Hypothalamic Histamine Neurons Activate Lipolysis in Rat Adipose Tissue

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The contribution of hypothalamic histamine neurons to the central regulation of peripheral lipid metabolism was investigated in rats using *in vivo* microdialysis system. A bolus infusion of L-histamine at doses of 10–10^3 nmol/rat into the third cerebral ventricle (I3vt) dose-dependently increased glycerol concentration in the perfusate from the epididymal adipose tissue. I3vt infusion of 10^2 nmol/rat thioperamide, an autoinhibitory H_3 receptor antagonist that activates histamine neurons to increase synthesis and release of neuronal histamine, convincingly mimicked histamine action in the augmented lipolysis. Intraperitoneal pretreatment with propranolol, a β-adrenoceptor antagonist, abolished the thioperamide-induced lipolytic action. An electrophysiological study demonstrated that efferent sympathetic nerves innervating the epididymal fat were activated after the I3vt infusion of thioperamide. Hypothalamic histamine neurons thus regulate peripheral lipid metabolism through the accelerating lipolytic action by activation of sympathetic β-adrenoceptor. [Exp Biol Med Vol. 227(3):208–213, 2002]

Key words: hypothalamic histamine; *in vivo* microdialysis; lipolysis; epididymal adipose tissue; sympathetic nerve activity

Energy expenditure is the consequence of myriad metabolic events at the cellular level. These events consist of those energy-consuming processes that are related to the support of cellular functions, physical work, and exercise on the one hand and those that exist exclusively to regulate energy balance, including adaptive thermogenesis, on the other. In mammals, the maintenance of energy stores in the form of triglyceride is of adaptive value, particularly in times of food scarcity. The lipolytic process in the adipose tissue is controlled by neural and humoral factors (1). A recent study using a viral trans-synaptic retrograde tract tracer has demonstrated that white adipose tissue (WAT) is innervated by the sympathetic nervous system (2). Several hypothalamic nuclei such as the medial preoptic area (MPOA) and the paraventricular nucleus (PVN) are identified as origins of efferent sympathetic nerve projection to the WAT (2). Cold exposure accelerated norepinephrine turnover in the WAT and increased the circulating concentration of free fatty acid (FFA) (3). Electrophysiological studies have demonstrated that intravenous infusion of glucose decreased sympathetic nerve activity in WAT, whereas 2-deoxy-D-glucose increased this activity (4). The foregoing anatomical and physiological evidence strengthens the hypothesis that the sympathetic nervous system and its originating upstream brain structures may regulate lipolytic activity in the WAT. There has been little direct evidence for central control of WAT lipolysis mediated by sympathetic nerve activation, although central effects on mobilization of circulating FFA have been reported (5, 6).

In previous studies on the brain functions of histamine neurons, hypothalamic neuronal histamine has been identified to suppress food intake through the histamine H_1 receptor in the ventromedial hypothalamic nucleus and the PVN, both of which are known as the essential nuclei of neural circuit regulating food intake (7–9). In addition, hypothalamic histamine neurons are shown to modulate peripheral glucose metabolism by causing catecholamine secretion from the adrenal medulla (10, 11). Central administration of histamine increased the concentration of circulating catecholamines (12). Histamine neurons were, on the other hand, activated by neuroglucoprivation such as starvation and insulin-induced hypoglycemia (13, 14), and by interleukin 1-β, a pyrogenic cytokine (15). These various effects of central histamine neurons led us to assume that histamine neurons may be critically involved in the activation of sympathetic nervous system for the purpose of maintaining energy metabolism.

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In recent progress of histamine research, it has been found that histamine neurons play a novel role as a target of leptin in the hypothalamus (16, 17). Central infusion of leptin accelerated sympathetic nerve activity (18), energy expenditure as assessed by oxygen consumption (19), and gene expression of uncoupling protein (UCP) 1 in brown adipose tissue (BAT) (17, 20), and UCP 2 and UCP3 in WAT (17, 21). Central administration of leptin elevated histamine turnover in the hypothalamus (16), which in turn inhibited *ob* gene expression in adipose tissue (22, 23). A negative feedback loop has thus been found between the function of histamine neurons and *ob* gene expression (16, 22, 23). Leptin-induced feeding suppression and upregulation of BAT UCP 1 mRNA were both attenuated in mice with targeted disruption of the histamine H_1 receptor (17). Based on these findings, a functional connection is suggested between hypothalamic neuronal histamine and leptin's effect on peripheral metabolism. However, much remains to be clarified about the functional roles of histamine neurons in regulation of energy metabolism, particularly as a downstream regulator of leptin's signals within the hypothalamus (24–26). The principal goal of the present study is to assess first, the effect of hypothalamic histamine neurons activity on lipolysis in the adipose tissue; and second, its modulation through sympathetic nerve activity.

Materials andMethods

Animals and Diet. Mature male Wistar King A rats weighing 280–320 g were used. They were housed in a room illuminated daily from 0800 hr to 2000 hr (a 12:12-hr light:dark cycle) and were maintained at 21°C ± 1°C with humidity at 55% ± 5%. They were allowed free access to pellet rodent chow (No. CE-2, Clea Japan Ltd., Tokyo, Japan) and tap water. 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. L-Histamine (Sigma, St. Louis, MO) thioperamide, a histamine H_3 antagonist (gifts from J.C. Schwartz, INSERM, France, and from Eisai Pharmaceutical Co., Tokyo, Japan), and propranolol, a β-adrenoceptor antagonist (Sigma) were all dissolved in phosphate-buffered saline (PBS) at concentrations of 10^{-3}–10^{-1} M, 10^{-2} M, and 0.056 M, respectively. Each solution was freshly prepared on the day of its administration. The pH of each solution was adjusted ranging from 6.4 to 7.2.

Surgery. Under sodium pentobarbital anesthesia (45 mg/kg, i.p.), the rats were fixed in a stereotaxic apparatus (Narishige Co., Japan) so that a stainless steel guide cannula (23 gauge) could be chronically implanted into the third cerebral ventricle (i3vt) at least 1 week before infusion of test solution. A stainless steel wire stylet (29 gauge) was left in the guide cannula to prevent the leakage of cerebrospinal fluid as well as obstruction of the cannula.

Under sodium pentobarbital anesthesia (45 mg/kg, i.p.), a small incision was made on the inguinal skin, and a stainless steel guide cannula (18 gauge) was implanted into the epididymal WAT 1 hr before starting of microdialysis. A probe was inserted and fixed in the epididymal WAT through the incision with aid of a guide cannula. Details of the surgical procedures are described elsewhere (27, 28).

***In Vivo* Microdialysis to Assess Lipolytic Activity.** Microdialysis study modified from the method of Tossman and Ungerstedt (29) was performed under sodium pentobarbital anesthesia (45 mg/kg, i.p.). A dialysis tube (0.5 mm long; dialysis with a molecular cut-off of 50 kDa molecular weight) surrounding a double-lumen microinjection stainless steel cannula (Eicom Co., Tokyo, Japan) was used. The perfusion solvent entered the probe through the inner cannula and passed down to a tip of the probe. Thereafter, it streamed upwards in the space between the inner cannula and the outer dialysis membrane. The perfusate left the probe through the outer cannula via a sidearm from which it was collected. During dialysis, the probe was connected to a microinjection pump (Eicom Co). When a 40-min equilibrium period had elapsed after the probe implantation, *in vivo* microdialysis study was started.

Matched on the basis of body weight, each testing group consisted of five rats. During perfusion with PBS in the adipose tissue, each testing rat was perfused with 10–10^3 nmol/rat histamine, 100 nmol/rat thioperamide, or control solution of PBS through an i3vt cannula at a speed of 1 μl/min for 10 min. A blocker study was performed by pretreatment with 0.017 mmol/rat propranolol (i.p.) before the i3vt infusion of thioperamide. Each dialysate was collected for glycerol assays through an outlet of polyethylene probe at every 15-min interval for 60 min before and 90 min after the i3vt infusion of histamine or thioperamide. A perfusate of 20 μl was employed for analysis of glycerol. An automated luminescence analyzer (Lumat LB9501; Berthold Co., Wildbad, Germany) was used for the glycerol assay (30, 31). To characterize the dialysis probe based on assessment by its recovery and to perform calibration, *in vitro* glycerol concentration was assayed in dialysate from probes placed in saline solution together with different glycerol concentrations up to 100 μM every 25 μM concentration interval. Test samples from *in vivo* dialysate were assayed according to the *in vitro* calibration standard (32).

Recording of Sympathetic Nerve Activity. An electrophysiological study was carried out under urethane anesthesia (1.0 g/kg). Additional subcutaneous injections ensured continued stability of anesthesia for at least 90 min. The rectal temperature was kept at 37°C ± 0.5°C by an automatically controlled electric blanket placed underneath the animals. After tracheostomy, the left epididymal adipose tissue was exposed through a left inguinal incision. Using a dissecting microscope, nerve filaments were isolated from the sympathetic nerves innervating the left epididymal adipose tissue. Efferent discharges were recorded from fine filaments of sympathetic nerve fibers that were dissected free from connective tissue. To differentiate efferent from afferent nerve activity, the nerve was cut at the distal end

adjacent to the epididymis so that afferent nerve activity could not be recorded by mistake. Nerve activity was detected by a pair of silver wire electrodes that were immersed in a mixture of liquid paraffin and white petroleum jelly to prevent dehydration. The action potential was amplified by means of a conventional differential amplifier and was filtered at low- and high-frequency cutoffs. The nerve signal was distinguished from background noise using window discriminator that enabled selection of action potentials right above a background threshold voltage level. All the nerve activities were analyzed based on the values obtained after conversion of raw data to standard pulses using an analogue-digital converter. Impulses were integrated by a rate meter with a reset time of 5 sec and they were recorded by a pen recorder. Succeeding to determination of the background firing rate of sympathetic nerves, changes in the nerve activity following a bolus i3vt infusion with thioperamide or PBS was measured up to a maximum of 90 min (n = 5 for each). Calculation of the nerve activity was carried out at 15 min, immediately before, and 30, 60, and 90 min after the thioperamide infusion. Each value after the infusion was expressed as the percentage of difference from 0 initial value. Details of the recording of nerve activity have been described elsewhere (4, 33, 34).

Statistical Analysis. Statistical analyses of microdialysis and electrophysiological studies were based on Student's t test and analysis of variance (ANOVA) for repeated measures followed by Scheffe's *post hoc* test. Evaluation of dose responsiveness was carried out by a single linear regression and ANOVA.

Results

Effects of i3vt Infusion of Histamine and Thioperamide on Lipolysis. Peak concentrations of glycerol for 30 min after i3vt infusion of histamine and thioperamide are shown in Figure 1. The histamine infusion at doses of 10–10^3 nmol increased glycerol concentration more potently than the appropriate PBS infusion ($P < 0.05$ for each) in a dose-dependent manner ($y = 33.267 + 0.001x$, $r = 0.826$, $P < 0.01$). Akin to histamine infusion, glycerol concentration was increased to reach its peak at 30 min after 100 nmol thioperamide infusion, and the elevation was significantly greater than that after PBS ($P < 0.05$; Fig. 1). The magnitude of thioperamide-induced lipolysis was confirmed to be equivalent to that of histamine-induced lipolysis at the same dose of 100 nmol. Figure 2 shows time-course changes in glycerol concentration in the perfusate of the epididymal adipose tissue following i3vt infusion of 100 nmol thioperamide. Glycerol concentration was elevated more predominantly following thioperamide infusion than PBS infusion ($P < 0.05$). The accelerating effect of thioperamide on lipolysis peaked at 30 min after the i3vt infusion ($P < 0.05$) and returned to the PBS control value at 60 min. The effect of propranolol on the thioperamide-induced lipolysis is illustrated in Figure 3. Pretreatment with 0.017 nmol propranolol showed no effect on glycerol concentration. The accelerating effect of i3vt thioperamide infusion on lipolysis was again confirmed, but this effect was largely abolished by pretreatment with propranolol injection ($P < 0.05$ vs thioperamide infusion pretreated with PBS; $P > 0.1$ vs PBS infusion pretreated with propranolol).

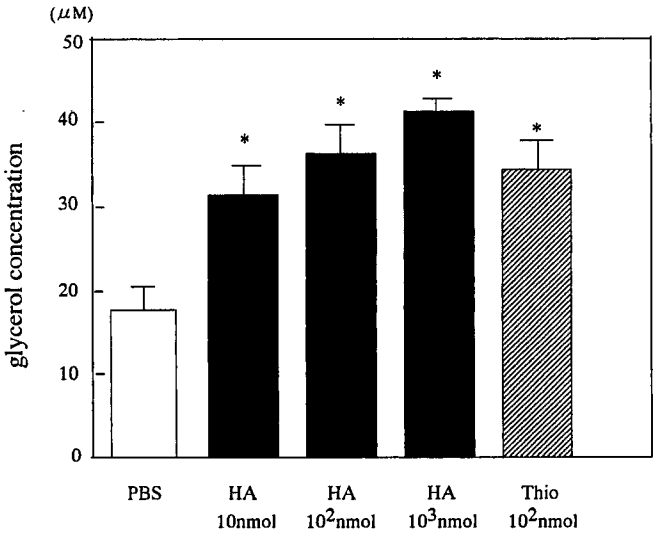

Figure 1. Effects of infusion of histamine (HA), thioperamide (Thio), or PBS in this and the succeeding figures into i3vt on glycerol concentration in the perfusates of the epididymal adipose tissue. Values and vertical bars, means ± SEM of five samples. *$P < 0.05$ vs PBS controls.

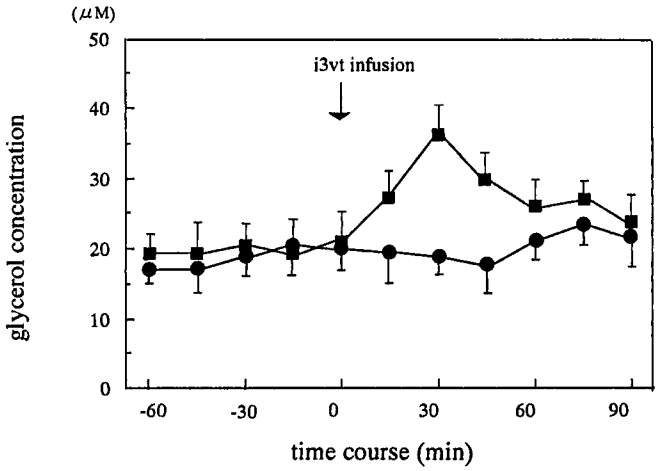

Figure 2. Time-course changes in glycerol concentration in adipose tissue perfusates after infusion of thioperamide or PBS into i3vt. Of note, thioperamide elevated outflow of glycerol into the perfusate. The arrow marks the infusion of test solutions. Values and vertical bars, means ± SEM of five samples. ■–■, the thioperamide group. ●–●, the PBS controls. $P < 0.05$ vs the PBS controls.

Effect of Thioperamide on Sympathetic Activity. Changes in activity of the sympathetic efferent nerves innervating the left epididymal adipose tissue following i3vt infusion of thioperamide are shown in Figure 4. Figure 4A illustrates one typical recording of the efferent nerve activity out of five samples. Following i3vt infusion of 100 nmol thioperamide, sympathetic efferent nerve activity showed a sustained increase throughout the infusion. The percentage

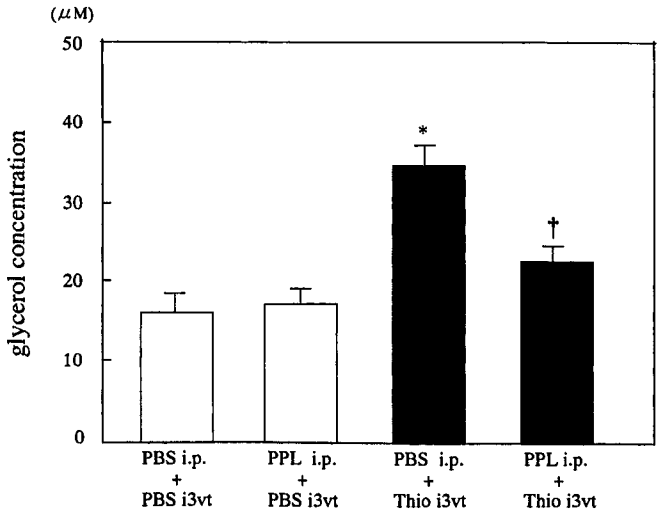

Figure 3. Antagonizing effect of pretreatment with propranolol into the peritoneum (i.p.) on lipolytic activity in the adipose tissue after infusion of thioperamide (Thio) into i3vt. Propranolol (PPL), a β-receptor antagonist, or PBS was injected i.p. 30 min before i3vt infusion of thioperamide or PBS. Glycerol concentration 30 min after the infusion of thioperamide was determined and plotted. Values and vertical bars, means ± SEM of five samples. *$P < 0.05$ vs the corresponding PBS controls. *$P < 0.05$ vs the i3vt thioperamide infusion group pretreated i.p. with PBS.

of change from the baseline value of the nerve activity after the infusion is shown in Figure 4B. The mean discharge rate was 78.5 ± 7.3, 76.8 ± 4.1, 116.3 ± 7.6, 121.2 ± 13.0, and 151.0 ± 11.7 impulses/5 sec at 15 min before, and 0 (immediately before), 30, 60, and 90 min after the infusion, respectively. PBS infusion did not show any remarkable change in sympathetic nerve activity. There was a significant difference in response of sympathetic nerve activity to thioperamide infusion compared with PBS infusion ($P < 0.001$).

Discussion

The present study has demonstrated that hypothalamic neuronal histamine reduces fat deposition in the adipose tissue by accelerating lipolytic activity. The triglyceride in adipose tissue is metabolized into fatty acids and glycerol in response to lipolytic substances such as adrenal catecholamine, glucagon, and so on. Glycerol released from the adipocytes is transferred into the circulation without reuptake by adipose tissue. For this reason, blood concentration of glycerol is a sensitive parameter of lipolytic activity. To directly assess the lipolytic activity in adipose tissue, we adopted an *in vivo* microdialysis system that enabled us to measure net changes of *in situ* glycerol concentration in adipose tissue, although the direct effect of local blood flow on the area using the probe was not entirely excluded in the present study. A recent neuroanatomical study has demonstrated that the WAT is innervated directly by sympathetic nerves, which originate from the spinal cord, the brain stem, and the hypothalamus (2). Cold exposure is shown to elevate both turnover rate of WAT norepinephrine and the concentration of circulating FFA (3). These findings implicate involvement of sympathetic nerve activity in lipolytic process of adipocytes.

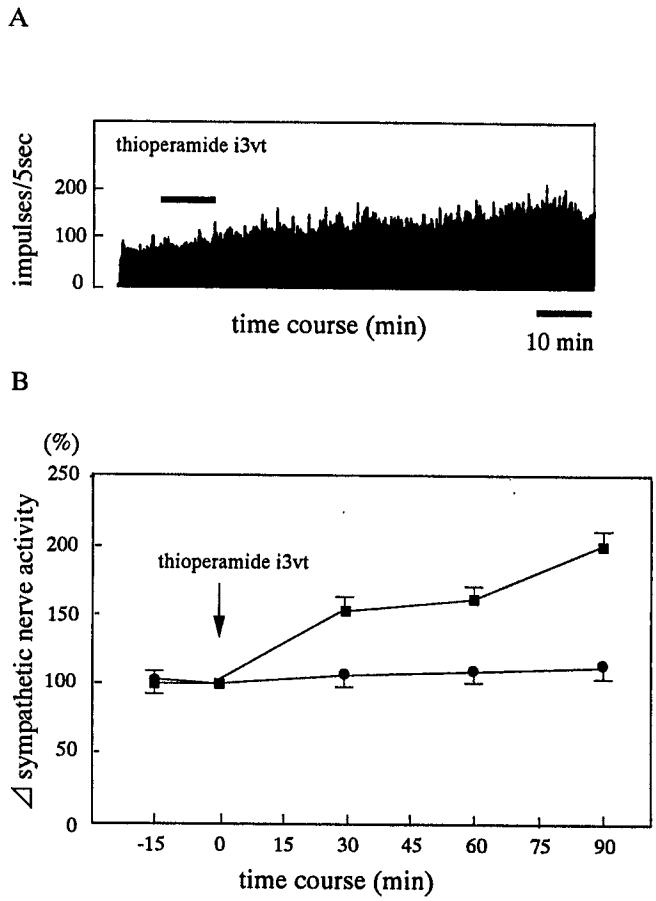

Figure 4. The effect of infusion of thioperamide into i3vt on activity of efferent sympathetic nerve innervating the left epididymal adipose tissue. (A) Changes in discharge of sympathetic nerve after i3vt infusion of thioperamide. Vertical axis, nerve impulses per 5 sec. Horizontal bars, a scale for 10 min. A bold horizontal bar, i3vt infusion of 10^2 nmol/rat thioperamide at a speed of 10 μl/10 min. (B) Percentage of difference in sympathetic nerve activity from baseline value (100%) after i3vt infusion of 10^2 nmol/rat thioperamide at a speed of 10 μl/10 min or infusion of PBS at the same volume with thioperamide. An arrow, infusion of test solutions. Values and vertical bars, means ± SEM of five samples. ■–■, the thioperamide group. ●–●, the PBS controls. $P < 0.001$ vs the PBS controls.

I3vt infusion of histamine increased glycerol concentration in the perfusate in a dose-dependent manner. As an alternate way to test a role of histamine in lipolytic generation in the adipose tissues, thioperamide, a histamine H_3 receptor antagonist that increases both synthesis and release of histamine from the nerve terminals (35), was infused i3vt. The thioperamide infusion convincingly mimicked histamine action in the enhancement of lipolysis, which concomitantly confirmed that the thioperamide-induced lipolysis was regulated by neuronal histamine per se, and not by mast cell-derived histamine.

Central administration of histamine was reported to increase blood concentration of catecholamines (12). The present study showed that β-adrenoceptor antagonist attenuated the augmented lipolytic response to thioperamide. These results strengthen the possibility that sympathetic nerve may

be involved in histaminergic modulation of lipolytic activity in the adipose tissue. However, we can not exclude indirect effects of histamine neurons on sympathetic nerve activity or a possibility that β-adrenoceptor antagonist may block a sympathomimetic action of thioperamide. The present electrophysiological study has made clear that there is a direct effect of thioperamide on the activity of sympathetic nerves innervating the WAT. Additionally, the effect of thioperamide on sympathetic activation was reproduced by i3vt infusion of histamine (our unpublished data).

A neuroanatomical study using a trans-synaptic retrograde tracer has identified a variety of hypothalamic origin of sympathetic nerve efferents to WAT, such as the PVN, the MPOA, and suprachiasmatic, dorsomedial, ventromedial, and arcuate nuclei (2). Among these nuclei, the PVN projects sympathetic preganglionic neurons directly to the intermediolateral cell column of the spinal cord (36). Polysynaptic pathways may exist from the MPOA to this column (2). Indeed, stimulation of these hypothalamic nuclei accelerates sympathetic nerve activity (34). Outside of the hypothalamus, an efferent pathway from several regions in the brain stem, such as C1 and A5 cell groups and the nucleus of the solitary tract (NTS), to the WAT has been identified (2). In parallel with these studies, hypothalamic histamine neurons are shown to project to the PVN (37), the MPOA (11, 37, 38), and the NTS (37) to regulate energy homeostasis. Based on these findings, these hypothalamic nuclei and the brain stem regions are the most likely loci through which the histamine neurons control lipolytic activity. Another possible mediation of histamine signal from the hypothalamus to WAT may be through the adrenal medulla because histamine increases catecholamine secretion from the adrenal gland (12). In addition, hyperglycemia induced by central administration of histamine was attenuated after adrenalectomy (10, 11).

A series of our previous studies on functional roles of histamine neurons in the brain has demonstrated that hypothalamic neuronal histamine is activated by a variety of states of energy deficiency aimed at inducing neuroglucoprivation in the brain, such as starvation, insulin-induced hypoglycemia, and glucoprivation due to 2-deoxy-D-glucose (13, 14). On the other hand, hypothalamic neuronal histamine induces hyperglycemia through elevation of catecholamine secretion from the adrenal medulla (10, 11). Hypothalamic histamine neurons thus play a counter regulatory role in protection of the brain from glucoprivation. Ultimately, the lipolytic process activated by histamine neurons results in production of FFA and ketone bodies that can be utilized in essential organs such as the brain and the heart as energy substrates. In other words, the lipolytic activation by histamine neurons contributes to maintenance of energy homeostasis under emergent energy deficiency.

In our previous reports, a variety of stimuli associated with energy deficiency, as well as leptin, were similarly found to activate hypothalamic histamine neurons. (13, 14, 16, 17) These findings seem contradictory from physiological point of view because leptin secretion is reduced under energy-deficient status. Precisely how this inconsistency can be accounted for is as yet unknown. One possible explanation is that the information on with energy deficiency or excess may be accepted by histamine neurons through different signal processing in the hypothalamus because histamine neurons play an essential role in homeostatic maintenance in energy metabolism (38). Based on this assumption, the present results imply that the leptin-histamine signaling system may contribute to the prevention of excessive fat accumulation.

Our present and other previous findings, i.e., activation of the sympathetic nerve driven by histamine neurons, promotion of lipolysis by histamine neurons, and activation of histamine neurons driven by leptin, raise the following two possible explanations: First, hypothalamic histamine neurons may mediate leptin-induced activation of the sympathetic nerve. Second, leptin may regulate peripheral energy metabolism by affecting not only expression of the UCP family, but also the lipolytic processes in adipose tissue through sympathetic nerve activation driven by histamine neurons. The present results provide a novel insight into central control of lipid metabolism mediated by a hypothalamic leptin-histamine signaling system.

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