CLINICAL STUDY

Circulating levels of incretin hormones and amylin in the fasting state and after oral glucose in GH-deficient patients before and after GH replacement: a placebo-controlled study

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Abstract

Objective: Hyperinsulinemia in association with GH excess is considered a compensatory response to insulin resistance, but the possibility of alternative insulinotropic mechanisms has not been investigated *in vivo*. It is also unknown how GH influences the secretion from pancreatic β -cells of amylin, a peptide which regulates prandial glucose homeostasis and may be linked to development of β -cell dysfunction. We therefore measured plasma concentrations of two gut insulinotropic hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulin-releasing peptide (GIP), and total as well as non-glycosylated amylin, in 24 GH-deficient adults before and after 4 months of GH replacement (daily evening injections of 2 IU GH/m²).

Design: Double-blind, placebo-controlled, parallel study.

Methods: All participants underwent an oral glucose tolerance test (OGTT) at 0 and 4 months.

Results: A 33% suppression of fasting GLP-1 concentrations was measured in the GH group at 4 months ($P = 0.02$), whereas a non-significant increase occurred in the placebo group ($P = 0.08$). Fasting levels of GIP and amylin did not change significantly after 4 months in either group. The incremental response in GLP-1 during the OGTT was significantly lower after GH treatment as compared with both baseline ($P = 0.02$) and the response in the placebo group ($P = 0.03$). The stimulation of GIP secretion following OGTT was similar on all occasions. The OGTT-induced incremental response in non-glycosylated amylin was moderately elevated after GH treatment as compared with placebo ($P = 0.05$). Plasma concentrations of glucose and insulin, both in the fasting state and after the OGTT, were higher after GH treatment, but the ratio between amylin and insulin remained unchanged.

Conclusions: GH-induced hyperinsulinemia is accompanied by proportionate elevations in amylin concentrations and a blunting of gut GLP-1 secretion. The mechanisms underlying the suppression of GLP-1 remain to be elucidated.

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Introduction

The effects of growth hormone (GH) on insulin secretion and glucose metabolism have been studied over several decades (1). Active acromegaly as well as sustained high-dose GH administration in normal adults is associated with hyperinsulinemia and insulin resistance (2, 3). By contrast, GH-deficient children may exhibit hypoinsulinemia and fasting hypoglycemia (4). Hyperinsulinemia associated with prolonged GH exposure is traditionally assumed to be secondary to induction of both hepatic and peripheral insulin resistance. Increased insulin levels and insulin resistance have also been documented in GH-deficient

adults during acute and prolonged replacement with GH in moderately supraphysiological doses (5, 6).

There is, however, also evidence to suggest alternative insulinotropic effects of GH. Co-administration of i.v. GH and glucose has been shown to exaggerate the increment in plasma insulin in normal and GH-deficient children (7), and i.v. GH administration in dogs acutely increases portal insulin levels (8). Moreover, GH stimulates insulin production in pancreatic β -cells *in vitro*, and these cells express functional GH receptors, which during activation stimulate insulin gene expression and mitotic activity (9). These studies suggest that stimulatory effects of GH on β -cell function *in vivo* may involve several independent pathways.

To further our understanding of this important aspect of GH action we have evaluated the circulating levels of pertinent markers and regulators of β -cell activity in GH-deficient adults. All patients underwent an oral glucose tolerance test (OGTT) before and after GH substitution in a placebo-controlled, parallel design.

We measured basal and glucose-stimulated plasma concentrations of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulin-releasing peptide (GIP). These gut factors, which are released by oral nutrients, are known to stimulate insulin secretion (10, 11). The secretion of GIP and GLP-1 accounts to a large extent for the so-called incretin effect, i.e. the phenomenon that oral glucose elicits a greater insulin response than an equivalent amount of i.v. glucose. Plasma amylin levels were also measured, since this pancreatic polypeptide, which is co-secreted with insulin, not only reflects islet cell function but has been shown to exert effects on insulin secretion and prandial glucose metabolism (12, 13).

Patients and methods

Patients

Twenty-four adults (6 females, 18 males) with a mean $\pm$ S.E. age of 38 ± 2 years, and a mean $\pm$ S.E. body mass index of 28.7 ± 1.0 were included. Pituitary disease was adult-onset in 17 cases and childhood-onset in 7 cases (9 non-functioning pituitary adenoma, 2 prolactinoma, 3 Cushing disease, 5 craniopharyngioma and 5 others). All patients except one had additional pituitary deficits for which conventional replacement therapy was continued unchanged during the study period (19 patients received hydrocortisone, thyroxine and sex steroids, one patient received hydrocortisone + thyroxine, whereas two received sex steroids and one patient received only desmopressin). Prior to study entry GH deficiency was verified or reconfirmed by a stimulated GH response less than $5 \mu\text{g/l}$ (mean $\pm$ S.E. peak GH response: $1.0 \pm 0.3 \mu\text{g/l}$). The tests included insulin-induced hypoglycemia, arginine infusion and heat exposure. Mean baseline serum insulin-like growth factor-I (IGF-I) concentration was $109 \pm 40 \mu\text{g/l}$.

Study design

In a randomized, double-blind, parallel design the patients were allocated to 4 months of either GH (Norditropin; Novo Nordisk, Gentofte, Denmark) or placebo. The medication was administered as daily s.c. self-injections performed in the evening. During the initial 6 weeks the GH dose was gradually increased to a daily target dose of 2 IU/m^2 . All measurements were performed both at baseline and after the 4 month treatment. The study was approved by the regional and national ethical committees, and by the national health authorities.

OGTT

The patients were admitted to hospital at 0800 h after an overnight fast of at least 10 h. An i.v. cannula was inserted into an antecubital vein for blood sampling. Baseline samples were drawn at -15 , -10 , and -5 min. At time 0, 75 g glucose monohydrate were administered orally in 300 ml tap water. Blood was sampled at 10 min intervals for the first hour, followed by 15 min intervals for the next 1.5 h, and finally after 3 h.

Assays

Total as well as non-glycosylated amylin immunoreactivity was measured as previously described (14). For both analytes the detection limit was $<2.0 \text{ pmol/l}$, and the intra- and interassay coefficients of variation (CVs) were less than 15%. GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol (v/v, final concentration). For the GIP RIA (15) we used C-terminally directed antiserum R65, which cross-reacts fully with human GIP but not with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion is uncertain. Human GIP and ^{125}I -human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured against standards of synthetic GLP-1 7-36 amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore does not react with GLP-1-containing peptides from the pancreas (16). The results of the assay accurately reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9-36 amide, into which GLP-1 is rapidly converted (17). For both assays sensitivity was below 1 pmol/l , and intraassay CV below 6% at 20 pmol/l . Corrections are made for an inherent 30% loss of standard in the extraction procedure. Plasma glucose was measured in duplicate by the glucose oxidase method (Beckman Instruments, Fullerton, CA, USA). C-peptide and insulin were determined by commercial assays (DAKO, Diagnostics Ltd, Cambs, UK).

Data on glucose, insulin and C-peptide have previously been published (18).

Statistical analysis

Basal levels of each parameter were compared using Student's *t*-test for paired (0 vs 4 months) and unpaired (GH vs placebo groups) data. To test for changes in plasma levels of each parameter with time during the OGTT, one-way ANOVA was performed. Comparisons between the temporal changes in each parameter at baseline and after 4 months were analyzed by two-way ANOVA (MANOVA) (time and condition). Area under the plasma curve (AUC) of each parameter was estimated according to the trapezoidal rule. Differences in AUC were analyzed within each treatment group (0 vs 4

Table 1 Fasting concentrations of GLP-1, GIP, total amylin and non-glycosylated amylin in placebo and GH-treated GH-deficient patients at baseline and after 4 months.

	Placebo			GH			GH vs placebo
	0	4	P	0	4	P	P
GLP-1 (pmol/l)	6.3±1.6	7.4±1.5	0.08	8.6±0.8	5.8±1.1	0.02	0.007
GIP (pmol/l)	17.4±6.4	18.0±3.4	0.93	15.8±3.0	10.3±1.1	0.15	0.39
Amylin _{tot} (pmol/l)	7.3±1.5	6.1±0.7	0.36	3.3±0.6	8.9±1.9	0.19	0.11
Amylin _{non-glyc} (pmol/l)	2.3±0.4	2.0±0.3	0.45	3.3±0.6	3.4±0.5	0.83	0.59

months) by Student's paired *t*-test, whereas the differences in AUC between GH and placebo, i.e. the true treatment effect, was analyzed by Student's unpaired *t*-test on Δ -values. If necessary, logarithmic transformations were performed to obtain normality. *P* values <0.05 were considered significant. Pearson's product moment correlation was used to measure the strength of association between GLP-1 and body composition variables. Results are expressed as means $\pm$ S.E.

Results

Fasting levels

Plasma levels measured prior to ingestion of glucose during the OGTT in each treatment group at baseline and after 4 months are presented in Table 1. At baseline no differences between the two treatment groups were recorded. After 4 months a significant 33% suppression of fasting plasma GLP-1 concentrations was measured in the GH group ($P = 0.02$), whereas a non-significant increase occurred in the placebo group ($P = 0.08$), which resulted in a highly significant treatment effect ($P = 0.007$). Fasting levels of GIP as well as total and glycosylated amylin did not change significantly after 4 months in either treatment group (Table 1).

Plasma concentrations of glucose, insulin, and C-peptide at baseline did not differ between the two groups. Four months of GH treatment induced significant increments in fasting plasma concentrations of glucose and insulin (glucose (mmol/l): 5.0 ± 0.1 (0) vs 5.4 ± 0.2 (4) ($P = 0.05$); insulin (pmol/l): 38 ± 9 (0) vs 55 ± 11 (4) ($P = 0.02$)). No significant changes occurred in the placebo group.

OGTT

Highly significant increments in plasma concentrations of GLP-1, GIP, and amylin were recorded within each group during the OGTT both at baseline and after 4 months (Figs 1–3). When comparing baseline and 4 month OGTT data within each group by two-way ANOVA highly significant differences ($P < 0.001$) in the responses were obtained in the GH group, but not in the placebo group. When expressed as AUC during OGTT the incremental response in

GLP-1 was significantly blunted after 4 months of GH as compared with baseline ($P = 0.02$), and the difference in Δ -AUC (0 vs 4 months) between the two treatment groups was also significant ($P = 0.03$) (Fig. 1). As compared with baseline data the AUC of non-glycosylated amylin during OGTT after 4 months tended to decrease in the placebo group ($P = 0.1$) and increase in the GH group ($P = 0.2$), which translated into a significant treatment effect ($P = 0.05$) (Fig. 3).

Figure 1 Mean $\pm$ S.E plasma concentrations of GLP-1 before and during an OGTT performed at baseline (open symbols) and after 4 months of treatment (solid symbols) with either GH (upper panel) or placebo (lower panel). Oral glucose was administered at time 0.

Figure 2 Mean $\pm$ S.E. plasma concentrations of GIP before and during an OGTT performed at baseline (open symbols) and after 4 months of treatment (solid symbols) with either GH (upper panel) or placebo (lower panel). Oral glucose was administered at time 0.

Plasma glucose_{AUC} during the OGTT was significantly higher after 4 months in the GH group, and a near-significant increment was observed in the corresponding plasma insulin_{AUC} (glucose_{AUC} (mmol/l $\times$ min): 1054 ± 14 (0) vs 1222 ± 17 (4) ($P = 0.02$); insulin_{AUC} (pmol/l $\times$ min): $17\,198 \pm 4660$ (0) vs $27\,067 \pm 1866$ (4) ($P = 0.07$)). No significant differences were recorded in C-peptide_{AUC}.

A correlation matrix including basal as well as Δ -values of GLP-1 and selected body composition variables did not disclose any significant correlations (data not shown).

Discussion

The main finding in the present study was a significant reduction in both basal and stimulated levels of GLP-1 following 4 months of GH replacement in hypopituitary adults, which occurred together with moderate elevations in insulin and amylin levels.

Hyperinsulinemia during GH exposure *in vivo* is a well-known feature, which traditionally is considered to

be secondary to induction of peripheral and hepatic insulin resistance. The mechanisms underlying the insulin-antagonistic actions of GH are not fully characterized, but at the level of substrate metabolism a key finding is stimulation of lipolysis and lipid oxidation, which may cause the reduced uptake and oxidation of glucose (1) in striated muscle through activation of the glucose/fatty acid cycle (19). In the present study GH treatment significantly impaired insulin sensitivity and glucose tolerance, as previously published in detail (18). There is, however, also evidence for a more direct tropic effect of GH on the pancreatic β -cell. Addition of GH to cultured β -cells has been shown to stimulate insulin secretion and biosynthesis through binding and activation of the GH receptor (9), but the effect of GH on gut insulinotropic hormones has not previously been investigated. GLP-1 is produced and secreted by L cells of the gut and is a product of post-translational processing of the proglucagon gene (11, 20). Pancreatic alpha cells predominantly produce larger GLP-1 moieties, which are not measured by our assay. A major stimulus of GLP-1 secretion from the gut is the presence of intraluminal glucose and other nutrients (11, 20). A major effect of GLP-1 is stimulation of insulin secretion, and this effect appears to be receptor mediated, inasmuch as a specific receptor for GLP-1 has recently been cloned from human islets (21). In addition, GLP-1 decelerates gastric emptying and lowers gastric acid secretion. Apart from this, GLP-1 is also produced in specific brain nuclei and may act as a satiety signal following binding to hypothalamic receptors (11). The incretin effect of GLP-1 has been experimentally verified by combined administration of glucose and exogenous GLP-1 in healthy subjects, which resulted in augmented insulin secretion (22). The contribution of GLP-1 to the overall incretin effect has, however, been questioned since the inhibitory effect of GLP-1 on gastric emptying appears to reduce substrate-mediated insulin secretion at least during experimental conditions in healthy subjects. The insulinotropic effects of GLP-1 depend on increased glucose concentrations (23, 24), and this glucose dependency has been substantiated by administering GLP-1 *i.v.* following a mixed meal in type 2 diabetic patients, which significantly amplified post-prandial insulin concentrations resulting in a prolonged lowering of plasma glucose concentrations (25).

GH treatment also resulted in predictable significant changes in body composition in terms of reduced fat mass and increased fat mass (18). No data exist regarding the possible influence of body composition on GLP-1 secretory dynamics. The effects of acute or short-term administration of GH would help to disclose possible direct effects on GLP-1. Endogenous GH secretion in healthy subjects depends strongly on nutrient intake. Short-term and more prolonged fasting is associated with pronounced hypersomatotropinemia (26), whereas oral glucose ingestion suppresses

Figure 3 Mean $\pm$ S.E. plasma concentrations of total amylin (upper panels) and non-glycosylated amylin (lower panels) during an OGTT performed at baseline (open symbols) and after 4 months of treatment (solid symbols) with either GH (left panels) or placebo (right panels). Oral glucose was administered at time 0.

endogenous GH release. In the present study, GH was injected s.c. between 2000 and 2200 h, which implies that the ambient GH concentrations during the OGTT, which was performed in the morning, were low, albeit not returned to pre-injection levels (27). The observed effects on GLP-1 could also be caused by elevated total IGF-I concentrations, but our study design does not allow firm conclusions about the mechanisms underlying the suppression of GLP-1 following GH exposure. Based on teleological considerations it is not unexpected that GH, which normally operates in the post-absorptive state to partition substrate metabolism from glucose utilization to mobilization of lipid stores, inhibits gut insulinotropic hormones. In all instances the study emphasizes that the effects of sustained GH exposure on glucose metabolism and insulin dynamics are not identical to those observed in other insulin resistant states such as type 2 diabetes, since fasting and stimulated GLP-1 levels are significantly elevated in newly diagnosed type 2 patients as compared with weight-matched controls (24).

The changes in plasma GIP concentrations following GH treatment were less clear since two-way ANOVA

revealed a significant difference when comparing the response to the OGTT at baseline and 4 months, whereas no differences were found when comparing changes in GIP_{AUC} between the two treatment groups. This reflects that a moderate reduction in the incremental GIP response was observed in both groups at 4 months. It is therefore concluded that GH treatment does not significantly affect GIP secretion. It is generally accepted that GIP is a major contributor to the incretin effect in normal subjects (10, 11), whereas this effect appears to be reduced in type 2 diabetes despite normal or elevated GIP levels (28).

As expected plasma amylin levels increased during the OGTT on all occasions, and this incremental response was moderately elevated following GH therapy. The amylin-to-insulin ratio (A/I) was, however, similar in all studies, which suggests that the amylin response in GH-treated subjects increases in proportion to enhanced β -cell secretion. An increased A/I has been observed during the early phases of hyperglycemia in diabetic rodents (29), and it has been suggested that circulating amylin may induce insulin resistance and hence contribute to the pathogenesis of type 2 diabetes

(13). In healthy adults infusion of a human amylin analog failed, however, to influence insulin sensitivity (30). It has also been speculated that deposition of islet amyloid, which is a known feature of type 2 diabetes, impairs β -cell function. Interestingly, co-administration of GH and dexamethasone in mice transgenic for human islet amyloid induced amyloid deposits and hyperglycemia (31). Our data do not, however, indicate that GH-induced insulin resistance and impaired glucose tolerance is associated with specific abnormalities in amylin secretion.

In summary, the insulinotropic effects of moderately supraphysiological GH exposure for 4 months in GH-deficient adults are accompanied by a reduction in basal and stimulated GLP-1 secretion, unchanged GIP levels, and a proportionate increase in amylin concentrations. These data suggest a hitherto unrecognized suppressive effect of GH on GLP-1 secretion the significance of which merits future studies. In particular, acute GH exposure timed according to GLP-1 stimulation could help to distinguish between possible direct and indirect effects of GH. We conclude that the effects of GH on β -cell function in human subjects involves other features than induction of insulin resistance.

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