Effect of acute elevation of IGF-I on circulating GH, TSH, insulin, IGF-II and IGFBP-3 levels in non-endocrine short stature (NESS)

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ABSTRACT. It is not clear whether acute and slight elevation of serum IGF-I, which does not affect blood glucose levels, modulates circulating GH levels. To clarify this, small doses of recombinant human IGF-I (rhIGF-I, 5 µg/kg, iv) were administered as a bolus to 10 children with non-endocrine short stature (NESS) (5 males and 5 females, 11.2±0.7 yr old) after an overnight fast. Physiological saline was administered intravenously to sex- and age-matched NESS controls (5 males and 5 females, 10.9±0.7 yr old). The changes of serum GH, TSH, PRL, IGF-I, IGF-II, IGFBP-3, T_4, T_3 and plasma glucose levels after the administration were compared to those of the control subjects. Serum IGF-I levels increased significantly from 15 to 150 min after injection compared to those in the control group. The peak value was observed at 15 min (Δ increment, 74.6±11.8 µg/l). At 15 min after the injection, serum insulin was suppressed significantly ($p<0.05$), although plasma glucose levels were not modified significantly. Serum TSH showed a significant decrease by rhIGF-I at 15 min and 60 min, whereas serum T_4 and T_3 levels were not modified. Serum GH was also significantly suppressed at 60 min ($p<0.02$) and showed a rebound increase at 120 min ($p<0.05$). Serum IGFBP-3 levels after rhIGF-I were higher than controls at 90 min and 150 min. No significant changes of serum PRL, IGF-II, (IGF-I plus IGF-II)/ IGFBP-3 ratios were observed after the IGF-I injection compared to controls. These results indicate that circulating IGF-I is a physiological regulator of GH secretion in normal children, since the changes of IGF-I after the small doses of rhIGF-I administration were within physiological ranges and did not affect plasma glucose levels.

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INTRODUCTION

Growth hormone secretion is mainly regulated by hypothalamic GHRH, somatostatin and IGF-I (1, 2). The physiological role of circulating IGF-I in humans is not well characterized.

The administration of large amounts of recombinant human IGF-I (rhIGF-I) stimulates circulating GH secretion through hypoglycemia in normal subjects (3). The effects of rhIGF-I on spontaneous GH secretion have therefore been studied under the prevention of hypoglycemia by either glucose clamp methods (1) or food intake (2). Recently, Ghigo *et al.* (4) reported that subcutaneous administration of 20 µg/kg of rhIGF-I inhibits either spontaneous GH secretion or stimulated GH secretion induced by GHRH and Hexarelin in normal young women without causing hypoglycemia.

So far, it has been reported that iv infusion of 3 µg/kg/h of rhIGF-I is the minimal dose to inhibit GH secretion in normal adults , but that this dose still has a glucose lowering effect in minor cases (5, 6). The physiological role of IGF-I on GH secretion in normal children is largely unknown. Limited studies have been carried out in patients with GH insensitivity syndrome. In these individuals, large amounts of rhIGF-I suppress fasting serum concentration of GH (7, 8). However, the activities of hypothalamic GHRH, SS neurons and pituitary somatotrophs, and IGF-I clearance are considered to be different from those of normal children due to the GH receptor abnormality and IGFBP-3 deficiency.

Key-words: Short stature, IGF-I, GH, TSH, IGFBP-3.

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Therefore, we examined circulating GH, TSH, PRL, Insulin, IGF-II and IGFBP-3 responses, in non-endocrine short stature (NESS) children, to a bolus injection of 5 μg/kg of rhIGF-I which has no effect on fasting plasma glucose levels. This dose causes a steep elevation of serum IGF-I levels and might be suitable to see acute and direct effects of IGF-I on pituitary somatotrophs.
This is the first report to examine serum GH responses to rhIGF-I in NESS.

MATERIALS AND METHODS

Ten NESS patients (5 males and 5 females, range 9-14 yr old, mean±SE=11.2±0.7 yr old) and age-and sex-matched 10 NESS patients as control (5 males and 5 females, range 9-13 yr old, mean±SE=10.9±0.7 yr old), all healthy and non-obese, were studied.
The study was approved by the independent Local Ethics Commitee in Sendai and informed consent was obtained from every parent and/or subject. In all NESS patients, height standard deviation score (HtSDS) was below -2SD, and Ht velocity for chronological age ranged from low to low-normal, but GH responses, at least to two provocative stimuli [classical tests, (clonidine, insulin tolerance test (ITT), arginine tolerance test (ATT), L-dopa and glucagon); and GHRH] were normal (>10 μg/l to classical tests, and >15 μg/l to GHRH), and plasma IGF-I levels were within normal ranges.
Intravenous cannula were inserted into the antecubital vein from 08:30 h after an overnight fast, and rhIGF-I (rhIGF-I, Fujisawa, Tokyo) (5 μg/kg, iv) was administered as a bolus at 09:00 h to these subjects. Physiological saline was administered intravenously to the control NESS patients. Blood samples were obtained 30 and 0 min before the IGF-I injection and 15, 30, 45, 60, 90, 120 and 150 min after the injection. Serum samples were kept frozen at -20 C until assay. Serum GH, TSH, PRL, IGF-I, IGF-II, IGFBP-3, T_4, T_3, insulin and plasma glucose were measured with commercial IRMA kits [Daiichi: GH, PRL, IGF-I (by acid etanol extraction method), IGF-II, IGFBP-3 and insulin], Immunofluorometric assay kits (Pharmacia: TSH, T_4, T_3) and the glucose dehydrogenase method, respectively.
The minimal detectable level, the intra- and interassay coefficients of variation were 0.006 μg/l, 2.2% and 3.5% for GH; 0.02 mU/l, 2.5% and 3.8% for TSH; 0.3 μg/l, 3.2% and 5.6% for PRl; 0.2 μg/l, 2.0% and 2.1% for IGF-I; 0.1 μg/l, 3.2% and 4.8% for IGF-II; 0.925 μg/l, 2.8% and 3.4% for IGFBP-3; 1.0 mU/l, 1.6% and 2.2% for insulin; 0.2 μg/dl, 3.0% and 6.1% for T_4; and 8.0 ng/dl, 2.9% and 6.7% for T_3, respectively (9, 10).
Data are expressed as the mean±SE. Statistical analysis was carried out using analysis of variance followed by Student/Neumann-Keuls test or Fisher's randomization test where appropriate for statistical comparisons between two groups or within a group.

RESULTS

After the administration of rhIGF-I, serum IGF-I levels in NESS increased significantly from 15 to 150 min compared to those in the control study (Fig. 1A). As -30 min and 0 min values of each parameter before IGF-I or saline study were quite similar (p=not significant=NS), 0 min values alone were shown in each figure. The peak value was observed at 15 min ranging from 87.4 to 541.6 μg/l, and the mean value was significantly higher than that of the control subjects (IGF-I *vs* saline, baseline 259.4±33.8 *vs* 247.5±34.3, p=NS; Δ increment, 75.2±9.9 *vs* -2.5±5.4 μg/l, $p<0.01$).
At 15 and 30 min after the injection, serum insulin was suppressed significantly (IGF-I *vs* saline, baseline, 5.2±0.9 *vs* 4.8±0.4, p=NS; Δ increment at 15 min, -2.3±0.7 *vs* -0.5±0.2, $p<0.05$; at 30 min, -1.6±0.5 *vs* 0.2±0.4 μU/ml, $p<0.05$) (Fig. 1B). Within the IGF-I study, 15 and 30 min values are also significantly lower than that of 0 min value (both $p<0.025$). Concomitant with the decrease of insulin, plasma glucose levels increased transiently at 30 min (IGF-I *vs* saline, baseline, 85.5±2.5 *vs* 83.0±1.7; Δ increment, 2.4±1.2 *vs* 0.2±0.6 mg/ml), but it was not statistically significant compared to control subjects (Fig. 1C).
Serum TSH was significantly suppressed by rhIGF-I at 15 min (IGF-I vs saline, baseline, 1.65±0.17 *vs* 1.36±0.22, p=NS; Δ increment, -0.18±0.04 *vs* -0.09±0.04 μU/ml, $p<0.01$) and 60 min (Δ increment, -0.44±0.10 *vs* -0.160±0.10 μU/ml, $p<0.05$) (Fig. 2A). Within the IGF-I study, all values from 15 to 150 min were significantly lower than that of 0 min value ($p<0.05$ to 0.005). In this study, NESS children showed -17.0±2.1% of TSH decrease at 30 min from the basal level and the nadir value (-26.7±3.5%) was at 90 min after the IGF-I injection.
However, serum T_4 and T_3 levels at 0 min and 150 min were not changed in either IGF-I (T_4: 7.8±0.3 *vs* 7.4±0.3μg/dl; T_3: 141±7 *vs* 128±7 ng/dl) or control groups (T_4: 7.7±0.3 *vs* 7.2±0.3 μg/dl; T_3:143±7 *vs* 128±7 ng/dl).
Serum GH showed a gradual decrease from 15 min and reached statistical significance at 60 min (IGF-I *vs* saline, baseline, 1.7±0.4 *vs* 1.8±0.4 μg/dl; Δ increment at 60 min, -1.0±0.3 *vs* 1.3±0.8, $p<0.05$).
Rebound increases occurred at 120 min (Δ increment, 8.7±4.2 *vs* 0.6±0.7 μg/dl, $p<0.05$) (Fig. 2B). Within

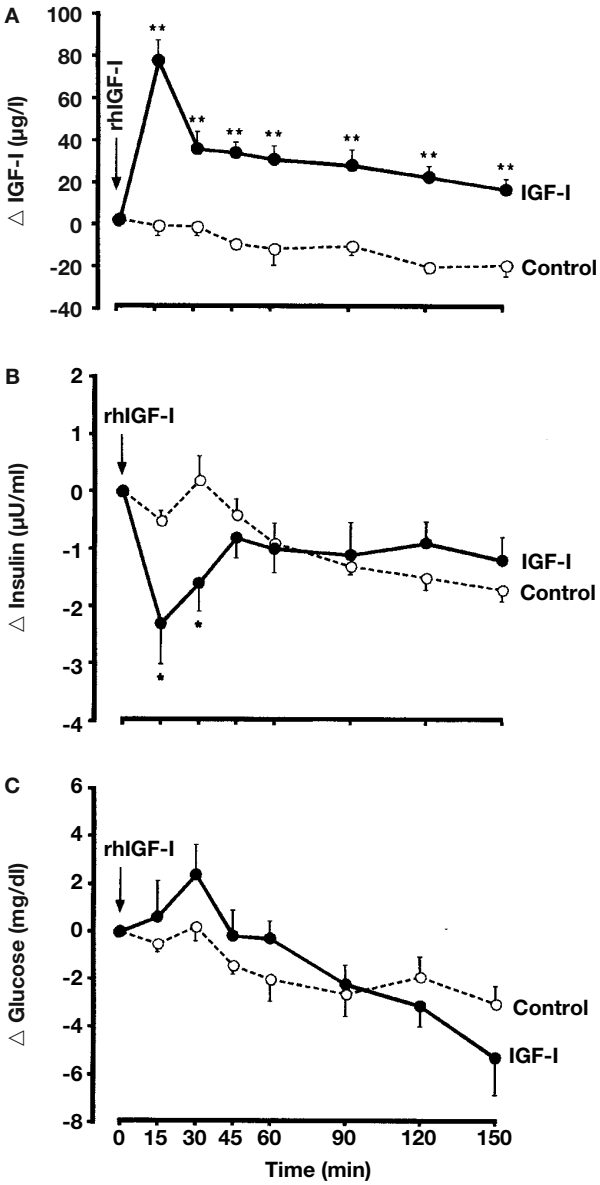

Fig. 1 - *Mean (±SE) serum IGF-I (A), insulin (B) and plasma glucose (C) levels after an iv bolus injection of recombinant human IGF-I (rhIGF-I) (5 µg/kg) in 10 subjects with non-endocrine short stature (NESS, closed circles). Open circles: saline administration in NESS controls, no.=10; *p<0.05; **p<0.01.*

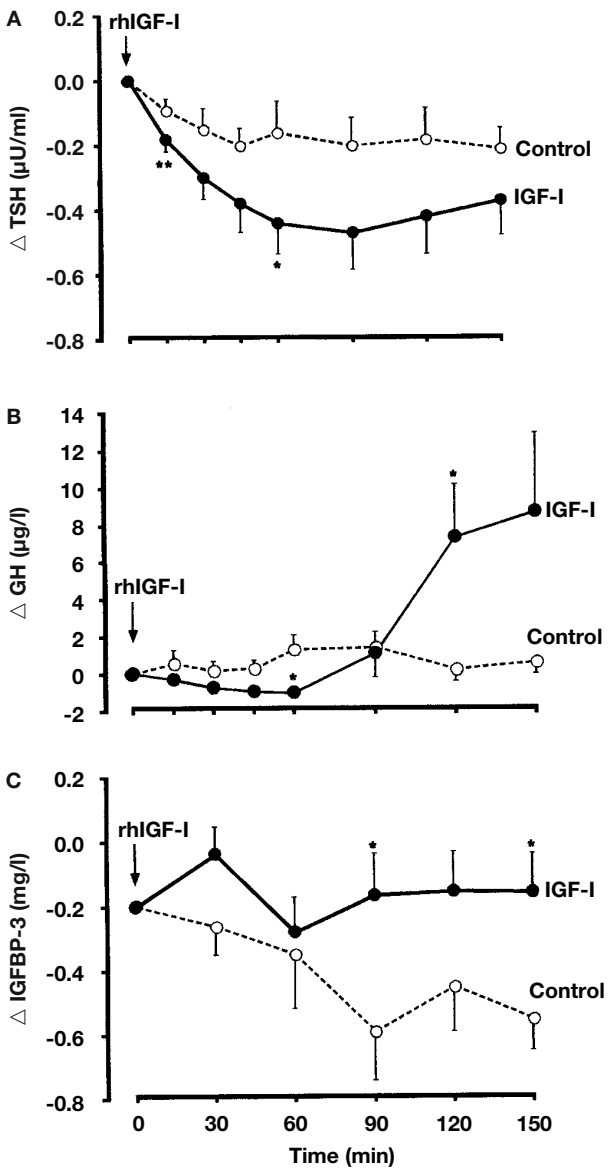

Fig. 2 - *Mean (±SE) serum TSH (A), GH (B) and IGFBP-3 (C) levels after an iv bolus injection of recombinant human IGF-I (rhIGF-I) (5 µg/kg) in 10 subjects with non-endocrine short stature (NESS, closed circles). Open circles: saline administration in NESS controls, no.=10, *p<0.05, **p<0.01.*

the IGF-I study, GH values were significantly lower [at 30 (p<0.01), 45 (p<0.01) and 60 min (p<0.005)] or higher [at 120 min (p<0.05)] than 0 min value. The % decrease of GH from the basal level at 30 min was -43.4±5.5 and the nadir was -58.8±7.6 at 60 min.
Serum PRL levels were not modified by the administration of exogenous rhIGF-I (data not shown).
Serum IGFBP-3 levels after rhIGF-I were higher than controls at 90 min (IGF-I *vs* saline, baseline, 2.728± 0.155 *vs* 2.674±0.181, p=NS; Δ increment at 90 min, 0.017±0.064 *vs* -0.196±0.075 mg/l, p<0.05) and 150 min (Δ increment, 0.021±0.060 *vs* -0.176±0.049 mg/l, p<0.05) (Fig. 2C).
No significant changes of serum IGF-II, and (IGF-I plus IGF-II)/IGFBP-3 ratios were observed after IGF-I injection compared with controls (data not shown).

DISCUSSION

In this study, a bolus injection of 5 μg/kg of rhIGF-I significantly increased serum IGF-I levels and decreased insulin levels transiently in NESS patients. It has been reported that circulating insulin in man is suppressed by much larger amounts of rhIGF-I (3, 11-13), probably by the direct action of IGF-I on pancreatic β-cells (14, 15). At the dose used in this study, plasma glucose levels are not suppressed. Rather, the levels were increased transiently, although it was not statistically significant. It appears that, with the doses used in this study, the insulin-suppressive action of IGF-I was more dominant than the insulin-like action of IGF-I.

Serum TSH secretion in this study was significantly suppressed by rhIGF-I injection. This is compatible with previous reports (2, 16, 17), although there is a report that IGF-I does not inhibit the release of TSH from the rat pituitary *in vitro* (18). After the beginning of rhIGF-I injection, TSH response occurred promptly, and it was followed by a slight rebound increase. It is well known that SS inhibits TSH secretion (2, 18, 19). Previously, we reported that normal young adults showed -34% of TSH decrease from baseline at 30 min after subcutaneous administration of SS-analog (SMS 201-995) with the nadir value (-54%) at 180 min (19). In this study, NESS children showed much smaller 30 min decreases (-17.0%) and a higher nadir value (-26.7% at 90 min). From these results we can not draw valid conclusions regarding the site of action of IGF-I, but it is conceivable that IGF-I exerts direct inhibition on thyrotrophs or indirect inhibition through the hypothalamic SS release.

As other mechanisms of IGF-I-induced TSH suppression, IGF-I-induced T_4 to T_3 conversion (5′ deiodination) in thyrotrophs should be considered (16) although we and Klinger *et al.* (17) did not observe any changes of peripheral T_4 and T_3 levels after IGF-I administration. However, since GH induces significant changes of thyroid hormone carriers, thyroxine binding globulin and transthyretin (20), measurement of free T_4 and free T_3 might provide better information regarding the effects of IGF-I on thyroid function.

Serum GH showed significant suppression 60 min after rhIGF-I administration followed by a rebound increase. It has been reported that IGF-I can inhibit GH release through different pathways, *i.e.* inhibition of hypothalamic GHRH (1, 21, 22) and the stimulation of hypothalamic somatostatin (2, 18) or through the direct inhibition of pituitary somatotrophs (18, 23-25). We previously showed that the percent decrease of GH at 30 min after an intravenous SS infusion in acromegalic patients [who are more sensitive to SS compared with normal young adults (19)] was -37% (26) and the maximal decrement was -68% at 75 min. In contrast, NESS patients showed much greater GH decrease at 30 min (-43.4%) and similar maximal decrement (-58.8%) at 60 min following the rhIGF-I injection. Therefore, it is plausible that IGF-I exerts the GH inhibitory effects directly on somatotrophs, although the coexistence of other GH inhibitory mechanism through the release of hypothalamic SS inhibition cannot be ruled out completely.

It is not clear whether IGF-I-induced inhibition of hypothalamic GHRH release is participating in the GH decrease or not (1, 21, 22). The rebound of the GH increase observed 120 min after IGF-I injection might be due to the steep IGF-I decline from 15 to 30 min after reaching peak and gradual decline thereafter, probably due to the short half life (approximately 10-16.6 min) of the free form of IGF-I (1, 27). Such rapid rebound GH phenomenon after suppression is also observed concomitantly with the cessation of IGF-I infusion (4).

As the peak IGF-I levels achieved (87.4 to 541.6 μg/l) were considered to be within the physiological range of age-matched controls (60-731 μg/L), it is highly conceivable that circulating IGF-I is a physiological regulator of GH secretion.

In this study, acute administration of rhIGF-I did not increase serum IGFBP-3 levels but blocked the physiological decrease of the levels despite the significant fall of serum GH (major regulator of IGFBP-3) (28-30). There are many controversial results regarding the effects of IGF-I on IGFBP-3 levels, *i.e.* inhibition (31, 32), stimulation (28, 33, 34), or no effects (4, 35). The reason of these contradictory observations are not clear. However, our acute examination suggests that exogenously administered IGF-I binds to circulating IGFBP-3 (and acid labile subunit: ALS) and such a complex may inhibit the degradation of circulating IGFBP-3. Related to this, the free form of IGFBP-3 has a very short half life, but when bound with IGF and ALS its half life is prolonged (34).

It is reported that high levels of IGF-I are associated with a correspondingly rapid decrease in IGF-II probably through a direct and indirect inhibition (via GH suppression) or by displacement from its binding proteins (9, 33, 34, 36, 37). However, in this study plasma IGF-II levels after the IGF-I injection were not statistically different from those of the control study. While the reason for these contradictory results is not clear, the study design including subjects, dose and mode of IGF-I administration might be factors.

The ratio of (IGF-I plus IGF-II)/IGFBP-3 has been reported to be constant (38, 39). This was confirmed in IGF-I and control groups throughout the study.

Whilst the PRL molecule has close similarity with GH, small doses of rhIGF-I did not lower serum PRL levels, although PRL responds to large iv bolus injection of rhIGF-I in patients with GH receptor deficiency (40). This may imply that somatotrophs and lactotrophs have distinctly different sensitivity to IGF-I, and that IGF-I does not have a significant physiological role in PRL secretion.

These results indicate that circulating IGF-I is a physiological regulator of GH secretion in normal children. The changes of IGF-I after the small doses of rhIGF-I administration did not affect plasma glucose levels and were thought to be within the physiological ranges. The possible regulatory roles of circulating IGF-I on plasma TSH, insulin and IGFBP-3 are also considered.

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