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Short communication

Central administration of α-melanocyte stimulating hormone inhibits fasting- and neuropeptide Y-induced feeding in neonatal chicks

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Abstract

In the present study, the effect of intracerebroventricular (i.c.v.) administration of α-melanocyte stimulating hormone (α-MSH) on food intake of neonatal chicks was examined. In experiment 1, i.c.v. injection of α-MSH (0.04, 0.2 and 1 μg) significantly inhibited food intake of 3-h fasted chicks in a dose-dependent manner. In experiment 2, α-MSH strongly inhibited neuropeptide Y-induced feeding when neuropeptide Y (2.5 μg) and several doses of α-MSH were given simultaneously i.c.v. These results suggest that α-MSH plays an important role in the regulation of food intake of neonatal chicks.

Keywords: α-MSH (α-melanocyte stimulating hormone); Feeding; Neuropeptide Y; (Chick)

1. Introduction

It is generally accepted that ingestive behavior is dominated by the central nervous system. A number of neurotransmitters have been identified, and function at various parts of the brain to integrate and process information from the internal and external environments. These neurotransmitters are considered to regulate feeding behavior by affecting stimulatory and/or inhibitory effects on food intake of animals (Flier and Maratos-Flier, 1998).

It has been reported that, in chicks, some neurotransmitters suppress food intake when given into the brain. Intracerebroventricular (i.c.v.) injection of neurotransmitters such as cholecystokinin, gastrin, glucagon-like peptide-1 and corticotrophin-releasing factor suppress feeding behavior in the chicks (Denbow and Myers, 1982; Denbow et al., 1999; Furuse et al., 1997a,b, 1999). Among neurotransmitters, α-melanocyte stimulating hormone (α-MSH), which is derived from a multifunctional precursor protein, proopiomelanocortin (POMC), plays an inhibitory role in food intake of mammals. The i.c.v. administration of α-MSH or its potential agonist, MTII (Ac-Nle^4-c[Asp^5, D-Phe^7, Lys^{10}] α-MSH-(4–10)-NH_2), suppresses food intake (Fan et al., 1997; Ludwig et al., 1998), and i.c.v. injection of a melanocortin antagonist, SHU9119 (Ac-Nle^4-c[Asp^5, D-2′Nal^7, Lys^{10}] α-MSH-(4–10)-NH_2) or HS014 (cyclic [AcCys^3, Nle^4, Arg^5, D-Nal^7, Cys-NH_2^{11}] α-MSH-(3–11)), stimulates feeding behavior of rats (Fan et al., 1997; Kask et al., 1998a; Seeley et al., 1997). In addition, targeted disruption of one of the melanocortin receptors, MC4R, results in obesity, hyperphagia, hyperinsulinemia and hyperglycemia (Huszar et al., 1997). These reports suggest that α-MSH is essential to maintain feeding behavior and body weight by reducing food intake in mammals. However, the function of α-MSH in food intake of avians has not been elucidated.

In the present study, therefore, the inhibitory effect of i.c.v. administration of α-MSH on fasting- and neuropeptide Y-induced food intake of neonatal chicks was examined.

2. Materials and methods

Day-old male broiler chicks (Cobb; Mori Hatchery, Fukuoka, Japan) were housed in a windowless room at a

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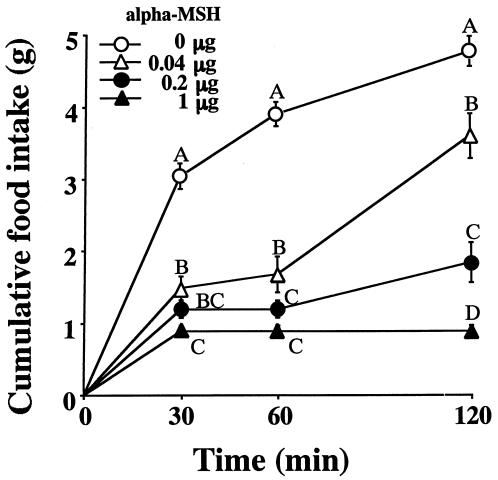

Fig. 1. Cumulative food intake (g) at 0.5, 1 and 2 h period after i.c.v. injection of α-MSH (0.04 μg: open triangles; 0.2 μg: closed circles; 1 μg: closed triangles) or saline (open circles) in 2-day-old chicks. Values are means ± S.E.M. Means without a common letter (A, B or C) at the same time are significantly different ($P < 0.05$). Food intake (g/30 min) = 1.990(SE 0.158) − 1.226(SE 0.298) X ($R^2 = 0.325$, $P < 0.001$), food intake (g/60 min) = 2.370(SE 0.221) − 1.671(SE 0.416) X ($R^2 = 0.315$, $P < 0.001$) and food intake (g/120 min) = 3.646(SE 0.234) − 2.993(SE 0.441) X ($R^2 = 0.569$, $P < 0.0001$).

constant temperature of 28°C. Lighting was provided continuously. The birds were given free access to a commercial starter diet (Toyohashi Feed and Mills, Aichi, Japan) and water, and were maintained in accordance with recommendations of the National Research Council (1985). The birds were distributed into experimental groups based on their body weight, so that the average body weight was as uniform as possible for each treatment. The birds were given the drugs (10 μl) i.c.v. using a microsyringe according to the method of Davis et al. (1979). In experiment 1, after being deprived of food for 3 h, the birds (2-day-old, 10 birds per group) were injected with α-MSH (0, 0.04, 0.2 and 1 μg) and cumulative food intake was measured for 0.5, 1 and 2 h. In experiment 2, the birds, which had free access to food (3-day-old, 8 birds per group), were given simultaneously neuropeptide Y (2.5 μg) and α-MSH (0, 0.04, 0.2 and 1 μg) to test the anorexigenic role of α-MSH on neuropeptide Y-induced feeding behavior. Human α-MSH and porcine neuropeptide Y were purchased from Peptide Institute (Osaka, Japan). Drugs were dissolved in a 0.1% Evans Blue solution, which was prepared in 0.85% saline. The doses were prepared by repeated dilution with saline solution. Saline solution was used as a control in all experiments.

At the end of experiment, the birds were decapitated. The brains were removed and cut with a razor blade to identify whether the injected Evans Blue dye was located in the lateral ventricle. Data pertaining to individuals not found to have the dye present in the lateral ventricle were discarded. The data were analyzed by one-way analysis of variance by the General Linear Model procedure using a commercially available package (SAS Institute, 1985). Regression equations with each time period were fitted to the data, and comparisons between means were made using Duncan's multiple range test. The results are presented as means ± S.E.M.

3. Results

The number of birds used was as follows: In experiment 1: control, 8; 0.04 μg, 9; 0.2 μg, 10 and 1 μg, 10. In experiment 2: saline, 8; neuropeptide Y, 8; neuropeptide Y plus 0.04 μg of α-MSH, 8; neuropeptide Y plus 0.2 μg of α-MSH, 8 and neuropeptide Y plus 1 μg of α-MSH, 8.

Fig. 1 gives the cumulative food intake of neonatal chicks at 0.5, 1 and 2 h after i.c.v. injection of α-MSH (0, 0.04, 0.2 and 1 μg, respectively). Food intake was significantly and linearly inhibited by α-MSH treatment in a dose-dependent manner.

Fig. 2 shows the effect of i.c.v. administration of α-MSH on inhibition of neuropeptide Y-induced food intake of neonatal chicks. With increasing α-MSH, neuropeptide Y-induced food intake was suppressed curvilinearly. When 1 μg of α-MSH was administered simultaneously with neuropeptide Y, food intake was significantly inhibited at all times tested. Injection of 0.2 μg of α-MSH signifi-

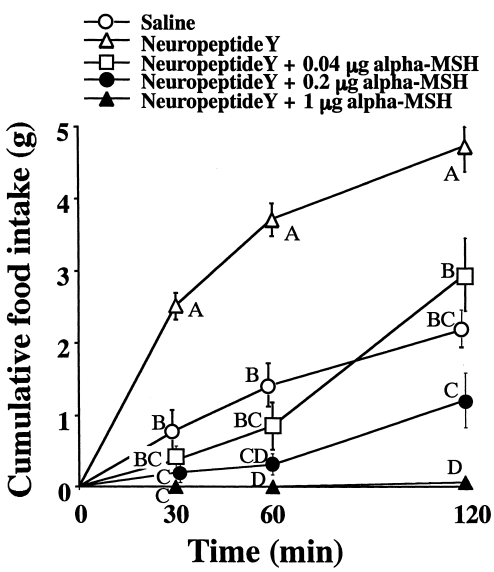

Fig. 2. Cumulative food intake (g) at 0.5, 1 and 2 h period after intracerebroventricular injection of mixture of neuropeptide Y (2.5 μg) with α-MSH (0 μg: open triangles; 0.04 μg: open squares; 0.2 μg: closed circles; 1 μg: closed triangles) or saline (open circles) in 3-day-old chicks. Values are means ± S.E.M. Means without a common letter (A, B or C) at the same time are significantly different ($P < 0.05$). Food intake (g/30 min) = 1.766 (SE 0.220) − 10.841 (SE 2.281) X + 9.085 (SE 2.163) X^2 ($R^2 = 540$, $P < 0.0001$), food intake (g/60 min) = 2.745 (SE 0.294) − 16.462 (SE 3.054) X + 13.730 (SE 2.897) X^2 ($R^2 = 0.608$, $P < 0.0001$) and food intake (g/120 min) = 4.276 (SE 0.308) − 18.976 (SE 3.200) X + 14.762 (SE 3.035) X^2 ($R^2 = 0.736$, $P < 0.0001$).

cantly suppressed neuropeptide Y-induced food intake at 30 and 60 min, with recovery from the inhibitory effect at 120 min. Administration of 0.04 μg of α-MSH did not suppress neuropeptide Y-stimulated food intake at any time.

4. Discussion

We used synthetic α-MSH in which the amino acid sequence is identical to that of human α-MSH (SYSMEHFRWGKPV) (Takahashi et al., 1981). According to the cloned sequence of chicken POMC (Takeuchi et al., 1999), the structure of α-MSH is identical in humans and chickens. Therefore, the α-MSH used in the present experiments is believed to be identical to endogenous chicken α-MSH.

The results showed that i.c.v. administration of α-MSH suppressed food intake of neonatal chicks in a dose-dependent manner. In both experiments, no chicks showed sleep-like or hyperactive behavior. Therefore, a central effect of α-MSH might be directly affecting the feeding behavior. The i.c.v. injection of α-MSH, or its agonist MTII, suppresses food intake of rats (Fan et al., 1997; Ludwig et al., 1998), suggesting that α-MSH plays a role in inhibition of food intake in both mammals and avians. Recent reports have indicated that, in mammals, α-MSH mediates the effect of leptin on food intake: i.c.v. pretreatment with the α-MSH receptor antagonist, SHU9119, abolishes the inhibitory effect of leptin on food intake (Seeley et al., 1997), and POMC neurons and mRNA of leptin receptor is colocalized in neurons of the hypothalamic arcuate nucleus (Cheung et al., 1997). In addition, i.c.v. administration of leptin increases POMC mRNA expression in the rostral part of the arcuate nucleus (Schwartz et al., 1997). However, i.c.v. injection of leptin does not inhibit food intake of neonatal chicks (Bungo et al., 1999). These reports suggest that α-MSH inhibits food intake through different mechanisms in mammals and in avians.

In the present study, α-MSH suppressed the neuropeptide Y-induced food intake of neonatal chicks. This suggests an interaction of α-MSH and neuropeptide Y in generation/modulation of feeding behavior. A recent report of Kask et al. (1998b) showed that the stimulatory effect of the α-MSH receptor antagonist, HS014, on food intake of rats was abolished by i.c.v. administration of the neuropeptide Y Y1 receptor antagonist, 1229U91. It was also reported that the level of neuropeptide Y mRNA expression was increased in the dorsomedial hypothalamus of obese MC4R knockout mice (Kesterson et al., 1997). These reports, together with our present results, suggest that α-MSH inhibits food intake by affecting neuropeptide Y in the brain. However, it is not yet known whether α-MSH affects the amount of neuropeptide Y release in the chick brain. In addition, the localization and projection of α-MSH-containing neurons in the brain of chicks have not been reported. Therefore, it is also possible that α-MSH and neuropeptide Y are not influencing each other and act independently to regulate the feeding behavior of chicks. In experiment 2, 1 μg of α-MSH completely inhibited the feeding induced by neuropeptide Y. This effect was somewhat different from the results obtained in experiment 1, where chicks ate small amounts of food even during treatment with 1 μg of α-MSH. It may be that neuropeptide Y released during fasting enhanced the food intake, which was partly inhibited by α-MSH with a slight time lag in experiment 1. On the other hand, in experiment 2, neuropeptide Y and α-MSH were given simultaneously i.c.v. The site of action for α-MSH may be nearer to the lateral ventricle than that for neuropeptide Y, i.e., the paraventricular nucleus of the hypothalamus. Thus, α-MSH may completely inhibit feeding before neuropeptide Y could reach the site of action in experiment 2. However, as the site of action of α-MSH in the chick brain has not been identified, further studies are needed to clarify the mechanisms of α-MSH-mediated feeding inhibition in the central nervous system of chicks.

The present results showed that central administration of α-MSH suppressed both fasting- and neuropeptide Y-induced food intake of neonatal chicks. In conclusion, α-MSH could play an important role for the regulation of food intake of neonatal chicks.

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