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The AMPK-mTOR signaling pathway is involved in regulation of food intake in the hypothalamus of stressed chickens

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ABSTRACT

Glucocorticoids (GCs) can stimulate the appetite and AMPK in broilers. The activation of hypothalamic mTOR has been proposed as an important anorexigenic signal. However, inhibitory effect of AMPK activity on appetite and AMPK downstream signaling pathway under stress has not been reported. In this study, we performed an intracerebroventricular (icv) injection of compound C, an AMPK inhibitor, in GC-treated birds to explore the regulatory mechanism on appetite and AMPK downstream signaling pathway. A total of 48 7-day-old broilers, which had received an icv cannula, were randomly subjected to one of two treatments: subcutaneous injection of dexamethasone (DEX) or saline. After 3 days of continuous DEX injection, chicks of each group received an icv injection with either compound C (6 μg/2 μL) or vehicle (dimethyl sulfoxide, 2 μL). The results showed that body weight gain was reduced by the DEX treatment. Compared with the control, icv injection of compound C reduced feed intake at 0.5–1.5 h. In the DEX-treated group, the inhibitory effect of compound C on appetite remained apparent at 0.5–1 h. The DEX treatment increased the gene expression of liver kinase B1 (*LKB1*), neuropeptide Y (*NPY*), and decreased p-mTOR protein level. In stressed broilers, inhibition of AMPK relieved the decreased mTOR activity. A significant interaction was noted in DEX and compound C on protein expression of phospho-AMPK. Taken together, in stressed broilers, the central injection of compound C could inhibit central AMPK activity and reduce appetite, in which the AMPK/mTOR signaling pathway might be involved.

1. Introduction

Currently, the term "stress" is widely used to describe a series of physiological and behavioral responses caused by adverse stimuli (Yang et al., 2015; Chen et al., 2020a; Chen et al., 2020b, 2021). As the end-product of the hypothalamus–pituitary–adrenal (HPA) axis (Song et al., 2011a), glucocorticoids (GCs) stimulate a series of essential physiological responses related to the mobilization and redistribution of stored energy toward the inhibition of nonessential functions, such as growth, maintaining homeostasis, and survival (Matteri et al., 2000). As orexigenic peripheral signals, GCs play a permissive role in regulating energy balance. The GCs is also involved in homeostasis control and the body's response to stressors, through glucose mobilization and lipolysis to release stored energy (Solomon et al., 2011; Magomedova and Cummins, 2016). In avian and mammalian species, GCs stimulate the expression of neuropeptide Y (NPY) (Asensio et al., 2004; Liu et al., 2014).

The central nervous system (CNS) participates in the regulation of satiety, hunger, and energy homeostasis (Morton et al., 2006). As an important part of the CNS, the hypothalamus plays a vital role in the regulation of feed intake, body temperature, and endocrine functions through different pathways (He et al., 2019). In mammals and birds, the hypothalamus can integrate peripheral signals that reflect energy balance (Liu et al., 2016). Appetite regulation is highly complex, and involves the brain-gut axis, hormonal and nonhormonal factors (Liu et al., 2013). The hypothalamus plays a major role in the control of the appetite (Abdalla, 2017). There are two primary populations of neurons in the birds' hypothalami that influence appetite by releasing of signaling molecules (Richards, 2003; Boswell, 2005). Several hypothalamic nuclei that express orexigenic or anorexigenic neuropeptides play key roles in appetite regulation and influence hypothalamic AMP-activated protein kinase (AMPK) activity (Song et al., 2012).

AMPK is a highly conserved serine/threonine kinase that functions as an energy sensor and transducer of cellular metabolism (Hardie, 2007). As a key regulator of cell energy homeostasis, AMPK regulates energy metabolism in response to nutrients, hormonal signals, and metabolic

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stresses at the cellular and whole-body levels (Steinberg and Kemp, 2009; Lim et al., 2010; Hardie, 2014). Recently, hypothalamic AMP-activated protein kinase (AMPK) signaling has become an important focus of interest in the control of food intake (Andersson et al., 2004; Minokoshi et al., 2004; Ropelle et al., 2007), and energy balance (Hurtado-Carneiro et al., 2012). Fasting increases and re-feeding decreases AMPK activity in several hypothalamic nuclei (Minokoshi et al., 2004). AMPK activation requires the phosphorylation of a threonine residue (T172) within the alpha catalytic subunit by upstream kinases, such as liver kinase B1 (LKB1) (Stein et al., 2000). Once activated, AMPK phosphorylates acetyl-CoA carboxylase (ACC) and switches on energy-producing pathways at the expense of energy depleting processes (Kahn et al., 2005; Hardie, 2006). Another target molecule for the control of food intake and energy homeostasis is the mammalian target of rapamycin (mTOR) catalytic activity, which has been suggested to be affected by the phosphatidylinositol 3-kinase/Akt pathway (Cota et al., 2006).

Mammalian target of rapamycin (mTOR) is an evolutionarily conserved serine–threonine kinase that modulates cell-cycle progression and growth by sensing changes in energy balance, growth factors, nutrients and oxygen (Dennis et al., 2001; Wullschleger et al., 2006; Jacinto and Hall, 2003). mTOR is a component of at least two multi-protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Guertin and Sabatini, 2007). mTOR signaling is inhibited under low nutrient conditions, such as decreased levels of glucose and amino acids (Proud, 2004; Wang and Proud, 2009) and low intracellular ATP levels (Dennis et al., 2001; Shamji et al., 2003). Activation of AMPK suppresses mTOR signaling through phosphorylation of tuberous sclerosis protein 2 (TSC2) (Inoki et al., 2003), phosphorylation of Raptor (Gwinn et al., 2008) and direct phosphorylation of mTOR at Thr2446 (Cheng et al., 2004). Consequently, mTOR activity changes inversely with that of AMPK, i.e. when intracellular energy is abundant, mTOR activity is increased and AMPK activity is decreased, and vice versa (Dennis et al., 2001; Kahn et al., 2005; Carling et al., 2008; Hardie, 2008; Lage et al., 2008).

Based on the above findings, we hypothesized that stress stimulated appetite by activating AMPK and consequently inhibiting mTOR activity in the hypothalamus. To test this assumption, we examined the influence of centrally applied AMPK inhibitor (compound C) on DEX-triggered hyperphagia and hypothalamic AMPK/mTOR signaling. This study will provide a theoretical basis for studying the regulation of AMPK-mTOR signaling pathway on appetite under stress in broilers.

2. Materials and methods

2.1. Animals

A total of 48 1-day-old male broilers with similar body weight were raised in a room. At the beginning, the ambient temperature was controlled at 35 °C, and then decreased by 2 °C to 3 °C per week. The ambient humidity was maintained at 40%–50%. The photoperiod was 23 h of light and 1 h of darkness. All broiler chicks received a basal diet containing 21.5% crude protein and 12.33 MJ/kg metabolizable energy. During the whole feeding period, all broilers were free to feed and water. The study protocol was approved by the Shandong Agricultural University and conducted in accordance with the Guidelines for Experimental Animals of the Ministry of Science and Technology (Beijing, China).

2.2. Implantation of icv cannulae

The procedure for implantation of the icv cannulae followed that of a previous report (Liu et al., 2014). At 7 days of age, chicks were anesthetized by an intravenous injection of pentobarbital sodium (Solarbio® LIFE SCIENCES, Beijing, China) at dose of 25 mg/kg body weight. A stereotaxic apparatus (Huai Bei Zheng Hua) was used to facilitate implantation of the icv cannulae. The thin-walled stainless steel guide cannula (Shenzhen ruiwode Life Technology Co., Ltd) was stereotaxically implanted into the third ventricle.

2.3. Intracerebroventricular injection and verification

During injection, an internal injection cannula (Shenzhen ruiwode Life Technology Co., Ltd) was inserted into, and extended 1 mm beyond the tip of the guide cannula. A 10 μL Hamilton syringe (Shenzhen ruiwode Life Technology Co., Ltd) was used for microinjection. Owing to diffusion of the drugs, the cannula was held in place for approximately 30 s after infusion, and a dummy cannula was reinserted. To locate the injection site, compound C (P5499, Sigma, USA) and dimethyl sulfoxide (D2650, Sigma, USA) contained 0.1% Evans blue solution.

2.4. Experiment 1

After implanting of the icv cannulae at 7 days of age, each chicken was placed in a separate cage. At 8 days of age, 48 broiler chicks were divided into two groups randomly, each of which had 24 replicates. The following treatments were separately applied to each group: the DEX-treated group (subcutaneous injection of DEX, 2.0 mg/kg BW); and sham-treated control (injection of saline). The treatment lasted for 3 days, once a day. Feed intake was recorded daily. Body weight gain (BWG) was measured daily. At 10 days of age, chicks were fasted for 3 h after a subcutaneous injection of DEX. Each group was subjected to two treatments as injected with compound C (6 μg/2 μL) or injected with vehicle (dimethyl sulfoxide, 2 μL) (Liu et al., 2014). The time point for each chick after compound C injection was accurately recorded during the test. After icv injection, the broilers were allowed to get feed immediately. The feed intake was recorded every 0.5 h.

2.5. Experiment 2

The experiment was conducted twice. During the second round of the experiment, the chicks were continuously fasted for 1 h after icv injection, and were then sacrificed by cervical dislocation and exsanguinated for resection of the hypothalami. The hypothalami were collected according to the methods of Hu and Wang (2019) and preserved at −80 °C until further use. The injection time for each chick was accurately recorded during the experiments.

2.6. RNA extraction and analysis*Figure 1, 2 and 3*

Gene expression in the hypothalamus was assessed by using quantitative real-time PCR with SYBR Green I labeling. Total RNA was extracted by the Trizol Reagent (15596026, Invitrogen, San Diego, CA, USA). The RNA quality was assessed through agarose gel electrophoresis, and its quantity was measured with a biophotometer (Eppendorf, Germany).

Reverse transcription was performed using transcription kits (0489, 703, 0001, Roche, Switzerland), and the reaction contained a total of 1000 ng RNA. Real-time analysis was conducted using the 7500 Real-time PCR System (Applied Biosystems, Foster, CA, USA). A volume of 20 μL of reaction mixture contained 0.2 μmol/L primers and the SYBR Green Master Mix (RR420, Takara, Dalian, Liaoning, PR China). The primer sequences are showed in Table 1. The primers were synthesized by Shanghai Sangon Company (Shanghai, China). Real-time PCR was performed according to the kit's manufacturer instructions.

The relative amount of mRNA was calculated in accordance with the method of Livak and Schmittgen (2001). The housekeeping gene was *β-Actin*. The mRNA levels of target genes were normalized to β-actin (ΔCT). ΔCT was calibrated against the average of the control chicks. The number of target molecules relative to the control was calculated by using $2^{-\Delta\Delta CT}$. Therefore, all gene transcription results are reported as the n-fold difference relative to the control. The results of the relative

Table 1
Gene-specific primers of related genes.

Gene	GenBank accession number	Primer sequence (5′ → 3′)	Product size (bp)
AMPKα2	DQ34039	F:GGGACCTGAAACCAGAGAACG R: ACAGAGGAGGGCATAGAGGATG	215
AMPKα1	NM_001039603	F: CGGAGATAAACAGAAGCACGAG R: CGATTCAGGATCTTCACTGCAAC	125
LKB1	NM_001045833	F: TGAGAGGGATGCTTGAATACGA R:ACTTGTCCTTTGTTTCTGGGC	158
NPY	NM_205473	F:CTCTGAGGCACTACATCAACC R:ACCACATCGAAGGGTCTTCAA	142
β-Actin	NM_205518.1	F:CTGGCACCTAGCACAATGAA R:CTGCTTGCTGATCCACATCT	123
FAS	J03860	F: CTATCGACACAGCCTGCTCCT R: CAGAATGTTGACCCCTCCTACC	107
GAPDH	NM_204305	F:ACATGGCATCCAAGGAGTGAG R: GGGGAGACAGAAGGGAACAGA	266

LKB1 = liver kinase B1; AMPKα1 = AMP-activated protein kinase α1; AMPKα2 = AMP-activated protein kinase α2; NPY = neuropeptide Y; FAS = fatty acid synthase; GAPDH = Glyceraldehyde phosphate dehydrogenase.

mRNA quantification were verified by glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) levels.

2.7. Protein preparation and Western blot analysis

Protein expression in the hypothalamus was measured according to the methods of a previous study (Liu et al., 2018). The hypothalamus was thoroughly homogenized in 0.8 mL lysis buffer (P0013B, Beyotime, China) and kept on ice during the trial procedure. Protein concentration was measured with the bicinchoninic acid assay kit (P0010, Beyotime, China). A total of 80 μg of protein were electrophoresed in 7.5%–10% sodium dodecyl sulfate polyacrylamide gels, after being boiled for 10 min. The separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, USA). The membranes were then blocked with blocking buffer (P0023B, Beyotime, China) at 25 °C for 1 h, and covered with primary antibodies at 4 °C overnight. The phospho-AMPKα^{Thr172} antibody (#2531); AMPKα antibody (#2532); phospho-acetyl-coenzyme A carboxylase (ACC)^{Ser79} antibody (#3661); ACC antibody (#3662); phospho-mTOR^{ser2448} antibody (#2971); mTOR antibody (#2983) (Cell Signaling Technology, Beverly, MA, USA); and anti-GAPDH (AG019) (Beyotime, China) were used as primary antibodies. These antibodies have been previously validated (Liu et al., 2014; Proszkowiec-Weglarz et al., 2006). The membranes were then incubated with horseradish peroxidase-linked anti-rabbit and anti-mouse secondary antibodies at 4 °C for 4 h. Membranes were subsequently visualized by exposure to Hyperfilm ECL (Beyotime, China). The band intensity was normalized to the GAPDH band in the same sample.

2.8. Statistical analysis

The effect of DEX injection on performance was analyzed using the SPSS version 11.0 software (SPSS, Chicago, IL), and one chicken was considered as one replicate (n = 24). The data were expressed as the mean ± SEM. A two-way ANOVA model was used to analyze the main effects of the DEX and compound C treatments and their interaction by using the SAS statistical software package. For the statistical analysis of cumulative feed intake after icv injection, one chicken was considered as one replicate (n = 12). For the measurement of gene and protein expression in the hypothalamus, one chick was used as one replicate (n = 8). $P < 0.05$ indicated statistical significance.

3. Results

3.1. Effects of DEX on performance in broilers

The BWG of DEX-treated broilers was reduced compared with that of the control at 8 and 9 days of age ($P < 0.05$; $N = 24$; Fig. 1A and C). However, DEX did not affect feed intake in either of the two phases ($P > 0.1$; N = 24; Fig. 1B and D).

3.2. Effects of compound C on feed intake in broilers

Cumulative feed intake was reduced after icv administration of compound C (6 μg) at 0.5–1.5 h, compared with that of the control ($P < 0.05$; $N = 12$; Fig. 2). In stressed chicks, this effect was observed at 0.5–1 h ($P < 0.05$; N = 12; Fig. 2).

3.3. Effects of DEX on appetite-related gene expression and the AMPK signaling pathway in broilers

DEX treatment significantly increased the hypothalamic *LKB1*, *NPY* mRNA levels ($P < 0.05$; Table 2) and tended to increase the expression of *AMPKα2* and *FAS* genes ($P = 0.0646$, $P = 0.0772$, respectively). However, DEX had no significant influence on the expression of *AMPKα1* ($P > 0.1$; Table 2). DEX treatment decreased p-mTOR protein level ($P < 0.05$; Fig. 3D). Furthermore, DEX treatment exerted no effect on the protein levels of phosphorylated AMPKα and ACC ($P > 0.1$; Fig. 3B).

3.4. Effects of compound C on appetite-related gene expression and the AMPK signaling pathway in broilers

The icv injection of compound C reduced *FAS* gene expression ($P = 0.0149$; Table 2) and showed a tendency to increase *AMPKα2* gene

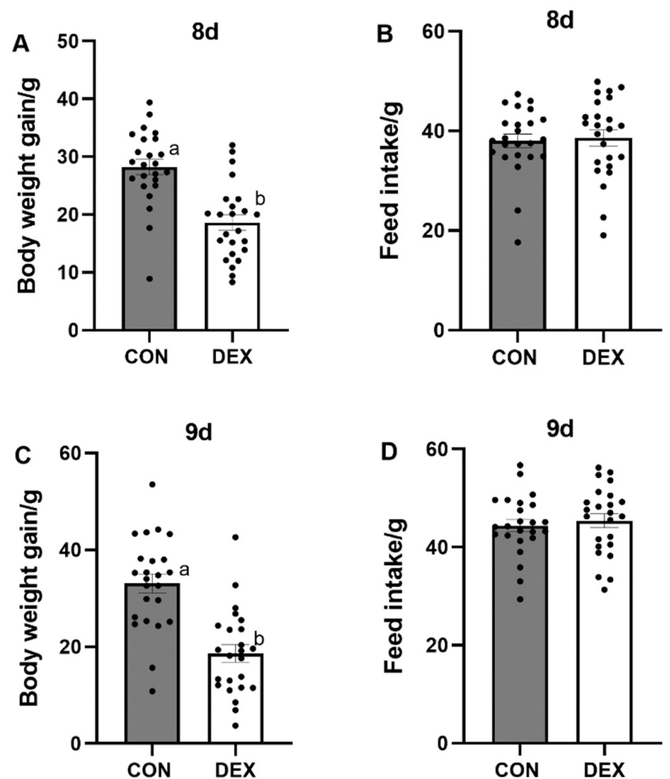

Fig. 1. Effects of subcutaneous injection of dexamethasone (DEX) (2 mg/kg BW) on (**A** and **C**) body weight gain (BWG) and (**B** and **D**) feed intake in chicks, compared with controls (saline). Values are expressed as the mean ± SEM (n = 24). ab, means differ significantly ($P < 0.05$).

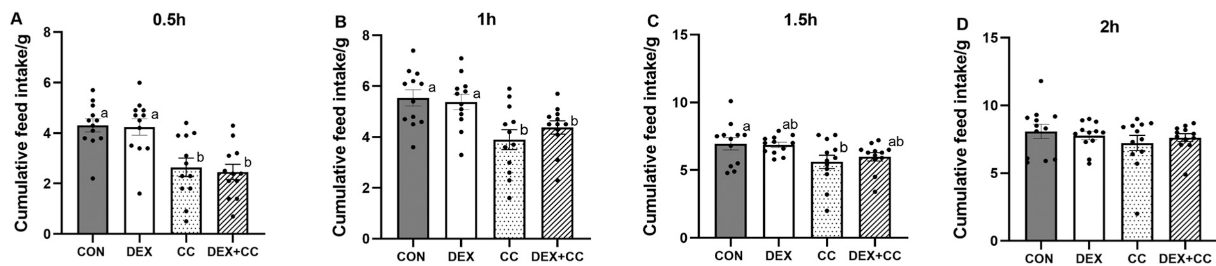

Fig. 2. Effects of central injection of compound C (6 μg) on the cumulative feed intake of chickens exposed to dexamethasone (DEX) or saline. Values are expressed as the mean ± SEM (n = 12). ab, means differ significantly ($P < 0.05$).

Table 2
Effects of central injection of Compound C (6 μg) on the mRNA levels of LKB1, AMPKα1, and AMPKα2 and NPY, and FAS in chickens exposed to DEX or saline.

	CON		DEX			*P*-value		
Items	CON	CC	CON	CC	SEM	DEX	CC	DEX*CC
LKB1	1b	1.05ab	1.18a	1.13a	0.04	0.0062	0.9539	0.3101
AMPKα1	1ab	1.18a	1.06ab	0.93b	0.07	0.2182	0.7596	0.0562
AMPKα2	1b	1.21ab	1.22a	1.28a	0.07	0.0646	0.0874	0.3289
NPY	1b	1.14b	2.41a	2.42a	0.06	<0.0001	0.1508	0.1859
FAS	1a	0.74ab	1.27a	0.47b	0.15	0.0772	0.0149	0.0921

SEM = standard error of the mean (n = 8, number of replicates).
DEX, dexamethasone (2 mg/kg body weight); CC, compound C ((6 μg/2 μL).

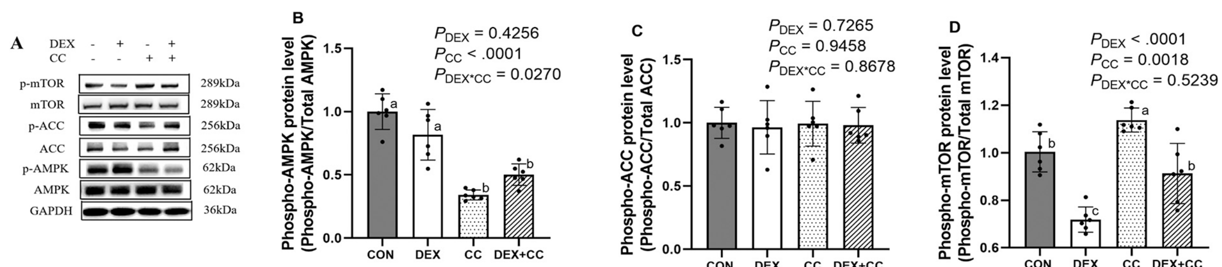

Fig. 3. Effects of central injection of compound C (6 μg) on the hypothalamic protein expression of (**B**) phospho-AMPKα$^{\text{Thr 172}}$; (**C**) phosphor-ACC$^{\text{Ser79}}$; and (**D**) phospho-mTOR$^{\text{ser2448}}$ in chickens exposed to dexamethasone (DEX) or saline. (**A**) Representative bands of hypothalamic proteins involved in the AMPK signaling pathway. Values are expressed as the mean ± SEM (n = 6). ab, means differ significantly ($P < 0.05$).

expression ($P = 0.0874$; Table 2), but exerted no effects on the expression of *LKB1*, *AMPKα1*, *NPY* ($P > 0.05$; Table 2). Compound C significantly reduced the protein level of phosphorylated AMPKα, and increased the protein level of phosphorylated m-TOR compared with the controls ($P < 0.0001$; Fig. 3B and D), but had no influence on protein levels of phosphorylated ACC.

3.5. Effect of interaction of DEX and compound c on appetite-related gene expression and AMPK signaling pathway in broilers

A trend of interaction was noted between the two treatment groups in the expression of *AMPKα1* and *FAS* genes ($0.05 < P < 0.1$; Table 2). Compound C significantly reduced the protein levels of phosphorylated AMPKα ($P < 0.0001$; Fig. 3B), and the inhibition remained in DEX-treated chicks. A significant interaction was observed between the two treatment groups ($P = 0.0270$; Fig. 3B). Injection of compound C relieved the inhibition of p-mTOR protein level induced by DEX ($P < 0.05$; Fig. 3). Moreover, the expression of the phosphorylated ACC protein was neither affected by DEX nor the compound C treatment ($P > 0.05$; Fig. 3C).

4. Discussion

4.1. The effect of DEX on appetite and central AMPK

Stress activates the HPA axis, to promote the release of GCs (primarily cortisol in humans and corticosterone in rodents), which are produced by the adrenal cortex, and induce a physiological response to the stressor (Shen et al., 2017). DEX is a synthetic GC that exhibits a high affinity for GC receptors and a delayed plasma clearance that enhances tissue exposure (Cai et al., 2009). The current study used DEX to induce a hyper-GC condition. The subcutaneous injection of DEX reduced BWG, consistent with our previous studies (Hu et al., 2019; Zhao et al., 2012). The decreased BWG indicated that the chicks were in stress condition. However, DEX treatment had no effect on feed intake. This result was consistent with our previous research (Hu et al., 2019). Many studies focused on the effect of glucocorticoids on FI in broilers, and their results were different. Song et al. (2011b) found that DEX increased feed intake. Previous studies have reported that feed intake decreased in chickens induced by DEX (Cai et al., 2009). The main reason may be that DEX reduced body weight, which leaded to the limitation of intestinal capacity, and finally concealed the promotion of DEX on feed intake.

The GCs can promote appetite by stimulating the expression of NPY in the arcuate nucleus (ARC) of the hypothalamus (Sato, 2005). The gene expression of NPY was increased by DEX treatment in this study, suggesting that DEX actually stimulated appetite. AMPK, as a key

indicator of energy state in the body, is allosterically activated by increased intracellular AMP/ATP ratio, and the phosphorylation of Thr172 by upstream kinases (Saha and Ruderman, 2003). Scerif et al. (2013) found that GC-treated WT mice display significantly increased hypothalamic AMPK activity. In the present study, DEX treatment significantly increased AMPKα2 gene expression but did not influence the protein level of total AMPKα and phosphorylation. Thus, short-term GC treatment partially activated AMPK in the hypothalamus.

4.2. The effect of compound C on appetite and central AMPK

A potent and selective small-molecule AMPK inhibitor has been identified as compound C (Zhou et al., 2001). Compound C is a common AMPK inhibitor. In poultry and mice, the icv injection of compound C leads to anorexia (Kim et al., 2004; Liu et al., 2014). Liu et al. (2014) found that intraventricular injection of 6 μg compound C remarkably reduces feed intake at 1.5–2 h after injection. In the current study, the icv injection of compound C under normal conditions reduced feed intake at 0.5–1.5 h. Study in mice has shown that the expression levels of NPY and AgRP are affected by blocking AMPK signaling in the hypothalamus (Shimizu et al., 2008). However, in the present study, the blockage of AMPK had no influence on the expression of NPY. These data suggest that a reduction in feed intake caused by the central injection of compound C may be attributed to promotion of the expression of anorexigenic neuropeptide. Thus, the underlying mechanism need to be investigated further.

The AMPK signaling pathway plays an important role in the regulation of energy homeostasis and food intake. Recent studies have shown that the AMPK signaling pathway in the hypothalamus is involved in the regulation of energy balance (Xue and Kahn, 2006). Moreover, AMPK overexpression in the hypothalamus increases food intake and body weight, and its down-regulation inhibits feeding (Minokoshi et al., 2004). In mice, the icv injection of the AMPK activator (AICAR) significantly activates NPY neurons in the ARC and increases food intake (Kohno et al., 2011). Inhibiting a hypothalamic AMPK activity through icv injection of compound C prevents the hypoglycemia-induced rise of counter-regulatory hormones and recovery from hypoglycemia (Han et al., 2005). In recent years, peripheral and central hormones, such as ghrelin and insulin, physiological changes (pathological changes or obesity), and certain drugs have been shown to affect AMPK activity in the hypothalamus (Minokoshi et al., 2008; Xue and Kahn, 2006). Compound C alters enzyme phosphorylation levels (Elamin et al., 2013), but has no apparent effect on AMPK complex gene expression (Zhou et al., 2001; Uerlings et al., 2018). These findings are consistent with those of the present study. In this study, the icv injection of compound C significantly reduced the protein level of phosphorylated AMPK, suggesting that compound C can inhibit AMPK activity in this condition.

4.3. The effect of compound C on appetite and central AMPK in stressed broilers

In the present study, the icv injection of compound C reduced feed intake within 1 h in the DEX-treated group, suggesting that the inhibitory effects of compound C on appetite remained apparent in stressed chickens. It was true that the inhibition of compound C on phosphorylated AMPK protein was still effective in the presence of DEX in this study. DEX treatment increased the gene expression of AMPK α2, but unfortunately did not affect the expression of AMPK phosphorylated protein. In view of the inconsistency of gene and protein results, we can only show that compound C can inhibit the partial activation of AMPK induced by DEX.

As a downstream target of AMPK (Bolster et al., 2002; Reiter et al., 2005), mTOR has two complex forms, mTORC1 and mTORC2 (Wullschleger et al., 2006). The hypothalamic mTOR/p70S6K pathway, as a detector of hormonal and nutritional signals, has been implicated in the control of feeding and the regulation of energy balances (Cota et al., 2006). Recently, it has been shown that intracerebroventricular administration of leucine, an mTOR activator with anorexigenic activity (Morrison et al., 2007), or leptin increases hypothalamic mTOR signaling and decreases food intake (Cota et al., 2006). Wang et al. (2006) found that excessive GC levels have a negative regulatory effect on the mTOR signaling pathway in muscle cells. The GCs inhibit mTOR signaling in hypothalamic organotypic cultures (Shimizu et al., 2010), which is consistent with the present results, owing to decreased p-mTOR protein levels in DEX-treated broilers. Shimizu et al. (2010) found that the hypothalamic regulation of appetite by GCs is mediated by TOR signaling in rats. In chicks, the TOR pathway may be involved in the regulation of GCs on appetite-related genes (Liu et al., 2015). Mounting evidence has implicated AMPK in the regulation of mTOR activity (Inoki et al., 2002; Kimura et al., 2003; Shaw et al., 2004). The activation of AMPK dependent mechanisms leads to the inhibition of mTOR activity (Inoki et al., 2003). AMPK and mTOR may have overlapping and reciprocal functions (Cota et al., 2006). High-protein diet is associated with decreased AMPK and increased mTOR activity that results in a reduction in food intake and weight loss in rats (Ropelle et al., 2008). AMPK/mTOR/ULK1-Beclin1 pathway partook in ammonia gas-induced energy metabolism disorder in chicken livers (Li et al., 2021). In the current study, DEX significantly downregulated p-mTOR protein level in the hypothalami. Furthermore, compound C treatment significantly alleviated the inhibition of the mTOR induced by DEX, indicating that the central injection of compound C activated mTOR activity.

In conclusion, the activation of appetite and AMPK induced by GCs can be partially inhibited by the injection compound C into the CNS, with increasing the mTOR activity. The AMPK-mTOR signaling pathway is involved in the regulation of food intake. These findings provide support for the hypothesis that AMPK and mTOR interact in the hypothalamus to regulate feeding during stress.

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Declaration of Competing Interest

The authors declare no competing or financial interests.

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