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Effects of chronic dexamethasone exposure on bile acid metabolism and cecal epithelia function in goats

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ABSTRACT

Bile acids (BAs) are synthesized in the liver via the oxidation of cholesterol and further metabolized by microbiota in the gut, where they simultaneously impact gut function. In the present study, 10 goats were randomly divided into 2 groups; 1 group was injected with dexamethasone (Dex; 0.2 mg/kg), and the other group was injected with saline as the control (Con) for 21 d. Expression levels of key genes and proteins in the liver and gut mucosa were analyzed and compared to investigate the impact of chronic stress on BA metabolism and related functions in ruminants. The results revealed that Dex decreased plasma total BAs (TBAs) concentration ($P < 0.05$) but increased TBA concentration in the cecal digesta ($P < 0.05$). Total cholesterol in the liver decreased moderately in response to Dex. The protein expression of cytochrome P450 family 7 subfamily A member 1 and cytochrome P450 family 27 subfamily A member 1, 2 enzymes that control BA synthesis in the liver, remained unchanged by Dex administration ($P > 0.05$). The expression of several genes in the cecal mucosa encoding epithelial tight junction proteins, including *occludin* ($P < 0.05$), *tight junction protein 1* ($P < 0.01$), and *claudin 1* ($P < 0.05$), increased significantly in response to Dex, and expression of *defensin beta 1*, which can strengthen the innate immune system, was also upregulated ($P < 0.05$). In addition, BAs increased the expression of the *Solute Carrier family 9 member A 2* ($P < 0.01$) that encodes a sodium hydrogen exchanger. These results suggest that the Dex-induced disruption of BA homeostasis might be mediated through a liver-independent pathway in goats, and the Dex-induced accumulation of TBAs in the cecal digesta may improve volatile fatty acid transportation and mucosal defense.

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is activated under stress conditions and consequently secretes cortisol into the circulation [1]. Dexamethasone (Dex) has been used experimentally to simulate stress situations in nonruminant and ruminant animals [2]. Our previous studies showed that subacute ruminal acidosis (SARA) induced by feeding a high-concentration diet to lactating dairy goats activates the HPA axis, with concomitant higher levels of plasma cortisol and disorders of lipid and glucose metabolism [3]. Activation of the HPA axis can also increase leakiness in the gut through a mast cell–dependent mechanism [4]. Animals with SARA show a considerably altered microbiota richness and composition in the rumen and hindgut [5]. Saturated free fatty acids are the predominant lipid material that leaves the rumen. However, the fatty acids must be released from digesta before they can be absorbed. Ruminants have evolved a number of key features for efficient absorption of fatty acids under the prevailing conditions, including bile salt metabolism, compared with nonruminants [6]. It is well established that disorders of the gut microbial flora in obese and diabetic patients experiencing chronic metabolic stress are at least partially mediated by bile acids (BAs) [7,8]. However, to the best of our knowledge, studies on the direct effect of stress hormones on BA metabolism in ruminants have not been reported.

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Bile acids are synthesized via the oxidation of cholesterol in hepatocytes, and BA synthesis is the major pathway for hepatic cholesterol catabolism. Bile acids are continuously stored in the gallbladder, secreted into the duodenum during the digestive period, and reabsorbed from the ileum through the enterohepatic circulation [9]. In the liver, cytochrome P450 family 7 subfamily A member 1 (CYP7A1) initiates the classical pathway of BA synthesis by catalyzing cholesterol, whereas cytochrome P450 family 27 subfamily A member 1 (CYP27A1) mainly participates in an alternative pathway for BA biosynthesis in the liver [10]. It is well known that BAs regulate various metabolic pathways by activating nuclear receptors, such as nuclear receptor subfamily 1, group H, member 4 (NR1H4) [9]. Activated NR1H4 alleviates the proinflammatory response by inhibiting cytokine production, thus attenuating disease severity and enhancing mucosal defense and intestinal barrier function [11–13].

Approximately 95% of biliary-secreted BAs are reabsorbed from the intestine, predominantly in the distal ileum. Bile acid in the gut lumen can profoundly alter mucosal functions, such as immune activation, inflammation, intestinal permeability, and intestinal dysmotility [14]. Unfortunately, no information has been reported about the effects of BAs on gut epithelial function in ruminants. Accordingly, the aim of this study was to investigate whether chronic exposure to Dex, a synthetic stress-inducing hormone, would affect BA metabolism in the liver and cecum and to document concurrent changes in epithelial barrier function in the cecum of goats. Our results will help gain better insight into BA metabolism in farm ruminants under pathophysiological conditions produced by chronic exposure to stressful situations or Dex.

2. Materials and methods

2.1. Ethics

The Institutional Animal Care and Use Committee of Nanjing Agricultural University approved all animal procedures. The “Guidelines on Ethical Treatment of Experimental Animals” (2006) No. 398 set by the Ministry of Science and Technology, China, and the Regulation regarding the Management and Treatment of Experimental Animals’ (2008) No. 45 set by the Jiangsu Provincial People’s Government were strictly followed during the slaughter and sampling process.

2.2. Animals and experimental procedures

Ten 6-month-old Chinese Boer goats (body weight 25 ± 1.0 kg) were raised in individual pens with free access to water and fed twice daily (08:00 h and 18:00 h). The diet contained 43% corn, 5% wheat bran, 17% mixed concentration, and 35% forage. The animals were acclimatized to all procedures of sampling and exposure before treatments. The Dex dose was chosen based on a previous study by Emikpe et al [15]. Ten goats were randomly assigned to 2 groups: 1 group (n = 5) was injected intramuscularly with saline as the control (Con group), and the other group (n = 5) was injected intramuscularly with 0.2 mg/kg Dex at 7:30 before the morning feeding for 21 d.

2.3. Sample collection

Blood samples were collected from the jugular vein of the goats on days 0, 7, 14, and 21 of the experiment, shortly before the injection and morning feeding, using heparin-containing vacuum tubes. The blood samples were centrifuged at 1,000 × *g* for 15 min at 4°C, and the plasma was extracted in Eppendorf tubes and stored at −20°C. Feces samples were collected after the morning feeding on days 0, 7, 14 and 21 d and stored at −20°C. At the end of experiment and after an overnight fast, all goats were weighed and killed by injections of xylazine (0.5 mg [kg body mass]$^{-1}$; Xylosol; Ogris Pharma, Wels, Austria) and pentobarbital (50 mg [kg body mass]$^{-1}$; Release; WDT, Garbsen, Germany). Immediately after death, the liver and cecal mucosal tissues were removed carefully. Digesta from the proximal cecum was aseptically collected and kept on ice, then centrifuged at 3,000 × *g* for 15 min, and the supernatants were stored at −20°C until they were analyzed. The cecum was washed in PBS and then laid flat on an inverted culture dish. The back of a double-sided blade was used to bluntly separate the mucosal layer of the cecum. After blunt separation of the cecum, the tissues were washed immediately 3 times in ice-cold PBS. The tissue and liver samples were frozen immediately in liquid nitrogen until used later for further analysis.

2.4. Bile acid analysis

The method used to analyze the BAs was described previously [16]. About 100 mg of the liver was used with 500 μL 95% ethyl alcohol (EtOH) for extraction, and about 200 mg feces (dried overnight) was mixed with 1 mL 95% EtOH. First, the tissue was homogenized and extracted by incubating it at 60°C in a water bath overnight. Then, the tubes were centrifuged at 8,000 rpm for 10 min, and the supernatant was collected in a 2 mL tube. The pellet was resuspended in 80% EtOH and extracted again by incubating it at 60°C in a water bath overnight. The tubes were centrifuged at 8,000 rpm for 10 min, and the supernatants were combined. The pellets were resuspended in 2:1 (v:v) chloroform: methyl alcohol and extracted overnight at room temperature. The tubes were centrifuged again at 8,000 rpm for 10 min, and the supernatants were combined. The total extract was centrifuged at 8,000 rpm for 10 min at room temperature to precipitate debris. Plasma was separated from jugular-collected blood, and cecal supernatant samples were used directly. Total BAs (TBAs) were determined with a BA assay kit (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China), strictly following the manufacturer’s instructions.

2.5. Cholesterol analysis

The cholesterol level in liver was measured with cholesterol kits (Boquan Co. Ltd, Nanjing, China), strictly following the manufacturer’s instructions. The level of the total cholesterol including free and esterified cholesterols in the liver was measured, and the changes were compared between Con and Dex-treated groups.