The Role of Corticotropin-Releasing Factor in Appetite Regulation in Broiler Chickens: Post-print

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Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment used live broiler chickens as experimental subjects, exogenously administered corticotropin-releasing factor (CRF) via intravenous injection, analyzed changes in feed intake and expression of appetite-regulating genes in the hypothalamus and intestine, and investigated the role of CRF in appetite regulation in broiler chickens. Twenty 1-day-old male Arbor Acres (AA) broiler chickens with similar body weight were selected and, at 7 days of age, randomly divided into 2 groups with 10 chickens per group, individually housed in single cages. Broiler chickens in the experimental group were injected with 1,000.0 g/kg BW of CRF via the wing vein at 08:00 on day 10 of age, while the control group was simultaneously injected with an equal volume of physiological saline. Two hours after injection, feed intake was recorded, and at 10:00 on day 10 of age, the chickens were slaughtered for sample collection. Real-time fluorescence quantitative PCR (RT-PCR) was used to detect the relative mRNA expression levels of appetite-related genes ghrelin, corticotropin-releasing factor receptor (CRFR) 1, CRFR2, and growth hormone secretagogue receptor-1 (GHSR-1) in the hypothalamus, duodenum, jejunum, and ileum. The results showed that, compared with the control group, intravenous injection of CRF significantly reduced feed intake in broiler chickens ($P<0.05$); significantly increased hypothalamic ghrelin and CRFR1 mRNA relative expression levels ($P<0.05$), while hypothalamic CRFR2 and GHSR-1 mRNA relative expression levels showed no significant changes ($P>0.05$); significantly increased CRFR1, CRFR2, and GHSR-1 mRNA relative expression levels in the duodenum, jejunum, and ileum ($P<0.05$), significantly decreased duodenal ghrelin mRNA relative expression level ($P<0.05$), while jejunal and ileal ghrelin mRNA relative expression levels showed no significant changes ($P>0.05$). These results indicate that intravenous injection of CRF reduces feed intake in broiler chickens, induces central ghrelin gene expression, and appetite suppression caused by upregulated expression of

hypothalamic CRFR1, ghrelin, and intestinal CRFR1, CRFR2, and GHSR-1a genes may be responsible for the reduction in feed intake.

Full Text

Effects of Corticotropin-Releasing Factor on Appetite Regulation in Broiler Chickens

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Abstract

This study investigated the role of corticotropin-releasing factor (CRF) in appetite regulation in broiler chickens by examining changes in feed intake and the expression of appetite-related genes in the hypothalamus and intestine following intravenous administration of exogenous CRF. Twenty 1-day-old male Arbor Acres (AA) broiler chickens with similar body weight were randomly divided into two groups at 7 days of age, with 10 birds per group housed individually in single cages. At 10 days of age, broilers in the experimental group received an intravenous injection of 1,000.0 g/kg body weight CRF via the wing vein at 08:00, while the control group received an equivalent volume of saline. Feed intake was recorded 2 hours post-injection, and all birds were slaughtered at 10:00 on day 10 for sample collection. Real-time quantitative PCR (RT-PCR) was used to measure the relative mRNA expression levels of appetite-related genes, including ghrelin, corticotropin-releasing factor receptor 1 (CRFR1), CRFR2, and growth hormone secretagogue receptor-1 (GHSR-1), in the hypothalamus, duodenum, jejunum, and ileum.

The results demonstrated that intravenous injection of CRF significantly reduced feed intake in broiler chickens compared to the control group ($P<0.05$). CRF administration significantly increased the relative mRNA expression levels of ghrelin and CRFR1 in the hypothalamus ($P<0.05$), while expression levels of CRFR2 and GHSR-1 in the hypothalamus remained unchanged ($P>0.05$). In the intestinal tract, CRF significantly elevated the relative mRNA expression of CRFR1, CRFR2, and GHSR-1 in the duodenum, jejunum, and ileum ($P<0.05$), but significantly decreased ghrelin mRNA expression in the duodenum ($P<0.05$). No significant changes in ghrelin mRNA expression were observed in the jejunum or ileum ($P>0.05$).

These findings indicate that intravenous administration of CRF reduces feed intake in broiler chickens and induces central ghrelin gene expression. The appetite suppression resulting from upregulated expression of hypothalamic CRFR1 and ghrelin, together with increased intestinal expression of CRFR1,

CRFR2, and GHSR-1 , likely contributes to the observed decrease in feed intake.

Keywords: broiler chickens; appetite; feed intake; CRF; ghrelin

Introduction

Stress response represents a disruption and restoration of physiological homeostasis, a process primarily mediated by activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis [1]. Corticotropin-releasing factor (CRF) is widely expressed in the central nervous system of vertebrates as well as in peripheral tissues including the gastrointestinal tract, playing crucial physiological roles in regulating the HPA axis and maintaining stress- and energy-related homeostasis [2]. Environmental changes and stress conditions activate CRF in vertebrates, leading to reduced feed intake and reproductive activity while increasing arousal and grooming behavior [3]. Studies have demonstrated that CRF participates in feeding and body weight regulation in mammals and fish [4-5]. Peripheral CRF plays an important role in stress-induced alterations in gastrointestinal motility, as peripheral injection of CRF inhibits gastric emptying in mice [6]. Furthermore, endogenous intestinal CRF is critical in mediating stress-induced changes in colonic motility and secretory function, with CRF knockdown in rat colon preventing these stress effects [7].

Ghrelin, a 28-amino acid peptide belonging to the brain-gut peptide family, serves as the endogenous ligand for the growth hormone secretagogue receptor (GHSR) [8]. Although primarily produced in the stomach of vertebrates, ghrelin is also expressed in various tissues including the brain, intestine, pancreas, gallbladder, kidneys, and gills [9-10]. Ghrelin increases feed intake and body weight gain in mammals through peripheral or central mechanisms [10] and induces foraging behavior and accelerates growth in trout [11]. In contrast to mammals and fish, ghrelin functions as an anorexigenic neuropeptide in poultry; intracerebroventricular injection of ghrelin in newly hatched chicks produces anxiety-like behavior and strongly suppresses feed intake [12]. Similarly, intracerebroventricular ghrelin administration in laying hens reduces both feed and water intake [13]. Studies using the glucocorticoid receptor antagonist RU486 and the CRF receptor antagonist astressin have revealed that peripheral ghrelin participates in appetite suppression in broiler chickens through the HPA axis [14].

Given that the HPA axis is regulated by CRF and that peripheral ghrelin is involved in appetite suppression via the HPA axis, we hypothesized that ghrelin might represent a molecular mediator linking CRF to appetite regulation. To address this question, the present study employed intravenous administration of exogenous CRF in live broiler chickens and analyzed the expression of relevant genes [ghrelin, corticotropin-releasing factor receptors (CRFR)1, CRFR2, and growth hormone secretagogue receptor type 1 (GHSR-1)] in the hypothalamus and intestine to explore the effects of CRF on appetite and ghrelin, thereby

providing a theoretical basis for understanding CRF's role in appetite regulation in broiler chickens.

1.1 Experimental Materials and Design

CRF was synthesized by Maituo (China) Biotechnology Co., Ltd. with a purity of 96.29%. Twenty 1-day-old male Arbor Acres (AA) broiler chickens with similar body weight were obtained from Shandong Dabao Poultry Industry Co., Ltd. Birds were housed in cages at 35°C with natural ventilation and relative humidity maintained at 55-65%. They were fed a basal diet formulated according to NRC (1994) nutrient requirements for broiler chickens (composition and nutrient levels shown in Table 1) and provided free access to feed and water. At 7 days of age, birds were randomly divided into two groups of 10 chickens each, with individual housing in single cages. The experimental group received an intravenous injection of 1,000.0 g/kg body weight CRF via the wing vein at 08:00 on day 10, while the control group received an equivalent volume of saline. Based on the body weight range in this experiment (256-272 g), a 1 mg/mL CRF solution was prepared, with a 260 g broiler receiving 0.26 mL of the CRF solution.

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Items	Content
Ingredients	
Corn	
Soybean oil	
Soybean meal	
Limestone	
CaHPO	
NaCl	
L-Lys · H SO	
DL-Met	
L-Thr	
Phytase (5,000 IU/g)	
Multi-vitamins[1]	
Multi-mineral[1]	
Choline chloride	
Total	
Nutrient levels[2]	
Crude protein (CP)	
Metabolizable energy (ME) (MJ/kg)	
Non-phytate phosphorus (NPP)	
Lysine (Lys)	
Methionine (Met)	
Methionine + Cysteine (Met+Cys)	

Items	Content
Threonine (Thr)	
Tryptophan (Trp)	

[1] Multi-vitamin and multi-mineral provided the following per kg of diet: VA 9,000 IU, VD 2,000 IU, VE 11.0 IU, VK 1.00 mg, thiamine 1.20 mg, riboflavin 5.80 mg, niacin 66.0 mg, pantothenic acid 10.0 mg, pyridoxine 2.60 mg, biotin 0.20 mg, folic acid 0.70 mg, VB 0.012 mg, Mn 100 mg, Zn 75.0 mg, Fe 80.0 mg, I 0.65 mg, Cu 8.00 mg, Se 0.35 mg.

[2] Nutrient levels were calculated values.

1.2 Sample Collection and Measurement

Feed intake was recorded for each bird during the 2-hour period following injection. At 2 hours post-injection, both experimental and control groups were simultaneously sampled for hypothalamus, duodenum, jejunum, and ileum tissues, which were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Total RNA was extracted from hypothalamic and intestinal tissue samples using an Animal Tissue/Cell RNA Extraction Kit (Beijing Kangwei Century Biotechnology Co., Ltd.) according to the manufacturer' s instructions. RNA concentration and purity were assessed using a micro-ultraviolet spectrophotometer (DS-11, DeNovix, USA) at 260 nm wavelength. cDNA synthesis was performed using the Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany) in a 20 L reaction volume with the following parameters: 25°C for 10 min, 55°C for 30 min, and 85°C for 5 min. The synthesized cDNA was stored at -20°C until use.

Real-time quantitative PCR (RT-PCR) was conducted with an initial denaturation step at 95°C for 10 s, followed by 40 cycles of PCR amplification at 95°C for 5 s and 60°C for 40 s. All primers were designed across introns using Dnaman 5.0 software based on published sequences from GenBank and synthesized by Shanghai Sangon Biotech Co., Ltd. Primer sequences are listed in Table 2 . The relative mRNA expression levels of target genes were quantified using the $2-\Delta\Delta CT$ method [15] with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene.

Table 2 Primer sequences

Gene names	GenBank accession No.	Primers sequences (5 →3)	Product size/bp
Ghrelin	AB075215	F: CCTTGGGACA-GAAACTGCTCR: GCTTCC-CAAACCAGCACTTCT	
CRFR1	NM_204321.1	F: CCTCACCTATTC-CACCGACAAGR: CAC-CAATTTCAAAAG-GAACGC	
CRFR2	NM_204454.1	F: TGCTCCAAAT-GATAGACCACAAR: AGCCTTCCACAAA-CATCCAGAA	
GHSR-1	AB095995.1	F: TTTTTCCTGCC-CGTATTCTGR: GCTTGGTGCTGGA-GAGTCTT	
GAPDH	NM_204305	F: ACATGGCATC-CAAGGAGTGAGR: GGGGAGACA-GAAGGGAACAGA	

1.3 Statistical Analysis

Data were analyzed using SPSS 24.0 software with t-tests. The confidence interval was set at 95%, and $P<0.05$ was considered statistically significant.

2 Results

2.1 Effects of Intravenous CRF Injection on Feed Intake in Broiler Chickens

As shown in Figure 1 [Figure 1: see original paper], intravenous injection of CRF significantly reduced feed intake within the 2-hour period compared to the control group ($P<0.05$).

Figure 1 Effects of intravenous injection of CRF on feed intake of broilers.

2.2 Effects of Intravenous CRF Injection on Relative mRNA Expression of Appetite-Regulating Genes in Hypothalamus and Intestine

Figure 2 [Figure 2: see original paper] demonstrates that intravenous CRF injection significantly increased the relative mRNA expression levels of ghrelin

and CRFR1 in the hypothalamus ($P<0.05$), while having no significant effect on GHSR-1 or CRFR2 mRNA expression ($P>0.05$).

Figure 2 Effects of intravenous injection of CRF on relative mRNA expression levels of related genes in the hypothalamus of broilers.

As illustrated in Figure 3 [Figure 3: see original paper], compared to the control group, intravenous CRF injection significantly decreased ghrelin mRNA expression in the duodenum ($P<0.05$) while significantly increasing the expression of GHSR-1 , CRFR1, and CRFR2 mRNA ($P<0.05$).

Figure 3 Effects of intravenous injection of CRF on relative mRNA expression levels of related genes in the duodenum of broilers.

Figure 4 [Figure 4: see original paper] shows that intravenous CRF injection significantly elevated GHSR-1 , CRFR1, and CRFR2 mRNA expression levels in the jejunum ($P<0.05$), without significantly affecting ghrelin mRNA expression ($P>0.05$).

Figure 4 Effects of intravenous injection of CRF on relative mRNA expression levels of related genes in the jejunum of broilers.

Similarly, Figure 5 [Figure 5: see original paper] reveals that intravenous CRF injection significantly increased GHSR-1 , CRFR1, and CRFR2 mRNA expression in the ileum ($P<0.05$), while ghrelin mRNA expression remained unchanged ($P>0.05$).

Figure 5 Effects of intravenous injection of CRF on relative mRNA expression levels of related genes in the ileum of broilers.

3 Discussion

3.1 Effects of Intravenous CRF Injection on Feed Intake in Broiler Chickens

CRF modulates the endocrine system, autonomic nervous system, immune system, and behavior through regulation of the HPA axis and other CRF pathways beyond the hypothalamus under stress conditions. In mammals, intracerebroventricular injection of CRF reduces feed intake, induces anxiety-like behavior, and stimulates glucocorticoid release from the adrenal glands [16-18]. Research indicates that the CRF system directly participates in energy balance regulation through central mechanisms [19], and functional impairments in this system can lead to pathological obesity and eating disorders [20-22]. In poultry, CRF functions similarly, participating in the regulation of appetite and energy balance when homeostasis is threatened [23]. Previous studies have shown that CRF acts as a mediator of stress responses in chickens, with intracerebroventricular administration decreasing feed intake and consequently affecting body weight gain [24]. In 2-day-old broiler chicks, intracerebroventricular CRF injection significantly reduced feed intake [25]. The present study demonstrates that

intravenous CRF injection significantly decreased feed intake in broiler chickens, consistent with previous findings and confirming that CRF functions as an effective anorexigenic factor in this species.

3.2 Effects of Intravenous CRF Injection on CRFR1 and CRFR2 mRNA Expression in Broiler Chickens

The CRF family includes CRF and urocortins (UCN) 1-3, which are highly conserved in mammals and exert their functions through two G protein-coupled receptors, CRFR1 and CRFR2. CRF and UCN3 function as multifunctional neuropeptides involved in stress responses and appetite suppression [20]. The stress response comprises three phases: initiation, maintenance, and recovery [26], with the CRF-CRFR1 system considered essential for stress initiation [27]. Studies have shown that during stress responses, CRF can induce neuronal apoptosis through CRFR1, while the CRFR1 antagonist R317573 inhibits this apoptosis, suggesting potential therapeutic applications for stress-related anxiety disorders [28]. In the present study, intravenous CRF injection significantly increased CRFR1 mRNA expression in both the hypothalamus and intestine (duodenum, jejunum, and ileum), suggesting that CRF-induced anxiety disorders mediated by CRFR1 may contribute to reduced feed intake in broiler chickens.

Research in mice indicates that CRFR2 mediates CRF' s effects on satiety, with CRFR2-selective antagonists dose-dependently reversing CRF-induced reductions in feed intake, suggesting potential therapeutic value for anorexia nervosa [29]. Additionally, intracerebroventricular injection of a CRFR2-selective antagonist dose-dependently inhibits CRF-induced delayed gastric emptying in rats, whereas a CRFR1-selective antagonist does not, demonstrating that CRFR2 mediates CRF-induced gastric emptying delay [30]. In this study, intravenous CRF injection significantly increased CRFR2 mRNA expression in the intestinal tract (duodenum, jejunum, and ileum), suggesting that CRFR2 may reduce feed intake by influencing satiety signals and delaying gastric emptying.

3.3 Effects of Intravenous CRF Injection on Ghrelin and GHSR-1 mRNA Expression in Broiler Chickens

Ghrelin is a peptide hormone that plays important roles in stimulating pituitary growth hormone secretion and appetite regulation [31-32]. The present study found that intravenous CRF injection significantly decreased ghrelin mRNA expression in the duodenum, while having no significant effect on ghrelin expression in the jejunum or ileum. This indicates that CRF differentially affects ghrelin expression between the hypothalamus and intestine, with different response thresholds among intestinal segments, the duodenum exhibiting the lowest threshold. Research has shown that ghrelin exerts differential effects on feeding across species, stimulating food intake in rats, sheep, goldfish, and humans, but producing opposite effects in juvenile fish and quail [33]. In mammals, ghrelin injection increases food intake by activating neuropeptide Y (NPY) and

agouti-related protein (AgRP) [34]. In contrast to humans and mice, chickens exhibit strong appetite suppression following ghrelin injection, with inhibitory effects showing dose-dependency [35]. In this study, intravenous CRF injection significantly increased hypothalamic ghrelin mRNA expression, and elevated ghrelin expression is known to reduce feed intake in broiler chickens, representing a likely important mechanism underlying the observed decrease in feed intake.

The ghrelin receptor (GHSR) exists in two forms, GHSR-1 and GHSR-1 , with GHSR-1 being the functional receptor [36]. Studies have demonstrated that dexamethasone (a synthetic glucocorticoid) injection in adrenalectomized rats increases GHSR-1 mRNA expression, and dexamethasone treatment of rat pituitary cells also elevates GHSR-1 mRNA expression [37]. Research in Cobb broiler chickens has shown that ghrelin-induced anorexic responses are mediated by HPA axis activation [14]. In the present study, intravenous CRF injection significantly increased GHSR-1 mRNA expression in the intestinal tract, likely resulting from CRF-induced activation of the HPA axis, which stimulates the adrenal cortex to release glucocorticoids, and the subsequent increase in peripheral glucocorticoid levels enhances GHSR-1 mRNA expression.

4 Conclusion

Peripheral intravenous administration of CRF reduces feed intake in broiler chickens and induces central ghrelin gene expression. The appetite suppression mediated by upregulated expression of hypothalamic CRFR1 and ghrelin, together with increased intestinal expression of CRFR1, CRFR2, and GHSR-1 , likely represents the mechanism underlying the observed decrease in feed intake.

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Note: Figure translations are in progress. See original paper for figures.