Antagonistic effects of two novel GIP analogs, (Hyp$^3$)GIP and (Hyp$^3$)GIPLys$^{16}$PAL, on the biological actions of GIP and longer-term effects in diabetic ob/ob mice

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O’Harte FP, Hunter K, Gault VA, Irwin N, Green BD, Greer B, Harriott P, Bailey CJ, Flatt PR. Antagonistic effects of two novel GIP analogs, (Hyp$^3$)GIP and (Hyp$^3$)GIPLys$^{16}$PAL, on the biological actions of GIP and longer-term effects in diabetic ob/ob mice. Am J Physiol Endocrinol Metab 292: E1674–E1682, 2007. —This study examines the actions of the novel enzyme-resistant, NH$_2$-terminally modified GIP analog (Hyp$^3$)GIP and its fatty acid-derivatized analog (Hyp$^3$)GIPLys$^{16}$PAL. Acute effects are compared with the established GIP receptor antagonist (Pro$^3$)GIP. All three peptides exhibited DPP IV resistance, and significantly inhibited GIP stimulated cAMP formation and insulin secretion in GIP receptor-transfected fibroblasts and in clonal pancreatic BRIN-BD11 cells, respectively. Likewise, in obese diabetic ob/ob mice, intraperitoneal administration of GIP analogs significantly inhibited the acute antihyperglycemic and insulin-releasing effects of native GIP. Administration of once daily injections of (Hyp$^3$)GIP or (Hyp$^3$)GIPLys$^{16}$PAL for 14 days resulted in significantly lower plasma glucose levels ($P<0.05$) after (Hyp$^3$)GIP on days 12 and 14 and enhanced glucose tolerance ($P<0.05$) and insulin sensitivity ($P<0.05$ to $P<0.001$) in both groups by day 14. Both (Hyp$^3$)GIP and (Hyp$^3$)GIPLys$^{16}$PAL treatment also reduced pancreatic insulin ($P<0.05$ to $P<0.01$) without affecting islet number. These data indicate that (Hyp$^3$)GIP and (Hyp$^3$)GIPLys$^{16}$PAL function as GIP receptor antagonists with potential for ameliorating obesity-related diabetes. Acylation of (Hyp$^3$)GIP to extend bioactivity does not appear to be of any additional benefit.

GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE (GIP), along with its counterpart glucagon-like peptide-1 (GLP-1), is one of the major mediators of postprandial glucose-induced insulin secretion (4). As such, GIP is implicated as one of the factors responsible for hyperinsulinemia that occurs in obesity and type 2 diabetes, possibly as a result of an overactive enteroinsular axis (5). Elevated plasma GIP concentrations are reported in both obese diabetic ob/ob mice (14) and in subjects with type 2 diabetes (5, 35).

GIP is released postprandially following nutrient ingestion, especially fat, which makes it a potentially important anabolic agent in obesity etiology either directly by promoting hyperinsulemia or indirectly through effects on lipoprotein lipase (LPL) activity. LPL plays a key role in the hydrolysis of circulating triacylglycerol, liberating nonesterified fatty acids for uptake and storage within the adipocyte (40). GIP stimulates the synthesis and secretion of LPL in rat adipose tissue (10, 23). Therefore, high circulating concentrations of GIP accompanied by high-fat energy feeding facilitate the uptake of triacylglycerol, all of which may contribute to increased adiposity (9, 10, 23).

A likely link exists between GIP and obesity, which is further supported by studies that demonstrate that GIP enhanced insulin-stimulated glucose transport through increased adipocyte sensitivity (36), increased fatty acid synthesis in adipose tissue explants (1, 30), and reduced glucagon-stimulated lipolysis (8).

Recent studies using GIP receptor (GIP-R) knockout mice demonstrated a phenotype associated with intolerance and diminished insulin secretion (29). Furthermore, knockout of the GIP-R conferred resistance to diet-induced obesity and protection from insulin resistance (28). Cross-breeding with obese diabetic ob/ob mice resulted in decreased body weight gain and significant amelioration of adiposity and insulin resistance despite hyperphagia comparable with control ob/ob mice (28).

These studies suggest that disruption of the GIP signaling pathway by use of GIP-R antagonists could theoretically prevent obesity and insulin resistance, making it a target worth investigating for treatment of type 2 diabetes. NH$_2$-terminally truncated GIP fragment peptides such as GIP-(3–42) have been shown to act as antagonists in vivo (18, 22). Acute administration of (Pro$^3$)GIP with an intraperitoneal injection of glucose exacerbates glycemic and insulinoactive excursions thought to be due to both inhibition of the insulinoactive actions of high levels of endogenous GIP (14, 15) and blocking of other extrapancreatic glucose-lowering actions (31).

Long-term administration of (Pro$^3$)GIP to ob/ob mice resulted in reductions of basal glucose and insulin concentrations and improvement of glucose and meal tolerance together with...
a change toward normalization of islet morphology and β-cell function (20). These actions are underlined by significant improvement by (Pro3)GIP of insulin resistance that is otherwise particularly severe in this animal model of type 2 diabetes (2).

The present study has investigated the acute and long-term effects of another novel Glu3-substituted GIP-related peptide analog, (Hyp3)GIP. Furthermore, a daughter analog modified to contain a [C16]palmitate fatty acid at the position Lys16 residue was produced with the aim of providing a longer-acting molecule due to promotion of albumin binding in vivo. Evaluation of the in vitro properties of these novel GIP analogs was carried out alongside parallel studies using the established antagonist (Pro3)GIP (18). Finally, the effects of 14 days of treatment with daily (Hyp3)GIP and (Hyp3)GIPlys16PAL were examined in obese diabetic ob/ob mice to establish their antidiabetic potential.

MATERIALS AND METHODS

Reagents. HPLC grade acetonitrile was obtained from Rathburn (Walkersburn, Scotland). Sequencing grade trifluoroacetic acid (TFA), dipeptidyl peptidase IV (DPP IV), 3-isobutyl-1-methylxanthine (IBMX), adenine 3′-monophosphate (AMP), and α-fluorenlymethylxycarbonyl (Fmoc)-protected amino acids and peptide synthesis reagents were obtained from Calbiochem Novabiochem (Beeston, Nottingham, UK), α-Fluorenylmethylxycarbonyl (Fmoc)-protected amino acids and peptide synthesis reagents were obtained from Calbiochem Novabiochem (Beeston, Nottingham, UK). RPNI 1640 and DMEM tissue culture medium, fetal bovine serum, penicillin, and streptomycin were all purchased from Gibco (Paisley, Strathclyde, Scotland). The chromatography columns used for CAMP assay, Dowex AG 50W-X8 and neutral alumina AG7, were obtained from Bio-Rad (Life Science Research, Alpha Analytical, Larne, Northern Ireland). Tritiated adenosine [C16]palmitate fatty acid (Amersham, Bucks, UK). All water used in these experiments was purified using a Milli-Q water purification system (Millipore, Milford, MA). All other chemicals used were of the highest available purity.

Synthesis, purification, and characterization of GIP peptides. Native GIP was sequentially synthesized, as described previously (32), on an Applied Biosystems automated peptide synthesizer (Model 432A; Synergy, Foster City, CA) with a preloaded Fmoc-Gln(Trt)-OSu/Na system (Software version 2.1.5) and subsequently characterized using cAMP production.

Table 1. Molecular identity and susceptibility of GIP and related analogues to DPP IV degradation

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Theoretical Mr</th>
<th>Measured Mr</th>
<th>%Intact Peptide Remaining, h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>GIP</td>
<td>4,982.4</td>
<td>4,981.8</td>
<td>100</td>
</tr>
<tr>
<td>(Pro3)GIP</td>
<td>4,949.5</td>
<td>4,949.4</td>
<td>100</td>
</tr>
<tr>
<td>(Hyp3)GIP</td>
<td>4,965.5</td>
<td>4,966.4</td>
<td>100</td>
</tr>
<tr>
<td>(Hyp3)LysPAL46GIP</td>
<td>5,222.0</td>
<td>5,220.7</td>
<td>100</td>
</tr>
</tbody>
</table>

Data are means ± SE and represent molecular masses or % intact peptide remaining (following HPLC separation) relative to the major degradation fragment glucose-dependent insulinotropic polypeptide (GIP)-[3–42] after incubation for 0–8 h with dipeptidyl peptidase IV (DPP IV). The reactions were performed in triplicate. *P < 0.001 compared with native GIP.
then removed, and 1.0 ml Krebs-Ringer bicarbonate buffer (KRB, 115 mM NaCl, 4.7 mM KCl, 1.28 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM HEPES, and 10 mM NaHCO₃, pH 7.4 with NaOH) supplemented with 0.1% (wt/vol) BSA and 1.1 mM glucose was added to each well. The cells were then incubated for 40 min at 37°C, after which the buffer was removed and replaced with fresh KRB (1.0 ml) supplemented with 5.6 mM glucose and a range of concentrations (10⁻¹³ to 10⁻⁷ M) of GIP or GIP analogs. Likewise, varying concentrations of GIP peptides (10⁻¹³ to 10⁻⁷ M) were added with GIP (10⁻⁷ M) for the antagonist studies. After a 20-min incubation at 37°C, the buffer was removed from each well (900 μl) and (200 μl) aliquots used for measurement of insulin by RIA (13).

Acute in vivo biological activity. Following an 18-h fast, plasma glucose and insulin responses were evaluated using 14- to 18-wk-old obese diabetic ob/ob mice (2). Responses were measured immediately prior to and following administration by intraperitoneal injection (n = 8) of glucose alone (18 nmol/kg body wt) as control or glucose together with GIP or GIP peptides (25 nmol/kg body wt). These tests were performed to evaluate whether either of the two Hyp-substituted analogs had any GIP agonist activity. In addition, to assess the antagonistic properties of these peptides, a double dose of GIP (50 nmol/kg body wt) or GIP (25 nmol/kg body wt) in combination with each GIP analog peptide (25 nmol/kg body wt) was given by intraperitoneal injection. Fasted mice were used to maximize glycemic and insulin responses to enable evaluation of the effects of GIP peptides. All animal studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Protocol approval was granted under appropriate project licences by HPSS in Northern Ireland.

Longer-term in vivo studies. Obese diabetic ob/ob mice aged 14- to 18-wk (22) were randomly divided into groups that received once daily intraperitoneal injections (1700) of saline vehicle, (Hyp³)GIP, or (Hyp³)GIPlys¹⁶PAL (25 nmol/kg body wt) over 14 days. Food intake and body weights were recorded daily. Blood samples were taken at 0900 at 3- to 4-day intervals for plasma glucose and insulin analysis. A number of metabolic tests were carried out after 14 days treatment and again 14 days after cessation of treatment. These included evaluation of the effects in nonfasted mice of intraperitoneal glucose (18 mmol/kg body wt) as control or glucose together with GIP (25 nmol/kg body wt) in combination with each GIP analog peptide (25 nmol/kg body wt), or insulin (50 U/kg body wt). Mice fasted for 18 h were used to evaluate responses to 15-min refeeding. In one experimental series, the pancreases of mice treated for 14 days were resected for determination of insulin content and islet morphometry, as described previously (20).

In both acute and longer-term studies, test solutions were administered in a final volume of 5 ml/kg body wt. Blood samples were collected from the cut tip of the tail vein of conscious mice into chilled fluoride/heparin coated glucose microcentrifuge tubes (Sarstedt, Nümbrecht, Germany) at the times indicated in the figures. Blood samples were immediately centrifuged using a Beckman microcentrifuge (Beckman Instruments, High Wycombe, Buckinghamshire, UK) for 30 s at 13,000 g. The resulting plasma was then aliquoted into fresh Eppendorf tubes and stored at −20°C prior to glucose determination using a Beckman Glucose Analyzer II (37) and insulin determination by RIA (13).

Statistical analysis. Results are expressed as means ± SE. Data were compared using Student’s t-test or ANOVA, followed by the Student-Newman-Keuls post hoc test, as appropriate. Groups of data were considered to be significantly different if P < 0.05.

RESULTS

Degradation by DPP IV. As shown in Table 1, native GIP was rapidly degraded by DPP IV to the truncated metabolite GIP-(3–42), with an estimated in vitro half-life of 1.3 h. In contrast, (Pro³)GIP, (Hyp³)GIP, and (Hyp³)GIPlys¹⁶PAL remained fully intact following 8-h incubation with DPP IV (Table 1).

cAMP production in vitro. GIP analogs tested at 10⁻⁷ M concentrations were considerably less potent than native GIP in stimulating cAMP production in GIP-R-transfected CHL fibroblasts (Table 2). The cAMP production at 10⁻¹¹ M GIP was 758 ± 83 dpm, which was only 5.05 ± 0.55% of the maximal GIP response at 10⁻⁷ M GIP. EC₅₀ values for native GIP, (Pro³)GIP, (Hyp³)GIP, and (Hyp³)GIPlys¹⁶PAL were 0.47 nm, 2.07 nm, 13.4 nm, and 1.45 nm, respectively. Indeed, in the presence of 10⁻⁷ M GIP, (Pro³)GIP, (Hyp³)GIP, and (Hyp³)GIPlys¹⁶PAL significantly inhibited cAMP formation 34–52% (P < 0.01 to P < 0.001).

Insulin secretion in vitro. Table 2 shows that GIP analogs exhibited weak insulin-releasing activity compared with native GIP. (Pro³)GIP, (Hyp³)GIP, and (Hyp³)GIPlys¹⁶PAL inhibited maximal GIP-induced insulin secretion by 29–38% (P < 0.05 to P < 0.001). There were no significant differences between any of the in vitro properties of the three GIP analogs (Table 2). The basal insulin secretion as measured in an acute test was (means ± SE, n = 8) 1.82 ± 0.14 ng·10⁶ cells⁻¹·20 min⁻¹ at 5.6 mM glucose alone (control). This was 66 ± 2% of the maximum insulin response observed with 10⁻⁷ M native GIP.

Acute metabolic effects in ob/ob mice. Figure 1A shows the plasma glucose responses to intraperitoneal glucose alone or in combination with GIP or its analogs. Injection of GIP reduced the glycemic excursion at 30 and 60 min and significantly lowered (P < 0.001) area under the curve.

Table 2. cAMP production and insulin secretory effects of GIP and related analogues

<table>
<thead>
<tr>
<th>Peptide, 10⁻⁷ M</th>
<th>cAMP</th>
<th>Insulin Secretion</th>
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<tbody>
<tr>
<td></td>
<td>Agonist action</td>
<td>Antagonist action</td>
</tr>
<tr>
<td></td>
<td>cAMP response</td>
<td>in presence of 10⁻⁷ M</td>
</tr>
<tr>
<td>GIP</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>(Pro³)GIP</td>
<td>54±3</td>
<td>66±7*</td>
</tr>
<tr>
<td>(Hyp³)GIP</td>
<td>57±4*</td>
<td>56±4*</td>
</tr>
<tr>
<td>(Hyp³)GIPlys¹⁶PAL</td>
<td>51±4*</td>
<td>48±7*</td>
</tr>
</tbody>
</table>

Results are presented as a %maximum GIP response at 10⁻⁷ M (%means ± SE and means ± SE ng·10⁶ cells⁻¹·20 min⁻¹ for cAMP and insulin, respectively; n = 4). cAMP production and insulin-releasing activity were measured in 2 separate cell lines, namely GIP receptor-transfected Chinese Hamster Lung fibroblast cells and glucose-responsive BRIN-BD11 pancreatic β-cells, respectively. *P < 0.01; †P < 0.001 compared with native GIP. Basal unstimulated levels were 5.05 ± 0.55% and 1.82 ± 0.14 ng·10⁶ cells⁻¹·20 min⁻¹ at 5.6 mM glucose control, respectively. Agonist effects were evaluated in the absence of added GIP, whereas possible antagonist actions were evaluated in the presence of 10⁻⁷ M GIP.

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(AUC) values (Fig. 1B). In contrast, glucose concentrations and AUC measurements were significantly greater following (Pro³)GIP, (Hyp³)GIP, or (Hyp³)GIPlys¹⁶PAL ($P < 0.01$ to $P < 0.001$). The effects were significantly greater for (Pro³)GIP ($P < 0.01$ to $P < 0.001$) than other GIP analogs, indicating that (Pro³)GIP was the most potent GIP-R antagonist under the conditions tested. The corresponding insulin release data are shown in Fig. 1C. The overall insulin responses to (Pro³)GIP, (Hyp³)GIP, or (Hyp³)GIPlys¹⁶PAL were significantly less ($P < 0.05$ to $P < 0.01$) than to GIP (Fig. 1D). Native GIP caused significantly greater insulin release than glucose alone ($P < 0.05$).

Fig. 2. Antagonistic effects of GIP peptides on the glucose-lowering and insulinotropic effects of GIP in 18-h-fasted ob/ob mice. Plasma glucose (A) and insulin (C) were measured prior to and after ip administration of glucose alone (18 mmol/kg body wt) or in combination with GIP, (Pro³)GIP, (Hyp³)GIP, and (Hyp³)GIPlys¹⁶PAL (25 nmol/kg). The time of injection is indicated by the arrow (0 min). Plasma glucose (B) or insulin (D) AUC values for 0–60 min postinjection are shown. Values represent means ± SE for 8 mice. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with glucose alone; $\Delta P < 0.05$; $\Delta\Delta P < 0.01$; $\Delta\Delta\Delta P < 0.001$ compared with native GIP; $++P < 0.01$; $+++P < 0.001$ compared with (Pro³)GIP.
The effects of (Pro3)GIP, (Hyp3)GIP, and (Hyp3)GIPLys16PAL on the glucose-lowering and insulinotropic actions of GIP administered together with glucose are shown in Fig. 2, A–D. Glucose levels were significantly higher \( (P < 0.05 \text{ to } P < 0.001) \) at 15, 30, and 60 min after injection compared with native GIP alone. AUC values (Fig. 2B) showed that the overall glucose responses were 1.8-fold greater \( (P < 0.001) \) than for native GIP. Insulinotropic actions of GIP were diminished 1.2- to 1.8-fold \( (P < 0.05 \text{ to } P < 0.001) \) by coadministration of each of the three GIP analogs over the 0- to 60-min study period (Fig. 2D). The effects of (Hyp3)GIP, (Hyp3)GIPLys16PAL, and (Pro3)GIP on the antihyperglycemic and insulin-releasing effects of native GIP were not significantly different.

Longer-term effects on body weight, food intake, and nonfasting glucose and insulin in ob/ob mice. Body weight and food intake of ob/ob mice were not changed by daily injection of (Hyp3)GIP or (Hyp3)GIPLys16PAL (25 nmol/kg body wt) for 14 days (Fig. 3, A and B). Plasma glucose was significantly reduced \( (P < 0.05) \) following (Hyp3)GIP, but not (Hyp3)GIPLys16PAL, on day 12 (Fig. 4A). As shown in Fig. 4B, insulin concentrations were unaffected by treatment with the GIP analogs.

Longer-term effects on glucose and insulin responses to intraperitoneal injection of glucose in ob/ob mice. Figure 5 shows the effect of 14-day treatment with (Hyp3)GIP or (Hyp3)GIPLys16PAL on glucose tolerance and insulin responses. Glucose concentrations were significantly lower \( (P < 0.01) \) for both peptide-treated groups compared with saline-treated controls at both basal \( (t = 0) \) as well as 15-min postinjection. The glucose concentration for the (Hyp3)GIP-treated group remained significantly reduced \( (P < 0.05) \) at 15-min postinjection in the (Hyp3)GIP-treated group (Fig. 5B). However, plasma insulin was unaffected in the (Hyp3)GIPLys16PAL-treated group (Fig. 5B)

Longer-term effects on glucose and insulin responses to feeding and insulin sensitivity in ob/ob mice. No significant differences were observed in the glycemic or insulin responses to refeeding in ob/ob mice treated for 14 days with either saline, (Hyp3)GIP, or (Hyp3)GIPLys16PAL (Fig. 6, A and B).
As shown in Fig. 7, glucose-lowering effects of insulin at 30 and 60 min postinjection were also enhanced in the (Hyp3)GIP- and (Hyp3)GIPLys16PAL-treated groups (P < 0.05 to P < 0.001) compared with saline controls.

**Longer-term effects on glucose and insulin responses to intraperitoneal injection of GIP in ob/ob mice.** Glucose levels were significantly lower (P < 0.05 to P < 0.01) at basal and 15 min postinjection with GIP for both peptide-treated groups compared with saline controls (Fig. 8A). However, at 60 min, glucose was significantly decreased (P < 0.01) in (Hyp3)GIP-treated mice compared with the (Hyp3)GIPLys16PAL-treated group. There were no significant differences in the corresponding insulin responses following a GIP challenge, as shown in Fig. 8B. Thus this indicates that the action of the fatty acid-linked GIP-R antagonist (Hyp3)GIPLys16PAL may be acting through mechanisms other than blocking the direct insulinotropic actions to induce its potent hyperglycemic effects (Fig. 6B).

**DISCUSSION**

(Pro3)GIP has been developed as a powerful and effective specific GIP-R antagonist capable of completely abolishing the glucose-lowering and insulinotropic actions of GIP (18). Daily administration of (Pro3)GIP for 11 days has been shown to ameliorate features of the ob/ob syndrome in mice, suggesting a therapeutic utility of GIP-R antagonists in obesity and/or diabetes (20). This effect is pathology related, since chemical or molecular knockout of the GIP-R has little effect on glucose homeostasis in normal mice (22, 28).

The present study has investigated the actions of another Glu3-substituted analog of GIP, (Hyp3)GIP, as a potentially new GIP-R antagonist. Hyp was selected because of its structural similarity to proline and the fact that it is unlikely to form fragments in vivo. As shown in Fig. 7, glucose-lowering effects of insulin at 30 and 60 min postinjection were also enhanced in the (Hyp3)GIP- and (Hyp3)GIPLys16PAL-treated groups (P < 0.05 to P < 0.001) compared with saline controls.

**Fig. 5. Effects of daily injection of (Hyp3)GIP and (Hyp3)GIPLys16PAL on glucose tolerance and insulin response to glucose in nonfasted ob/ob mice.** Mice received daily injections of saline alone or GIP peptides (25 nmol/kg body wt) for 14 days prior to ip administration of glucose (18 mmol/kg body wt) and subsequent measurement of glucose (A) and insulin (B). The time of injection is indicated by the arrow. Values are means ± SE for 8 mice. *P < 0.05; **P < 0.01 compared with saline control.

**Fig. 6. Effects of daily injection of (Hyp3)GIP and (Hyp3)GIPLys16PAL on metabolic response to feeding in ob/ob mice.** Mice received daily injections of saline alone or GIP peptides (25 nmol/kg body wt) for 14 days prior to an overnight fast (18 h) and subsequent 15-min refeeding as indicated by the black bars. Plasma glucose (A) and insulin (B) are shown. Values are means ± SE for 8 mice.
a peptide bond that was susceptible to the action of DPP IV (33). In addition to DPP IV-mediated degradation, recent reports (6) highlight the importance of renal filtration in elimination of GIP from the circulation. Structural modification of GIP to delay removal by the kidneys may therefore lead to a longer half-life. Thus we synthesized a daughter analog with palmitate conjugated to the ε-amino group of Lys16 of (Hyp3)GIP. This was envisaged to facilitate binding to plasma proteins and thereby prevent clearance by the kidney. Already, such an approach has been used successfully with insulin (16, 25) and other peptide hormones, including GLP-1 (16, 24).

As suspected, substitution of the third NH2-terminal amino acid of GIP with Hyp resulted in a stable enzyme-resistant analog that was unaffected by 8-h incubation with DPP IV. (Hyp3)GIPLys16PAL and, consistent with previous findings (18), (Pro3)GIP also exhibited complete DPP IV resistance. All three GIP analogs only very weakly stimulated cAMP production and insulin secretion. However, in the presence of cAMP and a stimulatory concentration of native GIP, (Hyp3)GIP and (Hyp3)GIPLys16PAL retained some antagonistic activity in vitro. This is consistent with their actions as GIP-R antagonists, as also observed and previously described for (Pro3)GIP (18).

Consistent with this view, (Hyp3)GIP and its acylated analog (Hyp3)GIPLys16PAL and (Pro3)GIP increased basal glucose and decreased insulin levels in ob/ob mice when administered acutely. Coadministration of these peptides with native GIP significantly countered both the antihyperglycemic and insulin-releasing effects of GIP. Although the three GIP analogs appeared to have similar potency in the in vitro tests, (Pro3)GIP appeared to have more pronounced effects when administered in vivo to ob/ob mice.

To further evaluate the possible utility of (Hyp3)GIP and beneficial effects of acylation, longer-term studies were performed in which ob/ob mice received once daily injections of either saline, (Hyp3)GIP, or (Hyp3)LysPAL16GIP for 14 days. Food intakes or body weights were not significantly changed compared with saline-treated controls. This corresponds with previous observations made in ob/ob mice treated with (Pro3)GIP (20, 22). More importantly, (Hyp3)GIP but not the acylated analog induced a significant reduction in plasma glucose on days 12 and 14 of this study compared with saline-treated controls. Plasma insulin levels were not appreciably affected by either peptide, although there was a tendency toward lower levels. Such results indicate that peptide

Table 3. Pancreatic insulin content and islet number in ob/ob mice after 14 once daily injections of saline, (Pro3)LysPAL16GIP, or (Hyp3)GIPLys16PAL

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Pancreatic Insulin Content, μg/g tissue</th>
<th>Number of Islets/Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>9.37±1.18</td>
<td>6.29±0.53</td>
</tr>
<tr>
<td>(Hyp3)GIP</td>
<td>7.12±0.58*</td>
<td>6.09±0.40</td>
</tr>
<tr>
<td>(Hyp3)LysPAL16GIP</td>
<td>6.51±0.29†</td>
<td>5.84±0.86</td>
</tr>
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</table>

Values are means ± SE. *P < 0.05; †P < 0.001 compared with saline control.
half-life was significantly enhanced or that the dose was high enough to overcome a short half-life. Presently available GIP antibodies react poorly with these analogs, and necessary pharmacokinetic studies to discriminate between these two possibilities await generation of new assay methods. These results also are reminiscent of the effects of (Pro3)GIP (20) and have parallel effects with Roux-en-Y gastric bypass in morbidly obese type 2 diabetic patients (34). These individuals suffer loss of functional GIP secretory cells and demonstrate significantly decreased fasting plasma glucose within 2 wk. In contrast, insulin approached but never reached significantly reduced levels after 12 wk (3).

More convincing evidence for impact on blood glucose control comes from results of glucose tolerance tests conducted in ob/ob mice after 14 days treatment. This revealed significantly lower glucose concentrations in both (Hyp3)GIP and (Hyp3)GIPlys16PAL-treated groups. Plasma insulin levels in the (Hyp3)GIPlys16PAL group were significantly lower during the test, suggesting an increased effectiveness of insulin. This was indeed confirmed by increased hypoglycemic response to exogenous insulin in both of the groups treated with GIP analogs. These observations, together with previously published effects of (Pro3)GIP (20), indicate that GIP-R antagonists alleviate obesity-related insulin resistance, which is a major driving force behind glucose intolerance, hyperglycemia, and ß-cell dysfunction (19).

Plasma glucose and plasma insulin responses to feeding following 14-day treatment with either (Hyp3)GIP, (Hyp3)GIPlys16PAL, or saline gave similar plasma glucose concentrations. This was accompanied by unchanged insulin responses, arguing against an important inhibitory effect of GIP-R antagonism on intestinal glucose adsorption, as suggested by the results of some but not all relevant studies (11, 38). This observation may also account for less pronounced lowering of nonfasting glucose than the glycemic responses to a large intraperitoneal glucose load. The possible involvement of changes in the expression of GIP-Rs on target cells in the beneficial effects of these analogs may also be of relatively little benefit and resulted in marginal impairment of in vivo activity compared with the parent molecule. Finally, since it might be speculated that GIP has an effect on leptin expression in the adipocyte, it is important to recognize that use of ob/ob mice has the limitation of representing a leptin-deficient model. Nevertheless, preliminary unpublished data indicate similar benefits of GIP-R antagonism in normal mice fed high-fat diets.

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GRANTS

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REFERENCES


