

CONTRIBUTION OF GROWTH HORMONE-RELEASING HORMONE AND SOMATOSTATIN TO DECREASED GROWTH HORMONE SECRETION IN ELDERLY MEN

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Objective. The pathophysiology of the decline in circulating growth hormone (GH) concentrations that may occur with ageing remains elusive. We have investigated the potential contributions of decreased endogenous GH-releasing hormone (GHRH) and increased somatostatin secretion to this phenomenon.

Design and methods. The strategy used was to stimulate GH secretion in 8 young (20 - 24 years old, body mass index (BMI) $22.8 \pm 2.8 \text{ kg/m}^2$) and 8 elderly (68 - 82 years old, BMI $23.4 \pm 1.6 \text{ kg/m}^2$) male subjects on separate occasions by means of: (i) intravenous bolus $0.5 \mu\text{g/kg}$ D-Ala² GHRH(1-29)-NH₂ alone; (ii) $0.5 \mu\text{g/kg}$ GHRH after pre-treatment with two oral doses of 50 mg atenolol (to inhibit somatostatin secretion); (iii) 1.25 mg oral bromocriptine alone (to increase endogenous GHRH and/or inhibit somatostatin); (iv) 50 mg oral atenolol plus 1.25 mg oral bromocriptine; and (v) $0.5 \mu\text{g/kg}$ GHRH after pre-treatment with 1.25 mg oral bromocriptine.

Results. The elderly men had a significantly lower peak and area under curve (AUC) GH response to intravenous GHRH when compared with 8 young men (peak $3.1 \pm 1.0 \text{ ng/ml}$ v. $21.6 \pm 5.0 \text{ ng/ml}$, AUC $205 \pm 56 \text{ ng/ml/min}$ v. $1315 \pm 295 \text{ ng/ml/min}$, $P < 0.05$). Pre-treatment with atenolol before GHRH administration produced no significant increase in peak and AUC GH response in both groups, which remained lower in the elderly men than in their young counterparts (peak $5.5 \pm 1.8 \text{ ng/ml}$ v. $29.3 \pm 7.0 \text{ ng/ml}$, AUC $327 \pm 90 \text{ ng/ml/min}$ v. $2017 \pm 590 \text{ ng/ml/min}$, $P < 0.05$). Bromocriptine alone did not cause a

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significant rise in GH concentration in either elderly or young subjects (peak 3.1 ± 1.1 v. 8.8 ± 3.2 ng/ml, $P > 0.05$). When atenolol was administered before bromocriptine, both groups responded but the elderly subjects had a significantly greater peak and AUC response (peak 3.6 ± 0.7 v. 10.7 ± 2.1 ng/ml; AUC 191 ± 39 v. 533 ± 125 ng/ml/min, $P < 0.05$).

Bromocriptine given before GHRH failed to potentiate GHRH action on GH release in either group. Of 5 elderly men who underwent further evaluation of GH secretory ability, 2 subjects had GH levels > 10 ng/ml, either basally or after intravenous GHRH. The remaining 3 had an initially impaired GH response to bolus intravenous GHRH. After 100 μ g GHRH subcutaneously twice daily for up to 2 weeks the GH responses to intravenous bolus GHRH (0.5 μ g/kg) were reassessed. One exhibited a normal response (> 10 ng/ml) after 1 week of daily GHRH treatment, another had a near-normal response after 2 weeks (9.7 ng/ml), while the third still had an impaired response by the end of the 2-week treatment period (3.2 ng/ml).

Conclusions. The restoration of endogenous GH secretion in these elderly subjects by means of GHRH priming, and the failure of manipulation of somatostatinergic tone to restore a normal GH response to GHRH suggests that somatotroph atrophy due to a reduction in endogenous GHRH secretion is the principal cause of the diminished GH secretion with ageing.

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The ageing process is associated with a decline in both growth hormone (GH) secretion and plasma insulin-like growth factor-I (IGF-I) concentration.^{1,5} There is a concomitant reduction in lean body mass and an increase in adipose tissue.¹ Furthermore, administration of GH to elderly males with low IGF-1 levels results in increased lean body mass and decreased adipose tissue, supporting the hypothesis that the age-related changes in body composition are a consequence of diminished GH secretion.⁶

GH secretion is regulated by the interaction of two hypothalamic peptides, GH-releasing hormone (GHRH) and somatostatin.⁷ A reduction in GH release may therefore reflect decreased GHRH release, increased somatostatin secretion, primary somatotroph dysfunction, increased sensitivity to the negative feedback effects of IGF-1 or combinations of these factors. Studies to elucidate the mechanisms underlying the diminished GH secretion in the elderly have yielded conflicting results. Although most investigators have shown acute GH responses to GHRH to be diminished in the elderly,^{8,16} this is not a universal finding.^{4,17,18} Impaired GH response to an initial GHRH dose in the elderly may be corrected by repetitive GHRH administration (priming), a finding that suggests a

reduction in endogenous GHRH synthesis or release.¹⁰ Conversely, the notion of enhanced somatostatin tone in the elderly is supported by studies demonstrating correction of the diminished GH response to GHRH by pre-treatment with inhibitors of somatostatin release, such as pyridostigmine or arginine.^{11,12,16} The possibility of increased sensitivity to endogenous IGF-1 feedback was not supported by a recent *in vivo* study of GH suppression by IGF-1 infusion in elderly subjects.¹⁹

The present study was designed to elucidate further the mechanisms underlying the reduction in GH secretion in elderly men. The GH response to exogenous GHRH was evaluated using D-Ala²GHRH(1-29)-NH₂, a GHRH analogue with enhanced agonist activity.²⁰ As previous workers have suggested that beta-adrenergic antagonists are effective inhibitors of somatostatin release,^{21,22} the possibility of enhanced somatostatin tone in the elderly was examined by pre-treatment of subjects with atenolol before the administration of GHRH. In addition, the GH response to bromocriptine was studied in young and elderly subjects, both with and without atenolol pre-treatment, as there is evidence that oral dopamine agonists induce release of endogenous GHRH.²³ The ability of bromocriptine to enhance the GH response to GHRH was also evaluated in view of the suggestion that dopamine agonists may stimulate GH secretion by inhibiting somatostatin release.²⁴ Finally, a group of elderly subjects with low GH responses to GHRH underwent 1 - 2 weeks of priming with D-Ala²GHRH(1-29)-NH₂ in an attempt to evaluate the possibility that the poor GH response to GHRH and other agents in the elderly is due to somatotroph atrophy resulting from low endogenous GHRH secretion or increased somatostatin secretion.

MATERIALS AND METHODS

Subjects

Healthy men were recruited by newspaper advertisement. The 8 elderly male subjects were aged 68 - 82 years, with body mass index (BMI) 23.4 ± 1.6 kg/m². None had significant medical problems as detected by medical history, physical examination and an electrocardiogram. Eight young men aged 20 - 24 years with BMI 22.8 ± 2.8 kg/m² were also studied. No subject was taking medication known to alter GH secretion. All subjects had normal baseline thyroid-stimulating hormone (TSH) estimations. Subjects gave informed consent for the study, which was approved by the University of Cape Town Ethics and Research Committee.

Procedure

Subjects were fasted overnight and were recumbent and awake during each test. After 15 minutes at rest, blood was taken for GH determination every 15 minutes from 08h15 to 12h00 from



a forearm vein that was kept patent by heparinised saline. The following tests were performed in random order, at least 1 week apart (Fig. 1): (i) 0.5 µg/kg D-Ala²GHRH(1-29)-NH₂ given as an intravenous (IV) bolus at 10h00, preceded by the same volume of vehicle (mannitol) IV as control at 09h00; (ii) atenolol 50 mg orally given the morning before the test, and at 08h30 on the morning of the test (GHRH was administered as above); (iii) bromocriptine 1.25 mg orally at 08h30 on the morning of the test; (iv) atenolol 50 mg orally given the morning before the test, and at 08h30 on the morning of the test, together with bromocriptine as above; and (v) bromocriptine 1.25 mg orally at 08h30, followed by GHRH at 10h00.

Pulse and blood pressure were monitored during each test. Blood was taken for serial prolactin determination during protocol 5. We elected to use D-Ala²GHRH(1-29)-NH₂ as it is a readily available and potent analogue of GHRH which has never previously been utilised in the study of GH secretion in elderly subjects.

In a further study, 5 elderly men (aged 66 - 80 years, BMI 22.7 ± 3.2 kg/m²) underwent an IV D-Ala²GHRH(1-29)-NH₂

(hereafter termed GHRH) test as described in (i) above. One of these subjects (subject 3) had completed the previous set of tests. In all studies a peak GH level > 10 ng/ml was regarded as a normal GH response. Subjects with an initial peak serum GH response < 10 ng/ml were instructed in the use of a Novopen syringe with a GHRH cartridge, and then self-administered 100 µg GHRH subcutaneously twice daily. The IVI GHRH test was repeated weekly until the GH response exceeded 10 ng/ml or 2 weeks had elapsed.

Assays

Serum GH concentrations were measured using a commercial kit (Pharmacia Diagnostics AB, Uppsala) with a detection limit of 0.25 ng/ml. The intra-assay coefficient of variation (CV) was 4.8% and the interassay CVs at low, mid and high levels were 2.2, 3.4 and 2.7% respectively. Prolactin was measured using a commercial kit (Diagnostic Products Corporation, Los Angeles) with a sensitivity of 1.4 ng/ml, and inter- and intra-assay CVs of 6.3 and 2.3% respectively. All samples from a single subject were measured in duplicate in a single assay.

Statistical methods

Results are expressed as mean ± standard error of mean (SEM). For calculations, undetectable plasma GH levels were assigned the value of the detection limit of the assay. The GH secretory responses are expressed as either peak serum concentration or integrated areas under the curve (AUC) over 120 minutes, calculated by the trapezoidal method. Non-parametric analysis (Wilcoxon rank sum and Mann-Whitney) was used as the data were not normally distributed. Statistical significance was accepted at $P < 0.05$.

RESULTS

GHRH (Fig. 2A and F)

The mean GH response of both young and elderly subjects to 0.5 µg/kg IV GHRH bolus is illustrated in Fig. 2A. In elderly subjects, GHRH induced a poor GH increase (peak 3.1 ± 1.0 ng/ml, AUC 205 ± 56 ng/ml/min), which was significantly less than that observed in young subjects (peak 21.6 ± 5.0 ng/ml, AUC 1 315 ± 295 ng/ml/min, $P < 0.05$). The AUC and peak GH response to GHRH was significantly increased compared with baseline in the young ($P < 0.05$) but not in the elderly subjects.

GHRH with atenolol pre-treatment (Fig. 2B and F)

Following administration of atenolol (Fig. 2B and F), the GH response to GHRH was not significantly enhanced in the elderly (peak 5.5 ± 1.8 ng/ml, AUC 327 ± 90 ng/ml/min) or the young men (peak 29.3 ± 7.0 ng/ml, AUC 2 017 ± 590 ng/ml/min, $P > 0.05$), and the GH response in the elderly

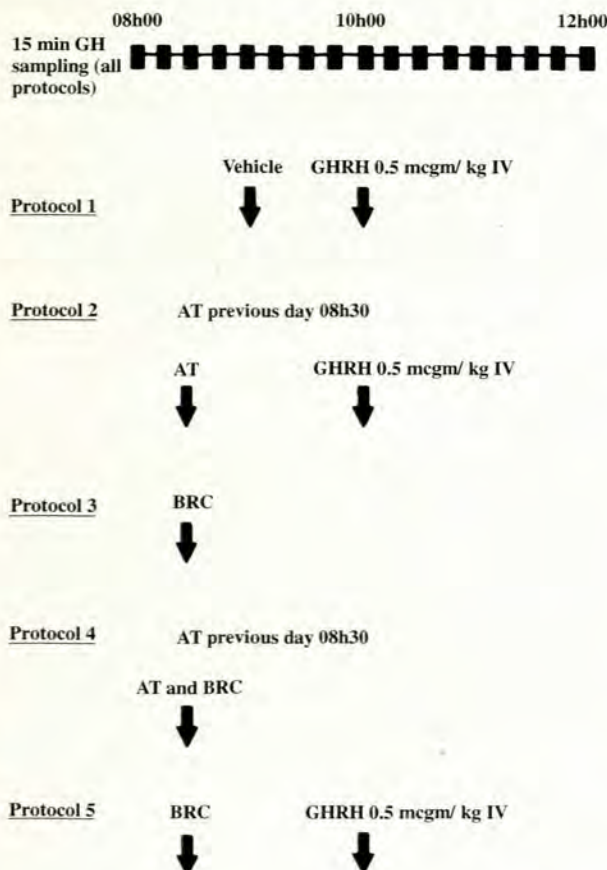


Fig. 1. Protocols for GH stimulation tests performed in random order on all subjects. (AT = atenolol 50 mg; BRC = bromocriptine 1.25 mg.)

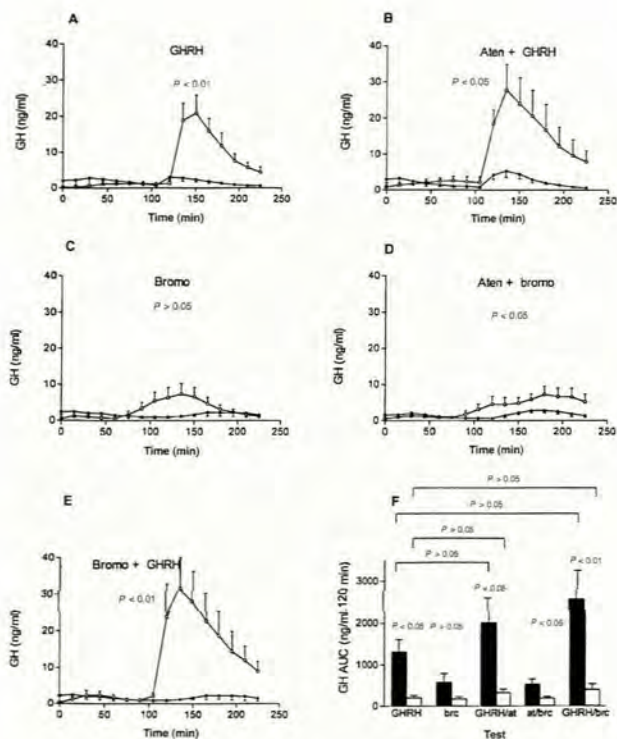


Fig. 2A-F. Mean \pm SEM GH response; (A) 0.5 μ g/kg GHRH; (B) 50 mg atenolol before 0.5 μ g/kg GHRH (C) 1.25 mg bromocriptine; (D) 50 mg atenolol before 1.25 mg bromocriptine; and (E) 1.25 mg bromocriptine before 0.5 μ g/kg GHRH in young (o) and elderly (●) subjects. The significance values refer to the peak GH response. Fig. 2F indicates corresponding GH AUC responses in young (shaded bars) and elderly subjects (unshaded bars).

remained significantly diminished when compared with that of the young men ($P < 0.05$). Atenolol had no significant effect on basal GH levels in either group. Peripheral bio-activity was confirmed by a reduction in the mean pulse rate in both young (from 65/min to 54/min, $P < 0.05$) and elderly subjects (from 71/min to 63/min, $P < 0.05$).

Bromocriptine (Fig 2C and F)

The GH response of the young and elderly to 1.25 mg oral bromocriptine alone is illustrated in Fig. 2C and F. This test in particular demonstrated the marked inter-individual variation of GH responses, which ranged from negligible to greater than 10 ng/ml. The mean increase in GH was not significant when compared with the basal levels in either group, and the response did not differ significantly between groups (in the elderly, peak 3.1 ± 1.1 ng/ml, AUC 177 ± 51 ng/ml/min v. the young 8.8 ± 3.2 ng/ml and 578 ± 217 ng/ml/min, $P > 0.05$).

Bromocriptine with atenolol pre-treatment (Fig. 2D and F)

Atenolol failed to modify the response to bromocriptine in either the young or the elderly groups ($P > 0.05$, Fig. 2D).

However, this combination resulted in significantly higher GH responses in the young compared with the elderly (peak 10.7 ± 2.1 ng/ml, AUC 533 ± 125 ng/ml/min v. peak 3.6 ± 0.7 ng/ml, AUC 191 ± 39 ng/ml/min, $P < 0.05$).

GHRH with bromocriptine pre-treatment (Fig. 2E and F)

Bromocriptine pre-treatment did not significantly enhance the peak or AUC GH response to GHRH in either the young or the elderly group ($P > 0.05$), and the response in the young subjects remained greater than in the elderly (peak 35.6 ± 10.3 ng/ml, AUC 2572 ± 693 ng/ml/min v. peak 6.3 ± 1.7 ng/ml, AUC 401 ± 141 ng/ml/min, $P < 0.01$). Bio-activity of bromocriptine at the dose used was confirmed by a fall in mean serum prolactin concentration in young (from baseline of 5.8 ng/ml to 2.5 ng/ml at time of GHRH administration and < 1.4 ng/ml at 120 min) and elderly subjects (7.1 ng/ml, 2.8 ng/ml and 1.8 ng/ml, respectively.)

GHRH priming

One of the 5 elderly subjects had a spontaneous GH peak during the baseline period (10.3 ng/ml), indicating preserved somatotroph function, and was therefore excluded from the priming study. Subject 2 had a vigorous GH response to the initial GHRH test (42.1 ng/ml), and was not primed further. The responses of the 3 remaining subjects to priming are illustrated in Fig. 3. Subjects 3 and 4 initially had low GH responses, which increased progressively during the priming period. Subject 3 attained a GH concentration > 10 ng/ml after 1 week of priming, and subject 4 nearly achieved this after 2 weeks (9.7 ng/ml). Subject 5 had a poor GH response to IV GHRH which failed to increase despite 2 weeks of priming (peak GH 3.2 ng/ml).

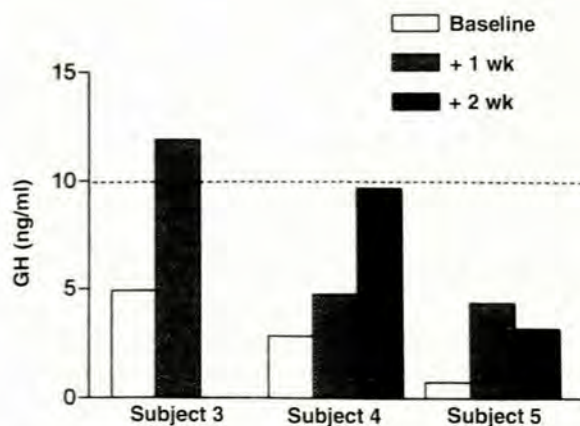


Fig. 3. The peak GH response to 0.5 μ g/kg GHRH before priming (baseline), and after 1 and 2 weeks' daily 100 μ g GHRH subcutaneously in 3 elderly subjects with an initial GH response to GHRH below 10 ng/ml. The line indicates a GH concentration of 10 ng/ml.



Side-effects

On occasion bolus IV doses of GHRH caused flushing and warmth of the face, transient tachycardia and a slight lowering of blood pressure. The administration of bromocriptine and atenolol in combination caused dizziness after the test in 2 elderly subjects, presumably due to hypotension. No adverse effects occurred during the priming study.

DISCUSSION

Increased awareness of the morbidity and possible mortality associated with GH deficiency in adults with hypopituitarism has stimulated interest in GH replacement therapy in these subjects.^{25,26} Furthermore, GH administration in normal elderly men with low IGF-1 concentrations produced a substantial increase in lean body mass and reduction in adipose tissue.⁶ Reduced GH secretion in the elderly may therefore warrant treatment, and studies of the underlying mechanisms are of particular relevance as modulators of endogenous GH secretion may potentially be used as alternatives to exogenous human GH administration.

This study, an attempt to evaluate GH secretion in the elderly, comprehensively illustrates the variable nature and extent of GH insufficiency. Previous reports have provided evidence for both enhanced somatostatin tone^{11,12} and diminished GHRH release^{10,27} to account for the diminished GH secretion in the elderly. We have further investigated the reasons for decreased GH secretion in the elderly by evaluating these postulated mechanisms. In an attempt to improve on the design of previous studies, the same subjects were examined when testing different sites of possible GH secretory dysfunction, as conflicting results may reflect inter-individual variation if different subjects are used. In addition, a group of elderly subjects with an initially diminished GH response to GHRH underwent daily GHRH priming for 2 weeks in order to evaluate whether endogenous GHRH deficiency, with consequent somatotroph atrophy, may be a factor contributing to the initially blunted GH response.

GHRH

The GH response to a GHRH bolus was diminished in all elderly subjects in the first test, consistent with most⁸⁻¹⁶ but not all previous reports.^{4,17,18} This diminished GH response was noted despite the use of an analogue of GHRH (D-Ala²GHRH(1-29)-NH₂) which is approximately twice as potent as the native peptide.²⁰ One of 8 subjects in the initial GHRH study, and 1 of 5 in the priming study, had a normal spontaneous GH peak during the baseline period. A further subject in the priming study had a normal GH response to the initial GHRH bolus before priming commenced. A decline in GH secretion is therefore not an inevitable consequence of the

ageing process, although a mean age-related decline in GH production rate of 14% per decade has been documented.²⁸

Atenolol

Atenolol at a dose of 100 mg increases the GH response to GHRH in both children and young men, presumably by inhibition of somatostatin tone.^{21,22} To the best of our knowledge it has not been tested in the elderly. However, in the present study a lower dose of atenolol (50 mg) failed to increase the GH response to GHRH (or bromocriptine) in either the young or the elderly, suggesting either failure of inhibition of somatostatin, or that somatostatin inhibition had a negligible effect on the GH response to GHRH. It is conceivable that despite evidence of peripheral bio-activity in the present study (induction of bradycardia), the 50 mg dose of atenolol utilised was insufficient for central inhibition of somatostatin tone. We chose the lower dose of atenolol as we were concerned that the combination of atenolol and bromocriptine, particularly in elderly subjects, would result in significant hypotension.

Previous studies of the effect of manipulating somatostatinergic tone on GH release in the elderly have yielded inconsistent results, including potentiation¹² or normalisation¹⁶ of the GH response to GHRH by pyridostigmine in the elderly and an equivalent response in both young and elderly to GHRH plus arginine.¹¹ Further evidence for enhanced somatostatinergic tone has arisen from studies in which free fatty acid (FFA) concentrations were manipulated in elderly subjects. FFAs inhibit GH release via an increase in somatostatin secretion.²⁹ Acipimox reduced initially normal FFA concentrations in elderly subjects and normalised the GH response to GHRH.³⁰ The possibility therefore exists that the elderly have increased stimulation of somatostatin secretion by normal FFA concentrations, accounting for increased somatostatinergic tone and GH deficiency. Although these studies support the hypothesis of increased somatostatin inhibition of GH secretion with ageing, our results failed to provide confirmatory evidence.

Bromocriptine alone or before GHRH

The mode of action of dopamine agonists in stimulating GH release remains controversial. Page *et al.*,³¹ using oral levodopa, suggested that the GH response to dopaminergic stimulation is GHRH-dependent. Studies demonstrating an increase in plasma GHRH in response to levodopa are, however, of dubious value as peripheral levels of GHRH may not reflect hypothalamic levels.^{23,31} Conflicting evidence for dopamine agonist-mediated inhibition of somatostatin was provided by workers demonstrating bromocriptine enhancement of the GH response to a maximal dose of GHRH.^{24,32} In the present study bromocriptine was therefore used alone, with GHRH, and with atenolol in order to evaluate its effect on both GHRH and somatostatin secretion.



We were unable to demonstrate significant potentiation of the GH response to GHRH in either age group, and therefore failed to confirm the bromocriptine-induced somatostatin inhibition suggested by previous studies.^{24,31} These studies, however, used 2.5 mg bromocriptine and it is therefore possible that 1.25 mg bromocriptine is an inadequate dose to inhibit somatostatin. As with atenolol, the lower dose of bromocriptine was used as we were concerned that the combination of bromocriptine and atenolol may induce significant hypotension. Although the significant decline in prolactin after bromocriptine confirms dopamine agonist activity in the anterior pituitary, we are uncertain whether this is a reliable surrogate marker of adequate exposure of the somatostatin-producing hypothalamic neurons to dopamine agonist. Furthermore, despite marked interindividual variation, we found no evidence that bromocriptine alone was a reliable GH secretagogue, arguing against a potent stimulation of endogenous GHRH release in either the young or the elderly subjects.

Priming

The finding that 70% of GH-deficient children respond to GHRH treatment has encouraged use of this agent in GH-deficient adults. Supportive evidence for GHRH deficiency in the elderly includes decreased peripheral GHRH concentrations, although this may inaccurately reflect hypothalamic GHRH secretion.¹³ Furthermore, alternate-day priming with 100 µg GHRH1-40 for 12 days in 7 elderly subjects has been found to restore partially the plasma GH response to GHRH, findings compatible with reversible somatotroph dysfunction in the elderly secondary to a decline in endogenous GHRH release.¹⁰ Finally, a recent study²⁷ documented a diminished GH response to withdrawal of a somatostatin infusion in the elderly, a result interpreted by the authors as suggesting an age-related decrease in endogenous GHRH secretion. The present priming study emphasises the spectrum of disordered GH secretion in the elderly. Two subjects (aged 66 and 80 years) had normal GH secretion initially, either spontaneously or in response to a GHRH bolus. Two subjects had poor initial GH responses to GHRH but exhibited normal responses after 1 or 2 weeks of GHRH priming. This supports the hypothesis that somatotroph dysfunction was secondary to deficient endogenous GHRH release. The fifth subject had a persistently low GH response despite 2 weeks of GHRH priming, suggesting either more severe somatotroph dysfunction (potentially responsive to more prolonged GHRH priming), enhanced somatostatin tone or alternatively poor compliance with GHRH administration. The small number of subjects who underwent priming, however, makes it difficult to draw firm conclusions regarding the pathogenesis of the decline in GH secretion in some elderly subjects.

The heterogeneity of GH response noted in elderly subjects may in part reflect the varying pathogenesis of diminished GH secretion in different elderly subjects. Moreover, the timing of stimulation by exogenous agents in relation to the last GH pulse and the prevailing somatostatin tone may affect the GH response of the somatotrophs, further contributing to the variability of GH response. Future studies may therefore benefit from prolonged baseline GH measurements before stimulation. In studying GH release in the elderly it may also be advantageous to examine subgroups divided on the basis of initial provocative tests to allow detailed study of homogeneous groups if the multifactorial nature of GH deficiency in the elderly is to be understood fully.

In conclusion, we have confirmed the diminished GH response to GHRH in the elderly, even to a more potent GHRH analogue not previously used in these subjects. We have demonstrated failure of consistent inhibition of somatostatin by low doses of atenolol in both young and elderly subjects, and found no evidence that bromocriptine acts by suppressing somatostatin or predictably stimulating GHRH. Finally, in a small group of elderly subjects, we have provided data supporting a reduction in endogenous GHRH secretion as the principal mechanism underlying the decline in stimulated GH secretion.

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