Estrogen receptor β mediates increased activation of PI3K/Akt signaling and improved myocardial function in female hearts following acute ischemia

Meijing Wang, Yue Wang, Brent Weil, Aaron Abarbanell, Jeremy Herrmann, Jianing Tan, Megan Kelly, and Daniel R. Meldrum

1Clarian Cardiovascular Surgery, Departments of 2Surgery and 3Cellular and Integrative Physiology, and 4Center for Immunobiology, Indiana University School of Medicine, Indianapolis, Indiana

Submitted 22 January 2009; accepted in final form 6 February 2009

Wang M, Wang Y, Weil B, Abarbanell A, Herrmann J, Tan J, Kelly M, Meldrum DR. Estrogen receptor β mediates increased activation of PI3K/Akt signaling and improved myocardial function in female hearts following acute ischemia. Am J Physiol Regul Integr Comp Physiol 296: R972–R978, 2009. —Females have a lower incidence of heart failure and improved survival after myocardial ischemia-reperfusion (I/R) compared with males. Although estrogen-suppressed cardiomyocyte apoptosis may be mediated through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway, it is unclear whether this action is mediated via estrogen receptor β (ERβ). Therefore, we hypothesized that ERβ mediates estrogen-induced cardioprotection through PI3K/Akt and antiapoptotic signaling in females but not in males. Isolated male and female hearts from ERβ (ERβKO) and wild-type (WT) mice (n = 5 mice/group) were subjected to 20-min ischemia followed by 60-min reperfusion (Langendorff). Ablation of ERβ significantly decreased postischemic recovery of left ventricular developed pressure in female, but not male, hearts. Reduced activation of PI3K and Akt was noted in female ERβKO hearts, which was associated with increased expression of caspase-3 and -8, as well as decreased Bcl-2 levels compared with WT. However, myocardial STAT3, SOCS3 (suppressor of cytokine signaling 3), VEGF, and TNF receptors 1 and 2 levels did not change in ERβKO of either sex following I/R. Furthermore, deficiency of ERβ increased myocardial JNK activation in females but increased ERK1/2 activity in males during acute I/R. We conclude that ERβ mediates myocardial protection via upregulation of PI3K/Akt activation, decreased caspase-3 and -8, and increased Bcl-2 in female hearts following I/R. These findings provide evidence of ERβ-mediated PI3K/Akt and antiapoptotic signaling in the myocardium and may lend insight into the mechanistic pathways behind the observed variation in clinical outcomes between males and females after myocardial infarction.

MYOCARDIAL ISCHEMIA-REPERFUSION (I/R) injury occurs during cardiac surgery and leads to production of inflammatory cytokines, as well as cardiomyocyte apoptosis and necrosis, all of which exacerbate postischemic myocardial dysfunction. Although there have been many studies focused on minimizing the adverse effects of I/R injury, few have resulted in significant clinical benefits. Sex differences have been noted in the myocardial response to ischemic injury with females exhibiting improved cardiac function, diminished inflammatory response, and reduced apoptotic signaling (17, 19). Studies from our group (25) and others (10) have further demonstrated that estrogen mediates cardioprotection in females following acute I/R. However, this notion was recently challenged by controversial results from clinical trials that did not demonstrate a cardioprotective effect of hormone replacement therapy on postmenopausal females (16). This led to a recognition that estrogen-mediated cardioprotection appears more complicated than originally thought and requires more research.

The effects of estrogen are mediated mostly through estrogen receptor α (ERα) and/or estrogen receptor β (ERβ), both of which are expressed in the heart and have been involved in regulating cardioprotection (9, 22). In fact, deficiency of the ERβ gene worsens cardiac dysfunction following myocardial ischemic injury (2, 6, 13). In addition, a selective ERβ agonist, 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN), protects the myocardium from acute ischemia in ovariectomized (OVX) females (11). Furthermore, 17β-estradiol (E2) has been shown to suppress cardiomyocyte apoptosis via activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway during myocardial infarction (12, 15). However, it is unknown whether this action is mediated through ERβ. On the other hand, our group has previously demonstrated TNF receptor 1 (TNFR1) signaling resistance as well as upregulated signal transduction and activator of transcription 3 (STAT3)/suppressor of cytokine signaling 3 (SOCS3)/VEGF cascade in female hearts following I/R (23, 24). It is important to elucidate whether ERβ plays a role in these pathways.

Therefore, with the use of mice containing a targeted deletion of ERβ, this study aimed to determine whether ERβ mediates protection of cardiac function through PI3K/Akt and antiapoptotic signaling in females, but not males, following acute I/R injury and whether ERβ may facilitate this cardioprotection via TNFR/STAT3/SOCS3/VEGF-mediated cascades.

MATERIALS AND METHODS

Animals. A total of 20 male and female C57BL/6J mice (16 ± 4 wk) with and without ERβ deficiency (ERβKO; Taconic Farms, Hudson, NY) were fed a standard diet and acclimated in a quiet quarantine room for more than 1 mo before the experiments. The animal protocol was reviewed and approved by the Indiana Animal Care and Use Committee of Indiana University. All animals received humane care in compliance with the “Guide for the Care and Use of Laboratory Animals” [DHEW Publication No. (NIH) 85-23, Revised 1996, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205].

All isolated mouse hearts were subjected to the same I/R protocol as described previously (23, 24). Mouse hearts were divided into four groups (25) and others (10) have further demonstrated that estrogen mediates cardioprotection in females following acute I/R. However, this notion was recently challenged by controversial results from clinical trials that did not demonstrate a cardioprotective effect of hormone replacement therapy on postmenopausal females (16). This led to a recognition that estrogen-mediated cardioprotection appears more complicated than originally thought and requires more research.

The effects of estrogen are mediated mostly through estrogen receptor α (ERα) and/or estrogen receptor β (ERβ), both of which are expressed in the heart and have been involved in regulating cardioprotection (9, 22). In fact, deficiency of the ERβ gene worsens cardiac dysfunction following myocardial ischemic injury (2, 6, 13). In addition, a selective ERβ agonist, 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN), protects the myocardium from acute ischemia in ovariectomized (OVX) females (11). Furthermore, 17β-estradiol (E2) has been shown to suppress cardiomyocyte apoptosis via activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway during myocardial infarction (12, 15). However, it is unknown whether this action is mediated through ERβ. On the other hand, our group has previously demonstrated TNF receptor 1 (TNFR1) signaling resistance as well as upregulated signal transduction and activator of transcription 3 (STAT3)/suppressor of cytokine signaling 3 (SOCS3)/VEGF cascade in female hearts following I/R (23, 24). It is important to elucidate whether ERβ plays a role in these pathways.

Therefore, with the use of mice containing a targeted deletion of ERβ, this study aimed to determine whether ERβ mediates protection of cardiac function through PI3K/Akt and antiapoptotic signaling in females, but not males, following acute I/R injury and whether ERβ may facilitate this cardioprotection via TNFR/STAT3/SOCS3/VEGF-mediated cascades.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
experimental groups: normal males (n = 5), ERβKO males (n = 5), normal females (n = 5), and ERβKO females (n = 5).

Isolated heart preparation (Langendorff). Experiments were performed with the use of a Langendorff apparatus as described previously for mouse hearts (24). The left ventricular developed pressure (LVDP) was continuously recorded using a PowerLab 8 pem amplifier/digitizer (AD Instruments, Milford, MA) and an Apple G4 PowerPC computer (Apple Computer, Cupertino, CA).

Western blotting. Western blot analysis was performed to measure PI3K/Akt signaling, STAT3 activation, SOCS3 expression, apoptotic proteins (caspase-3 and -8 and Bcl-2), and mitogen-activated protein kinases [MAPKs: p38 MAPK, c-Jun NH2-terminal kinase (JNK), and extracellular signal-regulated protein kinase p42/p44 (ERK1/2)]. Heart tissue was homogenized in cold radioimmunoprecipitation assay (RIPA) buffer (product no. R 0278; Sigma, St. Louis, MO) and was centrifuged at 12,000 rpm for 10 min. The protein extracts (15 μg/lane) were subjected to electrophoresis on a 4–12% Bis-Tris protein gel (Invtrogen, Carlsbad, CA) and transferred to a nitrocellulose membrane. The membranes were incubated in 5% dry milk for 1 h and then incubated with the following primary antibodies: PI3K, p-P38, Akt, p-Akt, STAT3, p-STAT3 (Thr180/Tyr182) (1:1,000 dilution; Cell Signaling Technology, Beverly, MA); caspase-3 and -8 and SOCS3 (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA); Bcl-2 (Oncogene Research Products, San Diego, CA); and GAPDH (1:5,000 dilution, Biodesign International, Saco, ME). Membranes were then incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG secondary antibody (Pierce, Rockford, IL), and detection was performed by examining caspase-3 and -8 and antiapoptotic protein Bcl-2 expression instead. ERβKO significantly increased myocardial LVDP in female, but not male, hearts. Results are means ± SE; n = 5 mice/group. *P < 0.05 vs. male WT. #P < 0.05 vs. female WT.

Effects of ERβ on myocardial apoptotic proteins after I/R injury. Given that the experimental period in this study is too brief (20-min ischemia followed by 60-min reperfusion) to detect significant apoptosis, we measured proapoptotic signaling by examining caspase-3 and -8 and antiapoptotic protein Bcl-2 expression instead. ERβKO significantly increased myocardial expression of caspase-3 and -8 and reduced Bcl-2 levels were noted in male ERβKO hearts (Fig. 3). It is possible that sex differences exist in ERβ-mediated apoptotic signaling following acute I/R.

Effects of ERβ on myocardial VEGF expression during acute I/R. Myocardial VEGF levels in either sex after acute I/R (Fig. 4).

DISCUSSION

Myocardial ischemia increases activation of the PI3K/Akt pathway, which in turn mediates cardiomyocyte survival in response to I/R injury. Ablation of the ERβ gene significantly decreased myocardial PI3K/Akt activation in females after I/R. ERβKO reduced activation of PI3K from 47.6% (percentage of p-PI3K/total PI3K) in WT to 14.6% (Fig. 2A) and Akt from 190% (percentage of p-Akt/total Akt) to 91.3% in female hearts (Fig. 2B). Although deficiency of ERβ decreased PI3K activation, the KO did not affect myocardial Akt activation in males (Fig. 2).

Effects of ERβ on myocardial apoptotic proteins after I/R injury. Although a trend of decreased STAT3 activation was observed in female ERβKO hearts, deficiency of ERβ did not significantly change myocardial STAT3 activation, TNFR1 and TNFR2 expression, SOCS3 levels, or VEGF production in either sex after acute I/R (Fig. 4).

Effects of ERβ on myocardial VEGF expression during acute I/R. Myocardial VEGF levels in either sex after acute I/R (Fig. 4).

DISCUSSION

Accumulating evidence has demonstrated that ERβ is involved in mediating E2-induced cardioprotection in females following I/R combined with isoproterenol-induced hypercon-
tractile condition (2, 11). In the present study, by utilizing mice lacking ERβ, we found that ERβ mediates acute myocardial functional protection likely through activation of PI3K/Akt pathway and decreased apoptotic signaling following I/R in females (presumably having a higher baseline levels of endogenous estrogen compared with males) but not in males (Fig. 6).

ERβ is a member of the nuclear receptor gene family of transcription factors and may play an important role in protecting against myocardial dysfunction caused by I/R injury. Previously, we indicated that E2, a nonselective ER ligand, improved functional recovery, reduced myocardial inflammation, and diminished proapoptotic signaling following acute ischemia in males as well as OVX females (25). Recently, deficiency of ERβ was shown to worsen postischemic myocardial dysfunction in female hearts with hypercontractile condition (2). In addition, DPN, a selective ERβ agonist, restored...
E2-mediated cardiac protection in OVX females under conditions of enhanced contractility following I/R injury (11). In our study, significantly decreased recovery of LVDP was noted in female ERβ/H9252 KO hearts compared with female WT after acute I/R, whereas ablation of ERβ did not affect postischemic myocardial functional recovery in males. This result further confirms that ERβ mediates protection of myocardial function not only in female hearts associated with isoproterenol-enhanced contractility (2, 11) but also in normal females during acute I/R.

ERβ has been shown to mediate E2-induced cardioprotection through genomic mechanisms including regulation of metabolic gene expression (2, 11) and modulation of ion channel expression and calcium-handling protein (6, 13). However, it is also possible that ERβ may be located in the plasma membrane and mediates nongenomic events triggered by E2 (18). Akt and its upstream signal, PI3K, play critical roles in mediating cell survival and apoptosis (12). It is evident that the E2-activated PI3K/Akt pathway functions as one of the acute nongenomic actions of E2 in various types of cells (18). Upregulation of PI3K/Akt by administration of E2 results in endothelial nitric oxide synthase activation via a transcription-independent mechanism (3). In addition, activation of the PI3K/Akt pathway is required for E2-suppressed apoptosis and

Fig. 4. Activation of myocardial STAT3 (A), expression of suppressor of cytokine signaling 3 (SOCS3; B), TNF receptor 1 and 2 (TNFR1 and TNFR2; C), and VEGF (D) in ERβKO and WT hearts of both sexes after I/R. ERβKO did not affect myocardial TNFR/STAT3/SOCS3/VEGF cascades following I/R. Representative immunoblots (2 lanes/group) are shown at left and densitometry bar graphs at right in A–C. ELISA data for VEGF are shown in D. Results are means ± SE.
E2-protected myocardial function in the heart following ischemia (12). Although most studies on the interaction of estrogen receptor with the PI3K/Akt pathway have indicated a crucial role for ERβ as a membrane-associated receptor engaged in this action (18), little information exists regarding the importance of ERβ in the E2-mediated nongenomic effect of PI3K/Akt activation. In this study, we found significantly reduced activation of the PI3K/Akt pathway in female ERβKO hearts, but not in males, following acute I/R. This provides evidence that ERβ is also involved in PI3K/Akt-regulated cardioprotection in females. In fact, ERβ has been shown to mediate E2-induced antiapoptotic effects through the PI3K/Akt pathway in skeletal muscle cells (21). In addition, administration of E2 protects cardiac H9c2 cells from oxidative stress-induced apoptosis through ERβ-activated Akt signaling (20). Consistent with these observations, we have demonstrated that deficiency of the ERβ gene significantly increases levels of proapoptotic proteins caspase-3 and -8 while reducing antiapoptotic protein Bcl-2 levels in female hearts in response to acute I/R.

E2-regulated Akt activation also may mediate myocardial protection via additional mechanisms. Previous studies have demonstrated, for example, that OVX-reduced Akt activation occurs with decreased myocyte contractile function and impaired intracellular calcium handling, whereas E2-upregulated Akt is associated with restored cardiac contractility and intracellular calcium homeostasis (15). This suggests that Akt signaling may play a role in E2-mediated protection of myocardial function in addition to reduction of apoptosis. Indeed, activation of the Akt pathway also has been linked to a lower incidence of arrhythmias after myocardial infarction (14). In addition, delivery of a constitutively active Akt mutant gene markedly improves myocardial function in vivo following acute I/R and protects hypoxia-induced cardiomyocyte dysfunction in vitro, likely by preventing hypoxia-induced abnormalities in calcium transients and shortening (7). Therefore, it is possible that decreased activation of Akt may lead to reduced myocardial recovery of LVDP in female ERβKO hearts following acute I/R in our study.

Fig. 5. Activation of myocardial p38 MAPK (A), JNK (B), and ERK1/2 (C) in ERβKO and WT hearts of both sexes after I/R. Ablation of ERβ did not affect myocardial p38 MAPK activation in either sex (A). However, ERβKO significantly increased JNK activation in female hearts (B) and elevated ERK1/2 activity in males following I/R (C). Representative immunoblots (2 lanes/group) are shown at left and densitometry bar graphs at right. Results are means ± SE. *P < 0.05 vs. corresponding WT. Experiments were repeated in 2 different sessions.

Fig. 6. Simplified schematic illustrates how the ERβ mediates activation of PI3K/Akt and apoptotic pathway and thus protects the female myocardium in response to I/R. ERβ-regulated JNK and ERK1/2 signaling is also shown.
TNFR1 signaling has been shown to mediate detrimental effects of TNF on the myocardium (8, 24), whereas the TNFR2 pathway conducts protective effects (4, 23). Evidence suggests that the balance of TNFR1 and TNFR2 signaling shifts in favor of TNFR2 and its beneficial effects in female hearts during I/R (23, 24). However, it is unclear whether ERβ is engaged in the regulatory balance of the TNFR1 and TNFR2 pathways. In addition, TNFR2-induced cardioprotection appears to be mediated through STAT3, SOCS3, and VEGF in female hearts. STAT3 has been shown to be a direct target gene for estradiol (1); administration of E2 upregulates STAT3 activation, which mediates nongenomic effects of E2 (1). Therefore, the administration of E2 upregulates STAT3 activation, which is mediated through STAT3, SOCS3, and VEGF in female hearts.

Perspectives and Significance

This study provides the direct evidence of ERβ-mediated cardioprotection in female hearts following acute I/R. Improved myocardial function is associated with ERβ-activated PI3K/Akt pathway and subsequently decreased apoptotic signaling in female hearts in response to I/R. Further investigation is required to elucidate the detailed mechanisms on the specific function of ER subtypes in the heart. Understanding effects of estrogen and estrogen receptors may help in advancing therapeutic manipulations in menopausal females and, potentially, males.

GRANTS

This work was supported in part by National Institutes of Health Grants R01 GM070628, R01 HL085595, and K99/R00 HL087607.

REFERENCES


