Hypothalamic Neuronal Histamine Modulates Febrile Response but Not Anorexia Induced by Lipopolysaccharide

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This study examined the contribution of hypothalamic neuronal histamine (HA) to the anorectic and febrile responses induced by lipopolysaccharide (LPS), an exogenous pyrogen, and the endogenous pyrogens interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). Intraperitoneal (ip) injection of LPS, IL-1β, or TNF-α suppressed 24-hr cumulative food intake and increased rectal temperature in rats.

To analyze the histaminergic contribution, rats were pretreated with intracerebroventricular (icv) injection of 2.44 mmol/kg or ip injection of 244 mmol/kg of α-fluoromethylhistidine (FMH), a suicide inhibitor of histidine decarboxylase (HDC), to deplete neural HA. The depletion of neural HA augmented the febrile response to ip injection of LPS and IL-1β and alleviated the anorectic response to ip injection of IL-1β. However, the depletion of neural HA did not modify the LPS-induced anorectic response or TNF-α–induced febrile and anorectic responses. Consistent with these results, the rate of hypothalamic HA turnover, assessed by the accumulation of *tele*-methylhistamine (*t*-MH), was elevated with ip injections of LPS and IL-1β, but unaffected by TNF-α at equivalent doses. This suggests that (i) LPS and IL-1β activate hypothalamic neural HA turnover; (ii) hypothalamic neural HA suppresses the LPS- and IL-1β–induced febrile responses and accelerates the IL-1β–induced anorectic response; and (iii) TNF-α modulates the febrile and anorectic responses via a neural HA-independent pathway. Therefore, hypothalamic neural HA is involved in the IL-1β–dominant pathway, rather than the TNF-α–dominant pathway, preceding the systemic inflammatory response induced by exogenous pyrogens, such as LPS. Further research on this is needed. Exp Biol Med 230:334–342, 2005

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Introduction

Hypothalamic histamine (HA) neurons originating from the tuberomammillary nuclei distributed in the posterior hypothalamus project diffusely throughout the brain (1). This neuronal system regulates a variety of physiologic functions involving energy metabolism, hormone regulation, mastication, thermoregulation, learning and memory, arousal state, and immunity (1, 2). Our studies of thermoregulation (2) have demonstrated that high ambient temperature increases the HA concentration in the hypothalamus (3). α-Fluoromethylhistidine (FMH), a suicide inhibitor of HA-synthesizing histidine decarboxylase (HDC), depleted neuronal HA from the hypothalamus, resulting in elevated body temperatures at high ambient temperatures (3). These results imply that hypothalamic HA neurons are activated by high ambient temperature to maintain a constant body temperature. The homeostatic thermoregulation maintained by hypothalamic neuronal HA was confirmed in studies of an endogenous pyrogen, interleukin-1β (IL-1β; Refs. 4, 5). Infusion of IL-1β into the third cerebral ventricle elevated both the HA turnover rate and the HDC activity in the hypothalamus (4). Pretreatment with FMH reinforced the elevated body temperature induced by IL-1β, indicating that neuronal HA prevents excessive body temperature elevation.

Studies showed that hypothalamic neuronal HA suppresses food intake via HA H_1-receptors in the ventromedial hypothalamic nucleus and paraventricular nucleus (6, 7). Moreover, elevated ambient temperature inhibits food intake, and injury to the hypothalamic preoptic area can abolish the negative relationship between appetite and temperature (8). In fact, high ambient temperature or administration of IL-1β, a pyrogenic cytokine, suppressed

food intake. The anorexia produced in this manner was alleviated by the depletion of neuronal HA after pretreatment with FMH (3). Therefore, thermoregulatory factors appear to be involved in regulating appetite together with body temperature via the modulation of hypothalamic neuronal HA.

Similar to IL-1β, tumor necrosis factor-α (TNF-α), another pyrogenic cytokine, is mobilized during inflammation, and elevates body temperature and suppresses food intake via the preoptic area (9). Lipopolysaccharide (LPS) is an exogenous pyrogen that liberates endogenous pyrogens, such as IL-1β and TNF-α, from monocytes, and provokes a febrile response and feeding suppression. Both IL-1β and LPS activate HA turnover or HDC activity in the rat hypothalamus (4, 10). Therefore, hypothalamic neuronal HA is likely involved in food intake and thermoregulation via the physiologic actions of the pyrogenic cytokines that are provoked during inflammation, such as with LPS administration. This study investigated how neuronal HA in the hypothalamus integrates anorectic and thermogenic information in the hypothalamus.

Materials and Methods

Animals. Mature male Wistar King A rats weighing 270–300 g were used. The rats were housed four to a cage in a soundproof room illuminated daily from 0700 to 1900 hrs (12:12-hr light:dark cycle) and maintained at 21 ± 1°C and 55 ± 5% humidity. They were fed standard rat chow (Clea rat chow; Japan Clea, Fukuoka, Japan), allowed access to tap water *ad libitum*, and handled for 5 mins daily. 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* (11).

Reagents. Lipopolysaccharide (serotype 055: B5; Sigma Chemical Co., St. Louis, MO) was dissolved in phosphate-buffered saline (PBS) to a concentration of 3, 4.5, or 6 μg/ml. Interleukin-1β (Otsuka Pharmaceutical, Tokushima, Japan) was dissolved in PBS to a concentration of 0.06, 0.12, or 0.24 μ*M*. Tumor necrosis factor-α (Dainippon Pharmacy, Tokyo, Japan) was dissolved in PBS to a concentration of 0.09, 0.18, or 0.36 μ*M*. The suicide inhibitor of HDC, FMH, was dissolved in PBS to a concentration of 67.2 m*M*, and was used at 224 μmol/kg for intraperitoneal (ip) injection or 2.24 μmol/kg for intracerebroventricular (icv) injection (a dose previously determined to deplete most neural histamine in the hypothalamus). Pargyline hydrochloride (Sigma Chemical), a monoamine oxidase B inhibitor, was dissolved in PBS to a concentration of 0.099 m*M* to estimate HA release in the hypothalamus. Each solution was freshly prepared immediately before testing. The pH of each solution was adjusted to 6.4–7.2.

Surgery. The implantation of a chronic cannula into the third cerebroventricle was performed (anterior/posterior axis: +6.0 mm from the ear bar zero; lateral: ±0 mm; depth: +7.7 mm from the cortical surface) with a 29-gauge inner cannula and a 23-gauge outer cannula at least 1 week before testing. All rats included in the study exhibited a clear liquid regurgitation from the implanted cannula at the time of cannula implantation, and indocian green dye was infused after each experiment to ascertain patency and placement of the cannula.

Measuring Food Intake and Rectal Temperature. To evaluate anorectic and febrile responses, 50 rats were divided into 10 equal groups: the PBS, LPS(3), LPS(4.5), LPS(6), IL-1β(0.06), IL-1β(0.12), IL-1β(0.24), TNF-α(0.09), TNF-α(0.18), and TNF-α(0.36) groups. Body weight and cumulative food intake were measured at 1700 hrs. They were administrated with 1.0 ml of LPS (3, 4.5, or 6 μg/ml), IL-1β (0.06, 0.12, or 0.24 μ*M*), TNF-α (0.09, 0.18, or 0.36 μ*M*), or PBS at 1800 hrs. To measure rectal temperature, another 10 groups of five rats each were treated with the same test solutions with a different time schedule for ip infusion: test solutions were infused at 1100 hrs to exclude the influence of increased activity on the core temperature of rats during the dark period.

To estimate relations between depleted neural histamine and inflammatory response, 40 rats were divided into eight equal groups: the PBS/PBS, FMH/PBS, PBS/LPS, FMH/LPS, PBS/IL-1β, FMH/IL-1β, PBS/TNF-α, and FMH/TNF-α groups. They were pretreated with an ip injection of 1.0 ml of 67.2 m*M* FMH or PBS, and then given an ip infusion of 4.5 μg of LPS, 0.12 μmol of IL-1β, or 0.18 μmol of TNF-α in a volume of 1.0 ml, or an equal volume of PBS. Body weight and cumulative food intake were measured at 1700 hrs. They were pretreated with FMH or PBS at 1730 hrs, and this was followed by an ip infusion of LPS, IL-1β, or TNF-α at 1800 hrs. To measure rectal temperature, another eight groups of five rats each were treated with the same test solutions with a different time schedule for ip infusion: FMH or PBS was infused at 1000 hrs and the test solutions infused at 1100 hrs.

To assess the effects of hypothalamic depletion of neural histamine on LPS-induced inflammatory response, 20 rats were divided into four equal groups: the PBS_{icv}/PBS, FMH_{icv}/PBS, PBS_{icv}/LPS, and FMH_{icv}/LPS groups. They were pretreated with icv infusion of 10 μl/rat of 67.2 m*M* FMH or PBS, and then given an ip infusion of 4.5 μg of LPS or PBS in a volume of 1.0 ml. Body weight and cumulative food intake were measured at 1700 hrs. The icv pretreatment was at 1730 hrs followed by the ip infusion at 1800 hrs. To measure rectal temperature, another four groups of five rats each were treated with the same test solutions with a different time schedule for infusion: FMH or PBS was infused by icv at 1000 hrs and the test solutions were infused by ip at 1100 hrs. Using a rectal thermometer (BAT-12; Physitemp Instruments, Clifton, NJ), the rectal temperature was measured immediately before the FMH treatment and every hour for 4 hrs after the cytokine treatment.

Measuring HA Turnover. The HA turnover rate

Table 1. Concentrations of Neuronal HA and Pargyline-Induced Accumulation of *t*-MH After ip Infusion of 4.5 μg of LPS, 0.12 μmol of IL-1β, and 0.18 μmol of TNF-α[a]

Treatment	No.	HA (nmol/g)	*t*-MH (nmol/g)
PBS[b]	5	3.181 ± 0.289	2.405 ± 0.211
LPS	5	3.086 ± 0.116	2.804 ± 0.093*
IL-1β	5	3.465 ± 0.311	3.882 ± 0.702*,**
TNF-α	5	3.022 ± 0.058	2.432 ± 0.443

[a] Each value, mean ± SEM. HA, histamine; *t*-MH, *tele*-methylhistamine; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor.
[b] PBS, a group with ip administration of phosphate-buffered saline.
* $P < 0.05$ vs. corresponding PBS group; ** $P < 0.05$ vs. LPS group.

was measured following the method of Oishi *et al.* (12). Hypothalamic HA and *tele*-methylhistamine (*t*-MH), a major metabolite of HA, were measured using high-performance liquid chromatography (HPLC; Shimazu, Osaka, Japan; Ref. 11). To assess the effects of pyrogens on hypothalamic HA turnover, 20 rats were divided into four equal groups: the PBS, LPS, IL-1β, and TNF-α groups. Each group was pretreated with ip infusion of 0.33 mmol/kg of pargyline hydrochloride in a volume of 1.0 ml at 0950 hrs, and this was followed by an ip infusion of 4.5 μg of LPS, 0.12 μmol of IL-1β, or 0.18 μmol of TNF-α in a volume of 1.0 ml or an equal volume of PBS at 1000 hrs. The dosages of these pyrogens were adjusted to the equivalent potency for feeding suppression in the subsequent experiments. To assume the effects of ip FMH administration on hypothalamic HA turnover, 20 rats were divided into four equal groups: the PBS/PBS, FMH/PBS, PBS/LPS, and FMH/LPS groups. Each group was pretreated by ip administration of 0.33 mmol/kg of pargyline hydrochloride in a volume of 1.0 ml at 0950 hrs, preceded by ip infusion of 1.0 ml of 67.2 m*M* FMH or PBS at 0930 hrs, and this was followed by an ip infusion of 4.5 μg of LPS or PBS in a volume of 1.0 ml at 1000 hrs. Each rat was decapitated 60 mins after beginning the ip injection of the test solution. All rats were served no food and water during the experiment for histamine measurement to exclude the influence of food and water intake on amine content. These procedures have been described in detail elsewhere (11).

Statistical Analysis. Food intake and rectal temperature were evaluated using two-way ANOVA with the replication method followed by the Scheffé test. Significance was defined as $P < 0.05$. The data were analyzed using the computer program StatView, version 5.0 9/99 (SAS Institute Inc., Cary, NC).

Results

Effects of HA Depletion on the Anorexia and Hyperthermia Induced by LPS, IL-1β, and TNF-α. Figure 1 shows the effects of FMH-induced HA depletion on the anorectic and hyperthermic actions of LPS. Administration of LPS suppressed 24-hr cumulative food intake (Fig. 1A) and elevated rectal temperature (Fig. 1B) in a dose-related manner. The PBS/LPS group treated with LPS had a lower 24-hr cumulative food intake (Fig. 1C) and higher body temperature (Fig. 1D) than the PBS controls (both $P < 0.05$). Depletion of HA augmented LPS-induced hyperthermia more than in the PBS/LPS control ($P < 0.05$; Fig. 1D), but did not affect LPS-induced anorexia (Fig. 1C). Administration of IL-1β suppressed 24-hr cumulative food intake (Fig. 2A) and elevated rectal temperature (Fig. 2B) in a dose-related manner. Administration of IL-1β *per se* decreased the 24-hr cumulative food intake (Fig. 2C) and elevated rectal temperature (Fig. 2D) compared with the PBS controls (both $P < 0.05$). In addition, the anorectic effect of IL-1β was attenuated (Fig. 2C), whereas its hyperthermic effect was augmented (Fig. 2D) with the depletion of neuronal HA in the hypothalamus resulting from FMH administration compared with the PBS/IL-1β controls (both $P < 0.05$). Administration of TNF-α suppressed 24-hr cumulative food intake (Fig. 3A) and elevated rectal temperature (Fig. 3B) in a dose-related manner. As with the LPS and IL-1β results, TNF-α *per se* decreased the 24-hr cumulative food intake (Fig. 3C) and elevated rectal temperature (Fig. 3D) compared with the PBS controls (both $P < 0.05$). In contrast, depletion of neuronal HA did not significantly affect the anorexia (Fig. 3C) or hyperthermia (Fig. 3D) induced by TNF-α compared with the PBS/TNF-α controls. Figure 4 shows the effects of FMH icv infusion-induced HA depletion on the anorectic and hyperthermic actions of LPS. The PBS_{icv}/LPS group treated with LPS had a moderate 24-hr cumulative food intake suppression (Fig. 4A) and higher body temperature (Fig. 4B) than the PBS_{icv}/PBS controls (both $P < 0.05$). Infusion of FMH by icv, which depleted hypothalamic HA, augmented LPS-induced hyperthermia compared with the PBS_{icv}/LPS group ($P < 0.05$; Fig. 4B), but did not affect LPS-induced anorexia (Fig. 4A).

Effects of LPS, IL-1β, and TNF-α on HA Turnover in the Hypothalamus. The turnover rate of HA expressed as the pargyline-induced accumulation of *t*-MH was elevated more by ip infusion of either LPS or IL-1β than by the PBS controls (both $P < 0.05$), as shown in Table 1. Moreover, the IL-1β–induced hypothalamic *t*-MH content was greater than in the LPS group ($P < 0.05$). By

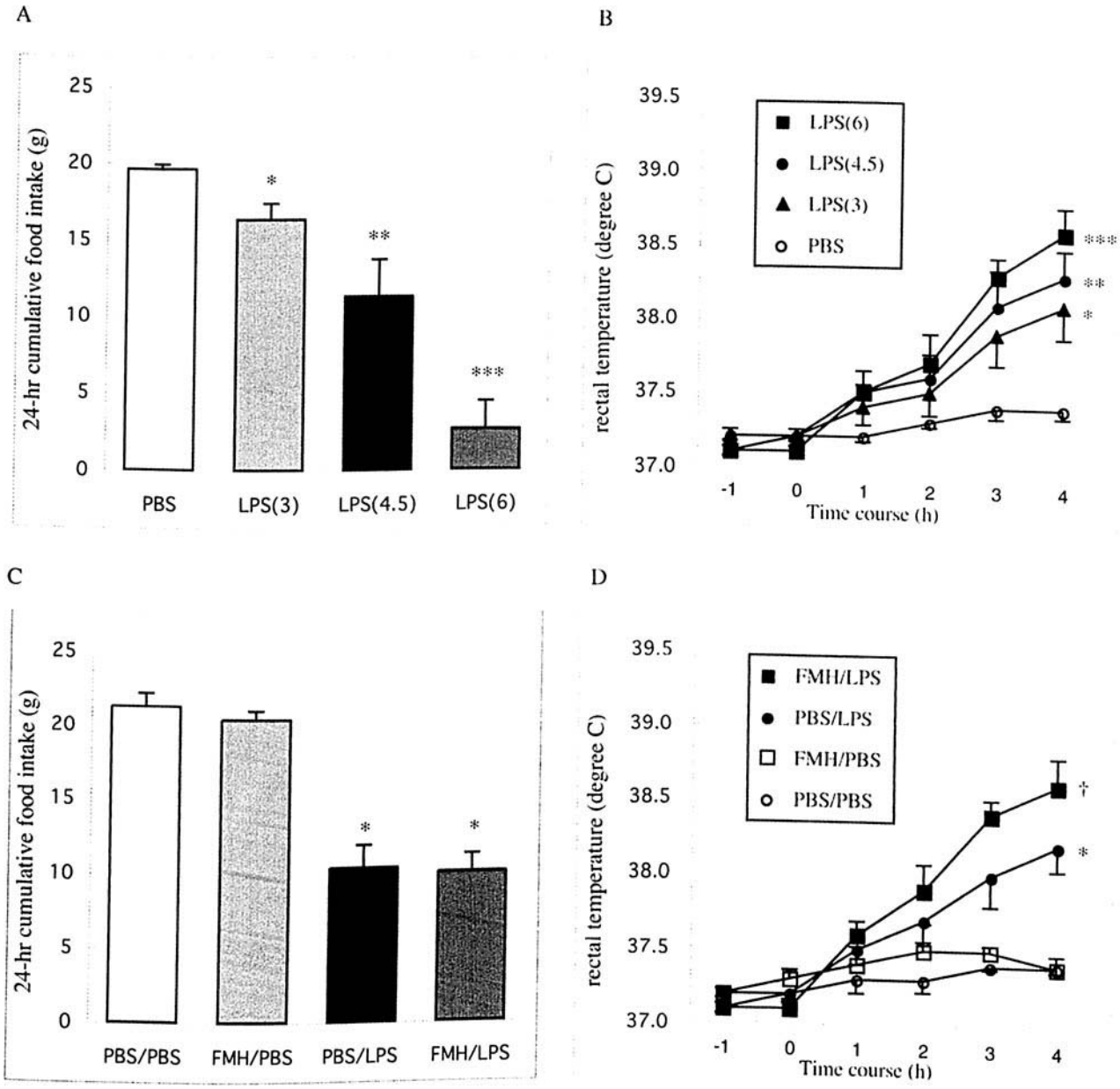

Figure 1. Changes in anorexia and hyperthermia induced by lipopolysaccharide (LPS) after neuronal histamine (HA) depletion by α-fluoromethylhistadine (FMH). (A) The 24-hr cumulative food intake treated with 3.0, 4.5, and 6.0 μg/rat of LPS. (B) The rectal temperature treated with 3.0, 4.5, and 6.0 μg/rat of LPS. (C) The effect of neuronal HA depletion by 244 mmol/kg of FMH on 24-hr cumulative food intake treated with 4.5 μg/rat of LPS. (D) The effect of neuronal HA depletion by 244 mmol/kg of FMH on rectal temperature treated with 4.5 μg/rat of LPS. Data from five rats is shown for each group; each value, mean ± SEM. PBS, a group with ip injection of phosphate-buffered saline (PBS); LPS(3), a group with ip injection of 3.0 μg of LPS; LPS(4.5), a group with ip injection of 4.5 μg of LPS; LPS(6), a group with ip injection of 6.0 μg of LPS; PBS/PBS, a group pretreated with ip injection of PBS followed by ip infusion of PBS; FMH/PBS, a group with ip administration of FMH followed by ip administration of PBS; PBS/LPS, a group with ip administration of PBS followed by ip administration of LPS; FMH/LPS, a group with ip administration of FMH followed by ip administration of LPS. * $P < 0.05$ vs. the corresponding control groups; ** $P < 0.05$ vs. the LPS(3) group; *** $P < 0.05$ vs. the LPS(4.5) group; † $P < 0.05$ vs. the PBS/LPS group.

contrast, the HA turnover rate in the hypothalamus was not significantly affected by ip infusion of TNF-α (Table 1). Table 2 shows the turnover rate of HA expressed as the pargyline-induced accumulation of *t*-MH with FMH ip infusion. The HA content was significantly decreased and *t*-MH content was not detected in the FMH/PBS and FMH/LPS groups (both $P < 0.01$), compared with the PBS/PBS or PBS/LPS groups.

Discussion

The main results of this study were as follows: (i) LPS administration produced anorexia and hyperthermia, but HA depletion resulting from FMH administration augmented the hyperthermia, whereas LPS administration elevated HA turnover; (ii) the IL-1β–enhanced anorexia and hyperthermia were significantly modified after HA depletion using FMH, with elevation of histamine turnover in the

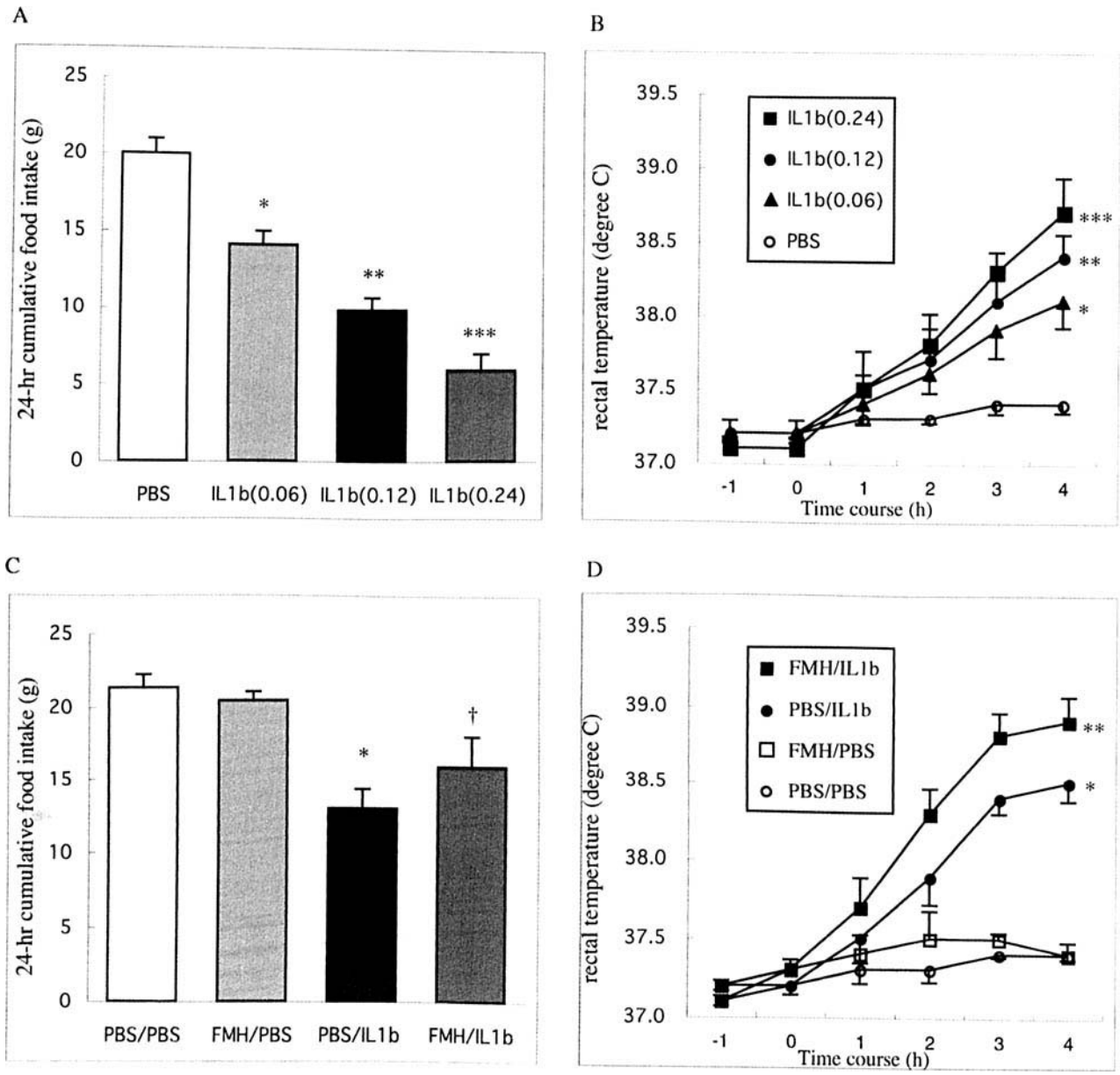

Figure 2. Changes in anorexia and hyperthermia induced by interleukin (IL)-1β after neuronal histamine (HA) depletion by α-fluoromethylhistadine (FMH). (A) The 24-hr cumulative food intake treated with 0.06, 0.12, and 0.24 nmol/rat of IL-1β. (B) The rectal temperature treated with 0.06, 0.12, and 0.24 nmol/rat of IL-1β. (C) The effect of neuronal HA depletion by 244 mmol/kg of FMH on 24-hr cumulative food intake treated with 0.12 nmol/rat of IL-1β. (D) The effect of neuronal HA depletion by 244 mmol/kg of FMH on rectal temperature treated with 0.12 nmol/rat of IL-1β. Data from five rats is shown for each group; each value, mean ± SEM. PBS/PBS, a group pretreated ip with phosphate-buffered saline (PBS) followed by ip infusion of PBS; FMH/PBS, a group with ip administration of FMH followed by ip administration of PBS; PBS/IL-1β, a group with ip administration of PBS followed by ip administration of IL-1β; FMH/IL-1β, a group with ip administration of FMH followed by ip administration of IL-1β. The treatment of procedures in the other groups is the same as in Figure 1, as applicable. * $P < 0.05$ vs. the corresponding control groups; ** $P < 0.05$ vs. the IL-1β(0.06) group; *** $P < 0.05$ vs. the IL-1β(0.12) group; † $P < 0.05$ vs. the PBS/IL-1β group.

hypothalamus; (iii) in contrast, the TNF-α–enhanced anorexia and hyperthermia were not affected by HA depletion using FMH, and this corresponded to the small augmentation of histamine turnover in the hypothalamus.

There is growing evidence that hypothalamic neuronal HA contributes to thermoregulation and feeding behavior (3, 11, 13). Previously, we demonstrated that hypothalamic HA neurons activated by a high ambient temperature contribute to homeostatic maintenance of the temperature of the human body (3). The hypothermic effect of HA prevents mammals from excessive thermogenesis (14, 15) via neural connections with the hypothalamic thermoregulatory center of the preoptic area (8, 16) or the brainstem, which is involved in heat loss mechanisms, such as polypnea (17). This study demonstrated that the depletion of hypothalamic neuronal HA augmented LPS-induced hyperthermia, but did not affect LPS-induced anorexia, although LPS administration accelerated the release of

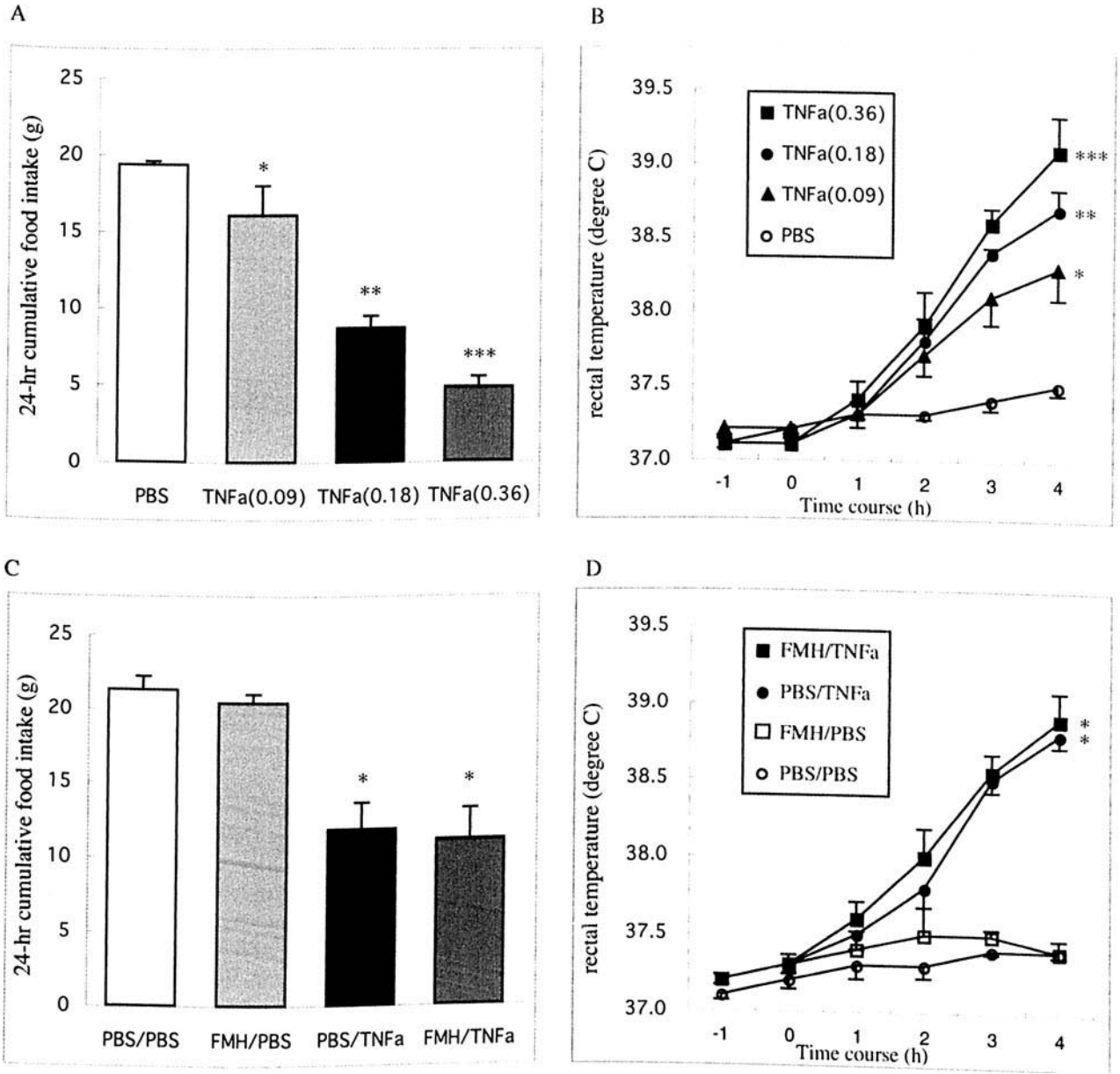

Figure 3. Changes in anorexia and hyperthermia induced by tumor necrosis factor (TNF)-α after neuronal histamine (HA) depletion by α-fluoromethylhistadine (FMH). (A) The 24-hr cumulative food intake treated with 0.09, 0.18, and 0.36 nmol/rat of TNF-α. (B) The rectal temperature treated with 0.09, 0.18, and 0.36 nmol/rat of TNF-α. (C) The effect of neuronal HA depletion by 244 mmol/kg of FMH on 24-hr cumulative food intake treated with 0.18 nmol/rat of TNF-α. (D) The effect of neuronal HA depletion by 244 mmol/kg of FMH on rectal temperature treated with 0.18 nmol/rat of TNF-α. Data from five rats is shown for each group; each value, mean ± SEM. PBS/PBS, a group pretreated ip with phosphate-buffered saline (PBS) followed by ip infusion of PBS; FMH/PBS, a group with ip administration of FMH followed by ip administration of PBS; PBS/TNF-α, a group with ip administration of PBS followed by ip administration of TNF-α; FMH/TNF-α, a group with ip administration of FMH followed by ip administration of TNF-α. The treatment of procedures in the other groups is same as in Figure 1, as applicable. * $P < 0.05$ vs. the corresponding control groups; ** $P < 0.05$ vs. the TNF-α(0.09) group; *** $P < 0.05$ vs. the TNF-α(0.18) group; † $P < 0.05$ vs. the PBS/TNF-α group.

neuronal HA in the hypothalamus. These results reinforce the hypothesis that hypothalamic neuronal HA plays an important role in preventing the excessive pyrogenic response induced by LPS. In contrast, LPS-induced anorexia might be regulated via HA-independent pathways. A previous report also showed that HA depletion caused by FMH did not affect LPS-induced anorexia (18). Lipopolysaccharide is reported to liberate endogenous pyrogens, such as IL-1β and TNF-α (19). Interleukin-1β and TNF-α induced by LPS act as signals to produce thermogenic responses via the brain (20, 21). As with LPS, in our study, IL-1β increased HA turnover in the hypothalamus, along with anorectic and hyperthermic responses. Anorectic and hyperthermic effects of IL-1β were attenuated and augmented, respectively, by peripheral treatment with FMH. These findings indicate that HA might be involved in both feeding suppression and hyperthermia via IL-1β. Although the effects of direct HA depletion by

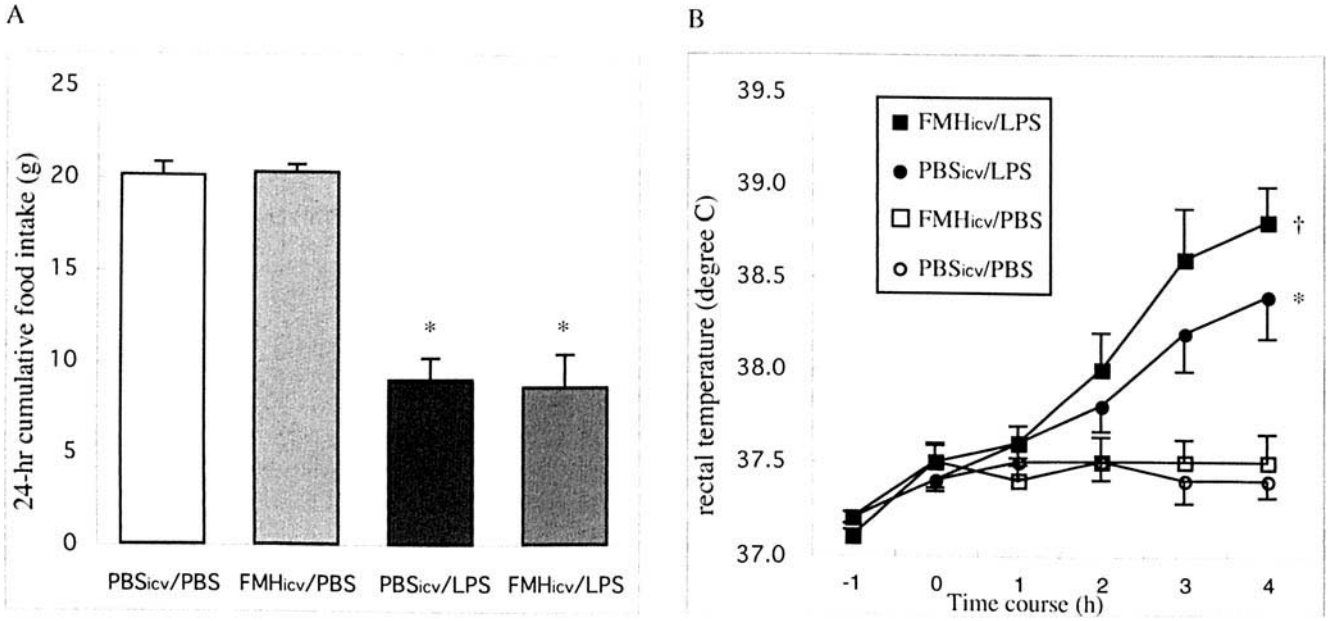

Figure 4. Changes in anorexia and hyperthermia induced by lipopolysaccharide (LPS) after neuronal histamine (HA) depletion by intracerebroventricular (icv) infusion of α-fluoromethylhistamine (FMH). (A) The effect of neuronal HA depletion by 2.44 mmol/kg of α-fluoromethylhistadine (FMH) icv infusion on 24-hr cumulative food intake treated with 4.5 μg/rat of LPS. (B) The effect of neuronal HA depletion by 2.44 mmol/kg of FMH icv infusion on rectal temperature treated with 4.5 μg/rat of LPS. Data from five rats is shown for each group; each value, mean ± SEM. PBS_{icv}/PBS, a group pretreated ip with phosphate-buffered saline (PBS) followed by icv infusion of PBS; FMH_{icv}/PBS, a group with icv administration of FMH followed by ip administration of PBS; PBS_{icv}/LPS, a group with icv administration of PBS followed by ip administration of LPS; FMH_{icv}/LPS, a group with icv administration of FMH followed by ip administration of LPS. * $P < 0.05$ vs. the corresponding control groups; † $P < 0.05$ vs. the PBS_{icv}/LPS group.

icv FMH administration on IL-1β–induced anorexia and hyperthermia were not evaluated in this study, the results are consistent with our previous studies (4, 5, 15). From the perspective of functional linkages involving LPS, IL-1β, and HA, it seems probable that IL-1β transmits thermoregulatory signals from LPS to hypothalamic HA neuronal systems. This signal pathway may not function for LPS-induced anorectic signals. This raises the question of why HA depletion has an inconsistent effect on the anorexia induced by LPS and IL-1β, although they both activate HA turnover. One possible explanation is that the levels at which LPS and IL-1β activate hypothalamic HA release differ, and the LPS-induced hypothalamic HA release might be insufficient for a massive HA-dependent reduction in the daily food intake. Another important cytokine that is released as an effect of LPS, TNF-α, produces both anorexia and hyperthermia (16, 22). The critical difference between the function of TNF-α and the functions of LPS and IL-1β was the effect on neuronal HA. We found that systemic TNF-α administration did not affect HA turnover in the hypothalamus. Consistent with this result, HA depletion did not influence either the anorexia or hyperthermia induced by TNF-α, implying that there is no functional relation between feeding behavior and the febrile response between the TNF-α signal pathways and HA neurons (23). Therefore, the hyperthermia induced by TNF-α may not be the main result of the thermogenesis promoted by LPS, because neuronal HA, which is unaffected by TNF-α, contributed to the

Table 2. Concentrations of Neuronal HA and Pargyline-Induced Accumulation of *t*-MH After ip Infusion of 4.5 μg of LPS or Same Volume of PBS Followed by ip Infusion of 10 μl/rat of 67.2 mM FMH or PBS[a]

Treatment	No.	HA (nmol/g)	*t*-MH (nmol/g)
PBS/PBS[b]	5	2.931 ± 0.311	2.288 ± 0.249
FMH/PBS[c]	5	1.349 ± 0.291**	ND
PBS/LPS[d]	5	2.863 ± 0.375	2.758 ± 0.198*
FMH/LPS[e]	5	1.427 ± 0.191**	ND

[a] Each value, mean ± SEM; ND, not determined. HA, histamine; *t*-MH, *tele*-methylhistamine; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; FMH, α-fluoromethylhistadine.
[b] PBS/PBS, a group with ip administration of PBS followed by ip administration of PBS.
[c] FMH/PBS, a group with ip administration of FMH followed by ip administration of PBS.
[d] PBS/LPS, a group with ip administration of PBS followed by ip administration of LPS.
[e] FMH/LPS, a group with ip administration of LPS followed by ip administration of FMH.

hyperthermia induced by LPS in this study. Interleukin-1β is likely a main mediator of the LPS-induced hyperthermia, although the possibility that TNF-α contributes to LPS hyperthermia, at least in part, cannot be excluded (24, 25). Combined, HA-dependent and HA-independent pathways in the hypothalamus may both be involved in inflammatory responses simultaneously induced by LPS. Pathway selection may depend on the endogenous cytokines released in response to inflammation (26).

Another crucial question is how do the signaling pathways of these cytokines differ? One possible explanation is the difference in their tendency to activate the prostanoid cascade, which mediates the inflammatory signals of these proinflammatory cytokines. A central infusion of prostaglandin E_2 (PGE_2) increased hypothalamic HA turnover in a dose-dependent manner (15). The administration of indomethacin, a cyclooxygenase inhibitor, abolished the increasing effects of IL-1β on HA turnover (15). Therefore, prostanoids serve as essential mediators of IL-1β, which induces thermogenic and anorectic responses (27–29). In contrast to the interaction of prostanoids with IL-1β, prostanoids are partially involved in the progression of anorexia and hyperthermia induced by TNF-α (30, 31). The obvious difference in the involvement of these cytokines in the prostanoid cascade is the difference in the abilities of IL-1β and TNF-α to stimulate HA release in the hypothalamus. Perhaps the inducible effect of LPS to accelerate hypothalamic HA turnover occurs because systemic LPS evokes sufficient prostanoid synthesis to accelerate the febrile response and hypothalamic HA release mediated mainly by IL-1β induction, but not by TNF-α.

Therefore, we suggest that, in the LPS-induced systemic inflammatory response, IL-1β–mediated systemic inflammatory signals to the central nervous system activate hypothalamic HA release to alleviate excessive hyperthermia, whereas TNF-α acts on the central nervous system via a hypothalamic HA-independent pathway, which progresses to a systemic inflammatory response, such as hyperthermia and anorexia. However, the details of hypothalamic HA-dependent or HA-independent regulation of the LPS-induced systemic inflammatory response remain unclear and need to be elucidated.

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