Satiation and Masticatory Function Modulated by Brain Histamine in Rats (44227)

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Abstract. **Both the ventromedial hypothalamus (VMH) and the mesencephalic trigeminal sensory nucleus (Me5) are densely innervated by histaminergic neurons. The depletion of neuronal histamine (HA) from the Me5 by the bilateral microinfusion of 448 nmol/rat α-fluoromethylhistidine (FMH), a specific suicide inhibitor of histidine decarboxylase, reduced the eating speed and prolonged meal duration, while leaving the meal size unaffected. HA depletion from the VMH increased the size of the meal and prolonged its duration, but not the eating speed. When the HA turnover rate was measured at 15 min after the scheduled feeding following fasting for less than 24 hr, the rate increased in the region including the Me5, but not in the hypothalamus. The turnover rate reached higher levels at 60 min in both regions. Gastric intubation of an isocaloric liquid diet or an equivolume of water with the liquid diet abolished the increase in HA turnover both in the Me5 region and the hypothalamus. The present findings indicate that brain HA thus modulates satiation through both the VMH and masticatory function as well as due to the action of the Me5. The HA function activated by mastication began earlier in the Me5 and later in the hypothalamus due to a signal originating from the oral proprioceptors and initiated by chewing.**

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In the mammalian brain, the cell bodies of histamine (HA) neurons are localized in the tuberomammillary nucleus of the posterior hypothalamus. Rich innervation was unevenly found in the hypothalamic areas (1) and at the mesencephalic trigeminal sensory nucleus (Me5) (2). Unique to the central nervous system, the somata of the Me5 were shown to possess synaptic formation from HA fibers (3). The Me5 receives proprioceptive sensory input from the periodontal ligament and the masseteric muscle spindles through the trigeminal sensory nerve, and also has efferent innervation to motor trigeminal nucleus (Mo5) relating to the jaw-jerk reflex (4, 5).

The posterior hypothalamus, which contains HA neurons, has been shown to be projected upon by neurons of the Me5 (6). Thus, this histological evidence suggests that HA neurons may be involved in the regulation of the masticatory function through the Me5.

Our previous studies demonstrated that the neuronal HA suppressed food intake through H_1 receptors in the ventromedial hypothalamus (VMH) and the paraventricular nucleus (PVN) (7, 8, 9, 10). In addition to these behavioral studies on the functions of brain HA, *in vivo* microdialysis studies also showed that the extracellular concentration of HA in the rat VMH was elevated following food intake (11). These findings indicate that feeding may activate the hypothalamic HA system as a cessational signal to prevent sustained food intake. However, it does raise the question as to what roles neuronal HA plays in the Me5, and also what kind of information in response to feeding initiates this increase of hypothalamic HA. Postprandial humoral factors such as hyperglycemia may not be a likely inducer, since hypoglycemia, but not hyperglycemia, has been shown to increase the HA turnover in the rat hypothalamus (12).

The aim of the present study is to elucidate the direct trigger for the activation of hypothalamic HA neurons in

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response to feeding, while particularly focusing on the HA function in the Me5 and the VMH.

Materials and Methods

Animals. Mature male Wistar King A (WKA) rats, 280–320 g, were housed in a sound-proof room that was automatically illuminated daily from 0800 hr to 2000 hr (a 12:12-hr light:dark cycle) and maintained at 21 ± 1°C with humidity at 55 ± 5%. The rats were allowed free access to standard pelleted rat chow (Clea rat chow, #CE-2, Japan Clea) and tap water. All procedures were performed in accordance with the Oita Medical University *Guide for the Care and Use of Laboratory Animals,* which was based on that of the NIH guide, and were approved by the Animal Care Committee of Oita Medical University.

Reagents. Alpha-fluoromethylhistidine (FMH, a gift from Dr. J. Kollonitsch, United States) was prepared at a dose of 224 m*M* dissolved in phosphate-buffered saline (PBS) and adjusted to 7.00–7.40 with 0.1 *N* sodium hydroxide. Pargyline hydrochloride (Sigma, St. Louis, MO) was dissolved in PBS at a dose of 0.33 *M*. PBS contained 137 m*M* NaCl, 2.68 m*M* KCl, 8.10 m*M* Na_2HPO_4, and 1.47 m*M* KH_2PO_4 (pH 7.35–7.40) and was used as a control solution. Each solution was freshly prepared on the day of administration.

Surgery. One week before testing, each rat was fixed in a stereotaxic apparatus (Narishige Co., Japan) under intraperitoneal (ip) pentobarbital sodium anesthesia (0.22 mmol/kg). Each rat for the microinfusion studies was chronically implanted bilaterally with 23-gauge stainless steel guide cannulae into the brain with the tip 1 mm above its target site, the Me5 (P 10.2, L 1.5, H 8.1 mm) or the VMH (P 2.4, L 0.6, H 9.2 mm) according to the atlas of Paxinos and Watson (13). P, L, and H represent, respectively, the distance behind the bregma, lateral to the midline and below the cortical surface (14). Through a guide cannula, a 29-gauge stainless steel wire stylet was left for insertion upon testing.

Evaluation of the Feeding Parameters Following Depletion of HA. A meal was defined to accommodate both the criteria (i.e., the consumption of > 0.5 g pellets and the intermeal intervals that exceeded 10 min) according to our previous study, which analyzed the distribution of either the meal sizes or the intermeal intervals (15). The following feeding parameters during feeding mentioned below were evaluated regarding: the size (g) and duration (sec) of the first meal after 1800 hr; eating speed (g/min) calculated from meal size divided by meal duration. All rats were handled for 5 min daily for 6 successive days before each experiment to equilibrate their arousal levels. Daily food intake and body weight were ascertained to have returned at least to the presurgical level by the start of testing. After the rats were acclimated to the 1-week experimental condition, the rats were fasted for 5 hr from 1300 hr to 1800 hr, and then the feeding parameters of the first meal after 1800 hr were measured on 1 day both before and after FMH infusion. Matched on the basis of the meal parameters and body weight on the last day of the adaptation period, 26 rats were then divided into 4 groups consisting of rats microinfused with FMH or PBS into the Me5 or the VMH on the infusion day (i.e., FMH-Me5, FMH-VMH, PBS-Me5, and PBS-VMH groups).

An infusion cannula (29-gauge) for the Me5 or the VMH was bilaterally inserted into the guide cannula 1 hr before testing so that the tip of the infusion cannula protruded 1 mm beyond the tip of the guide cannula. All test solutions were bilaterally infused at a volume of 1 μl and a rate of 0.2 μl/min for 5 min into the Me5 ($n = 7$) or the VMH ($n = 6$). The unilateral dosage of FMH into the nucleus resulted in 224 nmol per rat. The same volume of PBS was bilaterally microinfused into the Me5 ($n = 7$) or the VMH ($n = 6$) in the same manner as that of the controls. The microinfusion was started at 1300 hr under unanesthetized and unrestrained conditions (16).

On the next day of the infusion after completion of the behavioral testing, 60-μm frozen sections were cut for the injection site, and then the cannula location was verified histologically. All specimens were stained with Neutral red dye (8, 14), and the tip location of each infusion cannula was precisely verified according to a brain atlas (13). A statistical evaluation of the data was carried out by a two-way analysis of variance with replication.

Evaluation of the Brain HA in Response to Pellet Feeding. Since food deprivation of less than a 24-hr period did not affect the extracellular concentration of brain HA (11), the rats used in this and succeeding experiments were fasted for the preceding 24 hr so that they could start to eat immediately after the scheduled feeding at 1400 hr on a testing day.

Matched on the basis of body weight on the testing day, 48 rats were thus equally divided into pargyline and PBS groups, each of which was decapitated at 15 or 60 min after feeding at 1400 hr ($n = 12$ for each). Each group consisted of fed and nonfed subgroups ($n = 6$ for each). Pargyline was injected ip 10 min before the start of the scheduled feeding at 1400 hr. A statistical evaluation of the data was carried out by two-way analysis of variance.

Evaluation of Brain HA in Response to Tube-Feeding. Matched on the basis of body weight on the testing day after 24-hr fasting, 24 rats were equally divided into 4 groups (i.e., the *ad lib* feeding, the liquid tube feeding, the water tube loading, and the nonfed control groups) with $n = 6$ for each. All rats were pretreated with pargyline ip 10 min before the start of refeeding at 1400 hr. The *ad lib* feeding group was fed with pelleted rat chow *ad libitum.* To adapt to a gastric cannulation, the rats in the liquid- and water-intubated groups were administered 1 ml distilled water through a gastric cannula at 1400 hr for 1 week before testing. The tube-feeding group was administered an isocaloric liquid diet (#Clinimeal 2 kcal/ml, Eizai, Japan) through a gastric tube for 5 min at a speed of 1 ml/min. The caloric assessment for diet tubing in this group depended on the caloric intake in the preceding experiment in response to

pellet feeding. As a result, the rats consumed an average of 10 kcal based on the amount of food consumed and the nutritional contents. The water-intubated group was loaded with 5 ml of distilled water through a gastric cannula at the same speed as that in the tube-feeding group. The nonfed control group was allowed free access only to water. Brain samples were collected at 1500 hr, 60 min after the start of the scheduled feeding. A statistical evaluation of the data was carried out by two-way analysis of variance.

Measurement of HA and its Turnover. The linear accumulation of *tele*-methylhistamine (*t*-MH) after an ip injection of 0.33 mmol/kg pargyline, an inhibitor of monoamine oxidase B, was used to estimate the HA turnover rate (17) in the brain region including the rat Me5 and the hypothalamus. The brain was promptly removed and dissected on an ice plate. The hypothalamus was immediately separated according to the procedure of Glowinski and Iversen (18) (Fig. 1). With some substantial modifications of their procedure, the brain region including the Me5 was dissected by taking the line between the inferior colliculus and the interpeduncular fossa as the anterior limit (section 2 in Fig. 1) and the parallel line to section 2, which passed through the vestibulocochlear ganglion (section 3 in Fig. 1). The HA and *t*-MH concentrations were simultaneously measured by the method of Tsuruta *et al.* (19) as modified by Oishi *et al.* using high performance liquid chromatography. Brain samples were homogenized in 0.3 ml of 0.40 *M* perchloric acid containing 1.25 m*M pros*-methylhistamine as an internal standard. After centrifugation at 1000*g*, 0.25 ml of supernatant was used for the assay. These amines were extracted into n-butanol under NaCl by shaking with benzene. After adjusting the pH to 6.0, the extracts were then applied to P-cellulose columns. The columns were washed successively with 0.01 *M* phosphate buffer (pH 6.0, 2 ml × 2), distilled water (1 ml), and 0.12 *M* HCl (0.4 ml). The amines were eluted with *o*-phthalaldehyde at pH 10.0 in the presence of 2-mercaptoethanol. The resulting fluorophores were then injected into an HPLC system (20). The system consulted of an LC-6A pump (Shimadzu Co., Kyoto, Japan) and methanol (47:53 vol/vol). The excitation and emission wavelengths were set at 340 and 450 nm, respectively.

Results

Changes in Meal Parameters After HA Depletion from the VMH or the Me5. Figure 2-A shows changes in the parameters of the first meal one day before and after bilateral FMH or PBS microinfusion into the Me5. The depletion of neuronal HA from the Me5 due to bilateral FMH microinfusion decreased the eating speed [$F(1, 12) = 29.01$, $P < 0.01$] and prolonged the meal duration of the first meal [$F(1, 12) = 7.16$, $P < 0.05$] compared with those of the PBS controls, leaving the meal size unaffected. PBS infusion into the Me5 induced no significant changes in each parameter.

The depletion of the neuronal HA from the VMH increased with the size of the first meal [$F(1, 10) = 7.13$, $P < 0.05$] and prolonged its duration [$F(1, 10) = 5.26$, $P < 0.05$] compared with those of the PBS controls although there was no effect on eating speed. The PBS injection into the VMH produced no significant changes in each parameter (Fig. 2-B).

Histological Identification of FMH Microinfusion Studies. Figures 3-A and 3-B show typical infusion sites stained by Pontamine sky blue dye. The bilateral tips of these infusion cannulae were either in the Me5 or the VMH. The chronically implanted guide cannulae were thus found to neither touch nor damage the tissue of the bilateral target loci. The insertion of the thin infusion cannulae was thus confirmed not to cause any excessive tissue destruction.

Changes in the Brain HA and *t*-MH in Response

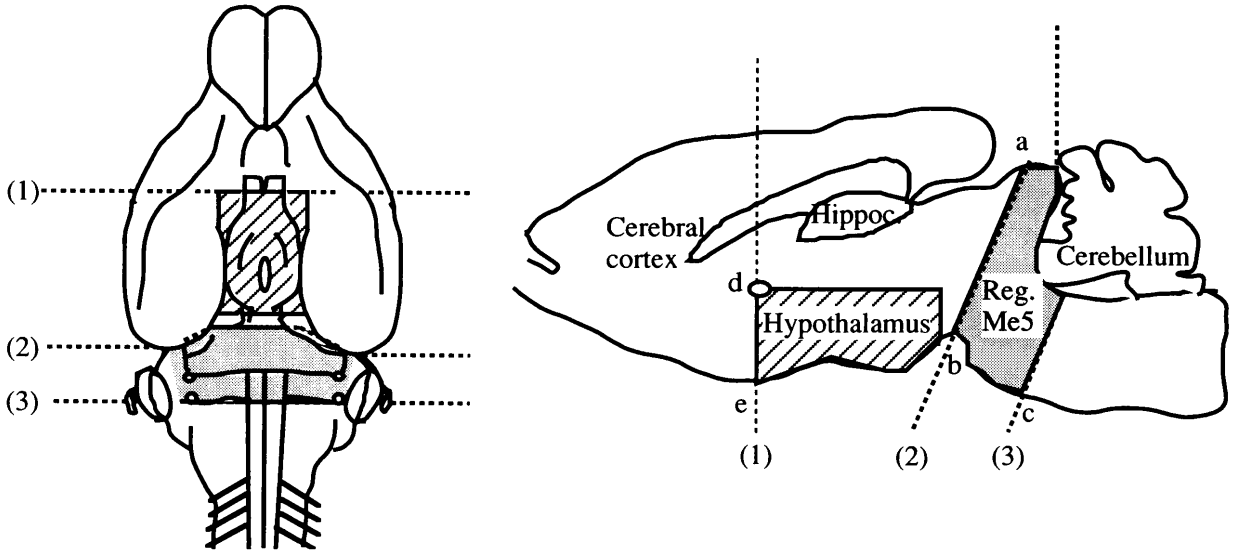

Figure 1. Diagrammatic representation of the dissection procedure for the rat brain. Dotted lines, positions of initial sections. a, inferior colliculus. b, interpeduncular fossa. c, vestibulocochlear ganglion. d, commissure anterior. e, chiasm opticum. Reg. Me5, region including the Me5. Hippoc., hippocampus.

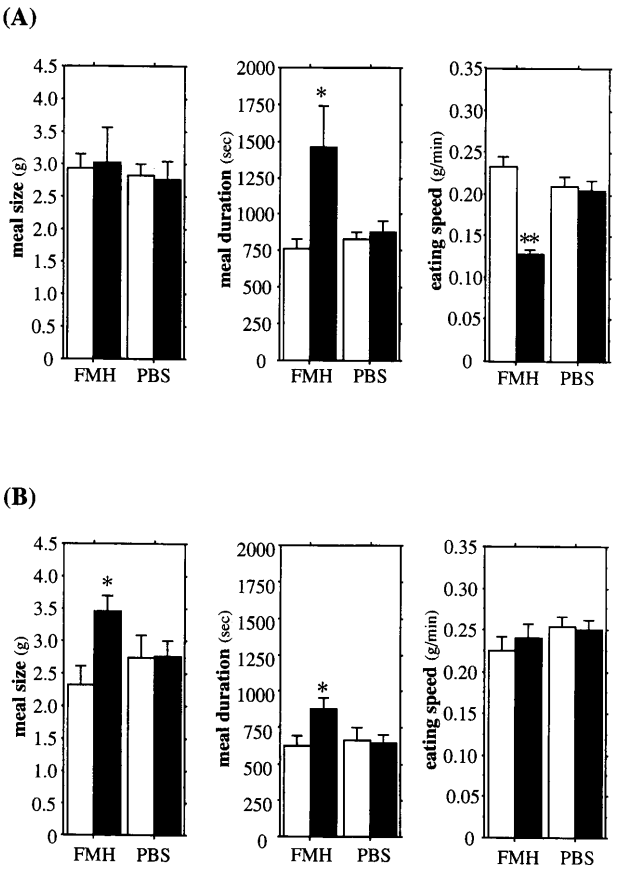

Figure 2. Changes in the meal size, meal duration, and eating speed after a bilateral microinfusion of α-fluoromethylhistidine (FMH) (224 nmol/rat for unilateral infusion) into the mesencephalic trigeminal nucleus (Me5) (A) or the ventromedial hypothalamus (VMH) (B). All values represent the mean ± SEM. Open columns, the control group before the infusion of test solution. Closed columns, the group after FMH or PBS infusion. $n = 7$ for the Me5 and $n = 6$ for the VMH. * $P < 0.05$, ** $P < 0.01$ vs. corresponding controls before the infusion and after PBS infusion.

to Pellet Feeding. Table I shows the changes in the HA and *t*-MH of the brain region including the Me5 and the hypothalamus in rats after pellet refeeding. Pellet feeding without pargyline pretreatment showed no significant effect on either the HA or *t*-MH steady-state level. After pargyline pretreatment, the HA levels in both brain regions were not affected at 15 or 60 min after feeding. The pargyline-induced accumulation of *t*-MH in the Me5 region increased more in the group fed with pelleted rat chow than the corresponding nonfed controls at both 15 ($P < 0.01$) and 60 min ($P < 0.05$) after feeding. However, in the hypothalamus the pargyline-induced accumulation in the group at 15 min after feeding did not significantly differ between the fed and the nonfed groups. At 60 min after feeding, the pargyline-induced accumulation of *t*-MH (i.e., the HA turnover rate increased in the fed rat hypothalamus compared with that in the nonfed control hypothalamus ($P < 0.05$)).

Changes in Brain HA and *t*-MH in Response to Tube Feeding. The effects of tube feeding on the concentration of HA and the accumulation of *t*-MH in the brain region including the Me5 and the hypothalamus at 60 min after feeding are shown in Table II. After pargyline pretreatment, the HA levels in both brain regions were not affected even at 60 min after feeding. However, the pargyline-induced accumulation of *t*-MH increased solely in the Me5 region of the *ad lib* fed group, when compared with those of the isocaloric tube-fed group ($P < 0.001$), the water tube-loading group ($P < 0.001$), and the nonfed control group ($P < 0.01$). In the hypothalamus, the pargyline-induced accumulation of *t*-MH in the pellet-fed group was higher than those in the isocaloric tube-fed group ($P < 0.05$), the water loading group ($P < 0.05$) and the nonfed control group ($P < 0.01$). However, the pargyline-induced accumulation of *t*-MH in the isocaloric tube-fed and the water loading groups did not differ from that in the nonfed control group.

Discussion

We previously showed that the infusion of FMH or H_1-receptor antagonists into either the third cerebroventricle or the VMH elicited feeding with short latency (7, 8, 14). These findings indicate that the sudden reduction of neuronal HA *per se* or the blockade of HA action at the H_1-receptor site in the satiety center thus induces feeding because of the attenuated HA action. To verify the physiological roles of endogenous HA in the Me5 and the VMH, we investigated the feeding parameters including meal size, meal duration, and eating speed after depletion of neuronal HA by FMH from those loci.

FMH is a specific "suicide" inhibitor of an HA synthesizing enzyme of histidine decarboxylase (HDC) (21). A "suicide" inhibitor is a substrate activated by its targeting enzyme, and their chemical reaction irreversibly inhibits the activity of the target enzyme (22). FMH inactivates HDC in a time- and concentration-dependent manner. Other pyridoxal-dependent enzymes, such as dopamine decarboxylase and glutamate decarboxylase, have been reported to be unaffected by FMH (10, 23, 24). FMH depletes brain neuronal HA almost completely from the nerve terminal within 1 hr after the intraperitoneal injection, and this effect persists for at least 48 hr after its administration (20, 21, 25). These findings support the fact that the direct infusion of FMH into either the VMH or the Me5 may inhibit HA synthesis and release neuronal HA more rapidly than an intraperitoneal injection.

A decreased eating speed and prolonged meal duration were observed when HA was depleted from the Me5 by bilateral microinfusion of FMH, and thus had no effect on meal size. Bilateral microinfusion of FMH into the VMH increased the size and the duration of the first meal without affecting the eating speed. The acute feeding induced by FMH (25) was designed to be prevented by a 5-hr food deprivation. In addition, the mechanical stimuli induced by the microinfusion of PBS did not affect any meal parameters. Taken together, these findings indicate that HA in the Me5 appears to control mastication through the modulation

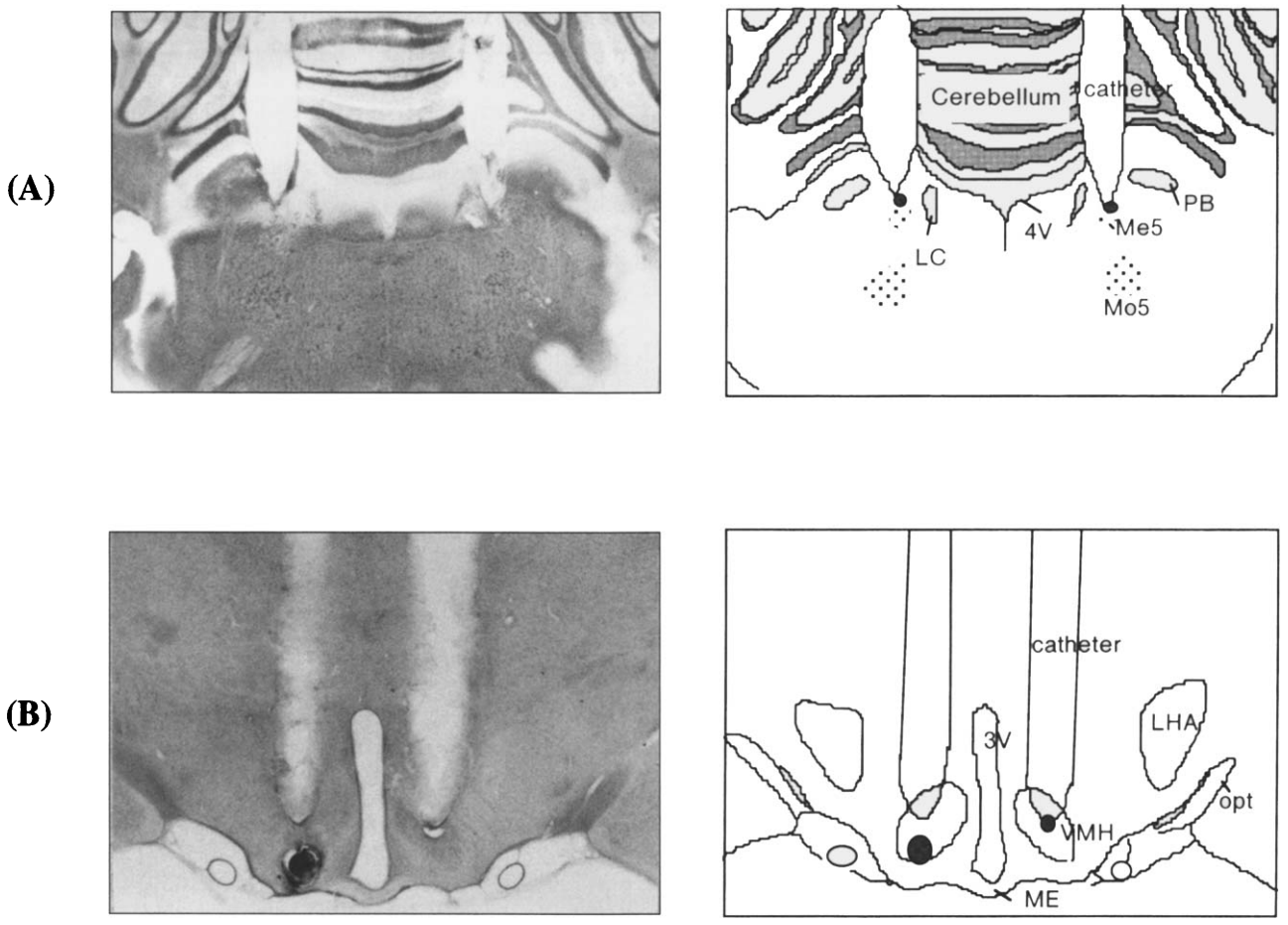

Figure 3. Histological identification of cannula tips stained by Neutral red in a frontal section (plate 56) of the rat mesencephalic sensory trigeminal nucleus (Me5) (A, left) and its schematic drawing (A, right), and in a frontal section (plate 27) of the rat ventromedial hypothalamus (VMH) (B, left) and its schematic drawing (B, right). Dark spots: the representative infusion sites stained by Pontamine sky blue. Dye was bilaterally localized in the Me5 (×40) or the VMH (×40). The distance from the interaural line according to the brain atlas by Paxinos and Watson was −0.68 mm (plate 56) and 6.70 mm (plate 27). LC, Locus coeruleus. Mo5, motor trigeminal nucleus. PB, parabrachial nucleus. 4V, 4th cerebroventricle. LHA, lateral hypothalamic area. ME, median eminence. opt, optic tract. 3V, 3rd cerebroventricle.

of signal transduction from the oral cavity to the Mo5 *via* the Me5, and the food intake activates hypothalamic HA to regulate meal consumption, which thus supports the essential role of hypothalamic HA in the cessation of sustained feeding.

It remains unclear as to why changes in the eating parameters induced by a depletion of FMH were differentiated between the VMH and the Me5. One possible explanation is that HA may exert a dual effect on feeding behavior. In fact, the feeding abnormalities observed in obese Zucker (*fa/fa*) rats also support this concept. Obese Zuckers failed to regulate food intake at levels of H_1- (7, 9, 14) and H_3-receptors (10, 26) in the brain. Simultaneously, the reduced activity of HDC in the brain was found in the obese Zuckers (27), which led to the insufficient production of brain neuronal HA (27, 28). This defect in the HA synthesizing enzyme of HDC produced HA dysfunction in regulation of the feeding parameters (27, 28). The HA dysfunction in obese Zuckers mimics the distorted meal patterns of FMH-treated rats (i.e., a larger meal size, longer duration, and slower eating speed (29)).

According to the present results of pargyline-induced accumulation of *t*-MH, HA turnover rate in the brain region including the Me5 increased at 15 min in the early phase of feeding, but the rate in the hypothalamus increased at 60 min in the late phase since the sampling times at 15 and 60 min correspond to the period during feeding and after feeding, respectively. The increase in the HA turnover rate in the VMH following feeding may be related to this increase in the pargyline-induced accumulation of *t*-MH in the hypothalamus. The HA nerve systems were actually activated in response to feeding in both the Me5 and VMH brain regions, although the time necessary for such activation differed for both regions. These findings also support the concept that HA in the Me5 is involved in the masticatory function while HA in the VMH is involved in the satiating function.

Regarding the process of satiety following feeding, gustatory information, baroceptive sensation such as gastric filling, chemosensitive information from the pancreas, the liver, and the duodenum, and humoral information in the blood and the cerebrospinal fluid have all been shown to

Table I. Histamine (HA) Concentration and Pargyline-Induced Accumulation of *tele*-Methylhistamine (*t*-MH) in the Brain Region Including the Mesencephalic Trigeminal Nucleus (Me5) and the Hypothalamus at 15 and 60 min After the Initiation of Pellet Feeding

Treatment	Pargyline-pretreated			PBS-pretreated		
	n	HA (nmol/g tissue)	*t*-MH (nmol/g tissue)	*n*	HA (nmol/g tissue)	*t*-MH (nmol/g tissue)
Brain region including the Me5						
15 min after feeding						
nonfed	6	0.24 ± 0.03	0.37 ± 0.05	6	0.22 ± 0.03	0.21 ± 0.03
fed	6	0.29 ± 0.03	0.69 ± 0.10[b]	6	0.20 ± 0.03	0.19 ± 0.04
60 min after feeding						
nonfed	6	0.27 ± 0.02	0.80 ± 0.05	6	0.22 ± 0.02	0.22 ± 0.04
fed	6	0.25 ± 0.02	1.07 ± 0.07[a]	6	0.22 ± 0.04	0.22 ± 0.04
Hypothalamus						
15 min after feeding						
nonfed	6	3.68 ± 0.14	2.49 ± 0.21	6	3.40 ± 0.19	2.02 ± 0.12
fed	6	3.78 ± 0.18	2.80 ± 0.17	6	3.45 ± 0.10	2.19 ± 0.11
60 min after feeding						
nonfed	6	3.68 ± 0.14	3.22 ± 0.21	6	3.72 ± 0.37	2.48 ± 0.13
fed	6	3.53 ± 0.40	3.94 ± 0.16[a]	6	3.67 ± 0.36	2.16 ± 0.16

Note. Values represent the mean ± SEM. *n*, number of rats tested.
[a] $P < 0.05$ vs corresponding nonfed controls.
[b] $P < 0.01$ vs corresponding nonfed controls.

Table II. Effects of Tube Feeding on the Histamine (HA) Concentration and Pargyline-Induced Accumulation of *tele*-Methylhistamine (*t*-MH) in the Brain Region Including the Mesencephalic Trigeminal Nucleus (Me5) and the Hypothalamus 60 min After Feeding

	Region including Me5			Hypothalamus		
	HA (nmol/g tissue)	*t*-MH (nmol/g tissue)	*n*	HA (nmol/g tissue)	*t*-MH (nmol/g tissue)	*n*
Ad lib feeding (pellet)	0.20 ± 0.02	1.07 ± 0.07[a,b]	6	4.10 ± 0.91	4.26 ± 0.38[c,d]	6
Tube feeding (liquid diet)	0.21 ± 0.02	0.66 ± 0.07	6	4.03 ± 0.19	3.46 ± 0.10	6
Tube feeding (water loading)	0.26 ± 0.03	0.69 ± 0.09	6	3.94 ± 0.30	3.53 ± 0.18	6
Nonfed control	0.24 ± 0.02	0.75 ± 0.04	6	3.97 ± 0.32	3.21 ± 0.12	6

Note. Values represent the mean ± SEM. *n*, number of rats tested.
[a] $P < 0.01$ vs nonfed control.
[b] $P < 0.001$ vs liquid diet and water loading.
[c] $P < 0.01$ vs nonfed control.
[d] $P < 0.05$ vs liquid diet and water loading.

regulate food intake (30). A question can be raised as to which of those signal messages may be a trigger for the activation of hypothalamic HA systems. Postprandial humoral factors are not likely inducers of feeding since an energy deficit in the central nervous system, such as starvation for more than 36 hr, insulin-induced hypoglycemia, glucoprivation in the neurons induced by 2-deoxy-D-glucose, or low level of *in vitro* glucose concentration in the medium, have all been shown to increase the HA turnover in the hypothalamus (12, 17, 20). Therefore, postprandial nutritional enrichment is not considered to be an inducer for the activation of HA neuron systems.

To answer this question, changes in the HA turnover rate, both in the hypothalamus and the Me5 region, were thus evaluated by the following 4 groups: the groups with *ad libitum* feeding of pellet chow, intubation of isocaloric liquid diet or water loading into the stomach, and nonfed treatment. Neither the group with tube feeding nor that with water loading failed to increase the HA turnover rate in the hypothalamus or the Me5 region. Thus, gastric loading or postprandial metabolic factors are not considered to be activators of the HA turnover in the hypothalamus or the Me5 region, but sensations originating from the oropharyngeal region can activate the HA systems in the hypothalamus and the Me5. The oral proprioceptive signals induced by mastication received at the Me5 may modulate hypothalamic HA neurons through the ascending pathway from the Me5 to the posterior hypothalamus (6) where the cell bodies of HA abundantly exist. In fact, our previous study demonstrated that a depletion of HA by FMH altered the meal parameters of the intake volume and its duration, which depended on the food consistency of the soft food and hard pellets (29). However, the possibility that such HA activation may be due to some other sensations such as gustation, textural sensation and so on, cannot be ruled out.

The activation of HA neurons modulates the mastica-

tory functions through the Me5, and then through the Mo5 in the early phase of feeding. On the other hand, the HA activation enhances satiation through the VMH in the late phase of feeding. The present results are thus considered to demonstrate another new function of hypothalamic HA related to the known mechanisms influencing feeding regulation.

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