General and Comparative Endocrinology 206 (2014) 51–59

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Distinct role of estrogen receptor-alpha and beta on postmenopausal diabetes-induced vascular dysfunction

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ARTICLE INFO

Article history:
Received 28 March 2014
Revised 6 June 2014
Accepted 10 June 2014
Available online 23 June 2014

Keywords:
Menopause
Diabetes
Vascular dysfunction
Estrogen receptor

ABSTRACT

Estrogen is known to influence vascular functions and insulin sensitivity, but the relative contribution of estrogen receptor (ER) isoforms in postmenopausal diabetes-induced vascular dysfunction is unclear. The aim of the present study was to delineate the distinct role of estrogen receptor-α and beta β on the vascular function in ovariectomized diabetic rats. Age matched 60 female sprague dawley rats (200–250 g) were divided in nine groups. Bilateral ovariectomy was performed and streptozotocin was used to induce experimental diabetes. Rats were administered with 10 μg/kg; s.c. of a nonselective estrogen receptor agonist, 17-β estradiol (E2), selective ER-α agonist (4,4′,4″-(4-propyl-[1H] pyrazole-1,3,5-triyl) tris phenol (PPT) and selective ER-β agonist, 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN) for 4 weeks after STZ injection. Treatment with selective ER-α agonist and E2 improved the impaired glycemic and lipid profile in ovariectomized diabetic rats, however selective ER-β agonist did not show any effect. Vascular endothelial dysfunction was assessed by acetylcholine and sodium nitroprusside-induced endothelium dependent and independent relaxation in isolated rat aortic ring preparation as well as by electron microscopy of thoracic aorta. Further, serum thiobarbituric acid reactive substances, tumour necrotic factor-alpha and interleukin-1 beta and C-reactive protein were estimated to assess oxidative stress and vascular inflammation. Treatment with ER-α agonist markedly and E2 partially improved vascular function and endothelial integrity along with reduction in serum TBARS and inflammatory cytokines. However, ER-β agonist did not show any improvement in vascular functions, oxidative stress or inflammation. These findings suggest that selective targeting of ER-α receptors results in vasculoprotection in the state of hypoestrogenicity and diabetes.

1. Introduction

Estrogens are classically regarded as important hormonal signals and their actions are not limited to hormonal responses and reproductive behavior, but encompass profound effects over many other organs as well as central nervous system (Kiss et al., 2012). Estrogen acts in our body by binding with two receptors mainly ER-α and ER-β to exert its classical effects (Barros et al., 2006). Receptors for estrogen have been identified both on the vascular smooth muscle and endothelium, reinforcing the fact that estrogen plays a key role in control of vascular function (Couse et al., 1997; Pau et al., 1998). Estrogen is known to increase NO bioavailability by mechanisms that involve either increase of NO generation directly or by decreasing O_2^- concentration, and thereby attenuating O_2^- mediated inactivation of NO (Novensa et al., 2010). In addition to NO, estrogen upregulates the production of endothelium-derived relaxing factors (EDRFs), such as PGI_2 and the endothelium-derived hyperpolarizing factors (EDHFs) Dantas et al., 2002; Tostes et al., 2003. Estrogen has also been demonstrated to suppress vascular inflammation by down-regulation of proinflammatory molecules including cytokines and adhesion molecules (Arenas et al., 2006; Stork et al., 2002). Although vasculoprotective effects of estrogen are well reported, however women with diabetes lose their estrogen mediated vascular protection and recently a consensus has been developed that estrogen displays dichotomous effects by changing the balance of estrogen receptors in distinct pathophysiological conditions including aging and diabetes (Dantas et al., 2012). A study by Cignarella et al. (2009) reported that in animal models of diabetes, the anti-inflammatory activity of estrogen is impaired in vascular smooth muscle cells which displays ER-β overexpression with respect to normoglycemic control. These observations have raised a speculation that vascular effects of estrogen may be influenced by distinct pathophysiological conditions, including menopause and diabetes.

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http://dx.doi.org/10.1016/j.ygcen.2014.06.013
0016-6480/

Various studies mentioned that expression pattern of both receptors differs between specific tissues, genders and species and even in pre/postmenopausal state (Bonomo et al., 2009; Matthews and Gustafsson, 2003; Merchenthaler et al., 2004). Both estrogen receptors are expressed in metabolic tissues such as adipose tissue, skeletal muscle, liver, pancreas as well as in the vascular tissue; however a study by Foryst-Ludwig and Kintscher (2010) showed that the beneficial effects of estrogen (such as antilipogenesis, improvement of insulin sensitivity, maintenance of insulin secretion and glucose intolerance and reduction in body weight) are mediated through ER-α receptors. In contrast, ER-β stimulation results in detrimental effects on the regulation of glucose and lipid homeostasis. The increased expression or activation of ER-β over ER-α is associated with higher oxidative stress, proinflammatory profile and increased atherosclerotic plaque formation (Cignarella et al., 2009). These evidences from literature thus suggest that ER-α and ER-β have to be selectively activated for targeting vascular dysfunction in postmenopausal diabetics.

With this background, the present study was designed to explore the effect of nonselective estrogen receptor agonist (17-beta estradiol), selective ER-α agonist (PPT an agent 410-fold more selective for ER-alpha than ER-beta) and selective ER-β agonist (DPN an agent 70-fold more selective for ER-beta than ER-alpha) on postmenopausal diabetes-induced vascular dysfunction in rats.

2. Material and methods

2.1. Animals

Sixty female sprague dawley rats (200–250 g) bred in Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a 12:12 h light:dark cycle and had free access to food (Ashirwad Industries, Mohali, India) and water. Animals were acclimatized to laboratory conditions before all the behavioral tests. All experiments were carried out between 0900 and 1700 h. The experimental protocols were approved by the Institutional Animal Ethics Committee of Panjab University (approval No. IAEC/346-356; dated:11/2/13) and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

2.2. Drugs

Streptozotocin, 17-β estradiol, PPT (selective ER-α agonist), DPN (selective ER-β), acetylcholine (ACh), phenylephrine, sodium-nitroprusside (SNP) were purchased from Sigma (St. Louis, MO, USA), insulin was procured from Biocon Limited, Bangalore, India. All other chemicals used for biochemical estimations were of analytical grade.

2.3. Induction of postmenopausal diabetes and drug treatment schedule

Female rats were divided in nine groups (n = 6–8). Group I, control; group II, Sham; group III, OVX; group IV, Dia; group V, OVX + Dia; group VI, OVX + Dia + E2; group VII, OVX + Dia + PPT; group VIII, OVX + Dia + DPN; group IX, OVX + Dia + Ins. Before starting the experiment, all animals except group I, II, IV underwent a bilateral ovariectomy. Bilateral ovariectomy was performed as described in the literature (Park et al., 2010; Merchenthaler et al., 2004). After 1 week of recovery diabetes was induced by a single intraperitoneal (*i.p.*) injection of streptozotocin (STZ; 45 mg/kg) in all groups except group I, II, III. Forty-eight hours after STZ injection, blood glucose concentrations were measured and rats with blood glucose ⩾ 200 mg/dl were deemed diabetic. 17-β estradiol, PPT, DPN (10 μg/kg; s.c.) dissolved in peanut oil were given for 4 weeks on alternate days after forty-eight hours of STZ injection in group VI, VII, VIII respectively. The dose of E2 was decided on the basis of previous reports (Frye and Rhodes, 2002; Rhodes and Frye, 2006; Walf and Frye, 2005). These studies reported that, 5–10 μg E2 s.c. is most effective in producing physiological levels of estradiol. DPN and PPT were used at the same dose as E2 and both of these agents did not change uterine weight and had minimal estrogenic side effects in the peripheral system at this dose (Irwin et al., 2012). Insulin (10 IU/kg; s.c.) was administered daily after STZ injection in group IX. Group II rats (Sham) were subjected to whole surgical procedure except for removal of ovaries. Taking into account, the clause mentioned in CPCSEA guidelines related to justified use of animals for experiments, we did not take Ovx + Dia + Vehicle group, as previous studies mentioned that hormones dissolved in peanut oil when given subcutaneously easily penetrates the blood stream, and peanut oil does not show any of its *perse* effect (Hennan and Diamond, 1998).

After 4 weeks of drug administration, blood samples were collected to determine level of blood glucose, glycosylated haemoglobin (Hb A1c%), insulin, lipid profile, estradiol, TNF-α, IL-1β, CRP and TBARS through biochemical and ELISA assay kits. After that animals were euthanized and aortas were harvested for evaluation of vascular functions and for histopathological studies, uteri were dissected and trimmed of connective tissue and fat to obtain uterus weight.

2.4. Assessment of vascular functions

2.4.1. Isolation of rat thoracic aorta

The rat was decapitated, thoracic aorta was removed, cut into a ring of 4–5 mm length and mounted in organ bath containing Krebs–Henseleit solution (NaCl, 119 mM; KCl, 4.7 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 1.0 mM; glucose, 11.1 mM; KH_2PO_4, 1.2 mM and $CaCl_2$, 2.5 mM) bubbled with carbonated oxygen (95% O_2 and 5% CO_2) and maintained at 37 °C. The preparation was allowed to equilibrate for 90 min under 1.5 g tension. The isometric contractions were recorded (Pieper et al., 1997) with a force- transducer (Ft-2147) connected to Physiograph (Medicaid, Chandigarh, India).

The aortic ring preparation was primed with 80 mM KCl to check its functional integrity and to improve its contractility. The cumulative dose responses of acetylcholine (ACh; 10^{-8} to 10^{-4} M) or sodium nitroprusside (SNP; 10^{-8} to 10^{-4} M) were recorded in phenylephrine (3×10^{-6} M) precontracted preparation with intact or denuded endothelium, respectively (Mittra and Singh, 1998). The intimal layer of aortic ring was rubbed gently with a moistened filter paper for 30 s to obtain endothelium free preparation (Ignarro et al., 1988). Loss of ACh (1×10^{-6} M)-induced relaxation indicates dysfunction of vascular endothelium.

2.4.2. Electron microscopic study

The longitudinal strips of thoracic aorta (3–4 mm) were fixed in 3% glutaraldehyde phosphate buffer (pH 7.4) and subsequently dehydrated in the series of alcohol and acetone concentrations. The tissue was embedded in CY 212 araldite and ultra thin sections of 60–80 nm thickness were prepared using an ultracut E (Reichert Jung, Vienna, Austria). The sections were examined using Morgagni 268(D) electron microscope (FEI Company, Hillsboro, OR, USA) attached with an image analyzer. Electron micrographs were critically examined for the integrity of vascular endothelial lining (David et al., 1973; Schiller et al., 1999).

2.5. Biochemical estimations and measurements of serum inflammatory markers

Plasma was used for estimation of glucose, triglycerides, total cholesterol, HDL-cholesterol using commercially available