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Research article

Role of leptin in conditioned place preference to high-fat diet in leptin-deficient *ob/ob* mice

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HIGHLIGHTS

- Conditioned place preference for a high fat diet was examined among *ob/ob* mice.
- Higher place preference for a high fat diet was shown in *ob/ob* mice.
- Leptin treatment decreased the preference in *ob/ob* mice independently of obesity.
- Leptin suppresses food intake by inhibiting the hedonic feeding pathway.

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ABSTRACT

Leptin is an adipocyte-derived anorexic hormone that exerts its effects via the hypothalamus and other brain regions, including the reward system. Leptin-deficient *ob/ob* mice that present morbid obesity, hyperphagia, insulin resistance, and infertility are one of the most investigated mouse models of obesity. Conditioned place preference (CPP) paradigm is a standard behavioral model to evaluate the rewarding value of substrates. While leptin is reported to decrease the CPP of lean mice for high fat diet (HFD), it is unknown how CPP toward HFD is affected by leptin replacement in the pathophysiological condition of *ob/ob* mice. In the present study, we performed the CPP test in order to clarify the effect of leptin on the preference of *ob/ob* mice for HFD. *Ob/ob* mice had a significantly higher HFD preference in CPP test when compared with wild-type (WT) mice and this preference was suppressed to the levels comparable to the WT mice by leptin replacement with or without normalization of body weight. These results demonstrate that leptin decreases the reward value of HFD independently of obesity, suggesting that leptin reduces food intake by suppressing the hedonic feeding pathway in *ob/ob* mice.

1. Introduction

The notion of hedonic regulation of food intake has gained increasing attention in the field of obesity research [9]. Brain reward systems play important roles in the hedonic control of food intake.

Leptin is an adipocyte-derived hormone that regulates food intake and energy expenditure [13]. Genetic mutations in the leptin pathway can cause obesity in humans and rodents [3]. The anorexic effect of leptin is primarily exerted through its action in the arcuate nucleus of the hypothalamus, but it is also known to act on several other regions and brain systems [10]. One such system is the brain reward system. We previously reported that postprandial suppression of neural activity is insufficient in the reward-related brain nuclei of hypoleptinemic lipodystrophic patients and that leptin replacement therapy effectively restores the impaired postprandial neural response in the patients [1]. We also reported that C57BL/6 mice fed on HFD for 16 weeks show the impaired leptin responsiveness in the hippocampus that is thought to be a part of the brain reward system [12]. These previous findings suggest the important role of leptin in the hedonic feeding regulation.

Leptin-deficient *ob/ob* mice are one of the most investigated mouse models of obesity. They show morbid obesity, hyperphagia, insulin resistance, and infertility. Conditioned place preference (CPP) is a widely used method for measuring the reward value of addictive drugs [8] that can also be used to measure the reward value of food. Leptin is thought to decrease the preference of lean

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mice for high fat diet (HFD) [4]. However, it is yet to be elucidated how CPP toward HFD is affected by leptin replacement in *ob/ob* mice that are supposed to have different pathophysiology from normal mice.

In the present study, we performed CPP test in order to clarify the effect of leptin replacement on the preference of *ob/ob* mice to HFD. As far as we know, there is no previous report examining the effect of leptin on the CPP of *ob/ob* mice to HFD.

2. Methods

2.1. Animals

Male C57BL/6J mice and *ob/ob* mice (5-week and 8-week old) were obtained from Japan SLC, Inc. (Shizuoka, Japan). They were housed under a 12/12 h light/dark cycle (lights on at 08.00 h), at a constant room temperature of 24 °C ± 1 °C and with free access to food and water. All experimental procedures were approved by the Kyoto University Graduate School of Medicine Committee on Animal Research. Every effort was made to optimize the comfort and to minimize the number of animals.

2.2. Leptin replacement

Three weeks or three days before the CPP test, all mice were subcutaneously implanted in the mid-scapular region with osmotic minipumps (Alzet; Palo Alto, CA) containing either saline or recombinant mouse leptin (200 ng/h; Amgen, Thousand Oaks, CA).

2.3. CPP test

The CPP test was performed according to the previous report [7]. The CPP test was carried out in a three-chamber apparatus with a small middle chamber that connected two large side chambers (MED Associates, St. Albans, VT). The two large chambers differed in floor and wall conditions. At day 0, mice were allowed to move freely in the three chambers for 20 min. At days 1–3, mice were confined to one large chamber with control diet (protein 20 kcal%; carbohydrate 70 kcal%; fat 10 kcal%: Research Diets, Inc., New Brunswick, NJ, USA; No. D12450K) for 30 min. Five hours later, they were confined to the other side chamber with a 60% HFD (protein 20 kcal%; carbohydrate 20 kcal%; fat 60 kcal%: Research Diets, Inc., New Brunswick, NJ, USA; No. D12492) for 30 min. At day 4, mice were placed in the middle chamber and allowed to move freely between the three chambers for 20 min. CPP was evaluated the difference in the time spent between the paired chambers, as follows: the time mice spent in the control chamber subtracted from the time spent in the HFD chamber.

2.4. Statistical analysis

All data were expressed as mean ± standard error of the mean. We performed *t*-test one-way ANOVA, or one-way repeated-ANOVA to analyze the data with JMP® 11 (SAS Institute Inc., Cary, NC, USA). ANOVA was followed by post-hoc Tukey-Kramer method or Fisher's least significant difference test for multiple comparisons.

3. Results

Ob/ob mice showed a significantly increased place preference for the HFD compared with the wild-type (WT) mice at baseline (WT mice vs. *ob/ob* mice; 317.0 ± 67.8 s. vs. 545.3 ± 70.7 s., P < 0.05) (Fig. 1).

To determine whether the leptin replacement influences HFD-induced CPP, we administered leptin (4.8 μg/day) to *ob/ob* and WT mice for 4 weeks (experiment 1). Leptin replacement significantly decreased the body weight of the *ob/ob* mice (saline-treated *ob/ob* mice vs. leptin-treated *ob/ob* mice; 44.9 ± 1.1 g vs. 25.9 ± 0.7 g, P < 0.001) while the leptin-treatment did not affect the body weight of WT mice (saline-treated WT mice vs. leptin-treated WT mice; 26.0 ± 0.8 g vs. 24.9 ± 0.4 g). After 4 weeks of the leptin replacement, the body weight of leptin-treated *ob/ob* mice (25.9 ± 0.7 g) was comparable to that of saline-treated WT mice (26.0 ± 0.8 g) (Fig. 2A). The saline-treated *ob/ob* mice showed a significantly higher place preference for the HFD compared with the saline-treated WT mice (saline-treated WT mice vs. saline-treated *ob/ob* mice 223.3 ± 51.0 s. vs. 443.3 ± 69.0 s., P < 0.05), confirming the baseline data. The leptin treatment significantly decreased CPP to the HFD in *ob/ob* mice (saline-treated *ob/ob* mice vs. leptin-treated *ob/ob* mice; 443.3 ± 69.0 s. vs. 289.5 ± 38.9 s., P < 0.05) to the level comparable to the saline-treated WT mice while the CPP tended to be decreased in the leptin-treated WT mice (saline-treated WT mice vs. leptin-treated WT mice; 223.3 ± 51.0 s. vs. 166.5 ± 52.9 s., p = 0.49) in this setting (Fig. 2B).

To further explore whether this decreased CPP to the HFD in *ob/ob* mice was due to the direct effect of leptin or the secondary effect of normalization of body weight, we administered leptin (4.8 μg/day) to *ob/ob* and WT mice for 1 week. One −week leptin treatment slightly decreased the body weight of *ob/ob* mice, however, the leptin-treated *ob/ob* mice were still markedly obese compared with the saline-treated WT mice (saline-treated WT mice vs. leptin-treated *ob/ob* mice; 26.6 ± 0.4 g vs. 38.6 ± 0.6 g, P < 0.001) (Fig. 3A). The saline-treated *ob/ob* mice showed a significantly higher place preference for the HFD compared with the saline-treated WT mice (saline-treated WT mice vs. saline-treated *ob/ob* mice 304.0 ± 49.4 s. vs. 627.1 ± 72.8 s., P < 0.05). The 1-week leptin treatment significantly decreased the CPP to the HFD in the *ob/ob* mice (saline-treated *ob/ob* mice vs. leptin-treated *ob/ob* mice; 627.1 ± 72.8 s. vs. 339.3 ± 68.6 s., P < 0.05) to the level comparable to the saline-treated WT mice while the CPP tended to be decreased in the leptin-treated WT mice (saline-treated WT mice vs. leptin-treated WT mice; 304.0 ± 49.4 s. vs. 245.3 ± 75.6 s., p = 0.93) in this setting (Fig. 3B).

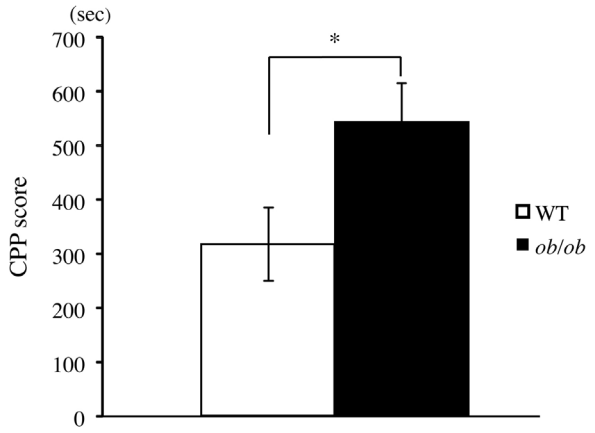

Fig. 1. HFD-induced CPP in *ob/ob* mice.
CPP was developed by repeated feeding with a 60% HFD for 3 days (n = 11 per group). *Ob/ob* mice showed a significantly higher preference to a HFD compared with the WT mice. White bar = WT mice; black bar = *ob/ob* mice. Data are shown as mean ± standard error of the mean; *P < 0.05
Abbreviations: CPP, conditioned place preference; HFD,high fat diet; WTwild type.

4. Discussion

In this paper, we demonstrated that CPP for HFD is higher among *ob/ob* mice than among WT mice, and that this CPP is suppressed

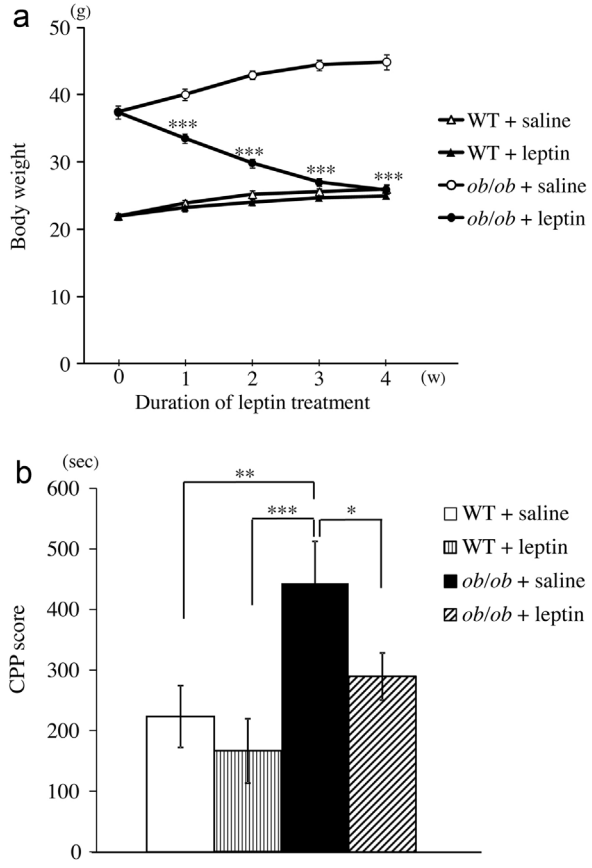

Fig. 2. Effect of 4-week leptin treatment on the CPP to HFD in the *ob/ob* mice.
(A) The change of body weight in *ob/ob* mice during 4-week leptin treatment. Mice were continuously given leptin (4.8 μg/day) or the same volume of saline. The body weight of *ob/ob* mice decreased to a level equivalent to that of WT mice following 4-week leptin treatment. Data are expressed as mean ± standard error of the mean. White triangles = WT mice treated by saline; black triangles = WT mice treated by leptin; white circles = *ob/ob* mice treated by saline; black circles = *ob/ob* mice treated by leptin. $^{***}P < 0.001$; *ob/ob* mice treated by saline vs. *ob/ob* mice treated by leptin.
(B) Effect of 4-week leptin treatment on HFD-induced CPP. CPP was developed by repeated feeding of a 60% HFD (n = 11–17 per group). Leptin-treated *ob/ob* mice spent significantly less time at the HFD-paired chamber than those treated by saline. In the WT mice, leptin treatment did not affect HFD-induced CPP. Data are expressed as mean ± standard error of the mean; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. White bar = WT mice treated by saline; vertically striped bar = WT mice treated by leptin; black bar = *ob/ob* mice treated by saline; diagonally striped bar = *ob/ob* mice treated by leptin.
Abbreviations: CPP, conditioned place preference; HFD, high fat diet; WT, wild type.

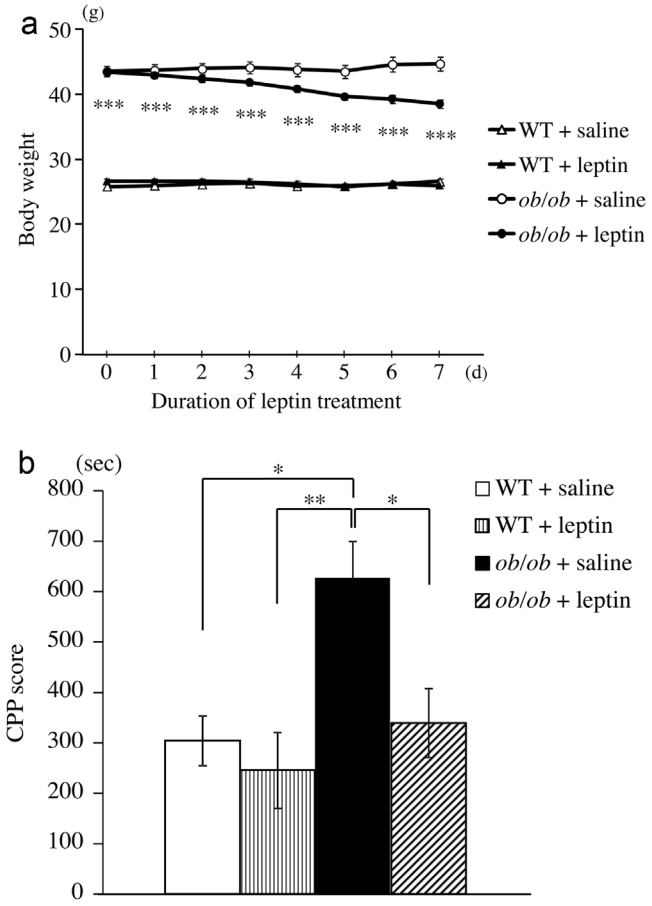

Fig. 3. Effect of 1-week leptin treatment on the CPP to HFD in the *ob/ob* mice.
(A) The change of body weight in *ob/ob* mice during 1-week leptin treatment. Mice were continuously given leptin (4.8 μg/day) or the same volume of saline. The body weight of *ob/ob* mice remained higher than that of WT mice following 1-week leptin treatment. Data are expressed as mean ± standard error of the mean. White triangles = WT mice treated by saline; black triangles = WT mice treated by leptin; white circles = *ob/ob* mice treated by saline; black circles = *ob/ob* mice treated by leptin. $^{***}P < 0.001$; WT mice treated by saline vs. *ob/ob* mice treated by leptin.
(B) Effect of 1-week leptin treatment on CPP induced by repeated feeding of a 60% HFD (n = 9-10 per group). Leptin-treated *ob/ob* mice spent significantly less time at the HFD-paired chamber than those treated by saline. In the WT mice, leptin treatment did not affect HFD-induced CPP. Data are expressed as mean ± standard error of the mean; $^{*}P < 0.05$; $^{**}P < 0.01$. White bar = WT mice treated by saline; vertically striped bar = WT mice treated by leptin; black bar = *ob/ob* mice treated by saline; diagonally striped bar = *ob/ob* mice treated by leptin.
Abbreviations: CPPconditioned place preference ; HFDhigh fat diet ; WTwild type.

by leptin replacement, suggesting that leptin decreases the hedonic feeding pathway in *ob/ob* mice. Leptin has been shown to reduce the conditioned preference of rats to both sucrose and HFD [4–6] in non-obese wild type animals. However, it has not been reported whether leptin can affect the CPP to HFD in *ob/ob* mice where many factors of obesity are involved in the different physiology from normal mice. The *ob/ob* mice had a significantly increased preference for HFD compared with the WT mice. Our data from 4-week and 1-week leptin treatment clearly showed that leptin decreased the CPP to HFD with or without normalization of obesity, indicating that leptin directly decreases the CPP to HFD. Our results from 1-week leptin treatment showed that leptin decreased the CPP independently of obesity, indicating that leptin can suppress hedonic pathway.

Compatible with our results, a previous study has shown that *ob/ob* mice showed a preference for fat by self-selecting from three food cups containing either carbohydrate, protein, or fat [11]. In addition, *ob/ob* mice have exhibited preference for sucrose in a two-bottle choice test [2]. Together, these data suggest that *ob/ob* mice have a higher preference for palatable high-energy food when compared with WT mice, which is a common feature of obese animals. Although it is considered that leptin treatment of *ob/ob* mice decreases food intake and that body weight mainly via its action in the hypothalamus, our data support the possibility that leptin suppresses food intake of *ob/ob* mice at least partially by decreasing their elevated preference for a more palatable HFD. *Ob/ob* mice are highly leptin-sensitive. In contrast, almost all other obese mice are leptin resistant. Thus, this preference-suppressing effect of leptin administration is not expected in other obese model mice, such as commonly used diet-induced obese mice (DIO mice). However, both *ob/ob* mice and DIO mice lack the action of leptin suggesting that lack of the suppressive effect of leptin on hedonic food intake is also important in the pathophysiology of DIO mice.

Mesolimbic dopamine system is considered as one of the major components of the reward system and dopamine plays an important role in the establishment of CPP. Leptin appears capable of decreasing the dopaminergic system in the nucleus accumbens.

Thus, leptin could suppress the CPP to HFD by inhibiting the mesolimbic dopaminergic system in *ob*/*ob* mice although there still remains controversy about the hyperactivity of their dopaminergic system. Further studies will be needed to elucidate the molecular mechanism.

In conclusion, we demonstrated that leptin replacement decreases the elevated preference of *ob*/*ob* mice for HFD independently of obesity, using the CPP methodology. This result indicates that leptin decreases food intake at least partially by suppressing the hedonic feeding pathway in *ob*/*ob* mice.

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