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Estrogen and the cardiovascular system

A.A. Knowlton [a,b,c,*], A.R. Lee [a,b]

[a] Molecular and Cellular Cardiology, Department of Medicine, University of California, Davis, United States
[b] Department of Medical Pharmacology, University of California, Davis, United States
[c] Northern California, VA, Sacramento, CA, United States

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ABSTRACT

Estrogen is a potent steroid with pleiotropic effects, which have yet to be fully elucidated. Estrogen has both nuclear and non-nuclear effects. The rapid response to estrogen, which involves a membrane associated estrogen receptor(ER) and is protective, involves signaling through PI3K, Akt, and ERK 1/2. The nuclear response is much slower, as the ER-estrogen complex moves to the nucleus, where it functions as a transcription factor, both activating and repressing gene expression. Several different ERs regulate the specificity of response to estrogen, and appear to have specific effects in cardiac remodeling and the response to injury. However, much remains to be understood about the selectivity of these receptors and their specific effects on gene expression. Basic studies have demonstrated that estrogen treatment prevents apoptosis and necrosis of cardiac and endothelial cells. Estrogen also attenuates pathologic cardiac hypertrophy. Estrogen may have great benefit in aging as an anti-inflammatory agent. However, clinical investigations of estrogen have had mixed results, and not shown the clear-cut benefit of more basic investigations. This can be explained in part by differences in study design: in basic studies estrogen treatment was used immediately or shortly after ovariectomy, while in some key clinical trials, estrogen was given years after menopause. Further basic research into the underlying molecular mechanisms of estrogen's actions is essential to provide a better comprehension of the many properties of this powerful hormone.

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Abbreviations: CEE, conjugated equine estrogen; DPN, dipropylnitrile, an ERβ agonist; E2, 17β-estradiol, the most potent form of estrogen; ER, estrogen receptor; HRT, hormone replacement therapy; HSF, heat shock factor; HSP, heat shock protein; HUVECs, human umbilical vein endothelial cells; mt, mitochondria; NB, Norway brown rat strain; NRF-1, nuclear respiratory factor 1; ovx, ovariectomy or ovariectomized; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; SHR, spontaneously hypertensive rat strain; TAC, transverse aortic constriction; Tfam, mitochondrial transcription factor A; VSMCs, vascular smooth muscle cells.

* Corresponding author at: Molecular & Cellular Cardiology, University of California, Davis, One Shields Avenue, Davis, CA 95616, United States. Tel.: 530 752 5461; fax: 530 754 7167.
E-mail address: aaknowlton@ucdavis.edu (A.A. Knowlton).

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1. Introduction

Estrogen is a potent steroid hormone present in high levels in females from adolescence to menopause and in low levels in men. Much anecdotal evidence accumulated over many years had supported the idea that estrogen post-menopause reduced cardiovascular disease. In the 1990s several double blind, controlled trials of estrogen replacement post-menopause were conducted including the Women's Health Initiative (WHI) and the Heart and Estrogen/progestin Replacement Study (HERS) (Hulley et al., 1998; Grady et al., 2002; Writing Group for the Women's Health Initiative Investigators, 2002). These studies showed an increased risk of both cancer and cardiovascular disease in those taking estrogen replacement. However scrutiny of these studies led to recognition of a possible cause of lack of estrogen efficacy based on the design of these studies, as a result of the effort to enroll women who definitely were post-menopause; 10 years had elapsed on average between menopause and the onset of estrogen replacement in these studies. This realization led to the development of the timing hypothesis (Grodstein et al., 2003; Dubey et al., 2005; Barrett-Connor, 2007). This hypothesis proposed that the prolonged delay after the onset of menopause led to significant tissue and gene expression changes, such that late estrogen replacement post-menopause was occurring in a markedly different tissue state than 10 years earlier, when estrogen production ceased.

1.1. Hormone replacement therapy controversies

A second issue in hormone replacement therapy (HRT) is the type and route of hormone replacement. The current review focuses on estrogen; however the combination of progesterone and estrogen has been addressed elsewhere by others (Canonico et al., 2010; Tchaikovski & Rosing, 2010). Estrogen is a potent steroid hormone with 17β-estradiol (E2) being the most active metabolite. There are two key issues in hormone replacement. First, the oral delivery of estrogen results in high hepatic levels of estrogen from first pass metabolism, which can stimulate protein synthesis and appears to be associated with increased risk of thrombosis compared to transdermal delivery, though this remains controversial (Bagot et al., 2010; Canonico et al., 2010; Tchaikovski & Rosing, 2010; Olié et al., 2011). Estrogen will, through its nuclear properties, change hepatic gene expression and can potentially effect expression of genes involved in coagulation. Estrogen replacement using a transdermal patch avoids the first pass surge in hepatic estrogen levels and may have less complications, though some controversy remains. Second, conjugated equine estrogen (CEE) is the most commonly used estrogen compound for HRT. CEE is derived from the urine of pregnant mares and includes estrogen compounds not found in humans. The composition of CEE differs significantly from the estrogens found in premenopausal women. Different estrogens differ in their binding affinities and selectivity for the estrogen receptors (ER); consequently they have different downstream effects. These differences could have a significant impact on the effects of HRT. CEE contains sodium estrone sulfate, sodium equilin sulfate, and sodium 17α-dihydroequilenin, but no 17β-estradiol, the major and most potent form of estrogen found in premenopausal women (Booth & Lucchesi, 2008). Recently estrone levels have been linked with thrombin generation, a central step in the coagulation cascade, in postmenopausal women (Bagot et al., 2010). Thus, estrones have the potential to greatly increase the risk of thrombosis, and as CEE contains a large amount of estrones, CEE would be expected to be associated with an increased risk of thrombotic events. However, the studies in this area are limited and it is premature to draw definitive conclusions.

Estrogen is a complex hormone with pleiotropic effects. Basic research is essential to understand the underlying cell and molecular mechanisms of estrogen's actions and to gain insight into the clinical effects of estrogen. This review will focus on basic cardiovascular research on estrogen, which provides the underpinning for our understanding of the clinical effects of this hormone.

2. Aging, inflammation and fibrosis

Aging and estrogen loss are indelibly linked. Aging is associated with inflammation and increased inflammatory serum cytokines such as TNF and IL-6 (Donato et al., 2008; Chung et al., 2009). Aging is also associated with increased oxidative stress and a blunting of the protective heat shock response in males and females (Fawcett et al., 1994; Locke & Tanguay, 1996; Gutsmann-Conrad et al., 1998; Jackson & McArdle, 2011; Stice et al., 2011). In the cardiovascular system, aging is accompanied by increased stiffness, increased fibrosis, loss of contractile reserve, increased ROS and endothelial dysfunction. All of these factors contribute to cardiovascular dysfunction. It is in this setting that estrogen, which is an antioxidant through indirect upregulation of antioxidant gene expression and increasing eNOS activity while decreasing superoxide production, is lost through menopause (Barbacanne et al., 1999; Florian et al., 2004; Siow et al., 2007). A key question is whether estrogen or a synthetic estrogen receptor modulator (SERM) can ameliorate any of these changes. SERMs have differing receptor specificity and tissue responses. Two SERMs are used clinically, tamoxifen and raloxifene, and many more are under development. SERMs have the potential to provide a selective activation profile of estrogen targets.

2.1. Summary

Estrogen's effects and the loss of estrogen must be considered in the setting of aging. There is potential for pharmacotherapeutics to eventually ameliorate some of the changes that occur with estrogen loss. In this review, we will focus on current understanding of the cellular and molecular changes associated with aging and estrogen loss and their implications for cardiovascular health.

3. Estrogen receptors

There are two established estrogen receptors, α and β (Fig. 1). Differences in tissue distribution in these receptors are thought to modulate tissue response to estrogen, but this remains to be proven. Post-translational modifications may be important modulators of receptor function, but studies in this area have been limited. Several differences between ERα and β have been identified in the cardiovascular system, as will be discussed. These differences are summarized in Table 1.

ERα and β are found both in association with the plasma membrane (not transmembrane), in the cytoplasm and in the nucleus. Palmitoylation of ERα regulates its localization to the plasma membrane, where it is found in caveolae (Chambliss et al., 2000; Acconcia et al., 2005; Levin, 2010). ERα complexes with caveolin 1, c-Src, Akt, PI3K, HSP90 and eNOS in caveolae in the plasma membrane (Haynes et al., 2003; Li et al., 2003; Kim & Bender, 2009). A number of studies indicate that an ERα splice variant, ER46, is present in this complex, rather than the full length receptor (Figtree et al., 2003; Li et al., 2003; Kim & Bender, 2009; Keung et al., 2011). ERα's co-localization with HSP90 and eNOS within the caveolae facilitates activation of eNOS by E2 (Chambliss et al., 2000; Mineo & Shaul, 2006; Levin, 2010). The chaperone protein, HSP90, assists with the interaction between the ER and eNOS and other signaling enzymes. Caveolin-1 actually inhibits eNOS, and caveolin-1 knockout mice have increased eNOS activity (Razani et al., 2001). The multiple proteins involved in the assembly of the ERα signaling complex in caveolae have been recently reviewed in detail (Boonyaratanakornkit, 2011). Activation of eNOS requires dissociation from caveolin-1; frequently this is mediated by calcium/calmodulin, but in some circumstances, such as shear stress, eNOS activation is