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Leptin treatment ameliorates anxiety in *ob/ob* obese mice

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Abstract

We investigated whether or not administered leptin influences anxiety-like behavior in *ob/ob* mice. Repeated intraperitoneal administrations of leptin were continued for 5 days. Anxiety was assessed in the standard elevated plus maze. Body weight was measured daily. Repeated administrations of leptin significantly increased the percentage of the total number of entries in the open arms and the number of total entries. The body weight was significantly reduced by 13.2% after treatment. Leptin treatment ameliorated not only obesity but also anxiety in *ob/ob* mice. Our results indicate that the treatment of obesity may lead to the solution of psychological problems.

Keywords: Leptin; *ob/ob* mice; Anxiety; Locomotor activity; Obesity

1. Introduction

Leptin, the *ob* gene product, is known to be a satiety signal from peripheral adipocytes to the hypothalamus, which is involved in control of energy balance (Inui, 1999; Zhang et al., 1994). Previous studies have shown that leptin decreases food intake and body weight, and increases metabolic rate (Pelleymounter et al., 1995). Circulating leptin downregulates the gene expression of anorexigenic peptides such as neuropeptide Y (NPY) and agouti-related peptide (AGRP), and upregulates that of anorexigenic peptides such as melanocortin and cocaine- and amphetamine-regulated transcript (CART) in the hypothalamus, thereby regulating energy metabolism (Inui, 2000a, 2001a, 2001b). It has also been reported that leptin-overexpressing mice show decreases in food intake and body weight with the complete disappearance of white adipose tissue and brown adipose tissue (Ogawa et al., 1999). However, little is known about the effect of leptin on anxiety. We investigated whether or not administered leptin influences anxiety-like behavior in *ob/ob* obese mice.

2. Methods

Obese (*ob/ob*) C57BL/6J mice (62–69 g; Shionogi, Shiga, Japan) were used. They were individually housed in a regulated environment (22 ± 2 °C, 55 ± 10% humidity, 12:12-h light:dark cycle with lights on at 7:00 a.m.). Food and water were available ad libitum. Recombinant mouse leptin was purchased from R&D Systems (Minneapolis, MN, USA). Just before administration, drug was diluted in 100 μl of physiological saline for intraperitoneal injection, which also served as the control solution. Repeated intraperitoneal injections were continued for 5 days in *ob/ob* obese mice. The mice were injected daily at 7:00 a.m. and 19:00 p.m. Body weight was measured daily at 7:00 a.m. Anxiety was assessed in the standard elevated plus maze 50 cm above the ground at the end of the experiment (8 h after the final

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intraperitoneal injection). The elevated plus maze was originally devised by Montgomery and improved by others for evaluation of anxiety. In this test, behavior is dependent on rodents' natural tendency to avoid open elevated space. The four arms were 27 cm long and 6 cm wide. Two opposing arms were enclosed by walls 15 cm high (closed arms), while the other arms were devoid of walls (open arms). Each mouse was placed in the center of the maze facing one of the enclosed arms. The cumulative time spent in each arm and the number of entries into the open or closed arms were recorded during a 5-min test session. The time spent in the open arms was expressed as a percentage of total entry time ($100 \times$ open/open + closed) and the number of entries in the open arms was expressed as a percentage of total number of entries ($100 \times$ open/total entries). Results are expressed as mean ± S.E.M. Analysis of variance (ANOVA) followed by Bonferroni's t test were used to assess the differences among groups. $P < .05$ was considered to be statistically significant. All experiments were approved by our university animal care committee.

3. Results

The effect of leptin on anxiety is shown in Fig. 1. Repeated intraperitoneal injections of leptin significantly increased the percentage of the total number of entries in the open arms (% entry) compared with saline-treated controls. The number of total entries, a crude measure of overall locomotor activity, was significantly increased (Table 1). The body weight of *ob/ob* obese mice was significantly reduced by 13.2% after 5 days of leptin treatment (Table 1).

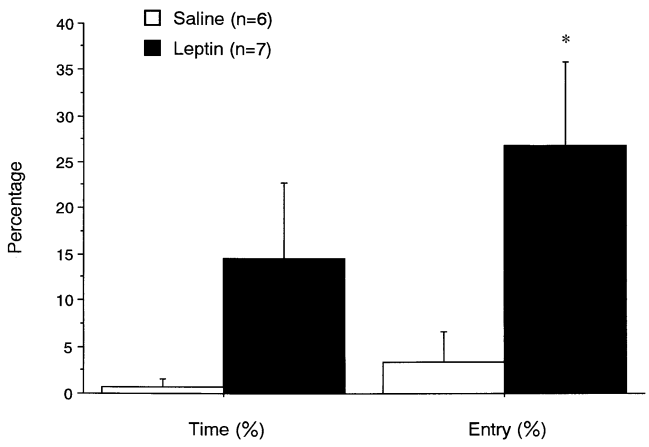

Fig. 1. Effects of repeated administration of leptin (3 nmol/mouse every 12 h for 5 days) on anxiety in the elevated plus maze in *ob/ob* obese mice. Results are expressed as mean ± S.E.M. n indicates the number of mice used. * $P < .05$ compared with the control group by Bonferroni's t test. Time (%), $100 \times$ open/open + closed. Entry (%), $100 \times$ open/total entries.

Table 1
Effects of repeated administration of leptin (3 nmol/mouse every 12 h for 5 days) on locomotor activity in the elevated plus maze and on body weight gain in *ob/ob* obese mice

	Saline ($n = 6$)	Leptin (3 nmol, $n = 7$)
Total entry number	1.833 ± 0.654	7.571 ± 0.922**
Body weight gain (g/day)	0.015 ± 0.006	− 0.147 ± 0.012**

Results are expressed as mean ± S.E.M. n indicates the number of mice used.
** $P < .01$ compared with the control group by Bonferroni's t test.

4. Discussion

Considerable evidence has been accumulated to demonstrate that feeding behavior is closely related to emotions (Greeno & Wing, 1994; Inui, 2001a; Schwartz & Seeley, 1997). Many obese patients tend to eat more when they are emotionally tense or depressed or simply bored (Vaswani, Tejwani, & Mousa, 1983). It has also been reported that obese patients with psychiatric manifestations ranging from depression to anxiety frequently have mild hypercortisolism (Chrousos, 2000). Corticotropin-releasing hormone (CRH) has been demonstrated to play a major role in anxiety-like behavior via activation of the hypothalamic–pituitary–adrenal (HPA) axis (Arborelius, Owens, Plotsky, & Nemetroff, 1999; Inui, 2001a). The *ob/ob* obese mice are known as the genetic model of obesity and diabetes, and show increases in corticosterone levels (Inui, 2000b; Picard, Richard, Huang, & Deshaies, 1998; Zhang et al., 1994). Previous reports suggest that *ob/ob* obese mice enhance adrenergic responses to environmental stress (Kuhn, Cochrane, Feinglos, & Surwit, 1987). In the present study, *ob/ob* obese mice injected with physiological saline showed decreased exploration of the open arms on elevated plus maze than did lean mice (Asakawa et al., 1999, 2001). In addition, leptin treatment increased exploration of the open arms and locomotor activity accompanied by significant decrease in body weight gain compared with saline-treated *ob/ob* obese mice. It has been shown that chronic treatment of *ob/ob* obese mice with leptin decreases plasma corticosterone levels (Picard et al., 1998). Moreover, adrenalectomy ameliorates obesity and hyperinsulinemia, and reduces the levels of corticosterone in *ob/ob* obese mice (Solomon & Mayer, 1973).

We showed here that leptin treatment ameliorated not only obesity but also anxiety in *ob/ob* obese mice. On the other hand, numerous studies have shown that obesity is associated with psychosocial aspects. These observations suggest that the treatment of obesity could lead to the solution of psychological problems.

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