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Review

Estrogen and the female heart

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ABSTRACT

Estrogen has a plethora of effects in the cardiovascular system. Studies of estrogen and the heart span human clinical trials and basic cell and molecular investigations. Greater understanding of cell and molecular responses to estrogens can provide further insights into the findings of clinical studies. Differences in expression and cellular/intracellular distribution of the two main receptors, estrogen receptor (ER) α and β, are thought to account for the specificity and differences in responses to estrogen. Much remains to be learned in this area, but cellular distribution within the cardiovascular system is becoming clearer. Identification of GPER as a third ER has introduced further complexity to the system. 17β-estradiol (E2), the most potent human estrogen, clearly has protective properties activating a signaling cascade leading to cellular protection and also influencing expression of the protective heat shock proteins (HSP). E2 protects the heart from ischemic injury in basic studies, but the picture is more involved in the whole organism and clinical studies. Here the complexity of E2's widespread effects comes into play and makes interpretation of findings more challenging. Estrogen loss occurs primarily with aging, but few studies have used aged models despite clear evidence of differences between the response to estrogen deficiency in adult and aged animals. Thus more work is needed focusing on the effects of aging vs. estrogen loss on the cardiovascular system.

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1. Introduction

Estrogens are potent steroid hormones present in high levels in women from adolescence to menopause. Estrogens have many properties, both protective and deleterious, and there have been numerous reviews on estrogen and cardiovascular disease (Xing et al., 2009; Deschamps et al., 2010; Farsetti et al., 2008; Knowlton and Lee, 2012; Bell et al., 2013; Korzick and Lancaster, 2013). The pace and depth of estrogen research has steadily increased since the identification of the estrogen receptors and the development

Abbreviations: ANT-1, adenine nucleotide translocator 1; CEE, conjugated equine estrogen; CHD, coronary heart disease; Cx43, Connexin 43; EMSA, electrophoretic mobility shift assay; eNOS, endothelial nitric oxide synthase; ER, estrogen receptor; E2, 17β-estradiol; E_2, estrogen; GPER, G-protein-coupled estrogen receptor; HSF, heat shock factor; HSP, heat shock protein; iNOS, inducible nitric oxide synthase; IPC, ischemic preconditioning; IR, ischemia reperfusion; MI, myocardial infarction; NB, Norway Brown; OVX, ovariectomy; PTM, post translational modification; VSMC, vascular smooth muscle cell.

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of knockout models, facilitating investigation of the many functions of estrogens.

Estrogen receptors, genomic/non-genomic effects – Estrogen binds two intracellular receptors, estrogen receptor (ER)α and ERβ. These two receptors are primarily found in the cytosol and in the nucleus (Fig. 1). They are also loosely associated with the plasma membrane, rather than transmembrane like many other receptors. Mitochondrial localization has also been reported. Genomic actions mediated by nuclear ERα are well-described (Deroo and Korach, 2006; Driggers and Segars, 2002) and involve ligand binding at estrogen (E_2) response elements. Non-genomic (rapid) effects of E_2 are thought to be mediated by ERα and/or ERβ localized to the plasma membrane (Alexaki et al., 2006; Yang et al., 2004; Zhai et al., 2001) and associated functions include Ca^{2+} homeostasis, anti-apoptotic effects and mitochondrial metabolism (Yang et al., 2004). Rapid ER signals are also known to regulate ER gene transcription in the myocardium (Meldrum, 2006). In this regard, ERs are subject to post-translational modification (PTM) through phosphorylation, acetylation and sumoylation, which not only has the potential to influence ER activity, but may also influence ER stability and localization, particularly with aging (for review see Keung et al., 2011; Nakamura et al., 2004; Watanabe et al., 2003; Takahashi et al., 2003; Chen et al., 2002; Faus and Haendler, 2006; Fu et al., 2004; Zhang and Trudeau, 2006).

A third receptor, G-protein-coupled estrogen receptor (GPER) or GPR30, has been more recently described. GPER is a classic membrane receptor with 7 transmembrane spanning domains (Revankar et al., 2005). This receptor is discussed below.

It is thought that at least part of the specificity of 17β-estradiol (E2) signaling is the result of differential expression and cellular localization of the ERs in different tissues and cell types. As ERα and ERβ have been studied the longest, much more is known about their localization than that of GPER. Deconvolution of rat endothelium (cerebral and coronary arteries) images demonstrated that about a third of ERα co-localized with caveolin-1 at the plasma membrane (Dan et al., 2003). In this study, ERβ was predominantly nuclear. In studies of the ovine uterine artery endothelial cells, ERα was present in the nucleus and associated with caveolin-1 at the plasma membrane (Liao et al., 2005). In contrast, ERβ mRNA levels were much less than those of ERα, and ERβ did not localize with caveolin-1. Thus in adult endothelial cells, ERα is nuclear and membrane associated, co-localizing with caveolin.

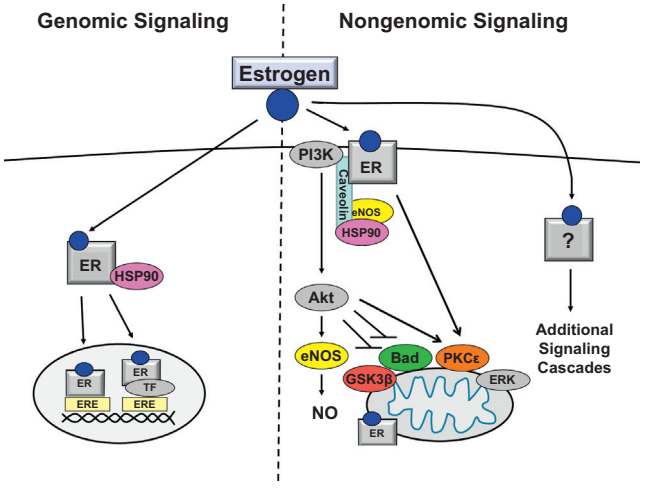

Fig. 1. Simplified schematic of cardioprotective cellular signaling mediated by genomic and non-genomic actions of estrogen receptor (ER) activation. Genomic actions mediated by nuclear ERs involve nuclear translocation and activation of protective gene transcription. Non-genomic ER actions include activation of downstream cardioprotective signaling pathways and possible mitochondrial localization. See text for specific details. Abbreviations: BAD, Bcl-2-associated death promoter; diacylglycerol, DAG; eNOS, endothelial nitric oxide synthase; ERE, estrogen response element; ERK, extracellular signal-regulated kinases; GSK-3β, glycogen synthase kinase-3β; HSP90, heat shock90; PI3 K, phosphoinositide 3-kinase; PLC, phospholipase C; PKCε, protein kinase Cε; TF, transcription factor. Updated from Stice and Knowlton (2008).

Considerably less is known about the ERs in cardiac myocytes and vascular smooth muscle cells (VSMCs), and few studies have investigated their precise subcellular localization in these specific tissues (Stice et al., 2011; Novotny et al., 2009). For cardiac myocytes, ERα has been reported to localize to nuclear, cytosolic, mitochondrial, and plasma membrane fractions, respectively (Novotny et al., 2009). ERβ expression has been reported in human cardiac mitochondria (Yang et al., 2004), however controversy exists regarding antibody quality and species dependency (Tomicek et al., 2013). Indeed, we were unable to identify ERβ mRNA in hearts of adult or aged Fisher 344 female rats (Tomicek et al., 2013). Much remains to be defined with regards to expression and localization of ERα and β in cardiac myocytes.

In contrast, more is known about VSMCs. In male SD rat mesenteric artery VSMCs ERα (the ER46 fragment) and ERβ associated with caveolin-1 (Keung et al., 2011). Both ERα and ERβ are found in adult human aortic VSMCs and both are predominantly nuclear (Keung et al., 2011; Nakamura et al., 2004). VSMCs from the aortic arch of 8 week old rats expressed ERα and ERβ based on RT-PCR (Watanabe et al., 2003). Similarly, primary adult human VSMCs contained both receptors, but less ERβ than ERα was found (Takahashi et al., 2003). VSMC cells lines had clearly different ERα and ERβ expression compared to primary cells and pathology specimens. Three different VSMCs cell lines expressed either ERα or ERβ, but not both, which makes these cell lines less than desirable models for investigation of ERα and ERβ in VSMCs (Nakamura et al., 2004; Watanabe et al., 2003). Multiple studies have shown that ERα and ERβ are both expressed in VSMCs and are primarily in the nucleus, however both ERα and ERβ are also at the plasma membrane and co-localize with caveolin-1. The receptors's relative quantities and intracellular allocation remains to be conclusively defined.

ER membrane complex, caveolae and caveolin – As noted above, ERα and β demonstrate both genomic and non-genomic effects, also referred to as nuclear and non-nuclear events. ERα is found in caveolae, where it complexes with caveolin 1, HSP90, eNOS, c-Src, Akt, and PI3K in the plasma membrane (Kim and Bender, 2009; Haynes et al., 2003; Li et al., 2003), and this is regulated by palmitoylation (Levin, 2010; Acconcia et al., 2005; Chambliss et al., 2000). ER46, an ERα splice variant, rather than the full length receptor, is present in the caveolar complex (Kim and Bender, 2009; Li et al., 2003; Levin, 2010; Figtree et al., 2003). The co-localization of ERα, eNOS and HSP90 within the caveolae facilitates eNOS activation by E2 (Levin, 2010; Chambliss et al., 2000; Mineo and Shaul, 2006). The chaperone protein, HSP90, promotes interaction of ER with signaling enzymes, such as eNOS. In contrast, caveolin-1 inhibits eNOS activity. Caveolin-1 knockout leads to increased eNOS activity in mice (Razani et al., 2001). The multiple proteins needed for the caveolar-ERα signaling complex have been reviewed in great detail (Boonyaratanakornkit, 2011). As would be expected given caveolin-1's inhibitory effects, eNOS activation entails separation from caveolin-1; this separation is usually mediated by calcium/calmodulin, but in special circumstances, including shear stress, eNOS activation does not require calcium. Several excellent papers have reviewed in details the steps involved in regulation of eNOS activation (Fleming, 2010; Dessy et al., 2010).

GPER – There persists some lingering controversy regarding whether GPER is an ER, and more work is necessary to fully delineate the role of GPER in estrogen signaling (Wu et al., 2011). Nonetheless, there is increasing acceptance of GPER as key receptor