Arginine Counteracts the Inhibitory Effect of Recombinant Human Insulin-Like Growth Factor I on the Somatotroph Responsiveness to Growth Hormone-Releasing Hormone in Humans*

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ABSTRACT

Insulin-like growth factor I (IGF-I) exerts a negative feedback effect on GH secretion via either direct actions at the pituitary level or indirect ones at the hypothalamic level, through stimulation of somatostatin (SS) and/or inhibition of GHRH release. In fact, recombinant human IGF-I (rhIGF-I) in humans inhibits spontaneous GH secretion as well as the GH response to GHRH and even more to GHRH-releasing peptides, whose main action is on the hypothalamus, antagonizing SS and enhancing GHRH activity. The aim of the present study was to further clarify in humans the mechanisms underlying IGF-I-inhibition-induced somatotroph secretion. In six normal young volunteers (all women; mean ± SEM: age, 28.3 ± 1.2 yr; body mass index, 21.3 ± 1.2 kg/m²) we studied the GH response to GHRH (1 μg/kg, iv, at 0 min), both alone and combined with arginine (ARG; 0.5 g/kg, iv, from 0–30 min), which probably acts via inhibition of hypothalamic SS release, after pretreatment with rhIGF-I (20 μg/kg, sc, at −180 min) or placebo. rhIGF-I increased circulating IGF-I levels (peak at −60 vs. −180 min: 54.9 ± 3.9 vs. 35.9 ± 3.3 mmol/L; P < 0.05) to a reproducible extent, and these levels remained stable and within the normal range until 90 min. The mean GH concentration over 3 h (from −180 to 0 min) before ARG and/or GHRH was not modified by placebo or rhIGF-I. After placebo, the GH response to GHRH (peak, 23.6 ± 2.9 μg/L) was strikingly enhanced (P < 0.05) by ARG coadministration (69.6 ± 9.9 μg/L). rhIGF-I blunted the GH response to GHRH (13.1 ± 4.5 μg/L; P < 0.05), whereas that to GHRH plus ARG was not modified (59.5 ± 8.9 μg/L), although it occurred with some delay. Mean glucose and insulin concentrations were not modified by either placebo or rhIGF-I. In conclusion, ARG counteracts the inhibitory effect of rhIGF-I on somatotroph responsiveness to GHRH in humans. These findings suggest that the acute inhibitory effect of rhIGF-I on the GH response to GHRH takes place on the hypothalamus, possibly via enhancement of SS release, and that ARG overrides this action. (J Clin Endocrinol Metab 85: 3604–3608, 2000)

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to further clarify the mechanism by which IGF-I inhibits GH secretion in humans. To this goal, in healthy young women we studied the effect of a low dose of rhIGF-I on the GH response to GHRH given alone or combined with arginine (ARG). In fact, there is evidence, although indirect, indicating that ARG acts via inhibition of hypothalamic SS release (31–33).

Subjects and Methods

Drugs

Vials containing 1000 μg lyophilized rhIGF-I were provided by Pharmacia & Upjohn, Inc. (Stockholm, Sweden). Vials containing 50 μg GHRH-29 were provided by Serono (Rome, Italy). Vials containing 30 g (in 100 mL solution) arginine hydrochloride (Arginine, Damor, Naples, Italy) were purchased from Damor (Naples, Italy).

Study protocol

Six normal young women (mean ± SEM: age, 28.3 ± 1.2 yr; body mass index, 21.3 ± 1.2 kg/m²) were studied in the early follicular phase. All subjects gave their informed consent to participate in the study, which had been approved by an independent ethical committee.

All subjects underwent the following six testing sessions in random order and at least 3 days apart: placebo (saline, 1 mL, sc, at −180 min), rhIGF-I (20 μg/kg, sc, at −180 min), placebo (at −180 min) followed by GHRH (1 μg/kg, iv, at 0 min), placebo (at −180 min) followed by GHRH (at 0 min) plus ARG (0.5 g/kg, iv, up to a maximum of 30 g from 0–30 min), rhIGF-I (at −180 min) followed by GHRH (at 0 min), and rhIGF-I (at −180) followed by GHRH (at 0 min) plus ARG (from 0–30 min). The tests were begun in the morning at 0830–0900 h after overnight fasting and 30 min after an indwelling catheter had been placed into an antecubital vein of the forearm and kept patent by slow infusion of isotonic saline. Blood samples were drawn basally at every 15 min from −180 to +90 min.

Serum GH levels were measured at each time point in all sessions. Serum IGF-I, serum insulin, and plasma glucose levels were measured every 30 min from −180 to 90 min in all sessions. Serum GH levels (micrograms per L) were measured in duplicate by immunoradiometric assay (hGH-CTK IRMA, Sorin, Saluggia, Italy). The sensitivity of the assay was 0.15 μg/L. The inter- and intraassay coefficients of variation were 2.9–4.5% and 2.4–4.0%, respectively.

Serum IGF-I levels (nanomoles per L; 1 μg/L × 0.1307 = 1 nmol/L) were measured in duplicate by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA). All samples were treated with acid-ethanol to avoid interference by binding proteins. The sensitivity of the assay was 0.01 nmol/L. The inter- and intraassay coefficients of variation were 10.1–15.7% and 7.6–15.5%, respectively.

Serum insulin levels (picomoles per L; 1 μU/L × 7.175 = 1 pmol/L) were measured in duplicate by immunoradiometric assays (Sorin). The sensitivity of insulin assay was 17.9 ± 2.2 pmol/L. Inter- and intraassay coefficients of variation were between 6.2–10.8% and between 5.5–10.6%, respectively.

Plasma glucose levels (millimoles per L; 1 mg/dL × 0.05551 = 1 mmol/L) were measured by the glucose oxidase colorimetric method (GLUCOFIX, Menarini Diagnostics, Firenze, Italy).

All samples from an individual subject were analyzed together. The hormonal responses are expressed as absolute values of circulating GH levels induced by stimuli. Statistical analysis was carried out using nonparametric ANOVA (Wilcoxon test). The results are expressed as the mean ± SEM.

Results

The administration of rhIGF-I increased circulating IGF-I levels (mean ± SEM peak at −60 vs. −180 min, 54.9 ± 3.9 vs. 35.9 ± 3.3 nmol/L; P < 0.05) to a reproducible extent, and IGF-I levels remained within the normal range until 90 min (Table 1). GH concentrations from −180 to 90 min after both placebo and rhIGF-I administration were similar. In fact, an overlapping significant (P < 0.05) decrease in basal GH levels was recorded after both placebo and rhIGF-I (Table 1). After placebo, the GH response to GHRH (23.6 ± 2.9 μg/L) was markedly potentiated (P < 0.05) by ARG coadministration (69.6 ± 9.9 μg/L; Fig. 1).

The GH response to GHRH was significantly blunted by pretreatment with rhIGF-I (13.1 ± 4.5 μg/L; P < 0.05), which, in turn, did not modify somatotroph responsiveness to the combined administration of GHRH and ARG (59.5 ± 8.9 μg/L). However, pretreatment with rhIGF-I induced a non-significant delay in the timing of peak GH after GHRH plus ARG treatment (Fig. 1).

When evaluated as the area under the curve (AUC), the results showed the differences reported above (Fig. 1), although the GH AUC recorded after the combined administration of GHRH and arginine preceded by rhIGF-I seemed slightly blunt.

Mean glucose and insulin concentrations from −180 to 90 min were not modified by either placebo or rhIGF-I (Table 1).

Side effects

Four subjects experienced transient discomfort at the injection site after rhIGF-I administration, but no major side-effects were recorded. Five subjects had transient facial flushing after GHRH administration. No side-effects were observed after ARG coadministration.

Discussion

The results of the present study show that arginine counteracts the inhibitory effect of rhIGF-I on somatotroph responsiveness to GHRH in humans. In agreement with previous studies (6, 10, 14, 23, 34), the rhIGF-I dose we administered in this study increased circulating IGF-I levels within the normal range, indicating that we were investigating the effects of physiological increases in IGF-I levels on stimulated somatotroph release.

The inhibitory effect of rhIGF-I on somatotroph secretion has clearly been demonstrated (1–14). In humans, high and low doses of rhIGF-I inhibit spontaneous GH secretion in

TABLE 1. Mean (±SEM) IGF-I, GH, insulin, and blood glucose levels after placebo or rhIGF-I (20 μg/kg, sc, at −180 min) in six normal subjects

<table>
<thead>
<tr>
<th></th>
<th>After placebo</th>
<th>After rhIGF-I</th>
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<tr>
<td></td>
<td>−180 min</td>
<td>0 min</td>
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<tr>
<td>IGF-I (nmol/L)</td>
<td>35.3 ± 2.6</td>
<td>35.1 ± 2.8</td>
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<tr>
<td>GH (μg/L)</td>
<td>7.3 ± 2.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Insulinemia (pmol/L)</td>
<td>48.1 ± 2.9</td>
<td>38.7 ± 3.6</td>
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<tr>
<td>Blood glucose (mmol/L)</td>
<td>4.1 ± 0.2</td>
<td>4.0 ± 0.2</td>
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*P < 0.05 vs. baseline levels at −180 min.
pathophysiological conditions such as Laron's syndrome, insulin-dependent diabetes mellitus, malnutrition, and critical illness and even in fasted normal subjects (7, 8, 23–25). Moreover, it has been shown that rhIGF-I administration inhibits both basal and stimulated GH secretion in normal fed subjects; in fact, low rhIGF-I doses were shown to blunt the GH response to ARG (35), GHRH (6, 13, 14), and peptidyl GH secretagogues (14). As anticipated, the marked inhibitory effect of rhIGF-I on the GH response to GHS is noticeable (14), because they mainly act at the hypothalamic level and show a GH-releasing effect that is generally refractory to inhibitory inputs, including exogenous SS (26–28).

In our experimental conditions, differently from other researches (9, 13), no inhibitory effect of rhIGF-I on spontaneous GH secretion was recorded, although the use of an ultrasensitive assay might have allowed us to disclose some

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**FIG. 1.** Mean (±SEM) GH curves (micrograms per L) and AUCs (micrograms per L/h) after GHRH alone (1 μg/kg, iv, at 0 min; upper panel) or combined with arginine (0.5 g/kg, iv, from 0–30 min; lower panel), preceded by placebo (○) or rhIGF-I (●; 20 μg/kg, sc, at −180 min) administration in six normal young adults. *, P < 0.05.
EFFECTS OF rhIGF-I ON GH SECRETION

Acknowledgments

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References

NIH-NIDDK Study

At the National Institutes of Health (NIH) in Phoenix, Arizona, we are studying the neurophysiology of eating behavior in successful dieters (18 yr or older, healthy, nonsmoker), i.e. people who were very obese (BMI ≥35 kg/m²), lost a significant amount of weight without the help of drugs or surgery, and have maintained a near-normal body weight (BMI ≈23 kg/m²) for at least 6 months. The NIH Institutional Review Board approved the study. We need referrals.

We offer a monetary compensation for time and participation, reimbursement of travel expenses, and a free medical check-up. The study requires a 5-day hospital stay at the NIH Research Unit in the Phoenix Indian Medical Center, Phoenix, Arizona.

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