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Feeding and drinking response following central administration of neuromedin S in chicks

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ABSTRACT

Neuromedin S (NMS) is recognized as an anorexigenic peptide in the brain of mammals. In chicks (*Gallus gallus*), however, the effect of NMS has not been investigated. Therefore, the purpose of the present study was to investigate whether intracerebroventricular (ICV) injection of NMS affected feeding and drinking behavior in chicks. The injection of NMS (0.01–1 nmol) significantly decreased food intake under both ad libitum and food deprivation-induced feeding conditions. However, NMS did not affect water deprivation-induced drinking behavior. ICV injection of NMS stimulated voluntary locomotion and wing-flapping behavior. In addition, we found that those effects of NMS might be related to the hypothalamus–pituitary–adrenal axis because ICV injection of NMS stimulated corticosterone release. The present study suggests that central NMS functions an anorexigenic factor in chicks.

1. Introduction

Neuromedin S (NMS) is a neuropeptide first isolated from the rat brain (Mori et al., 2005). The carboxyl-terminal 7 residues of mammalian NMS are identical to those of neuromedin U (NMU, Mori et al., 2005; Miyazato et al., 2008), which is regarded as an anorexigenic peptide in mammals (Howard et al., 2000; Nakazato et al., 2000). NMS has affinity for the NMU receptor-2 (FM4/TGR-1, Mori et al., 2005), suggesting that the action of NMS is at least partly similar to that of NMU. For example, intracerebroventricular (ICV) injection of NMS inhibits feeding behavior in rats (Ida et al., 2005; Miyazato et al., 2008) as is also the case for NMU (Howard et al., 2000; Nakazato et al., 2000). The anorexigenic effect is not observed in NMU receptor-2-deficient mice, demonstrating that central administration of NMS inhibits feeding behavior via this receptor (Peier et al., 2009). Furthermore, the anorexigenic effect of NMS is related to corticotropin-releasing hormone (CRH), which is a hypothalamic signal of the hypothalamus–pituitary–adrenal (HPA) axis in rats (Ida et al., 2005; Jászberényi et al., 2007).

In chicks (*Gallus gallus*), the effect of NMS has not been investigated. However, it is reported that ICV injection of NMU decreases food intake in chicks (Kamisoyama et al., 2007), suggesting that NMS also might cause an anorexigenic effect in chicks as has been shown in mammals. Previous studies revealed that most anorexigenic peptides found in mammals have similar effects in chicks. For example, CRH, glucagon-like peptide-1 (GLP-1), alpha-melanocyte-stimulating hormone (alpha- MSH) and pituitary adenylate cyclase-activating polypeptide (PACAP) decrease food intake of chicks (Furuse et al., 1997; Kawakami et al., 2000; Zhang et al., 2001; Tachibana et al., 2003) as has been shown in mammals. Additionally, ICV injection of NMS inhibits feeding behavior in adult quail (Shousha et al., 2006). Furthermore, chicken NMU receptor-2 is predicted (accession number: XP_425209). It is therefore possible that NMS might have an anorexigenic effect in chicks.

Therefore, the purpose of the present study was to investigate whether ICV injection of NMS affects feeding and drinking behavior in chicks. The effect of NMS on plasma glucose and non-esterified fatty acid (NEFA) was also examined because several anorexigenic neuropeptides affect them (Tachibana et al., 2007a,b,c). Furthermore, behaviors other than feeding and drinking were investigated. Finally, the effect of NMS on the HPA axis was assessed via measurement of plasma corticosterone concentration.

2. Materials and methods

2.1. Animals

Day-old male layer chicks (*Gallus gallus*, Julia, Ninobe Hatchery, Kagawa, Japan) were raised in a room kept at 30 °C with continuous lighting. A commercial diet (crude protein: 24%, metabolizable energy: 3050 kcal/kg, Toyohashi Feed Mills Co. Ltd, Aichi, Japan) and water were available ad libitum to the chicks. Chicks were transferred

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to their individual cages 1 day before each experiment. Before the experiment, body weight was measured and then chicks were distributed into experimental groups so that the average body weight was as uniform as possible between treatment groups. The chicks were maintained in accordance with the recommendations of the National Research Council (1996).

2.2. Peptides and ICV injections

Human NMS (Peptide Institute, Osaka, Japan) was dissolved in a saline solution that contained 0.1% Evans Blue dye. This saline solution was used as a vehicle and as the control treatment. ICV injections were performed according to a 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. The injection coordinates were 3 mm anterior to the coronal suture, 1 mm lateral from the sagittal suture, and 3 mm deep targeting the left lateral ventricle. Anatomical landmarks were determined visually and by palpation. The peptide solution was injected through the hole, using a microsyringe, at a volume of 10 μL. This procedure does not induce stress in neonatal chicks (Furuse et al., 1999; Saito et al., 2005). All injections were made between 10:00 and 12:00 under an ad libitum feeding condition (unless otherwise noted).

At the end of each experiment, the chicks were euthanized with an overdose of pentobarbital. The brain was then removed to confirm the accuracy of injection. Any chicks that did not have Evans Blue dye in the lateral ventricle were not used for further analyses.

2.3. Experiment 1: effect of NMS on ad libitum and food deprivation-induced feeding behavior

Six-day-old chicks were ICV injected with 0 (control, vehicle only), 0.01, 0.1 or 1 nmol NMS under an ad libitum feeding condition. Immediately after the injection, a pre-weighed feeder was then given to each chick. Their food intakes were measured at the accuracy of 0.001 g at 30 and 60 min after the injection. The dose of NMS was decided based on previous studies of rats and quail (Ida et al., 2005; Shousha et al., 2006; Miyazato et al., 2008). The number of chicks was as follows: 0 nmol, 9; 0.01 nmol, 9; 0.1 nmol, 10; and 1 nmol, 7.

In the food deprivation study, 5-day-old chicks were ICV injected with the same doses of NMS after 12-h food deprivation. Then food was given to chicks after the injection and food intake was measured at 30 and 60 min later. The number of chicks was as follows: 0 nmol, 9; 0.01 nmol, 9; 0.1 nmol, 10; and 1 nmol, 8.

2.4. Experiment 2: effect of NMS on water deprivation-induced drinking behavior

Before the injection, 6-day-old chicks underwent 12-h water deprivation to facilitate drinking behavior. Then the chicks were ICV injected with 0 (control), 0.01, 0.1 or 1 nmol NMS. Immediately after the injection, a pre-weighed water cup was given to each chick. Water intake was measured at the accuracy of 0.001 g at 30 and 60 min after the injection. Additional 6 water cups were put around cages to check water evaporation during experiment. The water cups were also weighed at 30 and 60 min and the change in the weight was regarded as the water evaporation. The evaporation of water was used for correction of water intake. The water intake was measured by weight and then was converted to volume. Food was removed after the injection so as to investigate the effect of NMS on drinking behavior itself. The number of chicks was as follows: 0 nmol, 7; 0.01 nmol, 8; 0.1 nmol, 6; and 1 nmol, 6.

2.5. Experiment 3: effect of NMS on plasma glucose and NEFA concentrations

Five-day-old chicks were ICV injected with 0 (control), 0.1 or 1 nmol NMS and then the chicks were deprived of food and water. Their blood was collected at 30 min following ICV injection from the jugular vein with heparin-containing micro-tubes. Blood was centrifuged (8000 *g* at 4 °C for 4 min) and the plasma was collected and stored at −80 °C. Plasma glucose and NEFA concentrations were measured using commercial kits (Wako Pure Chemical Industries, Osaka, Japan). The number of chicks was 8 in each group.

2.6. Experiment 4: effect of NMS on behavioral parameters

Behavioral observations were conducted for 30 min following ICV injection. Five-day-old chicks were injected with 0 (control) or 1 nmol NMS and then returned to their home cage. Their voluntary locomotion activity was quantified every 5 min with infrared beam sensors (NS-AS01, Neuroscience Inc., Japan) and analyzed by a digital data recording system software (DAS-008, Neuroscience Inc., Japan). The system counted more than 0.5 s movement as 1 unit of locomotion activity. Furthermore, the chick's behaviors were simultaneously recorded with a video camera. Those were categorized into standing and sitting and then these times (s) were measured. In addition, time spent for feeding during standing, the number of food pecks, water pecks, pecks except for food and water, wing-flapping and jumping also were measured. Food intake during the observation period was also measured. The number of chicks in 0 and 1 nmol groups were 7 and 6, respectively.

2.7. Experiment 5: effect of NMS on plasma corticosterone concentration

Five-day-old chicks were ICV injected with 0 (control), 0.1 or 1 nmol NMS and were then deprived of food and water. Their blood was collected at 30 min following ICV injection and the plasma was collected as described in Experiment 3. Plasma corticosterone concentration was measured by enzyme-immunoassay using rabbit anti-corticosterone antibody (420E, Cosmo Bio Co., Ltd., Tokyo, Japan). The intra- and inter-assay variations were 6.7 and 5.3%, respectively. The number of chicks in 0, 0.1 and 1 nmol group was 8, 7 and 8, respectively.

2.8. Statistical analysis

Data of Experiments 1 and 2 were statistically analyzed with one-way repeated measures analysis of variance (ANOVA). Locomotion activity of Experiment 4 was also analyzed with one-way repeated measures ANOVA. The Tukey–Kramer test was then used at each time point as a post hoc test. Data of Experiments 3 and 5 were analyzed with one-way ANOVA and then the Tukey–Kramer test was performed. Remainder data of Experiment 4 were analyzed with *t*-test. Data are expressed as means ± SEM. Statistical significance was set at $P<0.05$ for all experiments.

3. Results

3.1. Experiment 1: effect of NMS on ad libitum and food deprivation-induced feeding behavior

Fig. 1 shows the effect of ICV injection of NMS on ad libitum and food deprivation-induced food intake. The cumulative food intake increased with time [$F(1,31)=44.0$, $P<0.05$] but NMS significantly affected *ad libitum* feeding in chicks [$F(3,31)=10.3$, $P<0.05$]. The interaction was also significant [$F(3,31)=8.2$, $P<0.05$]. All doses of NMS significantly decreased ad libitum food intake at 30 min after the injection. The anorexigenic effect lasted to 60 min except for