CLINICAL STUDY

The effects of human leptin fragment(126–140) on pituitary functions in man

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Abstract

Objective: The effects of human leptin fragment(126–140) on pituitary function in eight healthy, non-obese men were studied.

Methods and design: The effects of the fragment on spontaneous secretion of pituitary hormones and endogenous leptin, as well as on GHRH-induced GH secretion were examined.

Results: After the administration of the fragment (50 µg i.v. for 150 min), the mean nadir value and 45 min value were significantly lower than that of the control study. Endogenous leptin levels did not decrease significantly following the administration of the leptin fragment. Other pituitary hormones were not affected by the fragment. The area under the curve of the GH response to GHRH(1–44)NH₂ (10 µg, i.v. from 0 to 75 min) was also significantly inhibited by the combined administration of the leptin fragment (100 µg i.v. from –30 to 75 min) ($P < 0.001$). Three subjects were re-examined with larger doses of the leptin fragment (200–400 µg), and even greater GH suppression was observed.

Conclusions: These results indicate that human leptin fragment(126–140) has an inhibitory role in GH secretion, since when administered exogenously this fragment significantly suppressed spontaneous and, in a dose-response manner, GHRH-induced GH secretion. Clear effects of the fragment on other pituitary hormones and an inhibitory effect on endogenous leptin secretion were not observed in this study.

European Journal of Endocrinology 149 407–412

Introduction

Leptin is the product of the obese (ob) gene, and is secreted from fat cells. Leptin was first thought to be a regulatory factor for appetite and energy expenditure (1–4). More recently, however, it was proposed that leptin has multiple actions, including the endocrine system, vascular system and immune system, bone metabolism and the hematopoietic system (5–10). Regarding endocrine action, leptin, its receptors and their mRNAs are reported to be expressed in the normal pituitary gland (6, 11).

Circulating leptin levels reflect the degree of adiposity, i.e. leptin levels are elevated in obesity, and decreased in emaciation (e.g. anorexia nervosa) (12). There are negative correlations between circulating growth hormone (GH) levels and leptin levels; GH secretion is decreased in hyperleptinemia (obesity), and is increased in hypoleptinemia (anorexia nervosa) (13, 14).

Recently, it has reported that patients with homozygous leptin gene mutations (obesity with low leptin levels) and simple obesity with high leptin levels similarly show impaired GH response to GH-releasing hormone (GHRH) and GH-releasing peptide-6 (GHRP-6), therefore factors other than leptin are proposed to be related to such GH inhibition (15).

However, it is still unclear whether human leptin has an inhibitory role in GH secretion in subjects with normal adiposity or not.

While the actions of circulating leptin on other pituitary hormones are also not well understood, hypothyroidism is noticed infrequently and hypogonadism frequently in patients with leptin or leptin receptor deficiency (5, 16–18).

Among several human leptin fragments, this C-terminal fragment(126–140) of full leptin was proved to possess immunological activity (19). In addition, mouse leptin fragment(126–140) causes significant reductions in body weight and food intake in mice, and the C-terminus of mouse leptin (in domains

between residues 106–140) contains functional epitopes (20, 21).

Therefore, human leptin fragment(126–140) was employed in this study to examine the effects on GH secretion, as well as on other pituitary hormones in normal healthy, non-obese subjects.

Subjects and methods

Eight healthy and non-obese adult males (age 24.1 ± 1.3 , range 20–30 years; body mass index 21.7 ± 0.4 , range 20.3–23.2 kg/m²) were studied after obtaining approval from the Local Ethics Committee in Sendai and informed consent from each subject. These subjects had no known illnesses and were not on any medication. These subjects received the following studies four to six times over the interval of 1 week.

After an overnight fast, synthetic human leptin fragment(126–140) (Bachem, Bubendorf, Switzerland; 50 µg) was infused i.v. from 0900 h for 150 min, and blood samples (to measure serum GH, thyrotropin (TSH), prolactin (PRL), adrenocorticotropin (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and leptin) were taken at 30 and 0 min before, and 15, 30, 45, 60, 90, 120 and 150 min after the infusion of the fragment.

Further, the effect of the human leptin fragment (100 µg in eight, 200 µg in two and 400 µg in one subject, i.v. from –30 to 75 min) on GH release induced by GHRH(1–44)NH₂ (Sumitomo, Osaka, Japan; 10 µg, i.v. from 0 to 75 min) was studied over 150 min in these subjects. As a control, these subjects were infused with normal saline i.v. from –30 to 150 min.

Serum GH, PRL, LH, FSH, ACTH and leptin were measured with IRMA kits (GH, PRL, LH, FSH, ACTH: Daiichi RI, Tokyo; leptin: Diagnostic Systems, Webster, TX, USA) and serum TSH with an immunofluorometric kit (Pharmacia, Uppsala, Sweden). The sensitivity of the IRMA for serum GH was 0.006 µg/l and interassay coefficients of variation ranged between 1.4 and 3.2%. The sensitivity of the leptin assay was 0.1 µg/l, and this assay did not show any cross-reactivity or interference between the following human leptin fragments: leptin (5–21), (22–42), (61–85) and (114–146). Therefore, the leptin fragment used in this study was not detected in this assay system, thereby allowing the accurate evaluation of changes of endogenous leptin levels.

Data are expressed as means $\pm$ S.E.M. and statistical analysis was conducted using ANOVA followed by Fisher's randomization test for the comparison of the two responses, and Student's paired *t*-test for the comparison of two nadir values or area under the curve (AUC), which was calculated by trapezoidal integration.

Results

Clinical effects: safety

No adverse effects of human leptin fragment were observed at the doses (50–400 µg) used in this study.

Effect of human leptin fragment(126–140) on basal pituitary hormone and leptin secretion

After the administration of leptin(126–140), serum GH showed a significant decrease at 30 min (mean $\pm$ S.E.M. 1.0 ± 0.4 µg/l) and 45 min (0.7 ± 0.2 µg/l) compared with 0 min (1.4 ± 0.4 µg/l), and the mean nadir value (0.3 ± 0.08 µg/l) was also significantly lower than the 0 min value (all $P < 0.025$ by randomization test; Fig. 1a). The value at 45 min and the nadir value were found to be significantly lower than the control study (1.0 ± 0.2 and 0.8 ± 0.2 µg/l) ($P < 0.005$ and $P < 0.05$ respectively).

Endogenous leptin levels (measured by the IRMA kit which did not cross-react with the leptin fragment used in this study) in the morning were fairly stable and

Figure 1 Serum GH (a) and native leptin levels (b) in eight normal subjects after the administration of 50 µg human leptin fragment(126–140). (In this IRMA, native leptin had no cross-reactivity or interference with the following human leptin fragments: leptin(5–21), leptin(22–42), leptin(61–85) and leptin(114–146).) * $P < 0.05$.

there were no significant differences from the control study at each time interval (Fig. 1b).

However, other pituitary hormones were not affected by the exogenously administered leptin fragment (TSH, PRL, LH, FSH and ACTH, Fig. 2a–e).

Effect of human leptin fragment(126–140) on GH response to GHRH

Serum GH response to GHRH was also distinctly inhibited by the combined administration of the leptin fragment (AUC, single GHRH vs leptin+GHRH: 847.0 ± 216.8 vs 524.1 ± 217.2 $\mu\text{g/l} \times \text{min}$, $P < 0.001$) (Fig. 3). Three subjects were re-examined with larger

Figure 2 Serum TSH (a), PRL (b), LH (c), FSH (d) and ACTH (e) levels in eight normal subjects after the administration of 50 μg human leptin fragment.

Figure 3 Serum GH responses in eight normal subjects to 10 μg GHRH with or without combined administration of 100 μg human leptin fragment.

Figure 4 The effects of different doses of leptin fragment ((Lep.) 100, 200 and 400 μg) on GHRH-induced GH releases in three normal subjects (a–c).

doses of the leptin fragment (200–400 µg), and even greater GH suppression was observed (Fig. 4).

Discussion

The exogenously administered human leptin fragment(126–140) significantly suppressed spontaneous and GHRH-induced GH secretion. A dose-dependent response correlation was observed between the different leptin doses and GH responses to GHRH.

It is reported that a negative relationship exists between the basal serum levels of leptin and GH in chronically hyponutritional children (22). Such negative correlations were also observed between the serum leptin concentration and basal 24 h GH secretory capacity (13) or stimulated GH release in children and adults (15, 22, 23). These results seem to indicate that circulating leptin has an inhibitory action on GH secretion.

Regarding the direct action of human leptin on human pituitary cells, it is reported that it seems to exert both a slight inhibitory effect on spontaneous GH secretion and a stimulatory effect on GHRH-induced GH secretion from GH-secreting adenomatous tissue (24). In animal studies, administration of leptin antiserum i.c.v. led to a decrease in spontaneous GH secretion in fed rats, while leptin administration (i.c.v.) to normal fed rats did not modify spontaneous GH secretion (25). Leptin administration (i.c.v.) to fasted rats, which show a marked decrease in GH responses to GHRH and GHRP-6, recovered the responses to those stimuli (26). These results are not always contradictory to this study, since the study design, route of leptin administration, nutritional states, tissues and species examined were different.

Some patients with congenital leptin or leptin receptor gene mutations show blunted GH responses to insulin-induced hypoglycemia and exercise, and show low serum insulin-like growth factor (IGF)-I and IGF-binding protein-3 levels (5, 18). However, growth retardation is generally not observed in these patients (5, 17, 18, 27). Such blunted GH responses observed in congenital leptin deficiency are possibly dependent on GH inhibitory effects induced by excessive insulin, free fatty acids and free IGF-I, which are increased in obese subjects and have an inhibitory action on GH release (28–30), rather than the loss of leptin action.

It has been postulated that leptin might regulate TSH secretion, since there is a synchronicity between leptin and TSH secretion in normal subjects, but such a pulsatile and circadian rhythm was disorganized in leptin-deficient patients (31). In addition, some patients with leptin gene or leptin receptor gene mutations have mild hypothyroidism, although this finding was not commonly seen in those reported patients (5, 16–18, 27, 31).

In this study, however, exogenously administered leptin did not affect TSH secretion. Similarly, Farooqi *et al.* (27) did not observe significant change in plasma TSH after treatment with recombinant methionyl human leptin (rmhLeptin) in three euthyroid children with congenital leptin deficiency, while plasma free thyroxine levels were significantly increased.

It seems that leptin has a minor role, if any, in TSH secretion, even though leptin and its receptor are present in human pituitary thyrotropes (6, 11).

While circulating leptin and PRL are both decreased in anorexia nervosa (32), and these hormone levels decline with age (33), there are no correlations found between the two hormones. In this study, no distinct influences of exogenously administered leptin on serum PRL levels were observed.

Patients with leptin deficiency and leptin receptor mutation(s) frequently have hypogonadism (5, 16, 18), but leptin-deficient patients show normal gonadotropin and testosterone responses to LH-releasing hormone (LHRH) and human chorionic gonadotropin respectively (5, 16). Therefore, it is possible that leptin may have some role in hypothalamic LHRH secretion in humans. In this study, however, the leptin fragment did not exert a distinct influence on basal gonadotropin secretion. Farooqi *et al.* (27) also reported that in two prepubertal children with congenital leptin deficiency, basal FSH, LH and sex steroid concentrations remained in the prepubertal range even after a maximum of 36 months of rmhLeptin therapy.

With respect to the role of leptin in the hypothalamo–pituitary–adrenal axis (HPAA) in humans, there are conflicting observations. Plasma leptin concentrations have been shown to be inversely related to ACTH and cortisol, and to modulate the levels of endogenous cortisol in normal subjects (34). In contrast, a positive correlation was observed between basal serum leptin concentration and ACTH response to naloxone in normal subjects (35, 36).

However, a substantial abnormality in the HPAA is not observed in human leptin deficiency or as a result of human leptin receptor mutations (16–18, 27). Plasma cortisol levels in three patients with congenital leptin deficiency are reported to be unchanged throughout the rmhLeptin therapy (27).

The fact that serum ACTH levels in this study were not modified by the administration of leptin fragment suggests that leptin does not play a major role in controlling the HPAA.

An auto-feedback phenomenon of leptin secretion was not observed in this study, since endogenous leptin levels were not modified by exogenously administered leptin fragment which had no cross-reactivity in the present assay system with the native, full-length leptin.

It is necessary to compare the biological activities of the above two leptins to clarify the physiological role in pituitary functions and the possible auto-feedback phenomenon. However, this study, together with the

fact of entrance of native leptin through the blood–brain barrier (37), might give an insight into the potential clinical applications of leptin fragment for the treatment of simple obesity and leptin-deficient patients, as well as to the elucidation of the leptin role in the hypothalamo–pituitary axis.

It is concluded that human leptin fragment (126–140) has an inhibitory activity on GH secretion in man. Clear effects of the leptin fragment on other pituitary hormones and the possible auto-feedback mechanism of leptin secretion were not observed in this study.

Acknowledgements

We thank Dr Steve Sugino for his careful review of this manuscript. We thank the nursing staff of Hanew Endocrine Clinic for their technical support.

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Received 17 April 2003

Accepted 29 July 2003