

CLINICAL STUDY

Glucagon inhibits ghrelin secretion in humans

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Abstract

Objective: It is well known that i.m. glucagon administration stimulates GH and cortisol release in humans, although the mechanisms are unclear. These effects are similar to those described for ghrelin on somatotroph and corticotroph function. The aim of the present study was to investigate the role of ghrelin in mediating the stimulatory effects of glucagon and to evaluate the effect of glucagon on ghrelin secretion.

Design and methods: We studied the endocrine and metabolic response to i.m. glucagon administration in 24 subjects (14 men, 10 women; age 19–65 years; body mass index, $25.3 \pm 1 \text{ kg/m}^2$), who were shown to have an intact anterior pituitary function as evaluated before enclosure.

Results: Serum ghrelin concentrations fell significantly at 30, 60, 120 and 180 min after glucagon administration (means \pm S.E.M.; baseline, $377.9 \pm 34.5 \text{ pg/ml}$; nadir, $294.6 \pm 28.3 \text{ pg/ml}$ (60 min); $P < 0.01$). Conversely, i.m. glucagon elicited an increase in GH (baseline, $1.5 \pm 0.4 \mu\text{g/l}$; peak, $14.2 \pm 2.7 \mu\text{g/l}$ (180 min); $P < 0.01$) and cortisol concentrations (baseline, $452.6 \pm 35.2 \text{ nmol/l}$; peak, $622.1 \pm 44 \text{ nmol/l}$ (180 min); $P < 0.01$). The changes in ghrelin concentration at both 120 and 180 min were still significant after correction for glucose and insulin ($P < 0.05$).

Conclusions: We show that i.m. glucagon decreases ghrelin significantly. Therefore, the already known stimulatory effects of i.m. glucagon on cortisol and GH are not mediated by a change in ghrelin concentrations. The mechanisms underlying the ghrelin suppression after i.m. glucagon are unlikely to include glucose or insulin variations and need to be further elucidated.

European Journal of Endocrinology 153 397–402

Introduction

Glucagon is a 29-amino acid peptide hormone processed from proglucagon in pancreatic α -cells and released from islets in a pulsatile fashion. Although the liver is considered to be the primary target for the action of glucagon, glucagon-binding sites have been identified in multiple tissues, including kidney, heart, brain, pancreatic β -cells, intestine, vascular tissues, adrenal glands and adipose tissues (1). The main physiological role of glucagon is to maintain glucose homeostasis, being a major counterregulatory hormone for insulin. Moreover, glucagon potently and reproducibly stimulates growth hormone (GH), corticotrophin (ACTH) and cortisol secretion in humans and has so far proved to be a classical test of GH secretion with a great diagnostic accuracy for the diagnosis of GH deficiency, both in childhood and adulthood (2–5).

These effects are similar to those of ghrelin, a 28-amino acid peptide produced predominantly by the enteroendocrine X/A-like cells in the oxyntic mucosa of the stomach and small bowel, although its expression has also been demonstrated in several other tissues including pancreas, kidneys, the immune system, placenta, testes, lung, pituitary and hypothalamus (6). Ghrelin is an endogenous ligand for the previously characterized GH secretagogue (GHS) receptor, which was isolated as the specific receptor for a family of synthetic peptidyl and nonpeptidyl molecules known as GHS. At the neuroendocrine level, ghrelin stimulates somatotroph, lactotroph and corticotroph function and suppresses the pituitary-gonadal axis pulsatility (6–8). In addition, ghrelin exhibits a wide range of biological activities, including the modulation of pancreatic exocrine and endocrine function, as well as effects on glucose metabolism (6).

Therefore ghrelin, like glucagon, may be an important regulator of glucose homeostasis, and a potential paracrine role for ghrelin to regulate insulin secretion is suggested by the observation that both ghrelin and its receptor are expressed in pancreatic islets (9–12).

The aim of the present study was to investigate the role of ghrelin in mediating the stimulatory effects of glucagon on the pituitary and to evaluate the effect of glucagon on ghrelin secretion.

Subjects and methods

Subjects

Prospectively, we recruited 24 patients (14 men and 10 women; age, 19–65 years; body mass index (BMI), $25.3 \pm 1 \text{ kg/m}^2$), who visited our outpatient Clinic for Endocrinology. Two female patients with well-controlled type 1 diabetes mellitus (age 22 and 31 years; BMI, 22 and 20 kg/m^2 , respectively), who were otherwise healthy, also participated. They were asked to skip their prandial insulin and to take the regular dose of their basal insulin the night before being studied (insulin glargine; Aventis Pharma, Frankfurt, Germany). All subjects gave written informed consent to participate in the study, which was approved by the hospital's ethical committee. The subjects got a full medical history, a physical examination and had height and weight recorded, from which BMI was derived. They underwent a series of standard endocrine tests for the assessment of hypothalamo-pituitary function to ensure intact pituitary function, including serum levels of thyroidstimulating hormone (TSH), 3,3',5-triiodothyronine (T_3), free thyroxine (T_4), insulin-like growth factor I (IGF-I), prolactin, luteinizing hormone, follicle-stimulating hormone, cortisol, and testosterone (males) and estradiol (females), followed by dynamic tests for gonadotrophin (gonadotrophin-releasing hormone (GnRH) test), TSH (TSHreleasing hormone (TRH) test), GH (insulin-tolerance test or arginine test) and ACTH secretion (insulin-tolerance test or metyrapone test).

Glucagon test

The subjects were asked to skip the administration of their medicine (i.e. antihypertensive medicine) on the morning of the test. The test started at 08.30 h after an overnight fast and 30 min after an indwelling catheter had been placed into an antecubital vein. Glucagon was administered intramuscularly (1 mg for subjects with a body weight $< 90 \text{ kg}$ and 1.5 mg for subjects with a body weight $> 90 \text{ kg}$) at 08.30 h and the subjects were kept supine until the end of the test. Blood samples were taken in serum and EDTA tubes at 30-min intervals from 08.00 to 12.30 (at -30 , 0, 30, 60, 90, 120, 150, 180, 210 and 240 min relative to the glucagon injections). Blood was centrifuged, and aliquots were frozen at -20°C until assay.

Hormone assays

Capillary blood glucose was measured using the glucose oxidase method on a glucometer (Biosen 5130; EKF-Diagnostic, Magdeburg, Germany). Insulin was measured with an ELISA (Mercodia, Uppsala, Sweden; intra- and inter-assay coefficients of variation were 4 and 3.6%, respectively). Plasma glucagon levels were assessed in duplicate with a RIA using ^{125}I -labelled glucagon as a tracer and antibody raised in rabbits against glucagon (DPC Biermann, Bad Nauheim, Germany; intra- and inter-assay coefficients of variation 4.8 and 8.6%, respectively). Serum ghrelin was analyzed in all samples in the same assay. Immunoreactive total human ghrelin was measured by a commercially available RIA (Phoenix Pharmaceuticals, Mountain View, CA, USA) using ^{125}I -labelled bioactive ghrelin as a tracer and a polyclonal antibody raised in rabbits against the C-terminal end of human ghrelin. This assay recognizes both acylated and de-acylated ghrelin and the antiserum does not cross-react with any relevant peptide according to the information provided by the manufacturer. Intra- and inter-assay coefficients of variation were 5.3 and 13.6% respectively. Serum GH concentrations were determined by a commercially available enzyme-labelled two-site chemiluminescent immunoradiometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA; sensitivity, 0.05 ng/ml; intra- and inter-assay coefficients of variation were 6.5 and 6.2%, respectively). Serum cortisol concentrations were determined by chemiluminescent immunometric assay (Diagnostic Products Corporation; total and intra-assay coefficients of variation were 10 and 8.8%, respectively).

Statistical analyses

Student's *t*-test for paired analysis was performed using SPSS software version 11.5 (SPSS Chicago, IL, USA). All significances are two-tailed. *P* values of < 0.01 were regarded as statistically significant (after Bonferroni correction for repeated measures). Data are presented as means \pm S.E.M. All baseline values are given as the mean value between -30 and 0 min. All data were analysed using repeated measures (General Linear Modeling) followed by Greenhouse–Geisser test to pinpoint specific differences on interaction means. The change in ghrelin concentrations at both 120 and 180 min were corrected for changes in insulin, glucose, glucagon, GH and cortisol concentrations using univariate analysis of covariance (ANCOVA; *P* values of < 0.05 were regarded as statistically significant).

Results

Plasma glucagon increased significantly with a peak after 30 min and returned to baseline levels after 240 min ($43.1 \pm 4.8 \text{ pg/ml}$ (baseline) versus 230.9 ± 11.2 (30 min)

and 51.6 ± 5.2 pg/ml (240 min); $P < 0.01$; Fig. 1). Serum ghrelin concentrations fell significantly at 30, 60, 120 and 180 min after glucagon administration and increased towards baseline level after 240 min (377.9 ± 34.5 pg/ml (baseline) versus 331.9 ± 31.6 (30 min), 294.6 ± 28.3 (60 min), 319.1 ± 25.7 (120 min), 323.6 ± 28.9 (180 min) and 336.3 ± 28.9 pg/ml (240 min); $P < 0.01$; Fig. 1). 30 min after i.m. glucagon administration glucose levels showed a maximal increase followed by a decrease to baseline level after 120 min (89.9 ± 3.7 (baseline), 140 ± 6.1 (30 min) and 89.6 ± 8.7 mg/dl (120 min); $P < 0.01$; Fig. 1). Insulin levels showed a similar increase with a peak after 30 min reaching the baseline level after 120 min (7.5 ± 2.5 (baseline), 39.2 ± 7 (30 min) and 9.6 ± 2.6 mU/l (120 min); $P < 0.01$; Fig. 1).

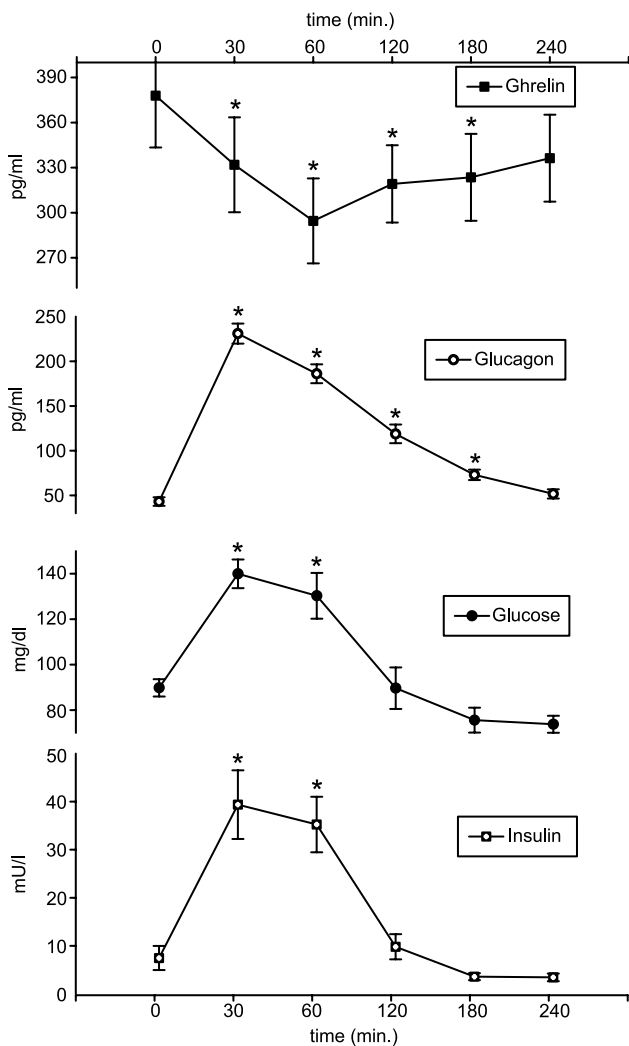


Figure 1 Mean \pm S.E.M. ghrelin, glucagon, glucose and insulin variations after the administration of glucagon (1–1.5 mg i.m.) in 24 subjects ($*P < 0.01$). All values are compared with the 0-min value using Student's *t*-test for paired analysis. The 0-min value is calculated as the mean from two baseline values (–30 and 0 min).

Glucagon elicited an increase in GH concentration (1.5 ± 0.4 μ g/l (baseline) versus a peak of 14.2 ± 2.7 μ g/l (180 min); $P < 0.01$) and cortisol concentration (452.6 ± 35.2 nmol/l (baseline) versus a peak of 622.1 ± 44 nmol/l (180 min); $P < 0.01$; Fig. 2). The change in ghrelin concentrations at both 120 and 180 min still proved to be significant after statistical correction for changes in glucose, insulin, GH and cortisol ($P < 0.05$). In the two type 1 diabetic subjects, similar changes occurred for ghrelin (411.9 ± 69.9 pg/ml (baseline) versus 369.3 ± 102.8 (30 min), 387.8 ± 99.2 (60 min), 364.1 ± 64.6 (120 min), 351.6 ± 61.6 (180 min) and 324.6 ± 12.6 pg/ml (240 min)), glucagon (72 ± 12.4 pg/ml (baseline) versus 382.3 ± 59.8 (30 min) and 75.5 ± 10.9 pg/ml (240 min)), GH (3.6 ± 2.7 μ g/l (baseline) versus a peak of 20.5 ± 19.5 μ g/ml (120 min)) and cortisol (497 ± 143.5 nmol/l (baseline) versus a peak of 616.5 ± 412.5 nmol/l (240 min)) concentrations. Glucose levels showed a maximal increase at 60 min followed by a decrease to baseline level after 180 min (101.4 ± 0.5 (baseline), 187.8 ± 18.6 (60 min) and 117.5 ± 3.5 mg/dl (180 min)), whereas no remarkable increase was seen in the insulin levels (3.4 ± 0.2 (baseline), 6.5 ± 1.2 (60 min) and 3.6 ± 0.5 mU/l (120 min); Fig. 3).

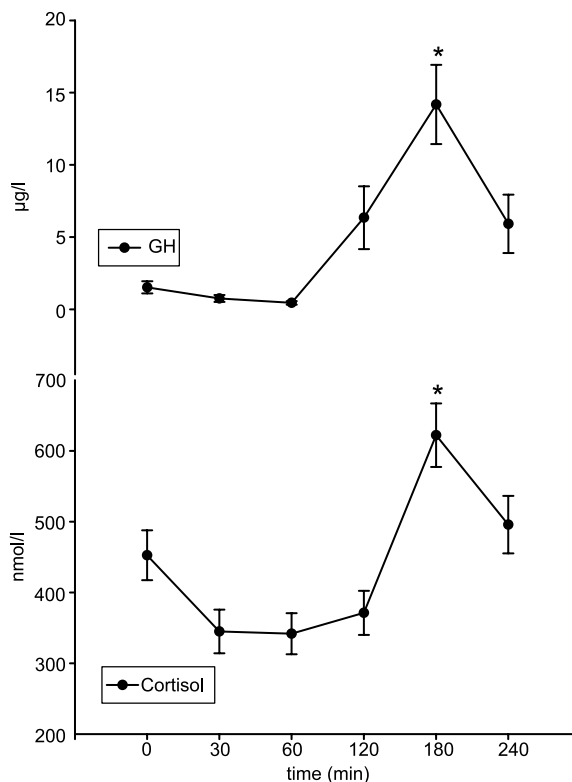


Figure 2 Mean \pm S.E.M. GH and cortisol variations after the administration of i.m. glucagon in 24 subjects ($*P < 0.01$). The 0-min value is calculated as the mean from two baseline values (–30 and 0 min).

Discussion

Our results demonstrate that glucagon, at least after i.m. administration, suppresses circulating ghrelin in addition to inducing the well-known increases in GH and cortisol levels. These responses therefore do not involve a ghrelin-mediated mechanism, even though ghrelin can stimulate both hormones independently (13). The role of ghrelin in driving the secretion of GH or cortisol under physiological conditions is strongly questioned. A regulatory feedback link between GH and ghrelin at physiological states has been suggested based on studies showing an increase of circulating ghrelin levels in rats following hypophysectomy (14) and an excessive accumulation of subcutaneous and visceral fat by dwarfism SMA1 mice in the presence of elevated ghrelin levels (15). Similarly, a role of ghrelin in driving the GH secretion in humans is suggested by studies demonstrating a positive association between GH and ghrelin levels in obesity and anorexia (16), a role of ghrelin in driving the GH response to fasting (17, 18) and by studies involving acromegalic patients, in whom ghrelin levels were lower than in normal subjects, but which increased with postsurgical treatment-induced normalization of GH and IGF-I (19, 20). However, no significant temporal association has been found between ghrelin secretion and GH pulsatility in animals (21) or humans (16), and ghrelin-knockout mice show no changes in body growth (22). The GH increase after

rigorous physical activity occurs without a change in the ghrelin levels (23), and ghrelin levels are unchanged during infusion of a GH-releasing hormone antagonist, suggesting no influence of GH on ghrelin secretion (24). Similarly, ghrelin is unlikely to play a major role in the control of corticotroph function since long-term treatment with synthetic GHS causes no significant increase in 24-h cortisol values (25). Moreover, the lack of any association between cortisol and ghrelin secretion during hypoglycaemia had already been reported (26, 27). The fact that ghrelin does not mediate the GH and cortisol response to glucagon is also confirmed by our study that demonstrates a clear suppression of circulating ghrelin after i.m. glucagon administration. Only a few determinants of circulating ghrelin concentration have been identified to date, indicating that ghrelin secretion is mostly associated with metabolic determinants (28). Information about the role of glucagon in ghrelin secretion is scant. A stimulatory influence of glucagon would be predicted based on a recent study showing that glucagon increases gastric ghrelin mRNA expression (29). Moreover, glucagon seems to have a stimulatory effect on ghrelin secretion from the isolated perfused rat stomach (30), and protein-rich meals stimulate both glucagon and ghrelin secretion (31, 32). All these data may argue somewhat against a direct inhibitory effect of glucagon on the gastric ghrelin-producing cells, at least in animals. In humans, the increase in glucagon due to hypoglycaemia doesn't seem to have an additional suppressive effect on circulating ghrelin (26). It has been suggested that insulin *per se* is an independent determinant of the circulating ghrelin concentration (26, 33, 34), or at least that it is essential for meal-induced ghrelin suppression in humans (35). Ghrelin decreased after oral and intravenous glucose, suggesting an inhibitory effect of glucose and/or insulin on ghrelin (17). Anyway, the acute increase in plasma glucose, but not the early insulin response, was suggested to induce the reduction in ghrelin concentration (36). Indeed, it is still a matter of debate whether insulin and hyperglycaemia *per se* inhibit ghrelin secretion directly or indirectly (37, 38) and the meal-induced suppression of ghrelin in diabetic rats occurs despite the absence of any postprandial increase of plasma insulin levels (39). Due to these findings, the assumption that glucose and insulin may regulate ghrelin secretion directly is more than questionable. In our study, ghrelin suppression was seen in some of the patients who showed minimal variations in glucose levels (data not shown) as well as in the two patients with type 1 diabetes mellitus, who showed no remarkable variations in the insulin levels (Fig. 3). Moreover, the decrease in ghrelin was still significant after statistical correction for the changes in both insulin and glucose. Therefore, it is unlikely that the changes in glucose and insulin levels are the main mediators in the ghrelin reduction induced by glucagon

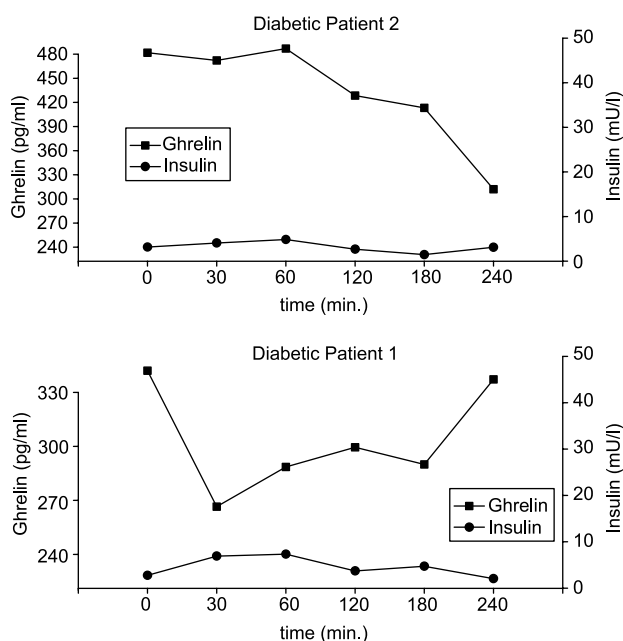


Figure 3 Mean \pm S.E.M. ghrelin and insulin variations after the administration of i.m. glucagon in two female patients with well-controlled type 1 diabetes mellitus. The 0-min value is calculated as the mean from two baseline values (-30 and 0 min).

administration. Furthermore, a role of GH and cortisol in mediating the glucagon-induced suppression of ghrelin can be excluded since no remarkable increase in their concentrations was seen during the first 2 h following the administration of glucagon, during which time ghrelin levels reached their nadir. Our data are contradictory to observations made in a recent study demonstrating that i.m. glucagon administration does not modify ghrelin secretion (29). However, our study involved a greater number of subjects, who were older, had a relatively higher BMI and in contrast to the previously mentioned study proved to have an intact hypothalamo-pituitary function. Moreover, our data are supported by Hirsh and colleagues (40), who showed a similar decrease in ghrelin levels after i.v. or i.m. glucagon administration in children as well as 6 min after i.v. or i.m. glucagon in adults. In physiological states, an inhibitory effect of glucagon on ghrelin levels can be assumed, regarding the fact that hypoglycaemia seen in type 1 diabetics may be responsible for the hyperphagia described in those patients. Furthermore, increased circulating ghrelin levels in rats with streptozotocin-induced diabetes and hypoglycaemia is shown to play a role in the pathogenesis of diabetic hyperphagia (39). Moreover, obese individuals known to have a further reduction in ghrelin concentrations after gastric bypass surgery (41) seem to show an increase in glucagon levels (42). According to our findings, glucagon represents the second most powerful inhibitor of ghrelin known to date after somatostatin and might therefore offer a treatment option for diseases presenting with hyperghrelinemia. In conclusion, ghrelin does not mediate the GH and cortisol responses to glucagon, and the mechanisms underlying glucagon's suppressive effect on ghrelin, like its other neuroendocrine effects, must be elucidated further.

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Received 8 March 2005

Accepted 7 June 2005