RAPID COMMUNICATION

Increased glucose-dependent insulinotropic polypeptide (GIP) secretion in acromegaly

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Abstract

Objective: Acromegaly is often associated with fasting and postprandial hyperinsulinemia, and the mechanisms involved are only partly understood. Hypersecretion of incretins such as glucose-dependent insulinotropic polypeptide (GIP) could play a role in determining hyperinsulinemia in acromegaly, but the available data are inconsistent. The aim of this study was to characterize the fasting and postprandial pattern of plasma GIP and insulin in a group of acromegalic patients.

Design and Methods: Eleven non-diabetic patients with newly diagnosed acromegaly and 11 sex- and age-matched healthy subjects were studied. Blood samples were taken at regular intervals in fasting conditions and for 3 h after a standard solid-liquid meal for growth hormone (GH), GIP and insulin measurements.

Results: Not only insulin, but also fasting and postprandial GIP levels were significantly higher in the patients with acromegaly than the healthy subjects ($P < 0.01$). In the former group fasting GIP levels and the integrated GIP response to the meal correlated significantly with GH basal levels ($r = 0.83$, $P < 0.01$ and $r = 0.65$, $P < 0.05$, respectively). Moreover, multivariate linear regression analysis showed that the presence of acromegalic status was associated with higher fasting and postprandial GIP levels independently of sex, age, fasting and postprandial plasma glucose and insulin levels, and the occurrence of normal or impaired glucose tolerance.

Conclusion: This study provides evidence that in patients with acromegaly fasting and postprandial GIP levels are abnormally high. GIP hypersecretion in turn might play a role in the pathogenesis of hyperinsulinemia that characterizes acromegaly.

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Introduction

Acromegaly is often associated with impaired glucose tolerance, insulin resistance, and fasting and postprandial hyperinsulinemia (1–4). However, the mechanisms involved in the effects of growth hormone (GH) excess on carbohydrate metabolism are only partly understood (5). Under physiological conditions, meal-induced insulin secretion is due to direct beta cell stimulation by absorbed nutrients, amplified by the concomitant release of gut insulinotropic hormones, or incretins, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7–36) amide (GLP-1) (6).

Recently, Pierluissi *et al.* (7) have demonstrated that coinfusion of GIP and glucose induces a significantly higher insulin response in patients with acromegaly compared with healthy subjects, and suggested that a greater activity of the entero-insular axis could play a

role in determining hyperinsulinemia in acromegaly. However, the available data on endogenous GIP levels in patients with acromegaly are scanty and controversial (7, 8).

The aim of this study was to characterize the fasting and postprandial pattern of plasma GIP, insulin and glucose in a series of patients with active acromegaly. The results were compared with those obtained in healthy subjects.

Materials and methods

Subjects

Eleven patients with newly diagnosed acromegaly, 4 men and 7 women, aged 22–58 years, mean 44, and 11 healthy subjects, 5 men and 6 women, aged 25–55 years, mean 39, (P : NS vs acromegalic patients), recruited among medical staff and acquaintances of

patients, volunteered for this study, which was approved by the local ethics committee.

Acromegaly was diagnosed on the basis of clinical features, elevated age-adjusted plasma insulin-like growth factor-I (IGF-I) concentrations (107 ± 44 (mean $\pm$ s.d.) nmol/l, range 46–186), and elevated serum GH levels (mean 13 ± 10.6 μ g/l, range 2.1–37.6) which were not suppressible to less than 1 μ g/l during a 75 g oral glucose tolerance test (9). No patient had diabetes mellitus, but 6 of them showed impaired glucose tolerance (10). None of the patients had been treated for acromegaly.

Procedures

Each subject was studied under basal conditions and after a mixed standardized solid-liquid meal (550 kcal: carbohydrate 48%, fat 33%, protein 19%) given at 0 time and eaten in 15 min. The studies started between 0900 and 1000 h, after an overnight fast and 1 h of bed rest. Blood was taken from a forearm through a venous cannula kept patent by slow saline infusion. Samples were collected in plain tubes for GH assay and in ice-chilled polypropylene tubes containing EDTA (1 mg/ml) and aprotinin (500 KIU/ml) for other assays at the following times: –30, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min. Plasma was separated immediately by centrifugation at 4 °C, whereas the serum was separated within 1–3 h; both plasma and serum were stored in aliquots at –80 °C until assayed.

Assays

IGF-I, GH, GIP and insulin levels were measured as previously described (11, 12) using commercially available kits (IGF-I: Mediagnost, Tübingen, Germany; GH: AutoDelfia hGH, EG&G Wallac, Finland; GIP: Peninsula Laboratories, Belmont, CA, USA; Insulin: Biodata, Guidonia Montecelio, Rome, Italy). Plasma glucose levels were determined by means of a glucose autoanalyzer with a hexokinase method (Beckman, Milan, Italy).

Statistics

Continuous variables were expressed as mean $\pm$ s.e.m. The integrated areas of secretion (AUC) were calculated with the trapezoidal method. All data were first tested for normality of distribution by the Kolmogoroff-Smirnoff test. If necessary, logarithmic transformations were done to approach normality. Univariate linear regression analysis was performed to evaluate the differences in mean GIP, insulin, and glucose levels between acromegalic and healthy subjects and to evaluate the possible effect of age and sex over these variables. The effect of acromegalic status (0 = no, 1 = yes) on GIP levels was then evaluated by multivariate linear regression analysis to control for possible

confounders such as fasting and postprandial insulin and glucose levels, and glucose tolerance. Relationships between variables were assessed by the Pearson's correlation test, using log-transformed data for GH values. A $P < 0.05$ was considered statistically significant.

Results

The results are summarized in Table 1 and Fig. 1. No age- or sex- related differences were observed in any of the variables considered in either group of subjects. In the patients with acromegaly fasting plasma glucose levels were similar to those of healthy subjects, whereas serum GH and plasma insulin and GIP levels were markedly elevated. All except 1 of the patients had GIP levels above the range found in healthy controls (49–87 vs 10–39 pmol/l) and 6 also had insulin levels above the normal range (143–244 vs 49–142 pmol/l). There was a significant correlation between log-transformed GH levels and GIP values ($r = 0.83$, $P < 0.01$), but insulin levels did not correlate with those of GH or GIP.

After the meal GH levels increased in 9 patients and were unchanged in two. Plasma glucose, insulin and GIP responses to the meal were significantly higher in the patients with acromegaly than in the healthy subjects. All except one of the acromegalic patients had GIP peak values above the normal range (136–273 vs 74–132 pmol/l), and 8 had insulin and glucose peak values above the normal ranges (581–2782 vs 162–513 pmol/l for insulin, and 6.8–8.1 vs 4.6–6.6 mmol/l for glucose, respectively). Patients with normal and impaired glucose tolerance showed similar postprandial peaks of glucose (7.1 ± 0.47 vs 7.3 ± 0.28 mmol/l,

Table 1 Pattern of fasting and postprandial plasma GIP, insulin, glucose and serum GH levels in healthy subjects and/or acromegalic patients. Data are mean $\pm$ s.e.m.

	Healthy subjects (n = 11)	Acromegalic patients (n = 11)	P value*
GIP			
Fasting (pmol/l)	22.7 $\pm$ 2.60	66.0 $\pm$ 6.16	<0.001
Peak (pmol/l)	98.0 $\pm$ 6.40**	178.7 $\pm$ 11.76**	<0.001
AUC (nmol/l/3 h)	14.1 $\pm$ 0.99	26.0 $\pm$ 1.82	<0.001
Insulin			
Fasting (pmol/l)	83.8 $\pm$ 7.75	157.8 $\pm$ 18.30	0.001
Peak (pmol/l)	283.5 $\pm$ 36.45**	1111.5 $\pm$ 231.32**	0.002
AUC (nmol/l/3 h)	33.1 $\pm$ 3.59	98.0 $\pm$ 14.42	<0.001
Glucose			
Fasting (mmol/l)	4.8 $\pm$ 0.09	5.1 $\pm$ 0.15	NS
Peak (mmol/l)	5.8 $\pm$ 0.17**	7.2 $\pm$ 0.25**	<0.001
AUC (mmol/l/3 h)	887.1 $\pm$ 24.42	1078.3 $\pm$ 37.47	<0.001
GH			
Fasting (μ g/l)	–	13.0 $\pm$ 3.2	
Peak (μ g/l)	–	19.5 $\pm$ 7.6	
AUC (ng/l/3 h)	–	2.9 $\pm$ 1.2	

*Univariate linear regression analysis; ** $P < 0.001$ vs fasting values (univariate linear regression analysis); –, no data; NS, not significant.

Figure 1 Plasma glucose, insulin and GIP responses to a standard solid-liquid meal in 11 healthy subjects (open squares) and in 11 acromegalic patients (closed squares). The meal (550 kcal) was given at time 0 and eaten within 15 min. Data are mean $\pm$ S.E.M.

NS), insulin (1019 ± 345.1 vs 1184 ± 338.7 pmol/l, NS) and GIP (172 ± 11.3 vs 184 ± 20.2 pmol/l, NS). In both the patients and healthy subjects there was a significant correlation between GIP-AUC and insulin-AUC ($r = 0.62$, $P < 0.01$). Moreover, in the patients with acromegaly log-transformed GH basal levels correlated significantly with GIP-AUC ($r = 0.65$, $P < 0.05$).

The multivariate linear regression analysis showed that the presence of acromegalic status was independently associated with higher GIP levels, both in the fasting state [regression coefficient = 40 pmol/l (95% CI 11.9–68.1), $P < 0.01$] and after the meal, as evaluated by AUC [regression coefficient = 8.6 nmol/l/3 h (95% CI 0.6–16.5), $P < 0.05$].

Discussion

Results of this study confirm that hyperinsulinemia is a characteristic feature of patients with acromegaly and demonstrate that in such patients fasting and postprandial GIP levels are abnormally high and correlate significantly with basal GH concentrations. Our data are at variance with those of Cassar *et al.* (8) who did not observe a significant difference in plasma GIP response to oral glucose between patients with acromegaly and control subjects. However, recently Pierluissi *et al.* (7) found elevated fasting GIP levels in 4 out of 5 patients with acromegaly. These discrepancies could be due to differences in radioimmunoassay reagents (13, 14). Indeed, until a few years ago porcine GIP, which differs from human GIP by two amino-acids (13), and antisera raised against porcine GIP were widely used for human studies, with a marked variability in results from different laboratories, mostly the postprandial results (14). Also Cassar *et al.* (8) used in their study porcine GIP (15), whereas we have employed a commercial kit with antibodies raised in rabbit against human GIP and human synthetic GIP as standard and tracer. Therefore, our assay should accurately measure the circulating human GIP concentrations both in the fasting state and after the meal.

Intestinal nutrient absorption is the major stimulus for GIP release (6, 16) and after a meal circulating hormone levels remain elevated for some hours, the pattern depending on both the rate of glucose and fat absorption in the duodenum and upper jejunum (16) and the relatively long half-life of GIP in the circulation, approximately 20 min (17). However, little is known about other factors that may influence both fasting and postprandial GIP release (12, 16). In this study multivariate linear regression analysis showed that acromegaly was associated with GIP hypersecretion independently of sex, age, fasting and postprandial plasma glucose and insulin levels, and the occurrence of normal or impaired glucose tolerance. Thus, the finding that in the patients with acromegaly both fasting and meal-stimulated GIP levels correlated significantly with basal serum GH concentrations suggest that GH by itself may stimulate GIP release or increase the mass of GIP releasing cells.

The role of GIP as an incretin is well known and depends on a sufficient postprandial increase of circulating glucose levels (18). Indeed, both *in vitro* and *in vivo* studies have demonstrated that GIP stimulates insulin release only in the presence of a

rise in glucose concentration of at least 1.1 mmol/l above basal values (16). Accordingly, in patients with acromegaly GIP hypersecretion may play an important role in determining postprandial hyperinsulinemia, but should not account for the high insulin levels in the fasting state. On the other hand, Pierluissi *et al.* (7) found that in acromegalic patients GIP infusion alone significantly increased plasma insulin levels, with a consequent decrease in circulating glucose levels, which strongly suggests an interplay between high GH and GIP levels in stimulating insulin release also at basal glucose concentrations.

In conclusion, we have shown that an up-regulation of GIP release is associated with acromegaly. This in turn may play a role in the pathogenesis of hyperinsulinemia that characterizes this disease.

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