# BRIEF COMMUNICATION

# Histidine Suppresses Food Intake through Its Conversion into Neuronal Histamine

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Hypothalamic neuronal histamine has been shown to regulate feeding behavior and energy metabolism as a target of leptin action in the brain. The present study aimed to examine the involvement of L-histidine, a precursor of neuronal histamine, in the regulation of feeding behavior in rats. Intraperitoneal (ip) injection of L-histidine at doses of 0.35 and 0.70 mmol/kg body weight significantly decreased the 24-hr cumulative food and water intakes compared to phosphate buffered saline injected controls ($P < 0.05$ for each). This suppression of feeding was mimicked dose-dependently by intracerebroventricular infusion of histidine at doses of 0.5, 1.0, and 2.0 μmol/rat ($P < 0.05$ for each). Pretreatment of the rats with an ip bolus injection of α-fluoromethylhistidine, a suicide inhibitor of a histidine decarboxylase (HDC), at a dosage of 224 μmol/kg blocked the conversion of histidine into histamine and attenuated the suppressive effect of histidine on food intake from 64.2% to 88.1% of the controls ($P < 0.05$). Administration of 0.35 mmol/kg histidine ip increased the concentration of hypothalamic neuronal histamine compared with the controls ($P < 0.05$). HDC activity was increased simultaneously by histidine administration compared with the controls ($P < 0.05$). The present findings indicate that L-histidine suppresses food intake through its conversion into histamine in the hypothalamus.

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A series of previous studies from our group on functions of brain histamine neurons has demonstrated that hypothalamic neuronal histamine suppresses food intake through $H_1$-receptors in the ventromedial hypothalamic nucleus and the paraventricular nucleus (1). Increase in hypothalamic histamine concentration raised peripheral glucose concentration (1, 2), accelerated lipolysis in the adipose tissue (3, 4) and decreased body temperature (1, 2). On the other hand, neuro-glucoprivation induced by starvation, insulin and 2-deoxy-D-glucose in the brain (5, 6), elevation of ambient temperature (1, 2, 7) and cytokines such as interleukin-1β(8) increased the concentration and turnover rate of hypothalamic neuronal histamine. These findings implicate the hypothalamic histamine neurons as an essential component in the regulation of energy intake and energy expenditure.

We have recently shown that histamine neurons were involved in the action of leptin on hypothalamic control of feeding behavior and energy metabolism (9, 10). Central administration of leptin increased histamine turnover in the hypothalamus (9). Depletion of neuronal histamine using α-fluoromethylhistidine (FMH), a suicide inhibitor of a histidine decarboxylase (HDC), the rate-limiting enzyme for histamine synthesis, attenuated the leptin-induced suppression of feeding (9). In addition, leptin-induced up-regulation of uncoupling protein 1 mRNA in the brown adipose tissue was attenuated in mice with targeted disruption of histamine $H_1$ receptors (10). Based on these findings, we proposed that hypothalamic neuronal histamine was a target of leptin's anti-obesity action in the brain. In fact, central exogenous

administration of histamine has been found to be effective in reducing body weight and adiposity in leptin resistant obese *db/db* mice, and mice with diet-induced obesity (11). Taken together, we have hypothesized that the treatment that activates endogenous histamine may be useful in the prevention and/or improvement of obesity.

Histidine is an essential amino acid that is a precursor of neuronal histamine. Neuronal histamine is synthesized in the brain by the enzymatic decarboxylation of histidine catalyzed by HDC. The present study aims to examine whether activation of endogenous histamine by L-histidine loading may be effective on suppression of feeding behavior.

## Materials and Methods

**Animals and Diet.** Mature male Wistar King A rats weighing 290 ± 10g were housed in a room illuminated daily from 0700 hr to 1900 hr (12:12 hrs light-dark cycle) and maintained at 21 ± 1°C with humidity at 55 ± 5%. They were allowed free access to standard solid rodent chow and tap water except as where otherwise described. All studies were conducted in accordance with the Oita Medical University Guidelines based on the NIH Guide for the Care and Use of Laboratory Animals.

**Reagents.** FMH (a gift from Dr. J. Kollonitsch, USA) and L-histidine (Sigma, USA) were dissolved in phosphate buffered saline (PBS) to concentrations of 0.07 M and 1.0 mM, respectively. Each solution was freshly prepared on the day of its administration. The pH of each solution was adjusted to be in the range of 6.4 to 7.2.

**Surgery.** Rats that were to receive central injections underwent surgery for placement of indwelling cannulas into the third cerebroventricle (i3vt). Under sodium pentobarbital anesthesia intraperitoneally (45 mg/kg, ip), rats were placed in a stereotaxic apparatus (Narishige Co., Japan). A 23G-stainless steel guide cannula was chronically implanted i3vt of each rats. Cannulas were implanted at least 1 week before the onset of infusions. A 29G-stainless steel wire stylet was inserted in the guide cannula to prevent leakage of the cerebrospinal fluid as well as obstruction of the cannula. Details of the surgical procedure have been described elsewhere (12).

**Behavioral Studies following Histidine Administration.** Rats were matched on the basis of body weight and food intake during the one-week adaptation period, and were equally assigned to one of eleven groups, with 5 rats per group. The first 7 Groups for peripheral administration of histidine were as follows: Group 1: Histidine 0.175 mmol/kg, ip; Group 2: Histidine 0.35 mmol/kg, ip; Group 3: Histidine 0.7 mmol/kg, ip; Group 4: PBS Control, ip; Group 5: FMH/Histidine 0.35 mmol/kg, ip; Group 6: PBS/Histidine 0.35 mmol/kg, ip; Group 7: PBS/PBS, ip. The remaining 4 Groups were assigned to central administration of histidine as follows: Group 8: Histidine 0.5 μmol/rat, i3vt; Group 9: Histidine 1.0 μmol/rat, i3vt; Group 10: Histidine 2.0 μmol/rat, i3vt; Group 11: PBS Control, i3vt.

Cumulative 24-hr food and water intake were measured for both 2 days before and after ip or i3vt infusion of histidine. The percentage difference in average daily food and water intake over I day following the histidine administration from the mean daily food and water intake measured for the 2 days prior to treatment in each group was compared with those in their corresponding PBS control group. The percentage difference in food and water intake was applied in these experiments because the baseline intake values were not identical in each group even if differences between baseline values were not significant. The percent change from the initial intake value enabled to assess effects of histidine on food and water intake precisely rather than absolute 24-hr ingestion. Group 5 rats were administered FMH at a dose of 224 μmol/kg ip (a dose previously determined to deplete most neuronal histamine in the hypothalamus), and Group 6 rats were administered the same volume of PBS, ip, 2 hr before 0.35 mmol/kg histidine or PBS injection. i3vt infusion studies were performed by infusing histidine at doses of 0.5 μmol/rat, 1.0 μmol/rat, 2.0 μmol/rat, and PBS (control), at an infusion rate of 1 μl/min for 10 min through the i3vt cannula at 1700 hr without FMH pretreatment. After the completion of the experiments, the animals were decapitated, the brains were carefully removed, and the location of the cannula was verified histologically.

**Studies to Measure Histamine and HDC Activity in the Brain.** An additional 20 rats were used to measure brain histamine levels and HDC activity following peripheral administration of histidine (5 rats for each) or PBS (5 rats for each). Sixty min following the ip administration of histidine (0.35mmol/kg) or PBS, 5 rats from each group were decapitated. The hypothalamus and the cerebral cortex were carefully dissected on an ice plate according to the method of Glowinski and Iversen (13). The tissue was immediately frozen on dry ice and stored at −80°C until the assays. Histamine concentrations in the hypothalamus and the cerebral cortex were measured by the method of Oishi et al. (14). 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 (HPLC).

The remaining rats treated with histidine (0.35mmol/kg, ip) and PBS (control) were used to measure the HDC activity in the hypothalamus. After homogenization and centrifugation, the supernatant of samples was dialyzed by Dialysis Merb Size 8 (Wako, Osaka, Japan). HDC activity was evaluated by measuring the formation of histamine from L-histidine in the dialysate. The reaction was started by addition of histidine (0.25 mM) to the dialysate (180 μl). Assay was carried out by HPLC in parallel with a mixture containing 0.1mM FMH as a blank. The details of the amine and HDC assays have been described elsewhere (6, 8, 14).

**Statistics.** Data for the behavioral study were analyzed using an analysis of variance (ANOVA) for repeated measures. Evaluation for a dose-responsiveness was done

by a single linear regression and ANOVA. The Mann-Whitney U test was used for the amines and HDC assays.

## Results

**Effects of Peripheral or Central Administration of L-Histidine on Feeding Behavior.** Administration of 0.35 mmol/kg and 0.7 mmol/kg but not 0.175 mmol/kg histidine significantly reduced 24-hr cumulative food and water intake of rats compared with PBS injection (control) ($P < 0.05$ for each) (Fig. 1) (data on water intake not shown). This suppression of food and water intake by histidine was dose related ($Y = 298.96 - 41.685X$, $r = 0.93$, $P < 0.0001$ for food intake, and $Y = 75.852 - 8.449X$, $r = 0.70$, $P < 0.05$ for water intake). The iI3vt infusion of histidine (0.5–2.0 μmol/rat) also decreased 24-hr cumulative food intake compared with PBS infusion (control) ($P < 0.05$ for each) in a dose responsive manner ($y = 50.417 - 31.92X$, $r = 0.96$, $P < 0.0001$) (Fig. 2).

**Effect of Histamine Depletion by FMH on Histidine-Induced Suppression of Feeding.** The percentage differences in daily food consumption in the ip-pretreated PBS/histidine and FMH/histidine groups compared to the PBS/PBS control group are shown in Fig. 3. Histidine administration pretreated with PBS decreased food intake to 64.2% of the baseline value (the mean daily food intake for 2 days before the administration) ($P < 0.05$ vs. PBS/PBS control). Following FMH pretreatment, histidine suppressed food intake to only 88.1% of the baseline value. Thus, FMH attenuated the histidine-induced suppression of feeding ($P < 0.05$ vs. PBS/histidine group). Pretreatment with FMH attenuated the histamine-induced suppressive effect on water intake ($P < 0.05$ vs. PBS/histidine group) similarly to that on food intake (data on water intake not shown in Fig. 3). FMH treatment per se showed no remarkable effect on food or water intake (Fig. 3, data on water intake not shown).

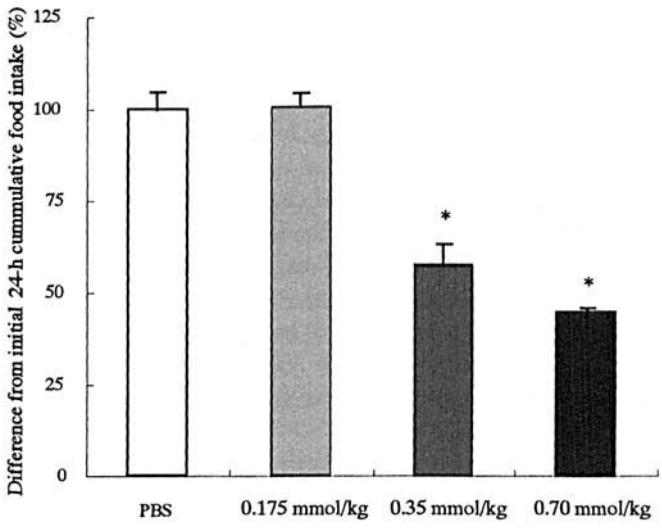


**Figure 1.** Effect of intraperitoneal injection of L-histidine on food intake. Values, mean ± SE for 5 rats per group. L-Histidine decreased food intake dose dependently. Ordinate, percentage difference between the initial value (the mean 24-hr cumulative food intake for the 2 days before the administration) and mean daily food intake over 1 day following the administration of L-histidine at doses of 0.175–0.70 mmol/kg. * = $P < 0.05$ vs. PBS controls.

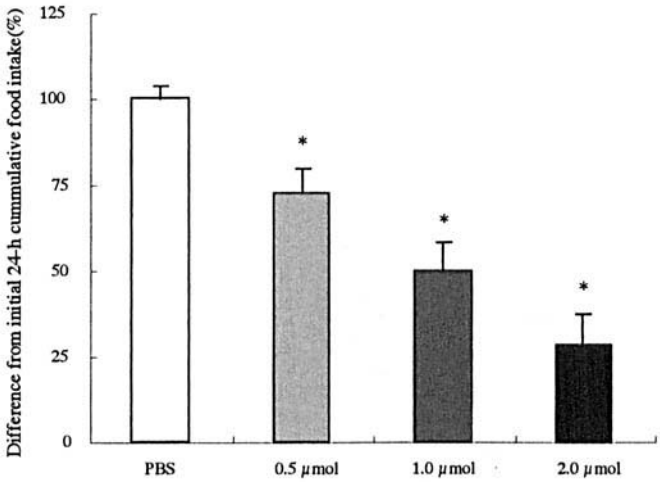


**Figure 2.** Effect of i3vt infusion of L-histidine on food intake. Values, mean ± SE for 5 rats per group. i3vt infusion of L-histidine decreased food intake dose dependently. Ordinate, percentage difference between the initial value (the mean 24-hr cumulative food intake for the 2 days before the administration) and mean daily food intake over 1 day following the administration of L-histidine at doses of 0.5–2.0 μmol/rat. * = $P < 0.05$ vs. PBS controls.

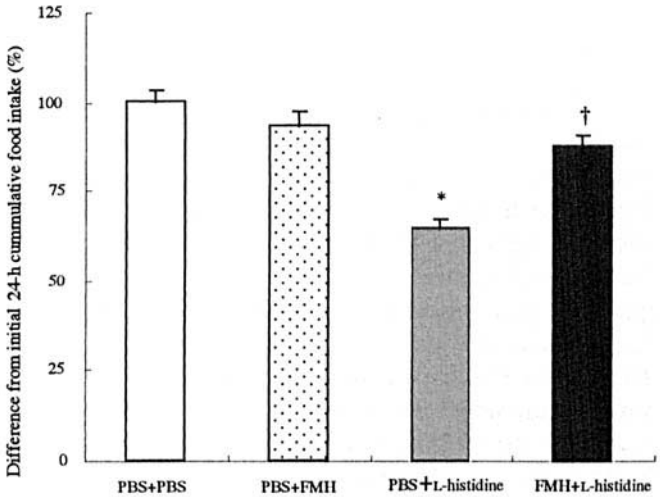


**Figure 3.** Effect of histamine depletion by FMH on histidine-induced suppression of feeding. Values, mean ± SE for 5 rats per group. L-Histidine decreased food intake to 64.2% of controls. FMH pretreatment by ip injection attenuated this suppression to 88.1% of controls. Ordinate, percentage difference between the initial value (the mean 24-hr cumulative food intake for the 2 days before the administration) and mean daily food intake over 1 day following the administration of L-histidine (ip) after depletion of histamine by ip injection of FMH. * = $P < 0.05$ vs. PBS (ip)/PBS (ip) group. † = $P < 0.05$ vs. PBS (ip)/histidine (ip).

**Effect of Histidine Administration on Histamine Concentration and HDC Activity.** Histidine administration ip increased the histamine concentration in the hypothalamus of rats ($P < 0.05$) (Fig. 4A). Correspondingly, the activity of HDC was also increased by administration of

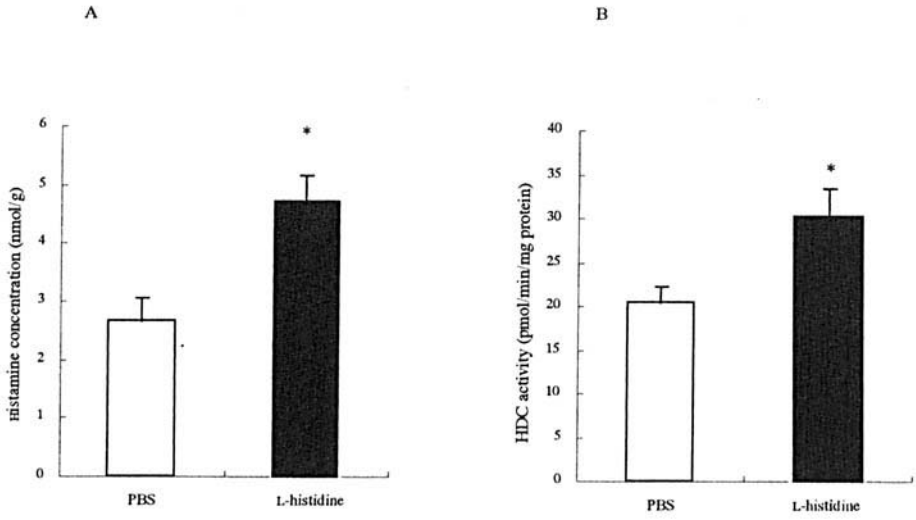


**Figure 4.** Effect of ip injection of 0.35 mmol/kg L-histidine on histamine concentration and HDC activity in rat hypothalamic tissues. Values, mean ± SE for 5 rats per group. Administration of L-histidine significantly increased histamine concentration (A) and HDC activity (B) in the hypothalamus. * = $P < 0.05$ vs. PBS controls.

histidine compared with PBS controls ($P < 0.05$) (Fig.4B). Histidine ip did not affect histamine concentration in the cerebral cortex (data not shown).

## Discussion

The present study demonstrated that peripheral administration of L-histidine suppressed food intake. This effect of peripherally administered histidine on the suppression of food intake and its magnitude were mimicked by i3vt infusion of histidine, implicating that the central nervous system may be a potent target of histidine action to suppress food intake. Some of histidine derivatives such as carnocine are endogenous antioxidants that are stimulated by stress (15). To confirm that hypothalamic neuronal histamine is involved in the suppressive effects of histidine on food intake, and to exclude the possibility that histidine may affect food intake through this stress-related pathway, we have carried out the experiments using FMH that depletes hypothalamic neuronal histamine almost completely (16). Depletion of neuronal histamine by FMH pretreatment attenuated almost completely histidine-induced suppression of feeding. Hypothalamic neuronal histamine has been shown to suppress food intake through histamine $H_1$ receptors in the ventromedial hypothalamic nucleus and the paraventricular nucleus, both known as satiety centers (1). Taken together, it can be concluded from these observations that conversion of histidine to histamine in the hypothalamus is necessary to produce the suppressive effect of histidine on feeding behavior. As for drinking behavior, activation of hypothalamic histamine neurons showed excitatory but not inhibitory effect on water intake (17, 18). In the present study, however, histidine decreased water intake. Additionally, pretreatment with FMH attenuated this suppressive effect on water intake similarly to that on food intake. Based on these findings, it is not likely that the suppressive effect on water intake may primarily be induced by histidine, but it may rather be caused secondarily by its suppressive effect on food intake.

The activation of histamine synthesis based on the conversion of histidine to histamine was confirmed by HPLC studies. In present study, peripheral administration of histidine increased hypothalamic histamine concentration. The activation of histamine synthesis was also supported by an increase in the activity of HDC following administration of histidine. Histamine concentration in the cerebral cortex showed no remarkable change in response to histidine administration. The result is consistent with our previous studies. The i3vt infusion of FMH or high ambient temperature, both of which affect feeding behavior oppositely, has demonstrated that changes of histamine concentration in the hypothalamus but not in the cerebral cortex regulate feeding behavior (7, 18, 19). The process of conversion of histidine to histamine in present study is considered to occur as follows: peripherally administered L-histidine is transported into the hypothalamus through the blood brain barrier. Uptake of histidine by histamine neurons and its subsequent decarboxylation by HDC are accelerated by the increase in local levels of histidine in the extraneuronal space. The histidine dosage used in the present behavioral and HPLC studies has been relatively large since sufficient supply of histidine may be necessary to overcome the several steps such as peripheral absorption, transportation into the brain, uptake by histamine neurons, and decarboxylation by HDC before histamine is synthesized. In fact, in previous studies, histamine concentration in the rodent brain was increased after systemic administration of histidine in large dosages (20, 21).

Recently, we have demonstrated the involvement of hypothalamic histamine neurons as targets for leptin action in the brain (9, 10). Central administration of leptin increased histamine turnover in the hypothalamus (9). Depletion of neuronal histamine using FMH attenuated the suppression of feeding induced by leptin (9). In addition, leptin-induced reduction of adiposity and up-regulation of uncoupling protein 1 mRNA in the brown adipose tissue

were attenuated in mice with targeted disruption of histamine $H_1$ receptors (10). We have previously shown low levels of brain histamine concentrations and HDC activity in Zucker fatty (*fa/fa*) rats (1, 2) that have dysfunctional leptin receptors (22). Similarly, the concentrations of histamine and *tele*-methylhistamine, a major metabolite of histamine, in the hypothalamus were significantly lower in the diabetes (*db/db*) mice, another leptin receptor-defective animal (22, 23), and obese (ob/ob) mice, a leptin deficient animal (24), compared to their lean littermates (9). In these genetically obese animal models, the lowered hypothalamic histamine concentration resulting from disruption of leptin signaling to histaminergic neurons because of their leptin-deficient or leptin-insensitive mutation may be one of the main causes for their development of obesity. Independently on leptin, the involvement of feeding-related neuropeptides in the hypothalamus and various metabolic, hormonal, cytokine, and growth factors need to be considered as well.

The findings that plasma leptin concentration is higher significantly not only in obese animals but also in obese patients have led to a concept that obese patients must be relatively insensitive to endogenous leptin signal (25, 26, 27). Exogenously sustained central administration of histamine has proved effective on reduction in food intake, body weight and adiposity even in leptin resistant *db/db* mice (11). Systemic administration of histamine, however, is inefficient on its centrally mediated anti-obesity action because exogenous histamine poorly penetrates the blood brain barrier. Therefore, the activation of endogenous histamine synthesis may be necessary to induce such physiologic effects of histamine. Indeed, the present study has convincingly suggested that treatment with histidine may be a useful tool for prevention or improvement of human obesity through its conversion to histamine in the hypothalamus. Natural foodstuffs such as marine products including tuna, sardine, Pacific saury and so on are quite rich in L-histidine. Although we are still away from clinical use of histidine, the outlines of some novel therapeutic approach to application of histidine to obese patients as one of diet therapies can now be seen. The idea, however, is still greatly in need of experimental verification to prove the effectiveness of histidine consumed as daily foodstuffs on food intake, energy expenditure, and adiposity.

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