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Stimulatory Effects of Ghrelin on Circulating Somatostatin and Pancreatic Polypeptide Levels

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Ghrelin, the recently identified endogenous ligand of the GH secretagogue receptor, is a gut-brain peptide with endocrine, orexigenic, and gastrointestinal effects. In rodents it increases circulating gastrin and insulin levels, whereas in man it appears to decrease insulin secretion despite a rise in blood glucose levels. The aim of the present study was to evaluate the effects of ghrelin administration on total circulating somatostatin (SS), pancreatic polypeptide (PP), and gastrin levels compared with those elicited on insulin, glucose, and GH. Eight healthy volunteers of normal weight (four women and four men) were injected with 3.3 μg/kg ghrelin or saline after an overnight fast on 2 different days. Blood was taken every 15 min for 1 h and then every 30 min for 2 h. As expected, ghrelin injection elicited a prompt GH and glucose increase with a peak at 30 min and an insulin decrease with a nadir at 60 min. Gastrin concentrations were not modified, whereas significant rises were observed in both SS (in a biphasic pattern with peaks at 15 and 120 min) and PP (which increased promptly with a peak at 15 min). A significant negative correlation was found between SS (first peak) and insulin changes ($r = -0.86$; $P < 0.01$). In conclusion, this study clearly demonstrates that ghrelin stimulates SS and PP release in man. Although the underlying mechanisms and biological significance of these pharmacological effects remain to be elucidated, a causal relationship between the SS increase and the insulin changes may be hypothesized. Finally, these findings strongly support ghrelin's postulated role in linking the endocrine control of energy balance and growth with the regulation of gastrointestinal functions. (*J Clin Endocrinol Metab* 88: 701–704, 2003)

GHRELIN WAS IDENTIFIED and purified from the rat and human stomach in 1999 because of its ability to bind the GH secretagogue receptor (1). Ghrelin is a gut-brain hormone, as it is synthesized in the hypothalamus (1), endocrine pancreas islets, and gastrointestinal tract (2–4). Although probably able to exert paracrine effects, it is released in the bloodstream from the gastric-enteric source, and most of its actions are believed to be due to endocrine effects (1). In analogy with synthetic GH secretagogues, ghrelin possesses powerful and dose-dependent GH-releasing activity, although its physiological role in GH secretion is probably less important than first believed (4). In the circulation, ghrelin levels mostly change in relation to the nutritional intake (5), thus suggesting a primary role in the regulation of food intake and absorption (6). In fact, it has been shown that ghrelin has orexigenic effects in both the rat and man, increasing food intake and fat deposition (7, 8). Moreover, in the rat, it modulates gastric motility and acid secretion (9, 10), inhibits gastric emptying (9), and increases insulin and gastrin secretion (11). All of these findings suggest important links with gastro-entero-pancreatic (GEP) hormones. In man, surprisingly, a decrease in insulin levels and an increase in glucose concentrations have been reported after ghrelin administration (12), whereas no data are as yet available on possible effects on other GEP hormones. Therefore, the aim of this study was to evaluate the effects of ghrelin administration on circulating levels of gastrin, somatostatin (SS), and pancreatic polypeptide (PP) compared with those elicited on GH, insulin, and glucose concentrations in healthy human subjects.

Abbreviations: AUC, Area under the curve; GEP, gastro-entero-pancreatic; NPY, neuropeptide Y; PP, pancreatic polypeptide; SS, somatostatin.

Subjects and Methods

Subjects

Eight healthy subjects of normal weight recruited among medical staff (four men and four women; mean age, 30 ± 9 yr; mean body mass index, 22 ± 2 kg/m^2) volunteered for this study, which was approved by the local ethics committee. All subjects had normal physical examination and no history of gastrointestinal or endocrine disorders.

Procedures

After an overnight fast, an iv catheter was inserted in a forearm vein between 0800 and 0900 h and was kept patent by slow saline infusion. After 1 h of bed rest, all subjects were injected with either human ghrelin (Europeptides, Argenteuil, France) at a dose of 3.3 μg/kg as an iv bolus at time zero or saline; the two tests were performed in random order on different occasions at least 7 d apart. This pharmacological ghrelin dose was chosen because it has previously been shown to be effective in eliciting GH release in humans (13), and in our hands it induced ghrelin peaks ranging from 1967 to 4933 pmol/liter. Blood samples were taken at –30, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min. Plasma samples for SS, PP, and gastrin assays were collected in ice-chilled polypropylene tubes containing EDTA (1 mg/ml) and aprotinin (500 kallikrein inhibitory units/ml), immediately separated by centrifugation at 4 C, and stored at –80 C until assayed. Serum for GH, ghrelin, and insulin and plasma for glucose were separated at room temperature and stored at –20 C.

Methods

Plasma SS and PP levels were measured after extraction on Sep-Pak C_{18} cartridges by RIA kits (Peninsula Laboratories, Inc., Belmont, CA) (14). The antibody employed for SS measurement bound equally well SS-14 and SS-28 (15); thus, total SS-like immunoreactivity was measured.

Plasma gastrin concentrations were assayed using a RIA kit (DiaSorin, Inc., Stillwater, MN).

Serum GH levels were measured by IFMA (AutoDelfia kit, Wallac, Inc. OY, Turku, Finland), serum insulin by ELISA (Medgenix-Ins-EASIA, Biosource Technologies, Inc. Europe, Nivelles, Belgium), and plasma glucose levels by glucose autoanalyzer with hexokinase method (Beckman, Milan, Italy). Ghrelin was assayed by RIA (Phoenix Pharmaceuticals, Inc., Belmont, CA) as previously described (16). The intra- and interassay coefficients of variation were less than 10% for all methods.

Statistical analysis

Statistical analysis was carried out using Friedman's test, followed by the Wilcoxon matched pairs test with Bonferroni's correction for multiple comparisons. The area under the curve (AUC) was calculated by trapezoidal integration, and comparison between AUCs was made using paired *t* test. Correlations were calculated by linear regression analysis. The results were expressed as the mean ± SD unless otherwise stated (Fig. 1). $P < 0.05$ was considered statistically significant.

Results

Saline

Mean serum GH and insulin levels and plasma SS, PP, and gastrin levels did not change significantly after saline administration (Fig. 1).

Ghrelin

The results are shown in Fig. 1. As expected, ghrelin elicited a marked GH rise with a peak of 53 ± 23 μg/liter at 30 min ($P < 0.01$), a significant decrease in insulin concentrations from 41.6 ± 21.5 to a nadir of 24.4 ± 13.6 pmol/liter at 60 min ($P < 0.05$), and a significant increase in glucose concentrations from 4.7 ± 0.2 to 5.1 ± 0.3 mmol/liter at 30 min ($P < 0.05$). Also, SS concentrations rose significantly, with a biphasic pattern. Namely, a first smaller peak (from 14 ± 6 to 21 ± 6 ng/liter; $P < 0.05$) was observed at 15 min, followed by a second, higher peak of 29 ± 11 ng/liter ($P < 0.05$) at 150 min. The mean integrated concentrations of plasma SS were significantly higher after ghrelin than after saline (ghrelin AUC, 3876 ± 1069; saline AUC, 1542 ± 361 ng/liter·180 min; $P < 0.01$). PP levels rose in all patients by about 3-fold, from 15.5 ± 5.3 to 51 ± 20.3 pmol/liter ($P < 0.01$) at 15 min (ghrelin AUC, 4905 ± 1922; saline AUC, 1230 ± 677 pmol/liter·180 min; $P < 0.005$). On the contrary, gastrin levels were not modified by ghrelin administration (ghrelin AUC, 7649 ± 1379; saline AUC, 7735 ± 1783 ng/liter·180 min). A negative correlation was observed between the changes in SS, first peak, and insulin levels ($r = -0.86$; $P < 0.01$).

Discussion

The present study demonstrates for the first time that under fasting conditions in man, ghrelin stimulates SS and PP release, whereas it does not modify circulating gastrin levels. In addition, it confirms that ghrelin has an inhibitory effect on insulin release despite increasing blood glucose concentrations (12). In this respect it is intriguing that both insulin and SS have an inhibitory effect on ghrelin secretion (17), suggesting complex interactive loops whose physiological significance remains to be clarified. The ghrelin-induced SS rise shows a biphasic pattern of response, and it is noteworthy that the first increase in SS correlated negatively with the decrease in insulin. Thus, it may be hypothesized that the initial SS release accounts for the subsequent decrease in insulin concentrations. This interpretation is in keeping with the recent finding by Broglio *et al.* (18) that combined ghrelin and SS administration produces no additive effect on the insulin decrease compared with individual treatments. Moreover, it is well known that SS inhibits insulin release and

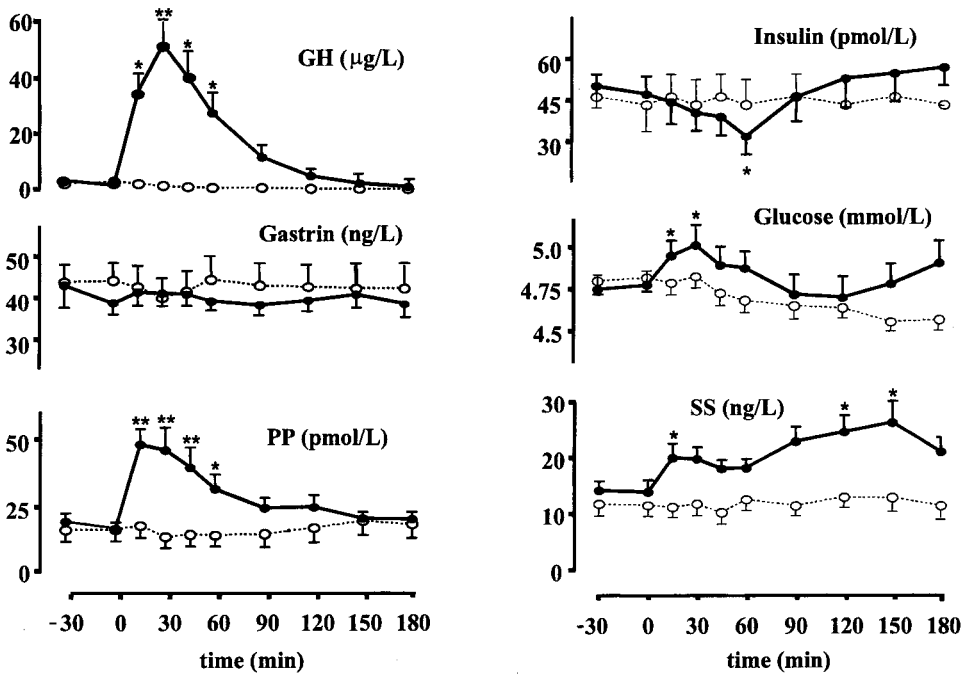

FIG. 1. Pattern of blood levels of GH, PP, gastrin, SS, insulin, and glucose after iv administration of ghrelin (3.3 μg/kg; *filled symbols*) or saline (*open symbols*), at time zero. Data are the mean ± SEM. *, $P < 0.05$; **, $P < 0.01$.

increases glucose concentrations (19). This suggests that even the glucose increase may be related to the SS rise. However, this hypothesis is not very consistent with the time course of glucose modifications that occurred simultaneously with those of SS, with a peak observed before any significant insulin decrease.

Therefore, it is conceivable that ghrelin itself has a direct, to date unknown, effect on glucose metabolism (by promoting glycogen breakdown or decreasing peripheral glucose uptake). Consistent with this view are the findings that ghrelin receptors are expressed in normal human liver (20, 21), and that in hepatoma cells, ghrelin has been shown to up-regulate gluconeogenesis (20), suggesting a role for ghrelin in regulating hepatic glucose metabolism. Alternatively, an indirect ghrelin effect on glucose could result from its influence on other hormone release. Thus, an increase in circulating epinephrine concentrations has been described to occur 10 min after ghrelin injection in man (22) and could cause the glucose increase. Also, the ghrelin-induced cortisol rise (4, 23) could play a role.

In the present study total SS immunoreactivity was measured by an antibody that recognizes both SS-14 and SS-28 with equal affinity (15). It is known that in man SS-28 is mainly released by the small intestine and is the predominant form of circulating SS both in the fasting state and after a meal (15, 24), but further studies are needed to clarify which form of SS is stimulated by ghrelin.

Ghrelin could elicit the first SS rise through different mechanisms. Both a direct effect on GEP D cells and indirect effects, mediated by vagal or β-adrenergic stimulation, may be hypothesized. In favor of the first hypothesis is the observation that in man, in contrast to rats, a relatively large number of D cells are present in oxyntic glands in close proximity with X/A-like, ghrelin-producing cells (25). In addition, as GH secretogogue receptors are expressed in the pancreas (26), ghrelin could directly stimulate D cells of the pancreatic islets in man. Consistent with a role of vagal tone, there are the observations that circulating SS levels are partly dependent upon vagal tone (27) and that ghrelin administration activates the neurons of the solitary tract and dorsomotor nucleus of the vagus (10), although there is evidence that some endocrine ghrelin actions are independent of cholinergic mediation (28). Finally, as ghrelin administration elicits an increase in circulating epinephrine levels, and β-adrenergic fibers stimulate SS release in perfused human pancreas (29), the activation of β-adrenergic receptors could also play a role.

The second SS peak could be the consequence of the important elevation of GH elicited by ghrelin administration. In fact, the observations that acromegalic patients tend to have higher fasting SS levels than healthy subjects (30) and that non-GH-deficient children treated with GHRH have increased plasma SS levels (31) support the hypothesis that GH itself might cause the increase in circulating SS levels, with a GEP source, besides increasing hypothalamic SS (32).

The powerful stimulating effect of ghrelin on PP release may be to some extent caused by vagal stimulation. In fact, vagal cholinergic mechanisms play the major role in the control of PP release (33). PP is a member of a brain-gut peptides family that also includes neuropeptide Y (NPY), with which it shares strong structural homology (34). It is well known that the stimulation of NPY is one of the mechanisms by which ghrelin stimulates appetite at the central level, at least in the rat (1, 6). Therefore, it is of interest that ghrelin is also a powerful stimulant of the pancreatic homolog of NPY. The genes encoding for these peptides have arisen from a common ancestral gene (34). It has been observed that ghrelin has direct activating effects on NPY genes in the arcuate nucleus (4). Thus, it might be worthwhile investigating whether ghrelin also has effects on pancreatic genes encoding PP. In rodents, PP participates in the control of food intake and gastric motility. When administered peripherally or when present in the circulation at high levels, such as in PP transgenic mice, PP decreases food intake and body weight (35, 36). PP and ghrelin could participate in a homeostatic loop that controls adaptive responses to starvation and the development of obesity.

In conclusion, the present study clearly demonstrates that ghrelin is able to stimulate some GEP hormone release in man. The underlying mechanisms and the biological significance of these effects remain to be elucidated. However, our findings strongly support and extend the role of ghrelin in linking the endocrine control of energy balance and growth with the regulation of gastrointestinal functions.

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