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Original Article

Chronic Stimulation of Leptin on Food Intake and Body Weight after Microinjection into the Ventromedial Hypothalamus of Conscious Rats

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Abstract

A very low dose of leptin (50 ng) was microinjected into the ventro-medial hypothalamus (VMH) of each rat daily once for three days. Food intake and body weight were measured after leptin injections. Microinjection of leptin into the VMH reduced food intake by 33.3 % significantly (*P*<0.01) during three days of leptin injection compared to the control. Body weight was measured after 24 h, 48 h and 72 h of leptin injection. After 24 h (*P*<0.01) and 48 h (*P*<0.05) of leptin injection, body weight was reduced significantly compared to that of rat before injection. Similarly, after 72 h of leptin injection, a significant reduced body weight was observed (*P*<0.1). A significant (*P*<0.001) reduced changes of body weight were found after 24 h, 48 h and 72 h after injection into the VMH when compared to the respective controls injected with saline. The results suggest that leptin has dramatic effect on reducing body weight by inhibition of food intake.

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Introduction

The hypothalamus is involved in regulation of obesity, caused by hyperphagia and decreased energy expenditure and motility. Among the hypothalamic structures, the VMH has been recognized as the satiety center. The sympathetic output from the VMH neurons innervates the peripheral tissues and regulates metabolic function. The destruction of the VMH produces severe hyperphagia and obesity (12) showing the VMH as the key center for regulation of body weight and food intake. Some of the peptides found in the hypothalamus and in other peripheral tissues. These peptides regulate hyperphagia and other syndromes of obesity. NPY was found in the arcuate nucleus and is believed to enhance feeding behavior of animal (18). The function of leptin in feeding is opposite (19). It is speculated that leptin inhibits NPY synthesis in the brain and inhibit feeding. Therefore, the mechanism of developing obesity and its prevention is not well defined. Therefore, the present study has been undertaken to find the role of leptin in feeding and body weight and whether the VMH is involved in this phenomena. This study was also undertaken to evaluate the possible role of the VMH region as a target for leptin's satiety signal. For this purpose, we microinjected very low doses of recombinant human leptin directly into the VMH to show the role of leptin in feeding behavior and body weight regulation.

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Materials and Methods

Male Sprague-Daweley rats weighing 200 g to 250 g were used. They were housed in plastic cages at 25±1 ^{0}C with a 12h light-dark cycle (lights on at 06.00-18.00 h) and given free access to laboratory chow and water.

The rats were anesthetized with sodium-pentobarbital (40 mg per kg body weight). They were placed in steriotaxic instruments and implanted double-walled cannula to the VMH of rats according to the coordinates (10). For the VMH, the stereotaxic coordinates used were AP 5.8 (5.8 mm anterior to the interaural line), L 0.5 (0.5 mm lateral to the sagital suture), and H 9.5 (9.5 mm below the surface of the skull). They were maintained in the cages with free access of water and ad libitum. When the body weight is recovered, the rats were used for experiment. The rats were repeatedly handled during the 5- to 7-day recovery period to habituate them to the injection procedure.

50 ng of leptin (recombinant human leptin) dissolved in 0.5 µl saline was given to the VMH of each rat. Control rats were given 0.5 µl saline only. Rats were injected with a single dose of leptin into the VMH once for three days daily at 3 P.M. Body weight was measured in the animal laboratory with balance (Yamato). Initial food given to each rat was weighed. After three days of leptin injection, the final food was weighed. Net food intake by each rat was calculated as the difference between the initial and final food weight. Body weight was measured for 24 h, 48 h and 72 h after leptin injection. All data, expressed as means ± SE, were analyzed using analysis of variance (pared t-test) by Stat View soft ware.

Results

Effects of microinjection of leptin into the VMH on food intake of rats: A single injection of leptin (50 ng in 0.5 µl saline) daily once for 3 days into the VMH significantly reduced food intake in rats. For saline treated control rats shown in Fig. 4 and Table 1, the initial average food weight was 470.2 ± 6.3 g and after three days of saline injection, the food weight was 401.5 ± 7.9 g. The net food intake after three days therefore was 68.7 ± 3.9 g. Similarly, the initial food weight and three days after leptin injection were 449.5 ± 26.1 g and 403.6 ± 27.5 g respectively. Therefore, the average food intake for leptin treated rats was 45.8 ± 2 .0 g. The average food intake was significantly reduced (P<0.01) after injection of leptin into the VMH in rats compared to the control injected with saline. Food intake was 33.3 % decreased in leptin treated rats.

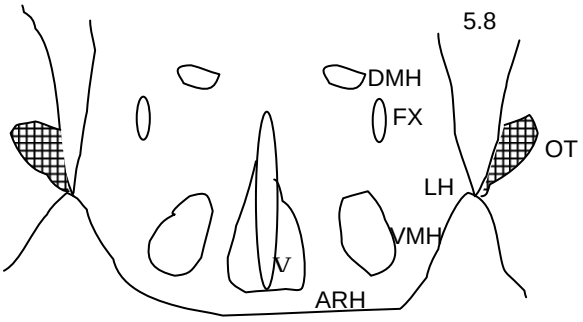

Fig. 1. Localization of the hypothalamic metabolic nuclei. Frontal sections of the brain were schematically illustrated at the level of 5.8 mm anterior to the interaural line according to the rat-brain atlas of Pellegrino et al. Brain cannulas were successfully implanted into the VMH of rats. ARH, arcuate nucleus of the hypothalamus; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus; FX, fornix; OT, optic tract; V, third ventricle.

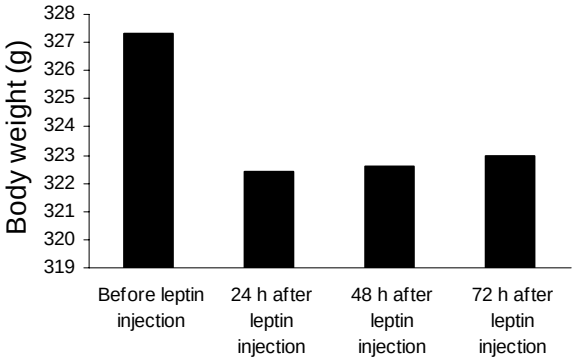

Fig. 2. 24 h-, 48 h- and 72 h- body weight were measured in conscious rats after single injection of leptin (50 ng) into the VMH, once daily and compared with body weight of rat before injection. The results are ± SE for 8 rats.

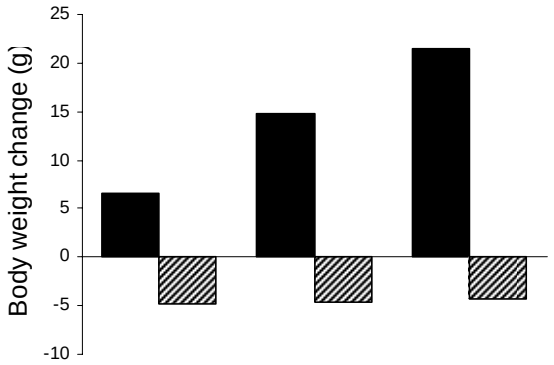

Fig. 3. Changes of body weight of conscious rats implanted with cannula in the VMH. 50 ng of leptin daily once injected in the VMH. 24 h, 48 h and 72 h after injection, average body weights were measured. The respective body weight changes for the above time periods were calculated. Control rats were similarly implanted with brain cannula into the VMH and injected with saline only. The net body weight changes for leptin were compared with the saline injected rats. The results are ± SE for 8 rats in each group.

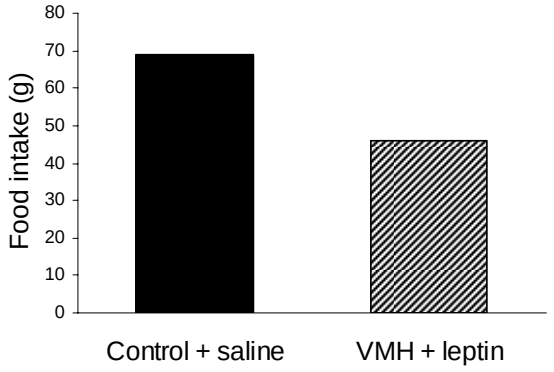

Fig. 4. Net food intake in rats after three days injections with leptin (50 ng) into the VMH was measured and compared with the saline injected control group. A significant difference (P<0.01) was observed. The results are ± SE for 8 rats in each group.

Effects of microinjection of leptin into the VMH on body weight of rats: Body weight was measured after 24 h, 48 h, and 72 h of leptin injection into the VMH of rats. As shown in Fig 2 and Table 1, the average body weight before injection of leptin was 327.2 ± 22.8 g and 24 h after leptin injection, body weight was 322.3 ± 23.2 g. Similarly, after 48 h and 72 h of leptin administration, the average body weights were 322.6 ± 23.0 g and 323.0 ± 23.3 g respectively. Injection of leptin produces significant decrease in body weight after 24 h (P<0.01) and 48 h (P<0.05) compared to that of rat before injection. After 72 h of leptin injection, a significant (P<0.1) reduced body weight was also observed. Higher potency of leptin on body weight reduction was observed after 24 h. On the contrary, injection of saline into the VMH did not prevent body weight and increased steadily up to 72 h. Here, the average body weights for 24 h, 48 h and 72 h of saline injection were 272.2 ± 11.3 g, 280.5 ± 10.4 g and 287.2 ± 10.0 g respectively. As shown in table 1, body weight was gradually increased significantly in the control rats injected with saline after 24 h, 48 h and 72 h (P<0.01) suggesting the normal weight gain of rat. A significant reduced body weight changes were found after 24 h (P<0.001), 48 h (P<0.001), and 72 h (P<0.001) of leptin injection compared to the respective controls injected with saline shown in Fig. 3.

Table 1. Effect of microinjection of leptin injected into the VMH on food intake and body weight. Conscious and trained rats were injected with leptin daily once. Three days after injection of leptin, the net food intake was calculated and compared with the control groups injected with saline. Body weights after 24 h, 48 h and 72 h of leptin injection were measured. Control rats were similarly injected with saline only. ***, ** and * indicate significance of difference at P<0.01, P<0.05 and P<0.1 respectively in reducing body weight compared to that of rat before injection. § indicates significance of difference at P<0.01 in net food intake by leptin compared to the control injected with saline. 24 h-, 48 h- and 72 h- body weight were measured after leptin injections and control rats were injected with saline and body weights were taken for the respective durations. Results are means ± SE. n= number of rats examined.

	Control (saline) (n=8)	VMH (leptin) (n=8)
Body Weight (g):		
Before saline/leptin injection	265.75 ± 11.81	327.25 ± 22.83
24 h after saline/leptin injection	272.25 ± 11.32	322.37 ± 23.26***
48 h after saline/leptin injection	280.50 ± 10.48	322.62 ± 23.08**
72 h after saline/leptin injection	287.25 ± 10.00	323.00 ± 23.30*
Food intake (g):		
Initial food weight	470.25 ± 6.39	449.50 ± 26.15
Final food weight	401.50 ± 7.90	403.62 ± 27.56
Net food intake after 3 days	68.75 ± 3.92	45.87 ± 2.02 §

Discussion

We found that microinjection of leptin into the VMH had reduced body weight and food intake in rats significantly. Leptin was injected into the VMH daily once for three days. Body weight was measured for 24 h 48 h and 72 h after leptin injection and compared to the respective controls injected with saline. After three days, the net food intake was calculated. Leptin has both central and peripheral effects on reducing body weight, food intake and other metabolic functions (29). However, the central effects of leptin are obtained from the peripheral injections. Because of the dominant expression of leptin receptors in the VMH, leptin directly goes to the hypothalamus and binds to the receptor after peripheral administrations. It is reasonable that higher potency of leptin on food intake and body weight should be observed after injections into the hypothalamus directly. We injected very low doses of leptin. It has been demonstrated that 50 ng of leptin enhances regional sympathetic nerve activity after injection to the VMH (16, 17). The increased sympathetic activity should be correlated with the increased energy expenditure of the subject. In fact, leptin increases motility of rat. The higher energy expenditure of rat and the reduced food intake could be responsible for the reduced body weight. In our study, the rat was chronically stimulated by leptin. It could be reasonable that the reduced body weight because of the increased degradation of body fats. It has been reported that leptin enhances fatty acid oxidation and lipolysis (30).

It has been demonstrated that chemical and electrical stimulation of the VMH activate the sympathetic neuron in this locus (21). The sympathetic out put from the VMH might inhibit the feeding of rat. Neuropeptide Y (NPY), the potent stimulator of feeding, is localized in the arcuate nucleus of the hypothalamus. It is probable, that the inhibition of food intake by leptin might be through the inhibition of the synthesis of NPY. In fact, there is growing evidence that leptin, at least in part, suppresses food intake by acting on hypothalamic NPY neurons. The *ob/ob* mice with knockout of the NPY gene are not as obese and are less hyperphagic than *ob/ob* mice with an intact NPY system (22). Leptin receptor messenger ribonucleic acid (mRNA) has been identified in NPY-expressing neurons, and leptin administered icv to rats suppresses NPY synthesis (23). Leptin may also act on hypothalamic POMC neurons to modulate food intake. Recent evidence indicates that activation of melanocortin-3 and -4 receptors (which bind POMC-derived peptides) inhibits feeding in a number of rodent models of hyperphagia (24). POMC neurons in the arcuate nucleus have been shown to express leptin receptor mRNA in rodents (25). The *ob/ob* mice have been reported to have significantly reduced levels of POMC mRNA in the arcuate nucleus, and leptin treatment restores POMC mRNA to normal levels (26, 27). Additionally, pharmacological blockade of melanocortin-4 receptors has been reported to prevent leptin-induced suppression of food intake in the rat (28).

Although we did not measure food intake in rats for 24 h, recent studies revealed that icv administration of leptin on food intake was apparent for more than 24 h. This is similar to results reported in rats in which a single injection of leptin icv suppressed food intake for 2–3 days, resulting in a loss of body weight that was sustained for at least 6 days (31). The prolonged effect of leptin administered icv on food intake may result from a slow rate of leptin degradation within the brain. Collectively, the reduced body weight and food intake by leptin is an important finding for the prevention of obesity and related disorder. The VMH may play the important role in the regulation of body weight and food intake.

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