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Chronic glucocorticoid treatment induced circadian clock disorder leads to lipid metabolism and gut microbiota alterations in rats

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ABSTRACT

Aim: Glucocorticoids (GCs), steroid hormones synthetized by the adrenal gland, are regulated by circadian cycles, and dysregulation of GC signaling can lead to the development of metabolic syndrome. The effects and potential mechanism of GCs in physiology were investigated in the present study.

Main methods: Male Wistar rats were orally administered dexamethasone sodium phosphate (DEX, 0.01 and 0.05 mg/kg body weight per day) for 7 weeks.

Key finding: DEX treatment attenuated body weight gain and reduced food intake, whereas it induced the accumulation of fat. Administration of DEX induced dysregulation of the expression of lipogenic genes in both fat and liver. Moreover, the mRNA levels of genes related to mitochondrial biogenesis and function were significantly downregulated in the liver and fat of DEX-treated rats. Furthermore, DEX treatment caused a significant reduction in the richness and diversity of the microbiota in the colon, as assessed using high-throughput sequencing of the 16s rRNA gene V3–V4 region, an increase in inflammatory cell infiltration, and a decrease in mucus secretion in the colon. Additionally, DEX administration induced phase shift or loss of circadian rhythmicity of clock-related genes in peripheral tissues. These results were associated with higher serum corticosterone levels and upregulation of GC receptor (GR) expression in peripheral tissues.

Significance: Our findings indicate that long-term administration of GC caused lipid accumulation, changes in the structure of the intestinal flora, and reduced colonic mucus secretion in vivo. The mechanism of these physiological changes may involve a circadian rhythm disorder and dysregulation of GR expression.

1. Introduction

In mammals, the master clock, which is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus [53], is synchronized with the sub-clocks in peripheral tissues by various regulatory mechanisms [1]. These include a series of molecular feedback mechanisms that promotes cyclical oscillations of circadian rhythm at the transcriptional and translation levels. Many of these cellular oscillators that can interact with the SCN are present in endocrine glands and in hormone-responsive target organs, and they appear to gate appropriate hormonal secretion in a time-controlled way (such as melatonin, insulin, thyroxine, and glucocorticoids) and in response to environmental stimuli [2].

Glucocorticoids (GCs) not only maintain the normal function of the peripheral clocks but are also related to a variety of physiological functions [3]. Patients with high corticosteroid levels may suffer from a variety of diseases, including obesity, fatty liver, dyslipidemia, and glucose intolerance [4]. GCs can stimulate the de novo synthesis of lipids, which includes almost all the circulatory pathways related to the anabolism and catabolism of triglycerides, and promotes an increase in free fatty acids (FFAs) in the circulation [5]. However, the detailed mechanism of how GC regulates lipid metabolism is still unknown because of the complexity of the whole pathway of lipid metabolism.

On the other hand, the microbiota plays vital roles in host metabolism, the immune system, and other physiological functions, such as obesity, diabetes and cancer [6,7]. Thus, a delicate balance between the host and the intestinal flora determines the state of symbiosis or disease [8,54]. Physiological and pharmacological levels of GCs can effectively promote the synthesis and secretion of mucins, which protect the intestinal mucosa from chemical substances, enzymes, and microbial damage [9,10]. Recent studies have shown that some part of the signaling pathways related to GCs is due to their effects on intestinal microbes [11]. Thus, GCs may regulate some physiological effects through the intestinal flora or the expression level of mucins. In addition, recent

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study have found that ablation of host molecular clock components caused aberrant microbiota diurnal fluctuations and dysbiosis [12]. Therefore, the GC-mediated gut microbiota alterations might be caused by the dysregulation of circadian rhythm of the host. The interactions among lipid metabolism, intestinal flora and circadian rhythm after the administration of GC might be intricate.

To clarify how GC regulates the lipid metabolism and gut microbiota, and the potential involvement of circadian clock in these physiological alterations, male Wistar rats were chronically administered with GCs to investigate the effects and potential mechanism of action of GCs on different tissues. The changes in biological rhythm, glycolipid metabolism, and intestinal flora were evaluated.

2. Materials and methods

2.1. Materials

Dexamethasone sodium phosphate (DEX; cat. number C22H28FNa2O8P, white powder form, CAS NO: 2392-39-4, purity: ≥ 98%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The DEX with high GC pharmacological potency was dissolved in sterile water for use in experiments.

2.2. Animals and experimental design

Seven-week-old male Wistar rats (200–220 g) were purchased from the China National Laboratory Animal Resource Center (Shanghai, China). They were fed and kept in a temperature-controlled (22 ± 1 °C) and light-controlled (200 lx, 12/12-h light/dark cycle) room, which represented the periodic day–night environment. The natural time was converted into *zeitgeber* time (ZT). The light went on at 8 o'clock in the morning (light on, ZT0) and off at 8 o'clock in the evening (light off, ZT12). All animals were allowed free access to standard rodent chow and sterile water. All rats were kept for 7 days to adapt to this environment before experiments were initiated.

In total, 90 male Wistar rats were divided randomly into three experimental groups (n = 30 each): a control group received sterile water daily at ZT0 (Con); the other two groups were gavaged with DEX (0.01 mg/kg body weight and 0.05 mg/kg body weight per day, respectively) at ZT0 and were named Dex0.01 and Dex0.05, respectively. The daily volume of water was 0.2 mL in the control group, which was equivalent to the dosage of the other two DEX-treated groups. Body weight and food consumption were assessed every week, and the feces were also collected and cryopreserved.

After 7 weeks of treatment, all rats were fasted for 12 h and euthanized after deep anesthesia by intraperitoneal injection of sodium pentobarbital (45 mg/kg body weight). All three groups of rats were sacrificed at 4-h intervals of a daily cycle starting at ZT0 (n = 5 per group at each time point). During the dark phase, dissection was carried out under dim red light. SCN, liver, epididymal fat, colon, cecum, and cecal contents were removed quickly. Liver and epididymal fat were weighed, and biopsy samples (liver, colon) were collected and fixed directly in 4% paraformaldehyde for histological analyses. The remaining isolated tissues were snap-frozen in liquid nitrogen and stored at − 80 °C for RNA extraction. Blood was also collected and then centrifuged (6000 *g*, 5 min, 4 °C), and serum was stored at − 80 °C. The experimental design is summarized in Fig. S1.

All experiments were performed according to institutional guidelines. The study was approved by the Research Committee of Zhejiang University of Technology.

2.3. Glucose tolerance test

In the last week of the experiment, rats were subjected to glucose tolerance tests [13]. Rats were fasted for 16 h before the test, and the glucose concentration in fasting blood was measured by tail blood sampling using a portable blood glucose meter (Optium Xceed). Rats were injected intraperitoneally with 2 g/kg body weight of D-glucose, and blood glucose concentrations were measured and recorded at 15, 30, 60, 90, and 120 min after injection.

2.4. Hormone and biochemical measurements

Hormones and biochemical measurements were performed as described previously [46]. Briefly, serum levels of corticosterone (CORT) were determined using kits purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (sensitivity: ≥ 1.0 ng/mL; CV: ≤ 15%; Shanghai, China). Serum levels of triglyceride (TG, sensitivity: ≥ 9.0 μmol/mL; CV: ≤ 8.0%) and free fatty acid (FFA, sensitivity: ≥ 9.1 μmol/mL; CV: ≤ 1.5%) were determined according to the manufacturer's protocol using commercial kits purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). Interferon-γ (IFN-γ, sensitivity: ≥ 1.03 pg/mL; CV: 4.1%–6.0%) and interleukin-2 (IL-2, sensitivity: ≥ 1.38 pg/mL; CV: 3.5%–6.8%) were determined according to the manufacturer's protocol using enzyme linked immunosorbent assay kit purchased from Lianke Biotechnology (Hangzhou, China).

2.5. Histopathological analysis

The collected liver and colon tissues were fixed in 4% paraformaldehyde, dehydrated, paraffin wax-embedded, and sectioned (at 10 μm) ([14]). The sections were deparaffinized and stained with Oil Red O and hematoxylin and eosin (H&E) or Alcian blue-periodic acid Schiff (AB-PAS), examined visually under a microscope (Olympus, Japan), and analyzed with an image analyzer (Image pro-Plus 6.0).

2.6. RT-qPCR analysis

Total RNA was isolated from the SCN, liver, epididymal fat, colon, and cecum using the TRIzol reagent (Takara Biochemicals, Dalian, China). cDNA was synthesized using a reverse transcriptase kit (Toyobo, Osaka, Japan). Real-time quantitative polymerase chain reaction (RT-qPCR) was performed on an Eppendorf MasterCycler ep RealPlex4 (Wesseling-Berzdorf, Germany) using the SYBR ExScript PCR Kit (Toyobo) as described previously [15]. The RT-qPCR primer sequences used for this experiment are listed in Table S1. *Glyceraldehyde 3-phosphate dehydrogenase* (*Gapdh*) was used as a housekeeping gene for normalization.

2.7. Total DNA extraction from cecal contents and bacterial 16S rRNA sequencing

Cecal contents and bacterial 16S rRNA sequencing was performed according to methods as described [16]. Microbial genomic DNA was extracted from cecal contents following the manufacturer's instructions using a commercial magnetic bead DNA isolation kit provided by Hangzhou Foreal Nanotechnology (Hangzhou, China). The V3–V4 region of the 16S rRNA gene was PCR-amplified from microbial gDNA using the following primers: forward primer: 5′-ACTCCTACGGGAGGC AGCAG-3′ and reverse primer: 5′-GGACTACHVGGGTWTCTAAT-3′. The composition of the gut microbiome was determined by dual-indexing amplification and sequencing on the Illumina MiSeq platform followed by QIIME (vision 1.6.0) bioinformatic analysis. Part of the microbial gDNA was amplified via RT-qPCR using bacterial phyla-specific primers (Table S1) under the following conditions: 50 °C for 2 min, 95 °C for 10 min, 95 °C for 15 s, 56 °C for 30 s, 72 °C for 1 min repeated for 40 cycles, and 72 °C for 10 min.

2.8. Statistical analyses

All experimental data were analyzed using Statview 5.0 software, and results are expressed as means ± standard errors (mean ± SEM).

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