Hypothalamic Neuronal Histamine as a Target of Leptin in Feeding Behavior

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Leptin, an *ob* gene product, has been shown to suppress food intake by regulating hypothalamic neuromodulators. The present study was designed to examine the involvement of brain histamine in leptin-induced feeding suppression. A bolus infusion of 1.0 µg leptin into the rat third cerebroventricle (i3vt) elevated the turnover rate of hypothalamic neuronal histamine (P < 0.05) as assessed by pargyline-induced accumulation of *tele*-methylhistamine (*t*-MH), a major metabolite of histamine. No remarkable change in the mRNA expression of histidine decarboxylase (HDC), a histamine-synthesizing enzyme, was observed in the hypothalamus after i3vt infusion of leptin. These results indicate that leptin increases histamine turnover by affecting the posttranscriptional process of HDC formation or histamine release per se. As expected, concomitant suppression in 24-h cumulative food intake was also observed after infusion of leptin. Systemic depletion of brain histamine levels by pretreatment with an intraperitoneal injection of 224 µmol/kg α-fluoromethylhistidine (FMH), a suicide inhibitor of HDC, attenuated the leptin-induced feeding suppression by 50.7% (P < 0.05). This attenuation of feeding suppression was mimicked by the i3vt infusion of 2.24 µmol/kg FMH before leptin treatment (P < 0.05). In addition, concentrations of hypothalamic histamine and *t*-MH were lowered in diabetic (*db/db*) mice, which are known to be deficient in leptin receptors (P < 0.05 vs. lean littermates for each amine), although the amine levels were higher in diet-induced obese rats (P < 0.05 for each amine). Leptin-deficient obese mice (*ob/ob*) showed lower histamine turnover (P < 0.05 vs. lean littermates), which recovered after leptin infusion. Thus, a growing body of results points to an important role for the hypothalamic histamine neurons in the central regulation of feeding behavior controlled by leptin. *Diabetes* 48:2286–2291, 1999

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CRH, corticotropin-releasing hormone; DIO, diet-induced obese; DMH, dorsomedial hypothalamic nucleus; FMH, α-fluoromethylhistidine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDC, histidine decarboxylase; i3vt, third cerebroventricle; NPY, neuropeptide-Y; PBS, phosphate-buffered saline; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus; *t*-MH, *tele*-methylhistamine; TMN, tuberomammillary nucleus; VMH, ventromedial hypothalamic nucleus.

Leptin, an *ob* protein secreted by adipose tissue, has received much attention in studies of the regulation of feeding behavior and fat deposition due to its actions in the central nervous system (1,2). Neuropeptides such as neuropeptide-Y (NPY), corticotropin-releasing hormone (CRH), and α-melanocyte–stimulating hormone, a pro-opiomelanocortin (POMC)-derived peptide, have been shown to be potent modulators of regulation of feeding behavior and peripheral energy metabolism. The actions of these neuropeptides on ingestion and energy metabolism have been found to be regulated partly via the central effects of leptin. Indeed, administration of leptin decreased NPY mRNA levels (3) and increased the expression of CRH (4) and POMC (5) mRNAs in the hypothalamus. Since it was initially believed that the feeding-suppressive effects of leptin might be explained by its downregulation of hypothalamic NPY, a behavioral study examined ingestive behavior in double-mutant mice deficient in both leptin and NPY ($NPY^{-/-}$, *ob/ob*) (6). Whereas the elimination of NPY attenuated the increased food intake and body weight typically observed in leptin-deficient mice, the effect was not complete (6), indicating that neuromodulators other than the NPY system may also be involved. The combined effects of defective POMC signaling, which results in obesity, and the absence of leptin were also examined in double-mutant lethal yellow/leptin-deficient (A^Y/a, *ob/ob*) mice (7). The weight gain observed in these double-mutant mice was independent and additive, revealing that the obesity induced by deficiency of POMC signaling is due at least in part to a leptin-independent mechanism. Thus, in spite of an intensive search for processes mediating leptin's actions in the brain, the complete details underlying its signaling mechanisms remain unclear.

Our previous studies on functions of histamine neuron systems in the brain (8) have demonstrated that hypothalamic neuronal histamine suppressed food intake through H_1-receptors in the ventromedial hypothalamic nucleus (VMH) and the paraventricular nucleus (PVN). In addition, an increase in hypothalamic histamine levels raised peripheral glucose concentrations (8,9), accelerated lipolysis in the adipose tissue (10) (K. Tsuda, H.Y., A. Niijima, S.H., M.K., T.S., unpublished observations), and decreased body temperature (8,9). Moreover, turnover or concentrations of hypothalamic neuronal histamine can be increased by many factors, including neuroglucoprivation in the brain induced by starvation, insulin, or 2-deoxy-D-glucose (11,12); elevation of ambient temperature (8,9); and cytokines such as inter-

leukin-1β (13). Together, these data suggest that the hypothalamic histaminergic neuron system is an important component in the regulation of energy intake and expenditure. In fact, studies in Zucker fatty (*fa/fa*) rats, which have a missense mutation in the leptin receptor gene (14), revealed decreases in both histamine concentrations and the activity of histidine decarboxylase (HDC), a key enzyme that synthesizes histamine from L-histidine, in the hypothalamus of these animals (8,9). Consequently, abnormalities in *fa/fa* rats, including disruptions in circadian feeding patterns, adaptive behaviors, and thermoregulation in response to high ambient temperature, mimicked those in the histamine-depleted rats induced by α-fluoromethylhistidine (FMH), a suicide inhibitor of HDC (8,9). Thus, we hypothesize that the leptin receptor deficiency in *fa/fa* rats may disrupt physiologic brain functions governed by hypothalamic histamine. The goal of the present study was to investigate potential leptin-induced changes in the turnover of hypothalamic histamine in regulation of feeding behavior of normal and diet-induced obese (DIO) rats and genetically obese mice.

RESEARCH DESIGN AND METHODS

Animals and diet. Animals used in these experiments were mature (12-week-old) male Wistar King A (WKA) rats weighing 290 ± 10 g (mean ± SE), male C57BL/KsJ diabetic (*db/db*) mice weighing 40 ± 2 g, their lean littermates (+m/+m) weighing 20 ± 1 g, 12-week-old male C57BL/6J obese (*ob/ob*) mice weighing 40 ± 2 g, and their lean littermates (+/+) weighing 20 ± 1 g. Animals were housed in a room illuminated daily from 0700 to 1900 (a 12:12 h light-dark cycle) and maintained at 21 ± 1°C with humidity at 55 ± 5%. They were allowed free access to standard solid rodent food and tap water unless otherwise noted. All studies were conducted in accordance with the Oita Medical University Guidelines based on the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

DIO rats. Diet-induced obesity was produced in WKA rats by simultaneous exposure to both a liquid diet of 34% sucrose and standard rodent food ad libitum. Body weight and total calorie intake, calculated by measuring consumption of both the sucrose solution and the rodent food, were recorded weekly. When the obese rats showed a significantly increased body weight compared with normal control rats (up to 20 weeks after the onset of the obese diet), rats were killed after intraperitoneal injection of sodium pentobarbital anesthesia (45 mg/kg), and serum and several tissues, including the hypothalamus and subcutaneous and visceral fat pads, were collected. For each rat, serum leptin concentrations were quantitated using a radioimmunoassay (RIA) kit (Linco, St. Louis, MO), and the weight of adipose tissue was measured. Hypothalamic histamine assays were performed as described below.

Reagents. FMH (a gift from Dr. J. Kollonitsch, Westfield, NJ) and pargyline hydrochloride (Sigma, St. Louis, MO) were dissolved in phosphate-buffered saline (PBS) to concentrations of 0.067 and 0.099 mol/l, respectively. FMH is a specific suicide inhibitor of histidine decarboxylase (HDC), which is a substrate activated by its specific enzyme, and the chemical reaction, in turn, irreversibly inhibits activity of the target enzyme because the substrate bounds the catalyzed metabolites throughout the catalytic processes (15). Recombinant murine leptin (a gift from Amgen Biologicals, Thousand Oaks, CA) was dissolved in PBS to concentrations of 0.1 and 1.0 µg/µl. Each solution was freshly prepared on the day of its administration. The pH of each solution was adjusted to 6.4–7.2.

Surgery. Under intraperitoneal sodium pentobarbital anesthesia (45 mg/kg), WKA rats were placed in a stereotaxis apparatus (Narishige, Tokyo), and a stainless steel guide cannula (23 gauge) was chronically implanted into the third cerebroventricle (i3vt) of WKA rats at least 1 week before the start of infusions. A stainless steel wire stylet (29 gauge) was inserted in the guide cannula to prevent leakage of the cerebrospinal fluid and obstruction of the cannula. Details of the surgical procedure have been described (16).

Assays for hypothalamic histamine. To compare the steady-state levels and turnover rate of histamine, both hypothalamic histamine and *tele*-methylhistamine (*t*-MH) were assayed in rats and mice that were obese or of normal control weight. Transmethylation of histamine into *t*-MH by histamine *N*-methyltransferase and subsequent deamination by monoamine oxidase B is the major metabolic breakdown pathway of histamine in the brain (12,17). Pretreatment with pargyline, an inhibitor of monoamine oxidase B, induces accumulation of *t*-MH in extraneuronal space as a major metabolite of released neuronal histamine (12). WKA rats, *ob/ob* mice, and their lean littermates were pretreated with intraperitoneal pargyline (0.33 mmol/kg) or PBS 10 min before i3vt infusion of either leptin or the same volume of PBS. WKA rats were infused with 1.0 µg leptin per rat and mice with 1.0 µg leptin per mouse for 10 min at an infusion rate of 1.0 and 0.1 µl/min, respectively. The dose and the infusion speed were selected on the basis of earlier results, including a dose-response relation between dose of leptin and food intake. Histamine turnover was estimated from the accumulation of *t*-MH over a 70-min period after pargyline treatment. All the animals were decapitated 60 min after the onset of the i3vt infusion. The hypothalamus was dissected on an ice plate according to the method of Glowinski and Iversen (18). The tissue was immediately frozen on dry ice and stored at –80°C until the assays. Histamine and *t*-MH concentrations were measured by the method of Oishi et al. (17). Homogenates of the brain were centrifuged at 1,000*g*, and the clear deproteinized supernatants containing the amine extracts were assayed by high-performance liquid chromatography. The details of the amine assays have been described (12). Assays of hypothalamic histamine and *t*-MH in both *db/db* mice and DIO rats were performed without pargyline pretreatment to estimate the basal levels of both amines in the obese model animals.

Northern blot analysis. Rats were killed 3 h after i3vt infusion of leptin to isolate hypothalamus and cortex for assessment by Northern blot analysis. Total RNA was extracted using Trizol reagent (Gibco, Grand Island, NY) according to the manufacturer's protocol. Northern blot analysis was performed as described (19). cDNA probes for the rat HDC were prepared by reverse transcription–polymerase chain reaction using the following primers: HDC-sense, 5′-GCT TGA GCT CCC TTG TGA AG-3′; HDC-antisense, 5′-GAA GGA TCC AAT CAC AAA CC-3′. Mapping with multiple restriction endonucleases and sequencing confirmed the identity of the appropriately sized product. The hybridization signals were analyzed with a BIO-image analyzer (BAS 2000; Fuji, Tokyo). The membrane was stripped by exposure to boiling 0.1% SDS and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe to control for small differences in RNA loading and transferring.

Behavioral analysis of WKA rats in leptin infusion study. Matched on the basis of body weight and food intake during the adaptation period, WKA rats were assigned to one of four groups: FMH/leptin, PBS/leptin, FMH/PBS, and PBS/PBS control. Cumulative 24-h food intake and body weight were measured both 2 days before and 2 days after i3vt infusion. Percentage difference in average daily food intake over 1 day after the i3vt infusion in each group was compared with the PBS/PBS control group. On the infusion day, FMH—either 224 µmol/kg intraperitoneally or 2.24 µmol/kg i3vt (a dose previously determined to deplete most neuronal histamine in the hypothalamus)—or the same volume of PBS was administered 2 h before 1.0 µg leptin per rat or PBS infusion (1.0 µl/min for 10 min) through the i3vt cannula at 1700. After the completion of the experiments, the animals were decapitated and the cannula location was verified histologically.

Statistic analysis. Statistical analyses for behavioral measures were carried out using a two-way analysis of variance with repeated measures; those for measurements of amines, leptin, body weight, and adipose tissue weight were done by the Mann-Whitney *U* test.

RESULTS

Effect of leptin administration on histamine turnover and expression of HDC mRNA. Bolus i3vt infusion of leptin increased pargyline-induced accumulation of *t*-MH, a major metabolite of brain histamine ($P < 0.05$), but not histamine in the hypothalamus of WKA rats (Fig. 1). In the absence of pretreated pargyline, an inhibitor of monoamine oxidase B, leptin did not affect steady-state levels of histamine or *t*-MH (data not shown). Messenger RNA expression of HDC, a histamine-synthesizing enzyme, was detected in the hypothalamus but not in the cortex. The i3vt infusion of leptin did not affect HDC mRNA expression in the hypothalamus (Fig. 2).

Effect of histamine depletion by FMH on leptin-induced feeding suppression. Administration of i3vt leptin significantly reduced 24-h cumulative food intake ($P < 0.05$) (Fig. 3*A*) and body weight ($P < 0.05$) of WKA rats. However, when rats were depleted of histamine by intraperitoneal pretreatment with FMH, a suicide inhibitor of HDC, this leptin-induced feeding suppression was attenuated, from an average daily food intake of 12.5 ± 1.1 g to 16.3 ± 1.3 g ($P < 0.05$) (Fig. 3*A*). The leptin-induced reduction in body weight was attenuated as well, from an average body weight reduction of 9.2 ± 2.7 to 2.3 ± 1.0 g ($P < 0.05$). FMH pretreatment per se or

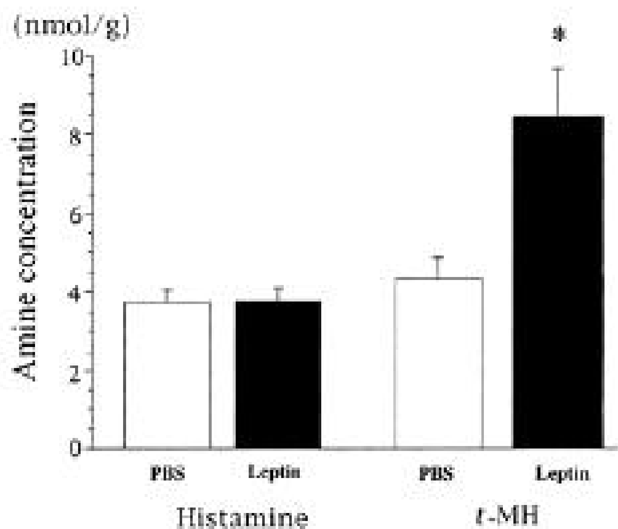

FIG. 1. Effects of i3vt infusion of 1.0 µg leptin per rat on pargyline-induced accumulation of histamine and *t*-MH in the hypothalamus of WKA rats. Values are means ± SE (*n* = 10 for leptin, *n* = 8 for control). **P* < 0.05 compared with PBS controls.

PBS infusion did not affect food intake or body weight. The percentage differences in daily food consumption in the intraperitoneally pretreated PBS/leptin and FMH/leptin groups compared with the PBS/PBS control group are shown in Fig. 3*B*. Leptin alone decreased food intake to 62.1 ± 12.2% of PBS/PBS controls ($P < 0.05$), and after FMH pretreatment, leptin suppressed food intake to only 81.3 ± 14.6% of the control animals ($P < 0.05$ compared with PBS/leptin group). Thus, FMH attenuated the leptin-induced feeding suppression by 50.7%. Figure 3*C* shows the effect of local depletion of hypothalamic histamine by i3vt infusion of FMH on leptin-induced feeding suppression. Leptin decreased food intake to 65.8 ± 6.5% of control animals ($P < 0.05$). After FMH infusion, however, leptin decreased food intake to 83.8 ± 9.2% of controls ($P < 0.05$ compared with PBS/leptin group). Thus, the effects of local depletion of histamine by i3vt infusion of FMH on leptin-suppressed ingestion were similar to those induced by intraperitoneal injection of FMH.

Concentration of hypothalamic histamine and *t*-MH in genetic obese and DIO animals. Concentrations of hypothalamic histamine and *t*-MH in *db/db* mice and DIO rats are shown in Fig. 4. Diabetic (*db/db*) mice revealed lower histamine and *t*-MH concentrations in the hypothalamus than their lean littermates ($P < 0.05$ for each) (Fig. 4*A*). In contrast to the *db/db* mice, the DIO rats revealed higher concentrations of histamine and *t*-MH in the hypothalamus than normal WKA control rats ($P < 0.05$ for each) (Fig. 4*B*). Table 1 shows body weight, weight of adipose tissue, and serum leptin concentrations in the DIO rats. Concomitant with the development of obesity, serum leptin concentrations increased, presumably as a result of the fat accumulation in these obese animals ($P < 0.01$). Figure 5 shows leptin-induced histamine turnover rate in *ob/ob* mice and their lean littermates. Pargyline-induced accumulation of hypothalamic *t*-MH after PBS infusion were lower in *ob/ob* mice ($P < 0.05$). The i3vt infusion of leptin restored pargyline-induced accumulation of *t*-MH to its normal control level ($P < 0.05$ compared with *ob/ob* mice with PBS infusion; $P > 0.1$ compared with normal controls with PBS infusion).

DISCUSSION

The present study demonstrated that i3vt infusion of leptin increased accumulation of *t*-MH. Leptin infusion did not affect concentration of hypothalamic histamine because histamine levels measured in the present study included released histamine in the extraneuronal space as well as intracellular histamine in the nerve terminal. Furthermore, histamine is rapidly converted to its metabolite, *t*-MH, in the brain. Therefore, it is much more informative to analyze histamine release by measuring its metabolite than to rely on a measurement of brain histamine level itself. The results show that leptin increases histamine turnover, since transmethylation of histamine into *t*-MH and its subsequent deamination is the major metabolic pathway of histamine in the brain (12,17). Expression of HDC mRNA in the hypothalamus, however, did not change in response to infusion of leptin. Neuronal histamine is synthesized from L-histidine by HDC, which is responsible for the one-step histamine formation in the brain. Histamine release is induced by depolarization of nerve endings through the opening of Ca^{2+} channels similar

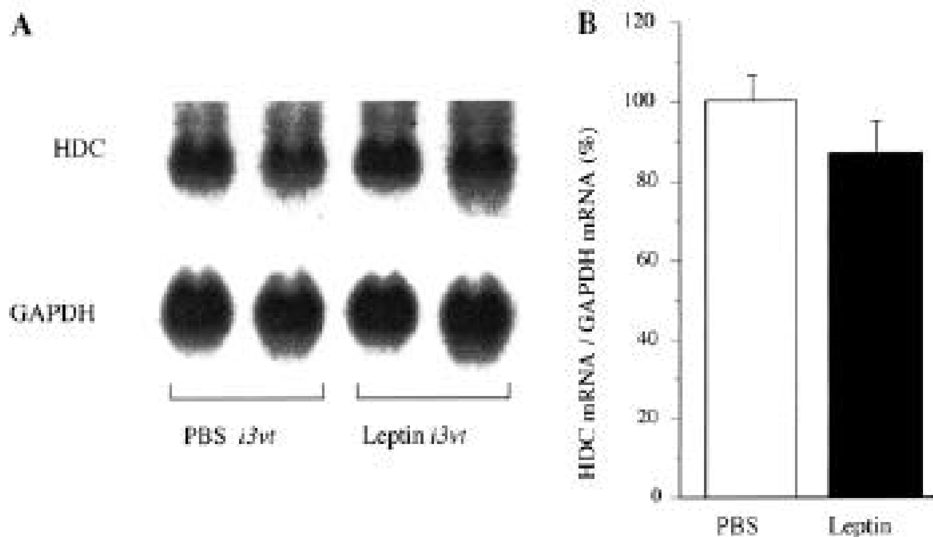

FIG. 2. Effects of i3vt infusion of leptin on the expression of HDC mRNA in the hypothalamus. *A*: Northern blots of hypothalamus from PBS-treated and leptin-treated rats. *B*: Quantitation of the percent changes in HDC/GAPDH levels in the leptin-treated hypothalamus versus those in the PBS-treated hypothalamus. Values are means ± SE (*n* = 5 for each).

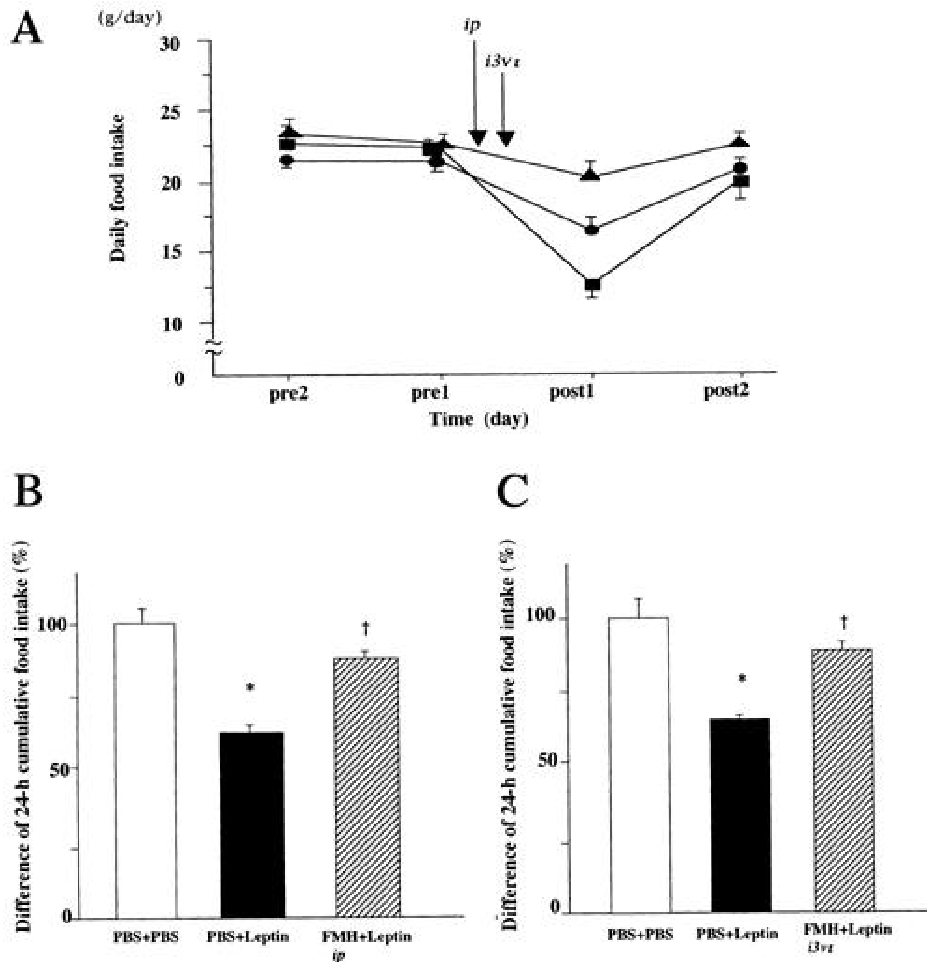

FIG. 3. Effects of histamine depletion by FMH on leptin-induced feeding suppression. *A*: Time course of daily food intake.Values are means ± SE (*n* = 5 per group). ip, pretreatment with intraperitoneal injection of 224 µmol/kg FMH or PBS; i3vt, i3vt administration of 1.0 µg leptin or PBS per rat; ▲, PBS (intraperitoneal)/PBS (i3vt); ■, PBS (intraperitoneal)/leptin (i3vt), *P* < 0.05 compared with PBS (intraperitoneal)/PBS (i3vt); ●, FMH (intraperitoneal)/leptin (i3vt), *P* < 0.05 compared with PBS (intraperitoneal)/leptin (i3vt). *B*: Percentage differences of 24-h cumulative food intake over 1 day following the administration of leptin after depletion of histamine by intraperitoneal injection of FMH. Values are means ± SE (*n* = 5 per group). **P* < 0.05 compared with PBS (intraperitoneal)/PBS (i3vt). †*P* < 0.05 compared with PBS (intraperitoneal)/leptin (i3vt). *C*: Percentage differences in 24-h cumulative food intake over 1 day following administration of leptin after local depletion of histamine by i3vt infusion of FMH. Values are means ± SE (*n* = 5 per group). **P* < 0.05 compared with PBS (i3vt)/PBS (i3vt). †*P* < 0.05 compared with PBS (i3vt)/leptin (i3vt). ip, intraperitoneal.

to those operating for other neurotransmitters (20). These findings indicate that the increase in histamine turnover induced by leptin may involve a posttranscriptional process of HDC formation in histamine synthesis, direct action on histamine release from the nerve terminal by depolarization of histaminergic neurons, or both.

The actual mechanism by which leptin enhances the release of neuronal histamine is unclear. One possible explanation is that leptin may activate the histamine neuron directly. This explanation is difficult to reconcile with the anatomically distinctive distribution of leptin receptor. The long form of leptin receptor is identified densely in the VMH, the dorsomedial hypothalamic nucleus (DMH), the arcuate nucleus, and the ventral premammillary nucleus but not in the tuberomammillary nucleus (TMN), where the cell bodies of histamine neurons exist (21). An alternative explanation is that leptin-induced activation of histamine neurons may be provoked by other leptin-responsive pathways. The DMH has been shown to provide a significant input to the dorsal TMN, together with a small, but well-formed, input to the ventral TMN (22). This neuroanatomic study using an anterograde tracer showed that DMH fibers projecting to the caudal hypothalamus overlapped significantly with the distribution of histaminergic cell bodies (22). This observation is very much in line with an assumption

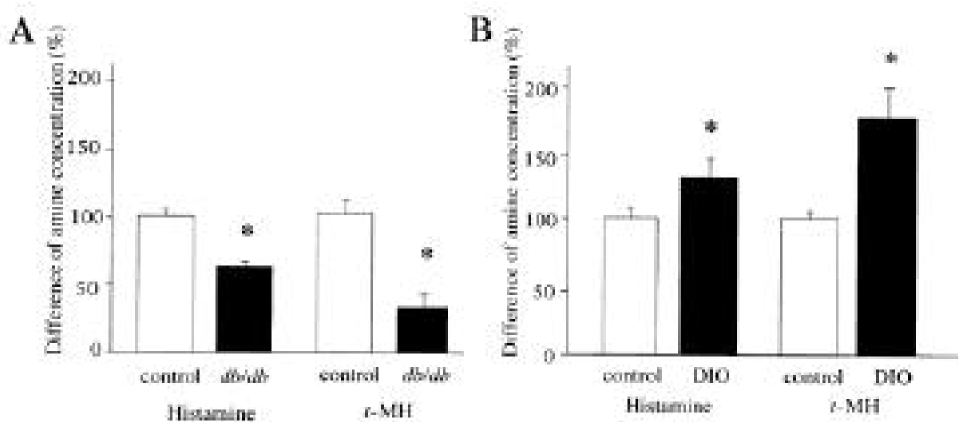

FIG. 4. Percentage differences in concentrations of hypothalamic histamine and *t*-MH in *db/db* mice (*A*) and DIO WKA rats (*B*). Values are means ± SE (*n* = 5 each for *db/db* mice and controls, *n* = 8 each for DIO rats and controls). **P* < 0.05 compared with lean littermates.

TABLE 1
Body weight, adipose tissue weight, and serum leptin levels in DIO rats

	n	Body weight (g)	Adipose tissue weight (g)	Serum leptin (ng/ml)
DIO rats	8	583.9 ± 14.6*	41.3 ± 4.3†	13.8 ± 3.5*
Controls	8	535.9 ± 6.9	17.1 ± 0.6	1.7 ± 0.2

Data are means ± SE. Body weight, adipose tissue weight, and serum leptin levels significantly increased in DIO rats. *$P < 0.01$ compared with controls. †$P < 0.001$ compared with controls.

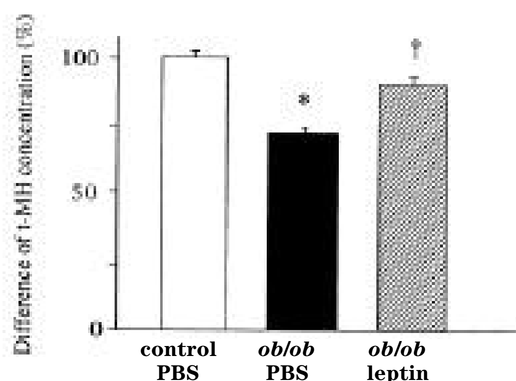

FIG. 5. Percentage difference in concentration of hypothalamic *t*-MH after pargyline treatment in *ob/ob* mice. Values are means ± SE (*n* = 5 each). *$P < 0.05$ compared with lean littermates. †$P < 0.05$ compared with PBS infusion in *ob/ob* mice.

that leptin modulates neuronal histamine through the DMH projections. CRH and NPY have been proposed as candidate mediators of leptin actions in the hypothalamus, since their mRNA expressions are modulated by leptin administration (3,4). Thus, leptin affects synthesis of these neuropeptides. According to our preliminary data, i3vt administration of CRH, but not NPY, increased histamine turnover as assessed by high-performance liquid chromatography (E.I., H.Y., M.K., T.S., unpublished observations). However, a recent neuroanatomic study failed to demonstrate significant direct neuronal projection from the PVN, an origin of CRH-containing neurons, to the TMN (23). These results raise the possibility that polysynaptic signals from the PVN or other leptin-responsive neurons may be conveyed to the TMN.

Additional functional evidence of histamine's interaction with leptin-induced signals was provided by studies on leptin-induced feeding suppression in the presence or absence of FMH. FMH is a suicide inhibitor of HDC, which depletes hypothalamic neuronal histamine almost completely (24). Depletion of neuronal histamine by intraperitoneal or i3vt administration of FMH attenuated leptin-induced feeding suppression by ~50%. Systemic administration of FMH depletes neuronal histamine not only in the hypothalamus but also throughout the brain and peripheral organs. The fact that its effect and magnitude were mimicked by i3vt infusion of FMH strongly suggests that it is the hypothalamic neuronal histamine that plays an essential role in leptin-induced feeding suppression. The 50% decrease in leptin's actions following histamine depletion indicates that hypothalamic neuronal histamine is involved in about half of leptin's effects on ingestion, presumably acting via H_1 receptors in the VMH and the PVN, both known satiety centers (8).

In our previous studies, low levels of brain histamine concentrations and HDC activity have been shown to be present in *fa/fa* rats (8,9), which have dysfunctional leptin receptors (14). In the present study, concentrations of histamine and *t*-MH in the hypothalamus were significantly lower in the *db/db* mice, another leptin receptor–defective animal (14,25), compared with their lean littermates. In contrast, concentrations of both amines were elevated in DIO rats. Serum leptin level has been shown to increase in *fa/fa* rats and *db/db* mice (26,27). The present study confirmed that serum leptin level also increased in DIO rats in response to their fat accumulation. Although increases in serum leptin level are similarly observed in the three obese animal models, its signal transduction in the central nervous system is impaired in leptin receptor–defective animals but not in DIO rats. DIO animals have been shown to be resistant to exogenous administration of leptin (28). The development of leptin resistance is well known to depend on length of dietary treatment, amount of calories in the load, and animal species (28). Compared with the high-fat diet usually used for production of DIO animals, the use of sucrose to enhance intake led to an obesity that was rather mild and that progressed slowly (K.H., H.Y., M.K., T.S., unpublished observations). In addition, hypothalamic NPY mRNA expression, which was overexpressed in *ob/ob* as well as *db/db* mice (3) and *fa/fa* rats (29) owing to a deficiency of leptin action, showed no remarkable change in present DIO rats compared with controls (K.H., H.Y., S.H., M.K., T.S., unpublished observations). This result is consistent with the data suggesting that leptin resistance may not be developed sufficiently within a 20-week load of sucrose, because hypothalamic amine levels responded normally to hypersecretion of leptin in the present DIO animals. In view of this, the lower activity of hypothalamic histamine in *fa/fa* rats and *db/db* mice does not arise from species specificity or obesity per se, but rather from leptin receptor abnormalities. The concept is supported by the present study on leptin-deficient *ob/ob* mice (1,2). Histamine turnover in *ob/ob* mice was lower than in their lean littermates. Further, administration of leptin restored this lowered histamine turnover rate of *ob/ob* mice to the normal control level observed in lean littermates. These findings confirm that the disruption of leptin-histamine signal transduction in *ob/ob* mice depends on their leptin deficiency.

The results so far show that neuronal histamine plays an important role in regulation of a sleep/wake cycle. Direct neuronal projection from the ventrolateral preoptic neuron, which is specifically activated during sleep, to the TMN has been shown to be mainly involved in this regulation (23,30). The findings raise the possibility that deficit in hypothalamic neuronal histamine observed in genetic obese animals may cause disruption of a sleep/wake cycle, behavioral circadian rhythms, or both. Indeed, circadian rhythms including feeding, drinking, and ambulatory behavior were found to be disrupted in *fa/fa* rats (31) as well as histamine-depleted rats treated chronically with FMH (32). Disruption of a light-dark cycle in feeding behavior was ascertained in *ob/ob* and *db/db* mice (M.K., H.Y., T.S., unpublished observations). There is the possibility that depletion of histamine per se produced nonspecific effects on food intake by influencing the arousal system. However, sustained histamine depletion induced by

chronic infusion of FMH, not the acute depletion of histamine by bolus infusion of FMH as used in the present study, is necessary to produce changes in the circadian rhythm of feeding (32). In addition, FMH per se did not affect 24-h cumulative food intake as shown in the present study. These results indicate that a change of arousal level does not influence food intake, at least in the acute phase of histamine depletion.

Histaminergic projections to the PVN, VMH, and arcuate nucleus (33) indicate that neuronal histamine may influence neurohumoral networks via CRH, NPY, and POMC neurons. In fact, histamine has been shown to produce excitatory effects on the activity of CRH neurons (34) and to induce the release of α-melanocyte–stimulating hormone, a POMC-derived peptide (35). By influencing this neuronal network, histamine may also contribute to leptin's effects on feeding behavior and peripheral metabolism.

In summary, we have demonstrated that hypothalamic histamine has a role in the central signaling pathway that is involved in leptin's regulation of feeding behavior. Alterations in the activity of histaminergic neurons in genetically obese animal models, presumably caused by the impairment of centrally mediated leptin signal transduction, could contribute to the development of abnormal feeding behavior and lipid metabolism, which are known to induce obesity in these animals.

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