Hypothalamic Neuronal Histamine in Genetically Obese Animals: Its Implication of Leptin Action in the Brain

EMI ITATEYAMA, SEIICHI CHIBA, TOSHIIE SAKATA, AND HIRONOBU YOSHIMATSU[1]

Department of Internal Medicine I, School of Medicine, Oita Medical University, Hasama, Oita 879-5593, Japan

Leptin regulates feeding behavior and energy metabolism by affecting hypothalamic neuromodulators. The present study was designed to examine hypothalamic neuronal histamine, a recently identified mediator of leptin signaling in the brain, in genetic obese animals. Concentrations of hypothalamic histamine and *tele*-methylhistamine (*t*-MH), a major histamine metabolite, were significantly lower in obese (*ob/ob*) and diabetic (*db/db*) mice, and Zucker fatty (*fa/fa*) rats, leptin-deficient and leptin-receptor defective animals, respectively, relative to lean littermates ($P < 0.05$ for each). A bolus infusion of leptin (1.0 μg) into the lateral ventricle (ilvt) significantly elevated the turnover rate of hypothalamic neuronal histamine, as assessed by pargyline-induced accumulation of *t*-MH, in *ob/ob* mice compared with phosphate-buffered saline (PBS) infusions ($P < 0.05$). However, this same treatment did not affect hypothalamic histamine turnover in *db/db* mice. In agouti yellow (A^y/a) mice, animals defective in pro-opiomelanocortin (POMC) signaling, normal levels of histamine, and *t*-MH were seen in the hypothalamus at 4 weeks of age when obesity had not yet developed. These amine levels in A^y/a mice showed no change until 16 weeks of age, although the mice were remarkably obese by this time. Infusions of corticotropin releasing hormone (CRH), one of neuropeptide related to leptin signaling, into the third ventricle (i3vt) increased histamine turnover in the hypothalamus of Wistar King A rats ($P < 0.05$ versus PBS infusion). Infusion of neuropeptide Y (NPY) or α-melanocyte stimulating hormone (MSH), a POMC-derived peptide failed to increase histamine turnover. These results indicate that lowered activity of hypothalamic neuronal histamine in *ob/ob* and *db/db* mice, and *fa/fa* rats may be due to insufficiency of leptin action in the brains of these animals. These results also suggest that disruption of POMC signaling in A^y/a mice may not impact on neuronal histamine. Moreover, CRH but neither POMC-derived peptide nor NPY may act as a signal to neuronal histamine downstream of the leptin signaling pathway. Exp Biol Med 228:1132–1137, 2003

Key words: leptin; neuronal histamine; obesity; corticotropin releasing hormone

Leptin, the ob gene (*ob*) product, has been demonstrated to act as a fat tissue-derived hormone to regulate appetite and energy metabolism (1–3). Peripheral injections of recombinant leptin have been shown to reduce food intake, body weight, and adiposity of *ob/ob* mice (1–3). Leptin is also active when administered centrally (3) indicating that the brain is a major target for the leptin action. In the hypothalamus, leptin receptors have been localized to the ventromedial hypothalamic nucleus (VMH), the dorsomedial hypothalamic nucleus (DMH), the paraventricular nucleus (PVN), the arcuate nucleus (ARC), and the ventral premammillary nucleus (PMV) (4). These nuclei, excepting the PMV, have been implicated in the regulation of energy balance. In particular, corticotropin releasing hormone (CRH) in the PVN, neuropeptide Y (NPY), and pro-opiomelanocortin (POMC)-derived peptide in ARC have been shown to be involved in the leptin signaling processes that regulate feeding behavior (5, 6). In genetically obese animals, abnormal expression of these feeding-related peptides, caused by disruption of leptin signaling, has been shown to induce obesity and hyperphagia. For example, hypothalamic NPY mRNA expression was elevated in *ob/ob* as well as diabetes (*db/db*) mice and Zucker fatty (*fa/fa*) rats (7, 8).

Histamine-positive cell bodies are present in the tuberomammillary nucleus (TMN) in the posterior hypothalamus, which has been shown to be involved in a variety of physiologic functions. These functions include: central control of neuroendocrine responses, cardiovascular regulation, emesis and motion sickness, control of the sleep-wake cycle, and thermoregulation (9–12). Our previous work has demonstrated the importance of hypothalamic neuronal histamine in the regulation of feeding behavior and energy metabolism. Neuronal histamine has been shown to suppress food intake via H_1 receptor in the VMH and the PVN (13). In addition, an increase in hypothalamic histamine levels has been demonstrated to raise peripheral glucose concentrations (14, 15), accelerate lipolysis in adipose tissue (16, 17), and increase mRNA expression of uncoupling protein (UCP) in peripheral tissue (18). Together, these data suggest that the histaminergic neurons of the hypothalamus play an

[1] To whom requests for reprints should be addressed at Department of Internal Medicine I, School of Medical Oita Medical University Idaigaoka, Hasama, Oita 879-5593 Japan. E-mail: hirohy@oita-med.ac.jp

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important role in the regulation of energy intake and expenditure as well as adiposity. Moreover we have determined that histaminergic neurons play an important role in leptin signaling in the hypothalamus, which controls feeding behavior (19). Central administration of leptin increased histamine turnover, as assessed by accumulation of *tele*-methylhistamine (*t*-MH), a major metabolite of neuronal histamine. Leptin-induced suppression of feeding was attenuated in histamine-depleted rats using α-fluoromethylhistidine (FMH), a suicide inhibitor of the histamine-synthesizing enzyme, histidine decarboxylase (HDC), and in histamine H_1 receptor knockout mice (19, 20).

In the present study, we aimed to clarify the change in hypothalamic neuronal histamine in genetic obese animals known to have abnormal leptin signaling due to leptin deficiency, leptin receptor abnormality, or a defect in POMC signaling, one of the important downstream pathways of leptin action.

Materials and Methods

Animals and Diet. The following animals were used: 12-week old male C57BL/6J *ob/ob* mice weighing 40 ± 2 g and their lean littermates (+/+) weighing 20 ± 1 g, 12-week old male C57BL/KsJ *db/db* mice weighing 40 ± 2 g and their lean littermates (+m/+m) weighing 20 ± 1 g, 12-week old male *fa/fa* rats weighing 664 ± 12 g and their lean littermates (+/+) weighing 450 ± 11g, 4- and 16-week male C57BL/6J agouti yellow (A^y/a) obese mice weighing 16 ± 1 g, 38 ± 1 g and their lean littermates (a/a) weighing 14 ± 1 g, 27 ± 0.5 g, respectively, and Wistar King A (WKA) rats weighing 333.4 ± 4.3 g. Animals were housed in a sound-proof room illuminated daily from 0700 h to 1900 h (a 12:12 h light-dark circle) and maintained at 21 ± 1°C with humidity at 55 ± 5%. They were allowed free access to standard solid rodent chow and tap water. Body weight was measured weekly in A^y/a mice and their lean littermates. 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*.

Reagents. Pargyline hydrochloride (Sigma, St. Louis, MO), an inhibitor of monoamine oxidase B, was dissolved in phosphate-buffered saline (PBS) to a concentration of 9.9 mM; recombinant murine leptin (Pepro Tech EC Ltd., London, England) was dissolved in PBS at 1.0 μg /μl. Corticotropin releasing hormone (CRH), neuropeptide Y (NPY), or α-melanocyte stimulating hormone (MSH) was dissolved in PBS at 1.05 nM, 2.6 nM, and 6.0 nM, respectively. Each solution was freshly prepared on the day of its administration. The pH of each solution was adjusted to a range of 6.4 to 7.2.

Surgery. Under sodium pentobarbital anesthesia (1 mg/kg), a stainless steel cannula (29 ga for mice; 23 ga for rats) was chronically implanted into the lateral ventricle (ilvt) of *ob/ob* and *db/db* mice and into the third ventricle (i3vt) of WKA rats at least 1 week before the onset of infusions. A stainless steel wire stylet was inserted in the guide cannula to prevent leakage of the cerebrospinal fluid and to prevent obstruction of the cannula. Details of the surgical procedure have been described elsewhere (21).

Measurement of Histamine and its Turnover Rate. To assess histamine basal levels and turnover rate, both hypothalamic histamine and *t*-MH were assayed in genetically obese animals and lean littermates. Transmethylation of histamine into *t*-MH catabolyzed by histamine N-methyltransferase and subsequent deamination by monoamine oxidase B is the major metabolic breakdown pathway of histamine in the brain (11, 12, 22). Pretreatment with pargyline induces accumulation of *t*-MH in extraneuronal space as a major metabolite of released neuronal histamine (22). Assays of hypothalamic histamine and *t*-MH in *ob/ob* mice, *db/db* mice, *fa/fa* rats, A^y/a mice, and lean littermates were performed without pargyline pretreatment to estimate basal levels of both amines in each of the obese animal models. Using other *ob/ob* mice, *db/db* mice, and lean littermates, pargyline (80 mg/kg) or PBS pretreatment was given 10 min before infusion of the test solution to analyze the change in histamine turnover in these obese animals. Leptin (1 μg/mouse or the same volume of PBS) was infused ilvt of mice through implanted cannula at a rate of 0.1 μl/min for 10 min. WKA rats were also pretreated with pargyline (80 mg/kg) or PBS before i3vt infusion of CRH, NPY, and MSH at doses of 1.05 nmol, 2.6 nmol, and 6.0 nmol, respectively. These test solutions were infused i3vt through the injector (29 ga) at a rate of 1 μl/min for 10 min. The dose and the infusion speed were selected on the basis of previous results, including a dose-response relationship between doses of leptin or other neuropeptide and food intake. Histamine turnover was estimated from the accumulation of *t*-MH over a 70-min period after pargyline treatment. All animals were decapitated at 12:00 hr. The time was 60 min after the onset of the ilvt infusion for animals used for turnover assay. The hypothalamus was dissected on an ice plate according to the method of Glowinski and Inversen (23). 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.* (24). Homogenates of the brain were centrifuged at 1000 × g, and the clear deproteinized supernatants containing the amine extracts were assayed by high performance liquid chromatography. Details of the amine assays have been described elsewhere (25).

Blood Sampling. Using a different set of rats and mice than those used for high performance liquid chromatography (HPLC) assays, blood samples were collected from a chronically indwelling silicone catheter implanted in the right external jugular vein with its end at a point just outside of the atrium (26). Surgical catheterizations were performed under diethylether anesthesia. Blood samples were taken at 11:50 hr to 12:00 hr and immediately frozen at −20°C until humoral factor levels were measured.

Assays of Blood Humoral Factors. Serum insulin levels were quantitated using an insulin radioimmunoassay (RIA) kit (Rat insulin [^{125}I] assays system, Amer-

sham, Little Chalfont, UK). Serum glucose concentrations were measured with a commercially available kit (Merck-auto Glucose, Kant Chemical, Tokyo). Serum leptin concentrations were quantitated using an RIA kit (Linco, Inc., St. Louis, MO).

Statistical Analysis. All data were expressed as means ± SE. The statistical analyses were carried out using the unpaired Mann-Whitney U test.

Results

Basal Concentration of Hypothalamic Histamine and *t*-MH in *ob/ob*, *db/db* Mice, and *fa/fa* Rats. Figure 1 shows basal concentrations of histamine and *t*-MH without pargyline pretreatment in *ob/ob* mice. Concentrations of hypothalamic histamine and *t*-MH were lower in *ob/ob* mice compared with their lean littermates ($P < 0.05$ for each). Histamine and *t*-MH concentrations in *ob/ob* mice were decreased by 73.9 ± 5.6% and 44.3 ± 3.3%, respectively, relative to those in their lean littermates. Lowered levels of hypothalamic histamine and *t*-MH were also observed in *db/db* mice (Fig. 2A) and *fa/fa* rats (Fig. 2B). Histamine and *t*-MH concentrations were decreased by 58.4 ± 3.4% and 32.9 ± 10.7%, respectively, in *db/db* mice, and by 86.5 ± 5.1% and 71.2 ± 3.3%, respectively, in *fa/fa* rats (relative to relevant lean littermates; $P < 0.01$ for each).

Effects of ilvt Infusion on Hypothalamic Histamine Turnover in *ob/ob* and *db/db* Mice. Figure 3 shows leptin-induced change in histamine turnover rate in *ob/ob* and *db/db* mice. Ilvt infusion of leptin increased pargyline-induced accumulation of *t*-MH in *ob/ob* mice to 123.8 ± 6.6% of control PBS infusion ($P < 0.05$). Ilvt infusion of leptin induced no remarkable change in pargyline-induced accumulation of *t*-MH in *db/db* mice compared with the PBS infusion.

Basal Concentration of Hypothalamic Histamine and *t*-MH in A^y/a Mice. Figure 4 shows basal concentration of histamine and *t*-MH at 4- and 16-week-old A^y/a mice. Hypothalamic concentrations of histamine and *t*-MH showed no remarkable differences compared with those in lean littermates at these developmental time points in A^y/a mice.

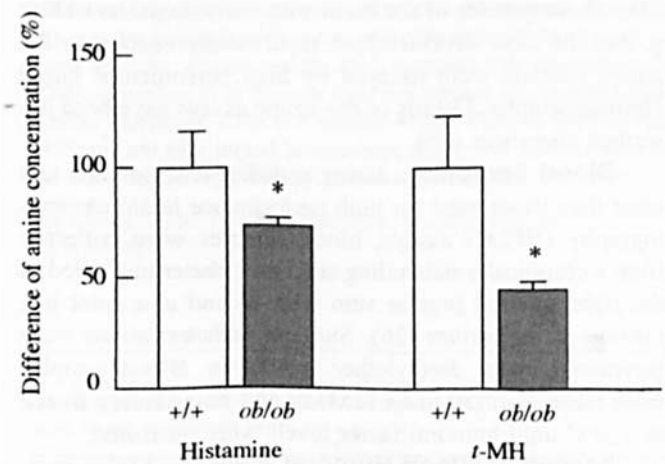

Figure 1. Percent difference in hypothalamic histamine and *t*-MH concentrations in *ob/ob* mice. Values are mean ± SE ($n = 7$ each for *ob/ob* mice and controls). *$P < 0.05$ compared with lean littermates.

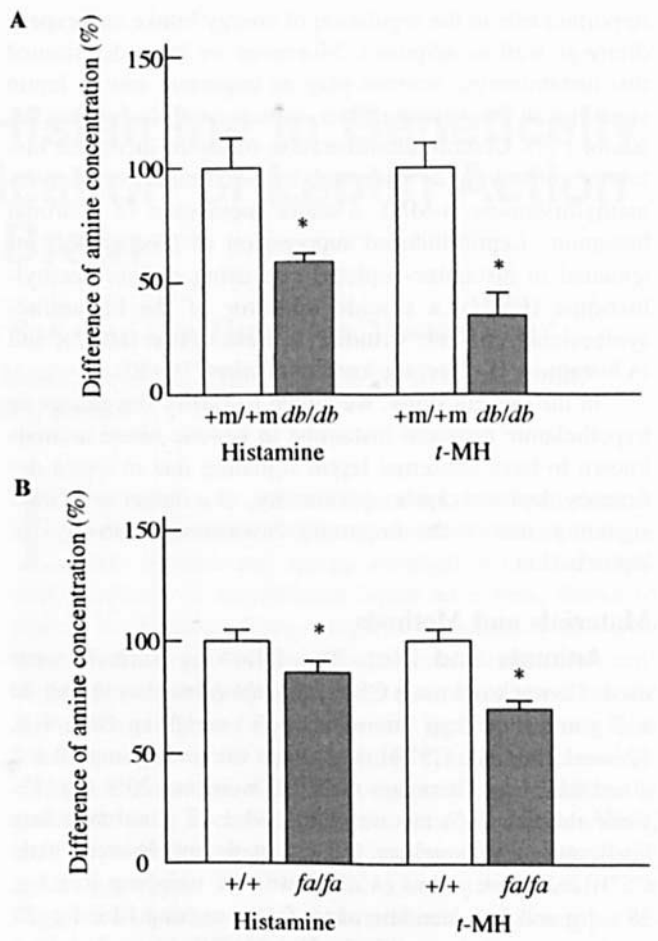

Figure 2. Percent differences in hypothalamic histamine and *t*-MH concentration in (A) *db/db* mice and (B) *fa/fa* rats. Values are mean ± SE ($n = 7$ each for *db/db* mice and controls; $n = 7$ each for *fa/fa* rats and controls). *$P < 0.01$ compared with lean littermates.

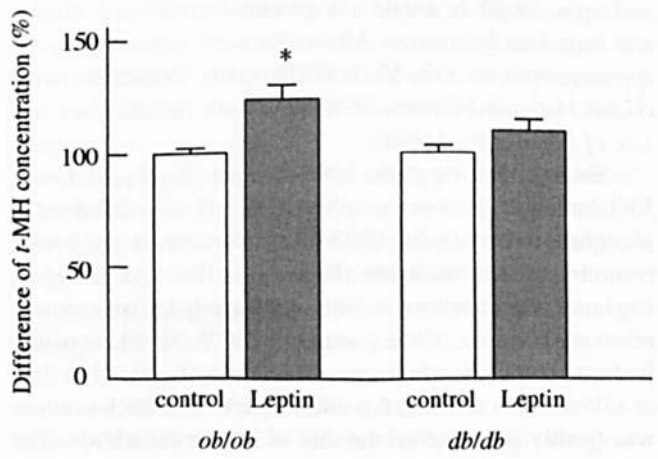

Figure 3. Effects of ilvt leptin infusion on pargyline-induced accumulation of *t*-MH in the hypothalamus of *ob/ob* mice and *db/db* mice. Values are mean ± SE ($n = 7$ each for leptin and controls). *$P < 0.05$ compared with PBS controls.

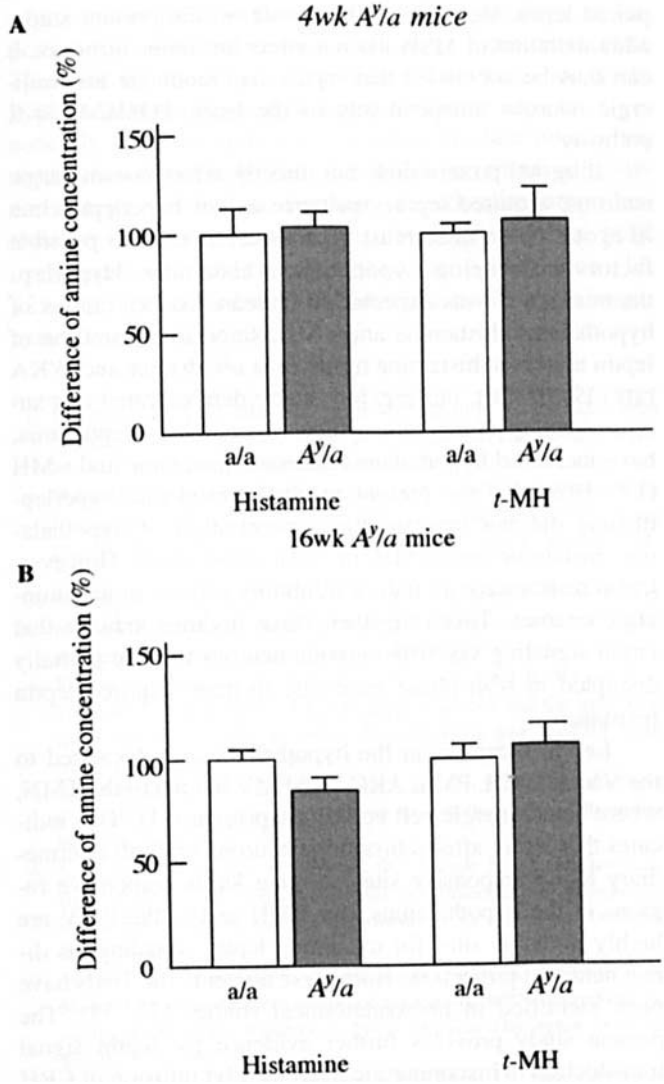

Figure 4. Percent difference in hypothalamic histamine and *t*-MH concentrations in (A) 4-week-old A^y/a mice and (B) 16-week-old A^y/a mice. Values are mean ± SE (n = 7 each for 4-week-old A^y/a mice and controls; n = 7 each for 16-week-old A^y/a mice and controls).

Table I. Body Weight, Serum Glucose, and Insulin Levels in Genetically Obese Animals

	Weight (g)	Glucose (mg/dl)	Insulin (μU/ml)
+/+	20 ± 1	125.3 ± 19.7	56.1 ± 7.5
ob/ob	40 ± 2[a]	349.5 ± 15.0[a]	425.0 ± 12.5[a]
+m/+m	20 ± 1	102.4 ± 8.5	44.1 ± 4.7
db/db	40 ± 2[a]	408.6 ± 22.1[a]	72.1 ± 9.0[a]
+/+	450 ± 11	107.8 ± 2.1	34.4 ± 4.0
fa/fa	664 ± 12[a]	107.3 ± 10.3[a]	838.0 ± 100.4[a]
a/a	27 ± 0.5	125.3 ± 19.7	56.1 ± 7.5
A^y/a (16 wks)	38 ± 1[a]	228.6 ± 22.1[a]	128.1 ± 9.8[a]

Note. Data are means ± SE. Body weight, serum glucose, and insulin significantly increased in genetically obese animals except glucose in *fa/fa* rats.
[a] $P < 0.05$ compared with each lean littermate.

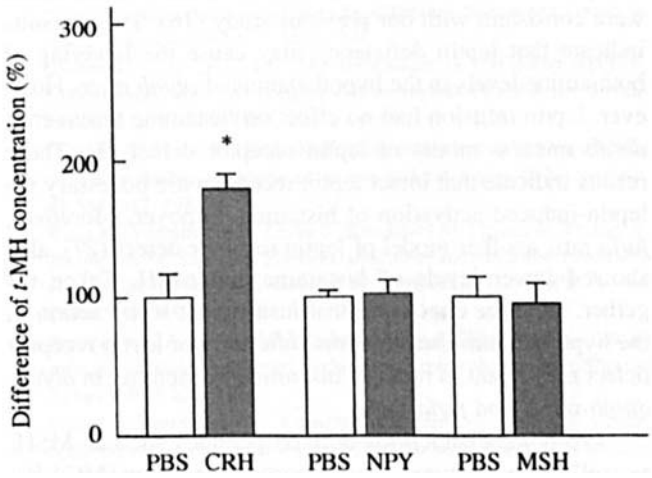

Figure 5. Effects of i3vt infusion of CRH, NPY, or MSH on pargyline-induced accumulation of *t*-MH in the hypothalamus of WKA rats. Values are mean ± SE (n = 5 each for CRH and controls; n = 5 each for NPY and controls; n = 5 each for MSH and controls). *$P < 0.001$ compared with PBS controls.

Body Weight, Serum Glucose, Insulin, and Leptin Levels in Obese Animal Models. Table I shows the body weight, serum glucose, and insulin levels in the obese animal models used in the present study. Hyperglycemia and hyperinsulinemia was observed in all obese animals, except *fa/fa* rats, which showed normal serum glucose levels.

Effects of i3vt Infusion of Feeding-Related Neuropeptides on Hypothalamic Histamine Turnover in WKA Rats. Figure 5 shows changes in histamine turnover rate in WKA rats in response to i3vt infusion of neuropeptides. I3vt infusion of CRH at a dose of 1.05 nmol/rat increased pargyline-induced accumulation of *t*-MH to 180.0 ± 9.4% relative to control PBS infusion ($P < 0.001$; Fig. 5). However, i3vt infusion of NPY at a dose of 2.6 nmol/rat or MSH at a dose of 6.0 nmol/rat did not affect pargyline-induced *t*MH accumulation in the hypothalamus.

Discussion

The present results demonstrate that hypothalamic histamine and its metabolite, *t*-MH, are decreased in genetically obese animals such as *ob/ob* and *db/db* mice, and *fa/fa* rats. Each animal model of obesity in the present study showed hyperglycemia and/or hyperinsulinemia. These abnormalities in serum glucose or insulin levels, however, are unlikely to underlie the abnormal histamine and *t*-MH concentrations in obese animals, as neither hyperglycemia nor insulin have been shown to affect histamine turnover in the hypothalamus (15). In fact, the hypothalamic concentrations of histamine and *t*-MH were not decreased in rats with sucrose loading diet-induced obesity (19), or in A^y/a mice (present study), both of which showed hyperglycemia and hyperinsulinemia. These results indicate that factors other than glucose and insulin may contribute to the change in hypothalamic histamine and its metabolite in genetically obese animals.

Recently, we identified hypothalamic neuronal histamine as a mediator of leptin action in the brain (19). Central administration of leptin has been shown to increase histamine turnover, as assessed by *t*-MH accumulation after pargyline treatment in WKA rats. It has also been observed that leptin-induced suppression of feeding is partially attenuated in histamine-depleted rats or in histamine H_1 receptor knockout mice (19, 20). We conclude from these results that leptin promotes the release of histamine, thereby suppressing food intake. The modulation of feeding behavior by neuronal histamine has been investigated in our previous studies, which have shown that neuronal histamine suppresses food intake through the H_1 receptor in the VMH and the PVN, two known satiety centers (13). In the present study, histamine and *t*-MH concentrations were lowered in *ob/ob* mice, a model of leptin deficiency, and ilvt infusion of leptin increased histamine turnover in these mice, which were consistent with our previous study (16). These results indicate that leptin deficiency may cause the lowering of both amine levels in the hypothalamus of *ob/ob* mice. However, leptin infusion had no effect on histamine turnover in *db/db* mice, a model of leptin receptor defect (3). These results indicate that intact leptin receptors are necessary for leptin-induced activation of histamine turnover. Moreover, *fa/fa* rats, another model of leptin receptor defect (27), also showed lower levels of histamine and *t*-MH. Taken together, it can be concluded that insufficient leptin action in the hypothalamus due to leptin deficiency or leptin receptor defect may result in reduced histaminergic activity in *ob/ob, db/db* mice, and *fa/fa* rats.

Pro-opiomelanocortin–derived peptides such as MSH, as well as its receptor, melanocortin 4-receptor (MC4-R), have received much attention in studies of the leptin signaling pathway, which controls feeding behavior. Leptin has been shown to increase POMC mRNA in the ARC (6), and administration of MSH or an MC4-R agonist induces intensive feeding suppression (28). In A^y/a mice, ectopic expression of agouti protein, which antagonizes MC4-R, induces obesity (29). These mice have been shown to be both hyperleptinemic and insensitive to exogenous leptin treatment, which suggests leptin resistance in this obese animal model (30). These findings indicate that leptin signaling is impaired by defect in the POMC signaling pathway in agouti mice. This led us to hypothesize that leptin's influence on histaminergic neurons may be disrupted in A^y/a mice. To clarify this possibility, we investigated changes in the amine levels using young non-obese, as well as obese A^y/a mice. Results showed that hypothalamic histamine and *t*-MH levels were normal in young non-obese A^y/a mice. It is well known that A^y/a mice show late onset of obesity and hyperleptinemia (29). Thus, it was not surprising that the amine levels were normal in these mice. However, the amine levels were also normal in obese A^y/a mice, which showed remarkable obesity and hyperleptinemia. These results indicate that defects in POMC signaling due to agouti protein do not affect histaminergic neurons, in spite of impaired leptin signaling. Furthermore, in the present study, administration of MSH did not affect histamine turnover. It can thus be concluded that leptin may modulate histaminergic neurons independently of the leptin-POMC-MC4-R pathway.

If agouti protein dose not directly affect histaminergic neurons, acquired leptin resistance and/or hyperleptinemia in agouti obese mice must be considered as other possible factors influencing hypothalamic histamine. Hyperleptinemia *per se* was expected to increase concentrations of hypothalamic histamine and *t*-MH, since administration of leptin increased histamine turnover in *ob/ob* mice and WKA rats (19). In fact, our previous study demonstrated that sucrose-loading obese rats, which showed hyperleptinemia, have increased hypothalamic neuronal histamine and *t*-MH (19). However, the present results showed that hyperleptinemia did not increase the concentration of hypothalamic histamine and *t*-MH in A^y/a obese mice. However, leptin resistance may induce inhibitory effects on histaminergic neurons. Taken together, these findings indicate that leptin signaling via histaminergic neurons may be partially disrupted in A^y/a obese mice due to their acquired leptin resistance.

Leptin receptors in the hypothalamus are localized to the VMH, DMH, PVN, ARC, and PMV but not to the TMN, where histaminergic cell bodies are present (31). This indicates that leptin affects histamine neurons through intermediary leptin responsive sites. Among leptin responsive regions in the hypothalamus, the DMH and/or the PMV are highly probable sites for mediating leptin signaling, as direct neuronal projections from these nuclei to the TMN have been identified in neuroanatomical studies (32, 33). The present study provides further evidence for leptin signal transduction in histaminergic neurons. I3vt infusion of CRH but not NPY or MSH increased histamine turnover in the present study. These three neuropeptides are identified as major targets of leptin action in the brain based on the fact that administration of leptin increases CRH or POMC mRNA expression and decreases NPY mRNA expression (5, 6, 34). The present study indicates that CRH is one of the mediators of leptin action that activates neuronal histamine. This hypothesis is supported by a retrograde tracer study demonstrating a projection from the PVN, site of CRH containing neurons, to the TMN (35). As described previously, neither defective POMC signaling in A^y/a obese mice, nor MSH administration affected neuronal histamine. These results indicate that leptin may affect histaminergic neurons via a pathway other than POMC-MC4-R. Therefore, CRH may be involved in a leptin-related but POMC-MC4-R independent pathway, which affects histaminergic neurons.

We recently demonstrated that hypothalamic neuronal histamine regulates adiposity and energy expenditure. In that study, we found that central administration of histamine or thioperamide, a histamine H_3 antagonist that activates endogenous histamine release, accelerated lipolysis in adipose tissue (17). Neuronal histamine also increased mRNA

expression of UCP in BAT and WAT, which plays an important role in thermogenesis and energy expenditure (18). These newly identified histaminergic functions led us to hypothesize that reduced neuronal histamine activity in genetically obese animals may contribute to their obesity via modulation of not only feeding but also peripheral adiposity and energy metabolism. Furthermore, other important histaminergic functions such as regulation of the sleep-wake cycle, neuroendocrine systems, and thermoregulation (11, 12) may also contribute to abnormalities observed in genetically obese animals.

1. Pellymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science **269:**540–543, 1995.
2. Halaas JL, Gjiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. Science **269:**543–546, 1995.
3. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science **269:**546–549, 1995.
4. Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Trayhurn P. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS Lett **387:**113–116, 1996.
5. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of Leptin action in rat hypothalamus. J Clin Invest **98:**1101–1106, 1996.
6. Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, Baskin DG. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes **46:**2119–2123, 1997.
7. Mizuno MT, Kleopoulos SP, Bergen HT, Roberts JL, Priest CA, Mobbs CV. Hypothalamic pro-opiomelanocortin mRNA is reduced by fasting in ob/ob and db/db mice, but is stimulated by leptin. Diabetes **47:**294–297, 1997.
8. Sanacora G, Kershaw M, Finkelstein JA, White JD. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology **127:**730–737, 1990.
9. Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H, Terano Y, Tohyama M, Wada H. Distribution of the histaminergic neuron system in the central nervous system of rats; A fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. Brain Res **295:**13–25, 1984.
10. Panula P, Yang H-YT, Costa E. Histamine-containing neurons in the rat hypothalamus. Neurobiology **81:**2572–2576, 1984.
11. Schwartz J-C, Arrang J-M, Garbarg M, Pollard H, Ruat M. Histaminergic transmission in the mammalian brain. Physiol. Rev. **71:**1–51, 1991.
12. Onodera K, Yamatodani A, Watanabe T, Wada H. Neuropharmacology of the histaminergic neuron system in the brain and its relationship with behavioral disorders. Prog Neurobiol **42:**685–702, 1994.
13. Sakata T, Yoshimatsu H. Homeostatic maintenance regulated by hypothalamic neuronal histamine. Meth Find Exp Clin Pharmacol **17:**51–56, 1995.
14. Sakata T, Yoshimatsu H. Hypothalamic neuronal histamine: implications of homeostatic maintenance in its control of energy metabolism. Nutrition **13:**403–411, 1997.
15. Yoshimatsu H, Machidori H, Doi T, Kurokawa M, Ookuma K, Kang M, Sakata T. Abnormalities in obese Zuckers: defective control of histaminergic functions. Physiol Behav **54:**487–491, 1992.
16. Bugajski J, Janusz Z. Lipolytic responses induced by intracerebroventricular administration of histamine in the rat. Agents Actions **11:**147–150, 1981.
17. Tsuda K, Yoshimatsu H, Niijima A, Chiba S, Okeda T, Sakata T. Hypothalamic histamine neurons activate lipolysis in rat adipose tissue. Exp Biol Med **227:**208–213, 2002.
18. Masaki T, Yoshimatsu H, Chiba S, Watanabe T, Sakata T. Central infusion of histamine reduces fat accumulation and up-regulates UCP family in leptin-resistant obese mice. Diabetes **50:**376–384, 2001.
19. Yoshimatsu H, Itateyama E, Kondou S, Tajima D, Himeno K, Hidaka S, Kurokawa K, Sakata T. Hypothalamic neuronal histamine as a target of Leptin in feeding behavior. Diabetes **48:**2286–2291, 1999.
20. Masaki T, Yoshimatsu H, Chiba S, Watanabe T, Sakata T. Targeted disruption of histamine H1 receptor attenuates regulatory effects of leptin on feeding, adiposity and UCP family in mice. Diabetes **50:**385–391, 2001.
21. Sakata T, Tsutsui K, Fukushima M, Arase K, Kita H, Ookuma Y. Feeding and hyperglycemia induced by 1.5-anhydroglucitol in the rat. Physiol Behav **27:**401–405, 1981.
22. Oohara A, Yoshimatsu H, Kurokawa M, Oishi R, Saeki K, Sakata T. Neuronal glucoprivation enhances hypothalamic histamine turnover in rats. J Neurochem **63:**677–682, 1994.
23. Glowinski J, Inversen LL. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H] dopa in various regions of the brain. J Neurochem **13:**655–669, 1966.
24. Oishi R, Nishibori M, Saeki K. Feeding-related circadian variation in tele-methylhistamine levels of mouse and rat brains. J Neurochem. **49:**541–547, 1987.
25. Kang M, Yoshimatsu H, Chiba S, Kurokawa M, Ogawa R, Tamari Y, Tatsukawa M, Sakata T. Hypothalamic neuronal histamine modulates physiological responses induced by interleukin-1β. Am J Physiol **269:**R1308–R1313, 1995.
26. Steffens AB. A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animals. Physiol Behav **4:**833–836, 1969.
27. Cusin I, Jeanrenaud FR, Krongrad AS, Jeanrenaud B. The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese fa/fa rats Reduced sensitivity compared with lean animals. Diabetes **45:**1446–1450, 1996.
28. Huang QH, Hruby VJ, Tatro JB. Role of central melanocortins in endotoxin-induced anorexia. Am J Physiol **276:**R864–R871, 1999.
29. Huszar D, Lynch CA, Huntress VF, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell **88:**131–141, 1997.
30. Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci USA **94:**8878–8883, 1997.
31. Elmquist JK, Bjrbk C, Ahima RS, Flier JS, Saper CB. Distribution of leptin receptor mRNA isoforms in the rat brain. J Comp Neurol **395:**535–547, 1998.
32. Thompson RH, Canteras NS, Swanson LW. Organization of projections from the dorsomedial nucleus of the hypothalamus: A PHA-L study in the rat. J Comp Neurol **376:**143–173, 1996.
33. Canteras NS, Simerly RB, Swanson LW. Projections of the ventral premammillary nucleus. J Comp Neurol **324:**195–212, 1992.
34. Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Halung HM, Krianclunas A, Mackellar W, Rostack PR Jr, Schoner B, Smith D, Tiasley FC, Zhang X, Helman M. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature **377:**530–532, 1995.
35. Ericson H, Blomqvist A, Kohler C. Origin of neuronal inputs to the region of the tuberomammillary nucleus of the rat brain. J Comp Neurol **311:**45–64, 1991.