Effect of Intravenous Administration of Growth Hormone-Releasing Peptide on Plasma Growth Hormone in Patients with Short Stature

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Abstract. Synthetic hexapeptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ (GHRP) is known to have remarkable GH releasing activity in a variety of species, especially in men. In this study, we examined the effects of GHRP (3µg/kg BW, iv) on plasma GH in 41 patients with short stature (29 males and 12 females, aged from 8-28 yrs) due to different causes (one after pituitary adenomectomy, nine with perinatal abnormality, two with Turner syndrome, 28 due to unknown causes), and compared the GH responses to GHRP with those to GHRH, L-dopa and insulin. GHRP did not cause any symptoms after the injection. Among 35 patients who had no organic abnormality at the hypothalamo-pituitary region in MRI, plasma GH rises of more than 7µg/L were observed in 34 patients after GHRP injection, 32 patients after GHRH, 21 patients after L-dopa and 24 patients after insulin-hypoglycemia. The mean ±SE peaks of plasma GH were 26.6 ± 2.8 µg/L after GHRP, 24.0 ± 3.1 µg/L after GHRH, 7.1 ± 1.1 µg/L after L-dopa and 7.5 ± 1.1 µg/L after insulin. Plasma GH peaks appeared within 45 min after GHRP injection, which was earlier than the time of GH peaks in other provocation tests. On the other hand, GHRH slightly but consistently increased plasma GH levels in short patients with pituitary stalk transsection, whereas GHRP did not. Although the mechanism of this finding remains unclear, other factors may be necessary for GHRP to exert its full activity in GH release.

In summary, GHRP increased the plasma GH level in patients with short stature more safely, consistently and quickly than other GH stimulants. The GHRP test will therefore be a useful and practical way to examine the GH secretory reserve in short stature.

Key words: growth hormone-releasing peptide (GHRP), short stature, GH

Introduction

His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ (GHRP) is a potent stimulator of GH secretion from the pituitary somatotroph [1-3]. We have already reported that an intranasal
administration of GHRP increased plasma GH levels and consecutive administrations increased plasma IGF-I levels in normal men [4]. It has been suggested that GHRP mediates through different receptors from those for GHRH [5-11], and Cheng et al. have recently reported that GHRP stimulates GH release from the pituitary somatotroph through an activation of protein kinase-C [5]. Thus, GHRP may be useful not only for evaluating the secretory reserve of GH but also for differentiating the cause of deficient GH release by comparison with the results of conventional GH provocative tests.

In this study, we examined the effects of a bolus injection of GHRP on GH release in short patients with or without organic lesions, and subsequently compared the GH secretory profile in the GHRP test with those in other conventional GH stimulation tests.

**Materials and Methods**

**Subjects**

Forty-one patients (12 females and 29 males, aged 8-29 yrs; mean 13 yrs) with short stature (defined as 2.0 SD below the mean for age and sex) were examined. One patient had undergone operation for pituitary adenoma, and her remaining pituitary had become atrophic on MRI. Four patients had a history of breech delivery, and their pituitary stalks were obviously transected on MRI. Four other patients had a history of perinatal abnormalities such as asphyxia but, since MRI revealed no organic abnormalities in the hypothalamo-pituitary region, they were counted as patients without organic lesions. Two patients suffered from Turner syndrome. The others were short but otherwise normal patients, who showed no abnormalities on brain MRI, and no evidence of chronic disease, genetic bone disease or malnutrition.

In this study, patients with organic lesions were defined as those who had macroscopically visible abnormalities on MRI. All patients were euthyroid at the time of the examination. Patients who were receiving GH therapy stopped injections at least two weeks before the examination. All the subjects gave informed consent to participate in this study.

**Study**

Synthetic GHRP was dissolved in saline, and aliquots were sterilized by passage through 0.22 μm Millex-GV filter (Millipore Corp., Bedford, MA). After an overnight fast, all subjects were kept at bed rest from 08:30 h until the end of the test, and an indwelling catheter was placed in the antecubital vein. GHRP was given as a dose of 1 μg/kg BW. The samples for GH measurement were collected immediately before and 15, 30, 45, 60, 90 and 120 min after GHRP injection. Plasma GH responses to synthetic hGHRH (1-44)NH₂ (1 μg/kg BW), L-dopa (10 mg/kg BW po) or regular insulin (0.1 u/kg BW iv) were examined on different days in the same patients who had taken the GHRP test.

**Assay**

Serum GH was measured in duplicate using commercially available IRMA kits (Pharmacia Ltd., Osaka, Japan).

**Results**

Intravenous administration of either GHRP or GHRH did not cause any side effects in any of the patients examined. Some of the patients noticed hotness, sweating and drowsiness in the insulin-hypoglycemia test and nausea in the L-dopa test.

An i.v. injection of 1 μg/kg GHRP increased plasma GH levels in all short subjects without organic lesions. They were increased to 26.6±2.8 μg/L within 15 min after GHRP injection. The plasma GH peaks appeared earlier after GHRP injection than after GHRH, L-dopa or insulin (Fig. 1). There was no significant difference between the peak GH level after GHRP and after GHRH. This result is of some interest, since
GH Response to GHRP in Short Stature

Fig 1. Plasma GH responses to various stimuli in 35 short patients without organic lesion. Mean ± SE are shown. Plasma GH peaks appeared 15, 30, 45 and 60 min after GHRP (1 µg/kg BW, iv) (panel a); GHRH (1 µg/kg BW, iv) (panel b); L-dopa (10 mg/kg BW, po) (panel c); and regular insulin (0.1 u/kg BW, iv) (panel d) administration, respectively.

GHRP had a more potent effect on GH secretion than GHRH in normal men [12]. On the other hand, it was unique for a GHRP test that GHRP could increase the plasma GH level so rapidly that peaks appeared 15 min after injection in more than half of the patients and within 45 minutes in all the patients without organic lesions (Fig. 2).

As the times of GH peaks were various after GHRH, L-dopa or insulin administration, the average of each plasma GH peak was compared. They were 30.7±2.8, 30.3±3.0, 10.0±1.2 and 10.2±1.1 µg/L (Mean ± SE) after GHRP, GHRH, L-dopa and insulin administration, respectively. There was no significant difference between the GH peaks after GHRP and GHRH.

Plasma GH responsiveness to GHRP was compared with that to GHRH, L-dopa and insulin in the individual patients (Fig. 3). Plasma GH responsiveness to GHRP was not correlated with that to GHRH, L-dopa or insulin. When the plasma GH peak levels after GHRP were compared with those after GHRH, there were eight patients whose GH peaks were less than 10 µg/L after GHRH despite plasma GH rises to more than 10 µg/L after GHRP. On the other hand, there were only two patients in whom plasma GH peak levels were less than 10 µg/L after GHRP but GHRH could increase plasma GH up to more than 10 µg/L. When plasma GH peaks after GHRP were compared with those after L-dopa or insulin administration, the relation was similar to those after GHRH and GHRP.

It was of interest that GHRP failed to increase plasma GH levels in short patients with pituitary transsection (classical pituitary dwarfism) in whom a single injection of GHRH resulted in a slight but significant increase in plasma GH (Fig. 4). This phenomenon was observed in all four patients.
Fig 2. Time-related distribution of plasma GH peaks in various GH provocative tests. Numbers of patients with short stature whose GH peaks appeared at the indicated time after GHRP (panel a), GHRH (panel b), L-dopa (panel c) or insulin (panel d) are shown.

Discussion

GHRP, a synthetic hexapeptide designed by a combination of conformational energy calculation and evaluation of GH releasing potency [13,14], is known to stimulate GH secretion in many species [3] especially in men [4,12,15]. In this study we demonstrated that GHRP consistently stimulated GH secretion as compared with other provocative stimuli such as GHRH, L-dopa and insulin-hypoglycemia, and also observed that the plasma GH peaks appeared rapidly, within 45 min after injection, in all the subjects who responded to GHRP. Bowers et al. [16] measured plasma irGHRP levels after GHRP bolus injection and found that it rose immediately after injection and gradually decreased within 20 min of its half-life. The rapid effect of GHRP on plasma GH after administration in short patients is compatible with the evidence that GHRP acts directly on the pituitary. It is of interest that GHRH increased plasma GH levels in short patients with pituitary stalk transsection in whom GHRP failed to cause any increase. Though the reason of this finding remains unclear, it is possible that GHRP might require some other factor from the hypothalamus to exert its full activity in GH

with pituitary stalk transsection. Neither GHRP nor GHRH increased the plasma GH level in a patient who had undergone pituitary adenomectomy, whose remaining pituitary was atrophic and not visible on MRI.
Fig 3. Comparison of plasma GH peaks after GHRP and after GHRH, L-dopa, or insulin administration. There was no correlation between GH responsiveness to GHRP and that to GHRH, L-dopa or insulin. Open symbols represent patients with organic lesions, and closed symbols represent those without organic lesions.

Fig 4. Plasma GH responses to various stimuli in short patients with organic lesions. Each point represents the mean ± SE of five patients.

stimulation. Alternatively, the composition of the subpopulation of somatotrophs responsive to GHRH and GHRP might change in the pituitary after stalk transection. We recently demonstrated, using a double reverse hemolytic plaque assay method, that distinct classes of somatotrophs mediate GHRP- and GHRH-stimulated GH release in rats (our unpublished data). It is important for examination of short subjects to estimate how much GH secretory potency is in reserve as well as to explore the cause of their short stature. Since most of the patients are children under compulsory education, a safe, rapid and reliable test is desirable to assess the pituitary GH reserve. GHRP might be a useful agent for this purpose.
References


