The GH-releasing effect of acylated ghrelin in normal subjects is refractory to GH acute auto-feedback but is inhibited after short-term GH administration inducing IGF1 increase


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Abstract

Objective: GH secretion is regulated by an interplay between GH-releasing hormone (GHRH), somatostatin (SST), and other central and peripheral signals. Acylated ghrelin (AG) amplifies GH pulsatility acting, at least partially, independently from GHRH and SST. The GH response to GHRH is inhibited by recombinant human GH (rhGH), likely due to a SST-mediated negative GH auto-feedback. The effect of exogenous rhGH on the GH-releasing effect of AG has never been tested.

Design and methods: In six healthy volunteers, we studied the GH response to acute AG administration (1.0 mg/kg i.v.) during saline or rhGH infusion (4.0 mg/kg per h i.v.) or after 4-day rhGH (10.0 mg/kg s.c.) administration.

Results: Compared with saline, rhGH infusion increased GH levels (P<0.01). During saline, acute i.v. AG induced a marked increase (P<0.01) in GH levels similar to those observed after AG administration during rhGH infusion. During s.c. rhGH, IGF1 levels rose from day 0 to day 5 (P<0.01). After 4-day s.c. rhGH, i.v. AG increased (P<0.01) GH levels, though significantly (P<0.05) less than on day 0.

Conclusions: The marked somatotroph-releasing effect of AG is refractory to a direct GH auto-feedback whereas is markedly inhibited after 4-day rhGH administration, suggesting the possibility of a selective IGF1-mediated inhibitory feedback.

Introduction

The neural control of GH secretion mainly depends on the tight interplay between GH-releasing hormone (GHRH) and somatostatin (SST) (1, 2, 3, 4, 5, 6). In addition, several neurotransmitters, neuropeptides, metabolic fuels, and also peripheral hormones, in particular insulin-like growth factor 1 (IGF1), have an important influence on somatotropic function mainly acting via the modulation of GHRH and SST release (7, 8).

In particular, the negative feedback exerted by IGF1 on GH secretion involves direct effects at the pituitary level through activation of IGF1 receptors, leading to the inhibition of GH synthesis and release (9). Indirect CNS-mediated mechanisms have also been demonstrated. In fact, in animals, IGF1 has been shown to be able to stimulate SST and inhibit GHRH release acting at the hypothalamic level (9).

GH itself, directly and indirectly, modulates its own secretion, and this negative GH auto-feedback control involves many of the above-mentioned factors (1, 2, 3, 4, 5, 6, 7, 8). GH increases IGF1 and free fatty acid levels, which in turn inhibits GH secretion acting both at the pituitary and hypothalamic level (10, 11). However, the influence of IGF1 and free fatty acids does not account for the inhibitory effect shown by the acute administration of GH on the GH response to GHRH both in animals and in man that is instead mediated by SST (12). SST antiserum in rat and substances inhibiting hypothalamic SST release in man are able to completely restore the GH response to GHRH when inhibited by a previous recombinant human GH (rhGH) bolus (12, 13, 14, 15, 16, 17).

In recent years, the complexity of the regulation of the GH/IGF1 axis has been further enriched by the discovery of ghrelin, a peptidic hormone mainly deriving from gastric production that, in its acylated form, displays a potent GH-releasing activity via the activation of a specific receptor, the GH secretagogues (GHS) receptor type 1a (GHSR1a) (18, 19, 20, 21).
Although many years have passed since its isolation, the actual role of acylated ghrelin (AG) in the physiological control of GH secretion is still a matter of debate and is likely to act as an amplifier of GH pulsatility rather than a true GH pulse generator as an alternative GHRH (22, 23). Although the physiological role of AG in the control of the somatotropic axis is probably less relevant than originally expected, anyway it remains that the exogenous administration of AG as well as its synthetic analogues such as hexarelin or GHRP6 represents one of the most potent pharmacological stimuli of GH secretion in humans in vivo (20, 24, 25).

Notably, despite this evidence, no information is available in the literature on the influence of GH and IGF1 levels themselves on the somatotroph responsiveness to AG in physiological conditions.

Based on the foregoing, the aim of this study was to verify whether the GH-releasing effect of AG is influenced by the acute increase in GH levels induced by short-term infusion of rhGH and/or by the increase in IGF1 levels induced by 4-day s.c. rhGH administration in adult healthy volunteers. In order to test the specificity of GH or IGF1 feedback on the GH-releasing effect of AG, the effects on the prolactin (PRL) and cortisol responses to the gastric hormone were also evaluated.

Materials and methods

Six healthy young male volunteers (age (mean ± S.E.M.), 27.8 ± 3.1 years; BMI, 23.1 ± 2.3 kg/m²) were studied. All the subjects gave their written informed consent to participate in the study, which had been approved by the Ethics Committee of the University of Turin.

All the subjects underwent the following three testing sessions in random order at least 30 days apart: i) acute AG administration (1.0 μg/kg i.v. as a bolus at 0′) during saline infusion from −180′ to +60′; ii) acute AG administration (1.0 μg/kg i.v. as a bolus at 0′) during rhGH (4.0 μg/kg per h) i.v. infusion from −180′ to +60′; and iii) acute AG administration (1.0 μg/kg i.v. as a bolus at 0′) during saline infusion from −180′ to +60′ after 4-day rhGH (10.0 μg/kg) s.c. administration at 21:00 h.

After overnight fasting, the tests began in the morning at 0830–0900 h, 30′ after an indwelling catheter 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 time −180′ up to +60′. GH, PRL, and cortisol levels were assayed at each time point in all the sessions. IGF1 levels were assayed at −180′ and at +60′ in each session. IGF1, IGFBP3, GH, free fatty acids, insulin, and glucose were assayed at time 0′ in each session and at 0800 h on the following day after every evening rhGH s.c. administration.

GH levels (μg/l) were measured in duplicate by immunoradiometric assay (IRMA; hGH–CT, RADIM, Pomezia, Italy); sensitivity, 0.04 μg/l; inter- and intra-assay coefficient of variation (CV) range, 4.6 and 3.7% respectively. IGF1 levels (ng/ml) were measured in duplicate by IRMA (IGF1 SM-C-RIA-CT, PANTEC, Torino, Italy); sensitivity, 0.25 ng/ml; inter- and intra-assay CV range, 10.8 and 9.5% respectively. IGFBP3 levels (μg/ml) were measured in duplicate by IRMA (IGFBP3 IRMA, IMMUNOTECH SA, Marseille, France); sensitivity, 0.050 μg/ml; inter- and intra-assay CV range, 9.5 and 6.0% respectively.

Cortisol levels (μg/dl) were measured in duplicate by IRMA (RADIM); sensitivity, 0.09 μg/dl; inter- and intra-assay CV range, 7.2 and 8.0% respectively. PRL levels (ng/ml) were measured in duplicate by IRMA (PANTEC); sensitivity, 0.5 ng/ml; inter- and intra-assay CV range, 8.0 and 2.8% respectively. Plasma glucose levels (mg/dl: 1 mg/dl = 0.055551 mmol/l) were measured by the glucose oxidase colorimetric method (GLUCOFIX; Menarini Diagnostics, Florence, Italy).

Serum insulin levels (μU/ml) were measured in duplicate by IRMA (INSIK-5; SORIN Biomedica, Saluggia, Italy). The sensitivity of the insulin assay was 2.5 ± 0.3 μU/ml. The inter- and intra-assay CV values were 6.2–10.8 and 5.5–10.6% respectively.

Serum free fatty acid (FFA) levels (mEq/l) were measured by enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). The inter- and intra-assay CV values were 6.2–10.8 and 5.5–10.6% respectively. IGF1 levels (ng/ml) were measured in duplicate by IRMA (IGF1 SM-C-RIA-CT, PANTEC, Torino, Italy). The sensitivity of the insulin assay was 0.04 μg/l; inter- and intra-assay CV range, 4.6 and 3.7% respectively. IGF1 levels (ng/ml) were measured in duplicate by IRMA (IGF1 SM-C-RIA-CT, PANTEC, Torino, Italy); sensitivity, 0.25 ng/ml; inter- and intra-assay CV range, 10.8 and 9.5% respectively. IGFBP3 levels (μg/ml) were measured in duplicate by IRMA (IGFBP3 IRMA, IMMUNOTECH SA, Marseille, France); sensitivity, 0.050 μg/ml; inter- and intra-assay CV range, 9.5 and 6.0% respectively.

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Results

Mean (± S.E.M.) GH levels during saline infusion were 0.6 ± 0.1 μg/l corresponding to an AUC<sub>saline</sub> of 111.0 ± 56.0 μg/l×min. During rhGH infusion starting at −180′, GH levels progressively rose up to a steady-state level at time −135′; in particular, mean GH levels<sub>−135′−60′</sub> were 29.8 ± 2.6 μg/l corresponding to an AUC<sub>−135′−60′</sub> of 3336 ± 171.0 μg/l×min, which as expected, was significantly higher (P < 0.01) than that observed during saline at the corresponding time points (AUC<sub>saline</sub>−135′−60′: 93.0 ± 60.0 μg/l×min; Fig. 1).

During saline infusion, the acute i.v. AG administration induced a marked increase (P < 0.01) in GH levels (Δpeak<sub>saline+AG</sub>−135′−60′: 53.0 ± 5.8 μg/l; AUC<sub>Δpeak−135′−60′</sub>saline+AG−2054.0 ± 161.0 μg/l×min) that were not different from that observed after acute i.v. AG administration
during rhGH infusion (Δpeak⁰–⁶⁰ -rhGH+AG; 55.6± 10.0 µg/l; ΔAUC⁰–⁶⁰ -rhGH+AG; 1962.0±319.0; Fig. 2). IGF1, IGFBP3, glucose, and insulin levels both at time 0' and at time 60' were similar in both sessions (data not shown).

During treatment with 10.0 µg/kg per day rhGH, IGF1 levels progressively rose from day 0 levels (212.2±15.6 µg/l) reaching statistical significance on day 3 (287.5±26.6 µg/l; P<0.01 vs baseline) and then showed a further increase on day 4 (306.2±36.9 µg/l; P<0.01 vs baseline) (Fig. 3). As expected, a concomitant increase (P<0.05) in GH levels was also observed (day 0; 0.2±0.1 µg/l; day 3; 1.2±0.7 µg/l; day 4; 1.4±0.9 µg/l; Fig. 3). On the contrary, no significant variations in IGFBP3, insulin, free fatty acids, and glucose levels were observed after 4-day rhGH s.c. treatment (data not shown).

After 4-day s.c. rhGH treatment, the i.v. administration of AG induced a significant increase (P<0.01) in GH levels (Δpeak⁰–⁶⁰ -AG; 19.0±4.1 µg/l; ΔAUC⁰–⁶⁰ -AG; 753.0±174.0 µg/l/min) that was, however, significantly (P<0.05) lower than that observed on day 0 (Fig. 2).

As expected, the administration of AG on day 0 also induced significant increases in PRL and cortisol levels (P<0.05) that were similar to those observed after AG administration (1.0 µg/kg i.v.) during saline or rhGH infusion (4.0 µg/kg/h i.v.) or after 4-day rhGH (10.0 µg/kg s.c.) administration (Table 1). In all the sessions, baseline PRL and cortisol levels were similar (data not shown). Ghrelin administration induced a transient facial flushing in two subjects. No side effects during rhGH i.v. or s.c. administration were observed.

Discussion

The results of this study show that the somatotroph-releasing effect of AG is refractory to the direct inhibitory effect of a short-term elevation of GH levels without increase in IGF1 levels, while it is markedly inhibited in the presence of increased IGF1 levels induced by 4-day rhGH administration.

AG has been isolated as an endogenous ligand of the GHSR1a and emerged as one of the most potent and reproducible gender-independent stimuli of GH secretion in vivo during the lifespan (18, 19, 20, 21).

The GH-releasing effect of AG is mediated by actions on the pituitary and, mainly, within the hypothalamus, in particular, through a positive action on GHRH-secreting neurons and a concomitant functional antagonism of SST activity (26).

Different from GHRH, the GH-releasing activity of AG has already been reported to be refractory to the most important inhibitory inputs on GH secretion such as glucose, free fatty acids and β-adrenergic stimulation, all acting to increase the hypothalamic SST release (26), and even to be resistant to the inhibitory effect of SST administration itself (26, 27).

Thus, our present observation that a short-term increase in circulating GH levels induced by rhGH i.v. infusion fails to inhibit the GH response to AG is not surprising. In fact, it is well accepted both in animals and in humans that the negative GH auto-feedback mechanism is mainly mediated by the release of hypothalamic SST (28, 29).

On the other hand, interestingly, our data clearly show that the GH response to AG is markedly inhibited after 4-day s.c. rhGH administration, able to induce a significant increase in IGF1 levels, though still in the normal range, without sensible variations in circulating GH levels. Although a definitive proof cannot be drawn by our experimental model, the negative correlation we observed between the changes in the GH responses to AG and in IGF1 levels before and after s.c. rhGH treatment (data not shown) might suggest a causative relationship between the two phenomena, i.e. the

**Figure 1** Mean ± SEM GH responses to acute AG administration (1.0 µg/kg i.v.) during saline or rhGH infusion (4.0 µg/kg/h i.v.) or after 4-day rhGH (10.0 µg/kg s.c.) administration. AG, acylated ghrelin; rhGH, recombinant human GH.

**Figure 2** Mean ± SEM ΔAUC 0'-60' GH responses to acute AG administration (1.0 µg/kg i.v.) during saline or rhGH infusion (4.0 µg/kg/h i.v.) or after 4-day rhGH (10.0 µg/kg s.c.) administration. AG, acylated ghrelin; rhGH, recombinant human GH.

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Saline both the pituitary and the hypothalamic level, it is mediated by functional antagonism of SST activity at and/or stimulation of SST release (29, 31).

D0, day 0; D1, day 1; D2, day 2; D3, day 3; D4, day 4.

recombinant human GH; NS, not significant.
PRL, prolactin; AG, provided that IGF1 is administered at a dose not able to modify spontaneous GH secretion. Actually, in our study, the dose of 10 μg/kg rhGH was chosen as the one previously reported to be the minimal effective to increase IGF1 levels in both males and females (30). There is a wealth of in vitro and in vivo animal data showing that IGF1 inhibits GH release acting either at the pituitary or the hypothalamic level (31, 32) via inhibition of GHRH and/or stimulation of SST release (29, 31).

As the GH-releasing activity of AG is supposed to be mediated by functional antagonism of SST activity at both the pituitary and the hypothalamic level, it is unlikely that the inhibitory effect of rhIGF1 on the GH response to AG takes place via activation of somatostatergic pathways (33). Thus, as there is evidence that IGF1 inhibits hypothalamic GHRH release, it is likely that the inhibitory effect of IGF1 on the GH response to AG might be mediated by counteracting its stimulatory effect on GHRH neuron activity (33). Alternatively, the possibility of a direct effect at the pituitary level cannot be excluded. In fact, prolonged exposure to GH has been reported to be able to decrease GHSR1α mRNA levels both at the pituitary and the hypothalamic level in rats (34, 35, 36) through IGF1-independent mechanisms (36).

Unfortunately, our study model in vivo in humans does not allow to distinguish the pituitary from the hypothalamic effects, nor to fully define the distinct role of the 4-day GH exposure or of the increased IGF1 levels per se. However, the hypothesis of a mechanism involving the modulation of GHSR1α level seems unlikely as both the responses of PRL and cortisol (due to the ACTH-releasing effect) to AG are not modified by either acute or prolonged rhGH administration. In fact, though elicited at different sites (e.g. lactotroph cells in the pituitary and CRH/AVP-secreting cells in the hypothalamus respectively), both actions have been clearly shown to be mediated by the activation of GHSR1α itself (21, 37).

Similarly, the hypothesis that the impairment of the GH response to AG after 4-day rhGH treatment could be mediated by other factors than the increase in IGF1 levels has to be considered. In fact, prolonged GH treatment has been reported to increase glucose and free fatty acids levels (38). However, as reported before, the GH response to AG has been clearly shown to be refractory to the inhibitory effect of both glucose and free fatty acids (26), and moreover, in this study, no significant variation of glucose and free fatty acids levels were detected after rhGH treatment.

On the contrary, although circulating AG and unacylated ghrelin levels were not assayed, the theoretical possibility that the different GH responses could be related to inter-session differences in blood AG levels after i.v. administration is very unlikely as the test order was randomized and each subject received the same i.v. AG dose in each session. Similarly, the hypothesis that the impaired GH response to AG after 4-day s.c. GH administration is related to a random effect is very remote despite the lack of a control arm, taking into account the well known high intra-subject reproducibility of the somatotroph responsiveness to natural and synthetic GHS ((20) and F Broglio, unpublished results).

In conclusion, our results indicate that the somatotroph-releasing effect of AG is refractory to the direct inhibitory effect of a short-term elevation of GH levels while it is markedly inhibited in the presence of increased IGF1 levels induced by 4-day rhGH administration, opening the possibility, in agreement with
previous studies with synthetic GHS (33), of a selective IGF1-mediated feedback.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

13 Tannenbaum GS. Evidence for autoregulation of growth hormone secretion via the central nervous system. Endocrine 1980 107 2117–2120. (doi:10.1210/endo-107-6-2117)


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