

# Evidence that the major degradation product of glucose-dependent insulinotropic polypeptide, GIP(3–42), is a GIP receptor antagonist *in vivo*

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## Abstract

The incretin hormone glucose-dependent insulinotropic polypeptide (GIP) is rapidly degraded in the circulation by dipeptidyl peptidase IV forming the N-terminally truncated peptide GIP(3–42). The present study examined the biological activity of this abundant circulating fragment peptide to establish its possible role in GIP action. Human GIP and GIP(3–42) were synthesised by Fmoc solid-phase peptide synthesis, purified by HPLC and characterised by electrospray ionisation-mass spectrometry. In GIP receptor-transfected Chinese hamster lung fibroblasts, GIP(3–42) dose dependently inhibited GIP-stimulated ( $10^{-7}$  M) cAMP production (up to  $75.4 \pm 5.4\%$ ;  $P < 0.001$ ). In BRIN-BD11 cells, GIP(3–42)

was significantly less potent at stimulating insulin secretion (1.9- to 3.2-fold;  $P < 0.001$ ), compared with native GIP and significantly inhibited GIP-stimulated ( $10^{-7}$  M) insulin secretion with maximal inhibition ( $48.8 \pm 6.2\%$ ;  $P < 0.001$ ) observed at  $10^{-7}$  M. In (*ob/ob*) mice, administration of GIP(3–42) significantly inhibited GIP-stimulated insulin release (2.1-fold decrease;  $P < 0.001$ ) and exaggerated the glycaemic excursion (1.4-fold;  $P < 0.001$ ) induced by a conjoint glucose load. These data indicate that the N-terminally truncated GIP(3–42) fragment acts as a GIP receptor antagonist, moderating the insulin secreting and metabolic actions of GIP *in vivo*.

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## Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is a 42 amino acid peptide hormone (Jörnvall *et al.* 1981) synthesised in enteroendocrine K-cells (Buchan *et al.* 1978) via the proteolytic processing of a larger precursor polypeptide (Takeda *et al.* 1987). Like its sister incretin hormone, glucagon-like peptide-1 (GLP-1), GIP potentiates meal-induced insulin secretion (Creutzfeldt & Ebert 1985) which together with extrapancreatic actions on muscle and adipose tissue makes a significant contribution to the regulation of postprandial glucose homeostasis (Morgan 1996).

To date, numerous structure–function studies have attempted to elucidate the bioactive domain(s) within the GIP molecule (Moroder *et al.* 1978, Carlquist *et al.* 1984, Maletti *et al.* 1986, Maletti *et al.* 1987, Pederson *et al.* 1990, Gelling *et al.* 1997, Hinke *et al.* 2001). Truncation of GIP at the C-terminus to GIP(1–39) (Sandberg *et al.* 1986) or GIP(1–38) (Moroder *et al.* 1978) produced fragments with comparable activity to the native peptide. Further truncation to GIP(1–31) produced a fragment, which displayed weaker receptor binding affinity, but still

retained an equal cAMP stimulating potency compared with native GIP (Maletti *et al.* 1987). More recently, GIP(1–30)amide and native GIP were shown to be equipotent at stimulating insulin secretion (Morrow *et al.* 1996), although GIP(1–30)amide displayed a significantly reduced somatostatinotropic activity (Pederson *et al.* 1990), indicating that part of the C-terminal sequence is critical for its acid inhibitory activity (Rossowski *et al.* 1992).

The role of the N-terminus for GIP action evaluated by fragment peptide analysis suggested that the bioactive site resided between residues 19 and 30 (Morrow *et al.* 1996). However, these results were in apparent conflict with studies using Des-Tyr<sup>1</sup>-Ala<sup>2</sup>-GIP (Schmidt *et al.* 1987) and synthetic GIP(4–42) (Maletti *et al.* 1986) which exhibited similar receptor binding affinity to the native peptide, but displayed greatly reduced insulinotropic activity. Subsequently, another bioactive domain was suggested to reside within amino acid residues 1–14 (Hinke *et al.* 2001), but the overall importance of the N-terminal Tyr<sup>1</sup>-Ala<sup>2</sup> dipeptide of GIP in regulating biological activity was demonstrated by Brown *et al.* (1981). Thus early studies using GIP(3–42) originally

isolated from porcine intestinal extracts demonstrated that this truncated peptide lacked insulinotropic activity, therefore confirming the necessity of an intact N-terminus for retention of biological activity (Brown *et al.* 1981).

Subsequent studies with GIP(3–42) have shown it to be devoid of any antagonistic activity (Schmidt *et al.* 1987), however, parallel studies using the sister incretin hormone GLP-1 have recently shown that the truncated GLP-1-(9–36)amide fragment may act as an antagonist (Grandt *et al.* 1994, Knudsen & Pridal 1996). Furthermore, GLP-2 (3–33) which also arises from proglucagon processing in the intestine may also act as a competitive antagonist (Thulesen *et al.* 2002). Recently, using a newly developed radioimmunoassay, GIP(3–42) has been identified as the major component of endogenous GIP immunoreactivity in human plasma (Deacon *et al.* 2000). This arises from degradation of GIP by the ubiquitous enzyme dipeptidyl peptidase IV (DPP IV) highlighting the need for further evaluation of the possible antagonist actions of GIP(3–42). Accordingly, the current study has examined the *in vitro* effects of GIP(3–42) on GIP-stimulated cyclic AMP (cAMP) production and insulin secretion together with *in vivo* effects in a commonly employed animal model of type 2 diabetes.

## Materials and Methods

### Reagents

High performance liquid chromatography (HPLC) grade acetonitrile was obtained from Rathburn (Walkersburn, Scotland). Sequencing grade trifluoroacetic acid (TFA) was obtained from Aldrich (Poole, Dorset, UK). Isobutylmethylxanthine (IBMX), adenosine 3',5'-cyclic monophosphate (cAMP) and adenosine 5'-triphosphate (ATP) were all purchased from Sigma (Poole, Dorset, UK). Fmoc-protected amino acids were from Calbiochem Novabiochem (Beeston, Nottingham, UK). RPMI 1640 and DMEM tissue culture medium, fetal calf serum, penicillin and streptomycin were all purchased from Gibco (Paisley, Strathclyde, Scotland). The chromatography columns used for cAMP assay, Dowex AG 50 WX and neutral alumina AG7 were obtained from Bio-Rad (Alpha Analytical, Larne, N. Ireland). All water used in these experiments was purified using a Milli-Q, water purification system (Millipore, Milford, MA, USA). All other chemicals used were of the highest purity available.

### Peptide synthesis and purification

GIP and GIP(3–42) were sequentially synthesised on an Applied Biosystems automated peptide synthesiser (model 432A) using standard solid-phase Fmoc protocols (Fields & Noble 1990), starting from a pre-loaded Fmoc-Gln-Wang resin. The following side chain protected amino

acids were used, Fmoc-Gln(Trt)-OH, Fmoc-Thr(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Asp(OtBu)-OH and Fmoc-Tyr(OtBu)-OH. Deprotection and cleavage of the peptide from the resin was by trifluoroacetic acid:water:thianisole:ethanediol (90:2:5:5:2:5, a total volume of 20 ml/g resin), the resin was removed by filtration and the filtrate volume was decreased under reduced pressure. Dry diethyl ether was slowly added until a precipitate was observed. The precipitate was collected by low-speed centrifugation, resuspended in diethyl ether and centrifuged again, the procedure being carried out five times. The resulting pellets were then dried *in vacuo* and judged pure by reversed-phase HPLC on a Waters Millennium 2010 chromatography system (Software version 2.1.5).

### Electrospray ionisation-mass spectrometry (ESI-MS)

HPLC-purified GIP and GIP(3–42) peptides were dissolved (approximately 400 pM) in 100 µl water and applied to an LCQ benchtop LC mass spectrometer (LC/MS) (Finnigan MAT, Hemel Hempstead, UK). Samples (20 µl direct loop injection) were applied at a flow rate of 0.2 ml/min, under isocratic conditions in 35% (v/v) acetonitrile/water. Mass spectra were obtained from the quadripole ion trap mass analyser and spectra collected using full ion scan mode over the mass-to-charge ( $m/z$ ) range 150–2000. The molecular masses of each fragment were determined using prominent multiple charged ions using the following equation:  $M_r = iM_i - iM_h$  (where  $M_r$  is molecular mass,  $M_i$  is  $m/z$  ratio,  $i$  is the number of charges and  $M_h$  is the mass of a proton).

### Cells and tissue culture

Chinese hamster lung (CHL) cells transfected with the human GIP receptor (Gremlich *et al.* 1995) were cultured in DMEM tissue culture medium containing 10% (v/v) fetal calf serum and 1% (v/v) antibiotics (100 U/ml penicillin, 0.1 mg/ml streptomycin). BRIN-BD11 cells were cultured in sterile tissue culture flasks (Corning, Glass Works, UK) using RPMI-1640 tissue culture medium containing 10% (v/v) fetal calf serum, 1% (v/v) antibiotics (100 U/ml penicillin, 0.1 mg/ml streptomycin) and 11.1 mM glucose. The origin and characteristics of BRIN-BD11 cells have been described previously (McClenaghan *et al.* 1996). The cells were maintained at 37 °C in an atmosphere of 5% CO<sub>2</sub> and 95% air using a LEEC incubator (Laboratory Technical Engineering, Nottingham, UK).

### *In vitro* studies

Effects on cAMP production were evaluated as detailed elsewhere (Gault *et al.* 2002). Briefly, transfected CHL

cells plated in 12-well plates were loaded with 2  $\mu$ Ci tritiated adenine (TRK311, Amersham) for 6 h at 37 °C. The cells were then exposed for 10 min at 37 °C to forskolin (FSK, 10  $\mu$ M) or varying concentrations of GIP(3–42) in the absence or presence of GIP ( $10^{-7}$  M). After removal of the medium, cells were lysed and intracellular tritiated cAMP separated on Dowex and alumina exchange resins as described previously (Widmann *et al.* 1993).

Insulin releasing activity was measured in BRIN-BD11 cells as described previously (Gault *et al.* 2002). In brief, BRIN-BD11 cells seeded into 24-well plates were allowed to attach overnight at 37 °C. After pre-incubation (40 min) in 1.1 mM glucose, acute tests for insulin release were performed ( $n=8$ ) at 5.6 mM glucose using various concentrations of GIP(3–42) in the absence and presence of GIP ( $10^{-7}$  M). After 20 min incubation, insulin concentrations were measured by radioimmunoassay (Flatt & Bailey 1981).

#### *In vivo biological activity*

Plasma glucose and insulin responses were evaluated using 14- to 18-week-old (*ob/ob*) mice (Bailey & Flatt 1982) following intraperitoneal (i.p.) injection of GIP or GIP(3–42) (25 nmol/kg body weight) immediately following the combined injection of GIP (25 nmol/kg body weight) with glucose (18 mmol/kg body weight). All test solutions were administered to 18 h fasted mice in a final volume of 8 ml/kg body weight. Blood samples were collected at the times indicated from the cut tip of the tail vein of conscious mice into chilled fluoride/heparin microcentrifuge tubes (Sarstedt, Nümbrecht, Germany). Plasma glucose was assayed using a Beckman Glucose Analyser II (Stevens 1971) and plasma insulin determined by radioimmunoassay (Flatt & Bailey 1981). All animal studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986.

#### *Statistical analysis*

Data are expressed as means  $\pm$  S.E.M. and were compared using Student's *t*-test or ANOVA, followed by Student–Newman–Keuls *post hoc* test. Incremental areas under the curve (AUC) were calculated using a computer generated program employing the trapezoidal rule (Burington 1973) with baseline subtraction. Groups of data were considered to be significantly different if  $P<0.05$ .

## Results

#### *Structural identification of GIP and GIP(3–42)*

GIP and GIP(3–42) were synthesised by solid-phase Fmoc protocols and purified by HPLC. Figure 1 shows the

monoisotopic molecular masses obtained for GIP (Fig. 1A) and GIP(3–42) (Fig. 1B) upon ESI-MS analysis. After spectral averaging was performed, prominent multiple charged species ( $M+3H$ )<sup>3+</sup> and ( $M+4H$ )<sup>4+</sup> were detected from GIP at  $m/z$  1661.6 and 1246.8, corresponding to intact  $M_r$  4981.8 and 4983.2 Da respectively (Fig. 1A). This corresponds very closely to the theoretical mass 4984.2 Da. Similarly, for GIP(3–42), ( $M+3H$ )<sup>3+</sup> and ( $M+4H$ )<sup>4+</sup> were detected at  $m/z$  1583.9 and 1188.1, corresponding to intact  $M_r$  4748.7 and 4748.4 Da respectively (Fig. 1B), which corresponds closely with the theoretical mass of 4746.4 Da.

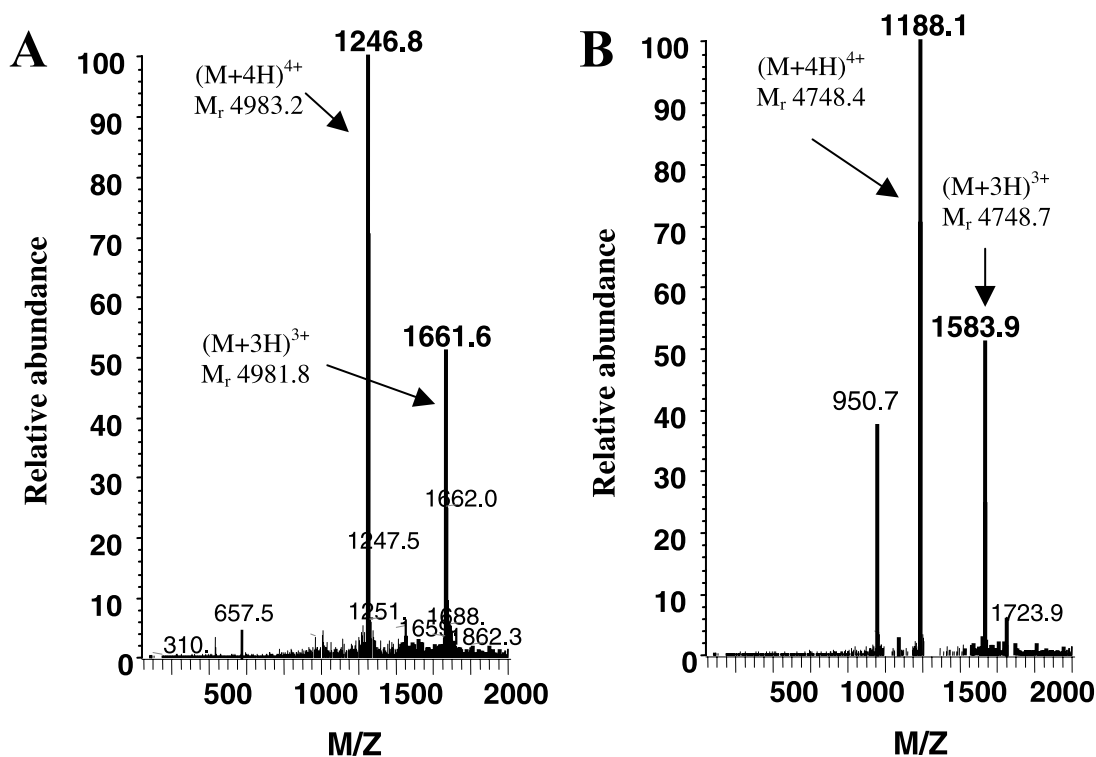
#### *In vitro actions of GIP and GIP(3–42)*

Coupling of the receptors to adenylyl cyclase was examined by production of intracellular cAMP after exposure of the GIP receptor transfected CHL cells to GIP and GIP(3–42). GIP dose dependently ( $10^{-12}$  to  $10^{-6}$  M) stimulated cAMP production with a half-maximal stimulatory concentration ( $EC_{50}$ ) value of 18.2 nM (Fig. 2A). In contrast, only very weak stimulation of cAMP production could be observed when GIP(3–42) was exposed to transfected fibroblasts (Fig. 2A). cAMP formation was less than 5% of the stimulation caused by native GIP even at concentrations as high as  $10^{-6}$  M. When incubated in the presence of  $10^{-7}$  M GIP, GIP(3–42) significantly inhibited cAMP production, with maximal inhibition ( $75.4 \pm 5.4\%$ ) observed at  $10^{-6}$  M (Fig. 2B).

Figure 3 shows the effects of GIP and GIP(3–42) on insulin secretion from clonal pancreatic BRIN-BD11 cells. From Fig. 3A, it can be seen clearly that native GIP dose dependently ( $10^{-11}$  to  $10^{-8}$  M) stimulated insulin secretion (1.2- to 1.8-fold;  $P<0.05$  to  $P<0.001$ ) compared with control incubations (5.6 mM glucose alone). GIP(3–42) did not significantly stimulate insulin secretion compared with the native peptide (1.9- to 3.2-fold lower;  $P<0.001$ ). In the presence of stimulatory GIP ( $10^{-7}$  M), GIP(3–42) dose dependently inhibited insulin release (1.1- to 1.5-fold;  $P<0.05$  to  $P<0.001$ ) compared with GIP, with maximal inhibition ( $48.8 \pm 6.2\%$ ;  $P<0.001$ ) observed at  $10^{-7}$  M (Fig. 3B).

#### *In vivo actions of GIP and GIP(3–42)*

Consistent with these effects, plasma insulin concentrations of (*ob/ob*) mice treated with GIP(3–42) were significantly lowered (2.2-fold;  $P<0.001$ ) at 60 min post injection compared with mice treated with GIP alone (Fig. 5A). Furthermore, the overall insulin response, estimated by AUC was significantly lower (2.1-fold;  $P<0.001$ ) following administration of GIP(3–42) compared with GIP-only treated mice (Fig. 5B). As expected, mice treated with GIP alone exhibited significantly improved insulin and glycaemic responses compared with glucose control (Figs 4 and 5).



**Figure 1** Electrospray ionisation-mass spectrometry (ESI-MS) of GIP (A) and GIP(3–42) (B). The peptides were dissolved (approximately 400 pM) in 100  $\mu$ l water and applied by direct loop injection to the LC/MS under isocratic conditions. Mass spectra were recorded using a quadrupole ion trap mass analyser and collected using full ion scan mode over the mass-to-charge ( $m/z$ ) range 150–2000. The  $M_r$  of GIP and GIP(3–42) were determined from ESI-MS profiles using prominent multiple charged ions and the following equation:  $M_r = iM_i - iM_h$  (see Materials and Methods).

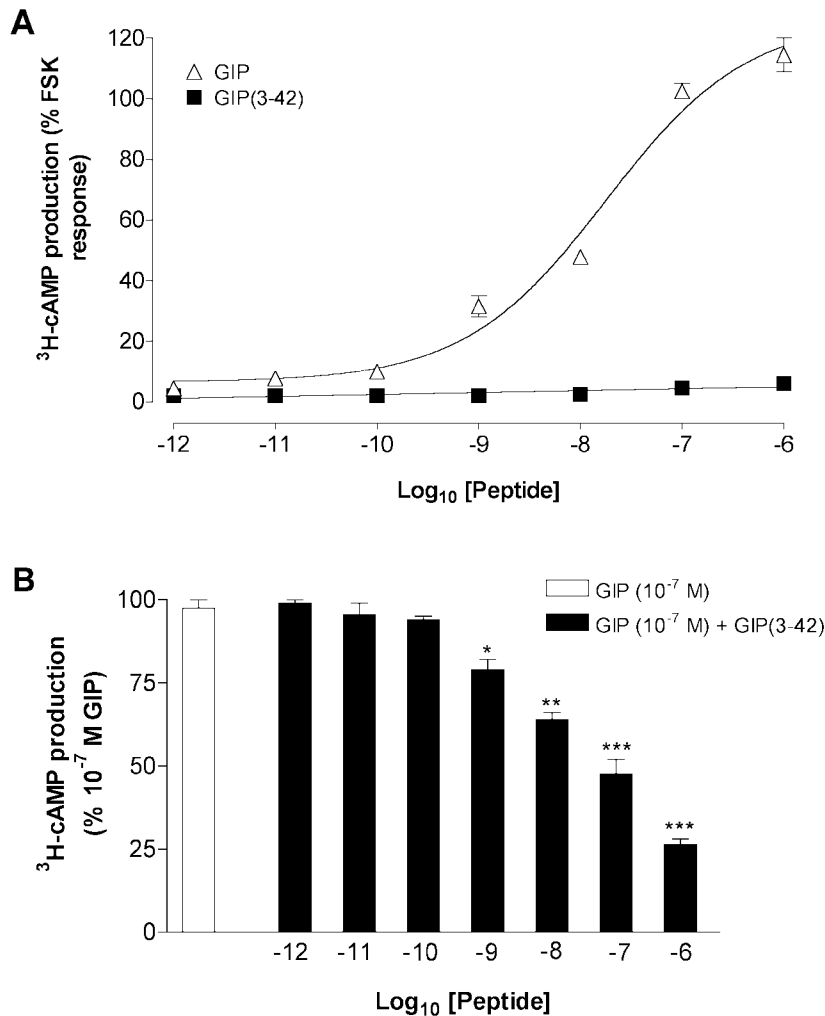
## Discussion

The rapid degradation of GIP by the enzyme dipeptidyl peptidase IV (DPP IV) and consequent short biological half-life (approximately 5–7 min) (Deacon *et al.* 2000) are major factors which hamper possible therapeutic use of the peptide for type 2 diabetes. The enzyme is ubiquitously expressed in mammalian tissues and organs (Wrenger *et al.* 2000) and circulates at relatively high concentrations in the blood (Lojda 1979). DPP IV's strict substrate specificity for removing dipeptides from the amino terminus of peptides with penultimate proline, alanine or hydroxyproline residues (Walter *et al.* 1980) means that, in the case of GIP, the amino-terminal Tyr<sup>1</sup>-Ala<sup>2</sup> dipeptide is removed producing the metabolite GIP(3–42).

In this study, the effects of the truncated GIP(3–42) fragment on GIP action were examined *in vitro* and in a commonly employed animal model of obesity–diabetes and enteroendocrine hyperactivity. The ability of GIP(3–42) to couple to the GIP receptor and stimulate intracellular cAMP production was examined using transfected CHL cells (Gremlich *et al.* 1995). Native GIP was shown to dose dependently stimulate cAMP production with an

EC<sub>50</sub> value of 18.2 nM, which is broadly in line with previous studies using this cell line (Gremlich *et al.* 1995). In comparison, GIP(3–42) demonstrated only very weak agonist activity, reminiscent of the effects of the novel GIP antagonist, (Pro<sup>3</sup>)GIP (Gault *et al.* 2002). Subsequent studies confirmed the antagonistic properties of GIP(3–42) by revealing that the truncated peptide inhibited GIP-induced cAMP production by as much as 75.4  $\pm$  5.4%.

Consistent with previous observations using clonal pancreatic BRIN-BD11 cells (O'Harte *et al.* 1998), GIP induced a concentration-dependent increase of insulin release at physiological glucose concentration. In contrast, GIP(3–42) lacked any significant insulinotropic activity, which corroborates previous findings in the perfused rat pancreas (Brown *et al.* 1981). However, when incubated with stimulatory GIP (10<sup>-7</sup> M), GIP(3–42) significantly countered the ability of the native peptide to enhance insulin secretion. An approximate 35% inhibition was observed with 10<sup>-8</sup> M GIP(3–42), making the metabolite comparable to established antagonists ANTGIP and (Pro<sup>3</sup>)GIP (Tseng *et al.* 1996, Gault *et al.* 2002). These data contrast with early observations where GIP(3–42) was recorded as being devoid of any antagonistic activity when applied to isolated islets at tenfold higher molar

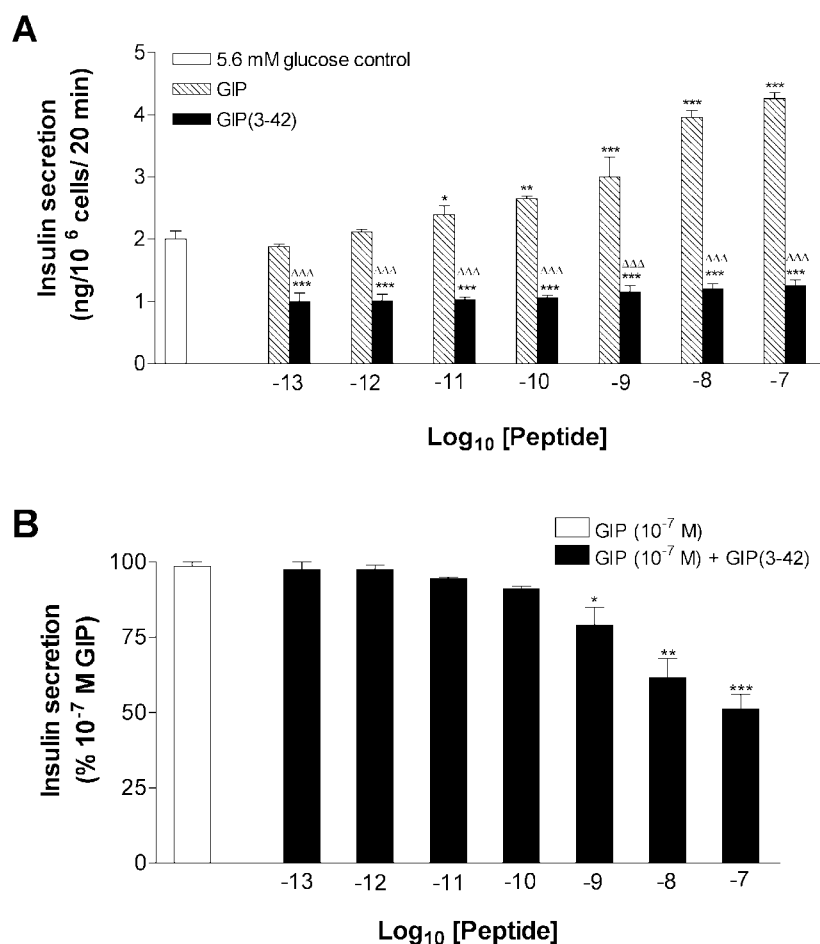


**Figure 2** Effects of GIP and GIP(3–42) on cAMP production in GIP receptor-transfected CHL cells. (A) GIP dose-dependently stimulated cAMP production with an EC<sub>50</sub> of 18.2 nM, whereas GIP(3–42) caused very small increases (5%). (B) Inhibition of GIP (10<sup>-7</sup> M)-stimulated cAMP production by varied concentrations of GIP(3–42). Data are expressed as a percentage of the forskolin (10 μM) response. Values are means ± s.e.m. of 3 observations. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 compared with GIP (10<sup>-7</sup> M) control.

concentrations than native peptide (Schmidt *et al.* 1987). The discrepancy may relate to the use of hyperglycaemic conditions in this earlier study or possibly improvements in peptide synthesis as the present data clearly demonstrate GIP(3–42) as an antagonist of cAMP production and insulin secretion.

To evaluate the biological actions of GIP(3–42) on normal islets and glucose homeostasis *in vivo*, we employed obese diabetic (*ob/ob*) mice. This is an extensively studied animal model displaying several abnormalities characteristic of type 2 diabetes, including obesity, insulin resistance, moderate hyperglycaemia and severe hyperinsulinaemia

(Bailey & Flatt 1982). The present results corroborate previous findings, as GIP displayed significant insulinotropic activity when administered in conjunction with glucose to (*ob/ob*) mice (Flatt *et al.* 1984, O'Harte *et al.* 2000). Such action resulted in a notable moderation in the glycaemic excursion. Consistent with the antagonistic properties of GIP(3–42) *in vitro*, GIP(3–42) significantly countered the insulinotropic and antihyperglycaemic action of GIP in (*ob/ob*) mice. In fact, administration of GIP(3–42) resulted in a worsening of the glycaemic and insulin responses compared with injection of glucose alone. These results demonstrate that GIP(3–42) acts



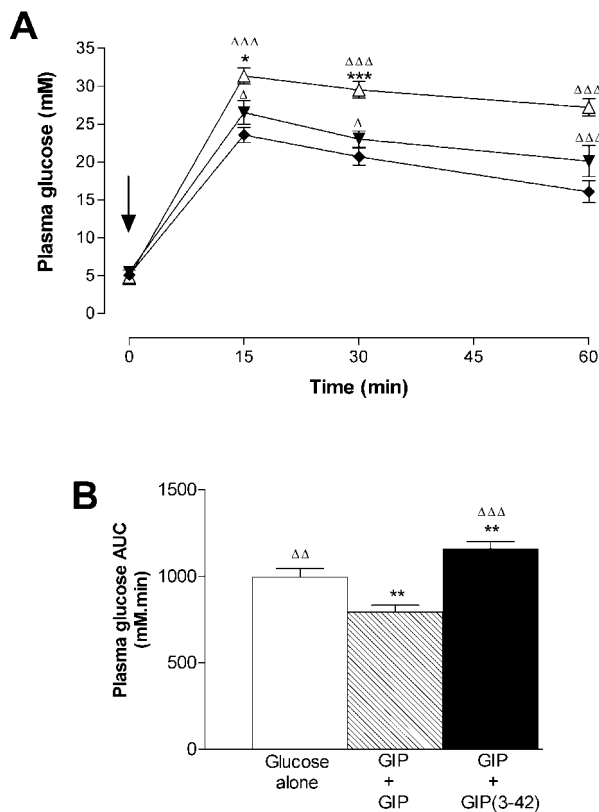
**Figure 3** Effects of GIP(3–42) on insulin secretion in clonal pancreatic BRIN-BD11 cells. (A) GIP dose-dependently stimulated insulin secretion, whereas GIP(3–42) lacked significant insulinotropic activity. (B) Inhibition of GIP ( $10^{-7}$  M)-stimulated insulin secretion by varied concentrations of GIP(3–42). Values are means  $\pm$  S.E.M. for 8 separate observations. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared with 5.6 mM glucose control.  $\Delta\Delta\Delta P < 0.001$  compared with GIP at the same concentration.

as a potent GIP receptor antagonist *in vivo*, and that endogenous GIP has a significant permissive effect on glucose-stimulated insulin release in *ob/ob* mice.

The observation that GIP(3–42) acts as an antagonist against the native peptide *in vivo* is significant since, using a specific N-terminally directed GIP radioimmunoassay, it has been shown that up to 80% of the circulating GIP is in the truncated GIP(3–42) form (Deacon *et al.* 2000). Therefore, it is likely that antagonism at the GIP receptor by the truncated GIP(3–42) metabolite greatly reduces the biological effectiveness of this glucincretin hormone, warranting further studies of GIP(3–42) concentrations in diabetes and different physiological states. The same scenario may also exist for GLP-1, since this peptide has been shown to exhibit an extremely short duration of action when administered subcutaneously to type 2

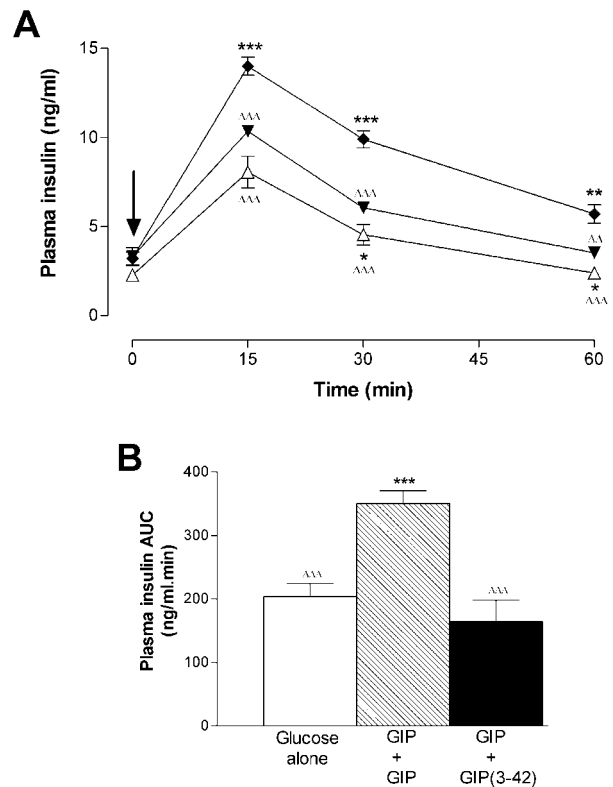
diabetic patients (Nauck *et al.* 1996). Consistent with this view, several studies have suggested that the truncated GLP-1 metabolite, GLP-1-(9–36) amide might, in fact, act as a GLP-1 receptor antagonist, acting not only on the pancreatic GLP-1 receptor, but also antagonising the gastrointestinal effects of GLP-1 (Grandt *et al.* 1994, Knudsen & Pridal 1996, Wettergren *et al.* 1998).

The concept that degradation of GIP and GLP-1 to their truncated forms leads to effective antagonists of their respective receptors has formed the basis for the proposition that inhibition of DPP IV may prove useful in the potential treatment of type 2 diabetes (Holst & Deacon 1998). This has led to the development of several DPP IV inhibitors which have been shown to inhibit N-terminal degradation of both GIP and GLP-1, while simultaneously augmenting the glucose and insulin responses (Balkan *et al.*



**Figure 4** Effects of native GIP and GIP(3–42) on plasma glucose homeostasis in (*ob/ob*) mice. (A) Plasma glucose concentrations after i.p. administration of glucose alone (▼, 18 mmol/kg) or in combination with either native GIP (◆, 2 × 25 nmol/kg) or native GIP (25 nmol/kg) plus GIP(3–42) (△, 25 nmol/kg). The time of injection is indicated by the arrow (0 min). (B) Plasma glucose AUC values for 0–60 min post injection. Values are means ± S.E.M. 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.

1999, Ahren *et al.* 2000, Deacon *et al.* 2001, Pospisilik *et al.* 2002). However, due to the presumed side effects of widespread DPP IV inhibition in the mammalian body, this method could prove problematic (Mentlein *et al.* 1993). Another alternative indirect approach of prolonging GIP or GLP-1 activity would be to structurally modify the molecule itself, thereby conferring the peptide with resistance to DPP IV activity. Since the strict substrate specificity of DPP IV is well characterised, it seems that small alterations at the N-terminus could render GIP and GLP-1 resistant to degradation. Several GIP, GLP-1 and GLP-2 analogues have already been synthesised and their DPP IV stability and biological activity tested (Drucker *et al.* 1997, Deacon *et al.* 1998, Siegel *et al.* 1999, O'Harte *et al.* 2000, Hinke *et al.* 2002). No matter which approach is chosen, inhibition of GIP and GLP-1 degradation would not only increase the availability of the biologically active



**Figure 5** Effects of native GIP and GIP(3–42) on plasma insulin responses in (*ob/ob*) mice. (A) Plasma insulin concentrations after i.p. administration of glucose alone (▼, 18 mmol/kg) or in combination with either native GIP (◆, 2 × 25 nmol/kg) or native GIP (25 nmol/kg) plus GIP(3–42) (△, 25 nmol/kg). The time of injection is indicated by the arrow (0 min). (B) Plasma insulin AUC values for 0–60 min post injection. Values are means ± S.E.M. for 8 mice. \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001 compared with glucose alone.  $\Delta$ *P*<0.01,  $\Delta\Delta$ *P*<0.001 compared with native GIP.

peptide, but would also reduce the effect of the feedback antagonism of the truncated forms at the level of the receptor.

In conclusion, this study has shown that N-terminal degradation of GIP to GIP(3–42) produces a peptide antagonist, which inhibits GIP-stimulated cAMP production and insulin secretion *in vitro*. Moreover, data presented here demonstrate that GIP(3–42) effectively countered the insulin releasing and antihyperglycaemic actions of native GIP *in vivo*. This antagonistic activity may have important implications for the use of GIP as a therapeutic agent in diabetes, since GIP(3–42) interaction with the GIP receptor may be important for regulating GIP activity *in vivo*.

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