Plasma Growth Hormone Responses to Repetitive Administrations of Growth Hormone Releasing Factor in Patients with Pituitary Dwarfism

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Abstract

Plasma growth hormone (GH) responses to the repetitive administrations of synthetic human pancreatic growth hormone releasing factor (hpGRF-44) were studied in 15 patients with GH deficiency (11 diagnosed as idiopathic and 4 diagnosed as secondary to hypothalamo-pituitary tumor). hpGRF-44 was administered by single iv bolus (2 fg/kg), repetitive im (100 fg, twice a day), and/or repetitive iv infusion (2.5 fg/min for 90 min, once a day) for three to six consecutive days.

Three of the eleven idiopathic GH deficient patients had plasma GH responses to both single iv bolus injection and repetitive administrations by im, or iv infusion of hpGRF. In four of the remaining eight, who had not had peak plasma GH levels above 5 ng/ml to a single iv bolus of the peptide, repetitive administrations of hpGRF-44 by im injection and/or iv infusion induced GH responses to the peptide. In the four patients with secondary GH deficiency, three had plasma GH response to hpGRF administration but one patient, who had indications of pituitary disorder, did not show any plasma GH response to either single iv injection or repetitive administrations of hpGRF-44.

These data show that repetitive administrations of hpGRF-44 can induce plasma GH responses in some GH deficient patients who do not respond to a single iv bolus of the peptide.

A majority of idiopathic pituitary dwarfs are thought to have defects in the hypothalamus, resulting in a lack of growth hormone releasing factor (GRF) synthesis or secretion (Kaplan 1975). The hypothesis has been tested by several research groups (Borges et al., 1983; Grossman et al., 1983; Takano et al., 1984a) using synthetic replicates of human pancreatic growth hormone releasing factors (hpGRFs) that have recently been isolated and characterized from pancreatic tumors in patients with acromegaly (Guillemin et al., 1982; Esch et al., 1982; Rivier et al., 1982). We have previously reported that forty percent of tested pituitary dwarfs have peak plasma GH responses higher than 5 ng/ml induced by a single iv bolus injection of 2 µg/kg of the 44 amino acid form of hpGRF (hpGRF-44). However, other patients who do not respond
to the single iv bolus injection of the peptide may not all have intrinsic pituitary defects. Since many laboratories have reported that repeated administrations of GnRH to patients with idiopathic hypogonadotropic hypogonadism were successful in restoring plasma levels of LH and FSH to normal (Yoshimoto et al., 1975; Reitano et al., 1975; Hoffman and Crowley 1982), similar repetitive administrations of hpGRF-44 may induce plasma GH responses in these non-responding pituitary dwarfs.

With the availability of large quantities of synthetic material, we have carried out such a study to investigate whether repetitive administrations of hpGRF-44 by im or iv infusion would induce plasma GH responses in patients with pituitary dwarfism who did not respond to a single iv bolus injection of the peptide.

Materials and Methods

Human subjects

Fifteen patients with pituitary dwarfism were chosen for this study. Eleven of them were diagnosed as idiopathic GH deficiency while the remaining four were secondary to hypothalamopituitary tumors (subjects 12 and 13 have a craniopharyngioma; 14 and 15 have an ectopic pinealoma). The clinical data on the 15 patients are summarized in Table 1. The diagnosis of GH deficiency was established by failure of peak plasma GH to rise above 5 ng/ml after insulin-induced hypoglycemia (ITT) (Roth et al., 1963), glucagon-propranolol (Parks et al., 1973), and/or L-dopa (Mims et al., 1973) tests. All of the 15 patients have been treated with hGH for periods ranging from 3 months to 7 years as well as medicated with the appropriate replacement therapy for other deficient pituitary hormones. They had no detectable hGH antibody in their plasma resulting from the hGH treatment.

Informed consent was obtained from each patient and/or the parents, and the study protocol was approved by our Department Review Committee at Tokyo Women's Medical College.

Peptide preparation

Human pancreatic growth hormone releasing factor (hpGRF-44) was synthesized by solid phase methodology as described (Guillemin et al., 1982). One hundred milligrams of the peptide were dissolved in 100 ml distilled water containing 1 mM ascorbic acid and sterilized by filtration through a 0.22 μm filter (Millipore Corp.). One hundred microliter aliquots of the peptide solution (100 μg) were prepared in sterile vials, lyophylized, and stored at −20°C. A vial was diluted with 2 ml physiological saline immediately before injection.

Test protocol

The patients were fasted and remained recumbent throughout the study. Each subject was fitted with a heparin-locked cannula in a forearm vein for drawing off blood and for the administration of hpGRF-44. The experiment was performed two to four days after the last injection of hGH in these patients.

I: Single iv bolus injection of hpGRF-44 (Standard GRF test)

Each subject received an iv bolus injection of 2 μg per kg BW of hpGRF-44, and 2 ml blood samples were drawn off at 0, 15, 30, 45, 60, and 90 min after injection. The samples were kept on ice and centrifuged immediately at 4°C after the last samples were taken. The plasma was separated and stored frozen at −20°C.

II: Repetitive administrations of hpGRF-44

a: One hundred micrograms of hpGRF-44 were injected intramuscularly twice a day at 0800 and 2000 hours for five consecutive days into six patients (No. 2, 3, 4, 12, 14 and 15). The standard GRF test was performed in these patients before the repetitive im injections and on the sixth day.

b: hpGRF-44 was infused at a dosage of 2.5 μg/min for 90 min for three to six consecutive days into ten patients (No. 1, 3, 5, 6, 7, 8, 9, 10, 11 and 13). The plasma GH responses during the infusion were measured at 0, 15, 30, 45, 60, and 90 min.

Radioimmunoassay for hGH

Plasma hGH was measured with a commercially available GH radioimmunoassay kit (Eiken Chemical Co., Ltd.). Human growth hormone
| CA, chronological age: | B4, bone age: | +, positive; | D, delayed response to TRH; | +, +, +, - | TRH, no change from the basal level; | 6.2 | 6.3 | 6.8 | 2.2 | 2.4 | 1.5 | 1.0 | 1.2 | 1.3 | 1.1 | 1.4 | 1.0 | 1.2 | 1.0 | 1.0 |
|------------------------|--------------|------------|-----------------|--------------|-----------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
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**Table 1.** Clinical data of 15 patients with GH deficiency.
Fig. 1. Plasma GH responses to one iv bolus administration of 2 μg/Kg hpGRF-44 before (●—●) and after (○—○) im injection of hpGRF-44 for five consecutive days in six patients with GH deficiency. Each patient received an im injection of 100 μg hpGRF-44 twice a day for five days. Inserted parentheses indicate the integrated area under the curve of plasma GH in ng/ml-h after the administration of 2 μg/Kg hpGRF-44 before and after im treatment (before→after). The number at the top of each panel corresponds to the patient shown in Table 1.

Results

I: Response of plasma GH to a single iv bolus injection of hpGRF-44
Two micrograms of hpGRF-44 per kg BW were given as an iv bolus injection to 15 patients with pituitary dwarfism. Nine patients (No. 1, 3, 4, 7, 8, 9, 10, 11 and 13) did not show significant responses because their peak plasma GH levels were never above 5 ng/ml at any time during the test (Table 1). In the remaining six patients (No. 2, 5, 6, 12, 14 and 15), maximal GH rises ranging from 6.2 to 8.7 ng/ml were detected (Table 1).

IIa: Response of plasma GH to repetitive im injections of hpGRF-44
Six patients (No. 2, 3, 4, 12, 14 and 15) received an im injection of 100 μg hpGRF-44 twice a day for five consecutive days. The standard GRF test was performed in
These patients before and after the repetitive im injections. All patients showed plasma GH responses after the repeated im administrations; their peak plasma GH levels ranged between 5.8 and 10.6 ng/ml (Fig. 1). In particular, two of these patients (No. 3 and 4) who had not responded to a single iv bolus injection of hpGRF-44, had their maximal plasma GH values increased from 4.1 to 10.6 and from 4.4 to 7.4 ng/ml, before and after the treatment, respectively. Likewise, the integrated area under the plasma GH curve increased from 4.8 to 12.0 and from 5.1 to 7.9 ng/ml-h, respectively.

**IIb: Response of plasma GH to repetitive iv infusion of hpGRF-44**

Ten patients (No. 1, 3, 5, 6, 7, 8, 9, 10, 11 and 13) received an iv infusion of hpGRF-44 at a dosage of 2.5 μg/min for 90 min for
three to six consecutive days (Fig. 2). In four patients (No. 1, 3, 5 and 6), greater GH responses to hpGRF-44 were induced by repetitive iv infusions of hpGRF-44 on the days following the first day of infusion (Fig. 2A and 2B), i.e. the peak plasma GH increased from 4.4 to 7.5, from 3.4 to 7.5, from 3.3 to 9.9, and from 3.8 to 12.5 ng/ml, on the first day versus the day when the greatest response was obtained respectively, and likewise the integrated area under the GH curve increased. In addition, one patient (No. 9) showed a minute GH response to hpGRF-44 on the day following the first day of infusion, i.e. the peak plasma GH level rose from 2.6 to 4.9 ng/ml, and the integrated GH increased from 2.6 to 5.4 ng/ml-h, on the first versus the third day of infusion (Fig. 2A).

However, in the remaining five patients (No. 7, 8, 10, 11 and 13), plasma GH responsiveness did not change significantly during repetitive iv infusion of hpGRF-44 (Fig. 2A).

Discussion

We have studied the responses of plasma GH to repetitive hpGRF-44 administrations to patients with pituitary dwarfism. An arbitrary value of 5 ng/ml has been used in Japan to evaluate GH response to provocative stimuli such as insulin induced hypoglycemia. Therefore we have used this value to temporarily evaluate the GH response to hpGRF-44 in this study. In eleven patients with idiopathic GH deficiency, three had a peak plasma GH level above 5 ng/ml in response to a single 2 μg/kg iv bolus injection of hpGRF-44, but eight did not. After repetitive administrations of hpGRF-44 by either im injection or iv infusion, four of these patients (No. 1, 3, 4 and 9) in whom there was no plasma GH response to a single iv bolus injection of hpGRF-44, showed plasma GH responses to hpGRF-44. Thus, repetitive administrations of hpGRF-44 can induce plasma GH response in some idiopathic GH deficient patients who do not respond to a single iv bolus of the peptide. This finding is similar to the induction of plasma gonadotropin response after repetitive administrations of GnRH in patients with idiopathic hypogonadotropic hypogonadism (Yoshimoto et al., 1975; Reitano et al., 1975; Hoffman and Drowley 1982).

The remaining four idiopathic pituitary dwarfs, who did not have a plasma GH response to the repeated administrations of hpGRF-44, may not all have intrinsic pituitary diseases. They had normal PRL responses to TRH, and delayed or normal TSH responses to TRH, and some of them had normal LH and FSH responses to GnRH, as shown in Table 1. In addition, they developed hypothyroidism during hGH therapy. These endocrinological data indicate that they may not have defects in the pituitary. Therefore, the frequency and duration of administration of hpGRF-44 in our present study may not be sufficient to induce plasma GH response to the peptide in these four patients. The other possible reason for them to fail to respond to repeated administrations of hpGRF-44 is the dose of hpGRF-44. However, there was no correlation between the dose per kg used and the GH responses in each experiment in this study. Furthermore, it has been reported that somatomedin inhibits pituitary GH secretion by stimulating somatostatin secretion (Berelowitz et al., 1981) and blunting the response to GRF (Brazeau et al., 1982). Therefore, their plasma somatomedin-C values may affect plasma GH responses to hpGRF-44. In the present study, the patients had been off hGH therapy for a very short time (2 to 4 days) and their plasma somatomedin-C levels varied from 0.1 to 1.0 U/ml (data not shown). However, there was no relationship between their plasma somatomedin-C values and their GH responses to hpGRF-44. In addition long
term treatment of hGH may cause atrophy of somatotrophs and then may affect hGH responses to hGRF. But, in the present study we could not find the relationship between plasma GH responses to hpGRF-44 and duration of hGH treatment.

In the four GH deficient patients with organic lesions of the hypothalamus or pituitary, three (No. 12, 14 and 15) responded to a single iv bolus injection of hpGRF-44. They also responded to the peptide after repetitive im injections of hpGRF-44 for five days, but these responses were not different from those before treatment. One patient (No. 13), who did not respond at all after repetitive administrations of hpGRF-44, had a craniopharyngioma that was resected and irradiated before he participated in this study. His clinical profile presented a case of panhypopituitarism because he showed no pituitary hormone responses to GnRH, TRH, and insulin induced hypoglycemia.

We have previously shown that the majority of normal children with short stature have a peak plasma GH level above 20 ng/ml in response to a single iv bolus injection of hpGRF-44 (Takano et al., 1984b). In this study, the maximal values of plasma GH induced by hpGRF-44 never rose above 20 ng/ml in the pituitary dwarfs even after repeated treatment with the peptide. To determine whether GH responses to hpGRF-44 are induced in pituitary dwarfs to the same degree as in normal children, further study will be required.

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References


