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Comparison of the subchronic antidiabetic effects of DPP IV–resistant GIP and GLP-1 analogues in obese diabetic (*ob/ob*) mice

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Abstract: Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are the two key incretin hormones released from the gastrointestinal tract that regulate blood glucose homeostasis through potent insulin secretion. The rapid degradation of GIP and GLP-1 by the ubiquitous enzyme dipeptidyl peptidase IV (DPP IV) renders both peptides noninsulinotropic. However, DPP IV stable agonists, such as *N*-AcGIP and (Val^8)GLP-1, have now been developed. The present study has examined and compared the metabolic effects of subchronic administration of daily i.p. injections of *N*-AcGIP, (Val^8)GLP-1 and a combination of both peptides (all at 25 nmol/kg bw) in obese diabetic (*ob/ob*) mice. Initial *in vitro* experiments confirmed the potent insulinotropic properties of *N*-AcGIP and (Val^8)GLP-1 in the clonal pancreatic BRIN BD11 cell line. Subchronic administration of *N*-AcGIP, (Val^8)GLP-1 or combined peptide administration had no significant effects on the body weight, food intake and plasma insulin concentrations. However, all treatment groups had significantly ($p < 0.05$) decreased plasma glucose levels and improved glucose tolerance by day 14. The effectiveness of the peptide groups was similar, and glucose concentrations were substantially reduced following injection of insulin to assess insulin sensitivity compared to control. These results provide evidence for an improvement of glucose homeostasis following treatment with enzyme-resistant GIP and GLP-1 analogues. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: glucose-dependent insulinotropic polypeptide (GIP); glucagon-like peptide-1 (GLP-1); dipeptidylpeptidase IV (DPP IV); analogue; glucose homeostasis; obese diabetic (*ob/ob*) mouse

INTRODUCTION

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are enteroendocrine gut hormones. GIP and GLP-1 regulate postparandial glucose homeostasis through interaction with specific receptors on the pancreatic beta-cell and subsequent potent insulin release [1]. In addition, both incretin hormones have shown beneficial effects on beta-cell proliferation, growth and survival [2–4]. Furthermore, the antidiabetic effects of GIP and GLP-1 are not limited to direct effects on pancreatic beta-cells, as antihyperglycaemic effects have been observed at several extrapancreatic sites [5,6]. Since many of these biological properties are directly involved in lowering blood glucose, much recent attention has focused on GIP and GLP-1 as therapeutic options for the treatment of type 2 diabetes [5,7,8].

However, the biological effectiveness of GIP and GLP-1 is countered by the action of the ubiquitous enzyme dipeptidyl peptidase IV (DPP IV; EC 3.4.15.5). DPP IV degrades numerous regulatory peptides, and, in the case of GIP and GLP-1, specifically hydrolyzes the aminoterminal Tyr^1-Ala^2 and His^7-Ala^8 residues from GIP and GLP-1 respectively, forming the truncated metabolites GIP (3–42) and GLP-1 (9–36) [9]. These truncated forms lack insulinotropic activity and were initially believed to be inactive [10]. However, recent evidence in *ob/ob* mice indicates that GIP (3–42) and GLP-1 (9–36) may even act as antagonists at high concentrations at their specific beta-cell receptors [11,12]. Thus, the pharmacokinetic profiles of native GIP and GLP-1 do not lend themselves readily to therapeutic administration. However, strategies to prevent enzymatic degradation of GIP and GLP-1 have recently emerged [13,14].

One such method adopts the approach of *N*-terminal modification to mask the potential binding site of DPP IV [13]. In general, these modifications are well tolerated, and the insulin-releasing and antidiabetic potential of several aminoterminally modified GIP and GLP-1 analogues has now been demonstrated [5,13]. Of these analogues tested in our laboratory, *N*-AcGIP and (Val^8)GLP-1 appear to be the most effective analogues. Substitution of Ala^8 with valine in GLP-1 confers resistance to DPP IV degradation, resulting in increased antihyperglycaemic activity in *ob/ob* mice [15]. In the case of GIP, addition of an acetyl adduct to the *N*-terminal of GIP results in a stable analogue with substantially augmented insulin-releasing and glucose-lowering properties in *ob/ob* mice [16].

The additive insulinotropic effects of GIP and GLP-1 have been observed previously [17]. These

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observations are supported by the fact that the signaling pathways of GIP and GLP-1, although utilizing the same K (ATP) channel, are mechanistically different [18]. Furthermore, GIP and GLP-1 have several distinguishing features. GLP-1 is known to decrease feeding activity and body weight and to inhibit gastric emptying and glucagon secretion, whereas GIP has no such effects [5,6]. GIP and GLP-1 are transcribed on separate genes [19,20] and each posses their own specific G-protein-coupled receptor [21].

The present study was thus designed to evaluate the relative efficacy and combined antidiabetic activity of two of the most promising GIP and GLP-1 analogues, (Val^8)GLP-1 and *N*-AcGIP, in obese diabetic (*ob/ob*) mice. Effects on basal plasma glucose and insulin, glucose homeostasis, insulin sensitivity and metabolic response to feeding were examined. The results illustrate significant antidiabetic actions of both *N*-AcGIP and (Val^8)GLP-1.

MATERIALS AND METHODS

Animals

Obese diabetic (*ob/ob*) mice derived from the colony maintained at Aston University, UK [22], were used at 15–19 weeks of age. Animals were housed in an air-conditioned room at $22 \pm 2\,^{\circ}C$ with a 12-h-light–12-h-dark cycle (08 : 00–20 : 00 h). Drinking water and standard rodent maintenance diet (Trouw Nutrition, Cheshire, UK) were freely available. All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. No adverse effects were observed following subchronic administration of *N*-AcGIP, (Val^8)GLP-1 or a combination of both peptides.

Synthesis, Purification and Characterization of GIP, GLP-1, N-AcGIP and (Val^8) GLP-1

GIP, GLP-1, *N*-AcGIP and (Val^8)GLP-1 were sequentially synthesized on an Applied Biosystems automated peptide synthesizer (Model 432 A, Foster City, CA, USA) using standard solid-phase Fmoc peptide chemistry as previously reported [16]. For *N*-AcGIP, an acetyl adduct was incorporated at the *N*-terminal Tyr^1 of native GIP and, in the case of (Val^8)GLP-1, a valine residue was substituted for alanine residue at position 8 of GLP-1. Peptides were judged pure by reversed-phase HPLC on a Waters Millenium 2010 chromatography system (Software version 2.1.5) and subsequently characterized using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry as described previously [23].

In vitro Insulin Secretion

BRIN-BD11 cells were seeded into 24-multiwell plates at a density of 1.0×10^5 cells per well, and allowed to attach overnight at 37 °C. Acute tests for insulin release were preceded by 40-min preincubation at 37 °C in 1.0 ml Krebs Ringer bicarbonate buffer (115 mM NaCl, 4.7 mM KCl, 1.28 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 10 mM $NaHCO_3$, 0.5% (w/v) BSA, pH 7.4) supplemented with 1.1 mM glucose. Test incubations were performed in the presence of 5.6 mM glucose with a range of concentrations (10^{-12}–10^{-8} M) of GIP, GLP-1, *N*-AcGIP and (Val^8)GLP-1. After 20-min incubation, the buffer was removed from each well and aliquots (200 ml) were used for measurement of insulin. The origin and functional characteristics of BRIN BD11 cells are detailed elsewhere [24].

Subchronic Effects of N-AcGIP, (Val^8)GLP-1 and Combined Peptide Administration in *ob/ob* Mice

Groups of *ob/ob* mice received once-daily intraperitoneal injections (17 : 00 h) of either saline (0.9%, w/v, NaCl), N-AcGIP, (Val^8)GLP-1 or a combination of both peptides (all at 25 nmol/kg body weight/day). Food intake and body weight were recorded daily from 4 days before commencement of the treatment regimes. Plasma glucose and insulin concentrations were monitored at 2- to 4-day intervals (10 : 00 h). On day 14, groups of animals were used to evaluate intraperitoneal glucose tolerance (18 mmol/kg) and insulin sensitivity (50 U/kg). All acute tests were commenced at 10 : 00 h. All 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, Galway, Ireland) for 30 s at 13 000 g. The resulting plasma was then aliquoted into fresh tubes and stored at −20 °C prior to glucose and insulin determinations.

Biochemical Analyses

Plasma glucose was assayed by an automated glucose oxidase procedure [25] using a Beckman Glucose Analyzer II. Insulin was assayed by a modified dextran-charcoal RIA as described previously [26].

Statistics

Results are expressed as mean ± SEM. Data were compared using repeated measures ANOVA or one-way ANOVA, followed by the Student–Newman–Keuls *post hoc* test. Incremental areas under plasma glucose and insulin curves (AUC) were calculated using a computer-generated program employing the trapezoidal rule [27] with baseline subtraction. Groups of data were considered to be significantly different if $p < 0.05$.

RESULTS

Stimulation of *In vitro* Insulin Secretion

All four peptides; GIP, GLP-1, *N*-AcGIP and (Val^8)GLP-1, significantly ($p < 0.05$ to $p < 0.001$) enhanced insulin release in a concentration-dependent manner compared to 5.6 mM glucose control (Figure 1). There was no significant difference in potency between the four peptides.

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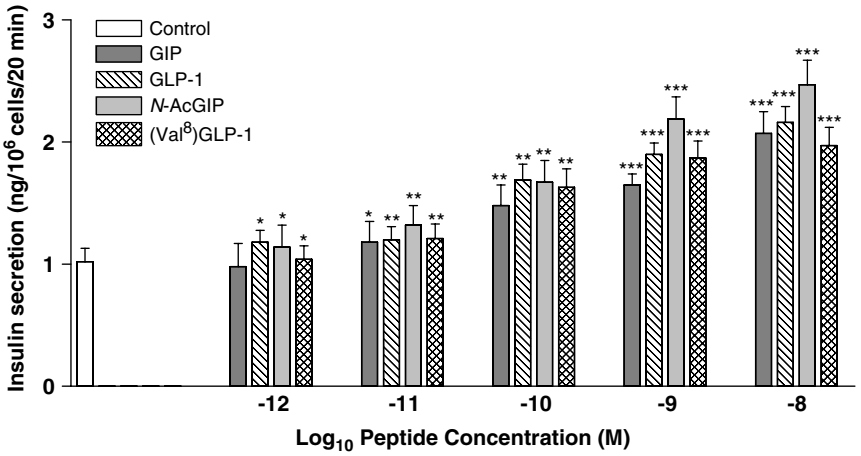

Figure 1 Insulin-releasing activity of GIP, GLP-1, *N*-AcGIP and (Val^8)GLP-1 in the clonal pancreatic beta-cell line, BRIN-BD11. After preincubation (40 min), the effects of various concentrations of peptide were tested on insulin release during a 20-min incubation. Values are means ± SEM for eight separate observations. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.01$ compared to 5.6 mM glucose control.

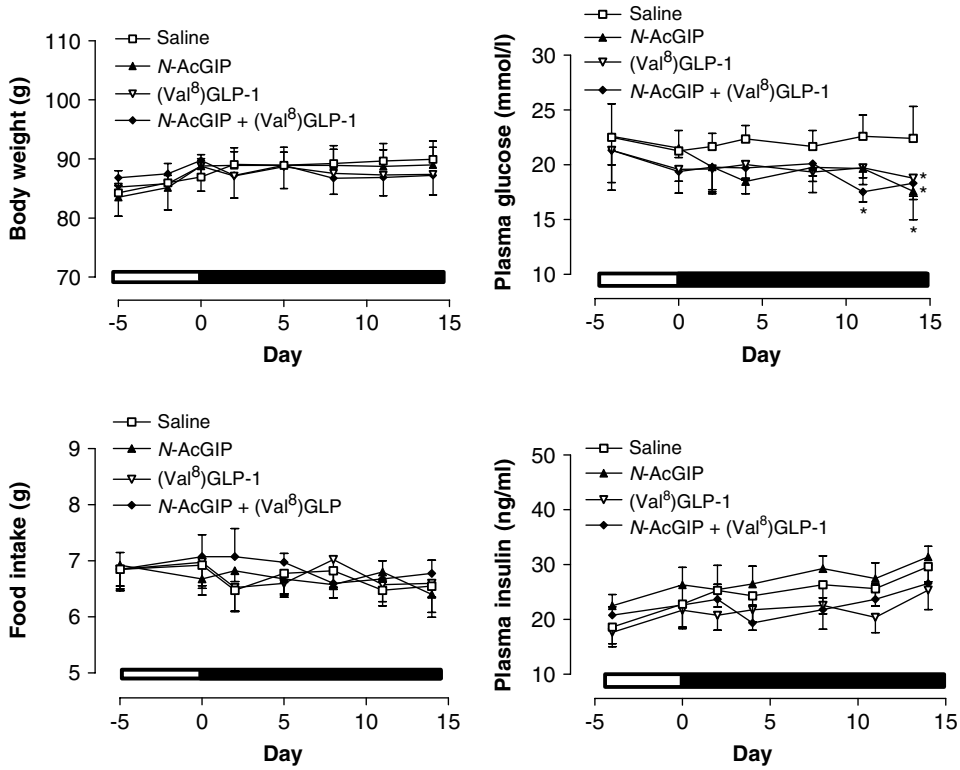

Figure 2 Effects of daily *N*-AcGIP, (Val8)GLP-1 and combined peptide administration on food intake, body weight and nonfasting glucose and insulin. *N*-AcGIP, (Val^8)GLP-1, a combination of both peptides (each at 25 nmol/kg/day) or saline vehicle (control) were administered for 14 days, as indicated by the horizontal black bar. Values are mean ± SEM for eight mice. $^*p < 0.05$ compared to control.

Subchronic Effects of N-AcGIP, (Val^8)GLP-1 and a Combination of Both Peptides on Food Intake, Body Weight and Nonfasting Plasma Glucose and Insulin Levels

Administration of *N*-AcGIP, (Val^8)GLP-1 or a combination of both peptides had no effect on food intake or body weight (Figure 2). Plasma glucose concentrations were progressively reduced, resulting in significantly ($p < 0.05$) lowered glucose concentrations on day 11 in the combined peptide-treated group, and on day 14 in all three treatment groups (Figure 2). These changes were not accompanied by significantly altered plasma insulin concentrations (Figure 2).

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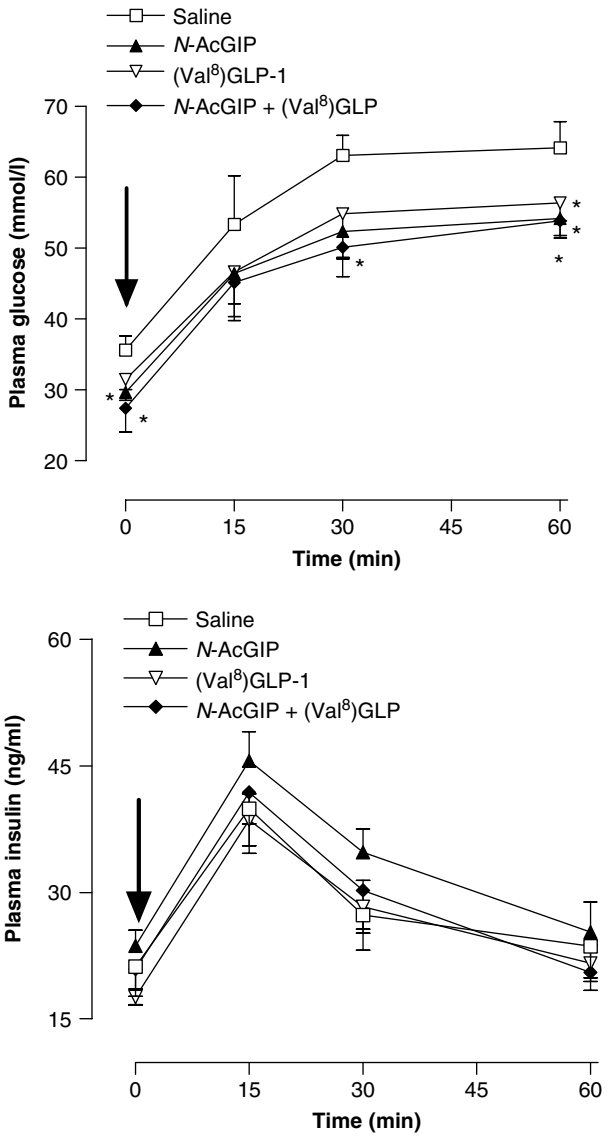

Figure 3 Effects of daily *N*-AcGIP, (Val^8)GLP-1 and combined peptide administration on glucose tolerance and plasma insulin response to glucose. Tests were conducted after 14 daily injections of either *N*-AcGIP, (Val^8)GLP-1, a combination of both peptides (each at 25 nmol/kg/day) or saline (control). Glucose (18 mmol/kg) was administered by intraperitoneal injection at the time indicated by the arrow. Values are mean ± SEM for eight mice. $^*p < 0.05$ compared to control.

Subchronic Effects of N-AcGIP, (Val^8)GLP-1 and a Combination of Both Peptides on Glucose Tolerance and Insulin Sensitivity

As shown in Figure 3, treatment with *N*-AcGIP, (Val^8)GLP-1 or a combination of both peptides for 14 days resulted in a significant improvement in glucose tolerance compared to controls (32, 26 and 29% respectively, $p < 0.05$ in all cases). This enhancement of glucose tolerance was associated with similarly augmented glucose-mediated insulin secretion in each of the treatment groups (Figure 3). The hypoglycaemic action of insulin was significantly augmented in terms of basal and postinjection values in *ob*/*ob* mice treated for 14 days with *N*-AcGIP, (Val^8)GLP-1 or a combination of both peptides (Figure 4).

DISCUSSION

GIP and GLP-1 are members of the glucagon peptide super family [1]. The primary function of these two incretin hormones is the glucose-dependent stimulation of insulin release from pancreatic beta-cells following nutrient absorption [17]. Since the insulin-releasing actions of GIP and GLP-1 are glucose dependent, there has been a great upsurge in stable incretin-based antidiabetic therapeutics [5,8,13]. Recently discovered analogues of GLP-1 such as exendin-4 and (Val^8)GLP-1 are resistant to DPP IV degradation. Exendin-4 has been approved for therapeutic use in the United States under the trade name Byetta [13]. (Val^8)GLP-1 and other *N*-terminally modified analogues have been shown to possess potent antidiabetic effects in animals [13]. *N*-terminal acetylation of the sister incretin GIP also yields a DPP IV–resistant analogue with enhanced bioactivity compared to the native peptide [16]. Thus, (Val^8)GLP-1, *N*-acetylGIP and other synthetic DPP IV–resistant forms are incretin-based molecules with antidiabetic potential [13,28]. Direct comparison of the subchronic antidiabetic properties of these stable incretin peptide analogues has not been carried out.

The present study confirmed the *in vitro* insulinotropic properties of *N*-AcGIP and (Val^8)GLP-1 preparations

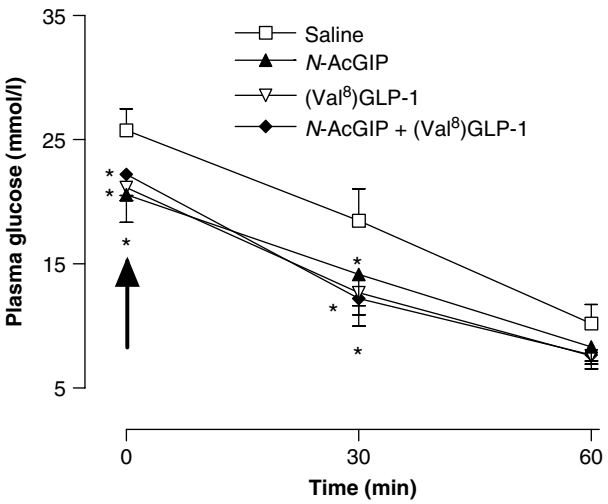

Figure 4 Effects of daily *N*-AcGIP, (Val^8)GLP-1 and combined peptide administration on insulin sensitivity. Tests were conducted after 14 daily injections of either *N*-AcGIP, (Val^8)GLP-1, a combination of both peptides (each at 25 nmol/kg/day) or saline (control). Insulin (50 U/kg) was administered by intraperitoneal injection at the time indicated by the arrow. Values are mean ± SEM for eight mice. $^*p < 0.05$ compared to control.

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used [15,16]. Subchronic administration of *N*-AcGIP, (Val^8)GLP-1 or combined administration to *ob*/*ob* mice for 14 days did not effect food intake or body weight. This accords well with the view that GIP lacks effects on feeding activity [29] but contrasts with the satiety effects of GLP-1 observed by others [30]. However, our earlier studies have shown that this dose of (Val^8)GLP-1 dose not affect feeding on a background of leptin deficiency [31].

Daily administration of *N*-AcGIP, (Val^8)GLP-1 or combination of both peptides significantly reduced plasma glucose and improved glucose tolerance by 14 days. These changes occurred without appreciable alterations of circulating insulin concentrations, possibly reflecting decreased stimulation of pancreatic beta-cells by lower ambient glucose concentrations. Most notably, (Val^8)GLP-1 and *N*-AcGIP had similar effects at the dose employed, indicating that the stable GIP analogue could overcome any resistance to native GIP observed characteristically in this mutant [5]. Treatment with either *N*-AcGIP or (Val^8)GLP-1 was also associated with substantially lower glucose concentrations during insulin sensitivity tests. This probably reflects either simple regression of glucose toxicity due to lower basal glucose concentrations or other glucose-lowering extrapancreatic effects of GIP and GLP-1 [5,13].

It has previously been shown that GIP and GLP-1 have additive insulinotropic actions [17] and that combined GLP-1 and GIP treatment can prime the pancreatic beta-cell to induce a greater insulinotropic response to subsequent secretory stimuli [32]. However, the present study did not see this effect at the dose employed. This could reflect the good efficacy of these stable incretins alone and the relatively high doses employed which could preclude an additive action. Such a benefit was clearly demonstrated when (Val^8)GLP-1 was substituted with exendin-4 (1–39)amide, which by itself has only very modest effects in *ob*/*ob* mice [33]. Further acute insulinotropic actions of (Val^8)GLP-1 and *N*-AcGIP maybe difficult to assess in the face of significant decrease of plasma glucose.

In conclusion, the present study indicates that once-daily injection of either *N*-AcGIP or (Val^8)GLP-1 represents an effective means of improving diabetes control in *ob*/*ob* mice. The various similar glucose-lowering effects observed demonstrate the potential of stable incretins as a new therapeutic approach to diabetes control.

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