The Inter- and Intra-Subject Variabilities of Plasma GH Response to Human Growth Hormone-Releasing Hormone (1-44) NH₂ in Men

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Abstract

The effects of intravenously given human growth hormone-releasing hormone (1-44) NH₂ (hGRH-44) on growth hormone (GH) secretion were studied in normal men.

A wide variability of intersubject GH response to hGRH-44 was observed. The peak plasma GH levels in response to 50, 100 and 200 µg hGRH-44 in 7 normal men were 9.1 ± 3.2 ng/ml (Mean $\pm$ SEM), 19.3 ± 3.3 ng/ml and 22.4 ± 4.0 ng/ml, respectively. Both the mean peak values for plasma GH response to 100 and 200 µg were significantly greater than that for 50 µg hGRH-44 injection ($p < 0.01$), although there was no significant difference of the mean peak plasma GH values and mean concentrations at each time point, except for those at 120 min, when 100 or 200 µg hGRH-44 was administered. A significant difference in the mean amount of plasma GH secreted in response to hGRH-44 was observed only between 50 and 200 µg hGRH-44 injection ($p < 0.01$). Furthermore, a dose-related plasma GH increase in response to hGRH-44 was not always observed in each subject.

In contrast to the wide intersubject variability, the difference among responses of plasma GH to 100 µg or 200 µg of hGRH-44 given at multiple times separated by intervals of at least 1 week in each individual was relatively small.

These results suggest that the wide intersubject variability could cause no dose-related GH response to hGRH-44 in doses of 50, 100 and 200 µg and that the intrasubject variability was small enough to evaluate the GH secretion by a single hGRH-44 injection, a procedure not adopted before.

Three peptides with growth hormone-releasing activity were originally isolated from human pancreas tumors that had

caused acromegaly. Amino acid sequence determination showed that they were closely related in structure and contained 37, 40 and 44 residues, respectively (Guillemin *et al.*, 1982; Rivier *et al.*, 1982). Recently human

hypothalamic growth hormone-releasing hormones (hGRHs) were shown to be identical to 44 and 40 residue GH releasing peptides isolated from pancreas tumors (Ling *et al.*, 1984).

Human GRHs have been demonstrated to be specific and potent secretagogues for GH release in normal human (Thorner *et al.*, Gelato *et al.*, 1983; Shibasaki *et al.*, 1984a, Vance *et al.*, Gelato *et al.*, 1984). However, the dose-related plasma GH response to hGRH injection was still controversial (Rosenthal *et al.*, Wood *et al.*, 1983; Vance *et al.*, Gelato *et al.*, 1984). These papers indicated the intersubject variability of plasma GH response to hGRH (Thorner *et al.*, Gelato *et al.*, Rosenthal *et al.*, Wood *et al.*, 1983, Vance *et al.*, 1984), but there was no report in which the intrasubject variability of plasma GH response to hGRH was studied. This prompted us to examine intra and intersubject variabilities of plasma GH response to a single dose of hGRH-44

in healthy men in order to confirm the reliability of hGRH injection test in evaluating GH secretion.

Subjects and Methods

Human subjects

Thirteen healthy men aged 21–41 years and weighing 48–68 kg were chosen for this study.

Informed consent was obtained from each subject before this study. The experimental protocol for this test was reviewed and approved by our departmental review committee of the Tokyo Women's Medical College.

GRH test

The GRH test was described earlier in our papers (Shibasaki *et al.*, 1984a; Shibasaki *et al.*, 1984b; Masuda *et al.*, Imaki *et al.*, 1985). Seven volunteers (Cases 1–7) were administered 50, 100 and 200 μ g hGRH-44 and another five (Cases 8–12) were injected with two doses of hGRH-44, 50, 100 or 200 μ g, at different times. Furthermore, five subjects (Cases 1, 2, 4, 5, and 14)

Fig. 1. Response of plasma GH to intravenously administered hGRH-44 in doses of 50, 100 and 200 μ g in 7 normal men.

participated in a 100 μ g hGRH-44 test and six subjects (Cases 1, 2, 4, 8, 9, and 13) received a 200 μ g hGRH-44 injection twice or three times separated by intervals of at least 1 week.

Radioimmunoassay for GH

Radioimmunoassay (RIA) for plasma GH was performed with human GH standard and anti-human GH serum generously supplied by NIADK's Pituitary Hormone Distribution Program. The intra- and inter-assay coefficients of variation were 6.5% and 7.9%, respectively. The minimal detectable dose was 70–80 pg/tube.

Peptides

The hGRH-44 in this study was synthesized by solid-phase methodology as described earlier (Ling *et al.*, 1984). The synthetic products have the correct amino acid composition and a purity level shown by reverse-phase high performance liquid chromatography to be higher than 95%.

Results

Figure 1 shows the responses of plasma GH to the intravenously administered 50, 100 and 200 μ g hGRH-44 in seven healthy men aged 22–31 years (mean 26) and weighing 57–69 kg (mean 64). It was apparent that there was a wide variation in GH response to the same dose of hGRH-44 in normal subjects. After the administration of 50 μ g hGRH-44, the plasma GH increased from the basal value of 1.8 ± 0.6 ng/ml (Mean $\pm$ SEM) to the peak value of 9.1 ± 3.2 ng/ml in the first 15–30 min. However, there were two volunteers in their twenties whose plasma GH failed to show a significant increase after the 50 μ g hGRH-44 injection. One hundred μ g hGRH-44 induced an increase in the plasma GH level from 1.4 ± 0.3 ng/ml to 19.0 ± 3.3 ng/ml at 15–45 min after the injection. The plasma GH level rose from the basal levels of 1.0 ± 0.3 ng/ml to the peak level of 22.4 ± 4.0 ng/ml at 45–60 min after the administration of 200 μ g hGRH-44. Both the mean peak values of plasma GH in res-

Fig. 2. Mean plasma GH concentrations in response to 50, 100 and 200 μ g hGRH-44 in 7 normal men.

▲—▲ 50 μ g, ●—● 100 μ g, ■—■ 200 μ g.

ponse to 100 and 200 μ g hGRH-44 were significantly greater than that in the 50 μ g hGRH-44 injection ($p < 0.01$). However, there was no significant difference between the plasma GH peak values for 100 and 200 μ g hGRH-44 administration.

The mean plasma GH changes after the injection of each dose of hGRH-44 are shown in Figure 2. There was no significant difference between plasma GH levels for 50 and 100 μ g hGRH-44 administration at each time point, whereas each mean plasma GH value at 30 ($p < 0.05$), 45 ($p < 0.005$),

Fig. 3. Each plasma GH response after hGRH-44 injection in doses of 50, 100 and 200 μ g in 12 normal men. $\blacktriangle$ - $\blacktriangle$ 50 μ g, $\bullet$ - $\bullet$ 100 μ g, $\blacksquare$ - $\blacksquare$ 200 μ g.

60 ($p < 0.005$) and 90 min ($p < 0.05$) after the 200 μ g hGRH-44 injection was significantly higher than that in response to 50 μ g hGRH-44. A significant difference in the mean plasma GH concentrations was obtained only at 120 min ($p < 0.005$) between 100 and 200 μ g hGRH-44 administration.

The mean total amounts of plasma GH response to 50, 100 and 200 μ g hGRH-44 for 2 hr were 446.1 ± 207.1 ng.min/ml (Mean $\pm$ SEM), 982.4 ± 174.2 and 1683.4 ± 304.7 , respectively. There was a significant difference in the mean total amounts of plasma GH only between the administration of 50

μ g and 200 μ g hGRH-44.

The plasma GH responses in 7 normal volunteers (No. 1-7) who were injected with 50, 100 and 200 μ g hGRH-44 and in the other 5 healthy volunteers (No. 8-12) administered two doses of hGRH-44 are demonstrated in Figure 3. The dose-related plasma GH increase in response to hGRH-44 was not always observed in each subject. Two subjects in their twenties (No. 1 and 4) showed no significant increase due to 50 μ g hGRH-44, though they had considerable plasma GH responses after both 100 and 200 μ g hGRH-44 administration. In the

Fig. 4. Plasma GH responses in two or three 100 μ g hGRH-44 tests in 5 normal men.

subject (No. 12) aged 41 years the plasma GH level did not rise in response to either 100 or 200 μ g hGRH-44.

The reproducibility of a subject's plasma GH response to intravenously administered hGRH-44 is illustrated in Figures 4 and 5. Five volunteers participated in the 100 μ g hGRH test twice or three times at intervals of at least a week. Although their plasma GH peak values in response to 100 μ g hGRH-44 were different in some cases, the amounts of plasma GH increase were alike in all subjects. Six subjects received 200 μ g of hGRH-44 twice or three times at intervals of at least a week. Plasma GH responses to hGRH-44 were similar in four subjects (Cases 1, 4, 8 and 9). Although the other two volunteers (Cases 2 and 13) showed varying plasma GH responses with different

peak values, the response patterns in all subjects were similar.

Among all these normal subjects there were no significant changes in plasma LH, FSH, TSH, PRL, cortisol or glucose levels following the administration of hGRH-44 (data not shown).

Almost all subjects felt flushing and a warm sensation in the face and neck between 30 sec and 5 min after injection of hGRH-44 at a dose of 100 μ g or higher and had observable facial and neck flushing within 2 min of administration which diminished in approximately 10 min. Thermography detected a 1°C increase in temperature in a subject's facial surface in response to hGRH-44 (data not shown). However, the blood pressure and pulse rate were unchanged. There was no abnormality in the complete

Fig. 5. Plasma GH Responses in two or three of 200 μ g hGRH-44 tests in 6 normal men.

blood cell count, general urinalysis or blood chemistry one week after a hGRH test in any of the subjects (data not shown).

Discussion

The intravenous administration of doses of 50, 100 and 200 μ g of hGRH-44 were performed in 7 normal subjects. These doses of 50, 100 and 200 μ g in our study corresponded to about 0.8, 1.6 and 3.0 μ g/kg body weight, respectively. We observed that there was a significant difference between the peak plasma GH values in response to 50 μ g hGRH-44 and those evoked by 100 or 200 μ g hGRH-44 and that the mean plasma GH level at 120 min after the injection of 200 μ g hGRH-44 was significantly

higher than that following 100 μ g hGRH-44, although there was no significant difference between the peak values of plasma GH for 100 and 200 μ g hGRH-44 administration. Concerning the dose-related GH response to the hGRH-44 injection, Rosenthal *et al.* (1983) reported that no significant relationship between the peak plasma GH levels and the dose of hGRH-44 could be demonstrated by using 0.5, 5 and 10 μ g/kg body weight hGRH-44. Vance *et al.* (1984) concluded that no dose-response relationship between 0.1, 0.33, 1.0, 3.3 and 10 μ g/kg body weight of hGRH-40 was found and these results might be caused by the considerable difference in response among subjects. On the other hand, Wood *et al.* (1983) indicated that hGRH-44 in doses of 10, 30 and 100 μ g dose-dependently stimu-

lated the release of GH. These results therefore suggest that the dose-related GH response to hGRH is observed only when the intersubject variation in plasma GH response is small and the dose of hGRH is less than 100 μg .

The considerable intersubject variation in plasma GH response after the hGRH-44 injection was demonstrated in this study as in others (Thorner *et al.*, Gelato *et al.*, Rosenthal *et al.*, Wood *et al.*, 1983; Vance *et al.*, 1984). The peak plasma GH values caused by 50 μg hGRH-44 in 8 subjects aged below forty years ranged from 2.3 ng/ml to 20 ng/ml and there were 2 non-responders, who showed a considerable GH increase in response to 100 and 200 μg hGRH-44. Thorner *et al.* (1983) also reported that 2 subjects out of the 6 normal in their twenties did not show a significant response of plasma GH after the 1 $\mu\text{g/kg}$ body weight hGRH-40 injection, but had a normal GH response to insulin-induced hypoglycemia. So these results indicate that 50 μg or 1 $\mu\text{g/kg}$ body weight hGRH was not enough to fully stimulate GH secretion in these young non-responders, who might have a decreased responsiveness of the pituitary to hGRH or increased somatostatin secretion. Therefore, the injection of at least 100 μg hGRH-44 is recommended in order to evaluate the GH secretion. In one subject (Case 12) aged 41 years neither 100 μg nor 200 μg hGRH-44 elicited a response in plasma GH. Since our previous paper (Shibasaki *et al.*, 1984a) indicated the existence of an age-related decrease in plasma GH response to hGRH-44 in men, especially in men over forty years old, the absence of plasma GH response in this subject (Case 12) might be due to the effect of aging.

In this study we demonstrated not only considerable intersubject variation but also a small intrasubject variation in hGRH-44-induced-GH secretion in normal subjects. The responses of plasma GH to hGRH-44 administration in doses of 100 μg and 200

μg showed similar patterns of plasma GH secretion in each case. It can be therefore concluded from this study that a single injection of hGRH-44 in a dose of at least 100 μg is reliable enough to determine whether a response of plasma GH secretion is obtained or not.

Acknowledgements

The research was supported by a grant from the Japanese Ministry of Education, Science and Culture as well as NIH grants HD-09690 and AM-18811 from the U.S.A. We thank Drs. Ihara and Minami, Nippon Medical School, for their cooperation and Miss N. Inoue for secretarial assistance.

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