

Peripheral Injection of Chicken Growth Hormone-Releasing Hormone Inhibits Feeding Behavior in Chicks

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Growth hormone-releasing hormone (GHRH), a stimulator of growth hormone (GH) secretion, is known to have several physiological roles such as the regulation of feeding behavior in mammals. Recently, we have reported that central injection of chicken GHRH decreased food intake in chicks, however, its peripheral role on feeding behavior has not been clarified. The purpose of the present study was to investigate the effect of peripheral injection of GHRH on feeding behavior in chicks (*Gallus gallus*). Intraperitoneal (IP) injection of GHRH47 (1 nmol), full length form of chicken GHRH significantly decreased food intake in chicks although the injection of GHRH27 and GHRH27-NH₂, short forms of chicken GHRH had no effect. The IP injection of GHRH47 did not induced any abnormal behavior, suggesting that GHRH47-induced anorexia might not be related to abnormal behavior such as sleeping, hyperactivity and convulsion. The anorexigenic effect of GHRH47 seemed not to be related to GH because IP injection of bovine GH did not affect feeding behavior in chicks. Collectively, these results suggest that peripheral GHRH is related to inhibit feeding behavior in chicks.

Key words: behavior, chick, feeding, growth hormone, growth hormone-releasing hormone, intraperitoneal injection

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Introduction

Growth hormone-releasing hormone (GHRH) is well known as a stimulator of growth hormone (GH) secretion (Sherwood *et al.*, 2000). The physiological role of GHRH is thought not to be limited in the release of GH, because GHRH receptor mRNA is widely expressed within the hypothalamus of rats (Takahashi *et al.*, 1995). In fact, brain GHRH is related to the regulation of feeding behavior because intracerebroventricular (ICV) injection of GHRH facilitates feeding behavior in rats (Vaccarino *et al.*, 1985; Vaccarino, 1990). However, the ICV injection of GHRH suppresses feeding behavior in rats at a higher dose (Imaki *et al.*, 1985), suggesting that the effect of GHRH on feeding behavior depends on the dose.

In chickens, GHRH has been identified and consists of 47 amino acids (GHRH47), but the short form of GHRH is also

suggested because GHRH47 has Lys-Arg sequence at 29th and 30th position (Wang *et al.*, 2007). In addition, the 28th amino acid Gly is expected to be amidated and thereby generate GHRH (1-27)-NH₂ (GHRH27-NH₂). GHRH27-NH₂ is thought to have similar physiological roles like GHRH47 because it shows high affinity to both chicken GHRH-receptor-1 and -receptor-2 *in vitro* (Huang *et al.*, 2012) and stimulates GH release from the pituitary of chickens *in vitro* (Meng *et al.*, 2014). In addition to these GHRHs, there is a peptide which has similar amino acid sequence in avian. This peptide is named as GHRH-like peptide (GHRH-LP) and is thought to be derived whole genome duplication (Wang *et al.*, 2007). The mRNA of chicken GHRH is predominantly expressed in the hypothalamus while GHRH-LP mRNA is widely expressed in the brain (Wang *et al.*, 2007). Interestingly, in contrast to rodents, ICV injection of both GHRHs and GHRH-LP suppresses feeding behavior in chicks (*Gallus gallus*) even though a lower dose was injected (Tachibana *et al.*, 2015). It is therefore possible that GHRH and GHRH-LP are related to the regulation of feeding in chicks but their effects would not be the same as that of mammals.

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In rodents, GHRH and its mRNA are distributed not only in the brain but also the peripheral tissues (Matsubara *et al.*, 1995). In addition, GHRH- or its receptor-immunoreactive cells are also found in several peripheral organs such as small intestine, kidney, liver and lung (Christodoulou *et al.*, 2006). Intraperitoneal (IP) injection of GHRH had no effect on food intake in rats (Vaccarino, 1990), however, consecutive subcutaneous injection of GHRH decreases food intake in 24-month-old LOU rats while it had no effect in 11-, 19-, and 28-month-old LOU rats (Veyrat-Durebex *et al.*, 2001). Thus, the role of peripheral GHRH on the regulation of feeding seems to be complex and remains to be studied. Similar to rodents, GHRH, GHRH-LP and their receptors mRNA have been identified in the several peripheral organs including small intestine in chickens (Wang *et al.*, 2007; 2010). Although IP injection of GHRH does not alter food intake in rats (Vaccarino, 1990), there is the possibility that this peptide may affect feeding behavior in chicks because central injection of GHRH showed opposite effect (Tachibana *et al.*, 2015). However, the physiological roles of peripheral GHRH on feeding regulation have not been investigated yet in chickens.

In the present study, we investigated the effect of IP injection of non-amidated GHRH27 (GHRH27), GHRH27-NH₂, GHRH47 and GHRH-LP on feeding behavior in chicks. As the result, since GHRH47 showed the anorexic effect, how GHRH47 suppressed feeding behavior was also investigated.

Materials and Methods

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 freely given to the chicks. Chicks were transferred to their individual cages 1 day prior to each experiment in order to accustom to the experimental condition. On the experimental day, chicks were weighed and distributed into experimental groups so that the average body weight was as uniform as possible between groups. The chicks were raised in accordance with the recommendations of the National Research Council (1996). This study was approved by the Committee of Animal Care and Use in Ehime University (No. 08-o3-10).

Peptides and Injections

Chicken GHRH27 and GHRH27-NH₂ were synthesized by Sigma-Aldrich Co. LLC, MO, USA and GL Biochem. Shanghai, China, respectively. GHRH47 was synthesized by a peptide synthesizer (Syo Wave, Biotage, Uppsala, Sweden). These peptides were dissolved in a saline solution which included 0.005N hydrochloric acid to facilitate the dissolution. Bovine GH (MP Biomedicals, CA, USA) was dissolved in a saline solution. Each vehicle was used for the control treatment. These solutions were IP injected at a volume of 0.2 ml per chick. All IP injections were per-

formed between 08:00 and 10:00.

Effect of IP Injection of GHRHs on Food Intake

Seven-day-old chicks (average body weight, 56.2±0.5 g; 6-day-old chicks for the GHRH47 study, average body weight, 54.1±0.5 g) were IP injected with 0 (control), 0.2 or 1.0 nmol GHRH27, GHRH27-NH₂ or GHRH47 under an *ad libitum* feeding condition. The dose of GHRHs was sufficient to reduce food intake of chicks when administered centrally (Tachibana *et al.*, 2015). 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.

Effect of IP Injection of GHRH-LP on Food Intake

Seven-day-old chicks (average body weight, 50.8±0.7 g) were IP injected with 0 (control), 0.2 or 1.0 nmol GHRH-LP under an *ad libitum* feeding condition. The dose of GHRH-LP was decided based on a previous study (Tachibana *et al.*, 2015). Then the food intake was measured at 30, 60 and 90 min after the injection.

Effect of GHRH47 on Behavioral Patterns

To investigate whether IP injection of GHRH47 induces abnormal behaviors, the effect of GHRH47 on behavioral patterns were investigated. Seven-day-old chicks (average body weight, 51.8±0.9 g) were IP injected with 0 (control) or 1.0 nmol GHRH47 under an *ad libitum* feeding condition. Then food and water were removed to exclude the effect of GHRH47-induced anorexia. Their voluntary activity was quantified for 30 min post-injection using infrared beam sensors (NS-AS01; Neuroscience Inc., Japan) and a digital data recording system software (DAS-008; Neuroscience Inc., Japan). The system counted more than 0.5 s of movement as 1 unit of voluntary activity. Additionally, the behavior was recorded with a digital video camera for 30 min after the injection, and the time spent for standing and sitting were measured. The numbers of preening, scratching, jumping and wing-flapping behavior were also counted.

Effect of IP Injection of Bovine GH on Food Intake

Six-day-old chicks (average body weight, 50.3±0.6 g) were IP injected with 0 (control), 0.03 or 0.15 nmol bovine GH under an *ad libitum* feeding condition. Food intake was measured as noted above.

Statistical Analysis

Data for food intake were analyzed using repeated two-way analysis of variance (ANOVA) with respect to peptide and time, and with the Tukey-Kramer test at each time point. Data of behavioral patterns were analyzed with t-test. Data are expressed as means±SEM and statistical significance was set at $p < 0.05$. The numbers of chicks are noted in the figure legends.

Results

Effect of IP Injection of GHRHs on Food Intake

IP injection of GHRH27 tended to decrease food intake in chicks, but the effect was not significant (Fig. 1). Similarly, the injection of GHRH27-NH₂ did not affect food intake (Fig. 1). On the other hand, GHRH47 significantly decreased food intake: 0.2 nmol GHRH47 significantly decreased it at

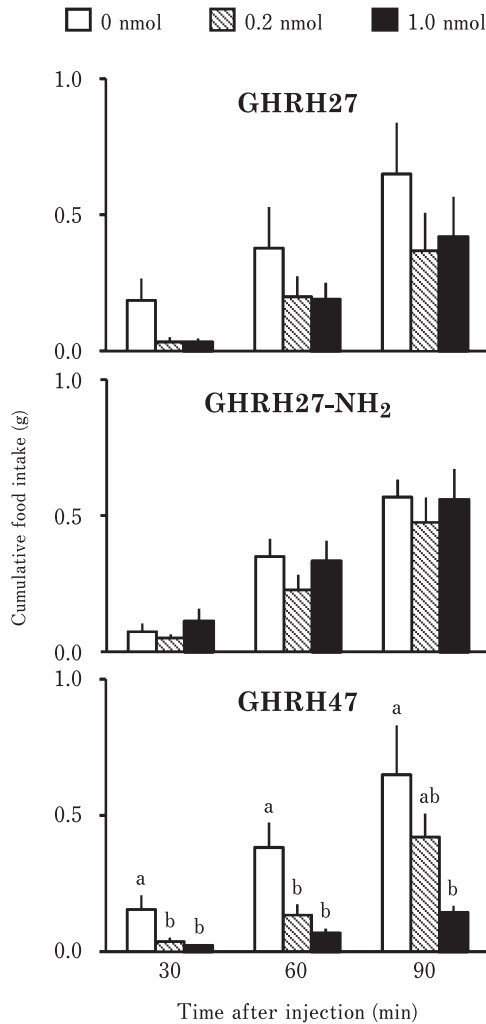


Fig. 1. Food intake after IP injection of GHRH27, GHRH27-NH₂ and GHRH47 in chicks under an *ad libitum* feeding condition. Data are expressed as means \pm SEM. The number of chicks was 8 in each group of GHRH27 study and 9 in each group of GHRH27-NH₂ and GHRH47 studies. Groups with different letters are significantly different at each time ($P < 0.05$).

30 and 60 min while 1.0 nmol decreased it at all times determined (Fig. 1).

Effect of IP Injection of GHRH-LP on Food Intake

IP injection of 0.2 and 1.0 nmol GHRH-LP did not affect food intake at all times (Fig. 2).

Effect of GHRH47 on Behavioral Patterns

IP injection of GHRH47 did not affect the voluntary movement during 30 min post-injection (Table 1). GHRH47 also did not affect the time spent standing and sitting, and the number of preening, scratching, jumping and wing-flapping behavior (Table 1).

Effect of IP Injection of Bovine GH on Food Intake

Food intake was not significantly altered by any doses of bovine GH (Fig. 3).

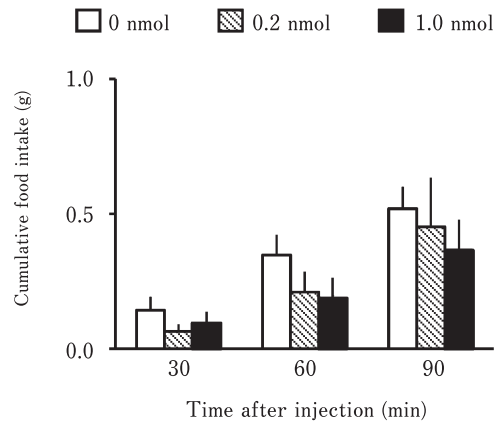


Fig. 2. Food intake after IP injection of GHRH-LP in chicks under an *ad libitum* feeding condition. Data are expressed as means \pm SEM. The number of chicks in 0, 0.2 and 1.0 nmol groups was 8, 8 and 7, respectively.

Table 1. Behavioral patterns after IP injection of GHRH 47 in chicks

	Experimental group	
	Control	GHRH47 ¹
n	6	6
Voluntary movement ³	207 \pm 54 ²	291 \pm 78
Standing time (s)	1227 \pm 332	1304 \pm 266
Sitting time (s)	573 \pm 332	496 \pm 266
Preening (times)	14 \pm 6	15 \pm 12
Scratching (times)	1 \pm 0	1 \pm 0
Jumping (times)	4 \pm 3	3 \pm 3
Wing-flapping (times)	0 \pm 0	0 \pm 0

¹ The injected dose of GHRH47 was 1 nmol.

² Data are expressed as means \pm SEM.

³ Arbitrary unit.

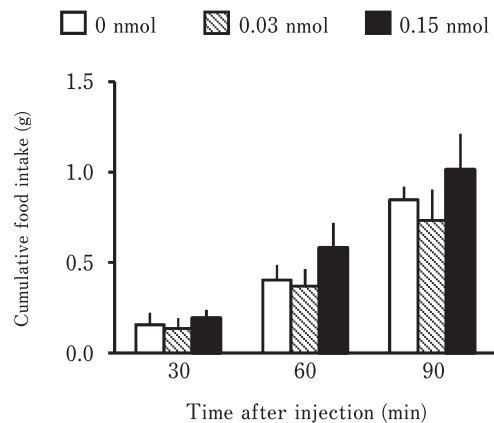


Fig. 3. Effect of IP injection of bovine GH on food intake in chicks under an *ad libitum* feeding condition. Data are expressed as means \pm SEM. The number of chicks was 9 in each group.

Discussion

We showed here that IP injection of GHRH47 significantly decreased food intake in chicks (Fig. 1). Since GHRH47 was demonstrated to inhibit feeding behavior when administered centrally (Tachibana *et al.*, 2015), both central and peripheral GHRH47 is expected to be related to the regulation of feeding behavior in chicks. On the other hand, IP injection of GHRH27 and GHRH27-NH₂ did not affect feeding behavior in chicks although GHRH27 tended to decrease it (Fig. 1). These results suggest that C-terminus amino acids would be important for the anorexigenic effect of GHRH. This feature is also observed when GHRH is administered centrally because ICV injection of GHRH47 shows strong anorexigenic effect rather than GHRH27 and GHRH27-NH₂ in chicks (Tachibana *et al.*, 2015). Although GHRH-LP suppresses food intake in chicks when administered centrally (Tachibana *et al.*, 2015), IP injection of GHRH-LP did not affect feeding behavior in chicks (Fig. 2). Thus the effect of GHRH-LP on feeding behavior is different between central nervous system and peripheral tissues.

In contrast to the present study, the IP injection of GHRH has no effect on feeding behavior in rats (Vaccarino *et al.*, 1985). Thus the effect of GHRH in peripheral is also different between chicks and rodents as well as the central nervous system (Imaki *et al.*, 1985; Vaccarino *et al.*, 1985; Vaccarino, 1990; Tachibana *et al.*, 2015). However, there remained a possibility that GHRH47 might induce abnormal behavior such as sleeping in chicks, and thereby inhibit feeding behavior as the side effect. Indeed, the injection of GHRH promotes non-rapid eye movement sleeping in rats (Obál *et al.*, 1996). In addition, the injection of anti-GHRH antibody suppresses spontaneous sleeping, demonstrating that GHRH is related to sleeping in rodents (Obál *et al.*, 1996). Furthermore, GHRH-deficient mice shows increase in activity and decreased anxiety- and depression-related behavior (Leone *et al.*, 2014). In order to elucidate the effect of GHRH47 on the behavioral patterns of chicks, chicks injected with GHRH47 were observed in the subsequent experiment. As the result, GHRH47 did not alter voluntary movement, standing time and sitting time (Table 1). The number of preening, scratching, jumping and wing-flapping behavior were not also affected by GHRH47 (Table 1). In addition, during the experimental period, the chicks did not show any specific behaviors such as sleeping behavior and vocalization although we did not quantify these behaviors. These results imply that the anorexigenic effect of GHRH47 might not be involved in any abnormal behavior.

It has been reported that the intravascular injection of GHRH increases plasma GH concentration in 4- to 5-week-old male White Leghorn chicken (Harvey *et al.*, 2014). Therefore, it might be a possibility that the GHRH-induced GH was related to the anorexigenic effect of GHRH in chicks. In fact, Buntin *et al.* (1988) demonstrated that mammalian GH stimulates feeding behavior in ring doves when administered centrally. However, we could not find the anorexigenic effect after IP injection of bovine GH (Fig. 3),

indicating that peripheral GH might not be related to the anorexigenic effect of GHRH in chicks.

Some previous studies revealed that the magnitude of the effect of some feeding regulatory peptides is different between the strains of chicks. For example, some anorexigenic peptides such as corticotropin-releasing hormone and glucagon-like peptide-1 showed weaker effect in broiler-type chicks than layer-type chicks (Tachibana *et al.*, 2006). Although the IP injection of GHRH47 inhibited feeding behavior of layer-type chicks in the present study, we have not examined the effect of GHRH on broiler-type chicks. It was reported that blood GH concentration and GH mRNA in the pituitary are different between layer-type and broiler-type chicks (Reiprich *et al.*, 1995), suggesting that the effect of GHRH might be different between the strains of chicks. Further studies using broiler-type chicks will clarify whether the effect of GHRH on feeding behavior is different between the strains of chicks.

In summary, the present study revealed that peripheral injection of GHRH47 inhibited feeding behavior in chicks. Although the anorexigenic mechanism of GHRH47 in chicks is still unknown but we found that GHRH-induced anorexia might not related to any abnormal behavior or GHRH-induced GH release.

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