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Central administration of metastin increases food intake through opioid neurons in chicks

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ABSTRACT

Metastin, an RFamide peptide, has been isolated from human placenta and possesses several physiological actions in mammals. However, little is known about this bioactive peptide in avian species. This study was conducted to assess the effect of metastin on feeding behavior of chicks (*Gallus gallus*). The food intake of chicks is significantly increased by the intracerebroventricular injection of metastin. Beta-funaltrexamine, a mu-opioid receptor antagonist, significantly attenuates metastin-induced food intake in chicks. In contrast, delta- and kappa-opioid receptor antagonists did not show any influence on metastin-induced food intake in chicks. In addition, administration of N^G-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, did not influence metastin-induced food intake. Taken together, this study shows the orexigenic effect of metastin in chicks and suggests that this effect is mediated by mu-opioid receptor.

1. Introduction

RFamides represent a group of peptides that bear exactly same amino acid sequence (arginine-R and amidated phenylalanine-F) at the C-terminus and regulate food intake in animals (Dockray, 2004; Bechtold and Luckman, 2007). The appetite regulation by these peptides is shown in a range of species, including invertebrates such as locusts (Hill and Orchard, 2005), jellyfish (Mackie et al., 2003) and nematodes (de Bono and Bargmann, 1998), higher vertebrates including avian (Tachibana et al., 2004, 2005; Cline et al., 2007, 2008), rodents (Murase et al., 1996; Lawrence et al., 2000; Sunter et al., 2001; Takayasu et al., 2006) and human (Bruzzone et al., 2006). These findings suggest that the feeding regulation by RFamide peptides arose early during evaluation and have been conserved. To date, four RFamide peptides, gonadotrophin-inhibiting hormone (GnIH), prolactin-releasing peptide (PrRP), neuropeptide FF (NPFF) and neuropeptide VF (NPVF) have been found to have an appetite regulatory effect in chicks (Tachibana et al., 2004, 2005; Cline et al., 2007, 2008).

Metastin, an RFamide peptide, isolated from human placenta and possesses several physiological actions in mammals (Kotani et al., 2001; Ohtaki et al., 2001; Horikoshi et al., 2003; Gottsch et al., 2004; Matsui et al., 2004). Metastin mRNA has been detected in several tissues such as testis, pituitary, spinal cord, pancreas, liver, small intestine and brain (Kotani et al., 2001; Ohtaki et al., 2001). The metastin receptor is reported in hypothalamus (Lee et al., 1999; Navarro et al., 2004) suggests the possible role in energy balance and appetite regulation. Castellano et al. (2005) has been reported that body energy stores impact the expression and function of the KiSS-1 (gene name of metastin) system at the hypothalamus. Since the information regarding metastin mainly derived from mammals, there is paucity of information about the effect of this peptide in avian species.

In mammals, it has been reported that central administration of kisspeptin-10, another name of metastin, failed to change food intake (Thompson et al., 2004; Castellano et al., 2005). On the other hand, the effect of metastin on feeding behavior in chicks has not yet been reported. Although metastin does not alter food intake in rats, there is the possibility that this peptide may affects feeding behavior in chicks because the appetite regulatory mechanism in avian is somewhat different from mammalian counterparts. For example, intracerebroventricular (ICV) injection of several mammalian orexigenic factors such as orexin, motilin, melanin-concentrating hormone, ghrelin or growth hormone-releasing hormone does not stimulate feeding behavior in chicks (Furuse, 2002). Other such factors, neuropeptide Y (NPY), galanin and opioid peptides stimulate feeding behavior in chicks when administrated centrally and opioid receptors are associated with their orexigenic mechanism (McCormack and Denbow, 1989; Bungo et al., 2004b; Dodo et al., 2005; Tachibana et al., 2008b). Nitric oxide (NO) is also recognized as orexigenic mediators and thought to related to feeding stimulatory effect because ICV injection of NO synthase (NOS) inhibitor decrease food intake in avian

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and as well as mammals and NO system mediates the orexigenic effect of NPY (Choi et al., 1994, 1995; De Luca et al., 1995; Morley et al., 1999).

In this study, first, we examined whether ICV injection of metastin affected food intake in chicks. Next, since some RFamide peptides (NPVF and GnIH) regulate feeding behavior via opioid receptors in chicks (Cline et al., 2007; Cline and Mathews, 2008; Tachibana et al., 2008a), we tried to elucidate the opioid receptor/(s) that is involved in appetite regulation by metastin. In addition, since NO plays an important role in the central regulation of feeding behavior in avian (Choi et al., 1995; Bungo et al., 2000; Yang and Denbow, 2007), we examined the effect of co-injection of metastin with NOS inhibitor in chicks.

2. Materials and methods

2.1. Animals

Day-old broiler chicks (*Gallus gallus*, Chunky, Mori Hatchery, Kagawa, Japan) were kept in a windowless room maintained at 30 °C and provided 24 h lighting. The chicks were given free access to water and a commercial diet (crude protein: 24%, metabolizable energy: 3050 kcal/kg, Toyohashi Feed Mills Co. Ltd, Aichi, Japan). They were maintained in accordance with the recommendations of the National Research Council (National Research Council, 1996). Chicks were placed in individual cages 1 day prior to the experiments. Chicks were weighed and divided into experimental groups.

2.2. Drugs and ICV injection

Either metastin (human, 45–54) (Peptide Institute, Inc., Osaka, Japan), beta-funaltrexamine (FNA: a mu-opioid receptor antagonist), ICI-174,864 (ICI: a delta-opioid receptor antagonist), nor-binaltorphimine (BNI: a kappa-opioid receptor antagonist) or N^G-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in a saline solution containing 0.1% Evans Blue solution to confirm the accuracy of the injection. The saline containing 0.1% Evans Blue dye was used as the control treatment for each experiment.

The ICV injection was performed according to the method reported previously (Davis et al., 1979). Briefly, the head of the chick was inserted into an acrylic box which had a hole at the top plate. A microsyringe was then inserted into the hole and the drug solution was injected in a volume of 10 µL. This procedure has been demonstrated as unstressful method (Furuse et al., 1999; Saito et al., 2005). The food intake was determined by measuring the reduction of diet from a pre-weighed feeder. The feeders were weighed using an electric digital balance of precision ± 1 mg.

At the end of each experiment, chicks were sacrificed with overdose of pentobarbital. The brain was then removed to confirm the accuracy of drug injection. Chicks which did not show Evens Blue dye in the ventricle were not used for further analyses.

Experiment 1. Effect of ICV injection of metastin on food intake in chicks.

Two-day-old broiler chicks were ICV injected with 0 (control), 0.4 or 1.5 nmol metastin under an ad libitum feeding condition and the food intake was measured at 30 and 60 min after injection. The number of chicks in each group was as follows: 0 nmol, 9; 0.4 nmol, 8; 1.5 nmol, 8.

Experiment 2. Effect of opioid receptor antagonists on the metastin-induced feeding.

In this experiment, food intake was measured at 30 min after the injection. The doses of opioid receptor antagonists used here were decided according to the previous reports (Dodo et al., 2005; Tachibana et al., 2008a).

In the mu-opioid receptor study, 2-day-old broiler chicks were injected with saline (control), 1.5 nmol metastin, 20 nmol FNA or metastin plus FNA. The number of chicks in each group was as follows: control, 8; metastin, 7; FNA, 8; metastin plus FNA, 7.

In the delta-opioid receptor study, 3-day-old broiler chicks were injected with saline, 1.5 nmol metastin, 6 nmol ICI or metastin plus ICI. The number of chicks in each group was as follows: control, 8; metastin, 7; ICI, 6; metastin plus ICI, 10.

In the kappa-opioid receptor study, 2-day-old broiler chicks were injected with saline, 1.5 nmol metastin, 2.7 nmol BNI or metastin plus BNI. The number of chicks in each group was as follows: control, 7; metastin, 9; BNI, 9; metastin plus BNI, 9.

Experiment 3. Effect of NOS inhibitor on the metastin-induced feeding.

The dose of NOS inhibitor, L-NAME was decided according to the previous reports (Tomonaga et al., 2005). In this experiment, food intake was measured at 30 min after the injection. Two-day-old broiler chicks were injected with saline, 1.5 nmol metastin, 400 nmol L-NAME or metastin plus L-NAME. The number of chicks in each group was as follows: control, 8; metastin, 7; L-NAME, 8; metastin plus L-NAME, 9.

2.3. Statistical analysis

Data were analyzed with one-way analysis of variance (ANOVA) and then a Tukey–Kramer test was performed as a post hoc test. Significant difference was set at $P<0.05$. Data are expressed as mean ± SEM.

3. Results

Experiment 1. Effect of ICV injection of metastin on food intake in chicks.

Fig. 1 shows the effect of ICV injection of metastin on food intake in chicks. The injection of metastin significantly stimulated food intake at 30 min.

Experiment 2. Effect of opioid receptor antagonists on the metastin-induced feeding.

The effect of ICV injection of FNA on the metastin-induced feeding is shown in Fig. 2. Metastin alone significantly increased food intake and the co-injection of FNA significantly suppressed the metastin-induced

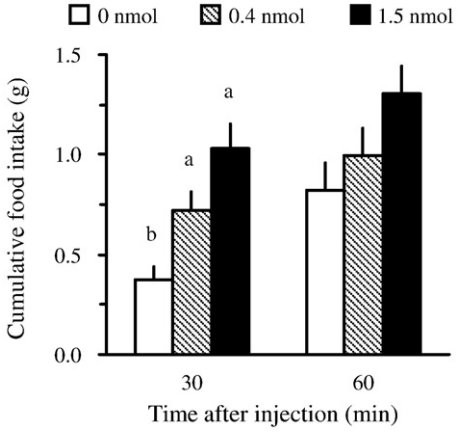

Fig. 1. Effect of ICV injection of metastin on food intake in chicks. Data are expressed as mean ± SEM. The number of chicks in each group was as follows: 0 nmol, 9; 0.4 nmol, 8; 1.5 nmol, 8. Groups with different letters are significantly different ($P<0.05$).