Effect of 15-day treatment with growth-hormone-releasing hormone alone or combined with different doses of arginine on the reduced somatotrope responsiveness to the neurohormone in normal aging

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It is well known that both spontaneous and growth hormone-releasing hormone (GHRH)-stimulated GH secretion undergo an age-related decrease; in addition, there is supportive evidence that the GH hyposecretory state of aging is of hypothalamic origin. The aims of the study in 35 normal elderly subjects (20 males and 15 females aged 65–89 years) were to verify whether the low somatotrope responsiveness to GHRH (1 µg/kg) can be primed by a daily GHRH treatment and whether the potentiating effect of both high intravenous (0.5 g/kg) and low oral (8 g) doses of arginine (ARG) on GH response to GHRH is maintained with time. In group A (N = 14) the GH response to GHRH on day 1 (AUC: 373.5 ± 78.5 µg·l⁻¹·h⁻¹) was unchanged after 7 (3720 ± 38 µg·l⁻¹·h⁻¹) and 15 days (377.9 ± 63.8 µg·l⁻¹·h⁻¹) of daily GHRH administration. In group B (N = 6) the GH response to GHRH co-administered with iv ARG on day 1 (1614.2 ± 146.2 µg·l⁻¹·h⁻¹) was higher (p < 0.05) than that of GHRH alone (group A) and persisted unchanged after 7 (1514.7 ± 366.5 µg·l⁻¹·h⁻¹) and 15 days (1631.7 ± 379.1 µg·l⁻¹·h⁻¹) of treatment. In group C (N = 15) the GH response to GHRH co-administered with oral ARG on day 1 (950.6 ± 219.4 µg·l⁻¹·h⁻¹) was higher (p < 0.03) than that of GHRH alone (group A) but lower (p < 0.05) than that to GHRH plus iv ARG (group B). It was unchanged after 7 (816.2 ± 208.5 µg·l⁻¹·h⁻¹) and 15 days (760.4 ± 165.0 µg·l⁻¹·h⁻¹) of treatment; these responses were still higher (p < 0.05) than that to GHRH alone. Insulin-like growth factor I levels were not modified by any of the treatments. In conclusion, our results demonstrate that in normal aging the low somatotrope responsiveness to GHRH is not improved by prolonged treatment with the neurohormone but it is enhanced by the combined treatment with ARG and this effect does not vanish after a 15-day treatment period. The effect of ARG is present even after a low oral dose, although less markedly than after a high intravenous dose.

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It is now widely accepted that the function of the growth hormone (GH)–IGF-I axis declines with age (1–4), likely contributing to changes in body composition (3, 5). These changes have been shown to be reversed in part by treatment with rhGH (5).

Besides the reduction of spontaneous GH secretion, in older humans the somatotrope responsiveness to several pharmacological stimuli, including GHRH, also is reduced (1, 3, 4, 6–9). There is evidence that this GH insufficiency is of hypothalamic origin (9–14). Arginine, which probably acts via inhibition of hypothalamic somatostatin release (9–11, 15, 16), strikingly enhances the reduced somatotrope responsiveness to GHRH in older humans and makes it similar to that of young adults and even normally growing children (9–11). These results show that the maximal secretory capacity of the somatotrope cells does not vary with age and, consistent with other data (17–20), suggest the existence of somatostatinergic hyperactivity in aging. This could be absolute or relative to a concomitant reduction of hypothalamic GHRH activity (7, 21–26) and/or GHRH receptors (26, 27).

Evidence that the GH pituitary pool is essentially preserved during aging implies that treatment with GHRH alone or in combination with arginine represents a possible approach by which to restore the defective function of the GH–IGF-I axis. We showed in a previous study that in aging the reduced GH response to GHRH is potentiated not only by a high intravenous but even by a low oral dose of arginine (28). Our aim in the present study was to verify, in elderly subjects, whether the low somatotrope responsiveness to GHRH can be primed by daily GHRH treatment and whether the potentiating effect of both a high systemic and a low oral dose of arginine on GH response to GHRH is maintained or vanishes with time.
Subjects and methods

Thirty-five normal elderly subjects (20 males and 15 females aged 65–89 years, BMI 21–25 kg/m²) were studied. All subjects were in good health and well taking any medication. Informed consent was obtained from all subjects. The study protocol had ethical approval by our Departmental Committee.

Subjects were divided into three groups that underwent different treatments. In group A (nine males and five females), subjects underwent a 15-day treatment with GHRH alone (1 µg/kg iv once daily at 09.00 h). In group B (four males and two females), subjects underwent a 15-day treatment with GHRH combined with iv arginine (ARG hydrochloride, 0.5 g/kg infused over 30 min from 09.00 to 09.30 h). In group C (seven males and eight females), subjects underwent a 15-day treatment with GHRH combined with oral arginine (ARG aspartate, Sargenor Chinoin, Italy; 8 g given at 08.00 h, 60 min before GHRH).

The GH response to different drug treatments was studied on days 1, 7 and 15. The acute GHRH test was performed 30 min after an indwelling catheter was inserted in a forearm vein kept patent by slow infusion of isotonic saline. Baseline blood samples were taken at 08.00 and 09.00 h (i.e. at times −60 and 0 min, respectively) and then every 15 min until 10.30 h.

Serum GH levels were measured in duplicate by immunoradiometric assay (hGH-CTK, Sorin Biomedica, Saluggia, Italy). The sensitivity of the assay was 0.1 µg/l. Ranges of inter- and intra-assay coefficients of variation were 4.9–6.5% and 1.5–2.9%, respectively. The GH responses were expressed either as absolute values (µg/l) or as areas under curves (AUC, µg·L⁻¹·h⁻¹) calculated by trapezoidal integration.

On days 1, 7 and 15 of various treatment periods, IGF-I levels also were measured. Serum IGF-I levels were measured in duplicate by radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, USA). To avoid interference by binding proteins, all samples were treated with acid ethanol. The sensitivity of the assay was 0.1 µg/l. Ranges of inter- and intra-assay coefficients of variation were 10.1–15.7% and 7.6–15.5%, respectively. The IGF-I values were expressed as absolute values (µg/l) with reference to a pure recombinant IGF-I preparation.

Routine blood clinical chemistry (urea, creatinine, total bilirubin, alkaline phosphatase, aspartate amino-transferase, gamma glutamyltransferase, sodium, potassium, calcium, phosphate) was monitored on days 1, 7 and 15.

Statistical evaluation was carried out by a non-parametric ANOVA (Kruskal–Wallis test) and by paired and unpaired Student’s t-test, where appropriate. Results are expressed as means ± SEM.

Results

Group A, B and C subjects had similar basal GH (1.6 ± 0.6 vs 0.8 ± 0.6 vs 1.2 ± 0.2 µg/l) and IGF-I levels (75.2 ± 4.2 vs 66.2 ± 7.2 vs 68.3 ± 2.9 µg/l).

The GH responses to various treatments are reported in Table 1. In group A the GH response to GHRH on day 1 was unchanged after 7 and 15 days of daily GHRH administration (Fig. 1). In group B the GH response to GHRH co-administered with iv ARG on day 1 was higher (p < 0.05) than that to GHRH alone (group A) and persisted unchanged after 7 and 15 days of treatment (Fig. 2). In group C the GH response to GHRH co-administered with oral ARG on day 1 was higher (p < 0.03) than that to GHRH alone (group A) and lower (p < 0.05) than that to GHRH plus iv ARG (group B). It persisted unchanged after 7 and 15 days of treatment and was still higher (p < 0.05) than that to GHRH alone (Fig. 3).

The IGF-I levels were similar on days 1, 7 and 15 in groups A (75.2 ± 4.2 vs 65.2 ± 2.2 vs 60.2 ± 4.8 µg/l), B (66.2 ± 7.2 vs 56.2 ± 8.2 vs 58.2 ± 7.9 µg/l) and C (68.3 ± 2.9 vs 60.8 ± 5.2 vs 61.2 ± 7.2 µg/l).

Side effects

Administration of GHRH induced a transient facial flushing in some but not all subjects; the association of oral ARG with GHRH injections did not affect this picture. In contrast, when given GHRH plus iv ARG, two subjects had increased levels of blood urea nitrogen (from 0.35 to 1.1 g/l in one subject and from 0.42 to 0.75 g/l in the other one) after 7 days of treatment. After treatment withdrawal, blood urea nitrogen returned to normal within 7 days. Owing to this side effect, no other subjects were studied with long-term administration of GHRH and iv ARG. No other alteration in the routine blood clinical chemistry was recorded.
Discussion

The results of this study demonstrate that in normal aging, the low somatotrope responsiveness to GHRH is not improved by prolonged treatment with the neurohormone, whereas it is enhanced by including arginine; this effect is preserved after 15 days of treatment. The GHRH-potentiating effect of arginine, albeit lower, is still noticeable even when the amino acid is co-administered orally at low doses. The high-dose iv regimen, however, frequently causes an increase in blood urea nitrogen; this phenomenon is probably attributable to the nitrogen load and possibly to a subtle, subclinical defect in kidney function pre-existing in some of our elderly individuals. As was suggested by previous results (28, 29), our study confirms that the lowest ARG dose able to enhance the GHRH-induced GH release is approximately 8 g.

Various mechanisms have been suggested to explain the impaired response of somatotrope cells to GHRH in vivo. Some data from animal studies support the possibility of an impairment in GHRH receptor and post-receptor mechanisms at the pituitary level (26, 27). In accordance with this view is the...
finding that theophylline, an inhibitor of phosphodiesterase, enhanced the low GH response to GHRH in elderly subjects (30). Other data favor the hypothesis of a reduced activity of GHRH-secreting neurons (7, 21–26). Thus, the low GH response to GHRH in elderly subjects was improved after pretreatment with GHRH (7, 23). In the present study though, we failed to demonstrate that the low somatotrope responsiveness to GHRH is improved significantly by a short-term treatment with the neurohormone. It is noteworthy that we administered 1 µg/kg GHRH iv daily for 15 days, while in one of the aforementioned studies (7) an improved response to GHRH was found in some subjects when the same GHRH dose was administered every other day for only 8 days. These contrasting results probably reflect the well-known variability of the GH response to GHRH (31, 32). In addition, it has to be emphasized that once-daily GHRH injections cannot be assumed to mimic the normal pattern of GHRH release occurring in the young adult. In agreement with our data, in a recent study, continuous or intermittent subcutaneous administration of 1 or 2 mg of GHRH daily for 14 days did not affect the original GH response to GHRH in older men, although it was able to increase GH pulsatility and IGF-I levels (24, 25). Overall, it would seem that impairment of GHRH-secreting neurons may not explain fully the GH insufficiency and the hyporesponsiveness to acute GHRH administration in aging.

Many data in animals and in humans indicate the existence of aging of a somatostatinergic hyperfunction (9–12, 17–20) that could be absolute or, more likely, relative to the concomitant reduction in the activity of GHRH-secreting neurons (7, 21–26). In this context it is worth recalling that, in the old rat, although somatostatin gene expression is decreased in the hypothalamus (33), an augmented secretion of somatostatin has been reported in cultures of hypothalamic neurons (19) or from the hypothalamus under potassium stimulation (20).

In keeping with the existence of an increased somatostatinergic function is the ability of a somatostatin antiserum (12) and ARG (9–11) to restore fully the reduced GH response to GHRH in old rats and humans, respectively. Our present results confirm these findings and, moreover, they show that the potentiating effect of ARG does not vanish with time. Based on the assumption that ARG acts via inhibition of somatostatin release (9–11, 15, 16), these results strengthen the view that a somatostatinergic hyperactivity is present in aging and show that the ARG effect does not undergo densitization.

Evidence that IGF-I levels were not increased by either GHRH alone or combined with ARG is not surprising, given the simple morning administration of our treatment schedule. This finding, however, contrasts with studies reporting an increase in IGF-I levels of aged subjects the day after a single GHRH administration (34).

In conclusion, our results show that in normal aging the reduced GH response to GHRH is not improved by prolonged treatment with the neurohormone but is enhanced markedly by combining this with ARG. As this effect does not fade after administration of the amino acid for 15 days, combined treatment with ARG and GHRH holds the promise of being a suitable approach to restore the function of the GH–IGF-I axis during aging. To this goal, however, only the low-dose orally administered ARG protocol is warranted, in order
to avoid the consequences of an exaggerated nitrogen load. Obviously, the availability of a long-acting orally effective GHRH analog makes this therapeutic option more feasible.

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