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Endocrine Activities of Cortistatin-14 and Its Interaction with GHRH and Ghrelin in Humans

FABIO BROGLIO, EMANUELA ARVAT, ANDREA BENSO, CRISTINA GOTTERO, FLAVIA PRODAM, SILVIA GROTTOLI, MAURO PAPOTTI, GIAMPIERO MUCCIOLI, AART JAN VAN DER LELY, ROMANO DEGHENGHI, AND EZIO GHIGO

Division of Endocrinology and Metabolism, Departments of Internal Medicine (F.B., E.A., A.B., C.G., F.P., S.G., E.G.), Biomedical Sciences and Oncology (M.P.), and Anatomy, Pharmacology, and Forensic Medicine (G.M.), University of Turin, 10126 Turin, Italy; Division of Endocrinology, Department of Internal Medicine (A.J.v.d.L.), Erasmus University of Rotterdam, 3015 The Netherlands; and Europeptides (R.D.), 95100 Argenteuil, France

Cortistatin (CST)-14, a neuropeptide with high homology with somatostatin (SS)-14, binds all sst subtypes but, unlike SS, also ghrelin's receptor. In six normal adults, we studied the effects of CST-14 or SS-14 administration (2.0 μg/kg/h iv) on: 1) GH and insulin secretion; 2) the GH response to GHRH (1.0 μg/kg iv); and 3) the GH, prolactin (PRL), ACTH, cortisol, insulin, and glucose responses to ghrelin (1.0 μg/kg iv). CST-14 inhibited GH and insulin secretion ($P < 0.01$) to the same extent of SS-14. The GH response to GHRH was similarly inhibited ($P < 0.01$) by either CST-14 or SS-14. Ghrelin released more GH than GHRH ($P < 0.01$); these responses were similarly inhibited ($P < 0.05$) by either CST-14 or SS-14, that made ghrelin-induced GH rise similar to that after GHRH alone. Neither CST-14 nor SS-14 modified PRL, ACTH, or cortisol responses to ghrelin. The inhibitory effect of CST-14 and SS-14 on insulin was unaffected by ghrelin that, in turn, reduced insulin secretion *per se* ($P < 0.01$). Ghrelin increased glucose levels ($P < 0.05$); CST-14 and SS-14 did not modify this effect. Thus, CST-14 inhibits both basal and stimulated GH secretion in humans to the same extent of SS-14. The GH-releasing activity of ghrelin seems partially resistant to CST-14 as well as SS-14. CST-14 and SS-14 do not affect PRL and ACTH secretion but, like ghrelin, inhibit insulin secretion; the ghrelin-induced inhibition is not additive with that of CST-14 or SS-14, suggesting a common mechanism of action on β cell secretion. (*J Clin Endocrinol Metab* 87: 3783–3790, 2002)

CORTISTATIN (CST) IS a recently described neuropeptide, the prepropeptide cDNA of which has been cloned from rat (1, 2) and more recently from mouse and human (1, 3, 4). Prepro-CST shows high structural homology with preprosomatostatin (-SS), particularly in the carboxyl terminus from which SS-14 and SS–28 are enzymatically processed (1, 3). Interestingly, rat prepro-CST may also be cleaved to pro-CST from which the two mature products CST-14 and CST-29 can be generated in rat (1, 2) and CST-17 and CST-29 in human (1, 3, 4). CST-14 shares 11 of the 14 amino acid residues with SS-14, although these peptides are encoded by distinct genes (1, 2).

SS exerts its biological effects via membrane-bound receptors of which five subtypes (sst 1 through sst 5) have been cloned (5, 6). SS receptors are expressed in the brain and periphery (5, 6) and mediate multiple SS activities including neurotransmission, neuromodulation, regulation of endocrine, and exocrine secretions and also the inhibition of tumor growth (5, 6).

CST-14, as well as CST-17 and -29, binds to all SS receptor subtypes with an affinity (1–2 nM) close to that of SS-14 and, therefore, was expected to have similar biological activities (1, 3, 7). The existence of specific receptors that selectively bind SS or CST has been hypothesized (1, 7), and indeed CST possesses central activities that are not shared by SS (1, 8, 9). For instance, CST, unlike SS, reduces locomotor activity and induces slow-wave sleep (1, 2), and CST and SS are often coexpressed in the same neurons but are regulated by different stimuli (1, 10, 11). Moreover, it has been recently demonstrated that in human tissues CST-14 as well as CST-17 but not SS-14 bind the GH secretagogue receptor (GHS-R) (Refs. 12 and 13; our unpublished results), an endogenous ligand, which includes ghrelin, a gastric peptide playing a major role in the control of somatotroph secretion (14, 15). Evidence that CST totally displaces the binding of iodinated ghrelin from its receptor would predict that CST but not SS has some interaction with ghrelin receptor and activity (12, 13).

Besides GHRH and SS receptors (16, 17), the GHS-R has been postulated to mediate a major stimulatory role in the control of GH secretion as well (18–20). GHS-R had been cloned following the evidence that synthetic peptidyl and nonpeptidyl molecules, named GHSs, show strong GH-releasing activity acting at the pituitary and mainly at the hypothalamic levels in which specific receptors are present (18–21). GHS-Rs are, however, present also in extrahypothalamic brain areas and peripheral animal and human tissues in which they mediate GH-independent activities including orexigenic and metabolic actions (18–25).

Ghrelin is a 28-amino acid peptide with an n-octanoyl ester in the serine 3 that is essential for its potent GH-releasing activity (14, 15). Ghrelin is mostly synthesized in the stomach, but it is also expressed in several other tissues including the hypothalamic arcuate nucleus (14, 15). Regarding GH secretion, ghrelin has no interaction with synthetic GHSs but shows synergic effect with GHRH and partial refractoriness to the inhibitory effect of exogenous SS (26–28). The activity

Abbreviations: CST, Cortistatin; GHS-R, GH secretagogue receptor; PRL, prolactin; SS, somatostatin.

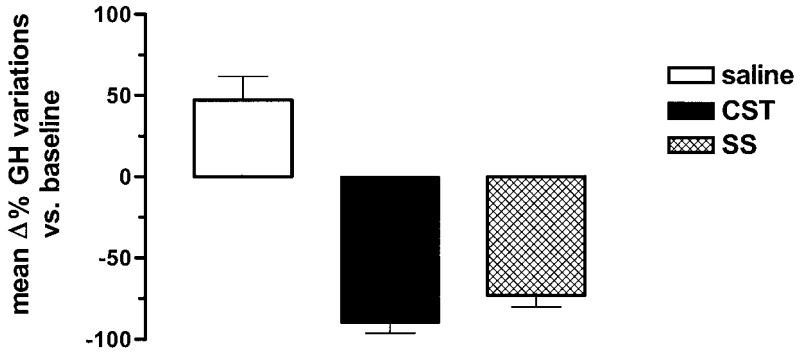

FIG. 1. Mean (±SEM) Δ% GH variations *vs.* baseline during infusion with CST-14 (2.0 μg/kg/h iv), SS-14 (2.0 μg/kg/h iv), or saline in normal subjects.

of ghrelin is not fully specific for GH, being able to stimulate also lactotroph and corticotroph secretion (26, 29) as well as to exert central and peripheral actions including influence on insulin secretion and glucose metabolism (30–32).

The endocrine activities of CST in humans have never been investigated so far, and to address this point, we studied the effects of CST-14 in comparison with those of SS-14 on basal GH and insulin secretion; the GH responses to GHRH; and the GH, prolactin (PRL), ACTH, cortisol, insulin, and glucose responses to ghrelin in normal young volunteers.

Given the ability of CST-14 but not SS-14 to bind the GHS-R, this study was also willing to verify whether CST-14 had some peculiar interaction with ghrelin or the activation of all SS receptor subtypes by CST-14 as well as by SS-14 leads to the same endocrine effects.

Subjects and Methods

Six healthy young male volunteers [age (mean ± SEM): 28.7 ± 2.9 yr; body mass index: 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 nine testing sessions in random order at least 3 d apart: 1) saline; 2) CST-14 [Pro-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys)-Lys-NH_2, Europeptides, Argenteuil, France; 2.0 μg/kg iv over 120 min from −30 to +90 min]; 3) SS-14 [Ala-Gly-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH, Serono, Geneva, Switzerland; 2.0 μg/kg iv over 120 min from −30 to +90 min]; 4) GHRH-29 (Serono, 1.0 μg/kg iv at 0 min); 5) Ghrelin-28 (Europeptides, 1.0 μg/kg iv at 0 min); 6) GHRH + CST; 7) GHRH + SS; 8) Ghrelin + CST; 9) Ghrelin + SS.

After an overnight fast, testing sessions began in the morning at 0830–0900 h, 30 min after an indwelling catheter had been placed into an antecubital vein of the forearm kept patent by slow infusion of isotonic saline. None of the subjects were under treatment with any drug. Smoking and alcohol consumption were not allowed in the 12 h preceding the testing sessions.

Blood samples were taken every 15 min from −30 up to +90 min. GH levels were assayed at each time point in all sessions. Insulin and glucose levels were assayed during CST and SS infusions both alone and in combination with ghrelin. PRL, ACTH, and cortisol levels were assayed after ghrelin administration both alone and during CST or SS infusion.

Serum GH levels (micrograms per liter; 1 μg/liter = 45.4 pmol/liter) were measured in duplicate by immunoradiometric assay (hGH-CTK IRMA, SORIN Biomedica, Saluggia, Italy). The sensitivity of the assay was 0.15 μg/liter. The inter- and intraassay coefficients of variation were 2.9–4.5% and 2.4–4.0%, respectively.

Serum PRL levels (micrograms per liter; 1 μg/liter = 43.5 pmol/liter) were measured in duplicate by immunoradiometric assay (PRL-CTK, IRMA, SORIN Biomedica). The sensitivity of the assay was 0.5 μg/liter. The inter- and intraassay coefficients of variation ranged from 3.9% to 6.8% and from 3.3% to 7.5%, respectively.

Plasma ACTH levels (picograms per milliliter; 1 pg/ml = 0.2202 pmol/liter) were measured by immunoradiometric assay (ACTH, Nichols Institute Diagnostic, San Juan Capistrano, CA). The sensitivity of the assay was 1 pg/ml. The ranges of inter- and intraassay coefficients of variation were 2.4–8.5% and 3.9–9.9%, respectively.

Serum cortisol levels (micrograms per liter; 1 μg/liter = 2.759 nmol/liter) were measured in duplicate by RIA (CORT-CTK 125, IRMA, SORIN Biomedica). The sensitivity of the assay was 4.0 μg/liter. The inter- and intraassay coefficients of variation ranged from 6.6–7.5% and from 3.8–6.6%, respectively.

Serum insulin levels (milliunits per liter; 1 mU/liter = 7.175 pmol/liter) were measured in duplicate by immunoradiometric assay (INSIK-5, SORIN Biomedica). The sensitivity of the assay was 2.5 ± 0.3 mU/liter. The inter- and intraassay coefficients of variation were 6.2–10.8% and 5.5–10.6%, respectively.

Plasma glucose levels (milligrams per deciliter; 1 mg/dl = 0.05551 mmol/liter) were measured by glucooxidase colorimetric method (GLUCOFIX, Menarini Diagnostici, Florence, Italy).

All samples from an individual subject were analyzed together.

The hormonal responses are expressed as absolute and delta areas under curves calculated by trapezoidal integration.

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

The results are expressed as mean ± SEM.

TABLE 1. Mean (±SEM) baseline and peak, AUC, and ΔAUC GH responses to GHRH (1.0 μg/kg iv) administration alone or during infusion with CST-14 (2.0 μg/kg/h iv) or SS-14 (2.0 μg/kg/h iv) in normal subjects

	Baseline (μg/liter)	Peak (μg/liter)	AUC (μg/liter/h)	ΔAUC (μg/liter/h)
GHRH	1.4 ± 0.7	25.0 ± 9.1	1511.1 ± 456.2	1383.9 ± 455.1
GHRH+CST	0.1 ± 0.1	4.1 ± 0.8	291.0 ± 37.1	289.2 ± 82.3
GHRH+SS	0.1 ± 0.1	3.6 ± 0.8	200.4 ± 35.9	195.2 ± 39.4

Results

CST inhibited basal GH secretion (delta area under the curve: −126.6 ± 96.3 *vs.* 6.0 ± 2.5 μg/liter/h, $P < 0.01$) to the same extent of SS (−151.5 ± 132.0 μg/liter/h, $P < 0.01$) (Fig. 1).

The GH response to GHRH was inhibited ($P < 0.01$) by either CST or SS to the same extent (Table 1 and Fig. 2). The percentage inhibitory effects of CST and SS on the mean GH responses to GHRH were similar (79% and 86%, respectively).

Ghrelin induced GH increase that was markedly higher ($P < 0.01$) than that elicited by GHRH. (Tables 1 and 2 and Fig. 2). CST and SS inhibited to the same extent ($P < 0.05$) the GH response to ghrelin, making it similar to that of GHRH alone but still higher ($P < 0.05$) than that after GHRH during CST or SS infusion (Tables 1 and 2 and Fig. 2).

The percentage inhibitory effects of CST and SS on the

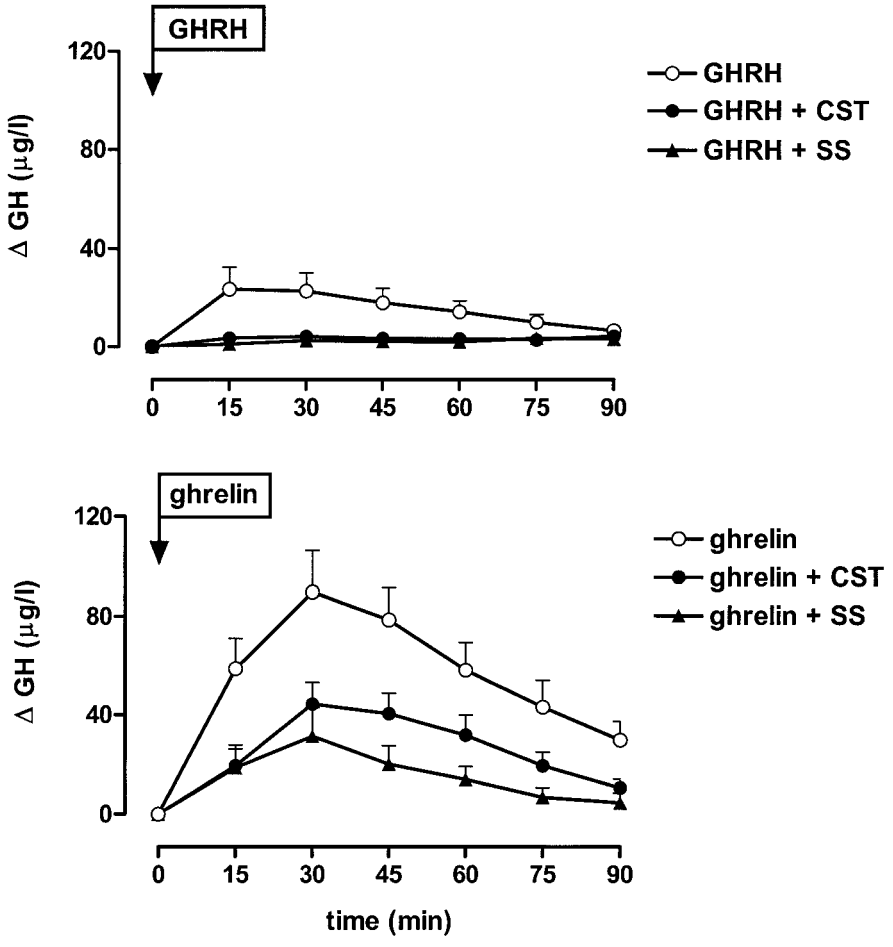

FIG. 2. Mean (±SEM) Δ GH responses to GHRH (1.0 μg/kg iv) or ghrelin (1.0 μg/kg iv) administration alone or during infusion with CST-14 (2.0 μg/kg/h iv) or SS-14 (2.0 μg/kg/h iv) in normal subjects.

mean GH response to ghrelin were similar (53% and 69%). The mean percentage inhibitory effects of CST and SS on the GH response to ghrelin were lower than those on the response to GHRH but the difference did not attain statistical significance.

The PRL, ACTH, and cortisol responses to ghrelin alone were not significantly modified by either CST or SS administration (Table 2 and Fig. 3).

CST as well as SS also inhibited ($P < 0.01$) basal insulin secretion (-407.7 ± 131.3 and -654.5 ± 152.8, respectively, *vs.* -50.4 ± 46.6 mU/liter/h) (Fig. 4). The inhibitory effect of CST and SS on insulin secretion was not modified by ghrelin coadministration that when given alone was able *per se* to significantly reduce insulin secretion ($P < 0.01$), although to an extent lower than that induced by SS ($P < 0.01$) and CST (*P*, NS) (Table 2 and Fig. 5).

CST and SS also induced a trend toward a transient reduction ($P < 0.05$ at 45 min after CST only) of basal glucose levels (-964.4 ± 723.1 and -417.0 ± 819.6, respectively, *vs.* -14.1 ± 270.4 mg/dl/h) (Fig. 4). On the other hand, ghrelin induced an increase in glucose levels ($P < 0.05$ from time +15 min) that was not modified by either CST or SS infusion (Table 2 and Fig. 5).

Side effects

A transient facial flushing was observed in two subjects after administration of GHRH. Two subjects sensed a peculiar sudden increase in appetite directly following ghrelin administration. CST and SS administration elicited no side effect.

Discussion

The results of the present study first show that CST-14 inhibits both basal and GHRH- or ghrelin-stimulated GH secretion in humans to the same extent of SS-14. The GH-releasing activity of ghrelin seems, however, partially resistant to CST as well as to SS that do not modify the stimulatory effect of ghrelin on both lactotroph and corticotroph secretion. On the other hand, CST-14, like SS-14, displays an inhibitory effect on insulin secretion. An inhibitory effect on insulin secretion is recorded also after acute administration of ghrelin that, however, does not modify that of CST and SS. CST and SS do not modify the ghrelin-induced increase in glucose levels.

CST-14 is a neuropeptide with a high structural homology with SS-14 though encoded by distinct genes (1–3). CST-14 and CST-17 and -29 bind to all SS receptor subtypes with an affinity (1–2 nM) quite close to that of SS (1, 3, 7). This evidence predicted that CST possesses the classical SS activities including regulation of both endocrine and exocrine secretions, neurotransmission, neuromodulation, and inhibition of tumor growth (5, 6).

Indeed, our present study shows that CST-14 displays the classical effects of SS-14 (5, 6) inhibiting to the same extent

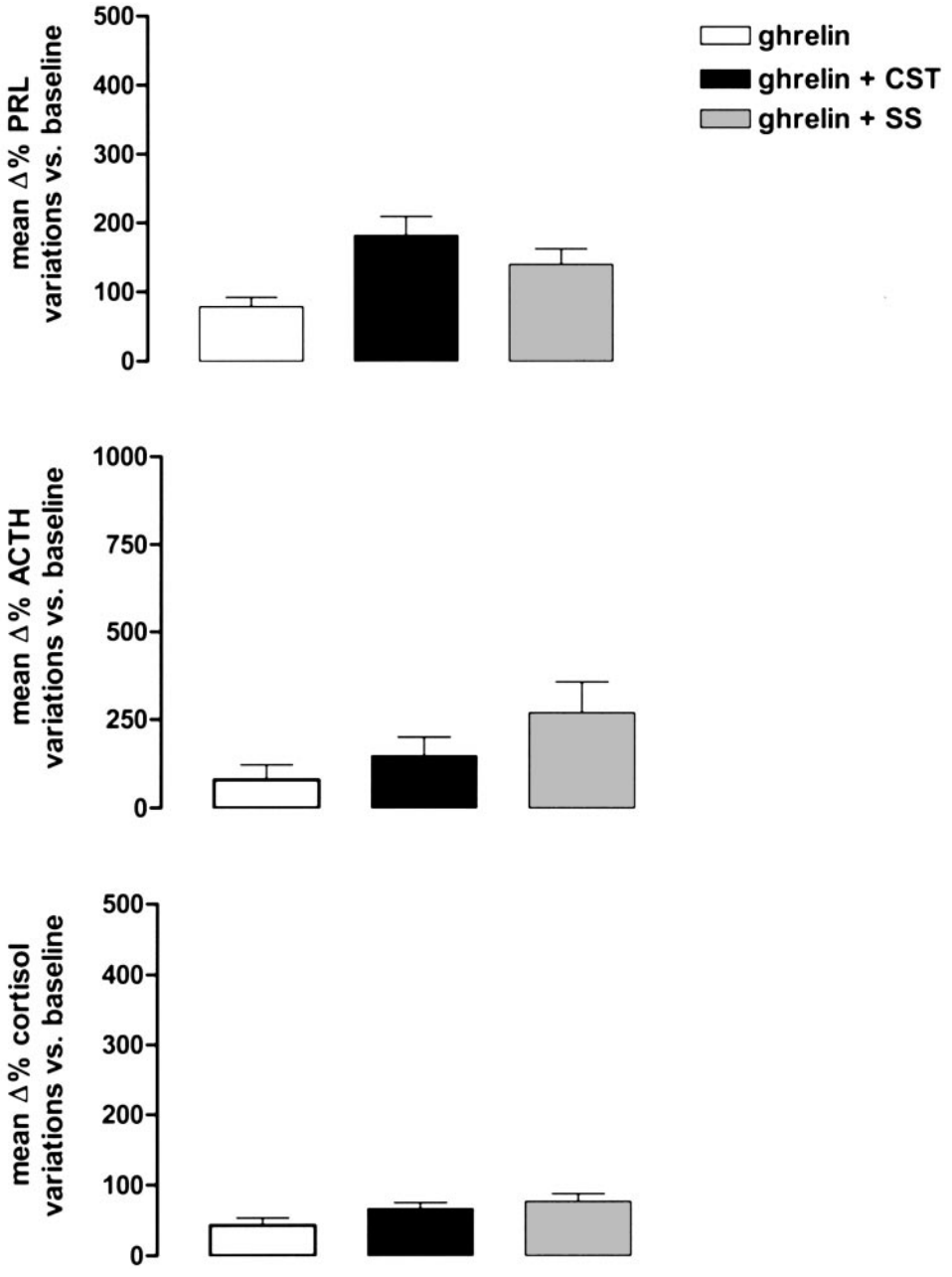

FIG. 3. Mean (±SEM) Δ% PRL, ACTH, and cortisol variations *vs.* baseline after ghrelin (1.0 μg/kg iv) administration alone or during infusion with CST-14 (2.0 μg/kg/h iv) or SS-14 (2.0 μg/kg/h iv) in normal subjects.

both basal and GHRH- or ghrelin-stimulated GH secretion as well as basal insulin secretion in humans. An inhibitory action of CST-14 on GH secretion similar to that of SS-14 has also been recently demonstrated by our group in dogs (33). From the physiological point of view, it was probably more appropriate to study the effects of the human CST-17 and -29 forms. However, the similar high affinity of CST-14, -17, and -29 to SS receptors predicts that these molecular forms share the same endocrine activity.

The observation that CST possesses central activities (such as reduction of locomotor activity and induction of slow-wave sleep) that are not shared by SS pointed to the existence of specific receptors for CST distinct from those of SS (1, 8–10). This hypothesis has been recently supported by the evidence that CST-14 and -17, but not SS-14, possess additional binding sites and bind to the GHS-R (Refs. 12 and 13; our unpublished results). Notice that CST-14 and -17 displace ghrelin and synthetic peptidyl GHSs from pituitary-binding sites with a 50% inhibitory concentration value about 50-fold higher than that required to inhibit the binding of radiolabeled SS-to-SS receptors (Refs. 1, 7, 12, and 13; our unpublished results). Thus, this evidence suggested the possibility that CST, at variance with SS, has peculiar interaction on ghrelin receptor and activity.

Like synthetic GHSs, ghrelin possesses potent stimulatory activity on somatotroph secretion (14, 18, 19, 26, 29, 34) but is able to also significantly stimulate PRL and ACTH secretion (26, 29). Ghrelin also possesses other central and peripheral actions including, for instance, stimulation of food intake and influence on insulin secretion

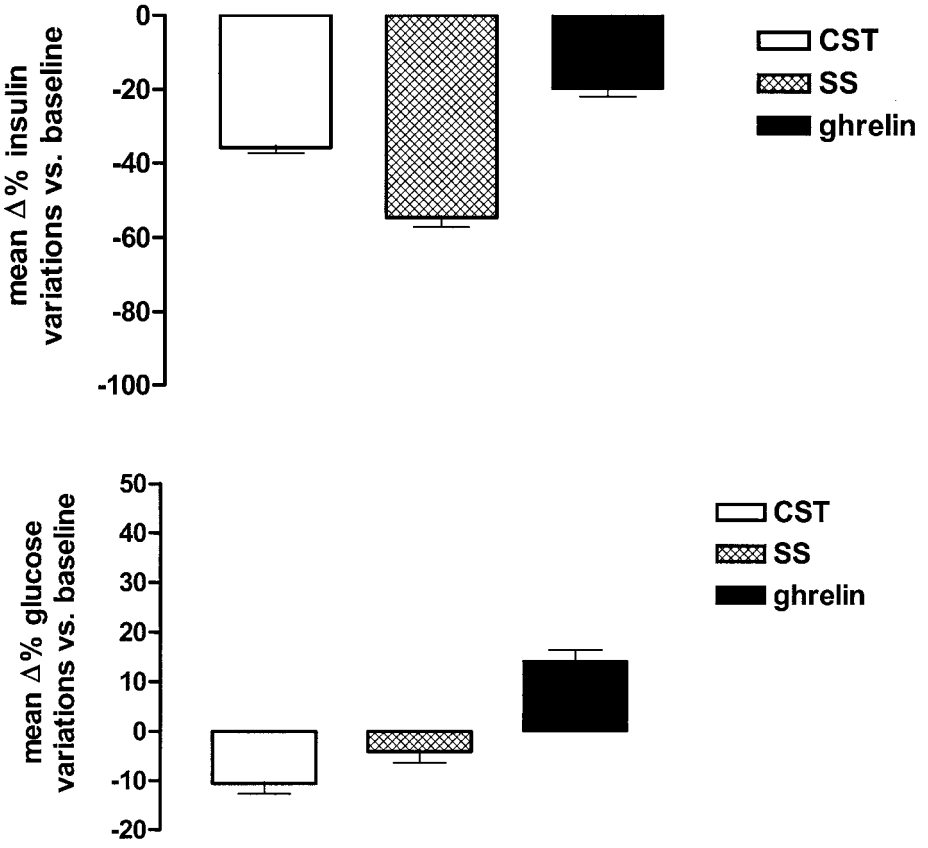

FIG. 4. Mean (±SEM) Δ% insulin and glucose variations *vs.* baseline during infusion with CST-14 (2.0 μg/kg/h iv) or SS-14 (2.0 μg/kg/h iv) or after acute ghrelin (1.0 μg/kg iv) administration in normal subjects.

and glucose metabolism (30–32, 35). These actions are mediated by the GHS-Rs that are mainly distributed within the hypothalamus-pituitary unit but also in other central and peripheral animal and human tissues (18–24). Regarding the GH-releasing activity, ghrelin has no interaction with synthetic GHSs, synergizes with GHRH, and seems partially refractory to the inhibitory effect of exogenous SS (26–28).

Our present findings show that either CST-14 or SS-14 almost abolishes the GH response to GHRH but only blunts the stimulatory effect of ghrelin on GH secretion. This suggests that ghrelin is partially refractory to the inhibitory effect elicited by the activation of SS receptors (1, 3, 7) in agreement with studies demonstrating that GHSs act, at least partially, as functional SS antagonists at both the pituitary and the hypothalamic level (18, 20, 36). In fact, ghrelin and synthetic GHSs neither bind SS receptors (12) nor decrease hypothalamic SS release (36, 37) but are able to counteract the hyperpolarizing effect of SS on the cell membrane (20, 36). The functional SS antagonism exerted by GHSs likely explains their synergic effect with GHRH (18, 20, 36).

Neither CST nor SS modified the lactotroph and corticotroph responsiveness to ghrelin, in agreement with previous studies showing that SS does not influence lactotroph and corticotroph responses to synthetic GHS (38). In agreement with *in vitro* studies, evidence that CST and SS do not affect the ghrelin-induced PRL increase is against the hypothesis that GHS action on lactotroph secretion takes place directly at the pituitary level (18, 39). On the other hand, the stimulatory effect of ghrelin on the hypothalamus-pituitary-adrenal axis is totally mediated by central actions that could include CRH- and vasopressin-mediated mechanisms but also other neurotransmitters such as NPY and γ-aminobutyric acid(18). Again, evidence that CST and SS do not affect the ghrelin-induced hypothalamus-pituitary-adrenal axis response agrees with a central nervous system-mediated action of ghrelin (18).

As anticipated, CST-14, like SS-14, infusion clearly inhibited insulin secretion which, though to a lower extent, was decreased also by the acute administration of ghrelin, in agreement with previous observation (30). Interestingly, ghrelin administration during CST or SS infusion did not modify its inhibitory action on insulin levels; these findings suggest that the negative influence of acute ghrelin administration on β-cell secretion would be mediated by enhanced somatostatinergic activity. Indeed, at least at the hypothalamic level, increased SS release has been reported after exposure to ghrelin in rats (37).

The present study confirms that the acute administration of ghrelin is followed by rise in glucose levels and shows that this administration is not abolished by CST or SS infusion. The hyperglycemic effect recorded after acute ghrelin administration is unlikely explained by a decrease in insulin levels that seems to occur later (30) and an increase in glucagon secretion that *per se* should increase insulin secretion (40). The hyperglycemic effect of ghrelin would reflect direct, non-GH-mediated action at the hepatic level (41).

The mechanisms underlying the influence of ghrelin on

TABLE 2. Mean (±SEM) basal and peak (or nadir, for insulin, only), AUC, and ΔAUC GH, PRL, ACTH, cortisol, insulin, and glucose responses to ghrelin (1.0 μg/kg iv) administration alone or during infusion with CST-14 (2.0 μg/kg/h iv) or SS-14 (2.0 μg/kg/h iv) in normal subjects

	Ghrelin				Ghrelin + CST
	Baseline	Peak (or nadir[a])	AUC	ΔAUC	Baseline
GH	1.3 ± 1.1	91.5 ± 16.7	5300.7 ± 922.9	5150.3 ± 917.9	0.1 ± 0.1
Prolactin	6.7 ± 0.7	14.4 ± 2.1	1021.8 ± 102.8	422.7 ± 142.8	5.7 ± 0.7
ACTH	25.1 ± 3.4	85.0 ± 20.9	3979.2 ± 897.2	1716.3 ± 812.3	18.5 ± 2.7
Cortisol	112.4 ± 18.0	175.9 ± 13.7	13097.4 ± 1456.2	2985.2 ± 893.1	81.8 ± 5.4
Insulin	11.9 ± 1.1	8.5 ± 0.6[a]	857.1 ± 58.5	−212.6 ± 43.3	7.7 ± 1.2
Glucose	93.6 ± 8.4	109.4 ± 9.3	9293.6 ± 705.7	1086.4 ± 426.0	72.8 ± 5.7

See text for units of measure.
[a] Nadir.

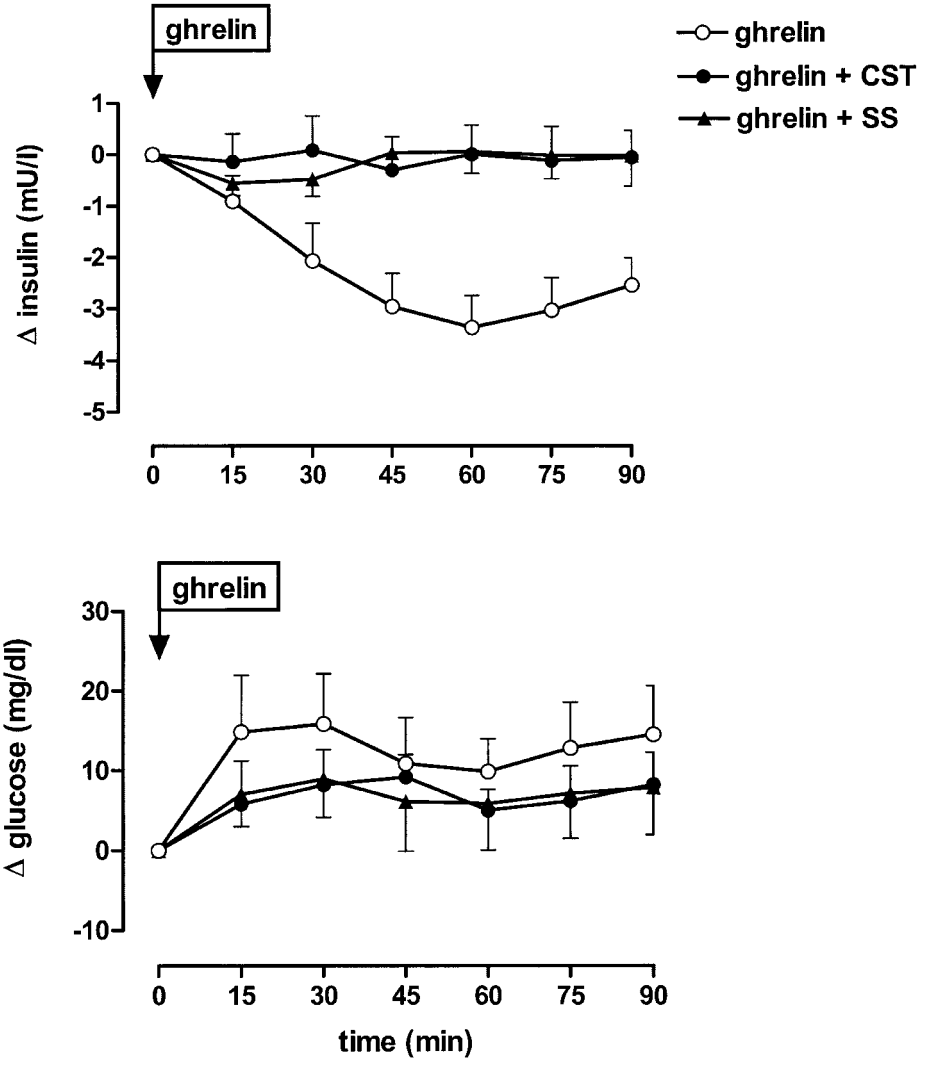

FIG. 5. Mean (±SEM) Δ insulin and glucose responses to ghrelin (1.0 μg/kg iv) administration alone or during infusion with CST-14 (2.0 μg/kg/h iv) or SS-14 (2.0 μg/kg/h iv) in normal subjects.

glucose metabolism and insulin secretion are, however, not surprising because it is expressed in the gut as well as in the pancreatic β cells (15, 31, 32, 42) and counteracts insulin action in hepatoma cell lines (41). Moreover, ghrelin modulates food intake and energy expenditure, and ghrelin concentration is increased by fasting and reduced by glucose load in rats (31, 32). Moreover, prolonged treatment with synthetic GHSs was often coupled with hyperglycemia in healthy elderly subjects (43). The understanding of the influence of ghrelin on the endocrine pancreas and glucose metabolism and its interplay with SS/CST requires further studies that are ongoing.

In conclusion, this study primarily shows that CST-14 is as effective as SS-14 in inhibiting both basal and GHRH- or ghrelin-stimulated GH release and insulin secretion in healthy normal individuals. This evidence indicates that these actions reflect classical activation of SS receptors that are bound by CST with an affinity similar to that of SS. The

TABLE 2. Continued.

Ghrelin + CST			Ghrelin + SS			
Peak (or nadir[a])	AUC	ΔAUC	Baseline	Peak (or nadir[a])	AUC	ΔAUC
44.5 ± 8.8	2407.7 ± 517.8	2400.3 ± 519.3	4.2 ± 2.7	35.6 ± 10.7	1777.8 ± 456.3	1403.7 ± 523.7
17.6 ± 4.2	1227.4 ± 257.7	745.1 ± 281.7	5.9 ± 0.9	17.9 ± 3.2	1211.4 ± 458.4	673.9 ± 193.8
99.4 ± 70.2	4821.2 ± 2914.5	3156.2 ± 2701.4	20.0 ± 4.8	103.2 ± 22.1	5014.4 ± 845.6	3208.0 ± 1075.9
152.8 ± 22.5	11960.6 ± 1657.4	4595.6 ± 1486.5	112.8 ± 27.1	192.8 ± 36.1	15176.7 ± 2259.2	5024.7 ± 1362.7
7.4 ± 0.9[a]	688.9 ± 100.9	−7.7 ± 84.7	4.9 ± 0.9	4.3 ± 0.9[a]	428.9 ± 81.9	−14.6 ± 56
82.0 ± 6.4	6946.5 ± 604.7	478.5 ± 187.5	71.0 ± 4.3	78.9 ± 4.9	6840.2 ± 416.3	450.2 ± 398.5

activation of SS receptors likely masks the ability of CST-14, but not of SS-14, to also bind the GHS-R with high affinity and thus to show some peculiar interaction with ghrelin receptor and activity. To clarify the impact, if any, of CST on ghrelin system, we are currently studying the effects of a CST analog that does not show any binding affinity to SS receptors but maintains its ability to bind the GHS-R.

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Address all correspondence and requests for reprints to: E. Ghigo, M.D., Divisione di Endocrinologia e Malattie del Metabolismo, Dipartimento di Medicina Interna, Ospedale Molinette, c.so Dogliotti 14, 10126 Torino, Italy. E-mail: ezio.ghigo@unito.it.

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References

1. **Spier AD, de Lecea L** 2000 Cortistatin: a member of the somatostatin neuropeptide family with distinct physiological functions. Brain Res Brain Res Rev 33:228–241
2. **De Lecea L, Criado JR, Prospero-Garcia O, Gautvik KM, Schweitzer P, Danielson PE, Dunlop CL, Siggins GR, Henriksen SJ, Sutcliffe JG** 1996 A cortical neuropeptide with neuronal depressant and sleep-modulating properties. Nature 381:242–245
3. **Fukusumi S, Kitada C, Takekawa S, Kizawa H, Sakamoto J, Miyamoto M, Hinuma S, Kitano K, Fujino M** 1997 Identification and characterization of a novel human cortistatin-like peptide. Biochem Biophys Res Commun 232: 157–163
4. **De Lecea L, Ruiz-Lozano P, Danielson PE, Peelle-Kirley J, Foye PE, Frankel WN, Sutcliffe JG** 1997 Cloning, mRNA expression, and chromosomal mapping of mouse and human preprocortistatin. Genomics 42:499–506
5. **Kreienkamp HJ** 1999 Molecular biology of the receptors for somatostatin and cortistatin. Results Probl Cell Differ 26:215–237
6. **Reichlin S** 1998 Neuroendocrinology. In: Wilson JD, ed. Williams textbook of endocrinology, ed 9. Philadelphia: WB Saunders; 165–248
7. **Siehler S, Seuwen K, Hoyer D** 1998 [125I]Tyr10-cortistatin14 labels all five somatostatin receptors. Naunyn Schmiedebergs Arch Pharmacol 357:483–489
8. **Vasilaki A, Lanneau C, Dournaud P, de Lecea L, Gardette R, Epelbaum J** 1999 Cortistatin affects glutamate sensitivity in mouse hypothalamic neurons through activation of sst2 somatostatin receptor subtype. Neuroscience 88: 359–364
9. **Sanchez-Alavez M, Gomez-Chavarin M, Navarro L, Jimenez-Anguiano A, Murillo-Rodriguez E, Prado-Alcala RA, Drucker-Colin R, Prospero-Garcia O** 2000 Cortistatin modulates memory processes in rats. Brain Res 858:78–83
10. **Calbet M, Guadano-Ferraz A, Spier AD, Maj M, Sutcliffe JG, Przewlocki R, de Lecea L** 1999 Cortistatin and somatostatin mRNAs are differentially regulated in response to kainate. Brain Res Mol Brain Res 72:55–64
11. **De Lecea L, del Rio JA, Criado JR, Alcantara S, Morales M, Danielson PE, Henriksen SJ, Soriano E, Sutcliffe JG** 1997 Cortistatin is expressed in a distinct subset of cortical interneurons. J Neurosci 17:5868–5880
12. **Muccioli G, Papotti M, Locatelli V, Ghigo E, Deghenghi R** 2001 Binding of 125I-labeled ghrelin to membranes from human hypothalamus and pituitary gland. J Endocrinol Invest 24:RC7–RC9.
13. **Deghenghi R, Papotti M, Ghigo E, Muccioli G** 2001 Cortistatin, but not somatostatin, binds to growth hormone secretagogue (GHS) receptors of human pituitary gland. J Endocrinol Invest 24:RC1–RC3.
14. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K** 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
15. **Kojima M, Hosoda H, Matsuo H, Kangawa K** 2001 Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 12:118–122
16. **Ghigo E, Arvat E, Gianotti L, Maccario M, Camanni F** 1999 The regulation of growth hormone secretion. In: Jenkins RC, Ross RJM, eds. The endocrine response to acute illness. Front Horm Res. Basel, Switzerland: Karger; 24:152–175
17. **Casanueva FF** 1992 Physiology of growth hormone secretion and action. In: Melmed S, ed. Endocrinology and metabolism clinics of North America. Philadelphia: WB Saunders; 483–517
18. **Ghigo E, Arvat E, Giordano R, Broglio F, Gianotti L, Maccario M, Bisi G, Graziani A, Papotti M, Muccioli G, Deghenghi R, Camanni F** 2001 Biologic activities of growth hormone secretagogues in humans. Endocrine 14:87–93
19. **Smith RG, Van der Ploeg LH, Howard AD, Feighner SD, Cheng K, Hickey GJ, Wyvratt Jr MJ, Fisher MH, Nargund RP, Patchett AA** 1997 Peptidomimetic regulation of growth hormone secretion. Endocr Rev 18:621–645
20. **Bluet-Pajot MT, Tolle V, Zizzari P, Robert C, Hammond C, Mitchell V, Beauvillain JC, Viollet C, Epelbaum J, Kordon C** 2001 Growth hormone secretagogues and hypothalamic networks. Endocrine 14:1–8
21. **Muccioli G, Ghe C, Ghigo MC, Papotti M, Arvat E, Boghen MF, Nilsson MH, Deghenghi R, Ong H, Ghigo E** 1998 Specific receptors for synthetic GH secretagogues in the human brain and pituitary gland. J Endocrinol 157:99–106
22. **Ong H, Bodart V, McNicoll N, Lamontagne D, Bouchard JF** 1998 Binding sites for growth hormone-releasing peptide. Growth Horm IGF Res 8(Suppl B): 137–140
23. **Muccioli G, Broglio F, Valetto MR, Ghe C, Catapano F, Graziani A, Papotti M, Bisi G, Deghenghi R, Ghigo E** 2000 Growth hormone-releasing peptides and the cardiovascular system. Ann Endocrinol 61:27–31
24. **Papotti M, Ghe C, Cassoni P, Catapano F, Deghenghi R, Ghigo E, Muccioli G** 2000 Growth hormone secretagogue binding sites in peripheral human tissues. J Clin Endocrinol Metab 85:3803–3807
25. **Cassoni P, Papotti M, Ghe C, Catapano F, Sapino A, Graziani A, Deghenghi R, Reissmann T, Ghigo E, Muccioli G** 2001 Identification, characterization, and biological activity of specific receptors for natural (ghrelin) and synthetic growth hormone secretagogues and analogs in human breast carcinomas and cell lines. J Clin Endocrinol Metab 86:1738–1745
26. **Arvat E, Maccario M, Di Vito L, Broglio F, Benso A, Gottero C, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Camanni F, Ghigo E** 2001 Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. J Clin Endocrinol Metab 86:1169–1174
27. **Hataya Y, Akamizu T, Takaya K, Kanamoto N, Ariyasu H, Saijo M, Moriyama K, Shimatsu A, Kojima M, Kangawa K, Nakao K** 2001 A low dose of ghrelin stimulates growth hormone (GH) release synergistically with GH-releasing hormone in humans. J Clin Endocrinol Metab 86:4552–4555
28. **Di Vito L, Broglio F, Benso A, Gottero A, Prodam F, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Ghigo E, Arvat E** The GH-releasing effect of ghrelin, a natural GH secretagogue (GHS), is only blunted by the infusion of exogenous somatostatin in humans. Clin Endocrinol 56:643–648
29. **Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, Mori K, Komatsu Y, Usui T, Shimatsu A, Ogawa Y, Hosoda K, Akamizu T,**

Kojima M, Kangawa K, Nakao K 2000 Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab 85:4908–4911
30. **Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R, Ghigo E** 2001 Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. J Clin Endocrinol Metab 86:5083–5086
31. **Inui A** 2001 Ghrelin: an orexigenic and somatotrophic signal from the stomach. Nat Rev Neurosci 2:551–560
32. **Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M** 2001 Minireview: ghrelin and the regulation of energy balance—a hypothalamic perspective. Endocrinology 142:4163–4169
33. **Deghenghi R, Avallone R, Torsello A, Muccioli G, Ghigo E, Locatelli V** 2001 Growth hormone-inhibiting activity of cortistatin in the rat. J Endocrinol Invest 24:RC31–RC33
34. **Arvat E, Di Vito L, Broglio F, Papotti M, Muccioli G, Dieguez C, Casanueva FF, Deghenghi R, Camanni F, Ghigo E** 2000 Preliminary evidence that ghrelin, the natural GH secretagogue (GHS)-receptor ligand, strongly stimulates GH secretion in humans. J Endocrinol Invest 23:493–495
35. **Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR** 2001 Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86:5992–5995
36. **Tannenbaum GS, Bowers CY** 2001 Interactions of growth hormone secretagogues and growth hormone-releasing hormone/somatostatin. Endocrine 14: 21–27
37. **Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I** 2000 Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology 141:4797–4800
38. **Massoud AF, Hindmarsh PC, Brook CG** 1997 Interaction of the growth hormone releasing peptide hexarelin with somatostatin. Clin Endocrinol 47: 537–547
39. **Shimon I, Yan X, Melmed S** 1998 Human fetal pituitary expresses functional growth hormone-releasing peptide receptors. J Clin Endocrinol Metab 83: 174–178
40. **Cryer P, Polonsky K** 1998 Glucose homeostasis and hypoglycemia. In: Wilson JD, ed. Williams textbook of endocrinology, ed 9. Philadelphia: WB Saunders; 939–972
41. **Murata M, Okimura Y, Iida K, Matsumoto M, Sowa H, Kaji H, Kojima M, Kangawa K, Chihara K** 2002 Ghrelin modulates the downstream of insulin signaling in hepatoma cells. J Biol Chem 277:5667–5674
42. **Volante M, Allìa E, Gugliotta P, Funaro A, Broglio F, Deghenghi R, Muccioli G, Ghigo E, Papotti M** 2002 Expression of ghrelin and of GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. J Clin Endocrinol Metab 87:1300–1308
43. **Chapman IM, Bach MA, Van Cauter E, Farmer M, Krupa D, Taylor AM, Schilling LM, Cole KY, Skiles EH, Pezzoli SS, Hartman ML, Veldhuis JD, Gormley GJ, Thorner MO** 1996 Stimulation of the growth hormone (GH)-insulin-like growth factor I axis by daily oral administration of a GH secretogogue (MK-677) in healthy elderly subjects. J Clin Endocrinol Metab 81: 4249–4257