Comparative Biochemistry and Physiology, Part B 162 (2012) 81–87

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part B

journal homepage: www.elsevier.com/locate/cbpb

Effect of Corticosterone on Gene Expression of Feed Intake Regulatory Peptides in Laying Hens

Lei Liu [a,1], Zhigang Song [a,1], Ardashir Sheikhahmadi [b], Hongchao Jiao [a], Hai Lin [a,*]

[a] Department of Animal Science, Shandong Agricultural University, Taian, Shandong 271018, China
[b] Department of Animal Science, Faculty of Agricultural, University of Kurdistan, Sanandaj, Iran

ARTICLE INFO

Article history:
Received 27 December 2011
Received in revised form 6 April 2012
Accepted 10 April 2012
Available online 24 April 2012

Keywords:
appetite
feed intake
glucocorticoid
laying hen

ABSTRACT

The present study was conducted to explore the effects of corticosterone (CORT) on the regulation of appetite-associated genes in laying hens. Forty eight laying hens were randomly divided into two groups: one received subcutaneous injection of CORT (2 mg/kg body weight, CORT-exposed) and the other received sham-treatment (Control). Treatment of hens with CORT stimulated an increase ($P<0.05$) in plasma CORT, glucose, uric acid (UA), insulin, cholesterol (Chol) and triiodothyronine (T_3), but the concentrations of plasma non-esterified fatty acids (NEFA) and triacylglycerol (TG) were decreased ($P<0.05$). CORT treatment had no significant effect ($P>0.05$) on the mRNA levels of neuropeptide Y (NPY), corticotropin-releasing hormone (CRH), melanocortin receptor 4 and 5 (MCR-4 and MCR-5) and cholecystokinin (CCK) in the hypothalamus when compared with control hens. However, the expression of pro-opiomelanocortin (POMC), agouti-related protein (AgRP) and melanocortin recepter 1 (MCR-1) were significantly ($P<0.05$) suppressed while the mRNA levels of ghrelin and cocaine-and amphetamine-regulated transcript (CART) were significantly upregulated ($P<0.05$) in CORT-treated hens. Treatment of laying hens with CORT had no significant ($P>0.05$) effect on the mRNA levels of CCK in the glandular stomach and the duodenum, and those of ghrelin in the glandular stomach, the duodenum and the jejunum. However, the mRNA levels of CCK in the jejunum and the ileum, and those of ghrelin in the ileum were significantly ($P<0.05$) suppressed by CORT treatment. In conclusion, these results suggest that CORT plays a unique role in some special neuropeptides (e.g., ghrelin, CART, POMC, CCK and MCRs) and a dynamic balance between these appetite-associated peptides in the hypothalamus and the gastrointestinal tract defines the feeding status of CORT-exposed laying hens.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

The regulation of feed intake in birds and mammals has been investigated by several groups over the past 30 to 40 years (Lacy et al., 1986; Drewnowski, 1998; Saito et al., 2002). Peptide hormones like neuropeptide Y (NPY), orexin-A, orexin-B, motilin, melanin-concentrating hormone (MCH), galanin, growth hormone releasing factor (GRF) and ghrelin have been found to modulate feeding in mammals and birds. Because feeding and energy homeostasis are both necessary for survival, mechanisms that regulate these processes can be expected to be highly conserved in all animals (Kuenzel et al., 1999). Although most of the peptides that control appetite are highly conserved, their function can sometimes differ between birds and mammals (Furuse, 2002). In birds, the hypothalamus plays a pivotal role in interpreting external environmental cues like stressors and generating the appropriate responses in feed intake (Richards et al., 2010). On the other hand, the hypothalamic-pituitary-adrenal (HPA) axis in mammals has an integral role in homeostatic regulation during stress via glucocorticoids, the final effectors of the HPA axis.

Glucocorticoids are the counter regulatory hormones with broad effects on carbohydrate, lipid and protein metabolism (Bamberger et al., 1996). Glucocorticoids participate in the control of whole body homeostasis and the response of the organism to stress by stimulating the release of energy stores via promoting glucose mobilization and lipolysis (Harvey et al., 1986). Glucocorticoid treatment is associated with increased levels of circulating FFAs in humans (Macfarlane et al., 2008) and rodents (Novelli et al., 2008). In birds it has been reported that corticosterone and insulin interact to

Abbreviations: AgRP, agouti-gelated protein; ARC, arcuate nucleus; BW, body weight; CART, cocaine-and amphetamine-regulated transcript; CCK, cholecystokinin; Chol, cholesterol; CRH, corticotropin-releasing hormone; FSH, follicular stimulating hormone; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; GRF, growth hormone releasing factor; GnIH, gonadotropin-inhibitory hormone; GnRH-I, gonadotropin-releasing hormone I; GT, gastrointestinal tract; HPA, hypothalamic-pituitary-adrenal; HPG, hypothalamo-pituitary- gonadal; MCH, melanin-concentrating hormone; MCR-1, melanocortin recrpter 1; MCR-4, melanocortin receptor 4; MCR-5, melanocortin receptor 5; MCRs, melanocortin receptors; NEFA, non-esterified fatty acid; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; T3, triiodothyronine; TG, triglyceride; UA, uric acid; VLDL, very low density lipoprotein.

* Corresponding author. Tel.: +86 538 8249203; fax: +86 538 8241419.
E-mail address: hailin@sdau.edu.cn (H. Lin).

[1] These authors contributed the same to this work.

1096-4959/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2012.04.005

regulate glucose and triglyceride levels during stress (Remage-Healey and Romero, 2001).

Glucocorticoids, especially corticosterone (CORT), are involved in the control of appetite in poultry (Yuan et al., 2008). El-Lethey et al. (2001) have shown that corticosterone, the main glucocorticoid in birds, can increase feed intake of chickens. Furthermore, there are reports of augmentation in plasma CORT after 24 h of feed deprivation in immature (Geris et al., 1999) or adult (Scanes et al., 1980) chickens. CORT responses to fasting can vary among individuals and depend on energy reserves and past experience (Webster, 2003). Additionally, the effect of CORT on feed intake in birds depends on the dosage of CORT as well as their diets. It has been reported that feed intake increased in chickens given higher doses of CORT but not in those given lower doses of CORT (Covasa and Forbes, 1995). Bartov (1985) showed that CORT stimulated feed intake in chickens that were fed with high-protein diets but not in those fed with low-protein diets.

Many neuropeptides, called feed intake regulatory peptides, are expressed in the hypothalamus and the gastrointestinal tract (GT), and are involved in the regulation of feed intake in birds (Richards and Proszkowiec-Weglarz, 2007). Gut peptides play an important role in gathering information about the presence or absence of feed or specific nutrients in the gut and sending them to the hypothalamus to control feed intake. Although there are a number of reports documenting the effect of CORT on feed intake of chickens (Bartov, 1985; Covasa and Forbes, 1995), the underlying neuroendocrine mechanism is virtually unknown. Therefore, the objective of our study was to investigate the alterations in mRNA levels of feed intake regulatory peptides (NPY, ghrelin, pro-opiomelanocortin (POMC), agouti-related protein (AgRP), corticotropin-releasing hormone (CRH), melanocortin receptor-1 (MCR-1), melanocortin receptor-4 (MCR-4), melanocortin receptor-5 (MCR-5), cocaine-and amphetamine-regulated transcript (CART), cholecystokinin (CCK)) in the hypothalamus and the GT (ghrelin and CCK) of CORT-exposed laying hens.

2. Materials and methods

2.1. Animal Management and Sample Collection

Laying hens (Hy-line brown, 24 weeks of age) were provided a diet with 16% crude protein and 11.3 MJ/kg of metabolizable energy, and a 16 h light/8 h dark light regime. The study was approved by the Shandong Agricultural University and carried out in accordance with the “Guidelines for Experimental Animal” of Ministry of Science and Technology (Beijing, P.R. China).

Forty eight laying hens with similar body mass (BW, 1.68 ± 0.04 kg) were selected and allocated to 8 pens. The hens in those 8 pens were randomly subjected to one of the following two treatments for 7 days: 1) subcutaneous injection of CORT (2 mg/kg BM, dissolved in corn oil, 4 mg/mL, twice per day; referred to hereafter as ‘CORT-exposed’) (Cai et al., 2009; Gao et al., 2010), and 2) sham-treatment (0.25 mL/kg BM of corn oil, twice per day; referred to hereafter as ‘control’). The injections of CORT and sham were performed at the same time each day (7:00–8:30 and 19:00–20:30).

After 7 days, the laying hens were fasted for 12 h before sampling to minimize the influence of feeding on blood metabolites concentrations. The blood was drawn from the wing vein using a heparinized syringe within 30 seconds and collected in ice-cold tubes (09:00 to 10:00 h). Plasma was obtained after centrifugation at 400 × g for 10 min at 4 °C and was stored at −20 °C for further analysis of glucose, insulin, cholesterol (Chol), triiodothyronine (T_3), CORT, non-esterified fatty acid (NEFA), triglyceride (TG) and urate (UA) levels. Immediately after the blood sample was obtained, hens were sacrificed by exsanguination (Close, 1997), and tissue samples of the hypothalamus, the glandular stomach, the duodenum, the jejunum and the ileum were collected. After snap freezing in liquid nitrogen, the tissue samples were stored at −80 °C for RNA extraction. 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. Then bilateral cuts were made 2 mm either side of the midline and the whole hypothalamus was removed according to the chicken brain atlas (Kuenzel and Masson, 1988). The samples of the whole intestinal tract were removed, and segments of were taken from the midpoint of glandular stomach, the midpoint of duodenum (duodenum), the midpoint between the bile duct entry and Meckel's diverticulum (jejunum), and the midpoint between the Meckel's diverticulum and ileo-cecal junction (ileum) (Awad et al., 2006).

2.2. Production Parameters

The following metrics were recorded daily during the trial: the number of eggs laid, egg weight, BW and the amount of feed intake. Feed conversion ratio (FCR; calculated as amount of feed/egg), egg laying rate, average egg mass, and egg production (g/hen/day) were then calculated based on our recorded data.

At the end of the experiment, random samples of 10 eggs from each treatment group were collected to measure shell thickness, which was calculated as a mean value of measurements at three locations on the egg (air cell, equator, and sharp end) by using a micrometer caliper (Um and Paik, 1999).

2.3. Plasma Metabolites and Hormones

Plasma concentrations of glucose, UA, TG, Chol and NEFA were measured spectrophotometrically using commercial diagnostic kits (Jiancheng Bioengineering Institute, Nanjing, P.R. China).

Plasma insulin level was measured by radioimmunoassay using guinea pig anti-porcine insulin serum (3 V Bio-engineering group Co., Weifang, P.R. China). In this assay, ^{125}I-labeled porcine insulin competes with chicken insulin for sites on insulin-porcine antibody immobilized to the wall of a polypropylene tube. A high degree of cross-reaction has previously been observed between chicken insulin and the guinea pig anti-porcine sera (Simon et al., 1974). The insulin used in this study is referred to as immune-reactive insulin. The sensitivity of the assay was 1 μIU/mL and all samples were included in the same assay to avoid inter-assay variability. The intra-assay coefficient of variation was 1.98%.

Plasma CORT was measured using a sensitive and highly specific RIA kit (IDS Inc, Boldon, UK), with a sensitivity of 0.39 ng/mL, and low cross-reaction with aldosterone (0.20%), cortisol (0.40%), and deoxycorticosterone (3.30%), and has been used in a previous study (Malheiros et al., 2003). Before conducting the assay, plasma samples were heated to 80 °C for 10 min to inactivate CORT-binding proteins. The intra-assay variability was 3.8%.

Plasma T_3 concentrations was measured by radioimmunoassay as described by Darras et al. (1992). The sensitivity of the assay was 1 nmol/mL and all samples were included in the same assay to avoid interassay variability. Intraassay coefficients of variation was 4.5%. Standards for antisera, T_3 was purchased from 3 V Bio-engineering group Co., Weifang, P.R. China.

2.4. RNA Isolation and Analysis

Expression of genes in the hypothalamus, glandular stomach, duodenum, jejunum and ileum was quantified using quantitative real-time PCR with SYBR Green I labeling.

Total RNA was isolated by the guanidinium isothiocyanate method with Trizol Reagent (Invitrogen, San Diego, CA, USA). The quality of RNA after DNase treatment was tested by electrophoresis on an agarose gel and the quantity of RNA was determined using a biophotometer (Eppendorf, Germany). RT reactions (10 μL) consisted of 500 ng total RNA, 5 mmol/L $MgCl_2$, 1 μL RT buffer, 1 mmol/L dNTP,

Download English Version:

https://daneshyari.com/en/article/1975475

Download Persian Version:

https://daneshyari.com/article/1975475

Daneshyari.com