Altered Neuroendocrine Control of GH Secretion in Normal Women of Advanced Reproductive Age

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Background. Previous studies have suggested that the neuroendocrine control of growth hormone (GH) secretion changes with increasing age in women with normal menstrual cycles and sex steroid levels.

Methods. In order to verify this hypothesis, 8 younger (22-32 years) and 8 older (41-45 years) women with normal menstrual function and gonadal steroid levels were tested with the serotonergic agent sumatriptan (6 mg in a subcutaneous bolus), the GABAergic agonist sodium valproate (800 mg orally), the dopaminergic compound L-Dopa (500 mg orally) and placebos. Furthermore, all women were tested with GH-releasing hormone (GH-RH 1 µg/kg body weight in an intravenous (i.v.) bolus) to determine whether GH secretion in response to its specific releasing factor was preserved. Serum GH levels were recorded over 2 hours in all tests and IGF-I levels in basal samples.

Results. Plasma IGF-I concentrations and the GH responses to sumatriptan, sodium valproate and L-Dopa were significantly lower in older than in younger women. Also, the GH-RH-induced GH response was significantly lower in older than in younger subjects. When peak GH responses to releasing stimuli were compared with age, significant negative correlations were found in all tests.

Conclusions. These data did not show a specific neurotransmitter change underlying defective GH secretion in older reproductive-aged women. On the other hand, the results indicated that age-related changes in the secretory machinery of GH, such as a reduced pituitary sensitivity to GH-RH and/or a reduction in the pituitary GH secretory capacity, affect women during the last years of the reproductive period.

During the last years of the reproductive period, women have been found to have lower circulating levels of insulin-like growth factor I (IGF-I) and lower integrated serum GH concentrations than younger subjects, despite normal menstrual cycles and normal sex steroid levels (1,2). An important role in the control of circulating IGF-I levels is played by 24-hour spontaneous GH surges (3), which in turn are regulated by central neurotransmitter activity (4,5). Therefore, we wondered whether a specific neurotransmitter alteration affects GH secretion in older reproductive-aged women. In the present study, we tested the GH response to stimulation with the serotonergic agent sumatriptan (6,7), the GABAergic agonist sodium valproate (8) and the dopaminergic compound L-Dopa (4) in younger and older women with normal menstrual cycles and sex steroid levels. At the end of the study, all subjects were recalled and tested with GH-RH to verify the possibility of a defective GH response to its specific releasing hormone in older reproductive-aged women.

Methods

Sixteen women, eight in younger (22-32 years) and eight in older (41-45 years) reproductive age participated in the study after giving informed consent. This study was performed in accordance with Helsinki II declaration. All women had had a history of regular menses of 26-32 days and recently had been shown to be ovulatory by midluteal plasma progesterone (P) concentrations greater than 6.9 ng/mL. All women were within 10% of their ideal body weight. They were fully ambulatory, well nourished and without clinical or laboratory evidence of endocrine, metabolic, hepatic, renal or neoplastic diseases. None of them were taking drugs before and during the period of the study or were engaged in excessive alcohol consumption (< 300 g ethanol/week).

Basal body temperature and plasma levels of ovarian steroids were elevated daily and served as criteria to determine the precise period of menstrual cycle.

Each subject was tested five times (with sodium valproate, sumatriptan, L-Dopa, saline and GH-RH) on luteal phase (22nd day of regular menstrual cycles). Tests were carried out in random order and followed a similar procedure.

Experimental procedure. — At 0800 hr of the experimental day, a 21-gauge cannula was inserted into the left antecubital vein of subjects lying in the recumbent position after an overnight fast and rest in bed, to be utilized for blood sampling. After 30 min (time 0), women were given:
sodium valproate (800 mg orally) with blood sampling at 30, 60, 90, 120, 150, and 180 min later, L-Dopa (500 mg orally) with blood sampling at 30, 45, 60, 90, 120, and 180 min later, sumatriptan (6 mg s.c. administration) with blood sampling at 10, 20, 30, 40, 60, 90, and 120 min later or GH-RH (1 µg/kg body weight as an i.v. bolus through an indwelling catheter placed into the right antecubital vein at time 0) with blood sampling at 15, 30, 45, 60, 90, and 120 min later. Drugs were given at doses usually utilized in GH stimulating tests (4-9-11).

Blood pressure and heart rate were measured at each sampling time during all tests. Samples from all experiments were used for measurements of serum GH. Blood glucose, free fatty acid, serum T3, T4, TSH, cortisol, 17b-estradiol (E2) and plasma insulin-like growth factor (IGF-1) concentrations were measured in the samples taken at time 0 of all tests. Urine samples were collected between 0800 hr of the day preceding each test and 0800 hr of the experimental day. During the 24-hour collection, urine samples were kept refrigerated; specimens were used for measurements of urinary cortisol levels.

Assays. — All samples from a single subject were analyzed in duplicate in the same assay. Serum GH concentrations were measured with a specific RIA, using materials supplied by Ares Serono (Milan, Italy). The sensitivity of the GH assay was 0.5 ng/mL; the intraassay and interassay coefficients of variation were 3.6% and 8%, respectively. Serum cortisol levels were measured by RIA, using a procedure developed by Medgenix Diagnostic (Bruxelles, Belgium). The sensitivity of the assay was 0.6 µg/dl and intra- and interassay coefficients of variation were 3.7% and 7.6%, respectively. Serum T3, T4, TSH, LH and FSH levels were measured by RIA, using kits provided by Ares Serono (the sensitivity of the assay was 0.4 mmol/l for T3, 12.9 mmol/l for T4, 0.02 mIU/l for TSH, 1.5 IU/L for LH, 1.5 IU/L for FSH; the intra- and interassay coefficients of variation were 4.5% and 7.8% for T3, 4.2% and 9.6% for T4, 4.8% and 6.7% for TSH, 6.9% and 9% for LH, 4.0% and 8.0% for FSH, respectively). Serum progesterone and E2 levels were measured by RIA with kits provided by Clone System (Bologna, Italy), (the sensitivity of the assays was 0.18 ng/ml for progesterone and 16 µg/ml for E2). The intra- and interassay coefficients of variation were 6.0% and 8.9% for progesterone, 5.4% and 7.6% for E2, respectively. The plasma concentrations of IGF-1 were measured by RIA, using kits obtained by Nichols Institute Diagnostic (San Juan Capistrano, CA) (the sensitivity of the assay was 0.1 ng/ml and intraassay coefficients of variation were 7.91% and 11.41%, respectively). Blood glucose levels were measured with an IL918 autoanalyzer (Instrumentation Laboratory, Milan, Italy).

Statistics. — Statistical analyses were performed with the Wilcoxon's matched pair rank sum test, Mann-Whitney U test, and two-way ANOVA for repeated measures, as appropriate. The area under the curve (AUC) was calculated by trapezoid method. Correlation studies between age of the subjects and peak GH responses to sodium valproate, sumatriptan, L-Dopa and GH-RH were performed. In addition serum estrogen levels were correlated with basal and peak GH levels in all tests. Spearman's correlation coefficient was utilized. Data are reported as means ± SE.

Results
Clinical, hormonal, and metabolic parameters in younger and older women are reported in Table 1. Each point represents the mean ± SE of values obtained at time 0 of all tests. No significant differences were observed between younger and older subjects in any examined laboratory parameter, except for plasma IGF-1 levels, which were higher in younger (234.5 ± 14.3 ng/mL) than in older (181.1 ± 16.8 ng/mL, p < .05) women.
Basal GH levels were similar in younger and older women (Figures 1-4). The administration of placebos did not change GH secretion (younger women, time 0: 1.0 ± 0.3 ng/ml, 30 min: 1.3 ± 0.2, 45 min: 1.4 ± 0.2, 60 min: 1.2 ± 0.2, 90 min: 1.1 ± 0.3, 120 min: 1.2 ± 0.2, 150 min: 1.3 ± 0.2, 180 min: 1.2 ± 0.2; older women, time 0: 1.1 ± 0.2, 30 min: 1.2 ± 0.2, 45 min: 1.3 ± 0.2, 60 min: 1.2 ± 0.3, 90 min: 1.2 ± 0.3, 120 min: 1.2 ± 0.2, 150 min: 1.4 ± 0.3, 180 min: 1.2 ± 0.2). In contrast, sumatriptan (Figure 1), sodium valproate (Figure 2), L-Dopa (Figure 3) and GH-RH (Figure 4) administration induced a striking increase in plasma GH levels in both groups. However, drug-induced GH increments were significantly higher in younger than in older subjects. (F = 11.84, p < .02 for sumatriptan, F = 11.88, p < .02 for sodium valproate, F = 11.78, p < .02 for L-Dopa and F = 11.95, p < .02 for GH-RH). The incremental areas of the GH response during tests are reported in Figure 5.
When peak GH responses in the various tests were compared with age, significant negative correlations were found for sodium valproate (r = -.768, p < .01), sumatriptan (r = -.706, p < .02), L-Dopa (r = -.779, p < .01) and GH-RH (r = -.785, p < .01) tests (Figure 6). In contrast, no signifi-
The results of this study agree with previous observations of a decline in GH secretion in women during late reproductive age (1). Our study failed to show a specific neurotransmitter change as the likely cause of a defective central control of GH secretion. In fact, in a similar manner the serotonergic agent sumatriptan, the GABAergic agonist sodium valproate, and the dopaminergic compound L-Dopa elicited lower GH responses in older than in younger women.

DISCUSSION
The results of this study agree with previous observations of a decline in GH secretion in women during late reproductive age (1). Our study failed to show a specific neurotransmitter change as the likely cause of a defective central control of GH secretion. In fact, in a similar manner the serotonergic agent sumatriptan, the GABAergic agonist sodium valproate, and the dopaminergic compound L-Dopa elicited lower GH responses in older than in younger women.
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women. Furthermore, all drug-induced peak GH levels were negatively correlated with age. We supposed that rather than representing the expression of different age-depending neurotransmitter alterations, these results were different aspects of a common neuroendocrine disorder. Both L-Dopa and sodium valproate are known to stimulate GH secretion through GH-RH mediation (12-15). The same mechanism is also supposed to mediate sumatriptan action (16,17), even though direct pituitary effects of the drug cannot be excluded (11,18). These considerations prompted us to test the GH stimulating effect of GH-RH in the same subjects. The lower GH response to GH-RH in older than in younger subjects indicated age-related changes in the secretory machinery of GH secretion, such as a reduced sensitivity to GH-RH or a reduction in the pituitary GH secretory capacity. Both conditions may be produced by diminished endogenous GH-RH activity or by enhanced somatostatinergic tone. Further studies are needed to clarify this issue.

The demonstration of a declined somatotropic axis activity in old reproductive-aged women with normal menstrual cycles and circulating sex steroid concentrations argues against the generally accepted opinion of an estrogen-dependent stimulation of GH secretion until menopause. In fact, no significant correlation was observed between estrogen levels and GH secretion in our subjects. As with men (19,20), also in women there is a reduced GH secretory activity with increasing age. In view of the anabolic properties of GH, the defective GH activity in the years preceding menopause might play a role in the development of the catabolic processes which characterize the following menopausal period. With increasing age, defective somatotropic function mainly contributes to osteopenia, muscle atrophy and decreased exercise tolerance (21,22). Therefore, age-related decline in GH secretion is considered one of the pace-makers of ageing and has been defined somatopause (21,22). These changes are worsened by the estrogen drop after menopause. In order to counter structural and functional alterations related to protein catabolism, restoration of endogenous GH secretion with pharmacological means has been attempted in senescent patients (21,22). It would be of interest to investigate whether similar treatments might be advisable even before menopause, particularly in women with lowest IGF-1 levels and GH responses to stimulatory tests.

Figure 6. Correlation between age and peak GH responses to sodium valproate, sumatriptan, GH-RH, and L-Dopa in 8 younger and 8 older normally cycling women.
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REFERENCES


