CLINICAL STUDY

The GH response to low-dose bolus growth hormone-releasing hormone (GHRH(1–29)NH₂) is attenuated in patients with longstanding post-irradiation GH insufficiency

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Abstract

Objective: Previous studies have suggested that post-irradiation GH insufficiency results from a loss of GHRH secretion, since many patients were able to release GH following exogenous GHRH stimulation. However, supramaximal doses of GHRH were used and the response may decline with time after radiotherapy. We re-evaluated the GHRH dose–response curve in patients post cranial irradiation and in controls.

Design: Randomized controlled study.

Methods: Five adult male long-term survivors of childhood brain tumours (median age 21.8 years (18.4–26.7); 13.7 years (11.4–15.7) post-radiotherapy, >30 Gy) and five matched controls were studied. An intravenous bolus of GHRH(1–29)NH₂ was administered in doses at the lower (0.05 µg/kg) and upper (0.15 µg/kg) range of the dose–response curves for young males, as well as the standard supramaximal dose (1.0 µg/kg). GH was measured before stimulation, every 2 min for the first hour and every 5 min for the second hour. All studies were conducted in a random fashion.

Results: Significantly lower peak and area under the curve (AUC) GH concentrations occurred in the irradiated group using 0.15 µg/kg (median peak Irradiated, 4.5 mU/l vs median Controls, 37.4 mU/l; $P<0.01$) and 1.0 µg/kg (median peak Irradiated, 4.8 mU/l vs median Controls, 15.2 mU/l; $P<0.05$) GHRH(1–29)NH₂. In irradiated subjects there was an incremental rise in GH output with increasing doses of GHRH(1–29)NH₂ (median AUC: 122 mU/l·min vs 179 mU/l·min vs 268 mU/l·min; $P=0.007$) reflecting altered pituitary sensitivity and reduced responsiveness.

Conclusion: The GH response to bolus GHRH(1–29)NH₂ is attenuated in adult long-term survivors of childhood brain tumours. This may reflect direct pituitary damage and/or the loss of the tropic effects of chronic GHRH deficiency.

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Introduction

Growth hormone (GH) insufficiency occurs frequently in children who have received high dose cranial irradiation used to treat primary brain tumours (1, 2). GH replacement is required, often for many years, and as more patients now reach adulthood, an increasing number of potentially severely GH-insufficient individuals will exist.

Post-irradiation GH insufficiency could result from dysregulation of the hypothalamic hormones GH-releasing hormone (GHRH) and somatostatin (SRIF), or from direct damage to the pituitary gland itself. Several studies have shown that patients with this condition are able to respond to an intravenous bolus injection of GHRH by releasing GH, despite poor

responses to other tests of GH stimulation. A primary defect in hypothalamic GHRH secretion has been suggested (3–6) but any response to bolus GHRH can be influenced by GH secretory status prior to stimulation (7, 8) and the peak levels obtained in most patients were less than in controls (3–6). Several studies have shown a decrease in the response to GHRH stimulation with time after radiotherapy (3, 4, 9). Such a decrease could represent direct pituitary damage or the effects of chronic GHRH insufficiency resulting in decreased GH synthesis (10) and somatotroph hypoplasia (11).

We have constructed dose–response curves for bolus GHRH(1–29)NH₂ stimulation in normal male volunteers and demonstrated that the standard doses of GHRH(1–29)NH₂ normally used (1–2 µg/kg) are supramaximal (12). Doses of just 0.08 µg/kg (range,

Table 1 Patient and control characteristics. Data shown as median with range in parentheses.

Characteristic	Irradiation (n=5)	Control (n=5)
Age (years)	21.8 (18.4–26.7)	23.3 (21.1–25.2)
Height (cm)*	174.7 (167.8–176.0)	176.0 (164.2–179.0)
Body mass index	24.0 (21.4–26.1)	23.7 (22.4–25.4)
Age at DXR	8.0 (4.6–14.6)	–
Time since DXR	13.7 (11.4–15.7)	–

*Predicted height was used if the patient had received DXR (radiotherapy).

0.06–0.12 µg/kg) are required to obtain a half-maximal response (ED₅₀). Constructing dose–response curves, as opposed to using supramaximal stimuli only, allows for a more adequate description of somatotroph physiology. Dose–response physiology can be classified as resetting (a shift in the ED₅₀ but no change in the gradient and maximum response), altered sensitivity (a shift in the ED₅₀ and a change in the gradient, but no change in maximal response), or reduced/limited maximal response ('resistance'). We have studied these measures in a group of adult male long-term survivors of childhood brain tumours and a group of matched controls.

Subjects and methods

Subjects

Five male adult long-term survivors (median age 21.8 years) of childhood brain tumours distant from the hypothalamo-pituitary axis were studied (Table 1). They had all received high dose (> 30 Gy) cranial irradiation at a median age of 8.0 years (range 4.6–14.6), 13.7 years (11.4–15.7) before this study. All patients had been treated with spinal radiotherapy; four patients had received chemotherapy. Four of them had previously been diagnosed as GH insufficient and treated with GH from a median age of 9.9 years (7.7–12.1) for 7.4 years (4.4–8.4) but stopped 4.3 years (2.3–5.3) before the study. All patients remained well and were on no other medication at the time, other than Patient 3 who was on fortnightly intramuscular testosterone replacement for primary gonadal failure (Sustanon 100 mg, Organon, Cambridge, UK). The studies were approved by University College London Hospitals Ethics Committee and informed consent was obtained.

Five male volunteers were recruited to act as a control group (Table 1). They were of similar age, body mass index and height. Parental target height was used to match those patients whose actual height had been compromised following spinal radiotherapy.

Study design and procedures

All subjects underwent three separate studies of GH release performed at least 1 week apart. Three separate

doses of bolus GHRH(1–29)NH₂ (Pharmacia-Upjohn, Stockholm, Sweden) were used: 0.05 µg/kg (Study 1), 0.15 µg/kg (Study 2) and 1.0 µg/kg (Study 3). These represent doses at the lower, upper and supramaximal parts of the dose–response curve for GHRH(1–29)NH₂ reported previously (12). All subjects were randomized to undertake these studies in a different order using a Latin-squares design. This was considered necessary since exposure of GHRH insufficient patients to a high dose of GHRH(1–29)NH₂ in one study could potentially 'prime' their GH response in subsequent studies.

Following an overnight fast, an indwelling intravenous cannula (20G) was inserted into a suitable vein at 0800 h for administration of GHRH(1–29)NH₂ and to allow regular blood sampling. At 0830 h a basal blood sample was withdrawn for the measurement of thyroxine, thyroid stimulating hormone, testosterone, follicle stimulating hormone, luteinizing hormone, prolactin, and cortisol to exclude an undiagnosed endocrinopathy or to assess adequacy of replacement treatment. Blood samples were then withdrawn every 15 min from 0830 h to 0930 h to measure GH secretory status prior to GHRH(1–29)NH₂ administration.

The bolus dose of GHRH(1–29)NH₂ was injected at 0930 h and discrete blood samples were then withdrawn every 2 min for the first hour (0930 h to 1030 h), and every 5 min for the second hour (1030 h to 1130 h) to measure the GH response. Samples were spun immediately and frozen at –20 °C prior to assay.

GH assay

Serum GH concentrations were measured using the Hybritech Tandem-R immunoradiometric assay (IRMA) (Hybritech, Liege, Belgium). This is specific for the 22 kDa GH isoform. The sensitivity of the assay was 0.5 mU/l. The inter-assay CV at serum GH concentrations of 7.8, 17.0 and 40.3 mU/l were 5%, 5.7% and 3.7% respectively, and the intra-assay CV at GH concentrations of 3.6, 7.0, 16.7 and 36.2 mU/l were 11.8%, 10.0%, 5.4% and 6.0% respectively. The standard used was HS2443E (NIH) which has been calibrated to mU/l (1 ng/ml = 2.6 mU/l) with the First International Standard (80/505).

Data analysis

Pre-stimulatory

Basal GH levels at the time of GHRH(1–29)NH₂ injection and mean GH levels in the hour before this were used to assess GH secretion prior to stimulation. GH secretory status was determined as reported by Devesa *et al.* (7) (secretory phase of GH release, $t_0 - t_{-15} > 1.0$ mU/l; secretory plateau, $t_0 - t_{-15} \leq 0$ mU/l with $t_0 > 3.9$ mU/l; refractory phase, $t_0 - t_{-15} \leq 0$ mU/l with $t_0 < 3.9$ mU/l).

Table 2 Median GH response characteristics following bolus GHRH(1-29)NH₂ stimulation.

Dose ($\mu\text{g/kg}$)	Mean pre-GH (mU/l)	Basal GH (mU/l)	Peak GH (mU/l)	Time to peak (min)	AUC (mU/l·min)
<i>Irradiation</i>					
0.05	0.5 (0.5–2.9)	0.5 (0.5–1.8)	2.2 (0.8–5.4)	12 (6–18)	122 (61–187)
0.15	1.0 (0.5–2.6)	0.5 (0.5–1.8)	4.5 ^a (3.4–5.4)	14 (12–20)	179 ^a (88–201)
1.0	1.1 (0.6–4.8)	0.6 (0.5–4.0)	4.8 ^b (1.6–16.5)	22 (10–38)	268 ^b (121–881)
<i>P</i>	0.04	0.35	0.09	0.16	0.007
<i>Controls</i>					
0.05	0.7 (0.5–1.5)	0.5 (0.5–0.9)	7.6 (1.0–10.1)	10 (8–14)	195 (62–289)
0.15	0.5 (0.5–6.7)	0.5 (0.5–5.9)	37.4 (7.8–71.0)	18 (12–32)	1515 (346–2722)
1.0	0.75 (0.5–26.7)	0.5 (0.5–19.1)	15.2 (7.8–43.0)	18 (16–46)	785 (554–1708)
<i>P</i>	0.52	0.70	0.03	0.05	0.02

Within-group differences, Friedman's two-way ANOVA; Between-group differences, Mann–Whitney U test.

^a $P < 0.01$, Controls vs Irradiation; ^b $P < 0.05$, Controls vs Irradiation.

Post-stimulatory

Peak GH responses and time to peak GH responses were determined for each study, area under the curve (AUC) for the GH response was calculated by the trapezoid method, and data array averaging was used to assess the GH response in each study and group from the point of GHRH(1–29)NH₂ stimulation.

Non-parametric statistics were used to compare within-group (Friedman's two-way ANOVA and Wilcoxon signed rank sum test) and between-group (Mann–Whitney U test) responses in each study (SPSS, Chicago, IL, USA).

Results

Pre-GHRH(1–29)NH₂ secretory status

Bolus injection of GHRH(1–29)NH₂ was an effective stimulus to GH release and significant incremental rises in GH greater than 1.5 mU/l (or three times the standard error of the assay) were seen in all cases except two (one patient and one control; 0.05 $\mu\text{g/kg}$ GHRH(1–29)NH₂; Table 2, Fig. 1A). Basal GH concentrations were not significantly different in any study or in any group. According to the criteria defined by Devesa *et al.* (7), an endogenous secretory phase of GH release was occurring in Control 5 in Study 2 and an endogenous secretory plateau was occurring in Patient 2, Study 3; Control 4, Studies 2 and 3; and Control 5, Study 3. In all other studies, GHRH(1–29)NH₂ was administered in the refractory phase.

Irradiated subjects vs controls

The lowest dose of GHRH(1–29)NH₂ (0.05 $\mu\text{g/kg}$) produced a small but predictable GH response

Figure 1 (A) Peak GH responses following GHRH(1–29)NH₂ stimulation in Study 1 (0.05 $\mu\text{g/kg}$), Study 2 (0.15 $\mu\text{g/kg}$) and Study 3 (1.0 $\mu\text{g/kg}$) for irradiated subjects (---●---) and matched controls (—○—). (B) Area under the curve (AUC) GH responses in the 2 h following GHRH(1–29)NH₂ stimulation in Study 1 (0.05 $\mu\text{g/kg}$), Study 2 (0.15 $\mu\text{g/kg}$) and Study 3 (1.0 $\mu\text{g/kg}$) for irradiated subjects (---●---) and matched controls (—○—).

Figure 2 Serial data array averaging (mean $\pm$ S.E.M.) of the GH response following bolus GHRH(1–29)NH₂ stimulation in irradiated subjects (lower panel) and matched controls (upper panel). ●, Study 1 (0.05 μ g/kg); ■, Study 2 (0.15 μ g/kg); ▲, Study 3 (1.0 μ g/kg). Note that different scales are used on the vertical axis.

which occurred soon after injection (median time: Irradiated, 12 min vs Controls, 10 min) and did not differ significantly between the groups (peak: Irradiated, 2.2 mU/l vs Controls, 7.6 mU/l) (Table 2). The dose of 0.15 μ g/kg produced greater GH responses which occurred later (time: Irradiated, 14 min vs Controls, 18 min) and differed significantly between the groups in terms of peak GH obtained (Irradiated, 4.5 mU/l vs Controls, 37.4 mU/l; $P < 0.01$; Fig. 1A) and area under the curve (Irradiated, 179 mU/l·min vs Controls, 1515 mU/l·min; $P < 0.01$; Fig. 1B). Significant differences in peak GH concentration and AUC were also obtained when the standard dose of GHRH(1–29)NH₂ (1.0 μ g/kg) was used (peak: Irradiated, 4.8 mU/l vs Controls, 15.2 mU/l, $P < 0.05$; AUC: Irradiated, 268 mU/l·min vs Controls, 785 mU/l·min, $P < 0.05$) (Fig. 1A and B).

Within-group differences

In the irradiated group, there was no significant difference in peak GH responses in each study, although a significant increase in GH AUC was seen with each increase in dose (Study 1, 122 vs Study 2, 179 mU/l·min vs Study 3, 268 mU/l·min; $P = 0.007$, Friedman's two-way ANOVA) (Table 2). The major effect appeared to be a reduced responsiveness to GHRH(1–29)NH₂ with an altered sensitivity (Fig. 2).

Significantly greater GH peaks were obtained in the control group in Studies 2 (0.15 μ g/kg) and 3 (1.0 μ g/kg) than Study 1 (0.05 μ g/kg) (37.4 mU/l and 15.2 mU/l vs 7.6 mU/l; $P = 0.03$) and a similar situation occurred when AUC GH was considered (1515 mU/l·min and 785 mU/l·min vs 195 mU/l·min; $P = 0.02$) (Table 2). No incremental change in response with dose was seen.

In fact, the peak GH response obtained with the 0.15 µg/kg dose was greater than that with the standard supramaximal dose (1.0 µg/kg) (peak: Study 2, 37.4 mU/l vs Study 3, 15.2 mU/l; $P = 0.05$).

Discussion

Pulsatile GH release is thought to result from the interaction of the hypothalamic hormones, GHRH and SRIF. A reduction in inhibitory SRIF tone is associated with an increase in GHRH stimulation, and stored pools of GH are secreted into the circulation (13, 14). The response to exogenous bolus GHRH administration is therefore dependent upon the endogenous secretory dynamics at the time of stimulation, the amount of stored GH available for release acutely, and, ultimately, the synthetic capacity of the pituitary gland itself. Studies in the rat (15) and man (7, 8) have shown that the response to bolus GHRH stimulation is greatest at times of endogenous GH secretion (when SRIF tone is low and stored GH is available for release), and lowest during the refractory phase between pulses (when SRIF tone is high and stored GH pools are depleted). Knowledge of the pre-stimulatory secretory state is therefore important for interpreting results, and, although many studies have shown a reduced GH response to GHRH stimulation in children with severe idiopathic GH deficiency (16–18), the variability in this test has limited its usefulness in differentiating children with less marked GH insufficiency from short normal controls (19).

GH insufficiency occurs progressively following the high-dose radiotherapy used to treat brain tumours in childhood (1, 2, 20, 21) so any effects are likely to be more marked in long-term survivors. Although previous studies have shown that many patients responded to GHRH stimulation by releasing GH and suggested that GHRH deficiency was the principle aetiology of this condition the response was less than in controls and some of the studies showed a decline in peak GH concentrations with time after radiotherapy (3–6, 9). By studying a group of long-term survivors we have shown that the peak GH response and AUC were both significantly reduced in irradiated subjects compared with controls, even when low doses of GHRH(1–29)NH₂ were used. Although the peak GH concentrations were similar when 0.15 and 1.0 µg/kg were used, data array averaging revealed differences in responses between these two doses suggesting a reduction in pituitary sensitivity to stimulation. Further, as it seems likely that the response obtained with 1 µg/kg is maximal (22, 23) there is also an overall reduction in pituitary secretory capacity.

It is unlikely that any of the patients had a profound SRIF dysregulation as there was little difference in pre-stimulatory GH levels. The reduced GH responsiveness for the group was more likely to result from direct pituitary damage limiting the

amount of GH that could be synthesized and released, or from chronic GHRH hyposecretion. GHRH has important tropic properties in regulating somatotrope development and integrity in rodents (24) as well as GH synthesis (10). A similar situation may be occurring here which could account for the progressive decline in the GH response to single doses of GHRH with time. Longer-term priming studies using repeated or continual GHRH stimulation would be needed to address this question (25).

An unexpected finding in the control group was the greater peak GH response obtained using 0.15 µg/kg GHRH(1–29)NH₂ compared with 1.0 µg/kg. These results are most likely to have arisen as a result of the small sample size, although the peak response obtained in Study 2 (0.15 µg/kg) was consistently greater or equal to that obtained in Study 3 (1.0 µg/kg) in all cases, a phenomenon which is difficult to explain. One individual was in the secretory phase of GH release in Study 2 which could have produced a greater response than expected. Also, two patients had received the highest dose of GHRH(1–29)NH₂ prior to the lower dose which could have had a priming effect, although the Latin-squares design of this study would have randomized for this and the time interval between studies makes such an effect unlikely in normal GH replete individuals. At a cellular level, the GHRH receptor is G-protein linked (26) and should not experience the inhibition seen at high ligand concentrations *in vitro* with receptors that require dimerization (27). There is no evidence that mobilization of stored GH pools is reduced by higher concentrations of GHRH and *in vivo* studies of cultured somatotropes have shown typical dose–response curves (26).

In conclusion, lower doses of GHRH may be useful in demonstrating subtle differences in GH secretory dynamics. This approach has been used here to show decreased responsiveness and an altered sensitivity to GHRH(1–29)NH₂ stimulation in a group of adult male long-term survivors of childhood brain tumours with post-irradiation GH insufficiency.

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