Plasma GH Response to hpGRF-44 in Normal Children of Short Stature and Patients with GH Deficiency

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Abstract

Synthetic human pancreatic GRF (hpGRF-44) was administered as an iv bolus to 139 normal children of short stature and 63 patients with GH deficiency. After a dose of 1 and 2 µg hpGRF-44/kg BW, mean plasma GH levels peaked at 15 and 30 min, respectively, with corresponding values of 32.2±3.6 and 31.8±2.4 (±SE) ng/ml in normal but short children. There were no differences according to sex or age in plasma GH response to hpGRF-44 between the ages of 4 and 18 years. A similar plasma GH response was observed when 2 µg hpGRF-44/kg BW was administered two hours after lunch. The overall plasma GH response was greater than that of insulin-induced hypoglycemia and was similar to that obtained in the glucagon-propranolol test.

Thirty-five of 63 patients with GH deficiency did not respond to a 2 µg hpGRF-44/kg BW. However, plasma GH increases to greater than 5 ng/ml occurred in the remaining 28 patients. Their mean GH level reached a peak at 90 min with a value of 8.8±0.8 ng/ml. The peak values ranged between 5.1 and 17.8 ng/ml with a mean of 9.5±0.8 ng/ml.

These results suggest that hpGRF-44 is useful for evaluating pituitary GH reserve in children of short stature and that some patients with GH deficiency, diagnosed on the basis of established tests, have GH responses to hpGRF-44.

A majority of the idiopathic GH deficient children are thought to have defects in the hypothalamus resulting in a lack of GRF synthesis or secretion (Kaplan 1975). Such a hypothesis could not be tested until recently, when Guillemin et al. (1982) reported the isolation and characterization of a GH-releasing peptide from a pancreatic tumor in a patient with acromegaly. This peptide contains 44 amino acids with an amidated carboxyl-terminus and is designated hpGRF-44. Synthetic replicates of hpGRF were found to be highly potent and specific for the release of GH in vitro (Brazeau et al., 1982a, 1982b) and in vivo (Wehrenberg et al., 1982a, 1982b). The similarity between hpGRF and human hypothalamic GRF is corroborated by the many reports stating that hpGRFs are active in man (Thörner et al., 1983; Rosenthal et al., 1983; Takano et al., 1984). With the availability of synthetic material, we undertook a study of the effects of hpGRF-44 on the release of GH in normal children of short stature and
patients with GH deficiency, and compared GH responses to other GH provocative tests.

Materials and Methods

Human subjects

One hundred and thirty nine normal children of short stature and 63 patients with GH deficiency were studied. Normal but short children had come to our hospital because of their short stature. Their height was below 2 SD of the mean height of Japanese boys and girls of the same age group in 86 subjects and between -2 SD and the mean height in 53 subjects. Bone age, GH, T4, T3 and other pituitary hormones were measured. Normal but short children were defined as children with no physical abnormalities except short stature and no abnormal findings in the above mentioned hormone tested. A total of 100 males and 39 females, aged 4-18 years, were in this group.

The patients with GH deficiency consisted of 49 males and 14 females, aged 5-42 years. The diagnosis of GH deficiency was established on the basis of the failure of plasma GH to respond to insulin-induced hypoglycemia, glucagon-propranolol, and/or L-dopa. The GH deficiency in these patients was considered to be idiopathic in 47 patients (IGD) and to be secondary in 16 patients (SGD) (craniopharyngioma in 9, pinealoma in 2, meningitis in 2, others in 3). Ten patients had never been treated with human (h)GH, and five patients had ceased hGH treatment. The rest of the patients were treated with hGH for between 6 months and 7 years. hpGRF-44 administration was performed 2-4 days after the last hGH injection in these latter patients. All were receiving appropriate replacement therapy for other pituitary hormone deficiencies and had no detectable hGH antibody in their plasma.

Informed consent was obtained from each subject and/or the parents, and the experimental protocol was approved by the Human Subjects Investigation Committee of our department.

Peptide preparation

Human pancreatic growth hormone releasing factor (hpGRF-44) was synthesized as described (Guillemin et al., 1982). One hundred mg of the peptide dissolved in 100 ml distilled water containing 1 mM HCl and 1 mM ascorbic acid and sterilized by filtration through a 0.22 μm filter (Millipore Corp.). A hundred μg aliquot of peptide solution (100 μg/100 μl) was put in each vial, lyophilized and stored at −20°C until used. The vial was diluted with 2.0 ml of physiological saline to give a final peptide concentration of 50 μg/ml immediately before use.

Test protocol

hpGRF-44 administration was performed in the morning (AM) and in the afternoon (PM). In the case of AM, the subjects were fasted overnight and, in the case of PM the test was performed at least 2 hours after lunch. Each subject remained recumbent throughout the study and was fitted with a heparin locked cannula in a forearm vein for drawing blood, and 1 or 2 μg hpGRF-44/kg BW was given as an iv bolus injection. Blood samples for hormone measurements were drawn 0, 15, 30, 60 and 90 min after injection. The specimens were centrifuged immediately at 4°C and the plasma was separated and frozen at −20°C until it was assayed. The subjects were asked to report any side effect the moment they experienced it.

The insulin-induced hypoglycemic test (IT) was performed using iv 0.05-0.1 U/kg BW doses of regular insulin. Plasma GH and blood glucose were determined 0, 30, 60 and 90 min after the administration of insulin. The test was considered effective if the blood glucose values declined to less than 50 mg/dl or by 50 % or more. In the glucagon-propranolol (G-P) test, glucagon (2 mg, im) and propranolol (10 mg, po) were simultaneously administered, and blood was collected immediately before and 90, 120, 150 and 180 min after drug administration. In the L-dopa (250 mg, po) test, blood was withdrawn before and 30, 60, 90 and 120 min after the ingestion.

Plasma GH was measured with a commercial RIA kit (Eiken Chemical Co., Ltd., Tokyo, Japan). Human growth hormone from Kabi (Stockholm, Sweden) was used for labelling and as standard. The intra- and inter-assay coefficients of variation were 6.6 % and 8.0 % respectively. The minimal detectable plasma GH level was 1.0 ng/ml.

Bone age was estimated according to the standards of Greulich and Pyle (1959). Student's t-test was used for statistical analysis.
Results

One microgram of hpGRE-44/kg BW was given to 20 normal children of short stature. The mean plasma GH level reached a peak value of 32.2 ± 3.6 (± SE) ng/ml at 15 min and declined thereafter (Table 1 and Fig. 1). However, the response varied considerably among these 20 children. Peak plasma GH levels occurred between 15 and 90 min after injection, and the peak values varied from 11.2 to 61.2 ng/ml, with a mean of 35.6 ± 3.6 ng/ml.

Doubling the dosage of 2 μg hpGRF-44/kg BW in 87 children of short stature shifted the mean plasma GH peak to 30 min, with a value of 31.8 ± 2.4 ng/ml (Fig. 1 and Table 1). Again, the individual responses were highly variable, with the peak plasma GH level occurring between 15 and 90 min at values ranging from 7.4 to 149.5 ng/ml, with a mean value of 37.3 ± 2.4 ng/ml. These values did not differ significantly from those after the administration of 1 μg of hpGRF-44/kg BW. Fourteen patients from this group were given 2 μg hpGRF-44/kg BW, again, on different occasions, and similar maximum responses of 32.1 ± 6.0 ng/ml in the first time and 41.7 ± 7.2 ng/ml in the second time were observed. Six children from this group were given both 1 and 2 μg of hpGRF-44/kg BW on different occasions, and similar maximum responses of 39.6 ± 5.4 ng/ml and 49.8 ± 20.5 ng/ml, respectively, were obtained.

<table>
<thead>
<tr>
<th>Time after hpGRF-44 (min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>mean peak values</th>
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<tr>
<td>Normal but short children</td>
<td></td>
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<tr>
<td>1 μg/kg (n=20) AM</td>
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<tr>
<td>Mean ± SEM (SD)</td>
<td>4.6 ± 0.7 (3.3)</td>
<td>32.2 ± 3.6 (16.0)</td>
<td>31.5 ± 3.8 (16.9)</td>
<td>19.3 ± 2.6 (11.7)</td>
<td>9.8 ± 1.3 (5.7)</td>
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<tr>
<td>Mean ± SEM (SD)</td>
<td>3.5 ± 0.2 (2.1)</td>
<td>27.4 ± 2.0 (18.8)</td>
<td>31.8 ± 2.4 (22.5)</td>
<td>31.3 ± 1.8 (16.9)</td>
<td>17.6 ± 1.2 (11.3)</td>
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<tr>
<td>Mean ± SEM (SD)</td>
<td>3.3 ± 0.3 (2.1)</td>
<td>27.8 ± 2.5 (19.4)</td>
<td>32.0 ± 3.1 (24.1)</td>
<td>23.6 ± 1.9 (14.6)</td>
<td>16.6 ± 1.4 (10.5)</td>
<td>36.7 ± 2.9 (22.6)</td>
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<tr>
<td>Mean ± SEM (SD)</td>
<td>3.9 ± 0.5 (2.4)</td>
<td>26.7 ± 3.4 (17.9)</td>
<td>30.9 ± 3.7 (19.0)</td>
<td>28.4 ± 4.1 (21.1)</td>
<td>19.9 ± 2.4 (12.6)</td>
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<tr>
<td>Mean ± SEM (SD)</td>
<td>4.1 ± 0.6 (4.3)</td>
<td>30.2 ± 4.0 (27.2)</td>
<td>35.0 ± 4.4 (30.4)</td>
<td>26.9 ± 2.7 (18.8)</td>
<td>20.1 ± 2.2 (14.2)</td>
<td>41.8 ± 4.3 (29.6)</td>
</tr>
</tbody>
</table>

| GH deficient children    |   |    |    |    |    |                 |
| Subgroup I               |   |    |    |    |    |                 |
| 2 μg/kg (n=35) AM        |   |    |    |    |    |                 |
| Mean ± SEM (SD)          | 2.4 ± 0.1 (0.6) | 2.7 ± 0.1 (0.5) | 3.0 ± 0.1 (0.7) | 3.2 ± 0.1 (0.7) | 2.8 ± 0.1 (0.5) | 3.4 ± 0.1 (0.6) |
| Subgroup II              |   |    |    |    |    |                 |
| 2 μg/kg (n=28) AM        |   |    |    |    |    |                 |
| Mean ± SEM (SD)          | 2.5 ± 0.2 (0.9) | 5.6 ± 0.5 (2.8) | 7.5 ± 0.8 (4.1) | 8.8 ± 0.8 (4.2) | 6.2 ± 0.5 (2.5) | 9.5 ± 0.8 (4.2) |

AM: performed in the morning
PM: Performed in the afternoon 2 hrs after lunch
There was no significance between the responses to hpGRF-44 in males and females in this group; the mean peak values after treatment with 1 μg/kg BW were 38.2 ± 4.2 ng/ml (n=14) for males and 30.3 ± 7.4 ng/ml (n=6) for females, while 2 μg/kg BW resulted in mean peak values of 36.7 ± 2.9 (n=60) and 38.6 ± 4.1 ng/ml (n=27) for males and females, respectively (Table 1).

The plasma GH responses to 2 μg hpGRF-44/kg BW were not affected by the children's age-groups, as shown in Fig. 2. There was no relationship between the maximum GH response and the ratio of bone age to chronological age (Table 2).

Forty-seven children of short stature received 2 μg of hpGRF-44/kg BW in the afternoon around 1300-1400 clock time two hours after lunch. The mean plasma GH level reached a peak value of 35.0 ± 4.4 ng/ml at 30 min, which was not significantly different from that after 2 μg of hpGRF-44/kg BW given in the morning (Table 1). Eleven children were given hpGRF-44 both a.m. and p.m. on different occasions, and similar

![Graph showing plasma GH response to hpGRF-44](image)

**Fig. 1.** Plasma GH response to hpGRF-44. One (right panel) and 2 (left panel) μg hpGRF-44/kg BW were administered to 20 and 87 normal children of short stature, respectively. Vertical lines indicate the SD.

**Fig. 2.** Plasma GH response to 2 μg hpGRF-44/kg BW at different age-groups between 4-18 years. Vertical lines indicate the SEM.

<table>
<thead>
<tr>
<th>BA/CA</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
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<td>8</td>
<td>57.4</td>
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<tr>
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<td>&lt;0.7</td>
<td>6</td>
<td>41.0</td>
<td>12.0</td>
</tr>
<tr>
<td>0.7 ≤</td>
<td>&lt;0.8</td>
<td>17</td>
<td>28.8</td>
<td>12.6</td>
</tr>
<tr>
<td>0.8 ≤</td>
<td>&lt;0.9</td>
<td>27</td>
<td>30.7</td>
<td>13.5</td>
</tr>
<tr>
<td>0.9 ≤</td>
<td>&lt;1.0</td>
<td>24</td>
<td>39.1</td>
<td>20.5</td>
</tr>
<tr>
<td>1.0 ≤</td>
<td>&lt;1.1</td>
<td>5</td>
<td>55.8</td>
<td>37.6</td>
</tr>
</tbody>
</table>
maximum responses of 40.5 ± 7.2 ng/ml and 37.1 ± 11.4 ng/ml, respectively, were obtained. There were two children who failed to respond to over 5 ng/ml of plasma GH in the first trial administration of 2 μg hpGRF-44/kg BW. One patient responded plasma GH to 16.0 ng/ml after IT and another responded with plasma GH to 65.5 ng/ml after 2 μg of hpGRF-44/kg BW administered in the afternoon.

The maximum plasma GH levels after IT, G-P and 2 μg of hpGRF-44/kg BW in AM are shown in Fig. 3. The mean maximum GH level after hpGRF in 56 subjects was 37.1 ± 3.0 ng/ml, which was greater than that obtained after IT (14.3 ± 1.1 ng/ml, p<0.001). However, the mean maximum GH level after hpGRF was not different from that obtained after G-P studied in 33 subjects, which was 31.2 ± 2.5 and 31.4 ± 2.3 ng/ml, respectively.

Two micrograms of hpGRF-44/kg BW were given to 63 patients with pituitary dwarfism in the morning (Table 1). Thirty five patients did not respond, i.e. their plasma GH levels never rose above 5 ng/ml and we designated these patients subgroup I. However a maximum GH response between 5.1 and 17.8 ng/ml occurred in 28 patients in this group, designated subgroup II. The mean plasma GH peak shifted to 60 min with a value of 8.8 ± 0.8 ng/ml. The maximum plasma GH response below 20 ng/ml was observed in 14 of 87 normal children of short stature (○) and in 28 pituitary dwarfs (●) who responded to hpGRF-44.
Intravenous bolus administration of hp-GRF-44 produced facial flushing in two normal short children and one pituitary dwarf. The flushing appeared 1–2 min after the injection and disappeared within 3 min. However, there was no accompanying change in the plus rate. All other blood analyses, including blood count, serum electrolytes, and serum chemistries, were normal.

**Discussion**

These results show that 1 and 2 µg of hpGRF-44/kg BW stimulated the secretion of GH in a number of normal but short children in a similar way. However, the magnitude of the response varied considerably from child to child. Variable responses to hpGRF-44 were also found in normal men (Gelato et al., 1983; Rosenthal et al., 1983) and were reported by Thorner et al. (1983), who used hpGRF-40. The responsiveness of plasma GH to hpGRF-44 injection in short children, aged 4–18 yr, was affected by neither sexuality, chronological age nor retardation of bone age. These results were different from the decreased responses in normal adult men after the age 40 found in this laboratory (Shibasaki et al., 1984). The peak GH response elicited by hpGRF-44 was much greater than that obtained after the IT and similar to that obtained after G-P tests. This may be due to the fact that IT stimulate GH secretion through a central nervous system pathway (Roth et al., 1963), whereas GRF acts directly on the pituitary. G-P tests are reported as a potent GH provocation test (Parks et al., 1973) and plasma GH responses are corresponding to those obtained by GRF administration.

The results from this study show that patients with GH deficiency can be classified into two subgroups according to their plasma GH responses to hpGRF-44 administration. In one subgroup, there was no increase in GH (Subgroup I), and in the other (Subgroup II), there was a subnormal but definite rise in GH levels. However, about one-fourth of these patients were moved to subgroup I to II or vice versa, when the 2nd hpGRF-44 administration was performed on different occasions. We checked whether there was any difference between the pituitary hormone reserves in subgroups I and II in 47 patients with IGD and found no difference between them. In patients with SGD, half of the patients in subgroup I did not respond with PRL to TRH, suggesting damage to the pituitary cells. The finding that there are some patients with GH deficiency who have GH responses to hpGRF was also reported in 4 patients by Grossman et al. (1983) and in 3 of 7 patients with isolated GH deficiency by Borges et al. (1983). In our study, 3 of 6 patients with isolated GH deficiency responded to hpGRF.

All patients with IGD, who did not respond to hpGRF-44 (Subgroup I), may not have intrinsic pituitary disease. Although the maximum plasma GH response did not reach 5 ng/ml, 9 of 25 patients with IGD showed a maximum GH response twice the basal values or more. In patients with idiopathic hypogonadotropic hypogonadism, repeated administration of GnRH was successful in restoring serum levels of LH and FSH to normal (Yoshimoto et al., 1975; Reitano et al., 1975; Hoffman et al., 1982). Therefore, daily im administration of hpGRF-44 for several days or pulsatile administration of the peptide by iv infusion may induce a response to hpGRF-44 in patients who do not respond to a single iv dose of the peptide. Recently, Borges et al. (1983) reported that the administration of 0.33 µg of hpGRF-40/kg BW every 3 hours for 5 days induced plasma GH response to hpGRF-40 in a patient with isolated GH deficiency.

Maximum plasma GH response below 20 ng/ml to 2 µg of hpGRF-44/kg BW was observed 14 of 87 normal children of short
stature, but there were no patients with GH deficiency whose plasma GH rose above 20 ng/ml. These data suggest that half of the patients with GH deficiency have little GH reserve in the pituitary.

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References


