EXPERIMENTAL STUDY

Leptin increases *in vivo* GH responses to GHRH and GH-releasing peptide-6 in food-deprived rats

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Abstract

Background: Leptin has recently been shown to have a stimulatory effect on basal GH secretion. However, the mechanisms by which leptin exert this effect are not yet clear. GHRH and GH-releasing peptide (GHRP)-6 are the two most potent GH secretagogues described to date.

Objective: To determine if leptin could also enhance *in vivo* GH responses to a maximal dose of GHRH. **Design:** Leptin (10 µg i.c.v.) or vehicle was administered at random before GHRH (10 µg/kg i.v.) or GHRP-6 (50 µg/kg i.v.), to freely-moving rats with food available *ad libitum* and to (48 h) food-deprived rats.

Methods: Leptin and GH concentrations were measured by radioimmunoassay. Comparison between the different groups was assessed by the Mann–Whitney test.

Results: In comparison with fed rats, food-deprived rats showed a marked decrease in GH responses to GHRH as assessed by the area under the curve (5492 ± 190 ng/ml in fed rats and 1940 ± 128 ng/ml in fasted rats; $P < 0.05$) and GHRP-6 (3695 ± 450 in fed rats and 1432 ± 229 in fasted rats; $P < 0.05$). In comparison with its effects in vehicle-treated rats, leptin administered to food-deprived rats markedly increased GH responses to both GHRH (6625 ± 613 ng/ml; $P < 0.05$) and GHRP-6 (5862 ± 441 ng/ml; $P < 0.05$).

Conclusions: These data suggest that the blunted GH response to GHRH and GHRP-6 in food-deprived rats is a functional and reversible state, and that the decreased leptin concentrations could be the primary defect responsible for the altered GH secretion in food-deprived rats.

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Introduction

In addition to stimulating body growth, growth hormone (GH) has an important role in metabolism. In turn, alterations in nutritional status, such as obesity or food deprivation, markedly influence GH secretion. As GH secretion is normalized after weight loss in obesity or after refeeding in states of food deprivation, there is no doubt that altered GH secretion develops as a consequence of altered metabolic status (1–3).

The *ob* gene is an adipocyte-specific gene that encodes leptin, a protein that regulates body weight (4–8). Recent data have clearly shown that the amount of leptin mRNA in adipocytes correlates with body weight (9, 10). Furthermore, serum immunoreactive leptin concentrations show a strong positive correlation with body fat, being increased in obesity and decreased in anorexia nervosa (6, 9, 11). Although the mechanisms by which leptin act are far from being understood, the presence of leptin receptors suggests that the brain is one of the main loci of the action of leptin. In addition, the recent demonstration of leptin receptors in some

specific hypothalamic nuclei such as the ventromedial, arcuate, paraventricular and periventricular, provides a basis for a neuroendocrine role of leptin in the control of anterior pituitary hormone secretion (12–16).

Other recent data suggest that leptin is a metabolic signal that regulates GH secretion, as the administration of leptin antiserum led to a decrease in spontaneous GH secretion, whereas administration of leptin to food-deprived rats reversed the inhibitory effect of fasting on basal GH secretion (17). However, the mechanisms by which leptin influences GH secretion are poorly understood. Thus, whereas some data suggest that leptin stimulates GH secretion by acting at the hypothalamic level regulating GH-releasing hormone (GHRH) and somatostatin-producing neurones via neuropeptide Y (18–22), others have reported an inhibitory effect of leptin on GHRH-stimulated GH secretion from the ovine pituitary gland (23).

GHRH and GH-releasing peptide (GHRP)-6 are the most potent GH secretagogues described to date. The stimulatory effect of GHRH is exerted directly at the pituitary level, whereas GHRP-6 acts mostly in the

hypothalamus (24–26). In order to gain further insight into the mechanisms by which leptin exerts its effects, we assessed the effect of leptin on GH responses to GHRH and to GHRP-6.

Methods

Animals and experimental procedure

Adult male Sprague–Dawley rats (200–250 g) were housed in a 12-h light:12-h darkness cycle in a temperature- and humidity-controlled room. Chronic i.c.v. and intracardiac cannulae were implanted with the animal under sodium pentobarbitone (50 mg/kg, i.p.) anaesthesia, as described previously (27). After surgery, the animals were placed directly in isolation test chambers for 5 days and were given free access to regular Purina rat chow and tap water. Thereafter, the animals continued to have food available *ad libitum* or were deprived of food for 48 h before blood sampling. On the day of the experiment, blood samples (0.3 ml) were withdrawn at the designated times. The animals ($n = 8$ rats/group) received vehicle, recombinant leptin (supplied by Eli Lilly), or both, through the i.c.v. cannula (17). GHRH (10 μ g/kg) and GHRP-6 (50 μ g/kg) were administered i.v.

Hormone assays

Plasma GH concentrations were determined by double-antibody RIA using materials supplied by the National Hormone Pituitary Program as described previously (27). Values are expressed in terms of the GH reference preparation (GH-RP-2). The intra- and interassay coefficients of variation were 7% and 10% respectively. Leptin was measured by radioimmunoassay as described previously (28). The intra- and interassay coefficients of variation were 2.4% and 4.8% respectively.

Statistical analysis

Data are expressed as means $\pm$ S.E.M. Comparison between the different groups was assessed by the Mann–Whitney test.

Results

Basal leptin concentrations were reduced in food-deprived rats (0.5 ± 0.02 μ g/l) compared with those in rats that had food available *ad libitum* (1.56 ± 0.1 μ g/l; $P < 0.001$). In fed rats, the administration of either GHRH or GHRP-6 led to a marked increase in plasma GH concentrations, as assessed by mean peak values (to 390 ± 14 ng/ml and 250 ± 23 ng/ml, respectively; Fig. 1). The administration of leptin 45 min before GHRH or GHRP-6 did not modify the mean peak GH response to either peptide (253 ± 54 ng/ml and 220 ± 19 ng/ml respectively) in these groups of fed rats (Fig. 1).

Figure 1 Serum GH concentrations (means $\pm$ S.E.M.) in normally fed rats ($n = 8$ per group) that received either vehicle (10 μ l i.c.v., $\square$) or leptin (10 μ g i.c.v., $\blacksquare$) 45 min before the administration of GHRH (10 μ g/kg administered i.v. at 0 min) or GHRP-6 (50 μ g/kg administered i.v. at 0 min).

In contrast, the mean peak GH response was notably reduced in food-deprived animals after administration of either GHRH or GHRP-6 (128 ± 12 ng/ml and 105 ng/ml respectively; both $P < 0.05$) in comparison with that in rats with food available *ad libitum* (Fig. 2). Interestingly, in these groups of fasted rats, the GH responses to both GHRH and GHRP-6 were markedly increased when leptin was administered 45 min before these secretagogues (GH concentrations respectively 430 ± 23 ng/ml and 381 ± 17 ng/ml; both $P < 0.05$ compared with rats treated with vehicle).

These effects were also apparent when expressed as area under the curve (Fig. 3, which also summarizes the mean peak effects).

Discussion

Although contradictory findings (29, 30) have been reported regarding the effect of prolonged (72 h) food

Figure 2 Serum GH concentrations in (48 h) food-deprived rats (means $\pm$ S.E.M.; $n=8$ per group) that received either vehicle ($10 \mu\text{l}$ i.c.v., □) or leptin ($10 \mu\text{g}$ i.c.v., ■) 45 min before the administration of GHRH ($10 \mu\text{g/kg}$ administered i.v. at 0 min) or GHRP-6 ($50 \mu\text{g/kg}$ administered i.v. at 0 min).

deprivation on GH responses to GHRH in the rat, there is a general agreement that it elicits a marked decrease in spontaneous GH secretion (17, 29, 31, 32). This later effect is due to a suppression of high-amplitude GH secretory bursts and a decrease in the duration of secretory episodes (31). Although the mechanisms mediating these alterations remain unclear, several possibilities have been put forward. Increased somatostatinergic tone has been implicated, because passive immunization of food-deprived rats with somatostatin antiserum restored spontaneous GH secretion to values similar to those in normally fed rats (31). Nevertheless, hypothalamic prepro-somatostatin mRNA levels and peptide content did not differ between food-deprived and control rats, and somatostatin immunostaining in middle and caudal regions of the median eminence has been reported to be decreased (33, 34). The finding that food deprivation led to decreased hypothalamic GHRH concentrations, reduced prepro-GHRH mRNA

Figure 3 Mean peak GH concentrations and area under the curve (means $\pm$ S.E.M.) in normally fed and food-deprived rats that received GHRH or GHRP-6 after the administration of either vehicle (–leptin, □) or leptin (+leptin, ■) (see also legends to Figs 1 and 2). * $P<0.05$, compared with leptin-untreated rats.

levels and decreased GHRH immunostaining in middle and rostral regions of the median eminence (33, 34) suggested that the marked suppression of GH secretory pulses observed in food-deprived rats could be due to decreased hypothalamic GHRH tone.

Alterations at the pituitary level are also possible. A small decrease (12%) in GH mRNA levels has been reported in food-deprived rats (34). In addition, there is a marked decrease in somatostatin binding sites in fasted rats. Assessment of mRNA levels for the different somatostatin receptor subtypes (SSTRs 1–5) have shown a decrease (up to 80%) in SSTR1, SSTR2 and SSTR3 mRNA expression, whereas SSTR4 and SSTR5 mRNA levels appeared to be unaffected in food-deprived rats compared with normally fed animals (35).

Taking into account that food deprivation is associated with a marked decrease in both plasma leptin concentrations and spontaneous GH secretion, we assessed the effect of leptin on GH responses to the two most potent GH secretagogues, namely GHRH and GHRP-6 (25, 26).

We found that, in fed rats, leptin administration did not modify *in vivo* GH responses to a maximal stimulatory dose of either GHRH and GHRP-6. These results are in agreement with previous findings of a similar ineffectiveness of leptin – acutely administered or infused for 3 days – on spontaneous GH secretion (17, 21), whereas central administration of leptin antiserum completely blunted episodic release of GH (17). Taken together, these data imply that, under normal physiological conditions, leptin is exerting a maximal stimulatory effect on GH secretion. However, it is possible that chronic sustained hyperleptinaemia – for example, as a result of infusion of leptin for 7 days – is able to increase GH secretion, even in fed rats (22).

After 48 h of food deprivation, GH responses to GHRH were markedly decreased compared with those in normally fed rats. Furthermore, we found that the administration of leptin 45 min before GHRH or GHRP-6 completely reversed this decrease in response. These findings indicate that the blunted GH responses to GHRH induced by food deprivation reflect a functional and reversible state, and suggest that the primary alteration could be mediated by decreased leptin activation of the hypothalamic–GH axis. It is also possible that the effect of leptin in food-deprived rats could be the result of increased gene expression of the long isoform of the leptin receptor in the hypothalamus, in response to fasting (36). However, this stimulatory effect of leptin on GH responses to GHRH resembles that obtained by others who assessed the same response after passive immunization with antisomatostatin antiserum (31). As there is both direct (37) and indirect (19, 20) evidence suggesting that leptin could act via somatostatin, this finding was compatible with an inhibitory effect of leptin on somatostatinergic tone as the mechanism by which it enhances GH responses to GHRH and GHRP-6.

In summary, these data suggest that blunted GH responses to GHRH and GHRP-6 in food-deprived rats reflect a functional and reversible state and that decreased leptin concentrations could be the primary defect responsible for altered GH secretion in this experimental setting.

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