METABOLISM CLINICAL AND EXPERIMENTAL 64 (2015) 814–825

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Chronic angiotensin AT2R activation prevents high-fat diet-induced adiposity and obesity in female mice independent of estrogen

*Sourashish Nag, Mohammad Azhar Khan, Preethi Samuel, Quaisar Ali, Tahir Hussain**

Department of Pharmacological & Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX 77204

ARTICLE INFO

Article history:
Received 21 August 2014
Accepted 5 January 2015

Keywords:
Obesity
Adiposity
AT2R
High-fat diet
Estrogen

ABSTRACT

Objective. Obesity is a known risk factor for various metabolic disorders and cardiovascular diseases. Recently we demonstrated that female angiotensin AT2 receptor (AT2R) knockout mice on high-fat diet (HFD) had higher body weight and adiposity with a parallel reduction in estrogen (17,β-estradiol/E2). The present study investigated whether the anti-adiposity effects of the AT2R are estrogen-dependent in female mice.

Methods. Female C57BL/6 ovary-intact (Ovi) mice were treated with an AT2R agonist (C21, 0.3 mg/kg, daily i.p.). Ovariectomized (Ovx) mice, supplemented with E2 (5 μg/day, pellets implanted subcutaneously), were treated with an AT2R agonist (C21, 0.3 mg/kg, daily i.p.) or vehicle. After 4-days of pre-treatment with C21, Ovi and Ovx mice were placed on either normal diet (ND) or HFD while the C21 treatment continued for the next 10 days. For a long-term study, Ovi mice were placed on HFD and treated with C21 for 12 weeks.

Results. Ovi mice fed the HFD had increased parametrial white adipose tissue (pWAT) weight, plasma free fatty acid and triglycerides compared to Ovi mice on ND. Ovariectomy alone caused similar changes in these parameters which were further increased by HFD-feeding. C21 treatment attenuated these HFD-induced changes in Ovi as well as Ovx mice. HFD also, increased the liver/body-weight ratio and decreased the liver expression of the β-oxidation enzyme, carnitine palmitoyltransferase 1 (CPT1-A). C21 treatment attenuated these changes as well. The long-term C21 treatment of Ovi mice lowered the HFD-induced body weight gain, increase in pWAT weight, parametrial adipocyte size and hyperinsulinemia induced by HFD. Finally, HFD drastically reduced urinary estrogen and the beneficial metabolic changes in response to C21-treatment occurred without significantly increasing urinary estrogen.

Conclusion. We suggest that the pharmacological activation of AT2R by the agonist C21 reduces adiposity and body weight gain independent of estrogen in female mice. Improvement in fatty acid metabolism is a potential mechanism by which the AT2R exerts anti-adiposity effects.

Abbreviations: AT1R, angiotensin type 1 receptor; AT2R, angiotensin type 2 receptor; AngII, angiotensin II; CPT1A, carnitine palmitoyltransferase-1A; CVD, cardiovascular diseases; C21, compound 21; E2, 17,β-estradiol or estrogen; FA, fatty acid; FFA, free fatty acid; HFD, high-fat diet; i.p, intraperitoneal; Kcal, kilocalorie; KO, knock out; ND, normal diet; Ovi, ovary-intact; Ovx, ovariectomized; pWAT, parametrial white adipose tissue; RAS, renin angiotensin system; TAG, tri-acyl glycerol or triglyceride; UCP1, uncoupling protein-1.

* *Corresponding author at:* 521 Science and Research Building 2, College of Pharmacy, University of Houston, Houston, TX 77204. Tel.: +1 713 743 1276; fax: +1 713 743 1229.
E-mail address: thussain@central.uh.edu (T. Hussain).

http://dx.doi.org/10.1016/j.metabol.2015.01.019
0026-0495/

1. Introduction

Obesity is a major risk factor for various metabolic disorders and cardiovascular diseases (CVD) namely diabetes, hypertension, atherosclerosis, etc. A positive balance in energy intake vs. energy expenditure is believed to be a primary cause for the development and maintenance of obesity, defined as the storing of fat in adipocytes resulting in enlargement of adipocyte size and mass (adiposity). The incidence of extreme obesity is much higher in women as compared to men [1]. Also, the relative risk of CVD in obese females is higher than in obese males [1]. In addition to obesity as an independent risk factor for CVD, menopause provides an added risk factor for such diseases. Estrogen, the female sex hormone, regulates adiposity/obesity and after menopause the absence of estrogen triggers adiposity in women.

Storing excessive fat by adipocytes is a normal physiological phenomenon. In addition to storing, the adipose also mobilizes fatty acids (FA) to generate heat by activating mitochondrial uncoupling protein 1 (UCP1). The activated UCP1 causes shunting of the proton away from the ATP synthase [2]. In obesity, increased adipocyte size and free fatty acids (FFA) are associated with non-alcoholic liver injury progression [3]. In liver cells, fatty acids undergo β-oxidation providing metabolites for gluconeogenesis and other metabolic pathways [4]. The enzyme, carnitine palmitoyl transferase, is the rate-limiting step of mitochondrial fatty-acid β-oxidation pathway [5] and the activity of this enzyme is used as an index of liver fatty acid β-oxidation. During adiposity and obesity, these metabolic markers are dysregulated further affecting the metabolism ensuing into dyslipidemia and fatty liver.

The renin-angiotensin system (RAS) regulates blood pressure and impacts various aspects of CVD [6]. Recently a local RAS in adipose tissue has been implicated in the pathophysiology of metabolic syndromes and adiposity [7–9]. Angiotensin II (AngII), a major agonist peptide of the RAS, exerts its physiological effects via two receptor subtypes, namely the angiotensin type 1 receptor (AT1R) and the angiotensin type 2 receptor (AT2R). Evidence suggests that activation of the AT1R contributes to adiposity and weight gain in male animal models [10,11]. The role of AT2R in obesity remains unclear. Male AT2R knockout mice were protected from high fat diet (HFD)-induced weight gain [12,13], while the double AT2R along with apolipoprotein-E knockout (a model of atherosclerosis) mice exhibited greater adiposity in response to HFD compared with single apolipoprotein-E knockout mice [14]. Further, the pharmacological stimulation of the AT2R in male mice reduces adiposity and improves insulin resistance [15,16]. On the other hand, the significance of AT2R in female obesity is suggested by the studies showing that AT2R polymorphism is associated with increased body mass index in Japanese women [17]. Recently, we observed that female AT2R-KO mice fed an HFD had increased body weight gain and adiposity, accompanied by a decrease in urinary 17,β-estradiol (E2) [13]. Although levels of AT2R expression and estrogen levels are positively correlated [18,19], and estrogen is a known regulator of adiposity [19], it is unknown whether the decrease in estrogen levels in female AT2R-KO mice contributes to the increased adiposity observed. To address this issue in the present study, we utilized bilateral ovariectomized and ovary-intact mice to assess the role of estrogen in AT2R-mediated effects on adiposity/obesity. Pharmacological activation of AT2R was achieved by treating the mice with the orally active preferential AT2R agonist C21.

2. Methods

2.1. Animals and experimental protocols

Eight-week old ovary-intact (Ovi) and bilateral-ovariectomized (Ovx) C57BL/6 female mice were obtained from Harlan (Indianapolis, IN). Another batch of 4-week old C57BL/6 Ovi female mice were used for a long-term study, as outlined below. The surgery for ovariectomy was performed at Harlan when the mice were 5 weeks old. Following 2 weeks to recovery from the surgery, the animals were shipped to the University of Houston's animal care facility. The animals were housed with free access to food and tap water and maintained under a 12-h light/dark cycle. The experimental protocols herein were approved by the Institutional Animal Care and Use Committee of the University of Houston. These protocols were:

a) *Two week protocol*: Starting on day 1, 8-week old Ovi and Ovx mice were pre-treated with vehicle or the AT2R agonist "Compound 21" (C21) at a dose of 0.3 mg/kg, daily i.p. for 4 days (Fig. 1A). In two additional groups of Ovx mice, supplementation with 17β-estradiol (E2) (5 μg/day) was provided by implanting E2-pellets subcutaneously. These Ovx E2 supplemented mice also were treated with C21 (0.3 mg/kg, daily i.p.) or vehicle for 4 day. On day 5 the mice were continued on a normal diet (ND) or placed on an iso-Kcal high-fat diet (HFD) while the treatments with C21 and/or E2 continued for 10 more days. Each treatment group had n = 8 mice. On day 15, in a non-fasting state, animals were euthanized by cervical dislocation under isoflurane anesthesia. Plasma was collected and stored at −80 °C until further use. The livers were removed, patted dry, weighed, snap-frozen and preserved at −80 °C. Parametrial adipose tissue (pWAT) also was removed, patted dried and weighed. Part of this pWAT was preserved in buffered formalin for histological examination while the remaining was snap-frozen and stored at −80 °C.
b) *Twelve week protocol*: Four week old female C57BL/6 mice were placed on ND or HFD and simultaneously treated with C21, 0.3 mg/kg, daily i.p., or vehicle for 12 weeks (Fig. 1B). Each treatment group had n = 9 mice. At the end of 12 weeks, the mice were sacrificed. Plasma was collected and stored at −80 °C until further use. Parametrial WAT was removed, patted dry and weighed. Part of this pWAT was preserved in buffered formalin for histological examination while the remaining was snap-frozen and stored at −80 °C.