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Dexamethasone and insulin stimulate ghrelin secretion of broilers in a different way

Xixi Song, Hongchao Jiao, Jingpeng Zhao, Xiaojuan Wang, Hai Lin*

College of Animal Science and Veterinary Medicine, Shandong Agricultural University, No. 61 Daizong Street, Tai'an 271018, PR China

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ABSTRACT

Ghrelin is one of the most important appetite regulating peptides, involved in the regulation of energy homeostasis. The role of ghrelin on the appetite and fat metabolism in chickens is different from that of ghrelin in mammals. Glucocorticoids and insulin are important hormones and work differently in energy regulation of body. In this study, the effects of dexamethasone (DEX, 2.0 mg/kg BW), subcutaneous insulin injection (40 μg/kg BW), and glucose load on ghrelin secretion and expression were determined in broilers. DEX treatment increased circulating ghrelin concentration in broiler fed with either a low-energy diet (11.05 MJ/kg of metabolizable energy) or a high-energy diet (14.44 MJ/kg of metabolizable energy). The expression levels of ghrelin were increased while both ghrelin and its receptor GHS-R1a expression levels were stimulated by DEX. A single subcutaneous insulin injection (40 μg/kg BW) or oral glucose infusion (2 g/kg BW) rise circulating ghrelin level. Ghrelin expression in the proventriculus was increased by insulin treatment but unchanged by glucose load. DEX had no detectable influence on ghrelin and GHS-R1a expression in the hypohtalamus, whereas insulin suppressed their expression. In conclusion, both insulin and glucocorticoid stimulate ghrelin secretion in chickens, in contrast to mammals. Glucocorticoids evoke peripheral ghrelin/GHS-R1a system while insulin increases peripheral ghrelin expression and suppress the activation of central ghrelin/GHS-R1a system. The result suggests that ghrelin involved in the modulating network of energy homeostasis in concert with glucocorticoids and insulin.

1. Introduction

Ghrelin is widely present in vertebrates and plays an important role in the regulation of appetite and energy homeostasis (Kojima and Kangawa, 2005; Kaiya et al., 2009; Sato et al., 2012; Abtahi et al., 2017). Ghrelin has two main molecular forms: desacyl-ghrelin and acyl-ghrelin, and only acyl-ghrelin can be combined with GHS-R1a to play its biological role (Yang et al., 2008; Zaniolo et al., 2011). Ghrelin can pass through the blood-brain barrier, linking the central organs and peripheral organs (Banks et al., 2002; Lopez et al., 2012; Stark et al., 2015; Howick et al., 2017).

In mammals, ghrelin is a hormone that promotes appetite (Nakazato et al., 2001; Abtahi et al., 2017). In poultry, however, central injection of ghrelin suppressed feed intake (Furuse et al., 2001; Saito et al., 2002; Dimaraki and Jaffe, 2006; Khan et al., 2006; Xu et al., 2011; Zendehdel et al., 2013; Kaiya and Al, 2013). Ghrelin inhibits food intake by interacting with the endogenous corticotropin releasing factor (CRF) and its receptor (Saito et al., 2005). Peripherally ghrelin serves as an antilipogenic factor in broiler chickens but not in rats (El-Magd et al., 2016). Glucocorticoid, the terminal regulator of the hypothalamic-pituitaryadrenal (HPA) axis, triggers a series of physiological reactions (Matteri et al., 2000), resulting in a new distribution of energy via insulin resistance and increased fat synthesis and deposits in chickens and mammals (Rebuffé-Scrive et al., 1992; Franco-Colin et al., 2000; Lin et al., 2006; Cai et al., 2009; Wang et al., 2010). Hence, we hypothesized that glucocorticoid effect the secretion and expression of ghrelin differently from that in mammals. Moreover, the glucoregulatory action of ghrelin receives recently increasing recognition (Poher et al., 2018). GCs and insulin make up a-bihormonal-system that regulates the overall energy balance (Dallman et al., 1995). Therefore, the effect of insulin on ghrelin secretion remains to be elucidated.

In the present study, three experiments were conducted to evaluate the regulating effect of GCs and insulin on the secretion of ghrelin and the gene expression of ghrelin and GHS-R1a in proventriculus of broilers. In experimental 1, the regulating effect of dexamethasone on ghrelin secretion was investigated in broilers fed with a low-energy or high energy diet. In experiment 2 and 3, the effects of subcutaneous injection of insulin and oral glucose administration were measured.

* Corresponding author.
E-mail address: hailin@sdau.edu.cn (H. Lin).

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2. Materials and methods

2.1. Animals

One-day-old healthy male broiler chicks (Arbor Acres) were obtained from a local hatchery (Dabao Breeding Technology Co., Ltd., Taian, P. R. China). The chicks were reared in an environmentally controlled room. The brooding temperature was maintained at 35 °C (65% relative humidity, RH) for the first 2 days and was then gradually reduced to 21 °C on day 21 and maintained the temperature until the end of the experiment. If it was not mentioned, the broilers received a starter diet (metabolic energy: 12.13 MJ/kg; 21% crude protein) until 21 d of age, thereafter received a grower diet (metabolic energy: 12.55 MJ/kg; 18% crude protein) until the end of the experiment.

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Shandong Agricultural University (No. 2001002) and performed in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, P. R. China).

2.2. Effect of dexamathasone and dietary energy levels

Forty 21-day of age broilers with similar body weight (BW) were randomly assigned to two groups and provided respectively with a high energy diet (14.44 MJ/kg of metabolizable energy, 21.9% crude protein, and 14.6% crude fat, HE) or a low energy diet (11.05 MJ/kg of metabolizable energy, 16.3% crude protein, and 6.9% crude fat, LE). After two-weeks feeding, the broilers in each dietary group were divided into two sub-groups and respectively subjected to a 7-day continuous subcutaneous injection of dexamethasone sodium phosphate (DEX, 2.0 mg dexamethasone/kg BW, 5 mg dexamethasone/mL; Cisen Pharmaceutical Co., Ltd, Jining, P. R. China) or the same amount of saline containing equal sodium phosphate (Control, Wang et al., 2010). Chickens were killed by cervical dislocation after 12 h feed withdrawal (Close et al., 1997). Before sacrificed, a blood sample was drawn from a wing vein and collected in pre-cooled tubes. Serum samples were obtained after centrifugation at 400*g* for 10 min at 4 °C and stored at −20 °C for further analysis. The breast muscle, thigh muscle, abdominal fat, liver, proventriculus, duodenum, and hypothalamus were sampled, snap froze in liquid nitrogen and then stored at −80 °C for further analysis. The whole hypothalamus was removed according to the method described in Liu et al (2014). Briefly, the hypothalamus was dissected from the ventral surface of the brain. Two transverse cuts were made at the apex of the optic chiasm and the rostral margin of the mammillary bodies. Next, 2-mm bilateral cuts were made on either side of the midline, the whole hypothalamus was removed and the cut was 4–5 mm deep parallel to the base of the brain according to the method described in previous studies (Yuan et al., 2009; Higgins et al., 2010).

2.3. Effect of subcutaneous insulin injection

Twenty 42-day-old AA broilers with similar BW were randomly divided into two groups and subjected to the following treatments after overnight fasting (12 h): subcutaneous injection of insulin (40 μg/kg BW, from porcine, dissolved in hydrochloric acid solution (pH = 3.5) and diluted with saline; I113907, Aladdin, Shanghai, P. R. China) or sham treatment with saline containing equal hydrochloric acid (Zhao et al., 2009). After one hour treatment, all the experimental broilers were sacrificed and tissue samples were obtained as forementioned.

2.4. Effect of oral glucose load treatment

Twenty 42-day-old AA broilers with similar BW were randomly divided into two groups and subjected to the following treatments after overnight fasting (12 h): oral administration of glucose (7.5 mL/kg BW, 0.27 g/mL, G7021, Sigma-Aldrich, U.S.A) or sham treatment with saline. After one hour treatment, all the experimental broilers were sacrificed and tissue samples were obtained as forementioned.

2.5. Blood metabolites, ghrelin, and insulin measurement

Serum concentration of glucose (GLU, F006), triglycerides (TG, F001), and uric acid (UA, C012) were measured with commercial diagnostic kits (Jiancheng Bioengineering Institute, Nanjing, P. R. China). The concentration of total ghrelin was measured with a commercial diagnostic ELISA kit (CSB-E14230C, Wuhan Huamei Bioengineering Co., Ltd, Wuhan, P. R. China), which has been successfully used in the study of chickens (Yu et al., 2016; Höhne et al., 2017; Vizcarra et al., 2018). The sensitivity of the assay was 25 pg/mL and all samples were included in the same assay to avoid interassay variability. The intraassay coefficient of variation was less than 15%. Insulin was measured by radioimmunoassay (RIA) with a guinea pig anti-porcine insulin serum (0704, 3 V, Bio-Engineering Group Co., Ltd, Weifang, P. R. China), according to Wang et al. (2012). The sensitivity of the assay was 1 μIU/mL and all samples were included in the same assay to avoid interassay variability. The intraassay coefficient of variation was 6.9%. A large cross-reaction has been observed between chicken insulin and this porcine anti-serum (Simon et al., 1974).

2.6. Detection of ghrelin concentration in proventriculus

100 mg tissue was homogenized in PBS (1 mL, P1020, Solarbio, Beijing, P. R. China) by a high-speed tissue homogenizer (KZ-II, Servicebio, Wuhan, P. R. China) and stored overnight at −20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged at 5000*g* for 5 min at 4 °C. The supernatant was removed and used for the measurement of protein concentration and ghrelin level immediately. The protein concentration was measured by the bicinchoninic acid assay (BCA, P0010, Beyotime Biotechnology, Shanghai, P. R. China). Ghrelin measurement was conducted with the commercial diagnostic ELISA kit forementioned.

2.7. RNA isolation and analysis

The gene expression of the ghrelin and GHS-R1a was determined by real-time PCR. Primers used in this study were designed using Primer 5.0 software and synthesized by Sangon Bioteach (Shanghai, P. R. China, Table 1). Total RNA of tissues collected above was extracted using Trizol (15596018, Invitrogen Life Technologies, Carlsbad, CA). The quantity and quality of the isolated RNA were determined using a biophotometer (BioPhotometer Plus, Eppendorf, Hamburg, Germany) and by agarose-gel electrophoresis, respectively. Total RNA (1 μg) was reverse transcribed into first-strand cDNA using Prime ScriptTM RT Master Mix (DRR019A, Takara, Dalian, P. R. China) following the manufacturer's instructions, and quantitative real-time RT-PCR was performed with a SYBR Green I master mix (DRR041A, Takara, Dalian, China) on ABI 7500 Real-Time PCR System (Q5, Applied biosystems, ABI, USA) using the following parameters: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 34 s. The specific products were confirmed by melting curve generated automatically using SDS analytic

Table 1
Quantitative real-time PCR primer sequences.

Gene	Sequences (5′→3′)	Accession NO.	Product size (bp)
Ghrelin	F: CCTTGGGACAGAAACTGCTC R: CACCAATTTCAAAAGGAACG	NM_001001131.1	203
GHS-R1a	F: TTTTTCCTGCCCGTATTCTG R: GCTTGGTGCTGGAGAGTCTT	NM_204394.1	397
GAPDH	F: ACATGGCATCCAAGGAGTGAG R: GGGGAGACAGAAGGGAACAGA	NM_204305.1	244