PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 74 (2000) 337–343

The Journal of Steroid Biochemistry & Molecular Biology

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Mechanisms of estrogen action in the cardiovascular system

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"The administration of estrogens in animals tends to inhibit the development of atherosclerosis otherwise produced by a high cholesterol diet. [Therefore], attempts are being made in human males to prevent further progress of the disease in cases of angina or previous coronary thrombosis by long-range estrogen therapy. This work is in its infancy …. It is hoped that, with further research, compounds might be found that eliminate the undesirable action of such hormones and yet retain its beneficial effects on arteries."

Samuel A. Levine, *Clinical Heart Disease*, 1958.

The incidence of cardiovascular disease differs significantly between men and women due to a number of complex factors [1,2]. The incidence of atherosclerotic diseases is low in premenopausal women, rises significantly in postmenopausal women, and is reduced to premenopausal levels by estrogen replacement therapy [1–3]. While the mechanisms responsible for these observations are incompletely understood, until recently the atheroprotective effects of estrogen have been attributed principally to the hormone's effects on circulating lipid levels [4]. However, estrogen-induced alterations in lipid levels account for only approximately one-third of the observed clinical benefits of estrogen [5–8]. Recent data suggest that direct actions of estrogen on blood vessels contribute significantly to the cardiovascular protective effects of estrogen (reviewed in [1,6,9]). The vasculature, like the reproductive tissues, bone, liver and brain, is now recognized as an important target for estrogen action. Direct effect of estrogen on the vasculature thus are of central importance to clinical medicine, and raise the possibility that vasospecific estrogen-like compounds of the type envisioned by Samuel Levine nearly 50 years ago will emerge soon as novel therapies for cardiovascular diseases in both women and men.

In the vasculature, estrogen has both rapid effects on vasomotor tone, and longer-term effects on the response of the vessel to injury and the development of atherosclerosis (Fig. 1). In the past decade tremendous advances have been made in our understanding of the molecular mechanisms by which steroid hormones exert biologic effects on gene expression [10]. *Longer-term, genomic* effects, such as the protective effects of estrogen on cell proliferation and matrix deposition during the vascular injury response and the development of atherosclerosis, occur over hours to days following estrogen treatment and depend on changes in transcription of vascular genes. Estrogen also leads to rapid, non-genomic vasodilation that take place within 5–20 min following estrogen exposure without any accompanying change in gene expression. Thus, the vascular signaling pathways that estrogen controls are complex. This article examines recent information regarding the mechanisms by which estrogen protects against vascular disease and the implications of this biology for clinical medicine.

2. Vascular estrogen receptors

Two estrogen receptors are currently known; ERα and ERβ, and their molecular biology and mechanism of action are reviewed in detail elsewhere in this issue. Normal blood vessels are complex structures, with walls comprised principally of smooth muscles cells and an endothelial cell lining (Fig. 1). Vascular endothelial cells and smooth muscle cells first were found to bind estrogen with high affinity two decades ago [6]. Estrogen receptors α and β recently have been identified in vascular smooth muscle and endothelial cells from both

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PII: S0960-0760(00)00110-2

men and women. However, the absolute level of expression of ERα and ERβ in different clinical settings, in different vascular beds, and in the vasculature of the two sexes is not yet well characterized. ERα likely mediates at least some estrogen-dependent activation of target genes in vascular smooth muscle [11–13] and endothelial cells [14–16] and the list of both confirmed and potential vascular target genes is now substantial [1]. Isoforms of ERα are also expressed in vascular smooth muscle cells[17,18], and may prove to be of physiologic relevance[18]. Additional estrogen-responsive genes important in the vasculature are being sought in many laboratories as potential targets for drug development. Estrogen receptors are activated by estrogen binding and can also be activated by growth factors in the absence of steroid hormone [19–22], which may be relevant when local growth factor concentrations are increased and/or when hormone levels are low (postmenopausal women, men). Hormone-independent activation of estrogen receptors likely occurs by different intracellular pathways in vascular and non-vascular cells [22]. ERβ is expressed in primate and human arteries and veins [23] and ME Mendelsohn, unpublished observations), as well as in the blood vessels of normal (wild type) mice and rats [24,25], and in mice in which the ERα gene has been disrupted [24]. Vascular cells are thus among the first examples of cells that express both estrogen receptors α and β.

3. Vascular cell exposure to estrogens

Vascular cells are exposed to estrogens from a number of sources. In premenopausal women, 17β-estradiol produced by the ovaries is the major circulating estrogenic compound, though a large number of other circulating estrogens and estrogen metabolites have been identified. Circulating estradiol concentrations, low in adolescent girls, begin to cycle in women with the onset of menses, ranging from less than 0.36 nM in the follicular phase to about 2.8 nM in midcycle. During pregnancy, circulating estrogen levels can be as high as 70 nM. E2 levels fall following menopause to low levels similar to those found in men (0.14–0.21 nM) [26]. Vascular cells also are exposed to estrogens from exogenous sources. In US, hormone replacement therapy (HRT) in postmenopausal women is given typically as a complex mixture of conjugated equine estrogens, though a number of other oral and/or transdermal preparations also are coming into use. A selective estrogen receptor modulator (SERM) for bone, raloxifene [27], recently became available for the treatment of osteoporosis. A SERM specific for the vasculature, such as that predicted by Samuel Levine years ago, has not yet been identified. Vascular cells are also exposed to exogenous estrogens from dietary sources. Phytoestrogens, a diverse group of compounds found in various plant-derived foods and beverages, can have both estrogenic and anti-estrogenic effects [28]. Vascu-

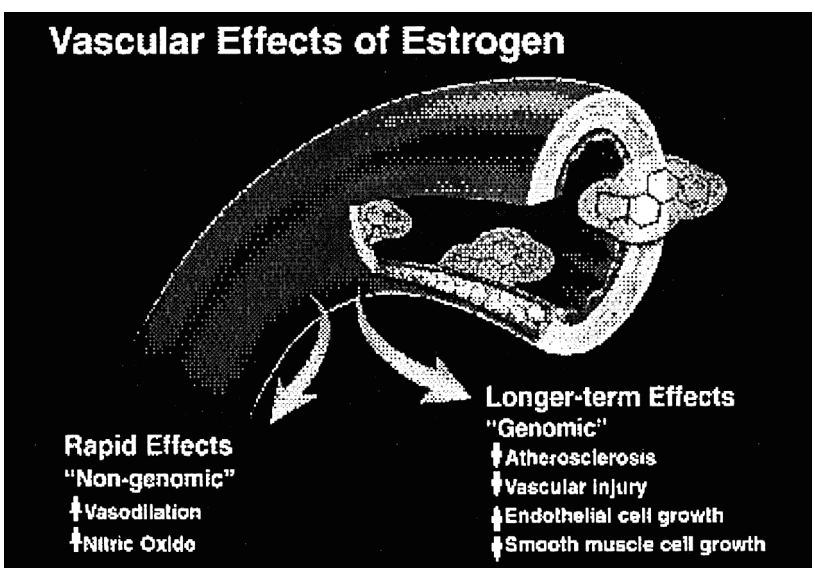

Fig. 1. Direct effects of estrogen on blood vessels. Estrogen has two direct effects on blood vessels; rapid (nongenomic) vasodilatory effects and longer-term (genomic) effects. The longer-term effect of estrogen contributes to the prevention of atherosclerosis and inhibition of the response to vascular injury. The vascular effects of estrogen effects are mediated by direct actions on estrogen receptors expressed in vascular endothelial cells (red) and smooth muscle cells (blue).

lar cells in both males and females also may be exposed to estrogens via local conversion of testosterone or Δ^4-testosterone to 17β-estradiol by the enzyme p-450 aromatase [29]. Vascular smooth muscle cells express aromatase [30], and may produce local vascular levels of estrogen that are physiologically relevant.

4. Rapid, non-genomic effects of estrogen

In normal blood vessels, the endothelium releases nitric oxide in response to a variety of stimuli, leading to vasodilation [31]. In diseased blood vessels with dysfunctional endothelium, release of nitric oxide is attenuated, leading to direct stimulation of smooth muscle contraction and 'paradoxical' vasoconstriction in response to the same stimuli [31]. Estrogen acutely enhances vasorelaxation via both endothelium-independent and endothelium-dependent pathways. These effects are non-genomic as they do not involve changes in gene expression. In the early 1990s, it was noted that estradiol, at supraphysiologic concentrations, could inhibit the influx of extracellular calcium into vascular smooth muscle cells through an effect on cell membranes and/or L-type calcium channels [32,33], causing vasodilation. However, the high levels of estrogen required and the lack of specificity of various estrogen derivatives for this effect underscore that this is largely a pharmacologic phenomenon [32,33]. At physiological levels, the principal acute effect of estrogen on ion channels is to open vascular smooth muscle calcium-activated potassium channels, which occurs via a nitric oxide/cGMP-dependent pathway [34–36]. A normal blood vessel with an intact endothelium secretes nitric oxide, which both relaxes vascular smooth muscle and inhibits platelet activation, forming a potent vasoprotective system[31]. In cultured endothelial cells, physiologic levels of estrogen cause the release of nitric oxide within five minutes, without alterations in gene expression [37,38]. Though the existence of rapid-acting membrane receptors for steroid hormones has been postulated for over a decade in both non-vascular and vascular cells [39], to date no new class of membrane steroid hormone receptor has been definitively isolated or cloned. However, very new data supports that the rapid effects of estrogen on vascular cells may be mediated by ERα somehow tethered to the plasma membrane [40], and able to stimulate NOS activity by estrogen in vascular endothelial cells in an ER-specific fashion that does not require changes in gene expression [37,38,41]. This ER-eNOS signaling module may be localized to the microdomain known to harbor eNOS, the caveolae. Thus current evidence supports that the rapid vasodilation caused by physiologic levels of estrogen is mediated at least in part by a novel action of the same receptors that act as transcription factors to mediate the longer-term effects of estrogen on vascular gene expression. Estrogen rapidly reverses vasoconstriction in vivo in cholesterol-fed ovariectomized female primates [42] and other animal models [43,44] and also acutely reverses vasoconstriction in arteries of postmenopausal women [45–49] and men [50,51]. In vivo data confirm that the acute vasodilatory effects on estrogen in human coronary arteries are mediated by increased bioavailability of nitric oxide [47] (see above).

5. Estrogen receptors and vascular injury

Estrogen increases levels of gene expression for many genes in the cardiovascular system (reviewed in [1]), such as the important vasodilator-producing enzymes, prostacyclin synthase and nitric oxide synthase [52,53]. Thus, eNOS is both rapidly activated by estrogen and the eNOS gene expression is also up-regulated by estrogen. Teleologically, this underscores the potential importance of the eNOS-NO system in estrogen's vascular effects (and vascular biology in general). Genes for both the endothelial and inducible forms of NOS are regulated by estrogen and estradiol also induces iNOS in rat aorta, which attenuates vasoconstriction [54], suggesting that estrogen may also enhance vascular bioavailability of NO by beneficial effects on this inducible form of NOS. Estrogen receptor α levels are important to regulation of eNOS in animal models and genetic disruption of ERα in mice leads to lower levels of vascular NO [55]. Furthermore, in a young man who carries a disruptive mutation of the ERα gene, brachial endothelium-dependent relaxation is impaired [56] and early coronary calcification is detectable [57], suggesting ERα is important for eNOS activity and perhaps atheroprotection in humans.

Vascular endothelial and smooth muscle cell growth both can be affected by estrogen. Estrogen has been shown in vitro to inhibit directly smooth muscle cell migration and proliferation [58,59]. Estrogen enhances endothelial cell growth in vitro [60] and in vivo [60,61] and increased local expression of vascular endothelial growth factor may result in an increased rate of re-endothelialization following vascular injury [61]. Early restoration of endothelial integrity by estrogen might contribute to attenuation of the response to injury by increasing NO availability, which can directly inhibit smooth muscle cell proliferation [62]. Estrogen also inhibits the human endothelial cell apoptosis in an ER-mediated manner [63]. In animal studies, estrogen enhances endothelial cell regrowth following denudation [61], attenuates vascular lesion size [24,64–72], and inhibits vascular smooth muscle cell proliferation [24,66,69,71]. Atheroprotective effects of estrogen have been shown in many normolipidemic and hypercholesterolemic animal models [1,6,9,24,65,68,69,71,73]. The protective effects of estrogen are apparent in animals in whom estrogen treatment has no effect on circulating

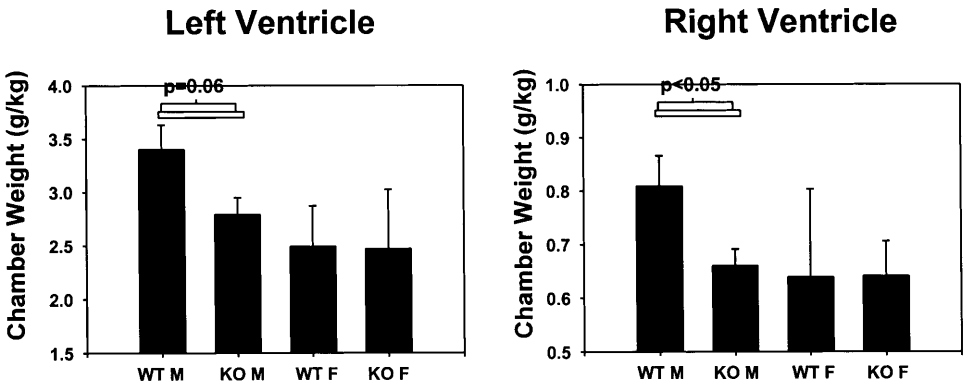

Fig. 2. Cardiac chamber weights in wild-type and ER knockout mice. Cardiac chambers were excised and weighed immediately and weights are expressed after normalizing for mouse weight. Note that the ER knockout mice have significantly smaller left and right ventricles, comparable in weight to the female mice.

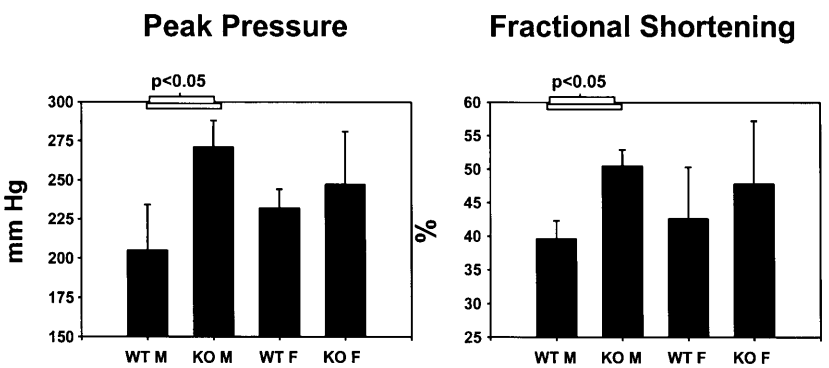

Fig. 3. Hemodynamic and echocardiographic measures of contractility in wild-type and ER knockout mice. Left panel shows invasive hemodynamic measurements of peak pressures generated by the left ventricle. Right panel shows echocardiographic measurement of fractional shortening, a non-invasive measure of cardiac contractility. Note that both approaches show that the male ER knockout mice are 'hypercontractile' compared to their wild-type littermates, comparable in contractility to female mice.

lipids [24,65,66,69,70], supporting further a direct effect of estrogen on the blood vessel wall.

Our laboratory has used mice developed recently in which estrogen receptors are genetically disrupted to explore the role of ERα and ERβ in mediated the vascular protective effects of estrogen. We first found that estrogen continues to protect against vascular injury in mice in which ERα has been genetically disrupted [24]. At the time these data were produced, ER, isolated from rat prostate cDNA, had just been described at the March 1996 Keystone Meeting on Nuclear Hormone Receptors. We, therefore, examined aortic tissues of the ERKO mice and found that ERβ is also clearly expressed in vascular tissues [24]. Identical experiments in ERβ knockout mice developed in the Smithies laboratory [74] have now been completed. Again, in ovariectomized female wild-type and ERβ knockout mice, 17-estradiol markedly and equally inhibited the increase in vascular medial area and the proliferation of vascular smooth muscle cells after vascular injury [75]. These results support that either of the two known estrogen receptors, ERα and ERβ, is sufficient to mediate estrogen protection against vascular injury, or that an unidentified receptor (i.e. 'ERγ') exists. This question will be explored shortly through studies of ERα, ERβ double knockout mice.

6. Estrogen receptors in the myocardium: preliminary studies in ERβ knockout mice

Myocardial cells also express ERα and ERβ [1]. In preliminary studies, we have examined the effects of ERβ disruption on myocardial function in 10–12-month-old male mice. A total of 20 mice were studied, five male and five female ERβ knockout mice, and five littermate controls of each sex. These are very small numbers and the data are thus quite preliminary, but the male ERβ knockout mice displayed an interesting myocardial phenotype: their hearts were smaller (Fig. 2) and 'hypercontractile' attaining higher peak pressures and great fractional shortening percentages by echocardiography than their wild type littermates (Fig. 3). Left ventricular end-systolic dimensions were also significantly shorter in the ERβ male knockout mice (Fig. 4). For all these

parameters, the ERβ male knockout mice were similar to the hearts of female animals. Invasive hemodynamic measurements revealed a decreased left ventricular/body weight (LV/BW ratio) in the male ERβ knockouts ($P = 0.06$), and an increase in the total pressure that the ventricle is able to generate ($P = 0.017$). Thus, though preliminary, these results suggest that estrogen receptors will have an important physiologic role in the function of the heart itself, as well as the blood vessels, and this is an area of investigation that is likely to grow rapidly in the next few years.

7. Summary

Estrogen, thus, has both rapid and longer-term effects on the blood vessel wall. Data regarding the mechanism(s) that mediate the rapid effects of estrogen on vasodilation support that liganded estrogen receptor increases the bioavailability of endothelial-derived nitric oxide in a non-genomic fashion, which, through NO-mediated increases in cyclic GMP, leads to vascular smooth muscle cell relaxation. The longer-term effects of estrogen, likely are due to ERα and/or ERβ-mediated changes in vascular cell gene and protein expression, the products of which then influence vascular function in an autocrine and/or paracrine fashion. The formal possibility that an ERγ exists is being actively explored in studies of ERα, ERβ double knockout mice. The distribution of ERα and ERβ in the normal and diseased vasculature, in different vascular beds, and in men, premenopausal, and post-menopausal women is not yet defined. The possible existence of an endogenous vasoactive estrogen-like compound(s) is not yet explored, nor is the physiologic relevance and function of estrogen metabolites, and of phytoestrogens well understood. However, it is likely that the rapid progress occurring in this field will allow therapies for cardiovascular disease like those envisioned by Samuel Levine 40 years ago to emerge in the near future.

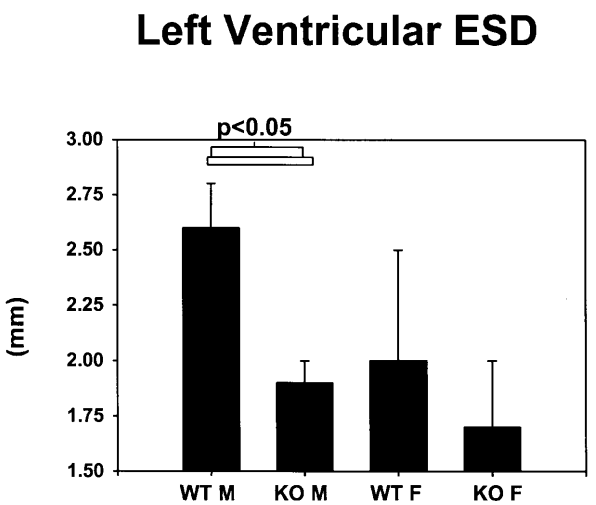

Fig. 4. Echocardiographic measurement of end systolic dimension in wild-type and ER knockout mice. The end systolic dimension (ESD) in ER knockout male mice is significantly smaller than the ESD in wild-type male littermates and similar to the ESD measured in females. The data of Figs. 2–4 are consistent with the presence of a smaller, hyperdynamic and hypercontractile heart in male ER knockout mice when compared with wild-type males.

Acknowledgements

Supported in part by NIH Grants HL55309, HL56069, and HL59953 to MEM. MEM is an Established Investigator of the American Heart Association. The author gratefully acknowledges Richard Karas and Rick Patten as well as the other investigators of the Molecular Cardiology Research Center, who have made the work described here possible.

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