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GHRELIN, A NATURAL GH SECRETAGOGUE PRODUCED BY THE STOMACH, INDUCES HYPERGLYCEMIA AND REDUCES INSULIN SECRETION IN HUMANS

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ABSTRACT Ghrelin, a 28 amino acid gastric hormone is a natural ligand of the GH Secretagogue (GHS) receptor (GHS-R) and strongly stimulates GH secretion though. like synthetic GHS, it shows other endocrine and non-endocrine activities. Aim of the present study was to clarify whether ghrelin administration influences insulin and glucose levels in humans. To this goal, we compared the effects of ghrelin, hexarelin, a synthetic GHS, or placebo on insulin and glucose as well as on GH levels in 11 normal young volunteers (age [mean±SEM]: 28.5±3.1 yr.; BMI: 22.2±0.9 Kg/m^2). Ghrelin induced very marked increase in GH secretion (ΔAUC^{0-180}: 5777.1±812.6 μg/l/h; $p<0.01$) which was not modified by placebo. Placebo administration did not modify insulin and glucose levels. On the other hand, ghrelin administration induced a prompt increase in glucose levels (ΔAUC^{0-180}: 1343.1±443.5 mg/dl/h; $p< 0.01$ vs. saline). Absolute glucose levels at +15' were already higher than those at baseline (93.9±7.1 mg/dl; $p< 0.01$) and persisted elevated up to 165' (90.3±5.8 mg/dl; $p<0.01$ vs. 0'). Ghrelin administration was also followed by a decrease in serum insulin levels (ΔAUC^{0-180}: -207.1±70.5 mU/l/h; $p<0.05$ vs. saline). Absolute insulin levels were significantly reduced from 30' (11.4±0.9 mU/l, $p< 0.01$ vs. 0'), showed the nadir at +45' (10.0±0.6 mU/l, $p< 0.01$ vs. 0') and then persisted lower ($p<0.01$) than baseline up to +105'. Hexarelin administration did not modify glucose and insulin levels despite its marked GH-releasing effect (ΔAUC^{0-180}: 4156.8±1180.3 μg/l/h; $p< 0.01$ vs. saline) that was slightly lower ($p<0.05$) than that of ghrelin. In conclusion, these findings show that, besides stimulating GH secretion, ghrelin is a gastric hormone possessing metabolic actions such as hyperglycemic effect and lowering effect on insulin secretion in humans, at least after acute administration.

Introduction

Ghrelin, is a new gastric hormone of 28 amino acids showing a unique structure with an n-octanoyl ester at its third serine residue (1). Ghrelin is a natural ligand of the GHS receptor through which it as well as synthetic GHS strongly stimulates GH secretion both in animals and in humans (1-13). The GH-releasing activity of ghrelin is mediated by activation on GHS-R at the pituitary and, mainly, at the hypothalamic level (1, 9, 14, 15) likely enhancing the activity of GHRH-secreting neurons and, concomitantly, acting as functional somatostatin antagonist (9-13). The GHS-R and its subtypes are not restricted to the hypothalamus-pituitary unit but are present also in other central and peripheral tissues (14) and the activity of ghrelin as well as of synthetic GHS is not fully specific for GH. In fact, ghrelin stimulates also lactotroph and corticotroph secretion, has orexigenic activity, exerts cardiovascular actions and antiproliferative effects on thyroid and breast tumors and has been shown able to regulate also gastric motility and acid secretion through vagal mediation (2, 12, 16-23). This latter evidence suggests that ghrelin like other gastro-entero-pancreatic hormones could affect also glucose metabolism and insulin secretion; in fact, some variations in glucose and insulin levels after administration of synthetic GHS have been already reported (24-26).

Aim of the present study was to clarify whether ghrelin administration influence insulin and glucose levels in humans. The effects of ghrelin were compared with those of hexarelin, a synthetic peptidyl GHS, or placebo.

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Subjects and Methods

Eleven healthy young male volunteers (age [mean±SEM]: 28.5±3.1 yr.; BMI: 22.2±0.9 Kg/m^2) were studied. All subjects gave their written informed consent to participate in the study, which had been approved by an independent Ethical Committee.

All subjects underwent the following 3 testing sessions in random order and at least 3 days apart: a) Ghrelin (1.0 μg/kg iv at 0'; human octanoylated-ghrelin, Europeptides, Argenteuil, France); b) Hexarelin (HEX, 1.0 μg/kg iv at 0'; Europeptides, Argenteuil, France); c) Placebo (saline 3 ml iv at 0').

After overnight fasting, the tests started in the morning at 08.30-09.00, 30' after an indwelling catheter had been placed into an antecubital vein, kept patent by slow infusion of isotonic saline.

Blood samples were taken every 15' from –15' up to +180'. Plasma glucose and serum insulin and GH levels were assayed at each time point in both sessions.

Plasma glucose levels (mg/dl) were measured by gluco-oxidase colorimetric method (GLUCOFIX, by Menarini Diagnostici, Florence, Italy).

Serum insulin levels (mU/l) were measured in duplicate by immunoradiometric assay provided by Sorin Biomedica, Saluggia, Italy. The sensitivity of insulin assay was 2.5±0.3 mU/l. The inter- and intraassay coefficients of variation were 6.2-10.8 % and 5.5-10.6 %, respectively.

Serum GH levels (μg/l) were measured in duplicate by immunoradiometric assay (hGH-CTK IRMA, SORIN,

Saluggia, Italy). The sensitivity of the assay was 0.15 μg/l. The inter-and intra-assay coefficients of variation were 2.9-4.5% and 2.4-4.0%, respectively.

All samples from an individual subject were analyzed together.

The responses are expressed as absolute or delta values or as delta areas under curves ($\Delta AUC^{0\text{-}180'}$) calculated by trapezoidal integration.

The statistical analysis was carried out using non parametric ANOVA (Friedman test) and then Wilcoxon test, as appropriate.

The results are expressed as mean ± SEM.

Results

Basal glucose, insulin or GH levels were similar in all testing sessions.

Placebo administration did not modify GH levels, which, as expected, were strongly stimulated by ghrelin (Δpeak: 1.9±1.2 vs. 90.5±12.0 μg/l; $\Delta AUC^{0\text{-}180}$: 16.8±27.6 vs. 5777.1±812.6 μg/l/h; $p<0.01$) and HEX (Δpeak: 67.4±15.8 μg/l; $\Delta AUC^{0\text{-}180}$: 4156.8±1180.3 μg/l/h; $p<0.01$) administration (Fig. 1, upper panel). The GH response to HEX was slightly lower than that elicited by ghrelin ($p<0.05$).

Placebo and HEX administration did not modify both insulin ($\Delta AUC^{0\text{-}180}$: 20.0±65.6 vs. –52.9±64.7 mU/l/h) and glucose ($\Delta AUC^{0\text{-}180}$: –35.6±164.1 vs. 91.1±287.7 mg/dl/h) levels (Fig 1, medium and lower panels).

Ghrelin administration induced an increase in plasma glucose levels ($\Delta AUC^{0\text{-}180}$: 1343.1±443.5 mg/dl/h; $p<0.01$ vs. saline). Absolute glucose levels at +15 min were already higher than those at baseline (93.9±7.1 mg/dl; $p<0.01$) and persisted elevated up to 165' (90.3±5.8 mg/dl; $p<0.01$ vs. 0') (Fig 1, medium and lower panels).

Ghrelin administration was also followed by a decrease in serum insulin levels ($\Delta AUC^{0\text{-}180}$: -207.1±70.5 mU/l/h; $p<0.05$ vs. saline). In fact, absolute insulin levels resulted significantly reduced from 30' (11.4±0.9 mU/l, $p<0.01$ vs. 0'), showed the nadir at +45' (10.0±0.6 mU/l, $p<0.01$ vs. 0') and then persisted lower ($p<0.01$) than baseline up to +105' (Fig 1, medium and lower panels).

Ghrelin, hexarelin or placebo administration were not followed by any relevant side effect; 6 subjects referred to be hungry approximately 2-3 h after ghrelin administration.

Discussion

The results of the present study demonstrate that, besides stimulating GH secretion, the acute administration of ghrelin is also able to induce prompt increase in plasma glucose levels, which is followed by reduction in insulin secretion in humans. On the other hand, the administration of hexarelin, a synthetic peptidyl GHS, is not followed by any change in glucose and insulin levels despite its potent GH-releasing effect.

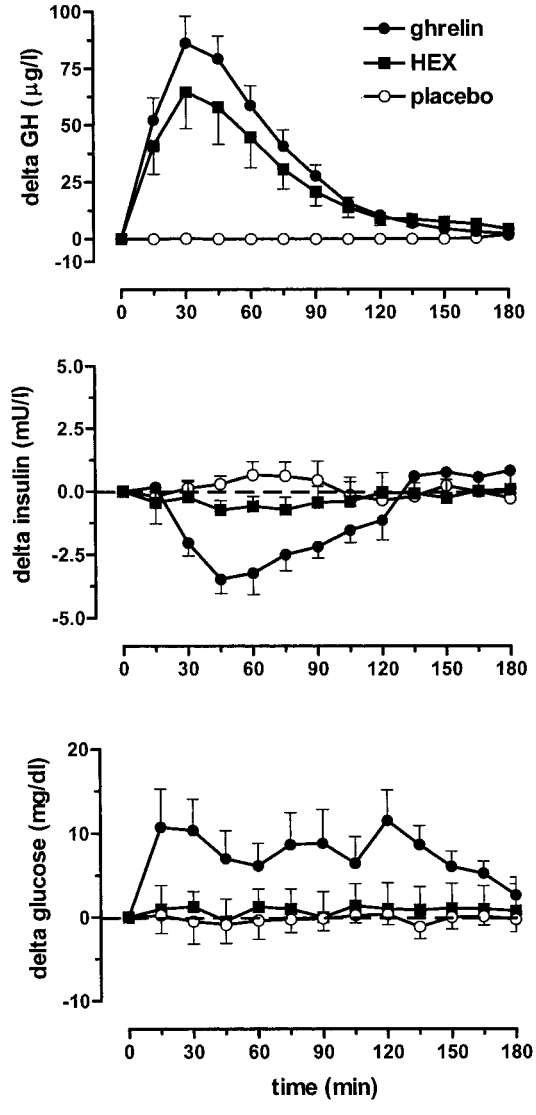

FIG 1. Mean (±SEM) ΔGH, Δ insulin and Δ glucose levels after ghrelin (1.0 μg/kg i.v. at 0') or placebo.

The strong, dose-dependent GH-releasing activity of ghrelin in humans has been already shown (2, 3, 6, 8) and is herein confirmed by the present study. In fact, ghrelin is a natural ligand of the GHS receptor through which it as well as synthetic GHS strongly stimulates GH secretion both in animals and in humans (1-13). The stimulatory effect of ghrelin and synthetic GHS on somatotroph secretion is mediated by the activation on GHS-R at the pituitary and, mainly, at the hypothalamic level (1, 9, 14, 15) likely via enhancement of the activity of GHRH-secreting neurons and, concomitantly, via functional somatostatin antagonism (9-13).

The presence of the GHS-R and its subtypes in the hypothalamus-pituitary unit but also in other central and peripheral tissues likely explains other activities of ghrelin and synthetic GHS (14). In fact, ghrelin as well as synthetic GHS stimulates also lactotroph and corticotroph secretion (2, 12), has orexigenic activity (17, 20), exerts cardiovascular actions (16, 21), possesses antiproliferative effects on thyroid and breast tumor cell lines (22, 23). More recently, it has been shown that, at least in the rat, ghrelin is able to increase also gastric motility and acid secretion (18, 19). Noteworthy, these effects are blocked by cholinergic antagonists suggesting that ghrelin effects take place through vagal mediation (19), which, in turn, is known to play a major role in the control of the endocrine pancreas (27).

Like many other gastro-entero-pancreatic hormones, it was likely that ghrelin, a new gastric hormone, could affect glucose metabolism and insulin secretion. Some influence of synthetic GHS on glucose and insulin levels in animals and humans had been already reported (24-26). Prolonged treatment with GHS had been followed by hyperglycemia in obese rats and this effect was supposed to reflect GHS-induced enhancement in the activity of HPA axis (24). Chronic treatment with MK-0677, a non-peptidyl GHS, in normal elderly, but not in obese subjects was coupled with hyperglycemia and hyperinsulinism in some subjects but this effect was supposed to reflect increased GH secretion (25, 28). However, in another study, it has been shown that during treatment with pegvisomant, a GH receptor antagonist, in fed conditions, GHRP-6 induces hyperglycemia though coupled with increased insulin levels (26) On the other hand, prolonged treatment with hexarelin, an hexapeptidyl GHS, in elderly subjects did not induce any significant increase in glucose levels though in presence of a significant trend toward increase in HbA_{1c} (29).

Our present findings firstly show that the acute administration of 1 μg/kg human acylated ghrelin elicits prompt increase in glucose levels which is then followed by slight but significant decrease in insulin secretion likely allowing further increase in glucose levels. Notice that increase in plasma glucose levels and decrease in insulin secretion persisted 2 h after ghrelin administration. This time course of glucose and insulin variations following ghrelin administration suggests that ghrelin has direct, non GH-mediated hyperglycemic effect. It could reflect glycogenolitic activity in the liver though GHS receptors have never been clearly shown in the liver (14). It is also unlikely that ghrelin stimulates glucagon secretion, which, in turn, should elicit increase in insulin secretion (27).

The mechanisms underlying the peculiar inhibitory effect of ghrelin administration on insulin secretion despite hyperglycemia are unknown. The presence of GHS receptors in the pancreas has been shown and thus a direct effect of ghrelin on insulin secretion from beta cells cannot be ruled out. Acetylcholine (Ach) mediates the stimulatory effect of ghrelin on gastric motility and acid secretion in animals (18, 19). As Ach plays a facilitating role on beta cell secretion (27), it is unlikely that Ach mediates the inhibitory influence of ghrelin on insulin secretion.

Evidence that, despite its strong GH-releasing activity, hexarelin does not share the same effects of ghrelin on glucose and insulin levels could be explained by the existence of GHS-R receptor subtypes that do not bind peptidyl GHS. Alternatively, as ghrelin is more potent than hexarelin even in stimulating GH (2 and present study), the lack of any metabolic effect of hexarelin would simply reflect its lower potency.

In all, though this study reports pharmacological rather than physiological effects of ghrelin, its influence on glucose metabolism and insulin secretion fits well with evidence that ghrelin plays a role in the control of food intake and is, in turn, modulated by fasting and re-feeding (30). Present data suggest that ghrelin would integrate the hormonal and metabolic response to fasting which, at least in humans, is connoted by clear-cut increase in GH secretion coupled with inhibition of insulin secretion and activation of mechanisms devoted to maintain glucose levels (27).

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