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Central administration of chicken growth hormone-releasing hormone decreases food intake in chicks

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HIGHLIGHTS

- Central injection of cGHRH and cGHRH-LP inhibits feeding in chicks.
- cGHRH and cGHRH-LP do not induce abnormal behaviors.
- Their anorexigenic effects are not related to PACAP and CRH.
- cGHRH and cGHRH-LP are expected to be one of anorexigenic peptides in the brain of chicks.

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ABSTRACT

Growth hormone-releasing hormone (GHRH) is well known as a stimulator of growth hormone (GH) secretion. GHRH not only stimulates GH release but also modifies feeding behavior and energy homeostasis in rodents. In chickens (*Gallus gallus domesticus*), on the other hand, two types of GHRH, namely, chicken GHRH (cGHRH) and cGHRH-like peptide (cGHRH-LP), have been identified. The purpose of the present study was to investigate the effect of central injection of cGHRH and cGHRH-LP on feeding behavior in chicks. Intracerebroventricular (ICV) injection of both cGHRH and cGHRH-LP (0.04 to 1 nmol) significantly decreased food intake without any abnormal behavior in chicks. Furthermore, the feeding-inhibitory effect was not abolished by co-injection of the antagonist for pituitary adenylate cyclase-activating polypeptide (PACAP) or corticotropin-releasing hormone (CRH) receptors, suggesting that the anorexigenic effect of cGHRH and cGHRH-LP might not be related to the PACAP and CRH systems in the brain of chicks. Finally, 24-h food deprivation increased mRNA expression of cGHRH but not cGHRH-LP in the diencephalon. These results suggest that central cGHRH is related to inhibiting feeding behavior and energy homeostasis in chicks.

1. Introduction

Growth hormone-releasing hormone (GHRH) is well known as a stimulator of growth hormone (GH) and belongs to the glucagon superfamily peptides [1]. In addition to GH release, GHRH is expected to have other physiological roles since GHRH mRNA is widely expressed within the hypothalamus of rats [2]. In fact, several studies revealed that brain GHRH is related to regulating feeding behavior in mammals. For example, intracerebroventricular (ICV) injection of GHRH stimulates feeding behavior in rats [3,4]. This effect cannot be observed by intraperitoneal injection [3], demonstrating that central but not peripheral GHRH is related to stimulating feeding behavior. On the other hand, a higher dose of GHRH inhibits feeding behavior in rats when injected centrally [5]. Thus, the effect of GHRH on feeding behavior depends on the dose and site of injection.

In chickens, GHRH-like peptide (cGHRH-LP) has been identified, but its amino acid sequence shows low homology to mammalian GHRH [1,6]. Furthermore, cGHRH-LP is less potent in stimulating GH release in chickens [7] while mammalian GHRH stimulates it in chickens [8]. Wang et al. [9] have identified novel chicken GHRH (cGHRH), which has higher affinity to chicken GHRH receptors (cGHRH receptor-1 and -2) than cGHRH-LP [10]. This cGHRH consists of 47 amino acids (Table 1), but the existence of a short form of cGHRH is also suggested

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Table 1
The amino acid sequences of cGHRH and cGHRH-LP.

Peptide	Amino acid sequence
cGHRH27	HADAIFTDNYRKFLGQISARKFLQTII
cGHRH27-NH_2	HADAIFTDNYRKFLGQISARKFLQTII-NH_2
cGHRH47	HADAIFTDNYRKFLGQISARKFLQTIIGKRLRNSESSPGEGVHKLLT
cGHRH-LP	HADGIFSKAYRKLLGQLSARKYLHSLMAKRVGGASSGLGDEAEPLS

Abbreviations are as follows: cGHRH27, cGHRH(1–27); cGHRH27-NH_2, cGHRH(1–27) amide; cGHRH47, cGHRH(1–47).

because cGHRH(1–31) still displays high potency in activating cGHRH receptors [9]. In addition, the 28th amino acid Gly is expected to be the amidation site which also exists in the precursors of other glucagon family peptides such as pituitary-adenylate cyclase polypeptide (PACAP) and vasoactive intestinal peptide, suggesting the existence of a short amidated form of cGHRH, namely, cGHRH(1–27)-NH_2 (Table 1). This idea is plausible because cGHRH(1–27)-NH_2 shows high affinity to both cGHRH-R1 and R2 *in vitro* [11] and stimulates GH release from the pituitary of chickens in vitro [12]. The mRNA of this cGHRH is predominantly expressed in the hypothalamus [9], suggesting that this novel GHRH is orthologous to mammalian GHRH in chickens [9,10,12]. However, the effect of GHRH on feeding behavior has not been investigated yet.

The 27 amino acids sequences at N terminus are similar between cGHRH and cGHRH-LP (Table 1). The cGHRH not only binds to cGHRH receptors but also to the cGHRH-LP receptor with a lower affinity [10]. Also, cGHRH-LP binds to both cGHRH receptors and the cGHRH-LP receptor while the affinity of cGHRH-LP to cGHRH receptors is notably lower than that of cGHRH [10]. These facts and previous studies suggest that the genes of cGHRH and cGHRH-LP originate from the same ancestral gene by gene duplication [1,9]. In fact, vertebrates other than mammals have both GHRH and GHRH-LP while mammals have only GHRH [1,9]. In non-mammalian vertebrates, GHRH-LP is encoded by the same gene as PACAP. In mammals, the PACAP gene includes the PACAP-related peptide (PRP) gene as an ortholog of GHRH-LP in non-mammalian vertebrates. In chickens, the physiological role of cGHRH-LP has not been investigated yet, but cGHRH-LP mRNA is widely expressed in the brain of chickens [9].

In the present study, we investigated the effect of ICV injection of cGHRH and cGHRH-LP on feeding behavior in chicks. We found that both peptides inhibited feeding behavior in chicks. We therefore performed additional experiments to clarify the mechanism underlying the anorexigenic effect of the peptides. For the additional experiments, cGHRH(1–27)-NH_2 was used because this peptide is thought to be a native ligand for cGHRH-Rs [9,11,12].

2. Materials and methods

2.1. Animals

Day-old male layer chicks (*Gallus gallus domesticus*, Julia; Nihon-Layer, Gifu, Japan) were raised in a room kept at 30 °C with continuous lighting. A commercial diet (crumble, crude protein: 24%, metabolizable energy: 3,050 kcal/kg; Toyohashi Feed Mills Co. Ltd, Aichi, Japan) and water were available ad libitum to the chicks. Chicks were transferred to their individual cages 1 day prior to 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 [13]. This study was approved by the Committee of Animal Care and Use in Ehime University (No. 08-o3-10).

2.2. Peptides and ICV injections

All injections were given between 8:00 and 10:00 a.m. Synthetic cGHRH(1–27)(cGHRH27), cGHRH(1-27NH_2)(cGHRH27-NH_2), cGHRH(1–47)(cGHRH47) and cGHRH-LP(1–46) were used for the present study. In addition, PACAP(6–38) and astressin were purchased from Peptide Institute (Osaka, Japan) and GenScript USA Inc. (NJ, USA), respectively. All peptides were dissolved in a saline solution containing 0.1% Evans blue dye, which included 0.005 N hydrochloric acid to support the dissolution of cGHRHs and cGHRH-LP. This vehicle was used for the control treatment.

ICV injections were performed according to a method reported previously [14]. In brief, the head of the chick was inserted into an acrylic box with a hole in the top plate. The injection coordinates were 3 mm anterior from the parietal bone, 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 micro-syringe at a volume of 10 μl. This procedure is quick and does not stress the neonatal chicks judging from food intake and corticosterone release [15,16]. 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 in which Evans blue dye could not be defined in the lateral ventricle were not used for further analyses.

2.3. Experimental procedures

2.3.1. Effect of ICV injection of cGHRH and cGHRH-LP on food intake

To investigate the effect of cGHRH and cGHRH-LP, food intake was measured under a 12-h fasting condition. Five-day-old chicks (6-day-old chicks for the cGHRH27 study) were ICV injected with 0 (control), 0.04, 0.2, or 1 nmol cGHRH27, cGHRH27-NH_2, cGHRH47, or cGHRH-LP after 12-h food deprivation. A pre-weighed feeder was then given to each chick, and food intake was measured at 30, 60 and 90 min after the injection using a digital balance with an accuracy of 1 mg.

Additionally, similar experiments were performed using 5-day-old chicks (6- and 4-day-old chicks for the cGHRH47 and cGHRH-LP studies, respectively) under an ad libitum feeding condition. After the ICV injection of 0 (control), 0.04, 0.2, or 1 nmol of each peptide, food intake was measured as noted above.

2.3.2. Effect of ICV injection of cGHRH and cGHRH-LP on behavior

Behavioral observations were carried out for 30 min following ICV injection of cGHRH and cGHRH-LP. Five-day-old chicks were ICV injected with saline (control, vehicle only), 1 nmol cGHRH27-NH_2, or 1 nmol cGHRH-LP and then returned to their home cage where diet and water were removed. Their voluntary activity was quantified with infrared beam sensors (NS-AS01; Neuroscience Inc., Japan) and analyzed using digital data recording system software (DAS-008; Neuroscience Inc., Japan). The system counted more than 0.5 s of movement as 1 unit of locomotion activity. Additionally, their behavior was recorded with a video camera, and the number of peckings of the cage and preening, scratching, jumping, and wing-flapping behavior was counted.

2.3.3. Effect of co-injection of PAC1 receptor antagonist on the anorexigenic effect of cGHRH and cGHRH-LP

The effect of ICV co-injection of PACAP(6–38), a PAC1 receptor antagonist, on cGHRH or cGHRH-LP-induced anorexia was investigated. Five-day-old chicks fasted for 12 h were ICV injected with saline (control, vehicle only), 1 nmol cGHRH27-NH_2, or 1 nmol cGHRH27-NH_2 plus 0.5 nmol PACAP(6–38). The dose of PACAP(6–38) was decided based on a previous study in chicks [17]. Food intake was then measured at 30, 60, and 90 min after the injection. The cGHRH-LP study was also performed using the same method.

2.3.4. Involvement of CRH on the effect of cGHRH and cGHRH-LP

First, the effect of ICV injection of cGHRH or cGHRH-LP on plasma corticosterone concentration was investigated. Seven-day-old chicks were ICV injected with 0 (control), 0.2, or 1 nmol cGHRH27-NH_2 under