Journal of Steroid Biochemistry & Molecular Biology 147 (2015) 1–9

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Significant role of estrogen in maintaining cardiac mitochondrial functions

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ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form 6 November 2014
Accepted 8 November 2014
Available online 13 November 2014

Keywords:
ATP synthesis
Reactive oxygen species
Mitofusin 2
Superoxide dismutase 2

ABSTRACT

Increased susceptibility to stress-induced myocardial damage is a significant concern in addition to decreased cardiac performance in postmenopausal females. To determine the potential mechanisms underlying myocardial vulnerability after deprivation of female sex hormones, cardiac mitochondrial function is determined in 10-week ovariectomized rats (OVX). Significant mitochondrial swelling in the heart of OVX rats is observed. This structural alteration can be prevented with either estrogen or progesterone supplementation. Using an isolated mitochondrial preparation, a decrease in ATP synthesis by complex I activation in an OVX rat is completely restored by estrogen, but not progesterone. At basal activation, reactive oxygen species (ROS) production from the mitochondria is not affected by the ovariectomy. However, after incubated in the presence of either high Ca^{2+} or antimycin-A, there is a significantly higher mitochondrial ROS production in the OVX sample compared to the control. This increased stress-induced ROS production is not observed in the preparation isolated from the hearts of OVX rats with estrogen or progesterone supplementation. However, deprivation of female sex hormones has no effect on the protein expression of electron transport chain complexes, mitofusin 2, or superoxide dismutase 2. Taken together, these findings suggest that female sex hormones, estrogen and progesterone, play significant regulatory roles in maintaining normal mitochondrial properties by stabilizing the structural assembly of mitochondria as well as attenuating mitochondrial ROS production. Estrogen, but not progesterone, also plays an important role in modulating mitochondrial ATP synthesis.

1. Introduction

The roles of female sex hormones in the regulation of cardiovascular function have been recently re-evaluated due to disputation in the benefits of hormone replacement therapy in postmenopausal women. [1]. Increased ventricular wall thickness but decreased fractional shortening in the hearts of postmenopausal females compared to age-matched premenopausal controls independent of hypertension suggested potential direct actions of female sex hormones on cardiac muscle [2,3]. Many clinical trials have previously demonstrated an improvement in cardiac performance in postmenopausal females who received hormone replacement therapy [4–6]. In addition, postmenopausal females who received hormone replacement therapy had a significantly improved in-hospital survival after coronary bypass [7], indicating the protective effects of female sex hormones against cellular damage. However, some studies found a small advantage in reducing heart disease incidence using hormone replacement therapy [8,9]. These inconclusive reports further challenged the benefit of hormone replacement therapy in cardiovascular medicine and indicated the need for more direct evidence of the regulatory roles of sex hormones in cardiac function.

Mitochondria may be one potential target of female sex hormone actions in the heart. It is well known that mitochondria play a fundamental role in regulating cell survival processes involved in ATP production, reactive oxygen species (ROS) generation, intracellular calcium mobilization, and the regulation of cell apoptosis [10–12]. Many previous studies have revealed the effects of estrogen and progesterone on mitochondria protein expression and respiratory function in various tissues [13–15]. However, only a few studies have determined the effect of female sex hormones on the mitochondria of cardiomyocytes [16], in which estrogen could prevent damage of the mitochondrial structure and a decrease in mitochondrial respiratory function induced after ischemia-reperfusion. Interestingly, the rate of ATP production in the heart of female rats was significantly lower compared to male hearts [17], whereas mitochondrial ROS production of male rat hearts was higher compared to female

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http://dx.doi.org/10.1016/j.jsbmb.2014.11.009
0960-0760/

hearts [18]. A decrease in the rate of mitochondrial oxygen consumption with a reduction of mitochondrial complex IV protein was observed in ovariectomized rats, [19]. An acute study showed that estrogen preincubation decreased the sensitivity of mitochondria to Ca^{2+}-induced permeability transition pore opening and the release of cytochrome *c* from cardiac mitochondria [20]. Another study demonstrated that estrogen treatment protected mitochondrial ATP production against trauma-hemorrhagic stress [21]. Importantly, all previous studies had indicated the cardioprotective effect of estrogen on mitochondrial function and did not highlight the cellular effect of progesterone. On the basis of the results of the Women's Health Initiative study [22], a significant contribution of progesterone to the increased risk of cardiovascular disease during hormone replacement therapy in postmenopausal females was indicated.

In the present study, we aim to demonstrate changes in the function of mitochondria in female rat hearts after chronic deprivation of ovarian sex hormones after estrogen and progesterone supplementation. Ten-week ovariectomized rats are used in this study, as previous reports had demonstrated significant suppression in cardiac contraction and relaxation after 10 weeks of sex hormone deprivation [23,24]. Two major functions of mitochondria, i.e., ATP production and ROS generation, are assessed using isolated a mitochondrial preparation. Our results demonstrate that chronic deprivation of ovarian sex hormones results in a decrease in mitochondrial ATP, but an increase in ROS production. Estrogen plays a regulatory role in ATP synthesis whereas progesterone contributes to suppressed ROS generation.

2. Materials and methods

2.1. Animal preparations

Female Sprague-Dawley rats weighing between 180 and 200 g (8–9 weeks old) were sham-operated or ovariectomized. All animals were fed *ad libitum* (C.P., Thailand) and had access to tap water during the entire experiment. Rats were individually housed in a shoe box under a 12:12 light-dark cycle with controlled temperature and humidity. Two days after the operation, ovariectomized rats were randomly divided into control and hormone-supplemented groups. Hormone supplementation was performed by subcutaneous injection of estrogen (5 μg/rat) and progesterone (1 mg/rat), three times a week as previously described [25]. Sham-operated and ovariectomized control rats were injected with corn oil (vehicle). Ten weeks after the operation, the rats were anesthetized and the hearts were quickly excised and placed in ice cold Krebs Henseleit (KH) buffer. Deficiency of ovarian sex hormones was confirmed by reduced uterine weight. Serum hormones were analyzed using ELISA test kit (Calbiotech) for estradiol and ECLIA system (IMMULITE 2000 XPi) for progesterone and testosterone levels. The animal protocol was approved by the Experimental Animal Committee, Faculty of Science, Mahidol University, in accordance with the National Animal Laboratory Centre, Thailand.

2.2. Isolation of cardiac mitochondria

Mitochondria were isolated from the left ventricle (LV) as previously described [26]. Briefly, the LV was weighed and minced in 8 mL of ice-cold homogenizing buffer containing 50 mM sucrose, 200 mM mannitol, 5 mM KH_2PO_4, 5 mM MOPS, 2 mM taurine, 1 mM EGTA, and 1% w/v BSA. The sample was transferred into a Glass–Teflon homogenizer for tissue homogenization by 12 strokes at speed 4 (800 rpm). The homogenate was centrifuged at 500 × *g* (1500 rpm) and 4 °C for 5 min, and the supernatant was kept at 4 °C. The total mitochondrion-containing supernatant was centrifuged at 15,000 × *g* (11,000 rpm), 4 °C for 5 min. The pellet was resuspended in 8 mL of homogenizing buffer, blended, and centrifuged at 10,000 × *g* (9100 rpm) and 4 °C for 5 min. The final pellet was washed with 1 mL of suspension buffer (50 mM sucrose, 200 mM mannitol, 5 mM KH_2PO_4, 5 mM MOPS, 2 mM taurine) and suspended in 400 μL of suspension buffer [26]. The protein concentration of the isolated mitochondria was determined using the Bradford assay [27]. The protein dilution was prepared for the ROS and ATP production assay.

2.3. Measurement of mitochondrial swelling

Cardiac mitochondrial swelling was determined by a decrease in light absorption as previously described [28], with modification. Isolated mitochondria were diluted with suspension buffer to a final concentration of 1 mg/ml. The optical density was continuously measured in 96-well microplate using a microplate reader at 540 nm wavelength.

2.4. Measurement of mitochondrial ATP synthesis

Measurement of mitochondrial ATP production was performed as previously described [26]. Complex I activity was determined in reaction buffer A (150 mM KCl, 25 mM Tris, 10 mM KH_2PO_4, 2 mM EDTA, 0.1 mM MgCl, and 0.1% BSA (pH 7.4)) with the addition of 1 mM pyruvate and 1 mM malate as substrates, 0.8 mM luciferin and 20 μg/ml luciferase (Promega), 0.15 mM di-adenosine pentaphosphate (to inhibit adenylate cyclase) and 30 μg mitochondria. Complex II activity was also determined in reaction buffer A using 5 mM succinate as the substrate and 40 μM rotenone as complex I inhibitor in 30 μg of mitochondria. In every reaction, ADP was added to a final concentration of 0.1 mM to initiate ATP synthesis

Table 1
Characteristics of sham-operated (SHAM) and ovariectomized (OVX) rats with and without estrogen or progesterone supplementation.

	SHAM	Ovariectomy		
		Oil	Estrogen	Progesterone
Body weight (g)	274 ± 3	351 ± 5*	242 ± 3**	343 ± 5*
Heart weight (g)	1.16 ± 0.02	1.39 ± 0.02*	1.06 ± 0.02**	1.34 ± 0.02*
Heart/body weight (×100)	425 ± 8	396 ± 7*	439 ± 6**	398 ± 7*
Uterine weight (g)	0.66 ± 0.03	0.14 ± 0.01*	0.51 ± 0.03**	0.18 ± 0.02*
Serum estradiol (pg/mL)	6.21 ± 0.73	2.68 ± 0.30*	6.65 ± 0.99**	2.59 ± 0.62*
Serum progesterone (ng/mL)	20.8 ± 1.1	6.4 ± 1.7*	12.4 ± 1.3*,**	12.6 ± 0.9*,**
Serum testosterone (ng/mL)	44.0 ± 4.9	34.3 ± 3.3	<20[NA]	<20[NA]

Data are mean ± SE from 10–12 rats of each group. [NA] indicates no statistical analysis.
* $P < 0.05$, significantly different from SHAM, using Student–Newman–Keuls test after ANOVA.
** $P < 0.05$, significantly different from OVX, using Student–Newman–Keuls test after ANOVA.