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Corticotropin-releasing factor (CRF) is involved in the acute anorexic effect of α-melanocyte-stimulating hormone: A study using CRF-deficient mice

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ABSTRACT

Alpha-melanocyte-stimulating hormone (α-MSH) and its receptors are critical and indispensable for maintaining appropriate feeding behavior and energy homeostasis in both mice and humans. Corticotropin-releasing factor (CRF) is a candidate for mediating the anorexic effect of α-MSH. In the present study, we examined whether CRF and its receptors are involved in the anorexic effect of α-MSH, using CRF-deficient (CRFKO) mice and a CRF receptor antagonist. Intracerebroventricular administration of NDP-MSH, a synthetic α-MSH analogue, suppressed food intake in wild-type (WT) mice. This effect was abolished by pretreatment with a non-selective CRF receptor antagonist, astressin, suggesting that the effect of α-MSH-induced anorexia was mediated by a CRF receptor. In CRFKO mice, administration with NDP-MSH did not affect food intake at an early phase (0–4 h). In addition, CRF mRNA levels in the hypothalamus were significantly increased in NDP-MSH-treated mice. Therefore, our findings, using CRFKO, strongly support evidence that CRF is involved in the acute anorexic effect of α-MSH. On the other hand, NDP-MSH administered to CRFKO mice led to suppressed food intake at the late phase (4–12 h), similar to the effect in WT mice. Further, NDP-MSH similarly reduced food intake during the late phase in all types of mice, including WT, CRFKO, and CRFKO with corticosterone replacement. The results would suggest that α-MSH-induced suppression of food intake at late phase was independent of glucocorticoids and CRF.

1. Introduction

Alpha-melanocyte-stimulating hormone (α-MSH) and its receptors, especially melanocortin-4 receptor (MC4R), are a major critical factor for energy homeostasis in the hypothalamus [8,12]. Activation of this system suppresses feeding behavior and facilitates energy expenditure, resulting in reduction of body weight [10,29,32,43], while inactivation of this system produces severe hyperphagia and obesity [11,20,21]. Therefore, α-MSH in the hypothalamus contributes to maintaining appropriate feeding behavior in both mice and humans.

Corticotropin-releasing factor (CRF), a 41-amino acid polypeptide isolated originally from the ovine hypothalamus, plays a central role in regulating stress responses [34,38]. Acute or chronic stress has been reported to enhance the concentration of CRF and CRF mRNA levels in several brain areas, such as the paraventricular nucleus (PVN), locus coeruleus, Barrington's nucleus and bed nucleus of stria

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terminalis [5,35]. CRF then coordinates neuroendocrine, behavioral, autonomic and immune responses, and controls the hypothalamic-pituitary-adrenal axis during stressful periods. Furthermore, CRF blunts the activity of the reproductive system [31] and induces anorexia [16]. On the other hand, central administration of a non-selective CRF receptor antagonist has been shown to attenuate some stress-induced behaviors, such as the suppression of feeding [15], exploratory behavior [2] and the stress-induced *c-fos* activation in the PVN [18]. Therefore, CRF and CRF receptors have been considered as pivotal systems against stress exposure.

CRF is a candidate for mediating the anorexic effects of α-MSH, because CRF neurons express MC4R in the hypothalamus [24]. CRF mRNA levels are increased by an α-MSH analogue via CRF receptors [24]. The release of CRF is also stimulated by α-MSH from hypothalamic explants [9]. Finally, central administration of CRF mimics anxious or anorexic behavior [17].

Both α-MSH and CRF are potent anorexic peptides. In addition, these peptides contribute to modify the metabolism or energy expenditure with a signal cross talk. It is important to elucidate the mechanism how CRF plays a role in causing the anorexia. Both α-MSH and CRF systems have been the focus as potential therapeutic target for eating disorders or metabolic syndrome.

Several approaches are useful to explore the roles of peptides on food intake. For example, food restriction paradigms and a liquid diet test meal have been used for this purpose [14,39]. We chose fasting for 24 h before each test to ensure food intake [19]. In the present study, we tested the hypothesis that CRF and its receptors are involved in the anorexic effect of α-MSH, using CRF-deficient (CRFKO) mice and a CRF receptor antagonist. We also used [Nie^4, D-phe^7]-α-MSH (NDP-MSH) as a substitute for α-MSH in this study, because this peptide is well known as a potent and enzymatically stable analogue.

2. Materials and methods

2.1. Animals

CRFKO mice were kindly donated by Dr. J.A. Majzoub (Harvard Medical School, Boston, USA). Wild-type (WT) and CRFKO mice were maintained under 12:12 h light:dark cycles (lights on at 07:00 h; lights off at 19:00 h). Food and water were available *ad libitum*. Mice utilized for all studies were 14–16 weeks old, 27–30 g body weights (no statistical significance between WT and CRFKO mice), and were of C57BL/6J × 129 Sv genetic background. All mouse experiments were carried out in accordance with the Guidelines for Animal Experiments, Hirosaki University.

2.2. Materials

NDP-MSH, astressin, and corticosterone were purchased from Sigma (St. Louis, MO, USA).

2.3. Intracerebroventricular (icv) surgery

Seven days prior to experiments, mice were anesthetized with sodium pentobarbital (Sigma), and 33-gauge stainless steel guide cannulae (Plastics One Inc., Roanoke, VA, USA) were implanted into the right lateral ventricles (0.3 mm posterior, 1 mm lateral to Bregma, and at a depth of 2 mm) with a stereotaxic instrument. Dummy cannulae were inserted into the guide cannulae and left in place until 24 h before any experiment to prevent obstruction of the guide cannulae by clots. Internal cannulae adjusted to the same height as the guide cannulae were used. Accurate cannula placement was confirmed by infusion of 2 μL methylene blue dye after the experiment. Mice used for data analysis had either dye in the ventricle, or a cannula tract ending in the ventricle.

2.4. Effects of icv administration of NDP-MSH on food intake

Experiments were performed 7 days after icv surgery. To ensure feeding behavior, mice were deprived of food 24 h prior to each experiment. Some CRFKO mice were provided with 10 mg/L corticosterone (CRFKO-B) or vehicle in drinking water 7 days before each experiment. One hour after lights on, mice were given preweighed food. After icv administration of 2 μL NDP-MSH, mice behavior and food intake were monitored over 12 h. The re-feeding period was divided into two phases, the first 4 h (early phase) and the subsequent 8 h (late phase).

Another set of WT and CRFKO mice was intracerebroventricularly pretreated with astressin or vehicle 30 min before administration of NDP-MSH. After icv administration of 2 μL NDP-MSH, mice behavior and food intake were monitored over 12 h. The re-feeding period was divided into two phases, the first 4 h (early phase) and the subsequent 8 h (late phase).

2.5. Plasma corticosterone and CRF mRNA assays

Blood samples were collected from mice retro-orbitally with heparinized tubes. Plasma samples were stored at −70 °C until the assay. Plasma corticosterone levels were measured using a commercial RIA kit (ICN Biochemicals, Costa Mesa, CA, USA).

To measure CRF mRNA levels in the hypothalamus, a set of mice was decapitated 4 h after icv administration of NDP-MSH or vehicle. Brains were then dissected, and stored at −70 °C until the assay. CRF mRNA levels in the hypothalamus were quantified by *in situ* hybridization between vehicle-treated mice (control) and NDP-MSH-treated mice, as described previously [40].

2.6. Statistical analysis

Each value is expressed as mean ± standard error of the mean (S.E.M.). Statistical analyses of the data were performed using either unpaired *t*-tests or analysis of variance (ANOVA), followed by the Fisher's PLSD test. The level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Effects of NDP-MSH on food intake in WT mice

To determine the effects of α-MSH on food intake, total food intake was measured at 2, 4, and 12 h after icv administration