Comparative Biochemistry and Physiology, Part A 260 (2021) 111018

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Effect of dexamethasone on gene expression of cannabinoid receptor type 1 and adenosine monophosphate-activated protein kinase in the hypothalamus of broilers (*Gallus domesticus*)

Linglian Kong [a], Chuanpi Xiao [a,b], Hai Lin [a], Johan Buyse [c], Xianlei Li [a], Zhigang Song [a,*]

[a] Department of Animal Science, Shandong Agricultural University, Taian, Shandong 271018, China
[b] Precision Livestock and Nutrition Unit, Gembloux Agro-BioTech, University of Liège, Gembloux 5030, Belgium
[c] Division Animal and Human Health Engineering, Department of Biosystems, KU Leuven, Kasteelpark Arenberg 30, Leuven 3001, Belgium

ARTICLE INFO

Editor: Michael Hedrick

Keywords:
Broilers
Stress
Cannabinoid receptor type 1
Adenosine monophosphate-activated protein kinase
Dexamethasone

ABSTRACT

Hypothalamic neural circuits play a critical role in integrating peripheral signals and conveying information about energy and nutrient status. We detected cannabinoid receptor type 1 (CB1) distribution in the hypothalamus, liver, duodenum, jejunum, and ileum among 7- and 35-day-old broilers. The effects of dexamethasone (DEX) on CB1 gene expression were evaluated by *in vitro* and *in vivo* experiments on glucocorticoid receptor (GR) and adenosine monophosphate-activated protein kinase (AMPK) in the hypothalamus of broilers. *In vitro*, hypothalamic cells from 17-day-old broiler embryos were incubated with either 0.1% dimethyl sulfoxide or DEX (100 nmol/mL) for 1 h. In the *in vivo* study, 28-day-old broilers were injected with DEX for 24 h or 72 h. Results showed that CB1 was mainly expressed in the hypothalamus, and 72 h DEX treatment increased the expression. One-day treatment of broilers with DEX did not change the hypothalamic CB1 gene expression. Moreover, DEX treatment for 24 h and 72 h increased the mRNA level of hypothalamic AMPKα2 and GR. However, no differences were observed on the gene expression of CB1, GR, and AMPKα2 in hypothalamic cells with DEX-treated for 1 h. In conclusion, CB1 is mainly expressed in the hypothalamus of broilers; 72-h DEX exposure can regulate the CB1 system and AMPK signaling pathway of the broiler hypothalamus.

1. Introduction

In poultry species, as in all animals, hypothalamic neural circuits play a critical role in integrating peripheral signals and conveying information about energy and nutrient status. Stressful conditions increase the activity of the hypothalamic-pituitary-adrenal (HPA) axis (Vandenborne et al., 2005). Glucocorticoids (GCs), the end products of the HPA axis, exert feedback effects at the hypothalamus and pituitary levels and counter-regulatory hormones by influencing carbohydrate, lipid, and protein metabolism. Corticotropin-releasing hormone is the primary feedback target of glucocorticoids at the hypothalamic level (Kretz et al., 1999). Dietary supplementation and subcutaneous injection of corticosterone can reduce the expression of the Corticotropin-releasing hormone gene in the hypothalamus; meanwhile, corticosterone can stimulate the food intake of broilers under high-energy diet (Song et al., 2011a).

Many of changes observed during GCs excess correspond to metabolic steps regulated by adenosine monophosphate-activated protein kinase (AMPK). Previous studies suggested that AMPK regulates cellular and systemic energy homeostasis and acts as a sensor of energy status in broilers (Song et al., 2012). In poultry, other candidates that target neuroendocrine energy balance mechanisms are currently under investigation. The endogenous cannabinoid is an important signaling molecule in the neuroendocrine control of homeostasis and is involved in the regulation of stress response, energy metabolism, and other processes (Di et al., 2005). Endogenous cannabinoids bind to cannabinoid receptor type 1 (CB1) receptors on presynaptic axon terminals and inhibit the release of γ-aminobutyric acid and glutamate. Moreover, γ-aminobutyric acid, the major inhibitory neurotransmitter in the central nervous system (CNS), can relieve stress (Freund et al., 2003). In this context, the endogenous cannabinoid system represents a new and promising research target in poultry.

Cannabinoids are natural lipophilic products from the flower of *Cannabis sativa* (Di Marzo et al., 2004). The two known types of

* Corresponding authors.
E-mail addresses: zhigangs@sdau.edu.cn, 245777398@qq.com (Z. Song).

https://doi.org/10.1016/j.cbpa.2021.111018
Received 3 April 2021; Received in revised form 24 May 2021; Accepted 11 June 2021
Available online 15 June 2021

cannabinoid receptors, CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993), have been identified. CB2 is predominately found in cells of the immune system, and it is only present in microglia within the brain (Núñez et al., 2004). With mounting evidence of more, CB1 is a G protein-coupled cannabinoid receptor (Matsuda et al., 1990), widely distributed in the CNS (Terry et al., 2010; Tsou et al., 1998). Besides, CB1 is highly abundant in the brain, involved in synaptic signaling (Stincic and Hyson, 2008), and can present in the metabolically relevant peripheral organs, such as gastrointestinal tissues, liver, pancreas, muscle, adipose tissue, and adrenal gland (Breivogel and Childers, 1998; Pagotto et al., 2006). Several studies suggest that CB1 activation shows high efficiency and leads to profound biological effects by modulating the activity of a wide variety of hypothalamic neuronal populations. GCs act rapidly, probably through membrane receptors, on corticotropin-releasing-factor-synthesizing cells to release endocannabinoids. These act locally at presynaptic axons *via* cannabinoid receptors to reduce excitatory glutaminergic input, and therefore induce fast feedback inhibition of corticotropin-releasing factor, by GCs (Dallman, 2003). Co-application of GCs and a CB1 inverse agonist block the GC-induced suppression of HPA axis responses to acute stress (Evanson et al., 2010), suggested GCs act nongenetically to provide rapid feedback inhibition of the paraventricular nucleus of hypothalamus by GC actions at membrane-associated GR and subsequent dendritic synthesis and release of CBs (Myers et al., 2012). Furthermore, in mammals, GCs can increase the number of endocannabinoids and stimulate AMPK activity in the hypothalamus (Di et al., 2003). However, there are few studies about GCs effects on the cannabinoid system in broilers. It is hoped that future research will give evidence for an interaction between GCs and food intake, possibly mediated by endocannabinoid secretion from target neurons in the hypothalamus (Dallman, 2003). Therefore, it is hypothesized that there is CB1 in broilers and the effect of exogenous injection of DEX on hypothalamic gene expression was studied. Specifically, we investigated CB1 distribution in the hypothalamus, liver, and intestine of broilers 7- and 35-days post-hatching. Dexamethasone (DEX), a synthetic GC, was employed to induce the hyper-GC status in the present study (Cai et al., 2011). We also examined the effects of DEX on the mRNA levels of AMPK, CB1, and GR in the hypothermic cell culture prepared from broiler embryos. We then elucidated the 24 h and 72 h effects of DEX administration to broilers on the mRNA levels of hypothalamic CB1, GR, and AMPK.

2. Materials and methods

2.1. Animal care, birds, and experimental design

The study was approved by Shandong Agricultural University and carried out following the Guidelines for Experimental Animals of the Ministry of Science and Technology (19,881,114, Beijing, People's Republic of China). Forty-eight 1-day-old male Arbor Acres broilers (n = 48) were obtained from a local hatchery (Processing Co., Ltd. Shandong Bao Farming). All birds were placed in raised-floor pens and raised in environmentally controlled rooms under standard conditions, and free access to feed and water during the breeding period. The experimental diets met the National Research Council (1994) requirements. The composition and nutrient levels of the diet are shown in Table 1. The temperature was 35 °C initially, which was gradually reduced by 2 °C per week according to the age of broilers, until it was decreased to 24 °C. The birds were given 23 h of light and 1 h of darkness in the first week, followed by 16 h of light and 8 h of darkness for subsequent research.

2.2. CB1 distribution

To investigate the distribution of CB1 in broiler tissues, eight birds with similar body weight (BW, 7 days: 154 ± 4.3 g, 35 days: 1540 ± 11.7 g) from each group were selected in 7 and 35 days of age. The tissue samples of the hypothalamus, liver, duodenum, jejunum, and ileum were collected, immediately cooled in liquid nitrogen, and stored at −80 °C for further analysis. The sampling method was in accordance with our previous research (Lei et al., 2013).

Table 1
Composition and nutrient levels of the diet used in this study (%, air-dry basis).

Item	Content, %
Ingredients	
Corn	52.194
Soybean meal	38.926
Soybean oil	5.129
Limestone	1.463
$CaHPO_4$	0.80
NaCl	0.30
L-Lys • H_2SO_4	0.267
DL-Methionine	0.302
L-Threonine	0.099
Phytase (5000 IU/g)	0.02
Vitamins premix[1]	0.20
Mineral premix[2]	0.20
Choline chloride	0.10
Total	100.00
Nutrient levels[3]	
CP	21.50
ME (MJ/kg)	12.81
Ca	0.95
Nonphytate phosphorus	0.44
Lysine	1.19
Methionine	0.59
Cysteine + Methionine	0.87
Threonine	0.76
Tryptophan	0.22

[1,2] Vitamin premix and mineral premix provided the following per kg of diet: VA 9000 IU, VD_3 2000 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_{12} 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. [3] Nutrient levels were calculated values.

2.3. DEX treatment

In vivo and *in vitro* experiments were designed to detect the effect of DEX on the gene expression of CB1, GR, and AMPKα2 in hypothalamus. At 28 days of age, twenty-four birds were assigned to two groups of twelve birds, which were randomly subjected to one of the following treatments for 24 h or 72 h: subcutaneous injection of DEX (2 mg/ kg BW) or sham-treated control (injection of saline). At the end of the experimental period (for each injection time), six birds were sacrificed from each group by exsanguination. Thereafter, 1 to 2 g of the sample was obtained from each hypothalamus according to our previous research (Hu et al., 2019), cooled in liquid nitrogen, and stored at −80 °C for further analysis.

In vitro treatment: hypothalamic cell culture and treatment. Fertile eggs (Arbor Acres, 41st week in the laying cycle) were obtained from a maternal flock in a commercial hatchery (Jinan SAIS Poultry). The eggs were incubated under standard conditions at the Department of Animal Sciences of Shandong Agricultural University. Neuron-enriched hypothalamic cell culture was prepared from birds at day 17 of incubation. The hypothalamus was obtained by following a previously described method (Song et al., 2012). The dissociated hypothalamic tissue was mechanically triturated. The cells were plated in six-well culture plate with a final density of 1.5×10^6 cells/well containing minimum essential medium (MEM) Earl salts (Invitrogen, Carlsbad, CA, USA) with 5% horse serum (HS, Invitrogen), 5% fetal calf serum (Biological Industries), B-27 supplement (1 lg/mL, Invitrogen), 0.6% glucose, gentamicin (20 lg/mL), and 2 mM glutamine (Invitrogen). The plates were precoated 1 day before the experiments with 0.01% poly-L-lysine solution (Sigma–Aldrich, St Louis, MO, USA). On the fourth day of *in vitro*

experiments, the medium was switched to MEM supplemented with 10% HS, 20 mg/mL 50-fluoro-20-deoxyuridine, and 50 mg/mL uridine (Sigma-Aldrich) to limit glial growth. Finally, the medium was changed to non-serum medium on seventh day of *in vitro* experiments, and cells were incubated for 3 days at 37 °C in a humidified incubator containing 5% CO_2. The hypothalamic cells were treated with 100 nmol/mL DEX (dissolved in non-serum medium with 0.1% dimethyl sulfoxide, DMSO) for 1 h, or received with non-serum medium contained 0.1% DMSO as a control. There are six replicates in each group, with one well of cells in six-well culture plate for each replicate.

2.4. Total RNA extraction and real-time PCR

Total RNA was isolated by the guanidinium isothiocyanate method with Trizol reagent (Invitrogen, San Diego, CA). The concentration and purity of each RNA sample were detected using a NanoDrop photometer (ND-2000, Thermo Scientific, Wilmington, USA). The integrity of RNA was detected by 1% agarose gel electrophoresis. Reverse transcription of 1 μg total RNA was performed using PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A, TaKaRa, Dalian, China). RT-PCR was performed to determine the gene expression using TB Green Premix Ex Taq (RR820A, TaKaRa) in ABI 7500 Real-Time PCR Systems (Thermo Scientific). The reaction program included the following: pre-denaturation at 95 °C for 10 s, then denaturation at 95 °C for 5 s for a total of 40 cycles, and finally annealing and extension at 60 °C for 40 s. Each reaction well was repeated 3 times, and the primer sequence is shown in Table 2. The amplification efficiency of primers was calculated with a standard curve. The specificity of the amplified product was verified by the melting curve. The geometric mean of the expression of *β-actin* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used to normalize the expression of the target gene. Relative gene expression levels of each target gene were analyzed by the $2^{-\Delta\Delta ct}$ method.

2.5. Statistical analysis

Data were presented as mean ± SD. All data were checked for normality with Shapiro–Wilk test (95% confidence level). Statistical differences between groups were analyzed with one-way ANOVA with Tukey's multiple comparisons. SPSS software (SPSS 26.0, SPSS, Chicago, USA) was used. Differences were considered significantly different at $P < 0.05$.

Table 2
Gene-specific primers used for broilers gene expression analysis.

Gene[a]	Association no.	Primer sequences	Amplicon size (bp)
CB1	NM_001038652.1	F: GGCTGTTTCCTTATGCCCCT R: GCTTGGCCTTTTGTGCTACC	262
GR	NM_001037826.1	F: AACCTGCTCTGGCTGACTTCTC R: CCCATCACTTTCGCATCTGTTT	121
AMPKα2	NM_001039605.1	F: GGGACCTGAAACCAGAGAACG R: ACAGAGGAGGGCATAGAGGAG	215
β-actin	NM_205518.1	F: CAGACATCAGGGTGTGATGG R: TCAGGGGCTACTCTCAGCTC	183
GADPH	NM_204305.1	F: GCCCAGAACATCATCCCA F: CGGCAGGTCAGGTCAACA	137

[a] Abbreviations: *CB1*, cannabinoid receptor type 1; *GR*, glucocorticoid receptor; *AMPKα2*, AMP-activated protein kinase α2; *β-actin*, beta-actin; *GAPDH*, glyceraldehyde phosphate dehydrogenase.

3. Results

3.1. Distribution of hypothalamic and peripheral CB1 gene expression

As shown in Fig. 1, the *CB1* expressed both in the hypothalamus, liver, duodenum, jejunum, and ileum of 7- and 35-day-old birds. The CB1 mRNA level in the hypothalamus was significantly higher than those in the liver, duodenum, jejunum, and ileum for 7- and 35-day-old broilers ($P < 0.001$; Fig. 1A and B).

3.2. In vivo and in vitro DEX treatment and hypothalamic CB1, AMPKα2, and GR gene expression

One- and three-days DEX treatments increased the hypothalamic *AMPKα2* and *GR* mRNA levels ($P = 0.001$, $P = 0.0007$; $P = 0.046$, $P = 0.04$, respectively; Fig. 2A, B, C, and D). The mRNA levels of *CB1* did not change in the hypothalamus of 24 h DEX-treated birds ($P = 0.505$; Fig. 2E). However,72 h DEX treatment increased the hypothalamic *CB1* gene expression ($P = 0.001$; Fig. 2F). Treatment of hypothalamic cells with DEX did not alter the gene expression of *AMPKα2*, *GR*, and *CB1* ($P = 0.581$, $P = 0.455$, $P = 0.293$, respectively; Fig. 3A, B, and C).

4. Discussion

4.1. Distribution of CB1 in 7- and 35-day-old broiler tissues

Previous reported indicated that CB1 mRNA is present in the CNS (Terry et al., 2010; Tsou et al., 1998) and in peripheral tissues, including adipose tissues, liver, and myocardium, of mammalian species (Butler and Korbonits, 2009). Furthermore, CB1 is expressed by neurons throughout the body, including the brain and spinal cord, autonomic, and enteric nervous systems (Núñez et al., 2004). However, CB1 in broilers has not been studied sufficiently (Soderstrom and Johnson, 2000). In the current study, CB1 was expressed in the peripheral tissue (liver and small intestine) of broilers, which was consistent with previous findings. Compared with the hypothalamus, the expression of CB1 in the liver, duodenum, jejunum, and ileum is significantly reduced, which was consistent with previous reports that CB1 is mainly expressed at the end of the cerebellum axon, and this distribution pattern has been confirmed throughout the brain (Pickel et al., 2012). It is reported that CB1 are heterogeneously expressed within subregions of the hypothalamus; in particular, CB1 are present at greater density within the paraventricular nucleus of hypothalamus relative to other hypothalamic regions (Wittmann et al., 2007). However, compared to other brain regions, CB1 density in the hypothalamus is lower (Herkenham et al., 1991) in spite of the fact that cannabinoids have many effects on hypothalamic-mediated processes (Fernández-Ruiz et al., 1997). Besides, there is evidence for CB1 expression at all levels of the HPA axis (Hillard et al., 2016). In birds, the expression pattern of CB1mRNA appears to be highly conserved across species in key regions such as parts of the cerebellum and forebrain, and displayed high levels of expression in a number of regions that are known to be involved in visual processing throughout the brain (Stincic and Hyson, 2008).

4.2. Effects of DEX-treatment in vivo and in vitro on CB1, GR and AMPKα2 gene expression in broiler hypothalamus

Although CB1 is highly expressed through the brain, the regulatory effects of GCs on CB1 expression are not ubiquitous throughout the CNS. In rodents, exogenous GCs cause different CB1 expression responses in diverse tissues (Hill et al., 2005; Wang et al., 2007). In the current study, the mRNA levels of CB1 did not change in the hypothalamus of 24 h DEX-treated birds, however, treated with DEX for 72 h showed an increased hypothalamic CB1 gene expression. Consistent with the present findings, increased CB1 expression was reported after 1 week of Glucocorticoid treatment in flushed bone and bone marrow cells of male

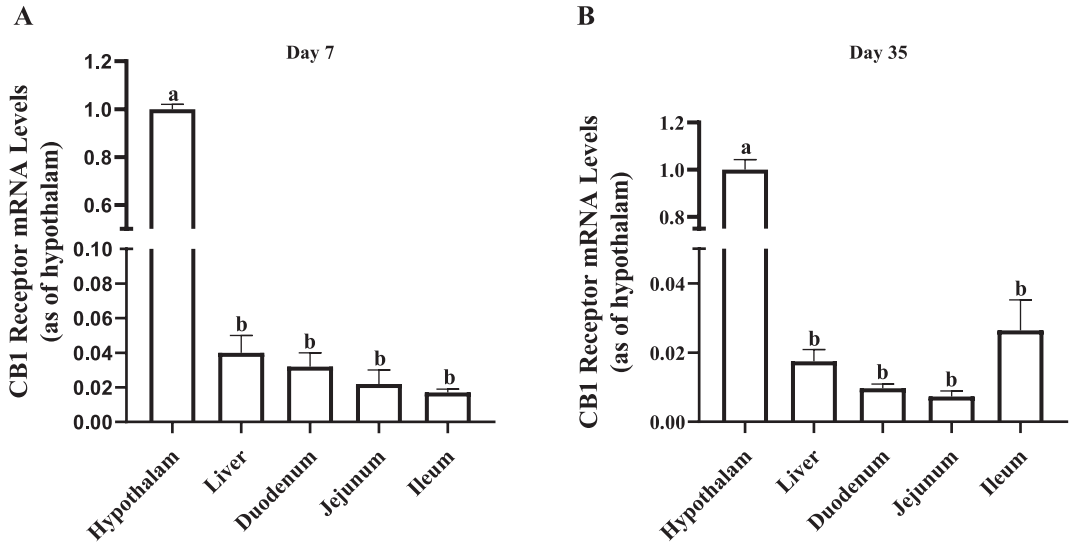

Fig. 1. Cannabinoid receptor type 1 mRNA levels in the hypothalamus, liver, duodenum, ileum, and jejunum of 7 (A) - and 35 (B) -days-old broilers. Values are means ± SEM ($n = 8$); [a, b] means with different letters differ significantly ($P < 0.05$).

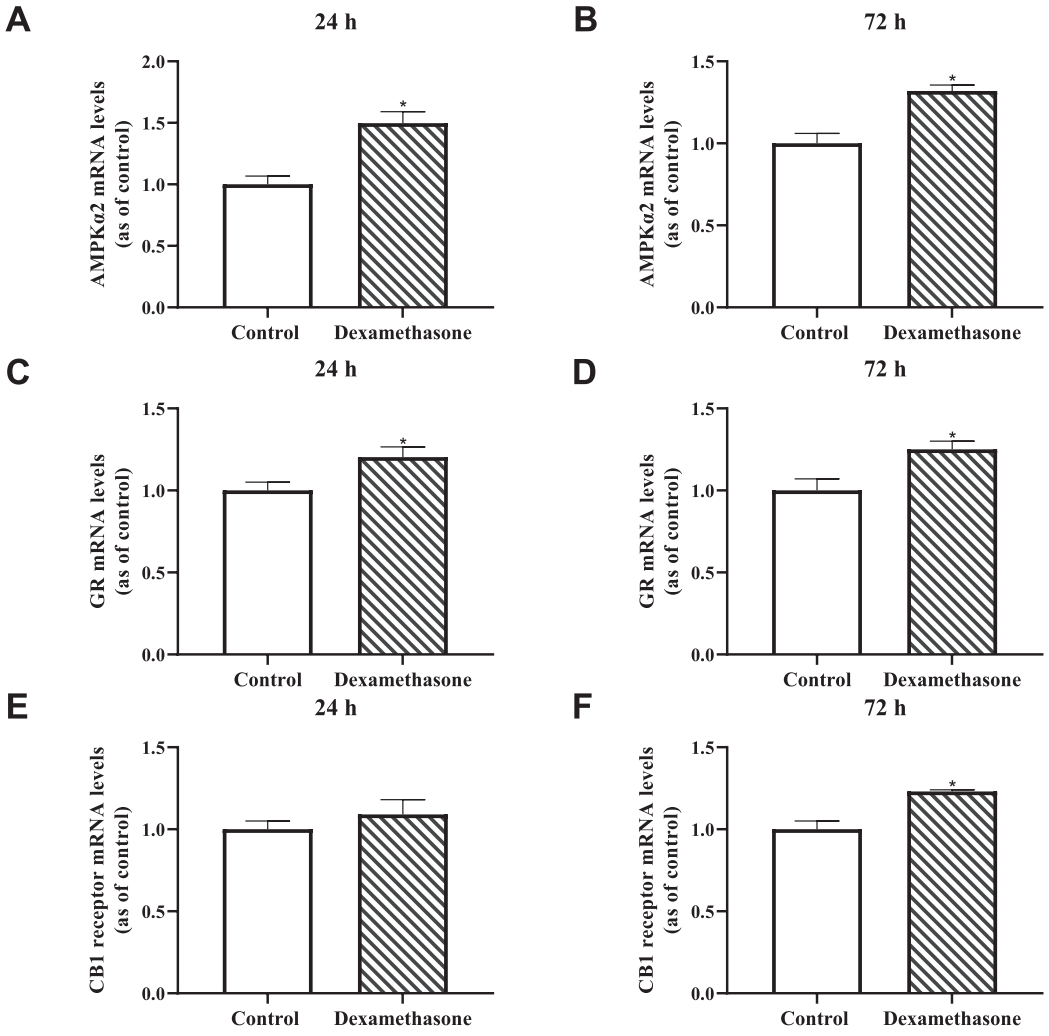

Fig. 2. Effect of 24 h (A) and 72 h (B) dexamethasone treatments on the hypothalamic AMPKα2 mRNA levels (*in vivo*); Effect of 24 h (C) and 72 h (D) dexamethasone treatments on the hypothalamic GR mRNA levels (*in vivo*); Effect of 24 h (E) and 72 h (F) DEX treatments on the hypothalamic CB1 mRNA levels (*in vivo*). Values are means ± SEM ($n = 6$); asterisks indicate values that are significantly different between the control and DEX-treated group (* $P < 0.05$).

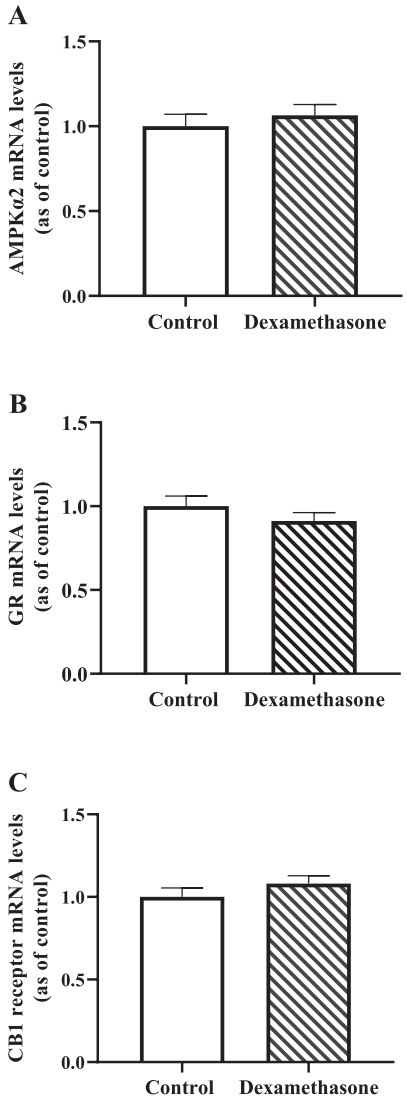

Fig. 3. A: Effect of dexamethasone treatment on AMPKα2 mRNA levels of the hypothalamic cells in broiler embryos (*in vitro*); B: Effect of dexamethasone treatment on GR mRNA levels of the hypothalamic cells in broiler embryos (*in vitro*); C: Effect of dexamethasone treatment on CB1 mRNA levels of the hypothalamic cells in broiler embryos (*in vitro*). Values are means ± SEM ($n = 6$).

rats (Ko et al., 2012). Treatment of cultured adipocytes with DEX for 24 h increased CB1 expression, suggesting that the effect of high GCs is mediated by enhanced CB1 expression (Pereira et al., 2014) Long-term corticosteroid treatment reduced the density of CB1 binding sites in the hippocampus and amygdala (Bowles et al., 2012). Repeated corticosteroid treatment resulted in a GR-dependent decrease in the CB1 protein expression in primary sensory neurons (Hong et al., 2011).

The endocrine response to physiological and psychological stress is mainly mediated by GCs by activating the HPA axis, which is released from the adrenal cortex. Glucocorticoid signaling is responsible for many physiological and psychological changes after stress, including increased food consumption, suppression of the immune system, and changes in memory and mood through effects on GRs (Hillard et al., 2016). This phenomenon was described in our early studies, wherein immune stress significantly reduced the food intake of broilers (Song et al., 2011b). Corticosterone treatment exhibits an immunosuppressive effect on the innate immune system of broilers, and such an effect could be enhanced by high dietary energy consumption (Yuan et al., 2008). A high-energy diet inhibits the appetite of broilers and reduces the gene expression of GR in the hypothalamus (Hu et al., 2019). Several lines of evidence suggest that ligand-dependent GR activation mediates the role of GCs, which are expressed in almost every cell type. Activated GR, as a transcription factor, controls the expression level of target genes and regulates intracellular signaling pathways (Hafezi and Nouhi, 2002; Karin and Chang, 2001). Glucocorticoid receptor activation can increase CB1 expression. For example, cannabinoid receptor type 1 upregulation in the spinal cord of rats with compressive nerve injury depends on GR gene expression (Wang et al., 2007). The glucocorticoid-induced CB1 expression in mouse osteoblasts increased through GR-dependent regulation of transcription and translation (Wu et al., 2011). After long-term treatment with GCs, CB1 expression in rat bone was upregulated (Ko et al., 2012). In the present study, the change in the CB1 mRNA level of cultured broiler embryo hypothalamic cells were not significant. However, cannabinoid receptor type 1 may be related to GR gene expression in the DEX-exposed broiler hypothalamus. An implication of this result is the possible increase in CB1 mRNA expression due to increased GR mRNA expression. These mechanisms may differ because of factors, such as DEX treatment time.

Many of the changes observed during excessive GC levels correspond to the metabolic steps regulated by AMPK (Scerif et al., 2013). The effect of GCs on AMPK activity is tissue-dependent (Wang et al., 2010). AMPK in broilers is a regulator of cellular and systemic energy homeostasis and acts as a sensor of energy states (Song et al., 2012). The AMPK signal in the hypothalamus is related to control of food intake (Minokoshi et al., 2004). This finding is under the present observation that fasting-induced AMPK activation in the hypothalamus of broilers (Song et al., 2012). Meanwhile, activated AMPK stimulates neuropeptide Y gene expression and food intake, whereas inactivated AMPK causes anorexia in broilers (Liu et al., 2014). Liu et al. (2015) proposed the potential role of the hypothalamic rapamycin pathway target in appetite-related gene regulation by GCs; however, this finding should be further verified. Glucocorticoids participate in appetite control (Yuan et al., 2008). Previous studies suggested that hypothalamic CB1 activation is a powerful orexigenic signal and acts as a key determinant of energy balance in mice (Cardinal et al., 2012). In mammalian species, cannabinoid receptor type 1 is required for the hypothalamic AMPK effects of GCs (Scerif et al., 2013). In the present study, the gene expression of AMPKα2 subunit and CB1 in 72 h DEX-treated broilers was significantly increased. CB1 that mediates AMPK regulation by GCs is one possible explanation, consistent with a previous report (Scerif et al., 2013). Different physiological and pathological stresses, such as glucose deprivation, ischemia, and hypoxia, leading to activation of AMPK (Kahn et al., 2005). Once AMPK is activated, it switches off anabolic pathways, such as fatty acid, triglyceride, and cholesterol synthesis, in favor of catabolic pathways, such as glycolysis and fatty acid oxidation, to restore energy balance.

In conclusion, CB1 is mainly expressed in the hypothalamus of broilers; long-term DEX exposure can regulate the CB1 system and AMPK signaling pathway of broiler hypothalamus. Moreover, hypothalamic cannabinoid signaling could be increased under conditions associated with hypersecretion of glucocorticoids, such as stress.

Conflicts of interest

We declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgment

This work was supported by the Natural Science Foundation of Shandong Province (ZR2020MC170), the National Key R&D Program of China (2018YFD0501401-3), and the Shandong Province Agricultural Industry Technology (SDAIT-11-08).

References

Bowles, N.P., Hill, M.N., Bhagat, S.M., Karatsoreos, I.N., Hillard, C.J., McEwen, B.S., 2012. Chronic, noninvasive glucocorticoid administration suppresses limbic endocannabinoid signaling in mice. Neuroscience. 204, 83–89.

Breivogel, C.S., Childers, S.R., 1998. The functional neuroanatomy of brain cannabinoid receptors. Neurobiol. Dis. 5, 417–431.

Butler, H., Korbonits, M., 2009. Cannabinoids for clinicians: the rise and fall of the cannabinoid antagonists. Eur. J. Endocrinol. 161, 655–662.

Cai, Y., Song, Z., Wang, X., Jiao, H., Lin, H., 2011. Dexamethasone-induced hepatic lipogenesis is insulin dependent in chickens (Gallus gallus domesticus). Stress. 14, 273–281.

Cardinal, P., Bellocchio, L., Clark, S., Cannich, A., Klugmann, M., Lutz, B., Marsicano, G., Cota, D., 2012. Hypothalamic CB1 cannabinoid receptors regulate energy balance in mice. Endocrinology. 153, 4136–4143.

Dallman, M.F., 2003. Fast glucocorticoid feedback favors 'the munchies'. Trends Endocrinol. Metab. 14, 394–396.

Di Marzo, V., Bifulco, M., De Petrocellis, L., 2004. The endocannabinoid system and its therapeutic exploitation. Nat. Rev. Drug Discov. 3, 771–784.

Di, S., Malcher-Lopes, R., Halmos, K.C., Tasker, J.G., 2003. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J. Neurosci. 23, 4850–4857.

Di, S., Boudaba, C., Popescu, I.R., Weng, F.-J., Harris, C., Marcheselli, V.L., Bazan, N.G., Tasker, J.G., 2005. Activity-dependent release and actions of endocannabinoids in the rat hypothalamic supraoptic nucleus. J. Physiol. Lond. 569, 751–760.

Evanson, N.K., Tasker, J.G., Hill, M.N., Hillard, C.J., Herman, J.P., 2010. Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. Endocrinology. 151, 4811–4819.

Fernández-Ruiz, J.J., Muñoz, R.M., Romero, J., Villanua, M.A., Makriyannis, A., Ramos, J.A., 1997. Time course of the effects of different cannabimimetics on prolactin and gonadotrophin secretion: evidence for the presence of CB1 receptors in hypothalamic structures and their involvement in the effects of cannabimimetics. Biochem. Pharmacol. 53, 1919–1927.

Freund, T., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol. Rev. 83, 1017–1066.

Hafezi, F., Nouhi, A.H., 2002. Abdominoplasty and seroma. Ann. Plast. Surg. 48, 109–110.

Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1991. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J. Neurosci. 11, 563–583.

Hill, M.N., Ho, W.S.V., Meier, S.E., Gorzalka, B.B., Hillard, C.J., 2005. Chronic corticosterone treatment increases the endocannabinoid 2-arachidonylglycerol in the rat amygdala. Eur. J. Pharmacol. 528.

Hillard, C.J., Beatka, M., Sarvaideo, J., 2016. Endocannabinoid signaling and the hypothalamic-pituitary-adrenal axis. Compr. Physiol. 7.

Hong, S., Zheng, G., Wu, X., Snider, N.T., Owyang, C., Wiley, J.W., 2011. Corticosterone mediates reciprocal changes in CB 1 and TRPV1 receptors in primary sensory neurons in the chronically stressed rat. Gastroenterology. 140.

Hu, X., Wang, Y., Sheikhahmadi, A., Li, X., Buyse, J., Lin, H., Song, Z., 2019. Effects of dietary energy level on appetite and central adenosine monophosphate-activated protein kinase (AMPK) in broilers. J. Anim. Sci. 97, 4488–4495.

Kahn, B.B., Alquier, T., Carling, D., Hardie, D.G., 2005. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 1, 15–25.

Karin, M., Chang, L., 2001. AP-1–glucocorticoid receptor crosstalk taken to a higher level. J. Endocrinol. 169, 447–451.

Ko, J.-Y., Wu, R.-W., Kuo, S.-J., Chen, M.-W., Yeh, D.-W., Ke, H.-C., Wu, S.-L., Wang, F.-S., 2012. Cannabinoid receptor 1 mediates glucocorticoid-induced bone loss in rats by perturbing bone mineral acquisition and marrow adipogenesis. Arthritis Rheum. 64, 1204–1214.

Kretz, O., Reichardt, H.M., Schütz, G., Bock, R., 1999. Corticotropin-releasing hormone expression is the major target for glucocorticoid feedback-control at the hypothalamic level. Brain Res. 818, 488–491.

Lei, L., Hepeng, L., Xianlei, L., Hongchao, J., Hai, L., Sheikhahmadi, A., Yufeng, W., Zhigang, S., 2013. Effects of acute heat stress on gene expression of brain-gut neuropeptides in broiler chickens. J. Anim. Sci. 91, 5194–5201.

Liu, L., Song, Z., Jiao, H., Lin, H., 2014. Glucocorticoids increase NPY gene expression via hypothalamic AMPK signaling in broiler chicks. Endocrinology. 155, 2190–2198.

Liu, L., Wang, X., Jiao, H., Zhao, J., Lin, H., 2015. Glucocorticoids inhibited hypothalamic target of rapamycin in high fat diet-fed chicks. Poult. Sci. 94, 2221–2227.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 346, 561–564.

Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y.-B., Lee, A., Xue, B., Mu, J., Foufelle, F., Ferré, P., Birnbaum, M.J., et al., 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature. 428, 569–574.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 365, 61–65.

Myers, B., McKlveen, J.M., Herman, J.P., 2012. Neural regulation of the stress response: the many faces of feedback. Cell. Mol. Neurobiol. 32, 683–694.

Núñez, E., Benito, C., Pazos, M.R., Barbachano, A., Fajardo, O., González, S., Tolón, R.M., Romero, J., 2004. Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. Synapse (New York, N.Y.). 53, 208–213.

Pagotto, U., Marsicano, G., Cota, D., Lutz, B., Pasquali, R., 2006. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. Endocr. Rev. 27.

Pereira, M.J., Palming, J., Svensson, M.K., Rizell, M., Dalenbäck, J., Hammar, M., Fall, T., Sidibeh, C.O., Svensson, P.-A., Eriksson, J.W., 2014. FKBP5 expression in human adipose tissue increases following dexamethasone exposure and is associated with insulin resistance. Metabolism: clinical and experimental. 63, 1198–1208.

Pickel, V.M., Shobin, E.T., Lane, D.A., Mackie, K., 2012. Cannabinoid-1 receptors in the mouse ventral pallidum are targeted to axonal profiles expressing functionally opposed opioid peptides and contacting N-acylphosphatidylethanolamine-hydrolyzing phospholipase D terminals. Neuroscience. 227, 10–21.

Scerif, M., Füzesi, T., Thomas, J.D., Kola, B., Grossman, A.B., Fekete, C., Korbonits, M., 2013. CB1 receptor mediates the effects of glucocorticoids on AMPK activity in the hypothalamus. J. Endocrinol. 219, 79–88.

Soderstrom, K., Johnson, F., 2000. CB1 cannabinoid receptor expression in brain regions associated with zebra finch song control. Brain Res. 857, 151–157.

Song, Z., Yuan, L., Jiao, H., Lin, H., 2011a. Effect of corticosterone on hypothalamic corticotropin-releasing hormone expression in broiler chicks (*Gallus Gallus domesticus*) Fed a high energy diet. Asian Australas. J. Anim. Sci. 24.

Song, Z., Zhao, T., Liu, L., Jiao, H., Lin, H., 2011b. Effect of copper on antioxidant ability and nutrient metabolism in broiler chickens stimulated by lipopolysaccharides. Arch. Anim. Nutr. 65, 366–375.

Song, Z., Liu, L., Yue, Y., Jiao, H., Lin, H., Sheikhahmadi, A., Everaert, N., Decuypere, E., Buyse, J., 2012. Fasting alters protein expression of AMP-activated protein kinase in the hypothalamus of broiler chicks (Gallus gallus domesticus). Gen. Comp. Endocrinol. 178, 546–555.

Stincic, T.L., Hyson, R.L., 2008. Localization of CB1 cannabinoid receptor mRNA in the brain of the chick (Gallus domesticus). Brain Res. 1245, 61–73.

Terry, G.E., Hirvonen, J., Liow, J.-S., Zoghbi, S.S., Gladding, R., Tauscher, J.T., Schaus, J. M., Phebus, L., Felder, C.C., Morse, C.L., et al., 2010. Imaging and quantitation of cannabinoid CB1 receptors in human and monkey brains using (18)F-labeled inverse agonist radioligands. J. Nucl. Med. 51, 112–120.

Tsou, K., Brown, S., Sañudo-Peña, M.C., Mackie, K., Walker, J.M., 1998. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience. 83, 393–411.

Vandenborne, K., De Groef, B., Geelissen, S.M.E., Boorse, G.C., Denver, R.J., Kühn, E.R., Darras, V.M., Van der Geyten, S., 2005. Molecular cloning and developmental expression of corticotropin-releasing factor in the chicken. Endocrinology. 146, 301–308.

Wang, S., Lim, G., Mao, J., Sung, B., Yang, L., Mao, J., 2007. Central glucocorticoid receptors regulate the upregulation of spinal cannabinoid-1 receptors after peripheral nerve injury in rats. Pain. 131.

Wang, X., Lin, H., Song, Z., Jiao, H., 2010. Dexamethasone facilitates lipid accumulation and mild feed restriction improves fatty acids oxidation in skeletal muscle of broiler chicks (Gallus gallus domesticus). Comp. Biochem. Physiol C Toxicol. Pharmacol. 151, 447–454.

Wittmann, G., Deli, L., Kalló, I., Hrabovszky, E., Watanabe, M., Liposits, Z., Fekete, C., 2007. Distribution of type 1 cannabinoid receptor (CB1)-immunoreactive axons in the mouse hypothalamus. J. Comp. Neurol. 503, 270–279.

Wu, R.-W., Lin, T.-P., Ko, J.-Y., Yeh, D.-W., Chen, M.-W., Ke, H.-C., Wu, S.-L., Wang, F.-S., 2011. Cannabinoid receptor 1 regulates ERK and GSK-3β-dependent glucocorticoid inhibition of osteoblast differentiation in murine MC3T3-E1 cells. Bone. 49, 1255–1263.

Yuan, L., Lin, H., Jiang, K.J., Jiao, H.C., Song, Z.G., 2008. Corticosterone administration and high-energy feed results in enhanced fat accumulation and insulin resistance in broiler chickens. Br. Poult. Sci. 49, 487–495.