Original Article

Sex differences play a role in cardiac endoplasmic reticulum stress (ERS) and ERS-initiated apoptosis induced by pressure overload and thapsigargin

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Received 12 February 2010; received in revised form 1 July 2010; accepted 26 July 2010

Abstract

Excessive endoplasmic reticulum stress (ERS) triggers myocardial apoptosis. Sex differences appear to be an important determinant in the occurrence of stress and apoptosis through many pathways, but the roles of sex differences in the cardiac ERS and ERS-initiated apoptosis are largely unknown. In the present study, we investigated the in vivo role of sex differences in the cardiac ERS and apoptosis elicited by ascending aortic banding surgery or thapsigargin (Thap) injection using male and female C57BL/6 JAX mice. The surgery significantly increased the expression levels of cardiac glucose-regulated protein (GRP)78 and CCAAT/enhancer binding protein homology protein (CHOP) protein, increased the myocardial apoptosis and decreased the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase isoform (SERCA)2 immunoreactivity in the male mice relative to female mice. Furthermore, during ERS induction using Thap, myocardial apoptosis and the expression levels of cardiac GRP78, inositol-requiring enzyme (Ire)\textsubscript{1α} and tumor necrosis factor receptor-associated factor (TRAF)2 were significantly increased in male mice relative to female mice. Sex differences significantly affected the above results. Our data suggest that sex differences affected the response of myocardial tissues in dealing with cardiac ERS and further result of ERS, apoptosis, at least in part through the regulation of SERCA2, CHOP, Ire\textsubscript{1α} and TRAF2. © 2011 Published by Elsevier Inc.

Keywords: Sex differences; Ascending aortic banding; Thapsigargin; Endoplasmic reticulum stress; Apoptosis

1. Introduction

The endoplasmic reticulum (ER) is involved in several important functions such as the folding of secretory and membrane proteins. Various conditions including ischemia, hypoxia, exposure to free radicals, elevated protein synthesis, hyperhomocysteinemia and gene mutation can cause pathological accumulation of unfolded proteins in the ER—a condition referred to as ER stress (ERS) [1–3]. To prevent deleterious effects of ERS, cells have various protective strategy termed as unfolded protein response (UPR) through the mediation of ER transmembrane receptors: pancreatic ER kinase-like ER kinase (PERK), activating transcription factor 6 and inositol-requiring enzyme (Ire) \textsubscript{1α} and tumor necrosis factor receptor-associated factor (TRAF)2 are maintained in an inactive state by glucose-regulated protein (GRP)78. However, if the stress cannot be resolved signaling switches from pro-survival to proapoptotic through the mediation of PERK and Ire\textsubscript{1α}-tumor necrosis...
factor receptor-associated factor (TRAF)2 pathways to downstream molecules such as CCAAT/enhancer binding protein homology protein (CHOP), c-Jun N terminal kinases (JNK) and caspases [1–5]. Accumulating evidence has demonstrated that apoptosis initiated by excessive ERS is involved in the pathogenesis of many diseases including diabetic cardiomyopathy [6], diabetic nephropathy [7], pressure overload-induced cardiac hypertrophy and thapsigargin (Thap)-induced cardiac ERS [8].

Sex differences have been reported to play an important role in the occurrence of cardiomyocyte apoptosis. The percentage of apoptotic cardiomyocytes was threefold higher in men’s than in women’s normal hearts [9]. In the failing heart, myocyte necrosis and apoptosis were markedly lower in women than in men [10]. Recently, a significantly higher level of apoptosis was found in male rats than in the female ones at 16 weeks after pressure overload elicited by the arteriovenous (AV) shunt surgery [11]. The same results were observed in the spontaneously hypertensive rats [12] and the ischemia reperfusion animal model [13]. Through these studies, sex differences appear to be an important determinant in the occurrence of apoptosis in both humans and animals. Many different signal transduction pathways that regulate apoptosis are reported to be sex differences-related. It is pointed out that protein kinase B (Akt) is considered to play a central role in cell survival and resistance to apoptosis and an increase in the activity of which has been reported in females [14]. Females have also been shown to be protected against ischemia-reperfusion injury through an increase in phosphorylated Akt and protein kinase C (PKC)-ε levels [15]. Recently, it has been shown that a significant increase in the expression of phospho Bcl-2 and BAX appeared along with lesser degree of myocardial apoptosis in the female AV shunt rats than in the male ones [11].

Despite our significant understanding of the role of sex differences in the occurrence of apoptosis, the roles of sex differences in cardiac ERS and ERS-initiated apoptosis are largely unknown. In the present study, we investigated the in vivo roles of sex differences in the cardiac ERS and apoptosis elicited by ascending aortic banding (AB) surgery or Thap injection using male and female C57BL/6 JAX mice.

2. Methods

2.1. Ascending AB surgery

To create pressure overload in the mice, we performed ascending AB surgery since this technique provides a more direct and rapid source of pressure overload on the left ventricle (LV) and a significant degree of hypertrophy after 48 hours [16]. Ten- to twelve-week-old male (male AB, n=7) and female (female AB, n=6) C57BL/6 JAX mice (Charles River Japan, Kanagawa, Japan) underwent ascending AB surgery. The ascending AB was performed using a previously described method [16]. In brief, the mice were anesthetized with Nembutal 50 mg/kg body weight (BW) given intraperitoneally. After an adequate depth of anesthesia was attained, each mouse was fixed in a supine position with tape. A 5-0 ligature was placed behind the front upper incisors and pulled taut so that the neck was slightly extended. The tongue was retracted and held with forceps, and a 20-G i/v catheter was inserted into the trachea. The catheter was connected to a volume-cycled ventilator supplying supplemental oxygen with a tidal volume of 2.5 ml and a respiratory rate of 120 beats per minute.

Prior to the incision, the chest was disinfected with povidone iodine solution and 70% ethyl alcohol, and 0.1 ml of 0.1% lidocaine was injected under the skin. The chest cavity was opened by an incision being made in the left second intercostal space. A chest retractor was then applied to facilitate visualization of the surgical field. The pericardial sac was opened and pulled apart, the ascending portion of the aorta was dissected from the surrounding tissues, and a 7-0 silk suture was passed underneath the ascending portion of the aorta and ligated against a 26-gauge needle. The latter was immediately removed to produce a lumen in the stenotic aorta. The lungs were overinflated, and the chest cavity, muscles, and skin were closed layer by layer with 6-0 nylon and 6-0 absorbable (for muscles) sutures. The duration of the whole procedure was about 20-30 minutes. Age matched male (male sham, n=6) and female (female sham, n=6) C57BL/6 JAX mice (Charles River Japan) underwent sham surgery and were used as controls. The procedure of the sham surgery was identical to that of the ascending AB surgery except that the ascending aorta was not ligated. The mice were then examined after 5 days.

All mice examined were maintained with free access to water and chow throughout the study period. The animals were treated in strict accordance with the recommendations of the Declaration of Helsinki and the guidelines for animal experimentation of our institute.

2.2. In vivo Thap injection

Ten- to twelve-week-old male (male Thap, n=6) and female (female Thap, n=6) C57BL/6 JAX mice (Charles River Japan) were immunized intraperitoneally with 1 mg/kg BW Thap, an effective inhibitor of Ca2+ ion pump proteins of the intracellular membranes of the sarcoplasmic reticulum (SR) and ER of skeletal and cardiac muscles and brain microsomes using a previously described method [17]. Age-matched male (male control, n=6) and female (female control, n=6) C57BL/6 JAX mice (Charles River Japan) were injected with phosphate-buffered saline and used as controls. The mice were examined after 8 h.

All mice examined were maintained with free access to water and chow throughout the study period. The animals were treated in strict accordance with the recommendations of the Declaration of Helsinki and the guidelines for animal experimentation of our institute.