Peptides 30 (2009) 2309–2315

Contents lists available at ScienceDirect

Peptides

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

17β-Estradiol modulates local cardiac renin-angiotensin system to prevent cardiac remodeling in the DOCA-salt model of hypertension in rats

V. Shenoy [a,1], J.L. Grobe [b,1], Y. Qi [a], A.J. Ferreira [c], R.A. Fraga-Silva [c], G. Collamat [a], E. Bruce [a], M.J. Katovich [a,*]

[a] Department of Pharmacodynamics, University of Florida, Gainesville, FL 32610, USA
[b] Department of Internal Medicine, University of Iowa, Iowa City, IA 52242, USA
[c] Department of Physiology and Functional Genomics, University of Florida, Gainesville, FL 32610, USA

ARTICLE INFO

Article history:
Received 16 July 2009
Received in revised form 28 August 2009
Accepted 3 September 2009
Available online 9 September 2009

Keywords:
Estrogen replacement therapy
Cardiac remodeling
Menopause
Hypertension

ABSTRACT

Ventricular remodeling can play a detrimental role in the progression of cardiovascular diseases, leading to heart failure. The current study was designed to investigate the effects of 17β-estradiol (E2) on cardiac remodeling. Cardiac fibrosis and hypertrophy were examined in deoxycorticosterone acetate (DOCA)-salt treated rats with chronic, six-week administration of two different doses of E2. Bilaterally ovariectomized (Ovex) female Sprague–Dawley rats were randomly assigned to one of the following groups: Ovex-control; Ovex-DOCA; Ovex-DOCA + low-dose E2 (1.66 μg/day); or Ovex-DOCA + high-dose E2 (2.38 μg/day). All DOCA-treated rats were uninephrectomized and drinking water was replaced by 0.15 M NaCl solution for the remainder of the study period. DOCA-salt treatment resulted in a significant increase in blood pressure, which was not altered by estrogen replacement. Histological examinations revealed marked cardiac remodeling (both ventricular hypertrophy and interstitial fibrosis) with DOCA treatment, which was attenuated in animals receiving estrogen therapy. Western blot analysis demonstrated increased cardiac levels of angiotensin converting enzyme (ACE) with DOCA treatment, which was attenuated by E2 replacement. Furthermore, increased levels of cardiac angiotensin converting enzyme 2 (ACE2) protein were observed in animals receiving high-dose E2 replacement. These findings suggest that physiologically relevant estrogen replacement therapy has blood pressure-independent cardioprotective effects, which are possibly mediated through modulation of the cardiac renin-angiotensin system.

1. Introduction

There are abundant data to demonstrate that men are more prone to cardiovascular disease (CVD) than premenopausal women of similar age [34]. Gender related differences in aspects of CVD have been attributed to several factors that include differences in endothelial function, lipid profile, functions of the vascular smooth muscle, and blood pressure [31]. The incidence of CVD, however, increases substantially following menopause, suggesting that female sex hormones may render some degree of cardioprotection. Similar effects have also been observed in animal studies as ovariectomy abolished the female-associated cardioprotection following ischemia/reperfusion injury or volume overload [9,3]. Conversely, cardiac protection was restored with exogenous estradiol administration in a pressure overloaded mouse model [43].

Though observational studies in post-menopausal women have found consistent, powerful protection of hormone replacement therapy (HRT) against CVD [21], results from the Women's Health Initiative (WHI) [45] and the Heart and Estrogen–progestin Replacement study (HERS) [18] showed no reduction in cardiovascular risk with HRT. There could be several possible explanations, both methodological and biological, such as (a) the mode of estrogen administration (transdermal vs. oral) [25]; (b) type of estrogen utilized (natural vs. synthetic) [30]; (c) age and duration of menopause before treatment [17]; (d) the dose and duration of estrogen administration [29] and; (e) the overall health of the women studied, that may account for the apparent discrepant results.

In view of the ongoing debate over the use of HRT, we investigated whether administration of 17β-estradiol (E2), immediately after ovariectomy, can offer cardioprotective effects in the deoxycorticosterone acetate (DOCA)-salt model of hypertension. In the DOCA model, initial volume overload and elevated sympathetic drive lead to chronic increases in blood pressure, causing end-organ damages. We have previously found that this particular model produces marked cardiac remodeling [14]. Among the most perilous forms of end-organ damage produced by chronic hypertension is ventricular remodeling, characterized

* Corresponding author. Tel.: +1 352 273 7690; fax: +1 352 273 7705.
E-mail address: katovich@cop.ufl.edu (M.J. Katovich).
[1] These authors contributed equally to this study.

0196-9781/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2009.09.005

by cardiomyocyte hypertrophy, apoptosis, and increased deposition of extracellular matrix (ECM) proteins, contributing to interstitial and perivascular fibrosis [36]. Cardiac remodeling leads to ventricular dysfunction, heart failure and ultimately death. Therefore, prevention or reversal of the remodeling process may have significant impact on organ function and survival.

The renin-angiotensin system (RAS) has been shown to play a pivotal role in producing structural alterations of the heart in response to hemodynamic overload. Activation of the cardiac angiotensin type 1 receptor (AT1R) by angiotensin II (Ang II), the main effector peptide of the RAS, stimulates myocyte growth, fibroblast proliferation, collagen synthesis and expression of ECM proteins [28]. Previous studies in animals have demonstrated that DOCA-induced activation of local RAS resulted in cardiac damage, which could be prevented by administration of angiotensin converting enzyme (ACE) inhibitor or AT1R blocker [4].

In the current study, we investigated the effects of two doses (1.66 and 2.38 μg/day) of estrogen replacement on cardiac remodeling in DOCA-salt treated animals, and further, we sought to determine whether the protective effect of E2 is mediated through modulation of the cardiac RAS. We chose to use two different doses that mimic physiologically relevant levels of circulating E2 since Zhan et al. [47], recently reported detrimental effects with higher levels of E2 treatment.

2. Materials and methods

2.1. Animals

Female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 180 and 250 g were used for this study. Animals were housed in a temperature and humidity-controlled room maintained on a 12:12 h light–dark schedule with free access to food and water. All animal procedures were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee conforming to US National Institutes of Health guidelines.

2.2. DOCA-salt model of hypertension

Animals were anesthetized with subcutaneous injection of a ketamine, xylazine, and acepromazine mixture (30, 6 and, 1 mg/kg, respectively). Eighteen animals underwent uninephrectomy and bilateral ovariectomy (Ovex) with subcutaneous implantation of a 40 mg pellet of DOCA (Sigma–Aldrich, USA). Immediately after removal of the ovaries, some of the animals were additionally implanted subcutaneously with either 0.05 mg [21 day release pellet delivering 2.38 μg/day, ($n = 6$)] or 0.1 mg [60 day release pellet delivering 1.66 μg/day, ($n = 6$)] of E2 (Innovative Research of America Florida, USA). Pellets were replaced after three weeks in the higher dose group. A separate group of age-matched rats ($n = 6$) underwent bilateral ovariectomy alone (without uninephrectomy), to serve as normotensive controls. After surgery (and for the remainder of the experiment), normotensive controls received plain drinking water while all DOCA implanted animals received 0.9% (0.15 M) sodium chloride (NaCl) solution.

2.3. Indirect blood pressure measurement

Systolic blood pressure was determined weekly by the indirect tail cuff method as described previously [14]. Briefly, animals were lightly heated for five minutes under a 200 W heat lamp before placing into a temperature-controlled restrainer to which the animals had previously become acclimated. A pneumatic pressure sensor was attached to the tail, distal to a pneumatic pressure cuff, both under the control of a programmed Electro-Sphygmomanometer (Narco Bio Systems, Austin, TX). Voltage outputs from the pressure sensor bulb and inflation cuff were recorded and analyzed electronically using a Power Lab signal transduction unit and associated Chart software (AD Instruments, Colorado Springs, CO). Systolic blood pressure values from each animal were determined by averaging a minimum of five separate indirect pressure measurements. All pressures were recorded in the morning between 9 AM and noon by the same individual.

2.4. Isolated heart preparation

After six weeks of treatment, the animals were decapitated 10–15 min after an intraperitoneal administration of heparin (400 IU). Their hearts were excised and mounted onto a Langendorff apparatus. Hearts were perfused using Krebs–Ringer solution (118.4 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 26.5 mM $NaHCO_3$and 11.7 mM glucose) at a constant flow rate of approximately 8 mL/min. The perfusate temperature was maintained at 37 °C and bubbled with 95% O_2/5% CO_2. To measure the intra-ventricular pressure, a balloon catheter was inserted into the left ventricle and connected to a pressure transducer (AD Instruments), which was interfaced to a PowerLab data acquisition unit (AD Instruments). Coronary perfusion pressure was measured by means of a pressure transducer connected to the aortic cannula and coupled to the recording system. After 30 min of stabilization, functional parameters were recorded for an additional period of 30 min. Data were analyzed using the Chart software.

2.5. Tissue collection and cardiac remodeling analysis

Following experiments on the Langendorff apparatus, the hearts were blotted dry and weighed. Cross-sections of the ventricles were obtained and fixed in 10% neutral-buffered formalin solution for 24 h, after which they were moved to 70% ethanol until processed. The hearts were embedded in paraffin, sectioned at 5 μm and sections were stained for collagen content using Picro-Sirius Red stain. Single sections from each animal were then viewed and photographed with a Moticam 1000 digital camera (Motic; Richmond, BC, Canada) under ×100 magnification for interstitial fibrosis. The collagen content of the left ventricular free wall was quantified using the ImageJ program from the National Institutes of Health [35], as described elsewhere [14]. Quantifications were carried out as the mean observations from three individuals blinded to the treatment groups. For collagen quantification, each observer examined a minimum of five separate images per section from different (non-overlapping) regions of the left ventricular free wall. The results for each animal from each of the three observers were then averaged for subsequent statistical analysis. The heart weights were normalized to tibial length to determine myocardial hypertrophy.

2.6. Estradiol measurement

Venous blood from the inferior vena cava was collected at the time of sacrifice into EDTA-coated collection tubes and centrifuged at 800 × g for 5 min. Plasma was separated and stored at −80 °C until assayed. Plasma estradiol levels were measured using an ELISA kit as per manufacturer's instructions (ALPCO, USA). The kit could measure E2 levels ranging from 10 to 3200 pg/mL.

2.7. Western blot analysis

Heart tissue was homogenized in radioimmuno-precipitation assay buffer (RIPA buffer) and the concentration of the protein extracted was measured using Bradford assay. The homogenates