General and Comparative Endocrinology 229 (2016) 74–83

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Chenodeoxycholic acid reduces feed intake and modulates the expression of hypothalamic neuropeptides and hepatic lipogenic genes in broiler chickens

Alissa Piekarski [a], Eddy Decuypere [b], Johan Buyse [b], Sami Dridi [a,*]

[a] Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701, United States
[b] Division of Livestock-Nutrition-Quality, KUL, 3001 Leuven, Belgium

ARTICLE INFO

Article history:
Received 16 December 2015
Revised 15 February 2016
Accepted 6 March 2016
Available online 7 March 2016

Keywords:
CDCA
Feed intake
Hypothalamic neuropeptides
Lipogenesis
Adipokines

ABSTRACT

Bile acids have recently become an emerging research hot spot in mammals due to their roles as metabolic regulators and molecular signatures controlling whole-body metabolic homeostasis. Such effects are still unknown in avian (non-mammalian) species. We, therefore, undertook this study to determine the effect of chenodeoxycholic acid (CDCA) on growth performance and on the expression of hypothalamic neuropeptides and hepatic lipogenic genes in broiler chickens. Chickens fed with diet-containing 0.1% or 0.5% CDCA for two weeks exhibited a significant and a dose dependent reduction of feed intake and body weight compared to the control (standard diet). These changes were accompanied with a significant decrease in plasma glucose levels at d10 and d15 post-treatment. At molecular levels, CDCA treatment significantly up-regulated the expression of feeding-related hypothalamic neuropeptides (NPY, AgRP, ORX, CRH, Ghrl, and MC1R) and down-regulated the hypothalamic expression of SOCS3. CDCA treatment also decreased the mRNA levels of key hepatic lipogenic genes (FAS, ACCα, ME, ATPcl, and SCD-1) and their related transcription factors SREBP-1/2 and PPARα. In addition, CDCA reduced the hepatic expression of FXR and the adipokine, visfatin, and adiponectin genes compared to the control. Together, our data provide evidence that CDCA alters growth performances in broilers and modulates the expression of hypothalamic neuropeptides and hepatic lipogenic and adipocytokine genes.

1. Introduction

As nutrient signaling hormones via activating specific nuclear receptors, bile acids play pivotal roles in nutrient transport, digestion, and metabolization (Li and Chiang, 2013). Chenodeoxycholic acid (CDCA), also known as chenodesoxycholic acid, chenocholic acid, or 3α 7α-dihydroxy-5β-cholan-24-oic acid, is one of the main bile acids produced by the liver (Russell, 2003). This end-product of hepatic cholesterol catabolism can be conjugated to taurine or glycine in hepatocytes to form taurochenodeoxycholate or glycochenodeoxycholate, respectively. During fasting state, CDCA travels to the gall bladder for storage, however during digestion, it reaches the intestine and regulates the solubilization and absorption of dietary lipids, cholesterol, and fat-soluble vitamins (Claudel et al., 2005; Distrutti et al., 2015). High portions of CDCA delivered to the duodenum are absorbed back into the blood within the ileum and circulate back to the liver through the portal vein. In the liver, CDCA promotes bile flow and cholesterol secretion (Li and Chiang, 2014).

Beyond essential roles in dietary lipid absorption and cholesterol metabolism, CDCA has emerged in recent years as a signaling molecule. Indeed, it binds to farnesoid X receptor (FXR), a transcription factor that regulates several genes involved in various cellular processes, and activates several signaling pathways including mitogen-activated protein kinases (MAPKs) and G-protein coupled receptors (Nakahara et al., 2002; Pellicciari et al., 2007). FXR

Abbreviations: ACC, acetyl-CoA carboxylase; ADPN, adiponectin; Adip-R1/2, adiponectin receptor 1 and 2; AgRP, agouti-related peptide; ATPcl, ATP citrate lyase; BW, body weight; CDCA, chenodeoxycholic acid; CRH, corticotropin releasing hormone; CXR, chicken xenobiotic-sensing receptor; FAS, fatty acid synthase; FI, feed intake; FXR, farnesoid X receptor; Ghrl, ghrelin; LXR, liver X receptor; MC1R, melanocortin receptor 1; ME, malic enzyme; NPY, neuropeptide Y; ORX, orexin; ORXR, orexin receptor; PPAR, peroxisome proliferator-activated receptor; SCAP, SREBP cleavage-activating protein; SCD-1, stearoyl-CoA desaturase 1; SOCS3, suppressor of cytokine signaling 3; SREBP, sterol regulatory element-binding protein.

* Corresponding author at: Center of Excellence for Poultry Science, University of Arkansas, 1260 W. Maple Street, Fayetteville, AR 72701, United States.
E-mail address: dridi@uark.edu (S. Dridi).

http://dx.doi.org/10.1016/j.ygcen.2016.03.007
0016-6480/

deficiency in mice resulted in increased hepatic bile acid levels, disruption of lipid metabolism, and caused hepatic steatosis, inflammation, and fibrosis (Kim et al., 2007; Sinal et al., 2000). FXR-mediated short heterodimer partner (SHP) expression has been shown to alter hepatic fatty acid and triglyceride biosynthesis and very-low-density lipoprotein (VLDL) production in mammals (Watanabe et al., 2004). Furthermore, CDCA has been reported to regulate energy homeostasis in mammals with inhibition of appetite and feed intake and induction of energy expenditure (Bray and Gallagher, 1968; Watanabe et al., 2006).

Feeding behavior is regulated by peripheral and central complex mechanisms and multiple contributing factors. Peripherally, eating behavior appears to be mediated by gut peptides and peripheral hormones such as cholecystokinin, leptin, insulin, ghrelin, and adiponectin to mention a few (King, 2014). Depending on the nutritional state, these peripherally-secreted hormones cross the blood–brain barrier and interact in a complex way with feeding-related (an)orexigenic hypothalamic neuropeptides including neuropeptide Y (NPY), agouti-related peptide (AgRP), proopiomelanocortin (POMC), cocaine and amphetamine regulated transcript (CART), orexin (ORX), corticotropin-releasing hormone (CRH), and melanocortin receptors (MCRs) (King, 2014). Recently CDCA has been shown to ameliorate insulin resistance through modulation of circulating adipokines in rats (Shihabudeen et al., 2015). On the other hand, Hyogo and colleagues showed that leptin controlled the enterohepatic circulation of bile salts and regulated feed intake in mice (Hyogo et al., 2002). Studies conducted by Yoneda and colleagues showed that central NPY enhanced bile secretion via vagal muscarinic and bicarbonate-dependent pathways (Yoneda et al., 1995, 1997).

Taken together, these studies indicated that bile acids play a key and complex role not only in the regulation of lipid and cholesterol digestion and absorption, but also in the regulation of hepatic fat metabolism and energy homeostasis. The bulk of these data originated from mammalian studies, however there is a paucity of information in avian (non-mammalian) species. Therefore, the present study aimed to determine the effect of CDCA on feed intake and on the expression of (an)orexigenic hypothalamic neuropeptides and hepatic lipogenic genes in broiler chickens.

2. Materials and methods

2.1. Animals

Experiments were conducted in accordance with the European Communities Council Directive (86/609/EEC) on the care and use of laboratory animals, and were approved by the Institutional Ethical Committee of KU Leuven.

2.2. Experimental design

Three week-old male Cobb 500 broiler chickens ($n = 30$) were received from Avibel (Halle-Zoersel, Belgium) and maintained in individual cages under controlled environmental temperature (22 °C ± 2 °C) and provided with individual feeders and drinking nipples. Food (12.6 MJ kg^{-1} ME, 21.4% protein) and water were provided *ad libitum*. After 5 days of adaptation, birds were divided into three homogenous body weight-matched groups ($n = 10$) and received three diets: control diet and diets supplemented with 0.1% or 0.5% chenodeoxycholic acid (CDCA, Sigma Aldrich, Bornem, Belgium) for two additional weeks. Feed intake and body weight were recorded daily at 10:00 am. Blood samples were drawn from the wing vein, using a 1 mL syringe and 27 gauge needle at day 1 (just before the experiment start) and every 5 days until the end of the experiment and collected in tubes with heparin (Sigma, Bornem, Belgium). After centrifugation (1500*g*, 10 min, 4 °C), plasma was collected and stored at −20 °C for later analyses of circulating metabolites and hormones. At the end of the experiment, liver and hypothalamus tissues were taken, snap frozen in liquid nitrogen and then stored at −80 °C for later RNA isolation. The whole brain samples were submerged in 2-methylbutane (Sigma, Bornem, Belgium) in dry ice for 1 min. This procedure preserves the brain structure and provides firmness necessary to make precise cuts for hypothalamus extraction. The hypothalamic dissection was based on the stereotaxic atlas of the brain of the chick (Kuenzel and Masson, 1988). Brain samples were placed on a cold metal plate with the ventral side of the brain exposed for dissection. Hypothalamus from each brain sample was dissected with an anterior cut at septopallio-mesencephalic tract (TSM) and a posterior cut at third oculomotor nerve (NIII). Laterally, 2 mm from the midline, two cuts were performed on either side. Dorsally, a 5 mm cut from the base of the brain was performed to have the whole hypothalamus block.

2.3. Plasma metabolites and hormone determination

As previously described (Dridi et al., 2006), commercial colorimetric diagnostic kits were used to measure plasma glucose (IL test kit, no. 182508-00), triglycerides (IL test kit, no. 181610-60), non-esterified fatty acid (NEFA, Wako Chemicals, Neuss, Germany), and uric acid (IL test kit, no. 181685-00) with an automated spectrophotometer (Monarch Chemistry Systems, Instrumentation Laboratories, Zaventem, Belgium). Circulating T3 levels were measured by RIA, as previously described (Dridi et al., 2006). The intra-assay coefficients of variation were 6.3%.

2.4. RNA isolation, reverse transcription, and real-time quantitative PCR

Total RNA was extracted from chicken liver and hypothalamus tissues by Trizol reagent (Invitrogen, Merelbeke, Belgium) according to manufacturer's recommendations, RQ1 RNAse-free DNAse treated (Promega, Leiden, Belgium) and reverse transcribed as previously described (Dridi et al., 2006). Briefly, after assessment of integrity and quality using 1% agarose gel electrophoresis, RNA (1 μg) was reverse transcribed using 10 units of AMV reverse transcriptase, 40 units of recombinant RNasin ribonuclease inhibitor, 1 mM dNTP mixture, and 0.5 μg of random hexamer primers. The reagents were purchased from Promega (Lieden, Belgium). The RT reaction was performed at 42 °C for 45 min followed by an incubation at 80 °C for 3 min.

The RT products were amplified by real-time quantitative qPCR (Applied Biosystems 7500 Real-Time PCR system) with Power SYBER green Master Mix (Life Technologies, Doornveld, Belgium). Oligonucleotide primers used for chicken neuropeptide Y (NPY), agouti-related peptide (AgRP), orexin (ORX), orexin receptor 1 (ORXR1), corticotropin releasing hormone (CRH), ghrelin (Ghrl), melanocortin receptor 1, 4, and 5 (MC1R, MC4R, and MC5R), suppressor of cytokine signaling 3 (SOCS3), adiponectin receptor 1 and 2 (Adip-R1/2) and 18S (reference gene) were previously described (Sintubin et al., 2014), acetyl-CoA carboxylase alpha (ACCα), fatty acid synthase (FAS), malic enzyme (ME), ATP citrate lyase (ATPcl), stearoyl-CoA desaturase 1 (SCD-1), sterol regulatory element binding protein 1 and 2 (SREBP-1/2), peroxisome proliferator-activated receptor alpha and gamma (PPARα/γ) and adiponectin (ADPN) (Nguyen et al., 2015). Primers used for chicken SREBP cleavage-activating protein (SCAP), liver X receptor (LXR), chicken xenobitic-sensing orphan receptor (CXR), farnesoid X receptor alpha (FXRα), visfatin, and PPARβ are summarized in Table 1. The qPCR cycling conditions were 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of a two-step amplification