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The local corticotropin-releasing hormone receptor 2 signalling pathway partly mediates hypoxia-induced increases in lipolysis via the cAMP–protein kinase A signalling pathway in white adipose tissue

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ABSTRACT

Our objective was to investigate the mechanisms by which the endogenous CRHR2 in white adipose tissue (WAT) regulates metabolic activities associated with lipogenesis and lipolysis under continuous exposure to hypoxia. We found that hypobaric hypoxia at a simulated altitude of 5000 m significantly reduced the body weight, food intake, and WAT mass of rats. Hypoxia also accelerated lipolysis and suppressed lipogenesis in WAT. Pretreatment with astressin 2B, a selective CRHR2 antagonist, partly but significantly attenuated the hypoxia-induced reductions in body weight and WAT mass by blocking the cAMP–protein kinase A (PKA)–hormone-sensitive lipase (HSL)/perilipin signalling pathway. Astressin 2B treatment failed to attenuate hypoxia induced lipogenic inhibition. In conclusion, activation of endogenous WAT Ucn2/3 autocrine/paracrine pathway was involved in hypoxia induced lipolysis via CRFR2 – cAMP–PKA signalling pathway. This study provides the novel understanding of local CRHR2 signaling pathway playing important role in WAT loss and lipid metabolism under hypoxia.

1. Introduction

People who ascend to high altitude for recreational activities, such as climbing, trekking, or skiing, or for work, often experience weight loss during their stay at high altitude (Benso et al., 2007; Rose et al., 1988). Accumulated evidence suggests that high altitude (HA) hypoxia-induced weight loss is predominantly due to a decrease in fat mass (Armellini et al., 1997; Boyer and Blume, 1984). White adipose tissue (WAT) was considered as the largest reservoir of fuel and played a crucial role in the regulation of whole-body energy homeostasis. Therefore, elucidating the cellular and molecular mechanisms that underlie the reduction in fat mass during hypoxic conditions is of great importance in preventing fat mass and maintaining energy homeostasis

WAT is a major endocrine organ that produces several hormones, known as adipokines, which influence thermogenic activities, food intake, and glucose homeostasis (Rosen and Spiegelman, 2006; Trayhurn and Wood, 2004). It is also well established that adipose tissue is sensitive to the circulating oxygen concentration. Exposure to hypoxia and a reduction in PaO_2 may disturb WAT activity and induce metabolism (Famulla et al., 2012; Trayhurn et al., 2008; Wang et al., 2008). Hashimoto et al. (2013) reported that 5% O_2 significantly reduced triglyceride

Abbreviations: CRHR2, corticotrophin-releasing hormone receptor type 2; Ucn2, urocortin 2; Ucn3, urocortin 3; WAT, white adipose tissue; BAT, brown adipose tissue; PKA, protein kinase A; PDE3B, phosphodiesterase 3B; ATGL, adipose triglyceride lipase; HSL, hormone sensitive lipase; peri, perilipin; PPAR-α, peroxisome proliferator-activated receptor-α; CPT-1, carnitine palmitoyltransferase-1; ACO, acyl-CoA oxidase; ACC1, acetyl CoA carboxylase 1; PPAR-γ, peroxisome proliferator-activated receptor-γ; SREBP-1c, sterol regulatory element-binding protein 1c; FAS, fatty acid synthase; SCD-1, stearoyl CoA desaturase; UCP-1, uncoupling protein-1; FFA, free fatty acid; TG, triglyceride.

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content and the size of lipid droplet by decreasing glucose uptake and the expression of lipogenic protein, and increases basal lipolysis in differentiated 3T3-L1 adipocytes. In a mouse model of chronic hypoxia, both AT mass and adipocyte size markedly reduced. Concomitantly, chronic hypoxia also induced significant alterations in AT metabolic gene expression and inflammation profile (van den Borst et al., 2013). In addition, training under hypoxia resulted in a greater reduction in body fat mass compared to exercise under normoxia (Wiesner et al., 2010).

The corticotropin-releasing hormone (CRH) family was composed of receptors encoded by two different genes (CRHR1 and CRHR2) and several related peptides (CRH, urocortin, urocortin 2, and urocortin 3). Both Ucn2 (Reyes et al., 2001) and Ucn3 (Lewis et al., 2001) selectively bind to corticotropin-releasing hormone receptor 2 (CRHR2) but weakly bind to CRHR1, which were considered as endogenous ligands for CRFR2. Extensive evidence showed that CRHR2 played critical role in the central homeostatic mechanisms that control food intake and energy balance (Bakshi et al., 2007; Chen et al., 2012; Yakabi et al., 2011).

Recently, more and more researchers began to focus on the biological effects of local CRH family in peripheral tissues and organs. It was found that CRH receptors and peptides were highly expressed in the skin tissue (Rassouli et al., 2011), skeletal muscle tissue and adrenal gland (Tsatsanis et al., 2007). Further studies suggested that endogenous CRH family were involved in the regulation of physiological functions and metabolism via autocrine/paracrine manner. For instance, Chen et al. (2006) found that Ucn 2 functioned as a local negative regulator of glucose uptake in skeletal muscle, which might provide benefits in insulin-resistant states such as type 2 diabetes. More recently, endogenous cardiac Ucn2/CRHR2 was found to mitigate against ischemia/reperfusion injury in autocrine/paracrine manner by activating AMPK signaling pathway (Li et al., 2013).

Although our knowledge of the functional roles of the CRH system in maintenance of energy homeostasis and metabolic pathways is increasing rapidly, little is known regarding the putative role of local CRH system in WAT. Whether local CRHR2 might play similar roles in WAT is not known, we therefore decided to test the hypothesis that endogenous WAT CRHR2 autocrine/paracrine pathway may also directly modulate the hypoxia induced lipid metabolism on the basis of the following: (1) WAT is sensitive to oxygen concentration and hypoxia could induce obvious alteration in lipid metabolism; (2) CRH receptors and its ligands were also expressed in WAT, with the most abundant receptor being CRHR2 (Seres et al., 2004); (3) CRHR2 agonists may protect muscle from excessive intracellular lipid storage and thus from lipotoxicity-induced insulin resistance (Solinas et al., 2006); (4) CRHR2 in the ventromedial nucleus of hypothalamus (VMH) could regulate peripheral lipid lipolysis metabolism in WAT (Chao et al., 2012); (5) Ucn3 transgenic mice (Ucn3^{+}) exhibit lean body composition and protection against high fat diet-induced obesity and fat deposits (Jamieson et al., 2011).

To address this issue, we investigated the effects of CRHR2 in hypoxia-induced fat loss using a CRHR2 antagonist, astressin 2B. The main objective of this study was to clarify the mechanism by which the CRHR2 signalling pathway regulates peripheral adipose tissue metabolism under continuous exposure to hypoxic conditions, equivalent to an altitude of 5000 m.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (150–200 g) were purchased from Weitong lihua Laboratory Animal Limited Company and Laboratory Animal Center of Zhejiang Province, China (Certification No. SCXK2008-0033). The rats were housed at room temperature (20–22 °C) and in a 12–12 h light–dark cycle with free access to food and water and adapted to the condition above for 1 week before experiment. All experiments were conducted in strict accordance with the laboratory animal care guidelines published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). All protocols concerning animal use were approved by the Institutional Animal Care and Use Committee of Institute of Basic Medical Sciences, Peking Union Medical College and School of Medicine, Zhejiang University.

2.2. Hypoxic challenge

Hypoxia group rats were placed in a hypobaric chamber (Guizhou Fenglei Air Ordnance Co., Ltd.) and subjected to hypoxia mimicking an altitude of 5000 m (54.02 kPa, 10.8% O_2) for 1, 2, 5, and 10 d. The chamber was opened daily for 30 min to clean and replenish food and water. Body weights and food intake were monitored daily. All the rats were sacrificed by decapitation and Plasma was obtained by centrifugation and stored at −80 °C. The perirenal fat pads were collected and weighed immediately, frozen in liquid nitrogen, and stored at −80 °C. Brown adipose tissue (BAT) were isolated and and stored at −80 °C.

2.3. CRHR2 antagonist treatment

The CRHR2 antagonist astressin 2B was commercially purchased (Sigma–Aldrich) and rats were given at 30 μg/kg body weight. Astressin 2B was double-distilled water (pH 7.0) by intra-peritoneal injection before hypoxic challenge.

2.4. Histology, staining, and immunostaining

WAT was fixed in 4% paraformaldehyde overnight and stained with hematoxylin and eosin. The WAT cell volumes were analyzed as described previously (Gerin et al., 2005). For immunostaining, sections were deparaffinized and rehydrated in xylene and ethanol. WAT sections were incubated in PBS containing 3% H_2O_2 for 15 min to block endogenous peroxidase activity, and then soaked immersed in a sodium citrate solution for antigen retrieval using pressure cooking method. After blocking with 5% normal bovine serum, the sections were incubated overnight at 4 °C with the primary antibodies CRHR2 (Abcam). After rinsing with PBS, the sections were incubated with the biotinylated secondary antibody, rabbit anti-rabbit IgG (Zhongshan, Beijing, China), at room temperature for 30 min. The streptavidin–peroxidase activity was visualized with diaminobenzidine method. Negative controls were incubated with preimmune serum instead of primary antibodies.

2.5. Reverse-transcription PCR and quantitative real-time PCR

Total RNA was prepared from frozen tissues with TRIZOL (Invitrogen) reagent and the cDNA was synthesized using TransScript TM First-Strand cDNA Synthesis Super-Mix (TransGenBiotech, AT301). The program was run on a S1000 Thermal Cycler. Quantitative real-time PCR was performed using the SYBR®Pre-mix Ex TaqTMkit (Takara, RR420A) and analyzed in a step-one plus RT-PCR system (Life Science, Applied Biosystems). The primer sequences are listed in Supp. 1.

2.6. Western blotting and densitometry analyses

Homogenized rat WAT were lysed in 200 μl RIPA lysis buffer (Beyotime, P0013B) with 1% phenylmethyl sulfonylfluoride and 4% complete protease inhibitor cocktail mix (Roche, Mannheim,