0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(10):4829–4832

doi: 10.1210/jc.2002-020956

GHRELIN SECRETION IS INHIBITED BY EITHER SOMATOSTATIN OR CORTISTATIN IN HUMANS

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ABSTRACT Ghrelin possesses endocrine and non-endocrine actions mediated by the GH Secretagogue (GHS)-Receptors (GHS-R). The regulation of ghrelin secretion is still largely unknown. Somatostatin (SRIF) modulates central and gastroenteropancreatic hormonal secretions and functions. SRIF actions are partially shared by cortistatin (CST), a natural SRIF analogue, that binds all SRIF receptors and also GHS-R. Herein, we studied the effects of SRIF-14 or CST-14 (2.0 μg/kg/h i.v. over 120 min) and of placebo on ghrelin, GH, insulin, glucagon and glucose levels in 6 normal young men. Placebo unaffected GH, insulin, glucagon, glucose and ghrelin levels. SRIF and CST similarly inhibited ($p<0.05$) spontaneous GH secretion of about 90%. After SRIF or CST withdrawal, GH levels recovered to baseline levels. Both SRIF and CST similarly inhibited ($p<0.01$) insulin secretion of about 45%. In both sessions, after SRIF or CST withdrawal, insulin overrode baseline levels. Both SRIF and CST similarly inhibited ($p<0.01$) glucagon levels of about 40%. After SRIF or CST withdrawal, glucagon persisted lower ($p<0.05$) than at baseline. Neither SRIF nor CST modified glucose levels. Both SRIF and CST similarly inhibited ($p<0.01$) circulating ghrelin levels of about 55%. Ghrelin levels progressively decreased from time +15 min, reaching the nadir at 120 and 105 min for SRIF and CST, respectively. Even 30 min after SRIF or CST withdrawal, ghrelin levels persisted lower ($p<0.05$) than those at baseline. In conclusion, this study first shows that SRIF and CST strongly inhibits ghrelin secretion that, differently from GH and insulin secretion, persists inhibited even after stopping the infusion of SRIF or CST.

Introduction

Acylated ghrelin displays strong GH-releasing activity mediated by the GH Secretagogue (GHS)-receptor (GHS-R) type 1a which had been shown specific for synthetic GHS (1-3). Notably, ghrelin as well as synthetic GHS has other remarkable activities including: a) stimulation of lactotroph and corticotroph secretion; b) orexant activity coupled with control of energy expenditure; c) control of gastric motility and acid secretion; d) influence on the exocrine and endocrine pancreatic function as well as on glucose metabolism; e) cardiovascular actions; f) anti-proliferative effects in neoplastic cell lines (2). Moreover, ghrelin, like leptin, is a hormone signaling the metabolic balance and managing the neuroendocrine and metabolic response to starvation (2, 4).

The regulation of ghrelin secretion is still largely unknown. Circulating ghrelin levels are increased by fasting and decreased by food intake and glucose administration but not by gastric distension (2, 5). Accordingly, circulating ghrelin levels are increased in anorexia and cachexia but reduced in obesity (2, 5).

Somatostatin (SRIF), both in its cyclic 14- (SRIF-14) and in the 28-amino acid (SRIF-28) form, is a peptide with a broad spectrum of biological activities in several tissues via a family of five receptors (6). Cortistatin (CST) is a natural SRIF analogue that binds with high affinity all five SRIF receptors (7). CST-14 and CST-29 are generated in rat and CST-17 and CST-29 in humans (7). CST-14 shares 11 of the 14 amino acid residues with SRIF-14 and inhibits to the same extent both GH and insulin secretion in humans and in animals (7-9).

Interestingly, CST does not share all SRIF activities and, surprisingly, CST, but not SRIF, also binds GHS-R (7, 10) suggesting a CST/ghrelin interaction.

In order to further investigate the regulation of ghrelin secretion, we studied in normal adult volunteers the effects on ghrelin secretion of either SRIF or CST given at a classical dose that has also already been shown able to inhibit the ghrelin-induced endocrine response in humans (9). The effects of SRIF and CST on GH, insulin and glucose levels were also studied in order to verify potential association between the effects on the secretion of ghrelin and of the other hormones that are classical targets of the inhibitory influence of SRIF (6).

Received 06/20/02. Accepted 08/27/02.

Subjects and Methods

Six healthy young male volunteers (age [mean±SEM]: 28.7±2.9 yr.; BMI: 23.4±0.8 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 at at least 3 days apart:

a) saline; b) somatostatin-14 (SRIF-14, Serono, Rome, Italy; 2.0 μg/kg i.v. over 120 min; c) cortistatin-14 (CST-14, Europeptides, Argenteuil, France; 2.0 μg/kg i.v. over 120 min).

After overnight fasting, the tests were begun in the morning at 0830-0900 h, 30 min after an indwelling catether had been placed into an antecubital vein of the forearm kept patent by slow infusion of isotonic saline.

Blood samples were taken every 15 min from 0 up to +150 min. Ghrelin, GH, insulin and glucose levels were assayed at each time point in all sessions.

Ghrelin levels (ng/l) were measured in duplicate, after extraction in reverse phase C18 columns, by radioimmunometric assay (Phoenix Pharmaceutical, Inc, Belmont, USA). Sensitivity of the assay: 30 pg/tube. Intra-assay coefficients of variation range: 0.3-10.7%.

Serum GH levels (μg/l) were measured in duplicate by immunoradiometric assay (hGH-CTK IRMA, SORIN Biomedica, Saluggia, Italy). Sensitivity of the assay: 0.15 μg/l. Inter-and intra-assay coefficients of variation: 2.9-4.5% and 2.4-4.0%.

Serum insulin levels (mU/l) were measured in duplicate by immunoradiometric assay (INSIK-5, SORIN Biomedica, Saluggia, Italy). Sensitivity of the assay: 2.5±0.3 mU/l. Inter- and intra-assay coefficients of variation: 6.2-10.8 % and 5.5-10.6 %.

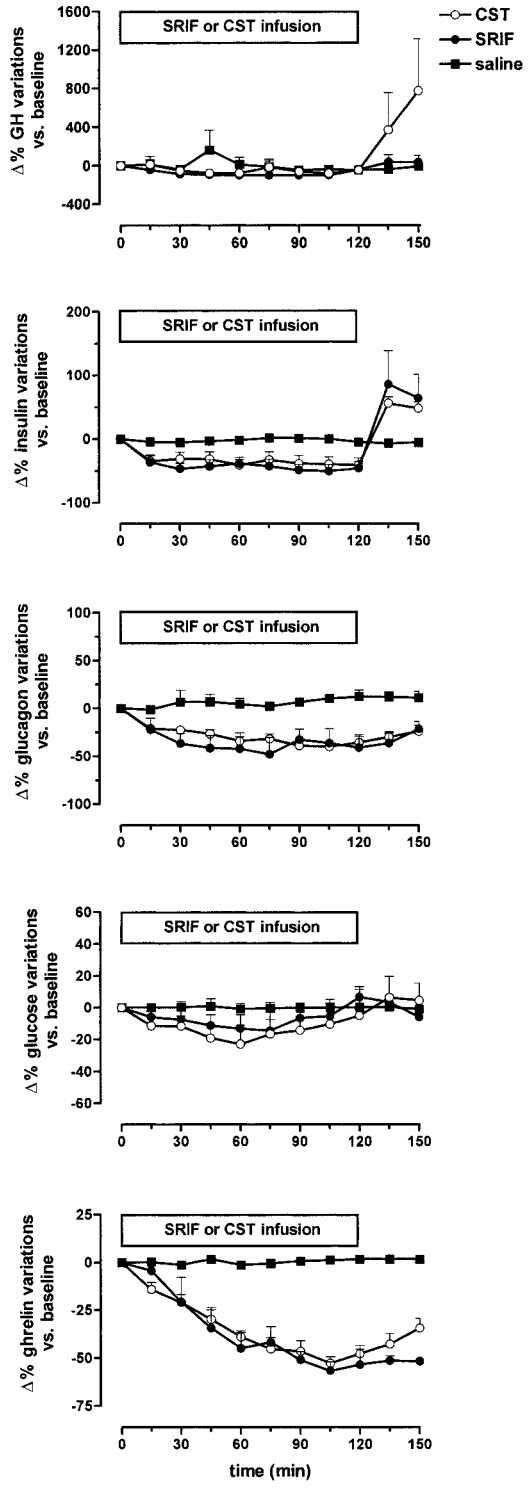

Fig. 1. Mean (±SEM) Δ% GH, insulin, glucagon, glucose and ghrelin variations vs. baseline during SRIF or CST (2.0 μg/kg/h) or saline infusion

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

All samples from an individual subject were analyzed together.

The hormonal responses are expressed as delta areas under curves (ΔAUC) calculated by trapezoidal integration or as percent variations versus baseline.

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

Placebo did not significantly modify GH, insulin, glucagon, glucose and ghrelin levels (Fig 1).

SRIF and CST inhibited ($p<0.05$) to the same extent spontaneous GH secretion (ΔAUC: -14.4±8.6 and -20.9±3.1 μg*min/l) of about 90%. After SRIF or CST withdrawal, GH levels showed recovery to baseline levels (Fig. 1).

Both SRIF and CST infusion induced similar significant ($p<0.01$) inhibition of insulin secretion (ΔAUC: -704.9±168.4 and -464.7±136.4 mU*min/l) of about 45%; insulin decrease was significant ($p<0.05$) from time +15 min up to the end of SRIF or CST infusion. In both sessions, after withdrawal of SRIF or CST infusion insulin levels overrode baseline levels (Fig. 1).

Both SRIF and CST significantly ($p<0.01$) inhibited to similar extent circulating glucagon levels (ΔAUC: -4829.5±1410.8 and -3415.1±525.6 μg*min/l) of about 40%. After SRIF or CST withdrawal, glucagon levels persisted significantly lower ($p<0.05$) than those at baseline (Fig. 1).

Neither SRIF nor CST infusion significantly modified glucose levels (ΔAUC: -1135.0±915.3 and -834.0±576.0 mg*min/dl) (Fig. 1).

Both SRIF and CST significantly ($p<0.01$) inhibited to the same extent circulating ghrelin levels (ΔAUC: -18993.0±6284.1 and -21411.4±4779.2 ng*min/l). Ghrelin levels underwent progressive decrease from time +15 min, reaching the nadir at +120 and +105 min for SRIF and CST, respectively (approximately 55% decrease). Even 30 min after SRIF or CST withdrawal, ghrelin levels persisted significantly lower ($p<0.05$) than those at baseline.

SRIF as well as CST administration elicited no side effect.

Discussion

The present study first shows that SRIF and CST remarkably inhibit (of approximately 55%) spontaneous ghrelin secretion in humans. The inhibitory effect of SRIF and CST on circulating ghrelin levels follows that on GH and insulin secretion. At the end of SRIF or CST infusion GH and insulin secretion almost immediately recover despite persistent inhibition of ghrelin levels.

It is well known that SRIF possesses a remarkable inhibitory effect on GH, insulin and glucagon release, as well as on many other gastroenteropancreatic (GEP) hormonal secretions and functions (6). CST, a natural SRIF analogue, binds with high affinity to all SRIF receptors and inhibits to the same extent both GH and insulin secretion in humans as well as in animals (7-9). CST does not share all SRIF activities and, surprisingly, CST but not SRIF can prevent specific binding of ghrelin to GHS-R (10) suggesting a CST/ghrelin interaction.

Ghrelin, a natural ligand of GHS-R, is mainly produced by the stomach but also by other peripheral and central tissues (1, 2). Similarly, GHS-R expression occurs in other central and peripheral tissues accounting for the other endocrine and non-endocrine ghrelin activities (2, 3, 11). Circulating ghrelin levels mostly reflect gastric secretion being reduced by 80% after gastrectomy (12). Within the stomach ghrelin is expressed by X/A-like cells whereas other endocrine cells including the SRIF positive D-cells are ghrelin negative (13). Ghrelin levels are increased by acute or chronic negative energy balance and reduced by acute or chronic positive energy balance (2, 5). Functional relationship between ghrelin and insulin is indicated by evidence that: a) there is negative association between their circulating levels in humans (14); b) ghrelin is expressed within human pancreatic beta cells (15); c) the acute administration of ghrelin induces hyperglycemia coupled with transient insulin decrease in humans (16) though insulin administration does not decrease ghrelin secretion, according to some (17) but not other Authors (18).
Though ghrelin drives the GH response to fasting in humans (19), ghrelin and GH secretion are unlikely linked by feedback mechanism since ghrelin secretion is unchanged in patients with severe GH deficiency either before or during rhGH replacement (20).

The present study shows that SRIF and CST inhibit ghrelin secretion in humans demonstrating that the activation of SRIF receptors remarkably suppresses the release of this new gastric hormone as well as that of other GEP hormones (6). At present, the mechanisms underlying the inhibitory effect of SRIF and CST on ghrelin secretion are unknown but likely reflect direct activation of SRIF receptors that are widely expressed in the gastric mucosa (6).

Ghrelin increases gastric contractility and acid secretion via a cholinergic-mediated mechanism being these action abolished by cholinergic blockade (21); on the other hand, SRIF exerts inhibitory effect on gastric contractility and acid secretion while SRIF secretion is enhanced by cholinergic blockade (6). Considering that an inhibitory effect of ghrelin on SRIF release in the rat has been reported (22), it seems likely that SRIF and ghrelin systems are linked by a functional relationship.

As anticipated, the inhibitory effect of SRIF and CST on circulating ghrelin levels followed that on GH and insulin secretion; moreover, SRIF or CST withdrawal was followed by GH and insulin recovery to baseline levels despite persistent inhibition of ghrelin secretion. These findings indicate that the inhibitory effect of SRIF and of CST on GH and insulin secretion as well as their rebound at the end of SS or CST infusion are not mediated by ghrelin.

The GH-releasing effect of ghrelin is not mediated by an inhibition of hypothalamic SRIF release. In fact, natural and synthetic GHS do not inhibit SRIF secretion that has been found even increased after hypothalamic exposure to these molecules (23). However, GHS probably act as functional antagonists of somatostatinergic action though via well distinct receptors (2). In fact, the GH-releasing effect of ghrelin and synthetic GHS is partially refractory to the inhibitory effect of SRIF and CST (2, 9, 24).

The present study cannot provide definitive information about the physiological relationship between ghrelin and SRIF also because it was performed by administering pharmacological doses to activate SRIF receptors. Nevertheless, our present findings suggest the existence of a physiologic feedback mechanism linking SRIF and ghrelin secretion. It should be also taken into account that, besides modulating GEP function, neuroendocrine actions of SRIF likely include centrally mediated influence on energy balance at the hypothalamic level (6, 25). Thus, some functional relationship between SRIF and ghrelin would be predicted even at the central level and could be devoted to modulate anterior pituitary function as well as eating behavior and/or modulation of energy balance.

At the peripheral level, a functional relationship between SRIF and ghrelin would be relevant in the control of the endocrine pancreas where ghrelin as well as SRIF is clearly expressed (6, 15). Obviously, the inhibitory effect of SRIF on insulin secretion is independent of ghrelin. However, given the negative association between ghrelin and insulin secretion (14), the persistent inhibition of ghrelin secretion after SRIF withdrawal would facilitate insulin rebound.

In conclusion, this study in humans first shows that SRIF and CST exert strong inhibitory effect on ghrelin secretion that, differently from GH and insulin secretion, persists inhibited even after stopping the infusion of SRIF or CST. Thus, the activation of SRIF receptors remarkably suppresses the release of this new gastric hormone as well as that of so many other GEP hormones. The inhibitory effect of SRIF and CST on ghrelin secretion also suggests that SRIF analogues would usefully affect hormonal secretion and growth of ghrelin-positive carcinoids of stomach and other parts of the GEP tract (26).

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Acknowledgements

The present study was supported by Ministero dell'Università e della Ricerca Scientifica (MURST), University of Turin,, Peptido Eureka Project (1923), SMEM Foundation and Europeptides.
The authors wish to thank Dr. S.W.J. Lamberts and F. Camanni for their collaboration to the study.
The skillful technical assistance of Mrs. M. Taliano and A. Bertagna is also acknowledged.

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