IGF-I does not affect the net increase in GH release in response to arginine

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IGF-I does not affect the net increase in GH release in response to arginine. Am J Physiol Endocrinol Metab 283: E702–E710, 2002. First published June 18, 2002; 10.1152/ajpendo.00075.2002.—Arginine stimulates growth hormone (GH) secretion, possibly by inhibiting hypothalamic somatostatin (SS) release. Insulin-like growth factor I (IGF-I) inhibits GH secretion via effects at the pituitary and/or hypothalamus. We hypothesized that if the dominant action of IGF-I is to suppress GH release at the level of the pituitary, then the arginine-induced net increase in GH concentration would be unaffected by an IGF-I infusion. Eight healthy young adults (3 women, 5 men) were studied on day 2 of a 47-h fast for 12 h (35th–47th h) on four occasions. Saline (Sal) or 10 µg·kg−1·h−1 recombinant human IGF-I was infused intravenously for 5 h from 37 to 42 h of the 47-h fast. Arginine (Arg) (30 g iv) or Sal was infused over 30 min during the IGF-I or Sal infusion from 40 to 40.5 h of the fast. Subjects received the following combinations of treatments in random order: 1) Sal + Sal; 2) Sal + Arg; 3) IGF-I + Sal; 4) IGF-I + Arg. Peak GH concentration on the IGF-I + Arg day was ~45% of that on the Sal + Arg day. The effect of arginine on net GH release was calculated as [(Sal + Arg) – (Sal + Sal)] – [(IGF-I + Arg) – (IGF-I + Sal)]. There was no significant effect of IGF-I on net arginine-induced GH release over control conditions. These findings suggest that the negative feedback effect of IGF-I on GH secretion is primarily mediated at the pituitary level and/or at the hypothalamus through a mechanism different from the stimulatory effect of arginine.

GROWTH HORMONE SECRETION is controlled by at least two hypothalamic hormones, growth hormone-releasing hormone (GHRH), which periodically stimulates GH release from the somatotrophs, and somatostatin (SS), which tonically inhibits GH release (27). Recent data suggest that ghrelin, a stomach-derived peptide, also plays a role in the stimulation of GH release (20). In addition, several findings suggest that GH secretion is under the negative feedback control of insulin-like growth factor I (IGF-I) (19). Experiments using exogenous IGF-I infusions in animals (25), as well as in humans (5, 14), show suppression of GH secretion. There are several conditions (including starvation, malnutrition, Laron-type dwarfism, and type 1 diabetes mellitus) in which plasma IGF-I concentrations are decreased as a result of GH resistance (16). The elevated serum GH concentrations in these conditions are consistent with a reduction of IGF-I negative feedback. Administration of exogenous IGF-I to these patients decreases serum GH concentrations (7).

It has been proposed that IGF-I acts on the hypothalamus and the pituitary to inhibit GH secretion. The direct inhibitory effect of IGF-I on the somatotroph is established (33). However, studies on the hypothalamic effects of IGF-I have yielded conflicting results. Some studies support effects of IGF-I to increase SS release or decrease GHRH release or both (3), whereas other studies have not observed these effects (11). We reasoned that if the dominant effect of IGF-I on GH release was via hypothalamic effects (particularly to increase SS release), then an infusion of IGF-I would attenuate the net increase in serum GH concentrations after an infusion of arginine, which is thought to act by decreasing hypothalamic SS release without any direct effect on the pituitary (1). However, if IGF-I suppresses GH release primarily via direct effects on somatotrophs, then the arginine-induced net increase in GH concentration would be unaffected by the additional IGF-I infusion, even if the absolute peak GH response to arginine was reduced. To investigate these hypotheses, we infused arginine together with IGF-I in healthy men and women. The subjects were fasted to enhance GH secretion and to avoid the confounding effects of meals.

METHODS

Subjects

The study was approved by the Human Investigation and General Clinical Research Center (GCRC) Advisory Committees of the University of Virginia and Genentech. All subjects...
studied: five men and three women, with a mean age of 25.8 ± 3.6 (SD) yr, a mean body mass index (BMI) of 25.7 ± 3.4 kg/m², and a mean height of 175.4 ± 5.1 cm. All subjects were nonsmokers, were taking no medications, had not undertaken transmeridian travel within 6 wk of study, and had unremarkable clinical histories and normal physical examinations. All had normal biochemical indexes of renal, hepatic, and hematologic function, glycated hemoglobin, thyroxine, thyroid-stimulating hormone, prolactin, luteinizing hormone, follicle-stimulating hormone, testosterone (men), and estradiol (women).

Study Design

Each subject was studied during four admissions to the GCRC, separated by ≥4 wk, and took ferrous gluconate between studies to prevent anemia. The young women were studied between day 1 and day 6 of the menstrual cycle (10) and had a negative β-human chorionic gonadotropin pregnancy test immediately before each study. For each study, the subjects consumed a standard dinner (consisting of 15% protein, 30% fat, and 55% carbohydrate) between 2000 and 2100 on the evening before study day 1 and then fasted for 47 h, during which they ingested only water, potassium chloride (20 meq/day), and multivitamins (one tablet/day). Compliance with the fast was monitored by daily measurement of weight and twice daily urinary ketone levels. On each morning of the fast and at the end of the study, blood was obtained for determinations of complete blood count, serum chemistries, and hepatic enzymes to monitor for adverse effects. The studies were performed on day 2 of the fast (37–42 h of fasting) (Fig. 1). During each of the four admissions, subjects received one of the following combinations of infusions in randomized order: 1) IGF-I and saline; 2) IGF-I and arginine; 3) saline and arginine; or 4) saline alone. At 0600 on day 2 of the fast, a cannula was placed in the antecubital vein of each arm for continuous infusion of test substances, and another was placed retrograde in a wrist vein for blood sampling. The latter was kept patent by a slow saline infusion, and the hand was kept in a heated box at 70°C to ensure arterialization of venous blood for estimation of plasma glucose concentrations (8). From 0700 on study day 2 to 1900 on study day 2, venous blood samples (1 ml) were obtained at 10-min intervals for measurement of GH. Serum glucose concentrations were measured at 0700, 0840, and 0850, every 10 min from 0900 to 1500, and then every hour until 1900 of study day 2. To prevent dehydration, 0.9% saline was infused intravenously at 50 ml/h from 0700 to 1900 on study day 2.

IGF-I + saline admission (IGF-I + Sal). Recombinant human IGF-I (rhIGF-I, 10 μg·kg⁻¹·h⁻¹) was administered intravenously from 0900 to 1400 on study day 2. The rhIGF-I (kindly provided by Genentech, South San Francisco, CA) was prepared in normal saline from a stock 10 mg/ml solution and was infused at 10 μl·kg⁻¹·h⁻¹ on study day 2, 20% dextrose was infused if plasma glucose fell >15% below the baseline values, defined as the mean of the plasma glucose concentrations at 0840 and 0850. The rate of the dextrose infusion was adjusted every 10 min to keep the plasma glucose level within 10% of this value on the basis of the plasma glucose measurements and a negative feedback algorithm (8). The actual glucose concentration of the 20% dextrose solution was measured for each experiment. A glucose analyzer (Beckman Analytical System Group, Columbia, MD) was used to measure plasma glucose concentrations during the study.

Dextrose infusions were delivered by a Harvard microprocessor pump controlled by a computer program (running on an IBM-compatible computer) written and supplied by Dr. David Krusch (University of Rochester, Rochester, NY). Normal saline (0.9%) was infused intravenously from 1200 to 1230 on day 2 of the study as a control for the arginine infusions on other admissions. Blood sampling was completed at 1900, and subjects were fed.

IGF-I + arginine admission (IGF-I + Arg). This second protocol was the same as the first, except that arginine (30 g; 300 ml of a 10% arginine hydrochloride solution, Pharmacia & Upjohn) was infused instead of saline from 1200 to 1230.

Saline + arginine admission (Sal + Arg). The third protocol was the same as the first, except that normal saline (0.9%) was infused instead of rhIGF-I from 0900 to 1400, and arginine (30 g) was infused instead of saline from 1200 to 1230.

Saline + saline admission (Sal + Sal). The fourth protocol was the same as the first, except that normal saline (0.9%) was infused instead of rhIGF-I.

Assays

GH. Serum GH concentrations were measured in duplicate by a commercially available chemiluminescence assay (Nichols Institute Diagnostics, San Juan Capistrano, CA); the assay was modified as previously described (6). The sensitivity of the assay is 0.002 μg/l. The interassay coefficients of variation (CVs) were 7.2% at 1.7 μg/l and 7.2% at 4.2 μg/l. The intra-assay CVs were 4.9% at 0.2 μg/l, 6.7% at 2 μg/l, and 6.4% at 4.9 μg/l.

IGF-I. Total plasma IGF-I was measured by RIA after acid ethanol extraction with a commercially available kit (Diagnostic Systems Laboratories, Webster, TX) by Dr. R. Hintz’s laboratory (Stanford University Medical Center). Interassay CVs were 1.5% at 53.84 μg/l and 3.7% at 255.9 μg/l. Intra-assay CVs were 3% at 55.35, 1.5% at 263.6 μg/l. The sensitivity of this assay is 0.8 μg/l.

Other assays. Other assays were performed in the Clinical Laboratories of the University of Virginia Health System by use of standard methods.

Statistical Analysis

To model GH response as a function of time, we used a bootstrap repeated-measures modeling procedure that has been previously used to model time-dependent GH secretion (described in detail in Ref. 4). To determine the effect of the
ARGinine AND IGF-I EFFECTS ON GH RELEASE

The results of various combinations of infusions on GH release, we computed a time profile of serum GH concentrations for each individual, which estimated at 10-min increments over the 12-h study period the within-subject effect of interest. The profile was generated in a way that any point on the 10-min increment the subject's measured GH concentrations under Sal + Sal infusion from the subject's measured GH concentrations under Sal + Arg infusion. The baseline-adjusted effect of arginine infusion on GH release was estimated by subtracting at each 10-min increment the subject's measured GH concentrations under Sal + Sal infusion from the subject's measured GH concentrations under Sal + IGF-I infusion. The effect of IGF-I on the maximal stimulation of GH release by arginine was estimated by [(IGF-I + Arg) − (Sal + Arg)]. The IGF-I effect on arginine-induced net increase of GH concentrations above control conditions was estimated as the effect of arginine in the presence of saline minus the effect of arginine in the presence of IGF-I [(Sal + Arg) − (Sal + Sal)] − [(IGF-I + Arg) − (IGF-I + Sal)].

Fig. 2. Insulin-like growth factor I (IGF-I) concentrations (means ± SE in μg/l; n = 8) measured in blood samples obtained every hour for 12 h. Arrow, start of 30-min arginine (Arg, 30 g) or saline (Sal) infusion; shaded area, 5-h infusion period of IGF-I or saline; ○, IGF-I + Sal infusion day; ●, Sal + Sal infusion day; ▲, IGF-I + Arg infusion day; ◊, Arg + Sal infusion day.

RESULTS

Total IGF-I Concentrations

The preinfusion total IGF-I concentration (in μg/l, means ± SE) was 197.9 ± 19.5 (range: 103–341) (Fig. 2). Mean IGF-I concentration at 1200, the starting time point of the arginine infusion, was significantly increased during the IGF-I infusion admission days: 346.3 ± 29.9 on the IGF-I + Arg admission day and 367.3 ± 37.7 on the IGF-I + Sal admission day, compared with 186 ± 14.6 on the Sal + Sal admission day and 196.1 ± 14.5 on the Arg + Sal admission day. The co-administration of arginine had no effect on IGF-I levels. The increase in total IGF-I was still present at 7 PM, 5 h after the infusion had stopped.

Plasma Glucose Concentrations and Glucose Infusion Rates

The baseline glucose concentration (in mmol/l, means ± SE) was 4.2 ± 0.1 (range: 3.3–4.9). Mean glucose concentration from 0900 to 1500 was 3.9 ± 0.2 mmol/l on the IGF-I + Sal admission day, 4.0 ± 0.2 mmol/l on the IGF-I + Arg admission day, 4.0 ± 0.1 mmol/l on the Sal + Sal admission day, and 4.3 ± 0.2 mmol/l on the Arg + Sal admission day. Arginine caused a slight but significant increase in mean glucose concentration when given either alone or in combination with IGF-I. This effect started 15–30 min after the start of the arginine infusion and was sustained until the end of the study at 1900 (6.5–6.75 h). Glucose was infused in only two subjects on two occasions. In one subject, we used a total glucose dose of 3.4 mg/kg from 1220 to 1240, and for the other subject we had to infused a total glucose dose of 47.5 mg/kg from 1240 to 1250. In
both cases this occurred during an IGF-I + Arg admission day.

**Serum GH Concentrations**

**Arginine effect on GH concentrations.** Figure 3A shows the serum GH concentrations (means ± SE in μg/l) over the entire 12-h study period during the Sal + Arg and the Sal + Sal admission days. The mean peak GH concentration was reached 1 h and 10 min after the start of the arginine infusion and was ~3.4-fold higher than during the control admission at that time point. The regression curve of the difference in serum GH concentrations between the two admissions [(Sal + Arg) - (Sal + Sal)] over time, with simultaneous 95% confidence bands, was calculated and is shown in Fig. 3B. It indicates that serum GH concentrations increased significantly 27 min after the start of the arginine infusion and remained significantly elevated for 1 h and 27 min, being the time period when the lower 95% confidence limit for the regression curve was >0.

**IGF-I effect on GH concentrations.** Figure 4A shows the serum GH concentrations (means ± SE in μg/l; n = 8) measured in blood samples obtained every 10 min for 12 h. Shaded area, 5-h infusion of IGF-I (10 μg·kg⁻¹·h⁻¹) or saline (Sal). Sal + Sal admission day; Sal + IGF-I admission day. B: change in serum GH concentrations in response to a 5-h infusion of IGF-I (10 μg·kg⁻¹·h⁻¹) compared with control (saline infusion, Sal) conditions [(IGF-I + Sal) - (Sal + Sal)] in fasted subjects. Regression curve (dotted line) and simultaneous 95% confidence limits (solid lines) are shown (see MATERIALS AND METHODS). Significant suppression of serum GH occurred when the lower 95% confidence limit for the regression curve was >0.

**Fig. 3.** A: serum GH concentrations (means ± SE in μg/l; n = 8) measured in blood samples obtained every 10 min for 12 h. Arrow, start of 30-min arginine (Arg, 30 g) or saline (Sal) infusion. Sal + Arg admission day; Sal + Sal admission day. B: change in serum GH concentrations in response to a 30-min arginine infusion compared with control (saline infusion, Sal) conditions [(Sal + Arg) - (Sal + Sal)] in fasted subjects. The regression curve (dotted line) and simultaneous 95% confidence limits (solid lines) are shown (see MATERIALS AND METHODS). A significant increase of serum GH concentrations occurred when the lower 95% confidence limit for the regression curve was >0.

**Fig. 4.** A: serum GH concentrations (means ± SE in μg/l; n = 8) measured in blood samples obtained every 10 min for 12 h. Shaded area, 5-h infusion of IGF-I (10 μg·kg⁻¹·h⁻¹) or saline (Sal). Sal + Sal admission day; Sal + IGF-I admission day. B: change in serum GH concentrations in response to a 5-h infusion of IGF-I (10 μg·kg⁻¹·h⁻¹) compared with control (saline infusion, Sal) conditions [(IGF-I + Sal) - (Sal + Sal)] in fasted subjects. Regression curve (dotted line) and simultaneous 95% confidence limits (solid lines) are shown (see MATERIALS AND METHODS). Significant suppression of serum GH occurred when the upper 95% confidence limit for the regression curve was <0.
infusion, a rebound increase of serum GH concentrations occurred 1–2 h later (1650–1725).

**IGF-I effect on the maximal GH response to the arginine infusion.** Figure 5A shows the serum GH concentrations (means ± SE in µg/l) over the entire 12-h study period during the IGF-I + Arg and the Sal + Arg admission days. The regression curve of the difference in serum GH concentrations between the two admissions [(IGF-I + Arg) – (Sal + Arg)] over time, with simultaneous 95% confidence bands, was calculated and is shown in Fig. 5B. The maximal GH response to the arginine infusion was significantly decreased during the IGF-I infusion compared with the saline infusion. This started 12 min after the beginning of the arginine infusion and lasted for 3.5 h. The maximal serum GH concentration after arginine infusion during

the Arg + IGF-I admission day was ~45% of that measured when arginine was given without an additional IGF-I infusion.

**IGF-I effect on arginine-induced net increase of GH concentration.** Figure 6A shows the serum GH concentrations (means ± SE in µg/l) over the entire 12-h study period during the IGF-I + Arg and the IGF-I + Sal admission days, and Fig. 6B shows them during the Sal + Arg and the Sal + Sal admission days (also shown in Fig. 3A). The regression curve of the difference in serum GH concentrations over time between the arginine effect in the presence of saline and the arginine effect in the presence of IGF-I infusion [(Sal + Arg) – (Sal + Sal)] – [(IGF-I + Arg) – (IGF-I + Sal)], with simultaneous 95% confidence bands, was calculated and is shown in Fig. 6C. Because neither the lower nor the upper 95% confidence limit for the regression curve crossed zero, no significant effect of IGF-I on net arginine-induced GH release over control conditions was demonstrated. The net effects of arginine on GH release were neither abolished nor significantly attenuated by IGF-I.

**Adverse Effects**

There were mild changes in serum concentrations of uric acid, creatinine, and bilirubin during the 47-h fast. Subjects did not experience adverse clinical effects from the IGF-I or arginine infusions.

**DISCUSSION**

In humans, the systemic administration of IGF-I inhibits GH secretion (5). Whether IGF-I inhibits GH secretion via negative feedback at the pituitary or at the hypothalamus through changes in SS release is unresolved. The major finding of the present study is that an IGF-I infusion attenuated the peak GH response, but not the net increase in GH release, in response to an arginine infusion. We interpret these results to suggest that arginine and IGF-I act through different mechanisms. We chose the model of fasting-enhanced GH secretion to study the mechanisms of action involved in the negative feedback effect of IGF-I on GH secretion. Fasting for 2 days enhances GH secretion 4- to 5-fold (15), probably by decreasing hypothalamic SS release (26). Even under the conditions of fasting-enhanced GH secretion, arginine induced a robust increase in GH release in the present study (Fig. 3), suggesting that arginine induced a further decrease in SS release. This assumption is based on the study published by Alba-Roth et al. (1), which indirectly shows that arginine acts through SS and does not have a direct in vitro effect at the pituitary. This view has been recently challenged by a paper published by Villalobos et al. (30) showing that arginine affects [Ca²⁺] in rat anterior pituitary cells in vitro. However, the study does not show specific arginine effects on GH-containing cells. Theoretically, our finding could also be compatible with an increased hypothalamic release of GHRH. Administration of a GHRH antagonist blocked the GH response to arginine, demonstrat-
ing that the presence of GHRH is necessary for the action of arginine on GH release (18). However, the potentiation by arginine of the GH response to exogenous GHRH administration strongly suggests that arginine increases GH release by reducing SS release (1).

In agreement with previous studies (5, 14), the IGF-I infusion alone resulted in a significant reduction of spontaneous GH secretion (Fig. 4). The rhIGF-I dose used in our experiment (10 μg·kg⁻¹·h⁻¹) increased circulating IGF-I concentrations significantly within the physiological range, as in our previous study (14). In the latter study, mean GH concentrations decreased 2 h after the infusion had started, whereas in our study this effect was present not earlier than 4.5 h after the start of the IGF-I infusion. The inclusion of women could explain this discrepancy, as it has been shown that IGF-I has a less suppressive effect on GH release in women than in men (17). In review of the time point of GH suppression by IGF-I in our experiment, it seems that the GH-releasing effect of arginine might have partially preceded the IGF-I-suppressing effect. However, the GH level measured in the blood at any given time point is a function of the amount of GH released from the pituitary and the GH clearance rate (29).

Extensive deconvolution studies (29) show that the GH increase in the peripheral blood follows the GH released in bursts from the pituitary with a certain delay. Taking this delay into account, it is expected that the negative feedback effect by IGF-I resulting in the decrease in GH release from the pituitary precedes the time point of the actual decline of peripheral GH levels. Thus it is likely that, at the time point of the arginine administration, the IGF-I negative feedback effect on GH release at the pituitary was fully effective, even though the peripheral levels were not fully suppressed at that time point. Gender-specific differences regarding the arginine as well as the IGF-I effect have been reported previously. The data of Jaffe et al. (17) suggest that the suppressive effect of IGF-I on GH release is ~20% greater in men than in women. The effect of arginine on GH release is also gender specific (31), with a greater serum GH area under the curve in women compared with men after arginine infusion. The statistical analysis in our set of data showed no significant difference between men and women; this analysis is limited by the small number of women in our study.

Fig. 6. A: serum GH concentrations (means ± SE) measured in blood samples obtained every 10 min for 12 h during IGF-I + Arg admission day (●) and control day (IGF-I + Sal; ▲). Arrow, start of 30-min arginine (Arg, 30 g) or saline (Sal) infusion. Shaded area, 5-h infusion of IGF-I (10 μg·kg⁻¹·h⁻¹). B: serum GH concentrations (means ± SE) measured in blood samples obtained every 10 min for 12 h during Sal + Arg admission day, ●, and control day (Sal + Sal; ▲). Arrow, start of 30-min arginine (Arg, 30 g) or saline infusion (Sal). Shaded area, 5-h infusion of saline. C: effect of a 5-h infusion of IGF-I (10 μg·kg⁻¹·h⁻¹) on net increase of serum GH concentrations above control conditions in response to a 30-min arginine (Arg, 30 g) infusion in fasted subjects. This was estimated as the difference of the arginine effect in the presence of saline (Sal) and the arginine effect in the presence of IGF-I [([Sal + Arg] – [Sal + Sal]) – ([IGF-I + Arg] – [IGF-I + Sal])]. Regression curve (solid line) and simultaneous 95% confidence limits (dotted lines) are shown (see MATERIALS AND METHODS). Because neither the lower nor the upper 95% confidence limit for the regression curve crossed 0, no significant effect of IGF-I on net arginine-induced GH release over control conditions was demonstrated.
However, an increase in the number of women in our study should not have changed the outcome of our analysis, because the gender differences of both the arginine and IGF-I effects have been shown to go in opposite directions (31, 17).

To avoid ≥15% decrease in plasma glucose concentrations during rhIGF-I infusion, two of eight volunteers required a dextrose infusion for brief time periods (10–20 min). Mean plasma glucose concentrations were slightly, but significantly, higher for several hours after the arginine infusion when given either alone or in combination with IGF-I. This effect of arginine on glucose concentrations is established (2). However, the mean increase in plasma glucose between the study days with and without arginine was small (6–9 mg/dl or 0.3–0.5 mmol/l) and was unlikely to have had an effect on GH secretion, because higher increments in plasma glucose are needed to affect GH concentrations (22). During the IGF-I infusions, pulses of GH continued at lower amplitudes. This suggests that interventions of endogenous GHRH pulses and troughs of SS release continued to generate GH pulses during the IGF-I infusion. This observation is compatible with either 1) a direct pituitary action of IGF-I, reducing the responsiveness of the somatotrophs to GHRH, or 2) hypothalamic actions of IGF-I to decrease GHRH or increase SS release.

IGF-I significantly decreased the maximal GH response to arginine. As is evident in Fig. 5, the negative feedback effect of the IGF-I infusion on GH release was present before the start of the arginine infusion, and this likely accounts for the lower peak GH response to arginine. Thus, simply comparing the maximal GH response to arginine during IGF-I vs. saline infusions does not take into account the lower basal GH concentrations before arginine administration that were present during the IGF-I infusion. Therefore, the effect of arginine on net GH release over time (change relative to control conditions) was calculated as [(Sal + Arg) − (Sal + Sal)] − [(IGF-I + Arg) − (IGF-I + Sal)]. As shown in Fig. 6C, this revealed that there was no significant effect of IGF-I on net arginine-induced GH release over control conditions. Arginine GH stimulation is used for the diagnosis of GH deficiency, especially under clinical circumstances when other, more invasive tests (i.e., insulin tolerance test) are contraindicated (23). Our results suggest that the net increase in GH in response to arginine is unaffected by ambient IGF-I levels. However, if one uses the peak GH response after arginine stimulation instead of the net effect on GH release, as is current standard practice, the endogenous IGF-I levels should also be considered because, according to our results, it does have an impact on the outcome of the arginine stimulation test in the commonly used test setting, i.e., measuring the absolute peak GH response (23).

Irrespective of whether the arginine infusion was given with or without an additional IGF-I infusion, GH levels were increased 3.4- to 3.3-fold compared with the control day. If both arginine and IGF-I act through the same GH-releasing mechanism (i.e., through SS), one would expect that the combined administration of both agents should result in an altered net GH-releasing effect of arginine. The fact that infusion of IGF-I had no impact on the net arginine effect suggests that IGF-I and arginine do not act on the same GH-releasing mechanism. The possibility that SS was maximally suppressed by fasting and arginine and, therefore, that no additional IGF-I effect was observed, is unlikely. The IGF-I infusion was started 3 h before arginine administration and resulted in a decrease in serum GH concentrations before the start of the arginine infusion. If IGF-I inhibits GH secretion solely by stimulating SS release, and if the combination of fasting and arginine maximally inhibits SS secretion so that it cannot be stimulated by IGF-I, we would have expected the net GH response to arginine to be greater during the IGF-I infusion than during saline infusion. This was not the case. Furthermore, the maximal GH response would not have been reduced by IGF-I. Although it is possible that arginine has multiple modes of GH-stimulating action, in human studies to date, there is only evidence for an effect to inhibit SS (1). Because arginine most likely acts exclusively via hypothalamic effects (1), these data favor a direct effect on the pituitary as a target for IGF-I negative feedback on GH secretion.

Although earlier animal studies suggested that IGF-I acts at the hypothalamus (25), Fletcher et al. (11) showed that intrapituitary infusion of IGF-I or IGF-II is followed by the suppression of GH secretion, whereas intracerebroventricular injection does not have an impact on GH release. Our hypothesis that IGF-I acts directly at the pituitary and reduces the available GH pool released by arginine is in agreement with the in vitro experiments of several other investigators (34). Their data demonstrate that IGF-I has a direct negative effect on GH mRNA levels in the pituitary.

In vivo studies in humans (3) have concluded that the suppression of GH secretion by IGF-I is, at least in part, due to its stimulation of hypothalamic SS secretion. Berman et al. (3) compared the inhibitory effect of IGF-I on thyroid-releasing hormone (TRH)-induced thyroid-stimulating hormone (TSH) release in humans with the inhibitory effect of SS on TRH-induced TSH release (32). However, it cannot be excluded that the blunted TSH release during the IGF-I infusion reported in that study (3) was caused by an increased thyroxine-to-triiodothyronine conversion during the IGF-I infusion, as shown in a later study (17). Gianotti et al. (12) studied the effects of a single bolus injection of IGF-I (20 μg/kg) before administration of GHRH with and without arginine in young women. They concluded that arginine is able to override the inhibitory effect of IGF-I on the response to GHRH, suggesting that IGF-I acts on the hypothalamus via enhancement of SS release. However, the authors do not provide data to show whether, after IGF-I administration, the GH response to arginine would differ from that after the combined injection of GHRH and arginine.

Although our data suggest that IGF-I and arginine act through different mechanisms, it cannot be ex-
cluded that IGF-I acts centrally through changes in hypothalamic GHRH release, which would also be consistent with our findings. Several groups have shown in vitro or in animal models that IGF-I changes hypothalamic GHRH release (24). Data published by Korbonits et al. (21) suggest that IGF-I, as well as IGF-II, inhibits the release of GHRH from the rat hypothalamus.

In summary, an IGF-I infusion attenuated the peak GH response but not the net increase in GH release in response to an arginine infusion. These in vivo data favor the hypothesis that arginine and IGF-I exert their opposing effects on GH release via different mechanisms. Because arginine stimulates GH secretion via nonpituitary effects, these results suggest that IGF-I inhibits GH primarily by a direct action on the pituitary. Additional effects of IGF-I on the hypothalamus cannot be excluded by this experiment.

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REFERENCES


