [14]. To circumvent degradation, DPP IV inhibitors are currently being developed for in vivo administration. Several positive effects on glycaemic control have been

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reported [15–17], but the effects of long-term interference on the metabolism of a plethora of other peptide substrates for DPP IV are as yet unknown [18]. Conceivably, a more attractive approach than widespread non-specific DPP IV inhibition involves the synthesis of specific GIP analogues modified at the enzyme cleavage site [11].

Another concern regarding the use of GIP in diabetes therapy stems from a study in which GIP was shown to exhibit marked reduction of insulinotropic activity in type 2 diabetic subjects [19]. Indeed, several studies have shown blunted insulin responses to GIP infusion in type 2 diabetes, albeit with differing degrees of beta cell resistance [19–22]. However, type 2 diabetes is associated with a global defect in insulin secretion not merely restricted to GIP, and encompassing other factors, including glucose and GLP-1 [23,24]. It has also been shown that beta cell sensitivity to GIP in type 2 diabetic patients improves with glyburide treatment [25]. Mutations of GIP receptors are rare in diabetes [21,26] and it is evident that any speculated abnormalities in GIP receptor binding can be overcome by enzyme-resistant analogues of GIP [11,27–33]. Furthermore, a recent study has shown that an N-terminally modified analogue of GIP, Tyr[1]-glucitol-GIP, evokes a substantially larger and more protracted insulin response to oral glucose than the native GIP in type 2 diabetic patients [34].

The attractiveness and potential of stable GIP analogues

A number of significant features indicate that enzyme-resistant analogues of GIP have unique potential for diabetes therapy. First, GIP is the major physiological incretin as indicated by greater hormone responses to feeding [35] and comparative studies using either receptor antagonists [36,37] or receptor knockout mice [38,39]. Second, structural modification of the sister incretin, GLP-1, invariably compromises biological activity at the GLP-1 receptor [40–44], whereas the opposite is true for many analogues of GIP [11,27–33]. Third, any effect of GIP on increasing glucagon concentrations is only observed at normal glucose concentrations [45,46] and thus is irrelevant following feeding or in treating type 2 diabetes [47]. Fourth, in contrast to GLP-1, GIP lacks significant effects on gastric emptying and is therefore well tolerated by human subjects. This has become an increasing and possibly damning difficulty with the use of GLP-1 and its analogues in patients, as evidenced by increased gastrointestinal side effects and nausea [48]. This, therefore, focuses increasing attention on the antidiabetic potential of GIP. To date, several studies have been published examining the in vitro activities of a range of GIP fragments and analogues [27,49–54]. Moreover, as reviewed in the following sections, the in vivo antidiabetic potential of a family of selective designer human GIP 1-42 analogues (Fig. 1) modified at positions Tyr[1] [11,28–30], Ala[2] [31–33], and Glu[3] [37,55] have been tested in animal models of type 2 diabetes and obesity.

Effects of modifications at position Tyr[1]

Several novel Tyr[1]-modified analogues of GIP have been developed, modelled on previous studies with the glucagon-secretin family of gastrointestinal peptides and knowledge of the substrate-binding specificity of DPP IV (Fig. 1). These analogues include N-acetyl-, N-Fmoc-, N-glucitol-, N-palmitate-, and N-pyroglutamyl-GIP [11,27–30]. All analogues modified at Tyr[1] exhibited complete resistance to DPP IV with in vitro half-lives greater than 24 h compared with 2.3 h for native GIP (Table 1), as illustrated for N-acetyl-GIP in Figs. 2A–B. This is in agreement with DPP IV substrate-binding specificity, which predicts the requirement for a bulky N-terminal amino acid (such as tyrosine in the case of GIP) possessing a free protonated α-amino group. By attaching amino acids or functional groups to the N-terminus of GIP, Tyr[1] becomes unprotonated, therefore, removing the explicit prerequisite required for DPP IV to act [18].

In assessing the biological activity of each analogue in vitro, cAMP formation in human GIP-receptor transfected cells [56] and insulinotropic activity in clonal BRIN-BD11 cells [57] was measured. All of the Tyr[1]-modified analogues studied exhibited an increased potency (2- to 10-fold increase in EC_{50} values) in stimulating cAMP production compared to native GIP (Table 1; Fig. 2C). Furthermore, the Tyr[1]-modified analogues exhibited significantly increased insulin secretory responses (1.1- to 1.4-fold) compared to the native peptide in BRIN-BD11 cells, indicative of improved biological activity (Table 1; Fig. 2D). However, subtle differences could be observed within this group, as both N-Fmoc- and N-palmitate-GIP appeared to be moderately (14–20%) less potent in vitro than N-acetyl-, N-glucitol-, and N-pyroglutamyl-GIP.

In determining the insulin releasing and antihyperglycaemic potential of the GIP analogues in vivo, we used *ob/ob* mice, an extensively studied animal model of spontaneous obesity and diabetes. Characteristically these mice exhibit hyperphagia, marked obesity, moderate hyperglycaemia, and severe hyperinsulinaemia [28]. All of the Tyr[1]-modified analogues were noticeably superior at stimulating insulin release (2.0- to 2.5-fold) and lowering blood glucose (1.4- to 1.9-fold) compared with native GIP (Table 1). Furthermore, it could be observed that N-acetyl-, N-glucitol-, and N-pyroglutamyl-GIP were slightly more potent than both N-Fmoc- and N-palmitate-GIP. Of the Tyr[1]-modified analogues tested, N-acetyl-GIP [29] appeared to be the most
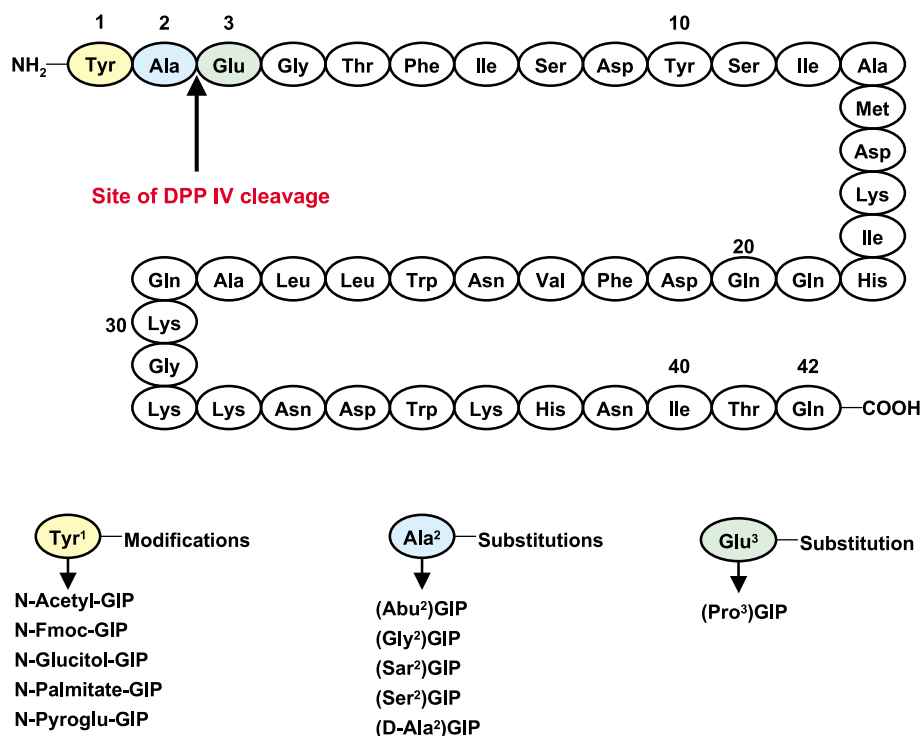
Fig. 1. A diagrammatic representation of the 42 amino acid sequence of human glucose-dependent insulinotropic polypeptide (GIP) with the solid arrow indicating the site of enzymatic degradation by dipeptidyl peptidase IV (DPP IV). The coloured amino acid residues (1–3) indicate the analogues synthesized and tested in animal models of obesity-diabetes [11,27–33], based on the entire human GIP 1-42 sequence.

impressive (Table 1). As shown in Fig. 3, this stable analogue significantly augmented the plasma insulin response and curtailed the glycaemic excursion following conjoint administration with glucose to obese diabetic *ob/ob* mice. The ability of *N*-acetyl-GIP to overcome the severe insulin resistance and beta cell defect (including poor response to native GIP) in this animal model is notable and affords proof of concept

that such analogues offer potential as future therapeutic agents for type 2 diabetes.

Effects of modifications at position Ala^2

A series of Ala^2-substituted analogues of GIP have also been synthesized and tested for their DPP IV

Table 1
Summary of the biological properties of designer human GIP analogues modified at the N-terminal Tyr^1, Ala^2 or Glu^3 amino acid residues

Modification	Peptide	DPP IV half-life (h)	cAMP production EC$_{50}$ (nmol/liter)	Maximal insulin response (% GIP max)	Plasma glucose AUC (% GIP max response)	Plasma insulin AUC (% GIP max response)
Native hormone	GIP	2.3	18.2	100 ± 3.1	100	100
Tyr^1-modification	*N*-Acetyl-GIP	>24	1.86	127 ± 4.0	62	251
	N-Glucitol-GIP	>24	2.03	141 ± 8.1	57	236
	N-Pyroglutamyl-GIP	>24	2.67	118 ± 1.6	61	224
	N-Palmitate-GIP	>24	10.0	122 ± 3.3	69	206
	N-Fmoc-GIP	>24	9.4	112 ± 2.5	69	208
Ala^2-substitution	(Gly^2)GIP	>8	15.0	107 ± 6.4	81	144
	(Ser^2)GIP	4.8	14.9	140 ± 4.7	75	126
	(Abu^2)GIP	1.9	38.5	62 ± 0.6	94	79
	(Sar^2)GIP	1.6	54.6	42 ± 3.8	106	96
Glu^3-substitution	(Pro^3)GIP	>24	Nil	52 ± 2.2	160	43

DPP IV half-lives were calculated by plotting the percentage of intact peptide remaining after incubation with DPP IV ($n = 3$) versus incubation time. EC$_{50}$ values were calculated from cAMP dose–response curves ($n = 3$) in human GIP-receptor transfected CHL cells using Graph pad Prism. Maximal insulin response values in BRIN-BD11 cells ($n = 8$) were calculated relative to the maximal percentage GIP response. Plasma glucose and insulin AUC values from *ob/ob* mice ($n = 7$–8) were calculated and recorded as a percentage of the maximal GIP response. Values represent means $\pm$ SEM. Data taken from [11,27–33].
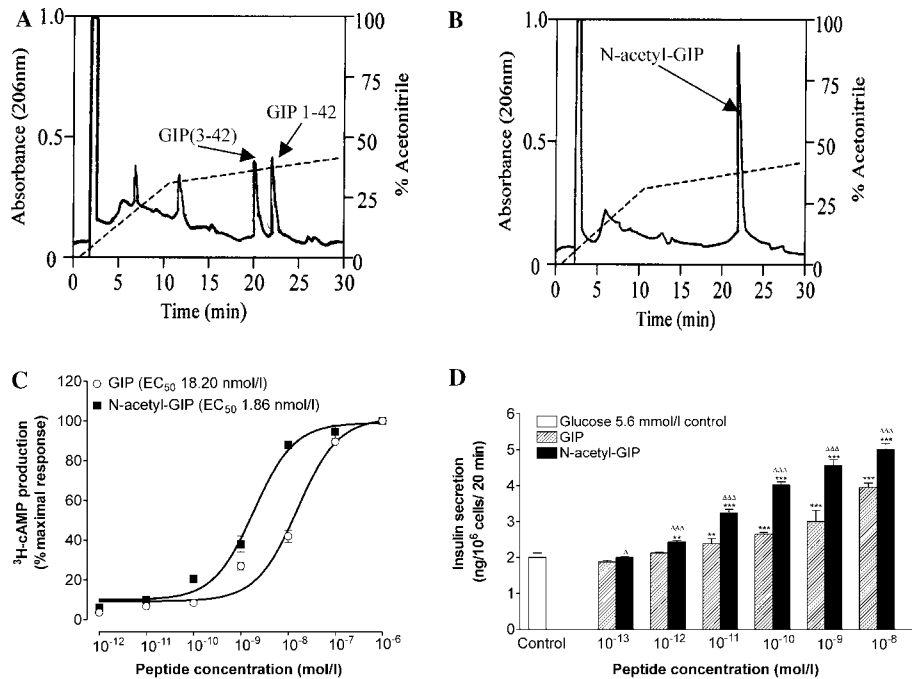
Fig. 2. (A,B) Representative HPLC profiles obtained after incubation of native GIP (A) and *N*-acetyl-GIP (B) with DPP IV for 2 h. Reaction products were separated on a Vydac C-18 column and peaks corresponding to intact GIP, *N*-acetyl-GIP, and GIP(3-42) are indicated. (C) Dose-dependent production of cAMP by native GIP and *N*-acetyl-GIP upon binding to CHL cells stably transfected with the human GIP receptor. (D) Dose-dependent effects of GIP and *N*-acetyl-GIP on insulin secretion from BRIN-BD11 cells. Values are means ± SEM ($n = 3$–8). **$P < 0.01$, ***$P < 0.001$ compared to control. $^{\Delta}P < 0.05$ and $^{\Delta\Delta\Delta}P < 0.01$ compared to native GIP. Data taken from [29].
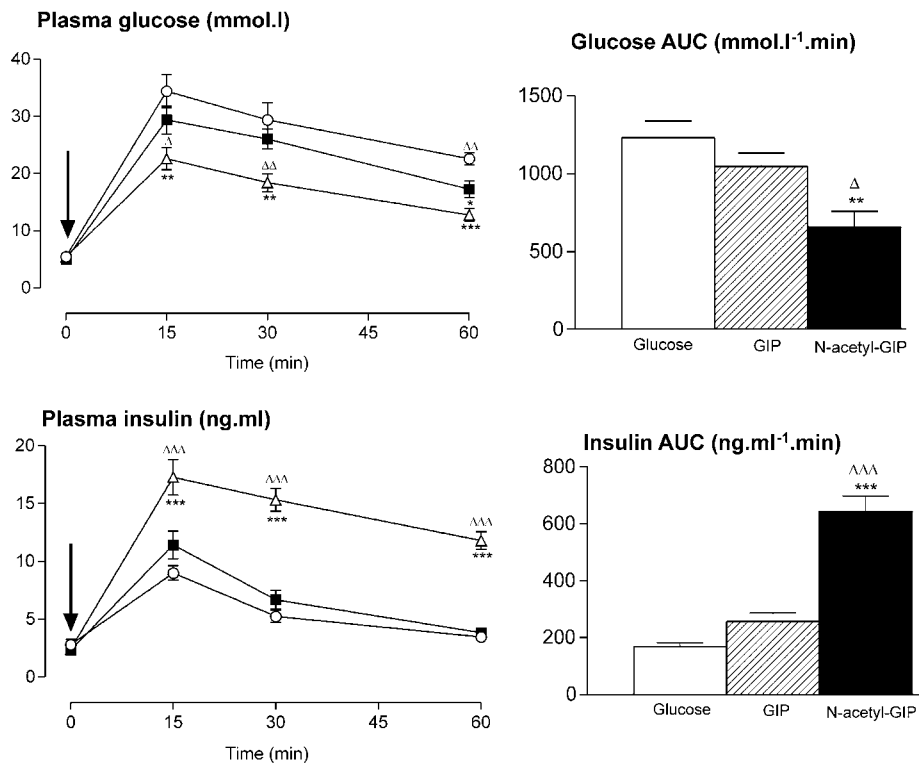

Fig. 3. Plasma glucose and insulin responses of 18 h fasted obese diabetic (*ob/ob*) mice after intraperitoneal administration of glucose alone (2 g/kg body weight; open circles), or in combination with either 25 nmol/kg GIP (solid squares) or *N*-acetyl-GIP (open triangles). Values are means ± SEM ($n = 7$–8). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to glucose alone. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, and $^{\Delta\Delta\Delta}P < 0.001$ compared to GIP. Data taken from [29].

stability and biological activity (Fig. 1). These include (Abu^2)GIP, (Gly^2)GIP, (Sar^2)GIP, (Ser^2)GIP, and (D-Ala^2)GIP [31–33]. Substitution of Ala^2 with either 2-aminobutyric acid (Abu) or sarcosine (Sar) did not appear to confer any increased resistance against DPP IV, as both analogues followed similar or slightly accelerated patterns of degradation to the native peptide (Table 1) [32]. Indeed, both of these substitutions impaired biological activity compared with native GIP when tested using the in vitro insulin secretion and cAMP models. Surprisingly though, both analogues displayed antihyperglycaemic and insulinotropic activity comparable to native GIP when administered to obese diabetic *ob/ob* mice (Table 1) [32].

In sharp contrast, substitution with either a glycine (Gly) or serine (Ser) residue for Ala^2 [31] produced more stable analogues with significantly prolonged DPP IV half-lives compared with native GIP (Table 1). Unlike the Abu and Sar substitutions, both (Gly^2)GIP and (Ser^2)GIP displayed enhanced abilities to elevate cAMP (1.2-fold increase in EC$_{50}$ values) and stimulate insulin secretion (1.2- to 1.4-fold) in vitro (Table 1). When tested in obese diabetic *ob/ob* mice, both analogues exhibited significantly improved insulinotropic activity (1.3- to 1.5-fold) and antihyperglycaemic activity (1.2- to 1.3-fold) compared with native GIP (Table 1), further supporting the idea that DPP IV resistant analogues of GIP may prove useful in the treatment of type 2 diabetes [31].

Using a similar approach, the research group of Pederson and McIntosh [33] recently investigated substitution of the L-alanine in position 2 of GIP with D-alanine. This enzyme resistant analogue exhibited moderately reduced biological activity in vitro, although it significantly decreased the glycaemic excursion (1.6-fold) in *fa/fa* VDF Zucker rats with an efficacy similar to that seen with either (Gly^2)GIP or (Ser^2)GIP [31]. However, while several of these Ala^2-substituted analogues demonstrated significantly improved biological activity compared with native GIP, their efficacy was not as impressive as that of the Tyr^1-modified analogues (Table 1).

Effects of modification at position Glu^3

Substitution of Glu^3 in GIP with a proline (Pro) residue [55] produced a novel GIP analogue, (Pro^3)GIP (Fig. 1), completely resistant to DPP IV (Table 1). Surprisingly though, (Pro^3)GIP inhibited GIP-stimulated cAMP production and insulin secretion with high sensitivity and specificity in vitro (Table 1). Furthermore, studies using *ob/ob* mice showed that (Pro^3)GIP effectively and specifically countered the insulin releasing and antihyperglycaemic actions of the native peptide in vivo [37,55], reminiscent of the effects of the major

DPP IV degradation product, GIP(3-42) [14]. (Pro^3)GIP has also recently been utilized to demonstrate that GIP is the major physiological incretin, accounting for approximately 80% of nutrient-induced enteroinsular pancreatic beta cell stimulation [37].

Interestingly, the therapeutic potential of such a selective GIP receptor antagonist has recently been demonstrated in a study by Miyawaki and colleagues [58], where they showed that GIP plays a central role in lipid metabolism and in the development of both genetically inherited and diet-induced obesity. Thus, growing evidence supports the long-held view that GIP is an important factor directly linking over-nutrition to fat deposition, obesity, and glucose intolerance [59,60].

Conclusion

Structural modification of GIP at Tyr^1, Ala^2 or Glu^3 resulted in analogues with greatly increased, moderately increased or antagonistic biological properties, respectively, both in vitro and in vivo. The Tyr^1-modified analogues, especially *N*-acetyl-GIP, exhibit a substantially enhanced potency and duration of action compared to native GIP. Accordingly, these novel agents provide the basis for exploration to realize the potential of GIP in diabetes therapy. However, despite the tremendous potential for GIP analogues in the treatment of diabetes-obesity, their peptidic nature effectively rules out the option of straightforward oral administration. Therefore, in order to further develop rational drug design for GIP, information on appropriate delivery systems, three-dimensional structure and molecular interactions of the peptide with its receptor are essential.

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