Rapid Publications

The Effect of Leptin Is Enhanced by Microinjection Into the Ventromedial Hypothalamus

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To determine whether changes in food intake produced by leptin involve targeting the hormone to distinct central nervous system regions, guide cannulas were positioned stereotaxically into three brain regions—the ventromedial hypothalamus (VMH) (bilaterally, $n = 6$), the dorsal raphe nucleus ($n = 3$), and the lateral ventricle ($n = 3$)—of nonobese male rats (400–500 g). Daily food intake and body weight changes were measured during twice-daily injections of saline (0.1 µl) followed by recombinant human leptin (0.05 µg) for 3 days via the brain cannulas. VMH-injected rats also were followed during a postleptin saline recovery interval. This small dose of leptin did not change food intake or body weight from that during the preceding saline injection period in ventricle-injected or dorsal raphe–injected rats. In sharp contrast, VMH-injected rats ate much less food (56 ± 8% basal) and lost 9 ± 3 g/day or 5% of their body weight during 3 days of leptin administration. VMH-injected animals fully recovered from leptin-induced effects within 3 days. We conclude that small doses of leptin that do not effect eating behavior when delivered to the ventricle or the dorsal raphe (another brain region believed to regulate feeding), suppress food intake when injected into the VMH. These data suggest that the VMH or a brain region in close proximity to it is a key target for the biological actions of leptin. *Diabetes* 46:150–152, 1997

Obesity and its resulting complications, such as heart disease, hypertension, dyslipidemia, and diabetes, have become increasingly significant public health concerns. The existence of a circulating satiety factor that contributes to the development of obesity has been suggested since the early parabiotic experiments of Coleman (1) showing reversal of obesity when the circulatory system of a morbidly obese (*ob/ob*) mouse was connected to that of a lean mouse. This hypothesis has been greatly strengthened by the recent cloning of the *ob* gene (2) and the identification of its product (leptin) as a 16-kDa protein secreted by adipose tissue into the circulation. In the *ob/ob* mouse, a mutation of the leptin gene results in the production of a truncated nonfunctional molecule. The importance of this genetic defect is underscored by data showing that systemic or intracerebroventricular (in smaller doses) administration of leptin produces reductions in food intake and body weight in leptin-deficient *ob/ob* mice (3–6). To produce similar effects in lean animals, much larger doses of leptin were required (3–6).

Although a wide variety of tissues appear to express leptin receptors and could potentially serve as target tissues, data demonstrating the greater effectiveness of intracerebroventricular administration of leptin (5,6) suggests its primary site of action lies within the central nervous system (CNS). The hypothalamus, in particular, is a likely target, since it is a major center for feeding control in rodents and humans and damage to it results in obesity and increased food intake (7). Moreover, in the parabiotic mouse model, lesions of the ventromedial hypothalamus (VMH) not only accelerate food intake in the lesioned animal but also decrease food intake in the nonlesioned animal (8). These findings are consistent with the hypothesis that a disruption of the VMH either directly or indirectly led to overproduction of leptin in the lesioned animal and a consequential suppression of appetite in the intact animal. The discovery of high-affinity leptin receptors in the hypothalamus provides further evidence for its importance as a site of leptin action (9).

This study was 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 and compared the effects on eating to those of similar small doses delivered into the lateral ventricle or into the dorsal raphe nucleus, another brain region believed to play a role in feeding behavior (10).

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats (Charles River Raleigh, Boston, MA) were anesthetized using 60 mg/kg i.m. ketamine and 21 mg/kg i.p. pentobarbital, and their brains were stereotaxically cannulated 1 week before study. The animals were divided into three groups depending on the area cannulated: *1*) VMH bilaterally ($n = 6$; from bregma, 20° medial-lateral [ML], –2.6 mm anterior-posterior [AP], ±3.8 mm ML, 8.2 mm dorsal-ventral [DV]); *2*) lateral ventricle ($n = 3$; from bregma, –0.8 mm AP, 1.5 mm ML, 3.2 mm DV); and *3*) dorsal raphe, ($n = 3$; from bregma, 20° ML, 7.4 mm AP, 2.0 mm ML, 6.0 mm DV). On the day after surgery, all animals were presented with a pellet diet consisting of 60% carbohydrates, 3.7% fat, and 24.1% protein (Noyes, Lancaster, NH) to facilitate accurate measurement of food intake. Thereafter, both food and rats were weighed daily (9:00

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CNS, central nervous system; VMH, ventromedial hypothalamus.

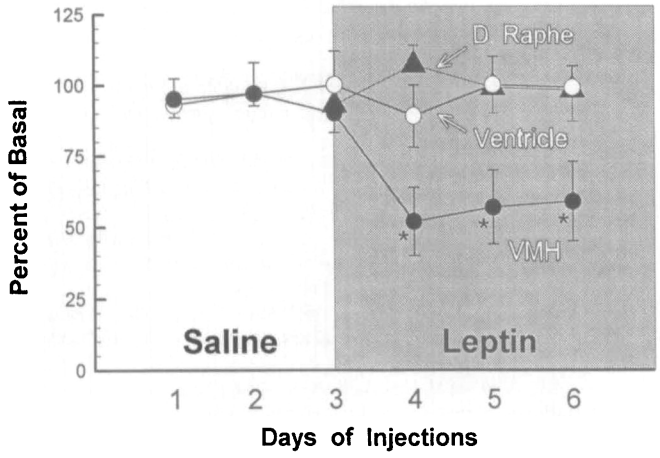

FIG. 1. Effect of leptin on daily food intake in normal rats. Leptin (0.05 μg twice daily for 3 days) or saline (0.1 μl twice daily) were injected directly into the lateral ventricle (n = 3), dorsal raphe nucleus (n = 3), or the VMH (each nucleus was injected daily, n = 6) via stereotaxically positioned guide cannulas. Results are calibrated as percent of intake during the basal (pre-injection) period. Data represent mean ± SE. *P < 0.05 vs. saline injection.

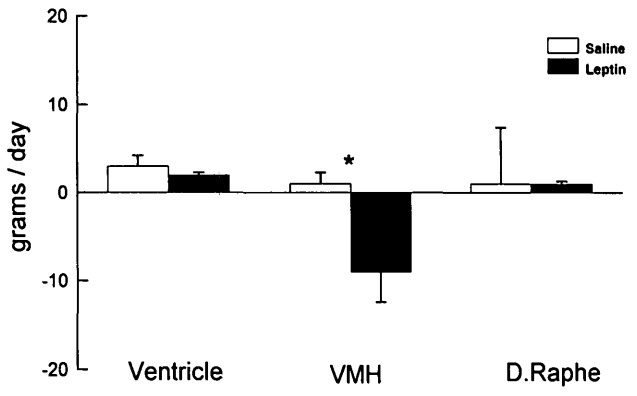

FIG. 2. The effect of leptin on daily body weight changes in normal rats receiving injections in the ventricle, VMH, or dorsal raphe nucleus. Results are calculated as the average change observed during each interval (saline or leptin). Data shown are mean ± SE. *P < 0.05 leptin vs. saline.

A.M.). Food was made available to the rats between 5:00 P.M. and 9:00 A.M. A 4-day basal period was continued until it was certain that the animals had fully recovered from surgery and that they had adjusted to the changes in diet and feeding interval. After completion of the baseline period, all three groups received local 0.1-μl injections of phosphate-buffered saline (pH 7.4) twice daily, at 9:00 A.M. and 5:00 P.M. The saline control period was continued for 3 days in the VMH- and ventricle-injected animals and for 1 day in the dorsal raphe–injected animals. The injections were delivered over a 2-min period via the brain cannulas using a Model 22 Harvard Pump (Harvard Apparatus, South Natick, MA). In the case of the VMH bilaterally cannulated animals, injections alternated between the left and right sides. During days 4–6 of the study, recombinant human leptin (provided by Chiron, Emeryville, CA) in a dose of 0.05 μg twice daily was added to the saline vehicle and administered at the same injection times as during saline alone. Three of the six VMH rats received saline injections (0.1 μl) for 3 additional days after the leptin injection period to examine their ability to recover from the effects of leptin.

Following the study, histological examination was performed to verify the placement of brain cannulas. Only animals with correct cannula placement as well as stable food intake and body weight during the saline control injection phase were used. All data, expressed as means ± SE, were analyzed using analysis of variance, followed by post-hoc Newman-Keuls tests to localize effects statistically (CRUNCH software, San Francisco, CA).

RESULTS

All animals (n = 12) stabilized their eating (28.5 ± 1.2 g/day) during the 4-day basal preinjection period. The effects on daily food intake during the saline and leptin periods in the three groups of animals are compared in Fig. 1. Data are expressed as percentages of basal food intake to control for body weight differences among the VMH-injected (435 ± 23 g), ventricle-injected (522 ± 54 g), and dorsal raphe–injected (497 ± 26 g) groups. There were no statistical differences among groups during the saline control period (94 ± 4, 97 ± 7, and 92 ± 6% of basal food intake, respectively). In addition, when low-dose leptin was administered to the ventricle and dorsal raphe groups, there was no significant change in daily food intake throughout the 3-day period (96 ± 6 and 102 ± 7%, respectively). In contrast, there was a sharp decrease in food intake when leptin was injected into the VMH; animals in this group ate only 56 ± 8% of their intake during the basal period (16 ± 2.3 g during leptin vs. 26 ± 1.3 g during saline). Furthermore, in the three rats in which the experiment was extended, recovery of food intake after leptin was complete within 3 days after initiating VMH saline injections, including in one rat that had virtually stopped eating during the VMH leptin injection period.

Body weight increased steadily during the saline control period in all three groups (VMH 1 ± 1 g/day, ventricle 3 ± 1 g/day, and dorsal raphe 1 ± 6 g/day) (Fig. 2). Weight gain continued in the animals who received intracerebroventricular and dorsal raphe injections of leptin for 3 days (average weight gain, 2 ± 0.3 and 1 ± 0.3 g/day, respectively). In contrast, VMH-injected animals lost a total of 5% of their body weight (from 444 ± 20 to 417 ± 27 g, or 9 ± 3 g/day) during the 3-day period of leptin administration. Each of the three VMH-injected rats participating in the saline recovery phase partially (n = 1) or completely (n = 2) regained the weight lost during the 3-day period of leptin injections.

DISCUSSION

In this study, we delivered leptin locally into specific regions of the brain via chronically implanted cannulas to awake rats. Our data demonstrate that small doses of leptin that do not have an effect when given intracerebroventricularly to nonobese rats do significantly decrease food intake and body weight when given directly to the region of the VMH. In addition, the effects of leptin within the hypothalamus are relatively selective. A comparable dose given to the dorsal raphe nucleus, another area of the CNS thought to be important in the regulation of feeding behavior (10), did not noticeably alter food intake or body weight. The differential response of these regions to our procedure, which bypasses the blood brain barrier, may be more significant from a physiological perspective, since the blood brain barrier is complete in the region of the dorsal raphe, whereas it is not in the region of the VMH (11).

Previous reports have shown that the administration of systemic injections of leptin to mice dramatically decreased food intake and body weight in a dose-response fashion. Although these effects were more pronounced in obese mice, large amounts of leptin significantly altered feeding in lean rodents as well. For example, when leptin was administered intraperitoneally to nonobese mice, very large doses (25 μg · g^{-1} · day^{-1}) were needed to produce significant changes in food

intake and body weight, which decreased by ~50 and ~10%, respectively. The importance of the CNS as a site of leptin action is supported by data showing that to achieve similar changes in lean mice, much smaller amounts of leptin were necessary when it was given via the intracerebroventricular route (0.06 $\mu g \cdot g^{-1} \cdot day^{-1}$). It is noteworthy that in the present study a much smaller leptin dose (~0.0002 $\mu g \cdot g^{-1} \cdot day^{-1}$) was sufficient to evoke comparable marked reductions in food intake (~50%) and body weight (~10%) when locally delivered to the VMH. This dose was not seen to have an effect when delivered intracerebroventricularly.

While these data imply that the region of the VMH is particularly sensitive to the biological effects of leptin and is therefore a key site of hormone action, it is possible that adjacent brain structures, particularly the arcuate nucleus, may have been exposed to leptin during these studies. It is noteworthy that the arcuate nucleus has a high level of leptin receptor gene expression and that neuropeptide Y, a known appetite stimulant produced in the arcuate nucleus, is suppressed by leptin (6,12). Thus, it is possible that our injections of leptin may have acted on the VMH or the arcuate or both structures.

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