Preserved Growth Hormone (GH) Secretion in Aged and Very Old Subjects after Testing with the Combined Stimulus GH-Releasing Hormone plus GH-Releasing Hexapeptide-6

DRAGAN MICIC, VERA POPOVIC, MIRJANA DOKNIC, DJURO MACUT, CARLOS DIEGUEZ, AND FELIPE F. CASANUEVA

Institute of Endocrinology, University Clinical Centre (D.M., V.P., M.D., D.M.), Belgrade Yugoslavia Y-11000; and Department of Physiology (C.D.) and Medicine (F.F.C.), School of Medicine, Santiago de Compostela University, Santiago de Compostela, Spain E-15780

ABSTRACT

Either spontaneous or pharmacological stimulated GH secretion is reduced with advanced age. This observation is an added difficulty for the biochemical diagnosis of GH deficiency in adults. Furthermore, the combined administration of saturating doses of GH-releasing hormone (GHRH) plus GH-releasing hexapeptide (GHRP)-6 is nowadays the most effective GH-releasing stimulus tested in a variety of settings related to altered somatotroph function. To understand whether the GH discharge elicited by the combined stimulus declines with age, 26 normal subjects of both sexes, divided into 3 age groups [adults 19–40 yr; aged 46–65 yr; and very old (75–96 yr) subjects] were studied. They were administered iv, as bolus and in combination, 90 μg GHRH plus 90 μg GHRP-6.

In the three groups, the combined administration of GHRH plus GHRP-6 elicited a GH area under the curve (μg/L per 120 min) of 3,127 ± 262, 3,409 ± 573, and 4,655 ± 737 for adults, aged, and very old subjects, respectively (nonsignificant differences). The mean GH peak was 47.5 ± 4.5 μg/L for adults, 52.9 ± 8.4 μg/L for aged subjects, and 76.0 ± 11.7 for very old subjects (nonsignificant differences). Individually examined, there were no nonresponders to the combined stimulus, and all subjects (independently of age) showed a GH peak over 25 μg/L (the lowest peak was 27.5 μg/L, and the highest peak was 119.2 μg/L).

In conclusion, the GHRH plus GHRP-6-induced GH release is well preserved in aged and very old subjects, which suggests that the GH secretory capability of the combined test is not reduced by age. This combined test may be useful for the diagnosis of GH-deficient states in adults. (J Clin Endocrinol Metab 83: 2569–2572, 1998)
Subjects and Methods

Twenty-six normal healthy volunteers (16 women and 10 men), 53.5 ± 4.5 yr old (range 19–96 yr), participated in this study after providing informed consent. All of them had normal life styles, were taking no medication, and were within 10% of their ideal body weight. The study was approved by the Hospital Bioethical Committee. Testing subjects had been previously divided, according to age, into 3 groups: 1) adults, n = 10 (6 women, 4 men), 29.0 ± 1.9 yr old (range 19–40 yr), body mass index (BMI) = 25.3 ± 1.0; 2) aged subjects, n = 8 (4 women, 4 men), 56.5 ± 2.1 yr old (range 46–65 yr), BMI = 22.2 ± 0.9; and 3) very old subjects, n = 8 (5 women, 3 men), 81.3 ± 2.9 yr old (range 75–96 yr), BMI = 21.0 ± 1.5.

Tests started at 0800 h, after an overnight fast, with the subjects recumbent. An indwelling catheter was placed in a forearm vein and kept permeable with a slow infusion of 150 mmol/L NaCl. The first blood sample was obtained at 0 min, and additional blood samples were obtained at appropriate intervals. Women were tested in the follicular phase of the menstrual cycle. As GH stimulant, it was administered the combined test of GHRH and GHRP-6 (His-DTrp-Ala-Trp-DPhe-Lys-NH2; Peninsula Laboratories, Madrid, Spain), immediately followed by an iv bolus injection of GHRH (90 μg GHRH (GRF 1–29 NH2, Genentech, South San Francisco, CA), at 0 min, and additional blood samples were obtained at appropriate intervals. Women were tested in the follicular phase of the menstrual cycle. As GH stimulant, it was administered the combined test of GHRH and GHRP-6 (His-DTrp-Ala-Trp-DPhe-Lys-NH2; Peninsula Laboratories, Madrid, Spain), immediately followed by an iv bolus injection of GHRP-6 (His-DTrp-Ala-Trp-DPhe-Lys-NH2; Peninsula Laboratories, Madrid, Spain), prepared as previously described (see Ref. 25).

Serum GH concentrations were determined by using a time-resolved fluoroimmunoassay (Delfia, Wallac Oy, Turku, Finland) with a GH sensitivity of 0.02 μg/L and coefficients of variation of 6.3% (0.4 μg/L), 5.3% (10.2 μg/L), and 4.2% (43.4 μg/L). Hormone levels are presented and analyzed as absolute values or as the mean GH peak. The areas under the curve (AUC) were calculated by a trapezoidal method and were compared between groups with the Wilcoxon rank test. The statistical level of significance was set at P < 0.05.

Results

As expected, the combined administration of GHRH, immediately followed by GHRP-6 (both at saturating doses), induced a clear-cut GH secretion in the three studied groups of subjects (Fig. 1), with a quite synchronized pattern of GH discharge. No differences were observed between men and women; therefore, values were pooled. The GHRH + GHRP-6-mediated mean GH peak was 47.5 ± 4.5 μg/L for adults, 52.9 ± 8.4 μg/L for aged subjects, and 76.0 ± 11.7 for very old subjects (Fig. 2). Compared with the adult group, no statistically significant differences were observed in either the aged or the very old group. Analyzed as area under the curve, the values in μg/L per 120 min were: 3,127 ± 262, 3,409 ± 573, and 4,655 ± 737 for adults, aged, and very old subjects, respectively, without statistical differences among the groups. Individually examined (Fig. 2), there were no nonresponders to the combined stimulus; and all subjects (independently of the age) showed a GH peak over 25 μg/L (the lowest peak being 27.3 μg/L; and the highest peak, 119.2 μg/L). The four higher GH peaks, in decreasing order, were observed in subjects who were 96, 77, 79, and 53 yr old. There was a positive correlation between the GHRH-GHRP-6-mediated GH response and age (r = 0.45, P < 0.01); however, the r value was too low to consider the correlation of biological relevance.

No side effects were reported in any of the tests.

Discussion

The signs and symptoms of GH deficiency in adults are similar to those of normal aging, making the diagnosis of GH deficiency a cumbersome and uncertain task in adult patients (1). The diagnosis of GH deficiency must rely on biochemical determinations, and provocative or pharmacological tests have been the only validated means for such diagnosis (6). Furthermore, because either spontaneous or stimulated GH secretion is reduced with the advancing of age, a new degree of uncertainty is shown in the diagnostic process. In fact, all provocative stimuli of GH secretion (including hypoglycemia, GHRH and GHRP-6 or hexarelin) show a lower effectiveness when aging progresses (19–23). So, the availability of a provocative test not affected by aging should be clinically useful.

The new GH secretagogues, among which GHRP-6 is the most widely studied, may represent a new physiological system that participates in the regulation of GH secretion in man (24–26), and these nonclassical secretagogues have been widely used in the last few years in the testing of the GH reserve in man (24–26). Although the mechanism of action is not fully understood, the combined administration of

![Fig. 1. Mean ± SE of plasma GH values after the administration iv of GHRH (90 μg) plus GHRP-6 (90 μg) at 0 min in three groups of healthy subjects with different age intervals: adults (19–40 yr), aged (46–65 yr), and very old subjects (75–96 yr).](image-url)
GHRH plus GHRP-6 (both at saturating dose) is nowadays considered the most potent stimulus of GH secretion in man (15), being able to restore the GH secretion in states associated with chronic blockade of somatotroph activity (as in obesity) (18). In the present work, the combined stimulus of GHRH-GHRP-6 has been studied in a group of very old subjects (age higher than 75 yr) showing no decline in the amount of GH secretion, as compared with both normal adults (less than 40 yr) and aged subjects (age 46–65 yr). A similar lack of age-related decline has been reported for normal subjects in their late adulthood (21). The GHRH-GHRP-6-mediated GH discharge was similar for the three groups of age, with similar mean GH peaks, AUC, and secretory pattern, which suggests a very synchronized type of secretion. However, the most interesting information came from the analysis of individual GH peaks. In fact, as Fig. 2 shows, all the subjects had a positive response to the stimulus, an unusual fact when facing a GH provocative test. Moreover, despite the dispersion in GH peaks in any of the age groups, no subject responded with a GH peak under 25 µg/L, a cut-off far from the highest level of GH secretion elicited in patients with GH deficiency, studied with similar tests (26).

When considering the suitability of GHRH plus GHRP-6 as a first-choice stimulus for assessing GH reserve, it is worthy to consider that it elicits a near-normal GH discharge in obesity (18) in patients with hypothyroidism (27) and in patients with type 2 diabetes mellitus (28). All of them are confusing situations when facing an individual diagnosis of GH deficiency in adults. Another positive fact is that it is a safe stimulus, without side effects, and that it does not require medical supervision under its realization. The lack of age-related decline in the GHRH-GHRP-6-mediated GH release is an added value to this promising test, and this opens the possibility of using it as a therapeutical tool to revert some deleterious manifestations of aging in man, as has been showed with the nonpeptidil GH secretagogues (29). It seems then that the combined test may be used for the diagnosis of GH-deficient states in adults, because it is scarcely affected by age, obesity, sex steroid levels, and so on. Because the maximal amount of GH that a given individual is able to release upon stimulation must have relationship with the pituitary reserve of the hormone, and because GHRH plus a GHS is the most powerful stimulus, it may have a relevant diagnostic role in the future.

Considerable work with larger groups of either normal or GH-deficient subjects is needed before the diagnostic advantages of this test, in confrontation with the ITT and the arginine-GHRH, may be established.

In conclusion, the GHRH plus GHRP-6-induced GH release is preserved in aged and very old subjects, suggesting that the GH secretory capability of the combined test is not reduced by age. This combined test may be useful for the diagnosis of GH-deficient states in adults.

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References


