Hypothalamic Melanocortin System Regulates Sympathetic Nerve Activity in Brown Adipose Tissue

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To clarify the neuronal mechanism of the hypothalamic melanocortin system in regulating energy metabolism, we investigated the effects of centrally administered α-melanocyte-stimulating hormone (α-MSH) and agouti-related protein (AGRP), an agonist and an antagonist for the melanocortin 4 receptor (MC4-R), respectively, on the activity of sympathetic nerves innervating brown adipose tissue (BAT) and on BAT temperature. A bolus infusion of α-MSH (1 nmol) into the third cerebral ventricle (i3vt) significantly increased sympathetic nerve activity and elevated BAT temperature ($P < 0.05$). The i3vt infusion of AGRP (1 nmol) gradually suppressed BAT sympathetic nerve activity and was accompanied by a significant reduction in BAT temperature ($P < 0.05$). In conclusion, the hypothalamic melanocortin system may regulate peripheral energy expenditure, as well as thermogenesis, through its influence on BAT sympathetic nerve activity. Exp Biol Med 229:235–239, 2004

Key words: melanocortin 4 receptor; agouti-related protein; α-melanocyte-stimulating hormone; brown adipose tissue; sympathetic nerve activity

Pro-opiomelanocortin (POMC) and the melanocortin 4 receptor (MC4-R) play important roles in the regulation of food intake. In particular, the POMC/MC4-R system mediates leptin signaling to induce anorectic effects in rodents (1, 2). The POMC/MC4-R system is regulated endogenously by hypothalamic neuropeptides such as α-melanocyte-stimulating hormone (α-MSH), a POMC-derived neuropeptide, and agouti-related protein (AGRP). The α-MSH suppresses food intake and AGRP stimulates food intake by acting agonistically or antagonistically on the MC4-R, respectively (3, 4).

In addition to their regulation of energy intake, leptin and hypothalamic feeding-related substances regulate peripheral energy expenditure. Uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) plays a major role in energy expenditure and thermogenesis under the control of sympathetic nerves and several humoral factors such as thyroid hormone and leptin (5–9). The implication of the hypothalamic melanocortin system in the regulation of energy expenditure has been demonstrated in several recent studies in which chronic central administration of MC4-R agonist or antagonist affected the expression of BAT UCP1 and/or oxygen consumption (10–12). In a neuroanatomical study, POMC neurons in the hypothalamic arcuate nucleus (ARC) were found to project directly to the thoracic intermediolateral cell column (IML) of the spinal cord, a site of sympathetic preganglionic neurons (13). Furthermore, MC4-R mRNA expression has been identified in the IML and in the dorsal motor nucleus of the vagus nerve, a site of parasympathetic preganglionic neurons (14). These observations suggest that α-MSH and/or AGRP likely regulate energy expenditure through the sympathetic nerves that innervate BAT. In fact, central administration of MTII, an MC4-R agonist, results in increased BAT sympathetic nerve activity, although the effects of α-MSH or AGRP remain unclear (15). In the present study, we investigated the effects of acute central administration of α-MSH and AGRP on sympathetic nerve activity in BAT to clarify how the melanocortin system regulates the efferent pathway from the hypothalamus to BAT.

Materials and Methods

Animals. Mature male Sprague-Dawley rats (8–10 weeks old; Seac Yoshitomi, Fukuoka, Japan) were maintained in a 12:12-hr light:dark photoperiod (lights on at 0700 hr) in a temperature (21°C ± 1°C) and humidity (55% ± 5%) controlled room. Rats were allowed free access to food (pelleted rodent chow CE-2; Clea Japan, Tokyo, Japan) and water. All studies were conducted in accordance

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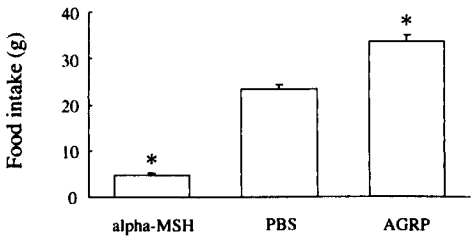

Figure 1. Effects of into the third cerebral ventricle (i3vt) infusion of α-melanocyte-stimulating hormone (α-MSH, 1 nmol); agouti-related protein (AGRP, 1 nmol); or phosphate-buffered saline (PBS, control group) on cumulative (24 hr) food intake. All data are the mean ± SEM ($n = 5$/group). *$P < 0.05$ versus control.

with the Oita Medical University Guidelines based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Reagents. The α-MSH (Sigma Chemical Co., St. Louis, MO) and AGRP (Peptide Institute, Osaka, Japan) were each dissolved in phosphate-buffered saline (PBS) to a final concentration of 1×10^{-4} M. Each solution was freshly prepared on the day of its administration. The pH of each solution was adjusted to 6.4–7.2.

Cerebroventricular Cannula Implantation. Each rat was fixed in a stereotaxic apparatus (Narishige, Japan) under sodium pentobarbital anesthesia (45 mg/kg, ip), and a stainless steel guide cannula (23 gauge) was implanted chronically into the i3vt. A stainless steel wire stylet (29 gauge) was left in the guide cannula to keep it patent and prevent leakage of cerebrospinal fluid. Surgery was carried out at least 1 week prior to the infusion of the test solutions; the details of the surgical procedure have been described elsewhere (16).

Measurement of Food Intake. All rats were handled for 5 mins daily on 3 successive days before each experiment to equilibrate their arousal levels. The rats that underwent surgery were allowed to recover for 5 days before the experiment. On the day of testing, the rats were confirmed to have had normal food intake and have normal body weight. Fifteen rats, matched on the basis of body weight, were divided into three groups ($n = 5$ for each) and were treated with α-MSH (1 nmol), AGRP (1 nmol), or PBS (control group). Test solutions were administered at 1500 hr to unrestrained, unanesthetized rats via the i3vt cannula at a rate of 1.0 μl/min for 10 mins. Immediately after injection of the test solution, animals were presented with a pre-weighed amount of chow. Cumulative food consumption was measured for 24 hrs.

Measurement of BAT Temperature. A plastic-coated thermocouple was inserted into the interscapular BAT of 12 additional rats, each with an i3vt cannula, under anesthesia (urethane, 0.8 g/kg; α-chloralose, 80 mg/kg). The temperature was measured at 10-min intervals for 60 mins after α-MSH (1 nmol), AGRP (1 nmol), or PBS was infused as described above.

Measurement of Sympathetic Nerve Activity. Electrophysiological recordings were made under anesthesia (urethane, 0.8 g/kg; a-chloralose, 80 mg/kg) using 12 additional cannulated rats. After the dissection of the fine branches of the sympathetic nerves that innervate the interscapular BAT, the nerves were transected where they entered the BAT. Nerve activity was measured using 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 and filtered at low- and high-frequency cutoffs. The nerve signal was distinguished from background noise using a window discriminator. All nerve activity was analyzed based on the values obtained after the conversion of the raw data to standard pulses using an analogue-to-digital converter. Impulses were integrated by a rate meter with a reset time of 5 secs and were recorded by a pen recorder. Details of this nerve recording technique have been described elsewhere (7, 17). After the background firing rate of sympathetic nerves had been determined, changes in nerve activity were measured for up to 60 mins after a bolus i3vt infusion with α-MSH (1 nmol), AGRP (1 nmol), or PBS ($n = 4$ per group). Nerve activity was measured at 10-min intervals beginning 20 mins before and continuing for 60 mins after the infusion of the test solution. Values recorded after the infusion of test solutions were expressed as the percentage difference from the initial value (0 min).

Statistical Analysis. Differences among groups were assessed using two-way analysis of variance (ANOVA) with repeated measures and the Dunnett's multiple comparison test for multiple comparisons. A two-sided P value of less than 0.05 was considered statistically significant.

Results

Effects of α-MSH and AGRP on Food Intake. Central administration of α-MSH induced no change in food intake during the first hour but caused a significant decrease over 24 hrs as compared with that of the control animals ($P < 0.05$; Fig. 1). In contrast, infusion of AGRP caused an elicitation in food intake during the first hour, and similarly increased food intake over 24 hrs ($P < 0.05$; Fig. 1).

Effects of α-MSH and AGRP on BAT Temperature. I3vt infusion of α-MSH produced a gradual, but significant, elevation in BAT temperature ($P < 0.05$; Fig. 2A), whereas AGRP infusion caused a gradual decrease in BAT temperature ($P < 0.05$; Fig. 2B).

Effects of α-MSH and AGRP on BAT Sympathetic Nerve Activity. Figures 3A and 4A illustrate the typical responses of BAT sympathetic nerve activity to the i3vt infusion of α-MSH or AGRP. Sympathetic nerve activity gradually increased after the central administration of α-MSH and decreased in response to AGRP. These responses appeared immediately upon infusion and lasted throughout the 60-min observation period. The mean changes in sympathetic nerve activity in response to α-MSH or AGRP were statistically different from those in response

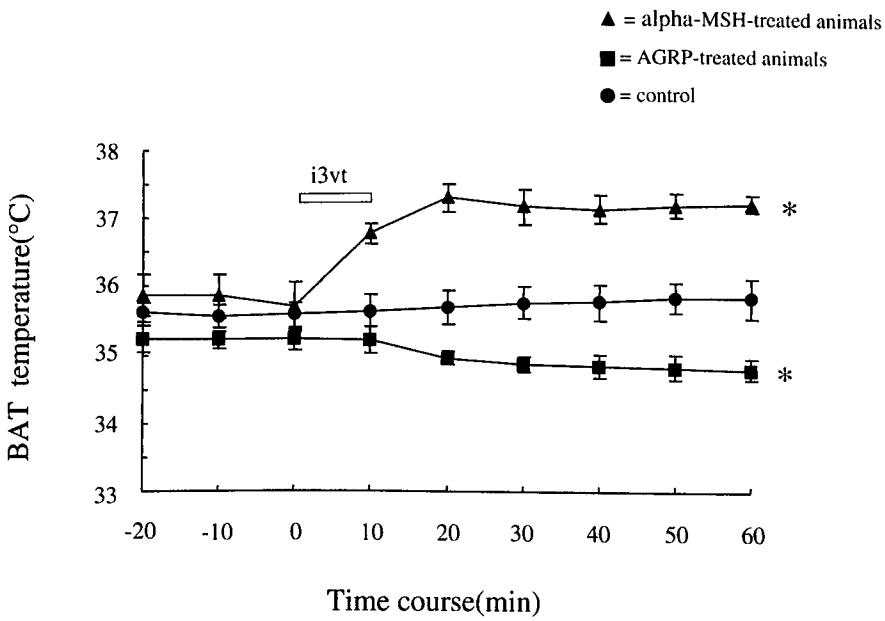

Figure 2. Effects of into the third cerebral ventricle (i3vt) infusion of α-melanocyte-stimulating hormone (α-MSH, 1 nmol); agouti-related protein (AGRP, 1 nmol); or phosphate-buffered saline (PBS, control group) on brown adipose tissue (BAT) temperatures. All data are the mean ± SEM (n = 4/group). Triangle = α-MSH-treated animals; square = AGRP-treated animals; circle = control. *P < 0.05 versus control.

to PBS ($P < 0.05$; Figs. 3B and 4B). Dose responsiveness of sympathetic nerve activity to each peptide was examined by additional infusion of 3 nmol, 6 nmol of α-MSH and 0.1 nmol, 0.3 nmol of AGRP (data not shown).

Discussion

In this study, we first investigated feeding responses to centrally administered α-MSH or AGRP to confirm the effects and dosages of these neuropeptides. The results were consistent with previous studies in which MC4-R agonists or antagonists suppressed or stimulated food intake, respectively (3, 4, 10–12, 18, 19). It has been shown that the blockade of MC4-R due to the ectopic expression of agouti protein or the overexpression of AGRP induces hyperphagia and obesity (20–22). The MC4-R and POMC knockout mice also express an obese phenotype (23, 24). Thus, the importance of the melanocortin system in the regulation of feeding behavior and body weight is generally accepted (1, 2).

In addition to the regulation of feeding, recent cumulative evidence indicates that the melanocortin system is also involved in the central regulation of energy expenditure. Treatment with an MC4-R antagonist inhibited a leptin-induced increase in UCP1 mRNA, a marker for energy expenditure in BAT (25). Chronic intracerebroventricular infusion of HSO14, an MC4-R antagonist, suppressed the expression of BAT UCP1 mRNA (10). Similarly, reductions in BAT UCP1 content and oxygen consumption were observed in animals treated with chronic central administration of AGRP (11). On the other hand, central administration of MTII, an MC4-R agonist, for 3 days up-regulated BAT UCP1 mRNA (19). These previous studies show that BAT UCP1 is a major target in the regulation of energy expenditure by the hypothalamic melanocortin system. BAT UCP1 is known to be under the control of the sympathetic nervous system and peripheral humoral factors (6–9), but it is still not clear how it is regulated by the hypothalamic melanocortin system. Chronic central administration of AGRP has been shown to suppress the plasma level of thyroid hormone (11, 18). Changes in body weight and adiposity induced by chronic treatment with MC4-R agonist or antagonist may affect the circulating leptin level. As thyroid hormone and leptin are intensive regulators of BAT UCP1 at the peripheral level (8, 9), the possibility that these humoral factors mediate melanocortin signals from the hypothalamus cannot be excluded. To provide direct evidence of neuronal mediation, we investigated the acute effect of centrally administered α-MSH or AGRP on sympathetic nerves. The results showed that α-MSH increased and AGRP decreased BAT sympathetic nerve activity. This is consistent with previous studies suggesting that increased food intake is usually associated with low levels of sympathetic activity and vice versa (26). In addition, these responses were accompanied by corresponding changes in BAT temperature, another parameter of UCP1 function. Taken together, these studies indicate that the melanocortin system may regulate energy expenditure and thermogenesis, at least in the acute phase, through efferent sympathetic nerves.

A recent neuroanatomical study demonstrated that MC4-R mRNA is expressed in the IML or the dorsal motor nucleus of the vagus (DMV), which are sites of sympathetic or parasympathetic preganglionic neurons, suggesting that the melanocortin system is involved in autonomic regulation (14). Corresponding to the distribution of the MC4-R, POMC neurons in the ARC project directly to the thoracic

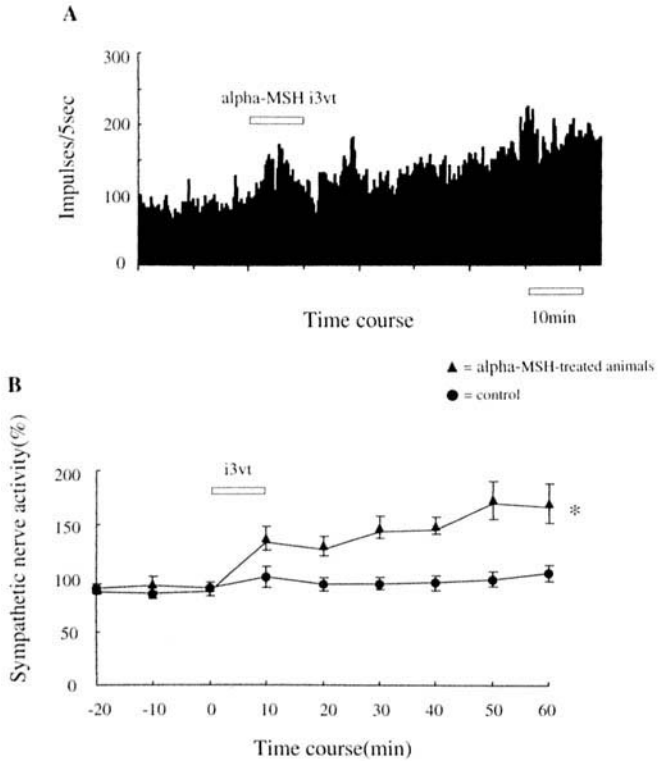

Figure 3. (A) Rate meter plots of brown adipose tissue (BAT) sympathetic nerve activity following into the third cerebral ventricle (i3vt) infusion of α-melanocyte-stimulating hormone (α-MSH, 1 nmol). Vertical axis: nerve impulses per 5 secs. Horizontal bars: 10-min time scale. Bold horizontal bar: α-MSH infusion. (B) Percentage differences in sympathetic nerve activity from baseline (100%) after i3vt infusion of α-MSH (1 nmol) or phosphate-buffered saline (PBS, control). All data are the mean ± SEM (n = 4/group). Triangle = α-MSH-treated animals; circle = control. *P < 0.05 versus control.

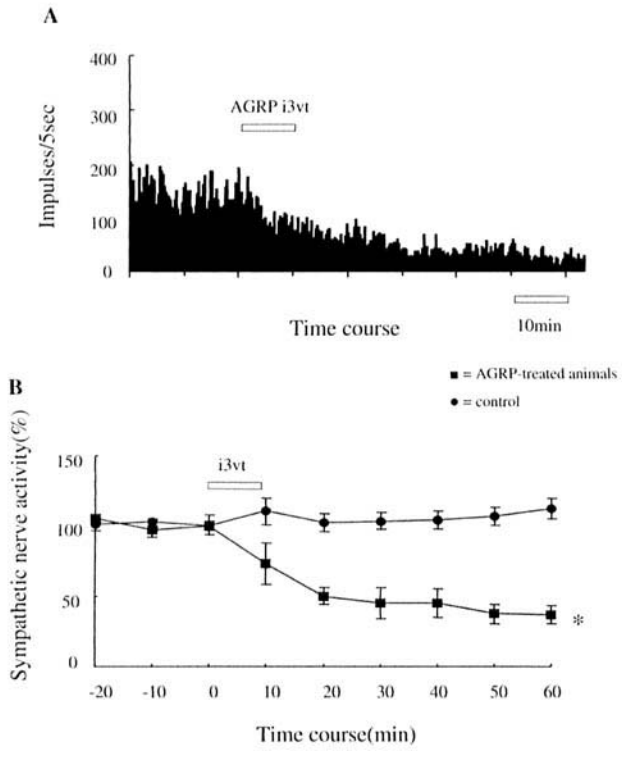

Figure 4. (A) Rate meter plots of brown adipose tissue (BAT) sympathetic nerve activity following into the third cerebral ventricle (i3vt) infusion of agouti-related protein (AGRP, 1 nmol). Vertical axis: nerve impulses per 5 secs. Horizontal bars: 10-min time scale. Bold horizontal bar: AGRP infusion. (B) Percentage differences in sympathetic nerve activity from baseline (100%) after i3vt infusion of AGRP (1 nmol) or PBS (control). All data are the mean ± SEM (n =

IML (13, 27), which in turn projects to the postganglionic neurons that innervate BAT (6, 28). The present study demonstrated that centrally administered α-MSH stimulates BAT sympathetic nerve activity. This observation, combined with previous findings (6, 13, 27, 28), suggests that α-MSH plays an important role in the sympathetic regulation of BAT energy expenditure; specifically, it acts as a signal transducer for efferent nerve pathways between the ARC and BAT. However, it is unlikely that the α-MSH infused into the third ventricle in the present study directly bound the MC4-R in the spinal cord, because the injection site was too far from the spinal cord. In the case of AGRP, centrally administered AGRP also affected sympathetic nerve activity, although AGRP neurons do not project directly to the spinal cord (29, 30). In light of these observations, it seems reasonable to suggest that the influence of α-MSH or AGRP may be mediated by other brain sites that express MC4-R. For instance, the paraventricular nucleus (PVN) and dorsomedial hypothalamic nucleus express MC4-R mRNA (14); receive inputs from α-MSH and AGRP neurons in the ARC (30, 31); and project directly to the IML in the spinal cord (32).

The functional correlation between leptin and hypothalamic neuropeptides, including α-MSH, AGRP, corticotropin-releasing hormone (CRH), and neuropeptide Y (NPY), suggests that the neuron network may regulate energy expenditure through connections with sympathetic nerves. As described above, leptin can stimulate BAT UCP1 and sympathetic nerve activity (33) and has stimulatory effects on CRH and α-MSH release, but it inhibits NPY and AGRP (34). The excitatory effects of CRH and the inhibitory effects of NPY on BAT sympathetic nerve activity have been established (35). The present study provides evidence that α-MSH stimulates and AGRP inhibits BAT sympathetic nerve activity. These observations suggest that the stimulatory effect of leptin on BAT sympathetic nerve activity and on UCP1 could be caused by the activation of factors that stimulate BAT sympathetic nerve activity, such as CRH and α-MSH, or by the suppression of inhibitory factors, such as NPY and AGRP.

In summary, we demonstrated that centrally administered α-MSH or AGRP regulates not only food intake, but also BAT sympathetic nerve activity. The reciprocal effects of α-MSH and AGRP in the regulation of energy balance through the MC4-R may contribute to the homeostatic control of energy metabolism and may be able to restore balance in animals with the excessive or deficient energetic conditions that characterize obesity or starvation, respectively.

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