Leptin Decreases Food Intake Induced by Melanin-Concentrating Hormone (MCH), Galanin (GAL) and Neuropeptide Y (NPY) in the Rat

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Abstract: Recent evidence suggests that leptin reduces food intake (FI) by acting at the hypothalamic level. Leptin decreases hypothalamic neuropeptide Y (NPY), melanin-concentrating hormone (MCH) and galanin (GAL) gene expression in rats. The purpose of the present study was to test the hypothesis that leptin decreases FI by additionally modulating the action of NPY, MCH or GAL in the hypothalamus. Intracerebroventricular (icv) administration of NPY, MCH or GAL induced FI in satiated rats. A prior icv injection of leptin (4 µg) completely prevented the increase of FI either by MCH, GAL or NPY. These results suggest that modulation of post-synaptic actions of MCH, GAL and NPY is one of the mechanisms of leptin signaling in the hypothalamus.

Introduction

Leptin, the obese gene product, is a 16-KD protein (1) that plays an important role in the regulation of food intake (FI) and body wt (2-4). Leptin administration either centrally or peripherally reduces FI in lean and obese mice (2-4). Central leptin administration also reduces FI in lean rats (5-9), but its effect on FI and body wt in genetically obese rats is reported to be either reduced (6,8) or not apparent (7). In addition, this protein may also correct obesity, per se, completely or in part, depending on the species considered (2-4, 10-12).

It is well established that the brain, and specifically the hypothalamus, is a major site where various central and peripheral signals are integrated to affect the expression of complex behavioral and neuroendocrine functions, such as feeding and energy homeostasis. Thus, the hypothalamus is thought to be the site of leptin action in modulating behavioral and neuroendocrine effects (3). Indeed, leptin receptors (13,14) have been localized in this region of the brain (5,15). However, the mechanisms of leptin signaling in the hypothalamus are not clearly understood. In this regard, hypothalamic neurons that produce neuropeptide Y (NPY), a potent stimulator of feeding (16,17), have been suggested to mediate, in part, leptin's action in the hypothalamus (5,6,9,18-21). Several lines of evidence also suggest that NPY is a physiological signal for the regulation of FI and energy homeostasis (17,22). While the role of hypothalamic melanin-concentrating hormone (MCH) and galanin (GAL), two other orexigenic signals (16,23-25), in long-term body wt regulation is not well established (25,26), evidence accumulated thus far clearly suggests a significant role for these peptides in daily FI (23-27). Moreover, since leptin reduces FI in NPY-knockout mice that maintain normal FI and body wt, the importance of other orexigenic neuropeptides, including MCH and GAL is becoming more apparent (28). Recently, it has been demonstrated that central administration of leptin decreased hypothalamic MCH and GAL gene expression in association with decreased FI and body wt (9). These results suggest that MCH and GAL are also the targets of leptin signaling in the hypothalamus.

Since leptin appears to be one of the most important peripheral signals that control FI and body wt gain, it is reasonable to suggest that this protein, in addition to its action to regulate gene expression of MCH, GAL and NPY neurons, may also influence the action of these peptides. The main purpose of the present study was to test this hypothesis by evaluating whether leptin can modulate the action of MCH, GAL and NPY on FI in the rat. 1

1A preliminary report of this research was presented at the 80th Annual Endocrine Society Meeting, New Orleans, LA, Abstract# F3-253, June 1998.

Materials and Methods

Animals:

Adult male Sprague-Dawley rats (Taconic, Germantown, NY), weighing 250-270 g, were housed individually in a temperature (22 C) and light (14 h light and 10 h dark, lights on 0500 h) controlled room with free access to food (rodent chow) and water.

Intracerebroventricular cannulation and injections:

After acclimatization, the rats were permanently implanted stereotaxically with 22-gauge stainless steel cannulae (Plastics One, Roanoke, VA) into the third cerebroventricle under pentobarbital anesthesia. The animals were allowed to recover for 2 weeks and handled daily for 7 days before the experiment in order to minimize nonspecific stress.

Leptin (r-MuLeptin, Amgen Inc, Thousand Oaks, CA) was dissolved in phosphate-buffered saline (PBS). Rat MCH (BACHEM California Inc, Torrance, CA), porcine NPY and rat GAL (Peninsula Laboratories, Belmont, CA) were dissolved in sterile saline (Abbott Laboratories, North Chicago, IL). The effects of leptin on FI induced by MCH, GAL and NPY were tested in two similar but separate studies. In the first study, rats showing efflux of cerebrospinal fluid were injected intracerebroventricularly (icv) with leptin (4µg in 4 µl PBS) or PBS alone followed

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1 h later by an icv injection of either MCH (5 μg), GAL (5 μg) or NPY (5 μg) in 4 μl saline or saline alone. Food was withdrawn during the 1-h period between injections. After the 2nd injection (time 0), rats were returned to their cages containing a preweighed aliquot of food (chow). Food intake was measured 2 and 4 h after the 2nd injection. The experiment, which was performed only once in each rat, was conducted between 0800 to 1300 h. In the second study with separate groups of rats, the effects of leptin (4 μg) on FI induced by a higher dose of MCH (10 μg), and by a lower dose of GAL (2.5 μg) or NPY (2 μg) were examined.

Statistical analysis
Food intake was expressed as the mean ± SEM. Statistical analyses were performed with ANOVA followed by Student-Newman-Keuls multiple range test (GB-STAT Software for the Macintosh, Dynamic Microsystems, Inc., Silver Spring, MD). Comparisons with P < 0.05 were considered to be significantly different.

Results
Administration of five μg of MCH produced an increase in FI of 275 ± 35 % and 240 ± 29 % at 2- and 4-h after injection, respectively (P < 0.001 vs PBS+SAL), and this was completely prevented by prior leptin injection [Fig. 1, left panel, 2 h: F(1,19) = 1.612, P = 0.22; 4 h: F(1,19) = 0.258, P = 0.617]. Similarly, leptin completely blocked the injection significantly decreased FI at both 2 and 4 h (Fig. 3, left panel, 278 ± 72 % at 2 h and 245 ± 43 % at 4 h vs PBS+SAL group). The FI in LEPT+NPY group, however, remained significantly increased at 2 [F(1, 20) = 11.318, P = 0.003] and 4 h [F(1, 20) = 13.289, P = 0.001] as compared to that of PBS+SAL group (Fig. 3). In the second study, FI induced by 2 μg NPY was completely prevented by prior leptin administration [Fig 3, right panel, 188 ± 30% at 2 h (F(1,9) = 4.48, P = 0.06) and 136 ± 23% at 4 h vs PBS+SAL group]. The increase in FI following NPY injection was significantly greater than that induced by same dose of either MCH (p < 0.001) or GAL (p < 0.001).

Although leptin administration alone had a tendency to decrease FI after vehicle injection, but it was not statistically significant [First study, 2 h: 99.96 ± 18.74% vs 57.75 ± 12.23% for PBS+SAL and LEPT+SAL groups, respectively;
F(1,24) = 2.82, P = 0.106; 4 h: 100.18 ± 18.74% vs 57.88 ± 10.94% for PBS+SAL and LEPT+SAL groups, respectively; F(1,24) = 2.92, P = 0.1; or second study, 2 h: 100.01 ± 28.28% vs 76.48 ± 14.45% for PBS+SAL and LEPT+SAL groups, respectively; 4 h: 100.13 ± 16.02% vs 72.26 ± 12.95% for PBS+SAL and LEPT+SAL groups, respectively, mean ± SEM).

Discussion

In this study, MCH, GAL and NPY, when injected centrally, stimulated FI in satiated rats confirming them as orexigenic agents (16,17,22-24). NPY, however, is the most potent orexigenic agent known (16,17), and this was also evident in this study. Leptin has been shown to inhibit NPY-induced FI in ob mice (29). The present study shows that leptin also inhibits NPY-induced FI in rat. In addition, the present results show, for the first time, that leptin inhibits MCH- and GAL-induced FI.

Since administration of the five or ten μg dose of MCH increased FI by the same amount, it appears that these are maximal doses for the orexigenic effects of MCH. This conclusion is maximalized by a recent study in rat (25). Thus, complete blockade of FI induced by either five or ten μg of MCH by leptin suggests that leptin can fully antagonize the action of MCH on feeding. In contrast, leptin was unable to completely prevent the effects of the highest dose of either GAL or NPY, although it completely blocked the FI induced by the lower dose of these peptides. Thus, in the presence of leptin, the feeding response to the highest dose of GAL or NPY was still partially operative. The mechanisms by which leptin modifies the actions of MCH, GAL or NPY are yet to be established. However, the complete antagonism of MCH’s orexigenic effect indicates that MCH may be acting solely via leptin sensitive pathway. On the other hand, orexigenic effects of NPY and GAL may involve multiple neuronal systems, including those that are not sensitive to leptin. It is also possible that icv leptin may not have access to some of the hypothalamic sites that mediate orexigenic effects of NPY or GAL. It is to be noted here that there was a trend of suppression (although not significant) in FI by leptin in the saline injected control rats. This general suppression may, partly, contribute to the suppressive effect of leptin on FI induced by the orexigenic peptides. The present results, however, reinforce the notion that leptin not only acts on MCH, GAL and NPY-producing neurons, it also modulates the action of the peptides they secrete at the level of the hypothalamus.

In summary, the results of the present study provide novel information about the possible mechanisms of leptin signaling in the hypothalamus. Leptin-induced decreases in enhanced FI by icv injection of either MCH, GAL or NPY in satiated rats suggest that leptin can antagonize the action of MCH, GAL or NPY on FI. It raises the possibility that leptin’s action at the level of the hypothalamus may, in part, be mediated by modulating the receptor mediated action of these neuropeptides.

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