Improved glycaemic control in obese diabetic ob/ob mice using N-terminally modified gastric inhibitory polypeptide

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Abstract
Gastric inhibitory polypeptide (GIP) is an important insulin–releasing hormone of the enteroinsular axis which is rapidly inactivated by the exopeptidase dipeptidyl peptidase (DPP) IV. The present study has examined the ability of Tyr1-glucitol GIP to be protected from plasma degradation and to enhance insulin–releasing and anti-hyperglycaemic activity in 20–25-week-old obese diabetic ob/ob mice. Degradation of GIP by incubation at 37 °C with obese mouse plasma was clearly evident after 3 h (35% degraded). After 6 h, more than 61% of GIP was converted to GIP(3–42) whereas N-terminally modified Tyr1-glucitol GIP was resistant to degradation in plasma (>99% intact after 6 h). The formation of GIP(3–42) was almost completely abolished by inhibition of plasma DPP IV with diprotin A. Effects of GIP and Tyr1-glucitol GIP were examined in overnight–fasted obese mice following i.p. injection of either peptide (20 nmol/kg) together with glucose (18 mmol/kg) or in association with feeding. Most prominent effects were observed in the former group where plasma glucose values at 60 min together with the area under the curve (AUC) for glucose were significantly lower following GIP (AUC, 874 ± 72 mmol/l.min; P < 0.01) or Tyr1-glucitol GIP (770 ± 134 mmol/l.min; P < 0.001) as compared with administration of glucose alone (1344 ± 136 mmol/l.min). This was associated with a significantly greater and more protracted insulin response following Tyr1-glucitol GIP than GIP (AUC, 491 ± 118 vs 180 ± 33 ng/ml.min; P < 0.01). Administration of Tyr1-glucitol GIP also enhanced the glucose-lowering ability of 50 units/kg insulin (218·4 ± 30·2 vs insulin alone 133·9 ± 16·2 mmol/l.min; P < 0.05). These data demonstrate that Tyr1-glucitol GIP displays resistance to plasma DPP IV degradation in a commonly used animal model of type 2 diabetes, resulting in enhanced antihyperglycaemic activity and insulin–releasing action in vivo.

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Introduction
Insulin secretion induced by the ingestion of glucose and other nutrients is due in part to hormonal and neural factors which constitute the enteroinsular axis (Creutzfeldt & Ebert 1985). Both gastric inhibitory polypeptide (GIP) and truncated glucagon-like peptide-1(7–36)amide (tGLP-1) are considered to be important physiologically active stimulants of postprandial insulin release (Brown 1994, Fehmann et al. 1995). However, recent years have witnessed a specific upsurge in interest in tGLP-1 as a result of its greater insulino-tropic potency (Suzuki et al. 1990, Siegel et al. 1992, Ørskov et al. 1993, Pederson 1994). Furthermore, studies in the rat using exendin(9–39), reported to be a specific GLP-1 receptor antagonist (Göke et al. 1993, Schirra et al. 1998), demonstrated a 48% reduction in postprandial insulin secretion indicating that tGLP-1 was the major physiological incretin hormone (Wang et al. 1995). Although tGLP-1 is undoubtedly an interesting peptide with potentially important extra-pancreatic actions (Fehmann et al. 1995), several recent observations suggest underestimation of the importance of GIP. First, physiological concentrations of tGLP-1 have been substantially overestimated due to cross-reaction in existing RIAs with inactive tGLP-1 metabolites (Deacon et al. 1995). Secondly, exendin(9–39) has been found to interfere with GIP receptor binding and cAMP generation (Wheeler et al. 1995) suggesting that its antagonist activity might not be confined to the GLP-1 receptor. Evidence for the importance of GIP has also been provided by the advent of a specific receptor antagonist GIP(7–30)amide which specifically inhibits GIP-induced insulin release (Tseng et al. 1996). Thus circulating insulin concentrations were reduced by 72% when GIP(7–30)amide was administered to rats, suggesting that GIP plays a key role in mediating postprandial insulin secretion (Tseng et al. 1996). The observation that GLP-1 receptor − / − mice show near normal glycaemic control due to compensatory
up-regulation of GIP secretion (Pederson et al. 1998a) also demonstrates the close interplay of both hormones in the enteroinsular axis (Nauck et al. 1989, 1993, Elahi et al. 1994, Jia et al. 1995).

Considerable effort has focused on the potential usefulness of tGLP-1 in therapy of diabetes since it has a range of desirable activities including its insulinotropic effectiveness and glucagonostatic action (Komatsu et al. 1989). Both activities are glucose-dependent, resulting in the control of blood glucose by a self-limiting mechanism, thus reducing the threat of postprandial hypoglycaemia (Kreymann et al. 1987, Gefel et al. 1990). A major weakness in the therapeutic use of GLP-1 is rapid degradation by dipeptidyl peptidase IV (DPP IV; EC 3.4.14-5) in serum (Mentlein et al. 1993, Orskov et al. 1993, Pederson 1994, Deacon et al. 1995, Kieffer et al. 1995), which removes the N-terminal dipeptide His7-Ala8 giving rise to the main inactive metabolite GLP-1(9–36)amide (Schmidt et al. 1987, Gefel et al. 1990, Grandt et al. 1994, Gelling et al. 1997). Efforts to prevent GLP-1 degradation have involved use of specific inhibitors of DPP IV (Deacon et al. 1998a, Holst & Deacon 1998, Pederson et al. 1998b) and production of N-terminally modified DPP IV analogues of tGLP-1 (Deacon et al. 1998b, Mooney et al. 1998, Burcelin et al. 1999). A significant extension of serum half-life is observed with tGLP-1 analogues substituted at the penultimate N-terminal position 8 with Gly, Aib, Ser or Thr (Deacon et al. 1998b, Ritzel et al. 1998, Burcelin et al. 1999). However, such alterations largely appear to compromise receptor binding and insulinotropic potency (Deacon et al. 1998h, O’Harte et al. 1998a, Ritzel et al. 1998, Burcelin et al. 1999).

GIP shares not only the same degradation pathway as tGLP-1 but many similar physiological actions, including stimulation of insulin and somatostatin secretion and enhancement of glucose disposal (Morgan 1996). In a recent study, we have shown that N-terminal glycation of GIP markedly enhances the insulin-releasing effect of the peptide on clonal pancreatic B-cells (O’Harte et al. 1998b). Furthermore, Tyr1-glucitol GIP showed improved DPP IV resistance in normal rats and thus its potential usefulness in type 2 diabetes therapy is enhanced (O’Harte et al. 1999). The present study has evaluated the antihyperglycaemic and insulin-releasing properties of GIP and this novel N-terminally modified GIP analogue in vivo in a commonly used and well-characterized animal model of type 2 diabetes (Bailey & Flatt 1997).

Materials and Methods

Materials

Human GIP was purchased from the American Peptide Company (Sunnyvale, CA, USA). HPLC grade acetonitrile was obtained from Rathburn (Walkersburn, Peeblesshire, UK). Sequencing-grade trifluoroacetic acid (TFA) was obtained from Aldrich (Poole, Dorset, UK). Diprotin A (DPA) was purchased from Calbiochem-Novabiochem (UK) Ltd (Beeston, Nottingham, UK) and rat insulin standard forRIA was obtained from Novo Industria (Copenhagen, Denmark). All other chemicals purchased including dextran T-70, activated charcoal, sodium cyanoborohydride and BSA fraction V were from Sigma Chemical Co. (Poole, Dorset, UK). Reversed-phase Sep-Pak cartridges (C-18) were purchased from Millipore-Waters (Milford, MA, USA). All water used in these experiments was purified using a Milli-Q Water Purification System (Millipore Corporation, Milford, MA, USA).

Preparation of Tyr1-glucitol GIP

Tyr1-glucitol GIP was prepared and purified by HPLC as described previously (O’Harte et al. 1998b). In brief, human GIP was incubated with glucose under reducing conditions in 10 mmol/l sodium phosphate buffer at pH 7.4 for 24 h. The reaction was stopped by addition of 0.5 mol/l acetic acid (30 µl) and the mixture applied to a Vydac (C-18) (4·6 × 250 mm) analytical HPLC column (The Separations Group, Hesperia, CA, USA). Gradient elution conditions were established using aqueous/TFA and acetonitrile/TFA solvents, as described previously (O’Harte et al. 1998b). Fractions corresponding to the glycated peaks were pooled, taken to dryness under vacuum using an AES 1000 Speed-Vac concentrator (Life Sciences International, Runcorn, Cheshire, UK) and purified to homogeneity on a Supelcosil (C-8) (4·6 × 150 mm) column (Supelco Inc., Poole, Dorset, UK). GIP and Tyr1-glucitol GIP were quantified by comparison of peak areas on HPLC (Supelcosil C-8 column) with a standard curve of known concentrations of GIP.

Degradation and HPLC analysis of GIP and Tyr1-glucitol GIP following plasma incubation

Pooled plasma (20 µl) taken from six 20- to 25-week-old fasted ob/ob mice was incubated at 37 °C with GIP or Tyr1-glucitol GIP (10 µg) for 0, 3 and 6 h in a reaction mixture made up to 500 µl, containing 50 mmol/l triethanolamine–HCl buffer pH 7.8. The reactions were terminated by addition of 5 µl TFA and resulting samples were applied to a C-18 Sep-Pak cartridge which was previously primed with 0·1% (v/v) TFA/water. Bound material was released by elution with 2 ml 80% (v/v) acetonitrile/water and concentrated using a Speed-Vac concentrator. Samples were reconstituted to 1·0 ml with 0·12% (v/v) TFA/water and stored at −20 °C.
Peptides were subsequently separated using a Vydac C-18 widepore column equilibrated with 0.12% (v/v) TFA/water at a flow rate of 1.0 ml/min. Using 0.1% (v/v) TFA in 70% acetonitrile/water, the concentration of acetonitrile in the eluting solvent was raised from 0 to 31.5% over 15 min, to 38.5% over 30 min and from 38.5 to 70% over 5 min, using linear gradients. The absorbance was monitored at 206 nm and peak areas evaluated using a model 2221 LKB integrator (Bromma, Sweden). Samples recovered manually were concentrated using a Speed-Vac concentrator and stored at −20 °C.

**Electrospray ionization mass spectrometry (ESI-MS)**

Samples for ESI-MS analysis containing GIP degradation fragments or Tyr1-glucitol GIP were dissolved (approximately 400 pmol) in 100 µl water and applied to an LCQ benchtop mass spectrometer (Finnigan MAT, Hemel Hempstead, Herts, UK) equipped with a microbore C-18 HPLC column (150 x 2.0 mm; Phenomenex UK, Ltd, Macclesfield, Cheshire, UK). Samples (30 µl direct loop injection) were injected at a flow rate of 0.2 ml/min, under isocratic conditions (35% (v/v) acetonitrile/water). Mass spectra were obtained from the quadrupole ion trap mass analyser and recorded. Spectra were collected using full scan mode over the mass-to-charge (m/z) range 150–2000. The molecular masses of GIP and Tyr1-glucitol GIP were determined from ESI-MS profiles using prominent multiply-charged ions and the following equation: M_i = iM_0 - iM_H (where M_r = molecular mass; M_i = m/z ratio; i = number of charges; M_H = mass of a proton).

**In vivo biological activities of GIP and Tyr1-glucitol GIP**

Effects of GIP and Tyr1-glucitol GIP on plasma glucose and insulin concentrations were examined using 20- to 25-week-old obese diabetic (ob/ob) mice. Animals at the age selected exhibit a relatively severe form of diabetes associated with well-established obesity, insulin resistance and pancreatic B-cell dysfunction (Flatt & Bailey 1981a,b, Bailey & Flatt 1982). These features may constitute a reasonably realistic situation to test for possible therapeutic usefulness of the peptide analogue. The genetic background and characteristics of the colony used have been described in detail elsewhere (Flatt & Bailey 1981a, Bailey & Flatt 1982). The animals were housed individually in an air-conditioned room at 22 ± 2 °C with a 12 h light:12 h darkness cycle. Drinking water and a standard rodent maintenance diet (Trouw Nutrition, Belfast, UK) were freely available. Food was withdrawn for an 18 h period prior to tests designed to evaluate in vivo biological potency of GIP and its N-terminally modified analogue.

**Metabolic effects of GIP and Tyr1-glucitol GIP following glucose administration**

Plasma glucose and insulin responses were evaluated following i.p. injection of glucose alone (18 mmol/kg body weight) or in combination with GIP or Tyr1-glucitol GIP (20 nmol/kg). Previous studies in ob/ob mice suggest that administration of approximately 10 nmol/kg GIP gives rise to physiological postprandial concentrations of immunoreactive GIP as measured by conventional RIA (Flatt et al. 1984).

**Metabolic effects of GIP and Tyr1-glucitol GIP following feeding**

In a second experimental series, fasted ob/ob mice were allowed to refeed for 30 min prior to i.p. administration of GIP, Tyr1-glucitol GIP (20 nmol/kg) or an equivalent volume of saline (9 g/l NaCl).

**Effects of GIP and Tyr1-glucitol GIP on glucose-lowering ability of insulin**

In the third set of experiments, the effects of GIP peptides on glucose-lowering ability of insulin was evaluated by i.p. injection of insulin (50 U/kg) alone or in combination with GIP or Tyr1-glucitol GIP (20 nmol/kg).

Test solutions for all three experimental series were administered in a final volume of 8 ml/kg body weight. Blood samples were collected from the cut tip of the tail of conscious mice into chilled fluoride/heparin microcentrifuge tubes (Sarstedt, Nümbrecht, Germany) immediately before injection/refeeding and at the times indicated in the Figures. Blood samples were immediately centrifuged using a Beckman microcentrifuge (Beckman Instruments, UK) for 30 s at 13 000 g. Plasma was aliquoted and stored at −20 °C prior to glucose and insulin determinations. All animal studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

**Analyses**

Plasma glucose was assayed by an automated glucose oxidase procedure using a Beckman Glucose Analyzer II (Stevens 1971). Plasma insulin was determined by dextran-charcoal RIA as described previously (Flatt & Bailey 1981a). Incremental areas under plasma glucose and insulin curves (AUC) were calculated using a computer program (CAREA) employing the trapezoidal rule (Burlington 1973) with baseline subtraction. Results are expressed as mean ± s.e.m. and values were compared using ANOVA, followed by the Student–Newman–Keuls post hoc test. Differences were considered to be significant at P<0.05.
Results

Degradation of GIP and Tyr¹-glucitol GIP by ob/ob mouse plasma

Figure 1 shows a set of typical HPLC profiles of the products obtained from the incubation of GIP or Tyr¹-glucitol GIP with obese mouse plasma for 0, 3 and 6 h. GIP (left panels) with a retention time of 30.19 min was progressively metabolized by plasma within a 3–6 h incubation (35–61% degraded) giving rise to the appearance of a major degradation peak with a retention time of 28.14 min corresponding to GIP(3–42). Addition of DPA, a specific inhibitor of DPP IV, to GIP during the 6 h incubation completely inhibited degradation of GIP by plasma. In contrast to the native peptide, the incubation of Tyr¹-glucitol GIP under similar conditions (right panels) did not result in the formation of any detected GIP(3–42) during this time with one major peak being observed with a retention time of 28.76 min corresponding to intact Tyr¹-glucitol GIP.

Identification of GIP peptides and degradation fragments by ESI-MS

Monoisotopic molecular masses were obtained for GIP, Tyr¹-glucitol GIP and the major plasma degradation fragment of GIP (GIP(3–42)) using ESI-MS. The peptides analysed were purified from plasma incubations as shown in Fig. 1. The exact molecular masses (M_r) of the peptides were calculated using the equation M_r = iM_o - iM_h as defined in Materials and Methods. After spectral averaging was performed, prominent multiply-charged species (M+3H)^3+ and (M+4H)^4+ were detected from GIP at m/z 1661.6 and 1246.8, corresponding to intact M_r.
4981·8 and 4983·2 Da respectively. Similarly, for Tyr1-glucitol GIP (M+4H)4+ and (M+5H)5+ were detected at m/z 1287·7 and 1030·3, corresponding to intact molecular masses of Mr 5146·8 and 5146·5 Da respectively. The difference between the observed molecular masses of the quadruply-charged GIP and the N-terminally modified GIP species (163·6 Da) confirmed that the latter peptide contained a single glucitol adduct (theoretical mass 164 Da) corresponding to Tyr1-glucitol GIP. Prominent multiply-charged species (M+3H)3+ and (M+4H)4+ were detected for GIP(3–42) at m/z 1583·8 and 1188·1, corresponding to an intact Mr of 4748·4 Da.

**Metabolic effects of GIP and Tyr1-glucitol GIP following glucose administration**

Figures 2 and 3 show the plasma glucose and insulin response to administration of glucose (18 mmol/kg) alone or in combination with either GIP or Tyr1-glucitol GIP in obese diabetic ob/ob mice. Compared with the control group, plasma glucose concentrations at 60 min and AUC from 0 to 60 min were significantly lower (P<0·05 to P<0·01, n=7 or 8) following administration of either GIP or Tyr1-glucitol GIP (Fig. 2A and B). Plasma insulin concentrations of ob/ob mice treated with Tyr1-glucitol GIP were significantly raised (P<0·05) at 60 min compared with those in animals treated with either GIP or glucose alone (Fig. 3A). Furthermore, the overall insulin responses, estimated as AUC, were also significantly greater for the Tyr1-glucitol GIP-treated group (Fig. 3B) compared with GIP-treated (P<0·05) and control groups (P<0·05). The significant elevation of plasma insulin at 60 min is of particular note, suggesting that the insulin-releasing action of Tyr1-glucitol GIP is more protracted than that of GIP (Fig. 3A).

**Metabolic effects of GIP and Tyr1-glucitol GIP following feeding**

The effects of GIP and Tyr1-glucitol GIP on the metabolic response to food ingestion are shown in Fig. 4A. Food
intake was similar in these three groups ($n=7$ or $8$) (control $0.60 \pm 0.09$ g/mouse per 30 min; GIP-treated $0.54 \pm 0.13$ g/mouse per 30 min; Tyr$^1$-glucitol GIP-treated $0.72 \pm 0.06$ g/mouse per 30 min). The glycaemic response of GIP and its N-terminal analogue were similar. A similar conclusion comes from glucose AUC data (Fig. 4B), although it is notable that plasma glucose at 60 and 120 min plus the overall glycaemic response were approximately 30% less for the analogue. However, plasma insulin concentrations showed a much clearer trend towards greater insulinotropic activity of Tyr$^1$-glucitol GIP (Fig. 5A). However, variable response of individual mice prevented AUC values from achieving significance (Fig. 5B).

**Effects of GIP and Tyr$^1$-glucitol GIP on glucose-lowering ability of insulin**

Figure 6 shows changes in plasma glucose concentration of ob/ob mice following insulin administration. Tyr$^1$-glucitol GIP combined with insulin was more effective than injection of insulin alone or insulin with GIP in reducing plasma glucose concentrations at 30 and 60 min ($P<0.05$ to $P<0.001$; Fig. 6A). A significant difference between controls (insulin alone) and the Tyr$^1$-glucitol GIP and insulin-treated groups was also confirmed from the AUC for the treatment-induced reduction in plasma glucose (Fig. 6B).

**Discussion**

GIP is a key hormone of the enteroinsular axis, which is released into the circulation from endocrine K-cells of the small intestine, producing a glucose-dependent stimulation of insulin secretion (Pederson & Brown 1976, Elahi et al. 1979). This glucose-dependency avoids hypoglycaemic episodes and thereby fulfils one of the most desirable features of any antidiabetic drug (Bailey & Flatt 1995).
DPP IV protection displayed by Tyr1-glucitol GIP was associated with significant enhancement of the antihyperglycaemic and insulin-releasing activity of the peptide when administered in conjunction with glucose to diabetic ob/ob mice. Native GIP reduced the glycaemic excursion as observed in previous studies (Pederson & Brown 1976, Flatt et al. 1984, Pederson 1994), but failed to evoke the same prominent insulin response as observed in younger mildly diabetic ob/ob mice (Flatt et al. 1984). However, the N-terminally modified GIP analogue increased both the antihyperglycaemic and insulin-releasing actions of the peptide by 2.5-fold and 1.5-fold respectively, as estimated from AUC measurements. The greater insulin concentrations following Tyr1-glucitol GIP as opposed to GIP at 60 min post-injection are suggestive also of a longer half-life, but detailed kinetic analysis was not possible due to limitations of sampling times in mice and questionable ability of existing RIAs to effectively discriminate between GIP(1–42) and inactive GIP(3–42). Since the insulinotropic actions of GIP are glucose-dependent (Pederson & Brown 1976, Elahi et al. 1979), it is possible that the relative insulin-releasing potency of Tyr1-glucitol GIP is underestimated in the present experiments. Furthermore, glucose sensitivity and the secretory function of pancreatic B-cells are compromised in ob/ob mice at the age range chosen (20–25 weeks) (Flatt & Bailey 1981b), making this study a reasonably severe test for possible antihyperglycaemic efficacy.

Administration of Tyr1-glucitol GIP to ob/ob mice after 30 min refeeding gave broadly similar results in terms of augmentation of circulating plasma insulin concentrations. However, in both sets of experiments the N-terminally modified analogue appeared more effective than GIP in promoting insulin release rather than in lowering blood glucose. This is not at all surprising given the extreme insulin resistance of this severely obese diabetic ob/ob mouse model of type 2 diabetes (Flatt & Bailey 1981b). The present findings are also in line with other observations in our laboratory using glucose-responsive BRIN-BD11 cells (McLenaghan et al. 1996), which revealed that the insulin-releasing potency of Tyr1-glucitol GIP was several-fold greater than native GIP (O’Harte et al. 1998b).

In vivo results in the present study indicate that N-terminal glycation of GIP confers resistance to DPP IV degradation in an animal model of type 2 diabetes while enhancing insulin secretory effects on the B-cell. The former action will impede degradation of the peptide to GIP(3–42), thereby prolonging the half-life and enhancing effective circulating concentrations of the intact biologically active peptide. Thus it appears likely that Tyr1-glucitol GIP enhances insulin secretion in vivo both by increased potency and by improved DPP IV resistance. Various other studies have shown that GIP(3–42)

The present results demonstrate that Tyr1-glucitol GIP displays profound resistance to degradation in obese mouse plasma. Consistent with rapid degradation of circulating GIP in ob/ob mice in vivo (Flatt et al. 1984), native GIP was cleaved to a major 4748.4 Da degradation product, corresponding to GIP(3–42), which confirms previous findings using human serum (O’Harte et al. 1999). DPA, a specific competitive inhibitor of DPP IV, completely inhibited degradation in ob/ob mouse plasma, confirming this as the main enzyme involved in GIP inactivation in the circulation (Mentlein et al. 1993, Kieffer et al. 1995). In contrast, Tyr1-glucitol GIP remained almost completely intact after incubation for up to 6 h. This indicates that modification of GIP at the N-terminal Tyr residue by glycation prevents DPP IV-induced removal of the Tyr1-Ala2 dipeptide and thus GIP(3–42) formation in diabetic ob/ob mice.
and other N-terminally modified fragments, including GIP(4–42), and GIP(17–42) fail to stimulate insulin release (Moody et al. 1981, Oektedalen et al. 1983, Maletti et al. 1986, Mentlein et al. 1993) and possibly exhibit receptor antagonist properties (Gelling et al. 1997). This suggests that inhibition of GIP catabolism may also have a positive effect by reducing possible feedback antagonism at the GIP receptor by truncated GIP3(4–42).

Another potentially important extra-pancreatic actions of GIP may contribute to the enhanced antihyperglycaemic activity and metabolic actions of DPP IV-resistant Tyr1-glucitol GIP. Such a view is clearly supported by the greater hypoglycaemic action of exogenous insulin when given to ob/ob mice conjointly with GIP, and most notably with Tyr1-glucitol GIP. Such actions are notable in overcoming the quite severe insulin resistance of ob/ob mice at 20–25 weeks and may include the stimulation of glucose uptake in adipocytes, increased synthesis of fatty acids and activation of lipoprotein lipase in adipose tissue (Eckel et al. 1978, Oben et al. 1991, Morgan 1996). GIP also promotes plasma triglyceride clearance in response to oral fat loading (Ebert et al. 1991). In liver, GIP has been shown to enhance insulin-dependent inhibition of glycogenolysis (Elahi et al. 1986). GIP also reduces both glucagon-stimulated lipolysis in adipose tissue and hepatic glucose production (Hartmann et al. 1986). Finally, other findings in our laboratory indicate that GIP has a potent effect on glucose uptake and metabolism in isolated mouse diaphragm muscle (O’Harte et al. 1998c). This latter action may be shared with tGLP-1 (Villanueva-Péñacarrillo et al. 1994, O’Harte et al. 1997) and both peptides have additional benefits of stimulating somatostatin secretion and slowing down gastric emptying and nutrient absorption (Brown 1994, Wahren et al. 1977).

In conclusion, this study indicates that Tyr1-glucitol GIP exhibits serum peptidase resistance, an extended half-life and an enhanced biological activity compared with GIP in an animal model of type 2 diabetes. This effect is accompanied in vivo by enhanced antihyperglycaemic activity and raised insulin concentrations in animals with well-established diabetes, pancreatic B-cell dysfunction and severe insulin resistance. Overall these observations support the idea that DPP IV-resistant GIP analogues such as Tyr1-glucitol GIP might prove to be useful agents for the therapy of type 2 diabetes.

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