Gender-based differences in mechanisms of protection in myocardial ischemia–reperfusion injury

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Received 21 December 2006; received in revised form 28 February 2007; accepted 28 March 2007
Available online 4 April 2007
Time for primary review 28 days

Abstract

Pre-menopausal women have reduced risk for cardiovascular disease, and cardiovascular disease rises after menopause. Studies in animal models have also suggested that females have reduced injury following ischemia and reperfusion (I/R). However, a large clinical trial, the Women’s Health Initiative, found an increase in cardiovascular incidents in women on hormone replacement therapy. Taken together, these data suggest that we need a better understanding regarding the mechanisms for the protection observed in the animal studies. In some studies, particularly in the rat, females show less I/R injury; however, in many animal studies no gender difference in I/R injury is observed. Under conditions where calcium is elevated or contractility is increased just prior to ischemia, females have been reported to have less I/R injury than males. Also, estrogen administration has been shown to reduce I/R injury. The protection observed under conditions of increased contractility has been shown to involve an increase in nitric oxide signaling leading to S-nitrosylation of the L-type calcium channel, which reduces calcium loading during ischemia and early reperfusion thereby reducing I/R injury. Estrogen binding to nuclear estrogen receptors results in altered expression of a number of cardioprotective genes such as nitric oxide synthase and heat shock proteins. Estrogen also alters a number of genes involved in metabolism such as lipoprotein lipase, prostaglandin D2 synthase, and peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1-alpha). The effects of these alterations in gene expression may depend on the context of other hormonal stimuli and gene expression as well as physiological stimuli. Furthermore, addition of estrogen has acute non-genomic responses that involve activation of the phosphatidylinositol 3-kinase (PI 3-kinase) pathway, which has been shown to be protective, at least when activated for short durations. This review will summarize the data showing protection in females in animal studies and will summarize the data on possible mechanisms of cardioprotection in females.

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Keywords: Calcium; Receptors; Estrogen; Ischemia; Signal transduction

1. Population-based studies in humans

There are considerable epidemiological data showing that pre-menopausal females have reduced risk for cardiovascular disease [1,2]. Studies have shown that compared to age matched males, pre-menopausal women have a lower incidence of left ventricular (LV) hypertrophy, coronary artery disease, and cardiac remodeling following myocardial infarction [3]. The incidence of cardiovascular disease increases in post-menopausal women [3]. Furthermore, an increase in coronary artery disease was reported in autopsy studies of pre-menopausal women who had undergone an oophorectomy [1]. Gender disparity is also evident in coronary heart disease (CHD) death rates with a rather consistent male to female ratio of 4.5 to 2.5 in countries with different lifestyles and CHD mortality [1]. A limitation of human population-based studies is the inability to distinguish sex-based differences due to a
difference in the underlying causes of ischemia (e.g. atherosclerosis, thrombosis etc.) versus sex difference in cardiomyocytes that can alter the response of the cell to ischemia. Furthermore, a recent large clinical trial failed to show cardioprotection for post-menopausal females on estrogen–progestin replacement [4]. In fact, the women’s health initiative study showed increased cardiovascular risk for females taking an estrogen–progestin combination. These studies suggest that we need a better understanding of the mechanisms responsible for cardioprotection in the female population.

A number of studies have examined sex differences in morbidity and mortality outcomes following coronary revascularization such as percutaneous coronary intervention (PCI) and coronary bypass surgery [5–10]. Studies in the 1980s and early 1990s reported that mortality was significantly higher in females than males [10]. Women undergoing percutaneous revascularization were older and had increased risk factors (diabetes, hypertension) compared to men. In these early studies, the poor outcomes persisted even after adjustment for these comorbidities [10]. In later studies, the sex-based difference in outcomes was reduced; this improvement in females was attributed to advances in techniques (use of stents etc.) and improved pharmacological treatment [5,6,8]. It is noted that women tend to be smaller, which has been taken as a surrogate for smaller vessels and therefore the procedures are more technically difficult in women. Interestingly, the trend toward improvement in outcomes in women has continued and recent studies correcting for higher prevalence of risk factors in women now report that female sex is associated with a reduction in long-term morbidity after PCI [7,9].

Consistent with a role for estrogen in the regulation of the cardiovascular system, several polymorphisms in the estrogen receptor (ER) have been associated with alterations in the cardiovascular system. Peter et al. [11] showed that in women, but not men, two polymorphisms in ER-β were associated with increased LV mass and LV wall thickness, particularly in women with hypertension. The Framingham Heart Study reported that polymorphisms in estrogen receptor-α were associated with measures of adiposity in men [12]. Schuit et al. studied ER-α polymorphisms (c.454–397T>C and c.454–351A>G) and found that the T–A haplotype was associated with an increased risk of myocardial infarction (MI) in post-menopausal women. This study is in contrast to Shearman et al. [13] who reported that the C–C haplotype increased the probability of myocardial infarction (MI) in men. It is possible that the variants have different effects in males versus females. Other possibilities for the discrepancy have been discussed such as differences in baseline covariates and environmental factors [14]. In spite of the discrepancy, both groups find polymorphisms in ER-α to correlate with increased risk of MI. Interestingly, these polymorphisms in ER-α have been reported to alter estradiol levels in post-menopausal females [15].

2. Sex differences in ischemia–reperfusion injury in animal studies

Gender differences in cardioprotection are observed in some animal studies suggesting that compared to males, intact pre-menopausal females (without exogenous estrogen treatment) have reduced ischemia–reperfusion injury [16,17]. Bae and Zhang using a global ischemia model in a perfused rat heart reported that females had smaller infarcts (37%) than males (48%). Wang et al., also using global ischemia in a perfused heart model, found improved post-ischemic recovery of the maximum rate of pressure development and relaxation (+/−dP/dt) in females compared to males. In contrast, other studies using an in vivo model of coronary artery ligation show that in wild type hearts, there is no male–female difference in ischemia–reperfusion injury [18–20]. Male–female differences were also not observed in a model of global ischemia in the perfused mouse heart, measuring either postischemic recovery of left ventricular developed pressure (LVDP) [15] or infarct size [21]. However, even when male–female differences in ischemia–reperfusion injury are not observed in wild type hearts, in a number of transgenic mouse models characterized by increased contractility, females have reduced ischemia–reperfusion injury [20,22,23]. Also under conditions of increased contractility/increased cell calcium such as that occurs with addition of isoproterenol or elevated perfusate calcium, females exhibit reduced ischemia–reperfusion injury compared to males [21,24,25]. It is possible that the inconsistencies regarding endogenous protection in females may be related to the level of catecholamines or contractility in the different models. Kam et al. [25] showed in Langendorff perfused hearts treated with isoproterenol that hearts from intact females had smaller infarcts than hearts from oophorectomized females, which they suggested was related to a higher expression of β1-adrenergic receptors (AR) in hearts from oophorectomized females. The lower expression of β1-AR in intact females would provide a mechanism for the reduced calcium overload [26] and reduced ischemia–reperfusion injury observed with isoproterenol; however the decrease in β1-AR in oophorectomized females would not easily account for the reduced injury observed in the transgenic models or WT hearts with increased extracellular calcium, which are observed in the absence of oophorectomy.

The reduced ischemic injury in hypercontractile females compared to males could be due to a reduction in calcium overload in females or alternatively females could have similar calcium loading but have less injury due to protection, perhaps mediated by increased PI-3 kinase activity [27]. It appears, however, that hypercontractile females have less calcium loading. For example, in transgenic hearts with overexpression of the plasma membrane sodium–calcium exchanger (NCX) [28] and WT hearts with addition of isoproterenol [26,29], females have been shown to have less calcium loading. These data suggest that estrogen reduces the
calcium load prior to ischemia in these genetic models and with isoproterenol, such that there is less calcium loading during ischemia and reperfusion, which results in less injury, since elevated calcium has clearly been shown to increase ischemia–reperfusion injury [30–33]. The mechanism by which estrogen modulates intracellular calcium is likely to be complex, but it appears to be mediated, at least in part, by nitric oxide synthase NOS (see Fig. 1). Estrogen is well known to upregulate NOS [34,35], and inhibitors of (NOS) or genetic deletion of eNOS or nNOS block the protection in females [22,35,36], suggesting a role for nitric oxide (NO). Neudling et al. [34] reported that transfection of COS7 cells with ER-β, but not ER-α, resulted in activation of eNOS and iNOS, suggesting that ER-β can regulate expression of NOS. Sun et al. [35] have reported that females have increased levels of eNOS associated with caveolin 3, the cardiomyocyte-specific caveolin, and that after ischemia–reperfusion, females have increased translocation of nNOS to caveolin-3. Sun et al. [35] further showed that under conditions in which females show less ischemia–reperfusion injury than males, that females have increased S-nitrosylation of the L-type-calcium channel, which results in decreased calcium entry via the L-type calcium channel. Thus, as illustrated in Fig. 1, the increase in NOS in females under conditions associated with increased calcium (which activates NOS) results in increased S-nitrosylation of the L-type-calcium channel, less calcium entry and therefore less calcium loading during ischemia. Previous studies have shown that reducing calcium levels during ischemia is cardioprotective [30,33].

There also are studies showing that treatment of rabbits with exogenous estrogen can reduce ischemia–reperfusion injury [19,37,38]. Booth et al. [39] showed that treatment of oophorectomized rabbits with 20 μg of estradiol 30 min prior to in vivo coronary occlusion reduced infarct size (19%) compared to vehicle (48%). Similarly, Das and Sarkar [38] reported that pretreatment of male rabbits with estradiol (10 μg/kg iv) prior to in vivo coronary artery ligation significantly reduced infarct size (19% versus 40%). They further showed that pretreatment with 5 hydroxydecanoate (an inhibitor of the mito KATP channel) blocked the infarct size reduction afforded by estradiol. Hale et al. reported that acute treatment of male and female rabbits with 1 mg of β-estradiol 15 min prior to in vivo coronary artery ligation reduced infarct size in females (10% in estradiol treated versus 23% in control) and in males (16% in estradiol treated versus 31% in control). Sbarouni et al. [40] showed in oophorectomized female rabbits that administration of conjugated estrogens (1 mg/day/3–4 kg for 4 weeks) reduced infarct size in an in vivo coronary artery ligation model. Lee et al. [41] have reported that administration of estradiol reduced infarcts size in dogs (38% in control versus 16% in estradiol treated). They also found that estradiol decreased phosphorylation of connexin 43 (Cx43) [41]. This is an interesting finding as Cx43 has been associated with cardioprotection. Mice lacking Cx 43 have been shown to have smaller infarcts [42]. However, mice lacking Cx-43 cannot be protected by preconditioning, and this lack of protection is independent of gap junctions, since isolated cardiomyocytes lacking Cx43 are also not protected by preconditioning [43].

Fig. 1. Estrogen has been suggested to increase expression of most isoforms of nitric oxide synthase (NOS). Nitric oxide (NO) generated from NOS has been shown to regulate a number of calcium transporters. Nitric oxide has been suggested to decrease Ca2+ entry via the L-type Ca2+ channel. NOS, localized in the sarcoplasmic reticulum (SR), has been suggested to lead to S-nitrosylation of the SR Ca2+ release channel (i.e. the ryanodine receptor, RyR2), which leads to an increase in open channel probability. Depending on the redox state, nitric oxide has been reported to either increase or decrease activity of the SR/endoplasmic reticulum Ca2+ ATPase (SERCA). Thus with an increase in NOS in females, an increase in Ca2+ will result in greater generation of nitric oxide in females (eNOS and nNOS are Ca2+ activated), which will alter Ca2+ handling. Females have less of an increase in Ca2+ following isoproterenol by a mechanism dependent of NOS. It is therefore proposed that the increase in NOS in females results in less Ca2+ overload in females during ischemia–reperfusion and therefore less injury in females.
The role for Cx43 in preconditioning appears to involve translocation of Cx43 to mitochondria [44]. Interestingly there is an age-dependent loss of Cx 43 with a decrease in mitochondrial Cx43 [45]. This age-dependent decrease in Cx43 is accompanied by a loss of protection by preconditioning [45]. The relationship between estrogen and Cx, and their role in cardioprotection will require further study.

3. A role for estrogen receptors

Although the sex difference in cardioprotection is likely to be multifactorial and involve effects of testosterone and other factors, this review will focus on the effects of estrogen. The basal blood estradiol levels in intact, wild-type C57BL/6 female mice are between 0.4 and 0.9 nM, and in healthy, pre-menopausal women are ~0.9 nM [46]. Peak estrogen levels in pre-menopausal women are ~2 nM. The effects of estrogen are largely if not entirely mediated by binding to receptors. Until recently most effects of estrogen were attributed to genomic effects mediated by estrogen binding to one of the classical nuclear estrogen receptors (ER), ER-α and ER-β. 17β-estradiol binds with similar binding affinity to both ER-α and ER-β (approximately 0.05 to 0.3 nM) [47,48]. Estrogen binds to an ER, which translocates to the nucleus where it functions as a ligand gated transcription factor resulting in altered gene expression. Recently it has been reported that estrogen can bind to receptors in other cellular membranes; either nuclear estrogen receptors in the plasma membrane or G-protein coupled receptors. Estrogen binding to these membrane localized receptors has been suggested to activate signaling cascades such as the PI3-kinase pathway and thereby acutely alter signaling pathways [27,49,50]; however the details of this issue are debated [51].

There are data suggesting that estrogen binds to ER in the plasma membrane [27,50]. Recent studies have suggested that estrogen can also bind to a heptahelical G protein coupled receptor, GPR30 [49,52,53]; however there is some question as to whether GPR30 localizes to the endoplasmic reticulum [52] or the plasma membrane [53]. It has been reported that estrogen binding to GPR30 results in transactivation of the epidermal growth factor receptor thereby activating PI3-kinase, mitogen activated protein kinase (MAPK), and NOS [49]. GPR30 is reported to bind 17β estradiol with an affinity of ~6 nM (compared to estradiol binding to ER at 0.3 nM in the same study) [47]. Thus estrogen binding to ER-α and ER-β can alter gene expression and estrogen binding to GPR30, or ER in the plasma membrane, can rapidly activate signaling pathways such as PI3-kinase, MAPK and NOS which have been shown to be cardioprotective. Interesting recent data show that ER can be post-translationally modified by phosphorylation, S-nitrosylation and O-GlcNAcylation [54–56]. It has been proposed that different modifications of the ER result in differences in activity. Activation of different signaling pathways could result in altered ER signaling via post-translational modification of ER. This will be an interesting area for future investigation.

There is also controversy regarding whether mitochondria contain functional estrogen receptors. Yang et al. [57] used immunocytochemistry, immunoblotting, and mass spectrometry to show that ER-β localizes to the mitochondria. However, Schwend and Gustaffson [58] have questioned the MALDI-TOF identification of ER-β. Others have also reported mitochondrial localization of ER. Pedram et al. [59] find ER-α and ER-β in MCF-7 and endothelial cells. They also find that estrogen inhibits a UV-induced release of cytochrome c, decrease in mitochondrial membrane potential, increase in ROS production, and increase in apoptosis. To determine whether these estradiol effects on mitochondria are mediated by ER localized to mitochondria [27], the ligand-binding domain of ER-α was targeted to the plasma membrane or the nucleus or the mitochondria in HCC-1569 or CHO cells. With nuclear localization of ER-α, addition of estrogen did not protect from UV irradiation. However both mitochondrial and plasma membrane localized ER-α provided protection. In another model Pedram et al. [59] showed that UV irradiation of mitochondria induces cytochrome c release which was blocked by addition of estrogen to the mitochondria. They further showed that the ER-β selective agonist, 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN) was more potent than the ER-α selective agonist 4,4′,4″-(4-propyl-1H-pyrazole-1,3,5-triy)tris-phenol (PPT) in inhibiting cytochrome c release, suggesting ER-β as the mediator of the action. Parkash et al. [60] report that estrogen addition to MCF7 cells can modulate mitochondrial calcium uptake. Similarly, Lobaton et al. [61] reported that several agonists and antagonists of estrogen receptors modulate calcium uptake into the mitochondria. Further study will be needed to establish the relative contributions of estrogen activation of receptors in different locations, which activate potentially different signaling pathways.

To define a role for classical ERs and to identify the specific estrogen receptor involved in cardioprotection in females, studies were done using mice lacking ER-α (αERKO) and mice lacking ER-β (βERKO) [17,21,62–65]. Studies have also been performed using ER-α and ER-β selective agonists [37,66,67]. One approach has been to study hearts subjected to ischemia following brief treatment with isoproterenol, and to measure postischemic recovery of contractile function and infarct size in wild-type (WT) male and female mouse hearts, and female αERKO and βERKO hearts. Under these conditions, WT males exhibited significantly poorer functional recovery and more necrosis than WT females [21]. αERKO females exhibited ischemia–reperfusion injury similar to that observed in WT females, whereas BERKO females exhibited significantly poorer functional recovery and more necrosis than WT females and were more similar to WT males. Using a model of trauma–hemorrhage shock injury, Chaudry and coworkers [66,67] found that males have depressed cardiovascular function which can be reversed by administration of 17 β-estradiol just following the trauma–hemorrhage. Hsieh et al. [67] found that 24 h following trauma hemorrhage, there is a decrease in
PGC-1α and ATP levels. They report that this decline in PGC-1α and ATP was reversed if estradiol or an ER-β selective agonist DNP was administered just following trauma–hemorrhage. These data suggest that the beneficial effects of estrogen in trauma–hemorrhage may be mediated by ER-β upregulation of PGC-1α. In another study by this group, Yu et al. [66] reported that trauma–hemorrhage resulted in a decrease in heat shock proteins 32, 60, 70 and 90 mRNA and heat shock factor-1 DNA binding and that these effects were blocked by administration of an ER-β agonist (DPN). In addition, Hsieh et al. [67] report that mitochondrial ER-β is important for the upregulation of mitochondrial respiratory complex proteins, and that DPN administration protects in a trauma–hemorrhage model by activation of mitochondrial ER-β. These data suggest that estrogen, through the β-estrogen receptor, plays a protective role in the female heart.

In contrast to these studies, Zhai et al. [63] found that hearts from αERKO mice showed increased injury in a model in which hearts were subjected to 45 min of global ischemia at 4° C followed by 180 min of oxygenated reperfusion at 37° C. Male αERKO hearts started beating later and had more fibrillation than WT hearts. Wang et al. [17] subjected Langendorff perfused mouse hearts to 20 min of ischemia and 60 min of reperfusion and found that male hearts had poorer recovery of contractile function (+dP/dt than females). They further showed that female mice lacking ER-α had a similar recovery of + and −dP/dt to WT and αERKO males, which was worse than that observed in WT females. Also consistent with a role for ER-α in cardioprotection, Booth et al. [37] reported that bolus iv administration of an ER-α selective agonist, PPT (3 mg/kg) 30 min prior to coronary occlusion, significantly reduced infarct size (18% PPT versus 45% vehicle) in rabbit hearts. The protection afforded by PPT was blocked by co-administration of the ER antagonist, ICI-182,780. Administration of the ER-β selective agonist DPN (3 mg/kg) did not reduce infarct size (45%). In oophorectomized rabbits, PPT at a 3 mg/kg dose also reduces infarct size, although it was not as protective as estradiol. However 10 mg/kg of PPT was as protective as estradiol.

Thus there is no clear consensus regarding the role of ER-α versus ER-β in cardioprotection. This discrepancy may be due to the different models of injury, the different end-points for protection, and the different doses of estrogen. Most of the studies examined I/R injury; however a large variety of endpoints were used making it difficult to compare. Infarct size was used as an endpoint in two studies with different conclusions; however the models used were very different. Gabel et al. [21] examined differences in I/R injury in female WT, and ERKO mice with no exogenous estrogen whereas Booth et al. [37] examined the effect of a bolus addition of PPT and DPN to female rabbits 30 min prior to coronary occlusion. The beneficial effects attributed to ER-β by Gabel et al. [21] are likely long-term effects of estrogen most likely attributable to upregulation of genes by ER-β. In contrast, the protection observed in the study of Booth et al. [37] is likely due to more short-term non-genomic effects of estrogen, since in the protocol used by Booth et al, there may not have been sufficient time for upregulation of ER-β dependent genes. Thus, although bolus addition of an alpha selective estrogen receptor agonist may be a useful therapeutic agent, it may not mimic the chronic effects of estrogen in hormone replacement therapy. Taken together these data suggest that both ER-α and ER-β may mediate protection against different end-points in different models of injury. The data would also be consistent with the hypothesis that some of the beneficial effects of estrogen may be mediated by ER-dependent upregulation of genes and some benefit may derive from estrogen mediated effects on acute signaling mechanisms, and these effects may rely on different ERs. In addition, in in vivo studies, it is not clear whether the protective effects of estrogen are mediated by direct effects on cardiomyocytes, the vasculature, or some other target tissue cell types. Clearly additional studies will be necessary to delineate the relative role of ERs in cardioprotection. Studies with cardiomyocyte specific loss of ER-α and ER-β would help to define the direct effects of estrogen in heart.

4. Gender differences in hypertrophy

Although hypertrophy is not the primary focus of this review, with hypertrophy induced by transaortic constriction, females have also been shown to have reduced cardiac hypertrophy compared to males [64]. Treatment of oophorectomized females with estrogen has also been reported to reduce hypertrophy [68]. In many animal models of hypertrophy/heart failure, females have improved survival and/or improved contractile function [69–71].

In contrast to the discrepancies regarding the role of ER-α and ER-β in protection from ischemia–reperfusion injury, there is good agreement that ER-β is important for the reduced hypertrophy observed in many models. Skavdahl et al. [63] performed transverse aortic constriction (TAC) and sham operations in male and female wild type (WT), αERKO, and βERKO mice [64]. WT male mice subjected to TAC showed a 64% increase in heart to body weight (HW/BW) ratio compared to sham. WT female mice subjected to TAC showed a 31% increase in HW/BW compared to sham, which was significantly less than their male counterparts, αERKO females developed an HW/BW ratio nearly identical to that seen in WT littermate females in response to TAC, indicating that ER-α is not essential for the attenuation of hypertrophy observed in WT females. In contrast, βERKO females responded to TAC with a significantly greater increase in HW/BW ratio compared to WT littermate females, indicating an important role for ER-β in attenuating the hypertrophic response to pressure overload. Similarly, Pelzer et al. [62] have reported that mice lacking ER-β have increased mortality and increased pro-atral natriuretic peptide levels in a cardiac hypertrophy/failure model resulting from myocardial infarction. Also consistent with a role for ER-β in hypertrophy, Babiker et al. [65] used ER-α-and ER-
β-deficient mice and showed that ER-β mediates the estradiol-dependent reduction in left ventricular hypertrophy following TAC. Thus, ER-β appears to be involved in attenuating pressure-overload hypertrophy observed in females. Interestingly, Peter et al. [11] showed that in women, but not men, two polymorphisms in ER-β (ERS2 rs1256031 and ERS2 rs1256059) were associated with increased LV mass and LV wall thickness. The protective effects of estrogen with regard to cardiac hypertrophy also appear to be age-dependent. Jazbutyte et al. [72] show that estrogen administration to young and senescent oophorectomized SHR rats inhibited uterine atrophy and gain of body weight, but cardiac hypertrophy was attenuated only in the young rats.

5. Cardiac genes regulated by estrogen

It is likely that much of the protection afforded by estrogen is due to altered gene expression. It should also be noted that estrogen mediated changes in gene expression may be beneficial in some contexts and detrimental in others. Although estrogen regulation of gene expression has been examined in many other tissues, particularly estrogen responsive organs such as breast and uterus, there are few reports on cardiac genes regulated by estrogen. Ottsuki et al. [73] examined the effect of long-term estrogen treatment on gene expression in the hearts of vehicle and estradiol treated oophorectomized females. Lipocalin-type prostaglandin D synthase (L-PDGS) was significantly upregulated, while procollagen Types I, III and XV were downregulated. Using mice lacking ER-α or ER-β, Ottsuki et al. [73] reported that ER-β was responsible for regulation of L-PDGS. Gabel et al. [21] used gene array profiling to examine genes differentially expressed in hearts from female mice lacking ER-β. Lipoprotein lipase, solute carrier family member 4, and SPOT 14 homologue were among the genes regulated by ER-β. Estrogen has also been reported to stimulate expression of the adenine nucleotide translocator (ANT1) in female rat hearts [74]. Furthermore, Stirone et al. [75] have shown in blood vessels that estrogen treatment increased the protein levels of cytochrome c, subunits I and IV of complex IV, and manganese superoxide dismutase. They suggest that estrogen regulates mitochondrial function by upregulating electron transport chain components and decreasing reactive oxygen species (ROS) production. Others also report sexual dimorphisms in mitochondrial biogenesis and levels of proteins involved in oxidative metabolism [76,77]. The effect of estrogen on ROS generation is debated. Estrogen has been suggested to be an antioxidant and some of the protection afforded by estrogen has been attributed to its antioxidant effects [78,79]. However, others studies suggest that estrogen increases mitochondrial ROS production, which initiates cell signaling pathways [60]. The effect of estrogen on mitochondrial ROS generation may depend on the level of estrogen and other signaling pathways that are activated in the cell. It is also possible that similar to cardiac preconditioning, estrogen may increase low, signaling levels of ROS, but reduce high, detrimental levels of ROS. Clearly additional studies will be necessary to clarify the role of estrogen in altering ROS production.

In the studies referred to above, it is unclear whether estrogen is having a direct or indirect effect on gene expression in the heart. For example, estrogen could mediate release of soluble factors from a tissue other than heart, which results in altered gene expression in the heart. However, Nuedling et al. [34] added estrogen to neonatal rat cardiomyocytes and showed upregulation of eNOS and iNOS, demonstrating the estrogen has direct effects on cardiomyocyte gene expression. In further support for a direct effect of estrogen, Nikolic et al. [80] perfused hearts from oophorectomized mice with DNP for 2 h to examine direct effects of the ER-β selective agonist on gene expression. DNP altered expression of 145 genes including cyclooxygenase 2, heat shock proteins and enzymes involved in regulating glycolysis. Furthermore, Jovanovic et al. [81] showed that cardiomyocytes pretreated with estradiol (10 nM) had reduced cytosolic calcium overload following metabolic inhibition (3 min exposure to dinitrophenol followed by washout of dinitrophenol). These data suggest that estrogen has direct protective effects on cardiac myocytes.

ER-α and ER-β have been suggested to regulate different genes and it has been proposed that ER-α and ER-β may oppose or counterbalance each other [82]. Thus altering the ratio of ER-α versus ER-β in the heart (or other tissues) could alter gene expression in the absence of a change in estradiol levels.

6. Potential cardioprotective mechanism of estrogen

Estrogen can alter cardiac function by effects on other tissues which leads to the release of factors that alter cardiac function or gene expression. Estrogen also has direct effects on heart. Estrogen binding to ER-α and ER-β leads to altered expression of several genes such as NOS isoforms [34,35], AKT [50], L-PGDS [73], and LPL [64] to name a few. The precise interaction between ER-α and ER-β is still not clear and it is suggested that activation of ER-α can oppose ER-β gene expression and vice versa. In addition, activation of signaling pathways can alter post-translational modification of ER which is suggested to alter ER activity. Thus even with similar estradiol levels, depending on the relative expression of ER-α and ER-β (not to mention interaction with other receptors such as progesterin receptors) and activation of other signaling pathways in the cell, there can be differential gene expression. In addition to binding to the ligand activated transcription factors (ER-α and ER-β), estrogen can also bind to a plasma membrane heptahelical G protein coupled receptor and activate additional signaling pathways such as activation of the PI3-kinase, AKT and eNOS pathways [53]. Of potential importance for cardioprotection is the synergy between the ER and GPR30 pathways. For example, ER pathways have been shown to lead to upregulation of eNOS, whereas GPR30 signaling results in activation of AKT and
increased phosphorylation and activity of eNOS. Thus estrogen acting via these two distinct receptors leads to increased levels of eNOS and increased activity of eNOS. Activation of NOS and production of NO have been shown to be cardioprotective. Increased NO can mediate protection via activation of guanylyl cyclase [83,84] or via S-nitrosylation of proteins such as the L-type Ca\(^{2+}\) channel or mitochondrial proteins [35,85,86]. As discussed earlier, increased S-nitrosylation of L-type Ca\(^{2+}\) channels in female hearts results in reduced Ca\(^{2+}\) entry during ischemia and therefore reduced calcium loading during ischemia–reperfusion, which has been shown to be cardioprotective. In addition estrogen via GPR30 results in activation of the PI3-kinase and MAPK pathways, which have been reported to be cardioprotective. This is an exciting time for studying cardiovascular effects of estrogen. As we learn more about the different mechanisms of estrogen action, we will be better able to design estrogen mimetics that elicit the beneficial effects of estrogen without potentially detrimental effects on estrogen-sensitive neoplasms.

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