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Impaired secretion of growth hormone-releasing hormone, growth hormone and IGF-I in elderly men

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Abstract. The GHRH test and L-dopa test were performed in 12 normal young men (24.1±1.1 years) and 12 normal elderly men (77.8±1.4 years) to investigate age-related changes in secretion of GHRH, GH and IGF-I. The basal plasma levels of GHRH and GH were not significantly different in young and elderly men, but the basal plasma level of IGF-I was higher in the young men (159.0±11.7 vs 86.7±11.6 μg/l). The area under the curve for plasma GH in the GHRH test was less in the elderly group (35.1±5.9 vs 11.2±2.1 $\mu g \cdot h^{-1} \cdot l^{-1}$, $p<0.001$). The AUCs for the plasma GHRH and GH responses in the L-dopa test in young and elderly men were 32.0±2.7 vs 20.3±1.8 $ng \cdot h^{-1} \cdot l^{-1}$ ($p<0.001$), and 21.8±4.6 vs 5.4±1.1 $\mu g \cdot h^{-1} \cdot l^{-1}$ ($p<0.01$), respectively, indicating decreased releases of GHRH and GH in the elderly. Correlations between the AUCs for plasma GHRH and GH responses in L-dopa were found in both groups, but the ratio of the AUCs for GH/GHRH was lower in the elderly group. The elderly group showed a significant correlation between the basal plasma IGF-I level and the AUCs for plasma GH in the GHRH and L-dopa tests. These results suggest that elderly men have a decreased reserve of hypothalamic GHRH, resulting in secondarily impaired GH release, which may lead to a lower level of IGF-I than in young men.

The age-related change in GH secretion in men is still controversial: the basal GH concentrations in elderly men have been reported to be either unchanged (1) or decreased (2). However, spontaneous 24-h secretion of GH in human subjects is reported to decrease with age (2,3). With regard to the plasma GH response to exogenous GHRH in the elderly, Shibasaki et al. (4) and Lang et al. (5) reported a marked decrease in men older than 40 years, but Pavlov et al. (6) observed no age-related decrease in the GH response to GHRH even in subjects in their eighties.

The development of a radioimmunoassay for plasma GHRH enabled us to evaluate hypothalamic-pituitary function by measuring plasma GHRH and GH in normal subjects and patients with various endocrine disorders (7). We have reported an increase in plasma GHRH after oral administration of L-dopa followed by GH release (8). These findings were confirmed by others (9-11).

In the present study, we compared the function of the GHRH-GH axis in young and elderly men by measuring the plasma GH response to GHRH and the plasma GHRH and GH responses to L-dopa, in addition to the basal plasma IGF-I level.

Subjects and Methods

Subjects and protocols

The subjects studied consisted of 12 young and 12 elderly males with no obesity or endocrine disorder (Table 1). The study was approved by the Human Subjects Protection Committee, School of Medicine, University of Tokushima, and informed consent was obtained from all subjects participating in the study.

Tests were performed on subjects in bed after overnight fasting. At least 30 min before the start of each test, a 21-gauge indwelling needle was inserted into the antecubital vein and blood samples were taken serially through a cannula before and after administration of GHRH or L-dopa.

Table 1.
Age, body mass index (BMI), area under the curve (AUC) for plasma GHRH and GH in the L-dopa test, AUC for plasma GH in the GHRH test, and basal plasma IGF-I levels in young and elderly men.

	Case No.	Age (years)	BMI (kg/m^2)	L-dopa test		GHRH test	basal IGF-I
				GHRH ($ng \cdot h^{-1} \cdot l^{-1}$)	GH ($\mu g \cdot h^{-1} \cdot l^{-1}$)	GH ($\mu g \cdot h^{-1} \cdot l^{-1}$)	($\mu g/l$)
Young	1	25	24.3	37.2	22.7	61.1	211.5
	2	24	17.9	41.2	21.2	41.8	191.0
	3	31	21.0	29.4	18.5	25.0	186.5
	4	23	22.3	34.7	31.5	17.4	165.7
	5	24	17.9	39.7	12.0	25.2	175.7
	6	27	22.5	31.0	18.1	49.4	159.7
	7	21	23.7	25.9	33.1	55.1	170.2
	8	20	17.8	53.5	57.0	68.9	145.3
	9	20	16.8	21.7	8.9	37.7	127.5
	10	23	18.0	22.9	9.3	9.5	100.5
	11	21	21.6	25.5	16.1	15.5	78.7
	12	30	21.1	21.3	13.1	14.7	195.7
	Mean	24.1	20.4	32.0	21.8	35.1	159.0
	±SEM	1.1	1.5	2.7	4.6	5.9	11.7
Elderly	1	74	20.1	14.6	7.2	19.7	101.8
	2	81	18.6	22.4	7.8	14.9	49.3
	3	77	23.8	28.2	11.0	12.8	91.1
	4	78	19.7	12.3	1.4	9.2	83.1
	5	79	23.2	25.3	1.7	4.2	34.4
	6	70	20.4	20.4	4.0	13.7	112.8
	7	84	20.0	26.3	9.6	15.3	130.5
	8	81	21.0	20.7	3.0	2.2	75.6
	9	80	20.3	10.9	1.6	0.8	46.9
	10	73	19.4	15.2	0.9	17.4	56.3
	11	85	22.5	19.5	5.2	2.8	85.1
	12	71	19.6	27.7	10.5	21.1	172.9
	Mean	77.8	20.7	20.3	5.4	11.2	86.7
	±SEM	1.4	0.9	1.8	1.1	2.1	11.6

GHRH and L-dopa tests

A synthetic preparation of GHRH(1-44)NH_2 (100 µg, Sumitomo Pharmaceutical Co, Ltd, Osaka, Japan), which is a registered drug for hormonal examination, were administered iv. Blood samples were obtained serially 0, 15, 30, 45, 60, 90 and 120 min after GHRH administration, and the plasma concentrations of GH were measured.

A dose of 500 mg of L-dopa was administered orally, and serial blood samples were obtained 0, 30, 60, 90 and 120 min later for measuring the plasma concentrations of GHRH and GH.

Radioimmunoassays of plasma GHRH, GH and IGF-I

GHRH was extracted from plasma as described previously (7,11). Briefly, 3.5 ml of plasma was mixed with 7 ml of cold acetic acid-acetone solution (3:100, vol/vol) and centrifuged. The supernatant was extracted twice with 20 ml of petroleum ether, and the ether layer was carefully removed. Remaining acetone was eliminated by evaporation, and the aqueous portion was lyophilized. The residue was dissolved in assay buffer and subjected to RIA for GHRH.

Synthetic GHRH(1-44)NH_2 (generously provided by Dr A. Felix, Hoffman-La Roche Inc, Nutley, NJ) was labelled with ^{125}I by the chloramine-T method and the iodinated product was purified on a 1 × 10 cm carboxymethyl cellulose column (CM 23, Whatman, Maidstone, England). The anti-GHRH serum (RAS-8061, Peninsula Lab, San Carlos, CA; lot No. 004118) used in this study did not cross-react with relevant neuropeptides and rec-

ognized the N-terminal and part of the middle portion of the amino acid sequence of GHRH(1-44)NH_2. When synthetic GHRH(1-44)NH_2 was used as standard, the sensitivity of this assay was 4 pg/tube. As the extract from 1 ml of plasma was dissolved in 100 µl of assay buffer and used for the assay as well as 100 µl of the standard, the least detectable value was 4 ng/l. Antibody-bound and free tracers were separated by the double-antibody method. The recovery of 30 pg of synthetic GHRH(1-44)NH_2 added to 1 ml of plasma was 59.5±2.1%. The intra- and inter-assay coefficients of variation were less than 10%.

The plasma GH concentration was measured with a radioimmunoassay kit, HGH-II (Dianabot Co, Ltd., Tokyo, Japan). The sensitivity of the assay was 0.3 µg/l, and the intra- and inter-assay coefficients of variation were 5.7-6.3% and 3.4-5.6%, respectively.

We have previously reported the basal plasma IGF-I level measured by radioimmunoassay (12). A biosynthetic homologue of IGF-I and its specific antiserum were kindly provided by Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan. The antiserum recognizes three portions of the IGF-I molecule: the N-terminal portion (the first five amino acid residues), middle portions (residues 13 to 20 and 21 to 33), and C-terminal portions (residues 47 to 53 and 60 to 70) (13). For RIA, this specific antiserum was used at a final dilution of 1:35 000. Acid-ethanol extraction of plasma was performed by a slight modification of the method of Daughaday et al. (14) for removal of the bulk of plasma proteins, especially the specific binding protein for IGF-I. The lower limit of detectability of IGF-I with 95% confidence was 1.5 µg/l, and the intra- and inter-assay coefficients of variation were less than 10%.

Statistical analysis

Data are expressed as means ±SEM. In calculating mean values and incremental rises, undetectable plasma hormone levels were assigned a value of the detection limit of the assay. Student's t-test was used to compare hormone levels in the two age groups and analysis of variance was used to compare difference at different time points.

Results

Basal levels of plasma GHRH, GH and IGF-I in young and elderly men

The plasma concentrations of GHRH, GH and IGF-I were 9.5±1.1 ng/l, 1.0±0.2 and 159.0±11.7 µg/l, respectively, in young men, and 8.3±0.7 ng/l, 0.9±0.2 and 86.7±11.6 µg/l, respectively, in elderly men. Only the difference between the basal plasma IGF-I levels of the two groups was significantly different ($p<0.001$).

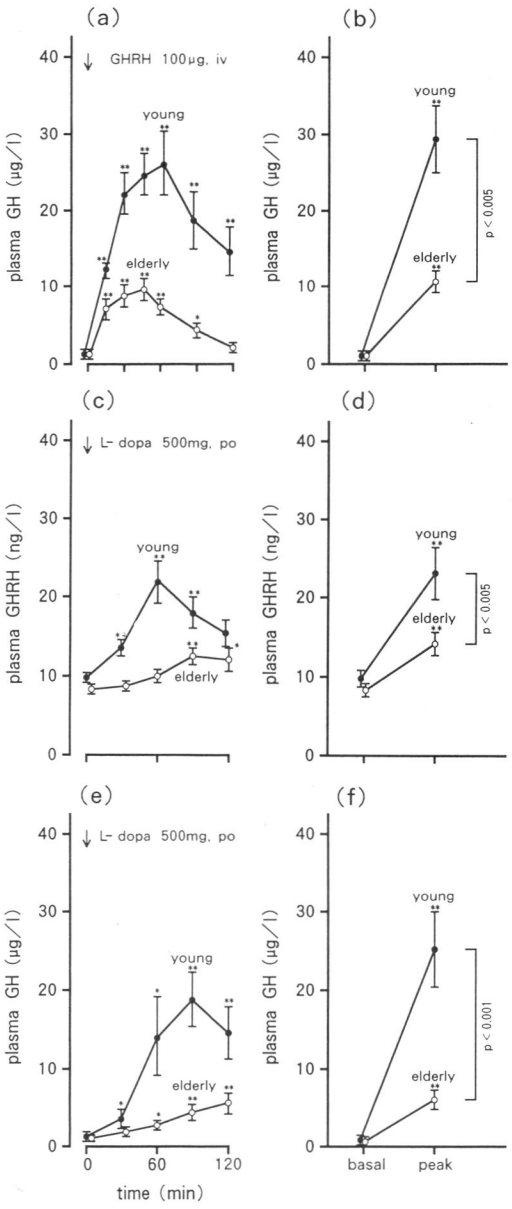

Fig. 1.

Plasma GHRH and/or GH responses to GHRH or L-dopa in young and elderly men.

a: Plasma GH response to GHRH.

b: Basal and peak plasma GH levels during the GHRH test.

c: Plasma GHRH response to L-dopa.

d: Basal and peak plasma GHRH levels during the L-dopa test.

e: Plasma GH response to L-dopa.

f: Basal and peak plasma GH levels during the L-dopa test.

Points and bars are means and SEM for 12 determinations. *$p<0.05$ and **$p<0.01$ vs basal value.

Plasma GH response to GHRH in young and elderly men

Both the young and elderly men showed a rise in plasma GH level in response to GHRH, but the elderly men showed a significantly lower peak value (29.4±4.2 vs 10.7±2.1 μg/l, $p<0.001$, Fig. 1a and 1b).

Plasma GHRH and GH responses to L-dopa in young and elderly men

The plasma GHRH responses to L-dopa in the young and elderly men are shown in Fig. 1c and 1d. The young men showed a higher rise in plasma GHRH level than the elderly men, the peak GH levels in the two groups being 22.8±2.5 and 13.5±1.5 ng/l, respectively ($p<0.005$).

The plasma GH responses to L-dopa in the two groups are shown in Fig. 1e and 1f. A rise in plasma GH level was observed in both groups, but the peak value was significantly lower in the elderly men (25.7±9.2 vs 6.0±2.6 μg/l, $p<0.001$).

Fig. 2 show the correlations between the area under the curve (AUC) for plasma GHRH and plasma GH concentrations in the L-dopa test in the young and the elderly men ($r=0.71$, $p<0.01$ and $r=0.69$, $p<0.05$, respectively).

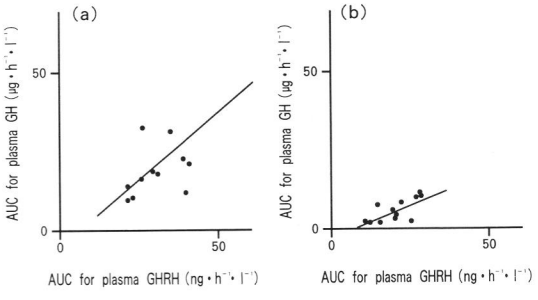

Fig. 2.
Correlation between area under the curve (AUC) for plasma GHRH and GH in the L-dopa test in young and elderly men.
a: A significant correlation
($y=-10.1+0.99x$, $r=0.71$, $p<0.01$) was observed in the young men.
b: A significant correlation
($y=-3.59+0.44x$, $r=0.69$, $p<0.05$) was observed in the elderly men.

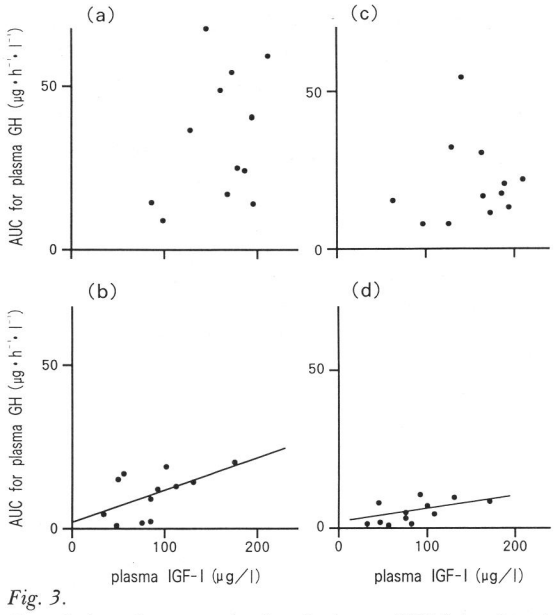

Fig. 3.
Correlations between the basal plasma IGF-I level and AUC for plasma GH in the GHRH and L-dopa tests in young and elderly men.
a: No significant correlation ($r=0.34$) was observed during the GHRH test in the young men.
b: A significant correlation
($y=1.99+0.11x$, $r=0.58$, $p<0.05$) was observed during the GHRH test in the elderly men.
c: No significant correlation ($r=0.13$) was observed during the L-dopa test in the young men.
d: A significant correlation
($y=0.12+0.063x$, $r=0.66$, $p<0.05$) was observed during the L-dopa test in the elderly men.

Correlations between basal plasma IGF-I levels and AUCs for plasma GH in the GHRH and L-dopa tests

The correlations between the basal plasma IGF-I levels and AUC of plasma GH in the GHRH test in young and elderly men are shown in Fig. 3a and 3b. No correlation was observed in the young men, but a significant correlation ($r=0.58$, $p<0.05$) was found in the elderly men.

The correlations between the basal plasma IGF-I levels and AUC for plasma GH in the L-dopa test in young and elderly men are shown in Fig. 3c and 3d. A significant correlation was found only in the elderly men ($r=0.66$, $p<0.05$).

Discussion

In this work we observed an age-related change in the plasma GH response to GHRH. This result is

compatible with those of Shibasaki et al. (4) and Lang et al. (5), who studied 37 men aged 20-75 years, and 63 men aged 18-95 years old, respectively, but contrasts the report of Pavlov et al. (6) of no age-dependent alteration in healthy men. Wealso found that the basal plasma IGF-I levels were lower in elderly men. This finding is consistent with a report by Florini et al. (15) of decrease in the basal plasma IGF-I level in elderly men and a positive correlation between the basal plasma IGF-I level and 24-h integrated GH level.

There are reports (7-10) of a GHRH-like substance that seems to be immunologically indistinguishable from synthetic GHRH(1-44)NH_2 in the plasma of patients with idiopathic pituitary dwarfism and acromegaly.

The source of plasma GHRH is uncertain, but the following observations suggest that the GHRH level in the peripheral blood reflects release of GHRH from the hypothalamus into the hypophyseal-portal vein and results in GH release from the pituitary. In humans, GHRH is mainly located in the hypothalamus and pituitary stalk, with only very small amounts in other organs, including the digestive tract, pancreas and adrenal gland (4). As reported earlier, episodic release of GHRH into the peripheral blood can be detected before or in association with the GH surge during slow-wave sleep (8). In addition, oral administration of L-dopa has been shown to stimulate the releases of GHRH and GH in normal subjects (8-10), but not in patients with hypothalamic disorders (10).

We also found significant differences between young and elderly men in the peak value of plasma GHRH, the increment of plasma GHRH concentration, and the AUC for plasma GHRH in the L-dopa test. These results suggest that GHRH secretion from the hypothalamus is impaired in elderly men, and may result in decreased secretions of GH and IGF-I. In fact, Leppaluoto et al. (16) reported that in young adult men GH release induced by heat exposure is mediated by GHRH, and that similar responses of GHRH and GH do not occur in older men. Our results are consistent with the finding of Leppaluoto et al. (16) that the GHRH response is impaired in elderly men. In this connection it is noteworthy that Iovino et al. (17) reported that repetitive GHRH administration restored the attenuated GH response in the GHRH test in elderly men.

In the L-dopa test, a positive correlation between the AUC for GHRH and AUC for GH was found in elderly men, indicating preserved function of the GHRH-GH axis. However, the fact that the slope of the correlation of the AUC for plasma GH with the AUC for plasma GHRH was lower in the elderly men also suggested that the capacity to secrete GH in response to endogeneous GHRH is decreased in elderly men.

GH secretion is also regulated by somatostatin released from the hypothalamus (18,19), and the somatostatin level is thought to increase with age (4). The secretory profile of somatostatin from the hypothalamus cannot be measured, so we cannot evaluate the participation of somatostatin in the release of GH during the GHRH and L-dopa tests. Thus, the possible involvement of somatostatin in the impaired GH release in elderly men cannot be excluded.

In this work we found that elderly men showed a lower GH response to GHRH, and lower GHRH and GH responses to L-dopa than young men. We also observed a significant correlation between the basal plasma IGF-I levels and AUC values for plasma GH in the GHRH and L-dopa tests. These results suggest that the function of the hypothalamus is decreased in elderly men, resulting in decreased releases of GH and IGF-I.

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