

Review Articles

*Mechanisms of Disease*FRANKLIN H. EPSTEIN, M.D., *Editor*THE PROTECTIVE EFFECTS
OF ESTROGEN ON THE
CARDIOVASCULAR SYSTEMMICHAEL E. MENDELSON, M.D.,
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THE incidence of cardiovascular disease differs significantly between men and women, in part because of differences in risk factors and hormones.¹ The incidence of atherosclerotic diseases is low in premenopausal women, rises in postmenopausal women, and is reduced to premenopausal levels in postmenopausal women who receive estrogen therapy.¹⁻³ Until recently, the atheroprotective effects of estrogen were attributed principally to the hormone's effects on serum lipid concentrations. However, estrogen-induced alterations in serum lipids account for only approximately one third of the observed clinical benefits of estrogen.³⁻⁵ Reviews of the data suggest that the direct actions of estrogen on blood vessels contribute substantially to the cardiovascular protective effects of estrogen.^{4,6} The vasculature, like the reproductive tissues, bone, liver, and brain, is now recognized as an important target of estrogen's action.

Estrogen increases vasodilatation and inhibits the response of blood vessels to injury and the development of atherosclerosis (Fig. 1). Estrogen-induced vasodilatation occurs 5 to 20 minutes after estrogen has been administered and is not dependent on changes in gene expression; this action of estrogen is sometimes referred to as "nongenomic." The estrogen-induced inhibition of the response to vascular injury and the preventive effect of estrogen against atherosclerosis occur over a period of hours or days after estrogen treatment and are dependent on changes in gene expression in the vascular tissues;

these actions are sometimes referred to as "genomic." This article reviews recent information about the mechanisms by which estrogen provides protection against vascular disease and the clinical importance of these mechanisms.

ESTROGEN RECEPTORS AND ESTROGENS

Estrogen Receptors

There are two estrogen receptors, estrogen receptor α and estrogen receptor β , both of which are members of the superfamily of steroid hormone receptors.^{7,8} Estrogen receptors α and β have considerable homology (Fig. 2) and, like all steroid hormone receptors, are transcription factors that alter gene expression when they are activated.^{7,8,10-12} Estrogen receptors are activated by estrogen binding and can also be activated by growth factors in the absence of estrogen.¹³ The latter mechanism of activation may operate when local concentrations of growth factors are high or when serum estrogen concentrations are low (as in men and postmenopausal women). Estrogen-independent activation of estrogen receptors may occur by different intracellular pathways in vascular and nonvascular cells.¹⁴

Blood vessels are complex structures, with walls containing smooth-muscle cells and an endothelial-cell lining (Fig. 1). Vascular endothelial and smooth-muscle cells bind estrogen with high affinity,^{4,6} and estrogen receptor α has been identified in both types of vascular cells in women and men,¹⁵⁻¹⁹ as well as in myocardial cells.²⁰ The levels of expression of estrogen receptors α and β in different vascular beds in normal women and men and in persons with abnormal vessels have not yet been well characterized. In one small study of premenopausal women, fewer α receptors were found in women with atherosclerotic coronary arteries than in those with normal coronary arteries.¹⁶ Variant forms of estrogen receptor α are expressed in vascular cells, and these findings may prove to be of clinical importance.^{21,22} Estrogen receptor α activates specific target genes in vascular smooth-muscle and endothelial cells (Table 1).^{15,17-19,32}

Estrogen receptor β is structurally and functionally distinct from estrogen receptor α (Fig. 2).^{8,33,34} In animals messenger RNA (mRNA) for estrogen receptor β is found in many tissues, including the prostate, uterus, ovary, testis, bladder, lungs, and brain.^{8,33} The receptor is present in nonhuman primate³⁵ and human arteries and veins (unpublished data), as well as in the blood vessels of normal mice and rats^{36,37} and in the blood vessels of mice in which the gene for estrogen receptor α has been disrupted.³⁶ Functional estrogen receptor β is also present

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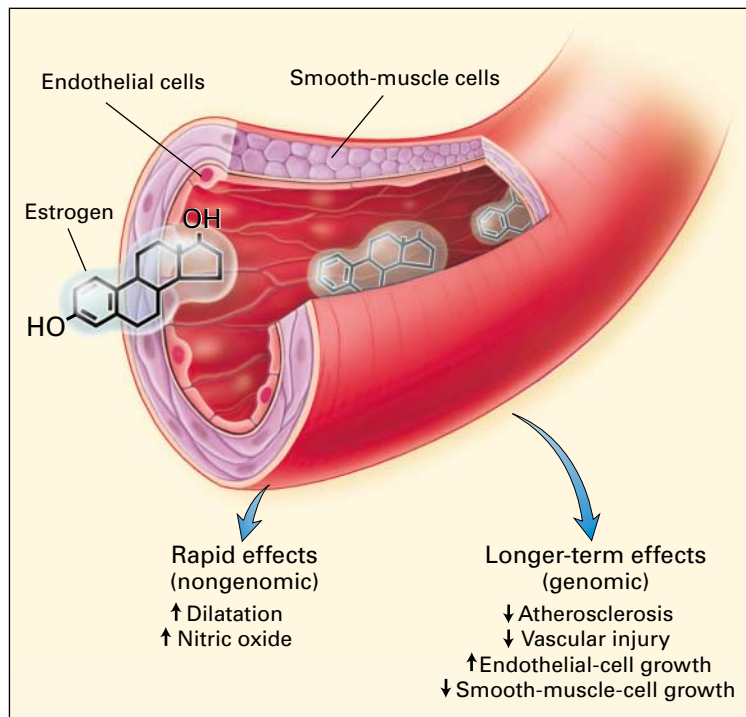


Figure 1. Direct Effects of Estrogen on Blood Vessels.

Vascular endothelial and smooth-muscle cells express the two known estrogen receptors. Estrogen has both rapid vasodilatory effects and longer-term actions that inhibit the response to vascular injury and prevent atherosclerosis. These effects are mediated by direct actions on vascular endothelial cells (red) and smooth-muscle cells (purple). The rapid effects of estrogen on the blood-vessel wall are believed to occur without any changes in gene expression (nongenomic effects), whereas the longer-term effects involve changes in gene expression (genomic effects) mediated by the estrogen receptors, which are ligand-activated transcription factors.

in myocardial cells, in which it regulates the expression of nitric oxide synthases (unpublished data). In addition to forming homodimers (Fig. 3), estrogen receptor α and estrogen receptor β can form heterodimers with each other,³⁸ adding a further degree of complexity to the regulation of gene expression by estrogen in cells expressing both receptors. Estrogen continues to provide protection against vascular injury in mice in which estrogen receptor α has been disrupted,³⁶ and the expression of estrogen receptor β , but not of estrogen receptor α , is stimulated after vascular injury in male rats.³⁷ Estrogen also provides protection against vascular injury in mice in which estrogen receptor β has been disrupted (unpublished data), suggesting that each of the two known estrogen receptors is sufficient to protect against vascular injury or that an unknown signaling pathway is involved.

Estrogens and Antiestrogens

In premenopausal women, 17 β -estradiol produced by the ovaries is the chief circulating estrogen. Serum estradiol concentrations are low in preadolescent girls

and increase at menarche. In women, they range from about 100 pg per milliliter (367 pmol per liter) in the follicular phase to about 600 pg per milliliter (2200 pmol per liter) at the time of ovulation. They may rise to nearly 20,000 pg per milliliter (70,000 pmol per liter) during pregnancy. After menopause serum estradiol concentrations fall to values similar to or lower than those in men of similar age (5 to 20 pg per milliliter [18 to 74 pmol per liter]).³⁹

Vascular cells are also exposed to estrogens from exogenous sources. In the United States, postmenopausal women are often treated with a mixture of conjugated equine estrogens, and this complex form of estrogen therapy has been used in most clinical studies involving postmenopausal women. Which form or forms of estrogen in this mixture directly contribute to the observed vascular protective effects of estrogen therapy is not known. In addition, other compounds in clinical use can activate vascular estrogen receptors. Tamoxifen, a nonsteroidal triphenylethylene derivative used in the treatment of breast cancer, acts as an estrogen agonist in some tissues (e.g., the uterus) but as an estrogen antagonist

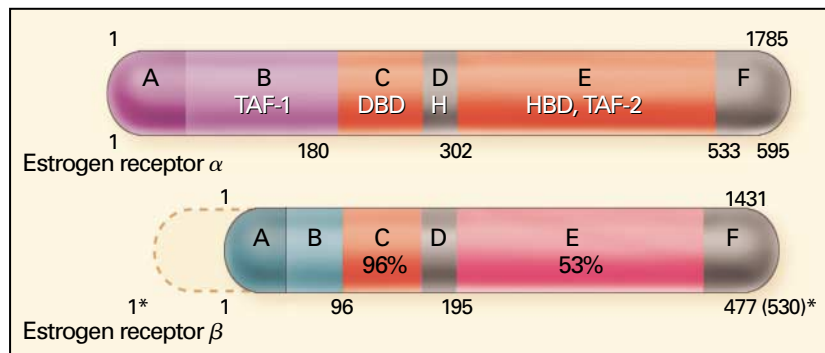


Figure 2. Structures of Human Estrogen Receptors α and β .

Estrogen receptors, like all steroid hormone receptors, are organized into domains. The amino-terminal A–B domains of the estrogen receptors contain a ligand-independent transcriptional-activation function (TAF-1). The DNA-binding domain (DBD) contains two zinc fingers that are highly conserved in all steroid hormone receptors. Domain D is the hinge region (H) of these receptors. Domain E contains the hormone-binding domain (HBD) and the hormone-dependent transcriptional-activation function (TAF-2). Domain F is a variable region that includes the sequence for helix 12 of the molecule, which is probably important for the difference in the responses of estrogen receptors to estradiol and selective estrogen-receptor modulators.⁹ The recently discovered estrogen receptor β is highly homologous with estrogen receptor α in the DBD (96 percent homology) and in the HBD (53 percent homology) but has a unique A–B domain. At least two forms of estrogen receptor β are currently thought to be expressed (broken line), because of the presence of several potential transcriptional start sites in isolated complementary DNA. The numbers above and below each receptor refer to the numbers of nucleotides and amino acids, respectively. The asterisks indicate the predicted 530-amino-acid protein that results from using an alternative transcriptional start site in estrogen receptor β .

in other tissues (e.g., the breast). Tamoxifen is thus an example of a selective estrogen-receptor modulator.⁴⁰ Another selective estrogen-receptor modulator, raloxifene, recently became available for the treatment of osteoporosis.⁴¹

The tissue-specific differences in the actions of these compounds are probably due in part to their ability to induce unique conformations in estrogen receptors^{9,42} and in part to cell-specific differences in proteins that interact with the estrogen receptors. However, a selective estrogen-receptor modulator that is specific for blood vessels or the heart 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 antiestrogenic effects.⁴³ Vascular cells in both men and women are also exposed to estrogens as a result of local conversion of testosterone to 17β -estradiol by the enzyme P-450 aromatase.⁴⁴

Estrogen-Receptor–Associated Proteins

Complexes of estrogens and estrogen receptors associate with and act in concert with other proteins, known as coactivators, to facilitate gene expression (Fig. 3).^{10,45} Coactivator proteins work in at least two ways. They recruit proteins of the general transcriptional apparatus (the multiprotein complex that transcribes DNA into RNA). They also have en-

zymatic activity that can facilitate the transcription of RNA by the general transcriptional apparatus — for example, histone acetyltransferase may participate in unwinding the tightly coiled DNA from its histone scaffold (Fig. 3).¹⁰

In addition, there are proteins, known as corepressors, that bind to steroid hormone receptors and silence transcription,¹⁰ and the first estrogen-receptor–specific corepressor was recently cloned.⁴⁶ The molecular mechanisms by which corepressors inhibit gene expression are not well understood. The presence of various types or amounts of estrogen-receptor–associated proteins (and perhaps cell-specific estrogen-receptor–associated proteins as well) may contribute to differences between the actions of estrogens in vascular and nonvascular cells. Control of gene expression by complexes of estrogens and estrogen receptors thus involves a series of specific molecular interactions among estrogens, estrogen receptors, estrogen-receptor–associated proteins, and the control regions for the different estrogen target genes present in each cell.

SYSTEMIC EFFECTS OF ESTROGEN

Estrogen alters serum lipid concentrations, coagulation and fibrinolytic systems, antioxidant systems, and the production of other vasoactive molecules, such as nitric oxide and prostaglandins, all of which can influence the development of vascular disease.

TABLE 1. ESTROGEN-REGULATED GENES OF POTENTIAL IMPORTANCE IN VASCULAR PHYSIOLOGY AND DISEASE.

| GENE PRODUCT | PHYSIOLOGIC OR PATHOPHYSIOLOGIC ROLE* |
|--|---|
| Candidate estrogen-regulated genes (vascular cells) | |
| Prostacyclin synthase | Vasodilatation |
| Endothelial nitric oxide synthase | Vasodilatation |
| Inducible nitric oxide synthase | Vasodilatation in response to vascular injury |
| Endothelin-1 | Vasoconstriction |
| Collagen | Vascular-matrix formation |
| Matrix metalloproteinase 2 ²³ | Vascular-matrix remodeling |
| E-selectin | Cell adhesion |
| Vascular-cell adhesion molecule | Cell adhesion |
| Vascular endothelial growth factor | Angiogenesis and endothelial-cell proliferation |
| Candidate estrogen-regulated genes (nonvascular cells) | |
| Growth- and development-related genes | |
| Transforming growth factor β , ²⁴ | Wound healing |
| Epidermal growth factor receptor | Cell growth in response to vascular injury |
| Platelet-derived growth factor ²⁵ | Cell growth in response to vascular injury |
| flt-4 tyrosine kinase | Angiogenesis and endothelial-cell proliferation |
| Coagulation- and fibrinolysis-related genes | |
| Tissue factor ²⁶ | Hemostasis in response to thrombosis |
| Fibrinogen | Hemostasis in response to thrombosis |
| Protein S | Hemostasis in response to thrombosis |
| Coagulation factor VII | Hemostasis in response to thrombosis |
| Coagulation factor XII ²⁷ | Hemostasis in response to thrombosis |
| Plasminogen-activator inhibitor 1 | Hemostasis in response to thrombosis |
| Tissue plasminogen activator ²⁸ | Fibrinolysis |
| Antithrombin III | Anticoagulation |
| Signaling-related and miscellaneous genes | |
| Estrogen receptor α | Hormonal regulation and gene expression |
| Estrogen receptor β | Hormonal regulation and gene expression |
| Monocyte chemotactic protein 1 ²⁹ | Monocyte recruitment and atherosclerosis |
| I _{SK} and HK2 (cardiac potassium channels) ³⁰ | Cardiac conduction |
| Connexin 43 | Cardiac conduction |
| Leptin ³¹ | Fat metabolism and obesity |
| Apolipoproteins A, B, D, and E and Lp(a) | Lipid metabolism and atherosclerosis |
| Angiotensin-converting enzyme | Vasoconstriction |
| Angiotensin II receptor, type 1 | Vasoconstriction |

*Data are from Mendelsohn and Karas,⁴ unless otherwise specified.

Effects on Serum Lipoproteins

The effects of estrogen therapy on serum lipid concentrations result largely from estrogen-receptor-mediated effects on the hepatic expression of apoprotein genes (Table 1). Many studies, including one large, randomized, controlled trial,⁴⁷ have documented that estrogen therapy in postmenopausal women decreases serum total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations, increases serum high-density lipoprotein (HDL) cholesterol and triglyceride concentrations, and decreases serum Lp(a) lipoprotein concentrations. The route of administration of estrogen influences its effects on serum lipids. Transdermally administered estrogen has less of an effect on serum lipid concentrations than does orally administered estrogen. Coadministration of a progestin can blunt the changes in serum lipids due to estrogen; the magnitude of this effect depends on the specific progestin.⁴⁷ Raloxifene has effects on serum lipid concentrations that

are similar to but less pronounced than those of estrogen, but raloxifene may lower serum Lp(a) lipoprotein concentrations more.⁴⁸ Whether raloxifene has cardiovascular protective effects remains uncertain^{49,50} and requires further study.

Effects on Coagulation, Fibrinolytic, and Other Vasoactive Proteins

Hepatic expression of the genes for several coagulation and fibrinolytic proteins is also regulated by estrogen through estrogen receptors (Table 1). Plasma fibrinogen concentrations are decreased by continuous estrogen therapy,⁵¹⁻⁵³ as are plasma concentrations of the anticoagulant proteins antithrombin III⁵² and protein S.^{51,52} Estrogen also decreases plasma concentrations of the antifibrinolytic protein plasminogen-activator inhibitor type 1,⁵⁴ and high serum estrogen concentrations are associated with an increased overall potential for fibrinolysis.⁵⁵ Estrogen therapy has also been associated with lower plasma

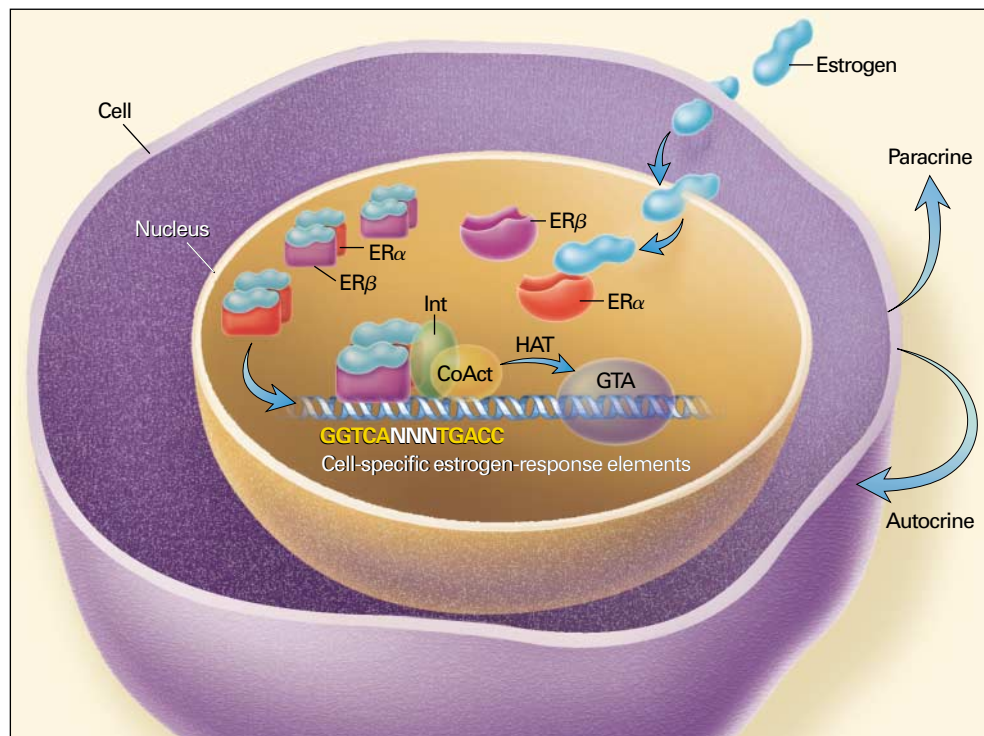


Figure 3. Mechanism of Estrogen-Receptor Activation of Gene Expression.

Estrogen enters target cells by passive diffusion and binds to high-affinity intracellular receptors. Estrogen receptors α and β (ER α and ER β , respectively) are transcription factors that undergo conformational changes after ligand binding. The estrogen–estrogen-receptor complexes form dimers and then bind to specific sites in the control regions of their target genes (estrogen-response elements). The simplest estrogen-response elements are mirror images separated by three nucleotides (N). The complexes associate with several proteins capable of activating the general transcriptional apparatus (GTA), the multiprotein complex containing RNA polymerase that transcribes DNA into RNA. These estrogen-receptor–associated proteins include coactivator proteins (CoAct) and general integrators of transcription (Int).¹⁰ Estrogen-receptor–associated proteins may have enzymatic functions as well, such as histone acetyltransferase (HAT) activity. Estrogen receptors may also suppress the transcription of selected target genes by interacting with corepressors.

concentrations of factor VII in some^{51,52} but not all⁵³ studies. The net effect of estrogens on coagulation depends on the form of estrogen used, the dose, and the duration of therapy.

Estrogen directly regulates vasomotor tone through both short-term and long-term effects on the vasculature. Long-term administration of estrogen is associated with decreased plasma concentrations of renin,⁵⁶ angiotensin-converting enzyme,⁵⁷ and endothelin-1⁵⁸ and decreased vascular expression of the gene for angiotensin II receptor type 1,⁵⁹ as well as an increased ratio of nitric oxide to endothelin-1 in plasma.⁶⁰ The net effect of these changes is to promote vasodilatation.

Antioxidant Effects

17 β -Estradiol has antioxidant effects *in vitro*, but related compounds have widely differing antioxidant or even pro-oxidant activities.^{61–63} Estrogen antagonists also have some antioxidant effects.⁶⁴ Near-phys-

iologic concentrations of estradiol may inhibit the *ex vivo* oxidation of LDL cholesterol in plasma.^{63,65} It remains unclear, however, whether the *in vitro* antioxidant effects of estradiol are physiologically important, since in most studies, very high concentrations of estrogen were used. In postmenopausal women, both long-term and short-term administration of 17 β -estradiol can decrease the oxidation of LDL cholesterol.⁶⁶ This antioxidant effect may be due to estrogen-receptor–mediated changes in the expression of genes for enzymes that regulate the local production and degradation of superoxide.⁶⁷

DIRECT EFFECTS ON VASCULAR CELLS AND TISSUES

Rapid, Nongenomic Effects

In normal blood vessels, the endothelium releases nitric oxide in response to a variety of stimuli, causing vasodilatation.⁶⁸ In diseased blood vessels with

dysfunctional endothelium in which the release of nitric oxide is reduced, these stimuli cause the contraction of smooth muscle and paradoxical vasoconstriction.⁶⁸ Estrogens can cause short-term vasodilatation by both endothelium-dependent and endothelium-independent pathways. These rapid effects do not appear to involve changes in gene expression. Two mechanisms for the rapid vasodilatory effects of estrogens have been explored in some depth: effects on ion-channel function and effects on nitric oxide.

Ion Channels and Rapid Effects

In vascular smooth-muscle cells, ion channels direct the flow of potassium, sodium, and calcium ions into and out of the cell, determining the electrical potential of the membrane at rest and the contractile state of smooth muscle.⁶⁹ At supraphysiologic concentrations, estrogen inhibits the influx of extracellular calcium into vascular smooth-muscle cells by an effect on cell membranes or L-type calcium channels.⁷⁰⁻⁷² However, the high concentrations of estrogen required and the lack of specificity of various estrogen derivatives for this effect on calcium channels^{71,72} suggest that it is largely a pharmacologic phenomenon. At physiologic concentrations, estrogen stimulates the opening of calcium-activated potassium channels through a nitric oxide- and cyclic guanosine monophosphate-dependent pathway,^{73,74} thus relaxing smooth muscle and promoting vasodilatation (Fig. 4).

Nitric Oxide and Rapid Effects

Normal endothelium secretes nitric oxide, which both relaxes vascular smooth muscle and inhibits platelet activation.⁶⁸ In cultured endothelial cells, physiologic concentrations of estrogen cause a rapid release of nitric oxide without altering gene expression.^{76,77} Are these rapid effects of estrogen mediated by an unidentified estrogen receptor or by one of the known estrogen receptors acting in a novel way? The existence of rapidly acting membrane receptors for steroid hormones in both nonvascular and vascular cells has been postulated for over two decades,^{78,79} but no such receptors have been isolated or cloned.

Alternatively, the rapid effects of estrogen on vascular cells could be mediated by a known estrogen receptor, perhaps located in the plasma membrane⁷⁹ and able to activate nitric oxide synthase rapidly in a nongenomic manner (Fig. 4). This suggestion is consistent with the observations that estrogen-induced stimulation of nitric oxide synthase activity in endothelial cells is blocked by specific estrogen-receptor antagonists⁷⁵⁻⁷⁷ and that estrogen receptor α can directly activate endothelial nitric oxide synthase,⁷⁵ perhaps through a tyrosine kinase pathway or the mitogen-activated protein kinase signaling pathway (Fig. 4).^{75,80} These rapid effects do not require changes in gene expression but may involve proteins

that interact with the estrogen receptor, such as heat-shock protein 90, which also binds to and activates endothelial nitric oxide synthase (Fig. 4).⁸¹ Thus, rapid vasodilatation caused by estrogen may be mediated at least in part by a novel action of estrogen receptor α ,⁷⁵ which also acts as a transcription factor to mediate the longer-term effects of estrogen on gene expression.

Estrogen rapidly causes coronary vasodilatation *ex vivo*^{4,6} and *in vivo* in cholesterol-fed ovariectomized primates⁸² and other animals.⁸³ Estrogen dilates coronary and brachial arteries in postmenopausal women⁸⁴⁻⁸⁹ and, in some studies, in men.^{88,90,91} Sublingual administration of 17 β -estradiol in postmenopausal women increases the duration of treadmill exercise before the onset of ischemia.⁹² The short-term coronary vasodilatory effects of estrogen in humans are largely mediated by the increased production of nitric oxide.⁸⁹

Longer-Term Effects on the Vasculature

Effects on Genes Regulating Vascular Tone

Estrogen increases the expression of genes for important vasodilatory enzymes such as prostacyclin synthase and nitric oxide synthase (Table 1).^{93,94} Some of the rapid effects of estrogen may therefore be due to longer-term increases in the expression of the genes for these enzymes in vascular tissues. For example, estrogens reverse vasoconstriction in vascular rings from animals in which long-term estrogen therapy has been discontinued,⁹⁵ and vascular rings from ovariectomized animals with long-term exposure to estrogen do not constrict in response to acetylcholine.⁹⁶ These effects are probably mediated at least in part by longer-term increases in the expression of a gene or genes for nitric oxide synthase. Estrogen may also increase the bioavailability of nitric oxide in vessels by increasing the expression of the gene for the inducible form of nitric oxide synthase.⁹⁴ Genetic disruption of estrogen receptor α in mice also leads to lower levels of vascular nitric oxide.⁹⁷ Long-term administration of estrogen increases acetylcholine-mediated coronary vasodilatation in nonhuman primates^{98,99} (though tamoxifen does not⁹⁹), male-to-female transsexuals,^{100,101} postmenopausal women¹⁰² (though the effect may be attenuated by concomitant progesterone therapy¹⁰³), and postmenopausal women with angina and normal coronary arteries.¹⁰⁴ Furthermore, a case has been reported of a young man who has no functional estrogen receptor α and has impaired brachial endothelium-dependent relaxation¹⁰⁵ and early coronary calcification,¹⁰⁶ supporting the hypothesis that estrogen receptor α is important for the activity of endothelial nitric oxide synthase.

Effects on the Response to Vascular Injury and on Atherosclerosis

Estrogen accelerates endothelial cell growth *in vitro*¹⁰⁷ and *in vivo*.^{107,108} The rapid reendothelializa-

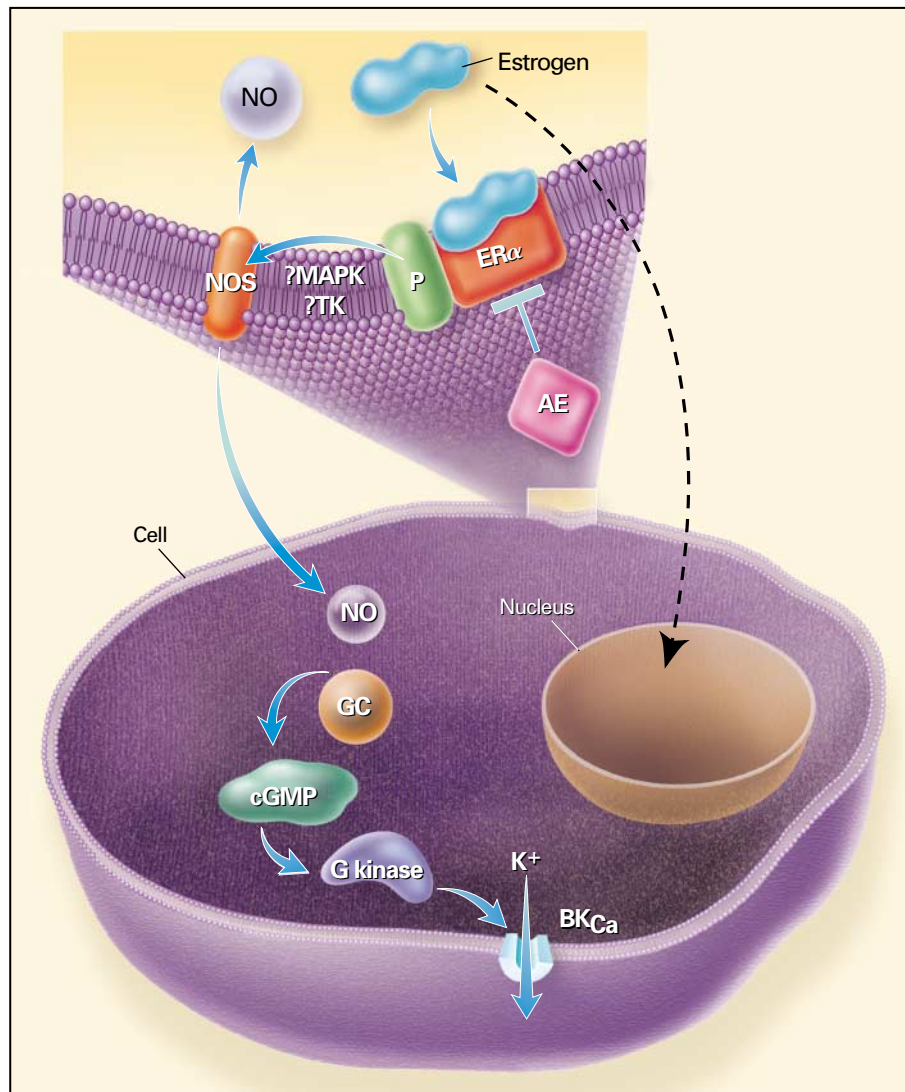


Figure 4. Mechanism of Rapid, Nongenomic Activation of Nitric Oxide Synthase by Estrogen in Endothelial Cells and Vascular Smooth-Muscle Cells.

Estrogen causes rapid activation of nitric oxide synthase (NOS) in a manner that does not require new gene transcription. In endothelial cells (upper portion of figure), this probably occurs by a novel action of estrogen receptor α ($ER\alpha$), is blocked by antiestrogens (AE), and may require a class of receptor-associated proteins (P) distinct from those that act with the receptors to mediate changes in transcription. The result is rapid activation of endothelial NOS, perhaps through signal-transduction pathways involving tyrosine kinase (TK) or the mitogen-activated protein kinase (MAPK).⁷⁵ In vascular smooth-muscle cells (lower portion of figure), estrogen rapidly activates calcium-activated potassium channels (BK_{Ca}), which hyperpolarize and relax smooth-muscle cells. BK_{Ca} activation in vascular smooth muscle occurs through a pathway that is dependent on nitric oxide (NO) and cyclic guanosine monophosphate (cGMP)^{73,74} though the form of NOS activated in smooth-muscle cells by estrogen has not yet been identified. GC denotes guanylate cyclase, and G kinase cGMP-dependent protein kinase.

tion induced by estrogen after vascular injury may be due in part to increased local expression of vascular endothelial growth factor.¹⁰⁸ Estrogen also inhibits apoptosis of cultured human endothelial cells in an estrogen-receptor–dependent manner.¹⁰⁹ Early restoration of endothelial integrity by estrogen may

contribute to the attenuation of the response to injury by increasing the availability of nitric oxide, which can directly inhibit the proliferation of smooth-muscle cells.¹¹⁰ Estrogen directly inhibits the migration and proliferation of smooth-muscle cells in vitro^{111,112} and, in some¹⁹ but not all¹¹³ studies, the expression of ad-

hesion molecules by vascular cells. Estrogen contributes to long-term vascular protection by inhibiting the proliferation of vascular smooth-muscle cells and accelerating the growth of endothelial cells (Fig. 1).

Estrogen also has atheroprotective actions in many normolipidemic or hypercholesterolemic animals,^{4,6,36,96,114-119} and it provides protection against vascular injury in mice in which estrogen receptor α ³⁶ or estrogen receptor β (unpublished data) has been genetically disrupted. Studies in animals have shown that estrogen increases the regrowth of endothelial cells after denudation,¹⁰⁸ reduces the size of vascular lesions in carotid arteries and the aorta,^{36,114-117,120} and inhibits the proliferation of vascular smooth-muscle cells in carotid arteries.^{36,114} These beneficial effects of estrogen are blocked by high but not by low doses of progesterone.^{116,117,120} Estrogen also appears to be protective in castrated male rats, but not in intact male rats,^{116,117} although it prevents atherosclerosis in intact, apolipoprotein E-deficient male mice.¹¹⁵ In a study of rabbits with cardiac allografts, estradiol inhibited myointimal proliferation and decreased the vascular expression of major-histocompatibility-complex antigens and the infiltration of immune cells.¹²¹ These protective effects of estrogen were apparent in studies in which estrogen treatment had little or no effect on serum lipid concentrations,^{4,6,36,114,115,120} observations that provide further support for a direct effect of estrogen on blood-vessel walls.

Abundant evidence from both prospective and retrospective observational studies demonstrates that estrogen therapy reduces the primary risk of cardiovascular disease in previously healthy postmenopausal women by 35 to 50 percent.^{1,3,4,122,123} Less is known about the effects of combined therapy with estrogen and progestin in postmenopausal women,³ but one large study suggests that it too is beneficial.¹²³ These observational data are extensive and consistent, but definitive evaluation of the efficacy of estrogen therapy for the primary prevention of coronary heart disease in women must await the results of ongoing randomized clinical trials such as the Women's Health Initiative. The few studies of estrogen therapy as secondary prevention also support a cardiovascular benefit.¹²⁴⁻¹²⁶ However, in the Heart and Estrogen/Progestin Replacement Study, the first randomized trial to examine the effect of therapy with conjugated equine estrogens plus medroxyprogesterone in postmenopausal women with coronary disease, treatment for an average of 4.1 years did not reduce the overall rate of events associated with coronary heart disease but increased the rates of thromboembolic events and gallbladder disease.¹²⁷ There was an increase in coronary events in the first year in the treated women and a decrease in coronary events in years 4 and 5. These findings may be due to the effects of the conjugated equine estrogens, medroxyprogesterone, or both on nonvascular tissues, such as hepatic effects

that may have altered coagulation profiles and increased thrombotic events in a subgroup of the women studied.

CONCLUSIONS

Estrogen has both rapid and longer-term effects on the blood-vessel wall. The mechanisms that mediate the rapid effects of estrogen are not fully understood. Current data suggest that estrogen influences the bioavailability of endothelial-derived nitric oxide and, through nitric oxide-mediated increases in cyclic guanosine monophosphate, causes the relaxation of vascular smooth-muscle cells. The longer-term effects of estrogen, about which more is known, are due at least in part to changes in vascular-cell gene and protein expression that are mediated by estrogen receptor α , β , or both. Estrogen-regulated proteins influence vascular function in an autocrine or paracrine fashion. However, additional vascular target genes regulated by estrogen receptors need to be identified.

A number of questions remain to be addressed in this evolving field. The direct effects of estrogen on the vasculature promote vasodilatation and inhibit the development and progression of atherosclerosis. However, some of the nonvascular effects of estrogen may offset its beneficial vascular effects. There are currently no identified estrogens with relative selectivity for the vasculature.¹²⁸ The potential clinical effects of estrogen metabolites, phytoestrogens, and local conversion of testosterone to estradiol all need to be explored. Direct myocardial effects of estrogen on cardiac structure and function are likely to be important as well and deserve greater attention. More prospective clinical trials of estrogen therapy for the primary and secondary prevention of cardiovascular disease are needed, including additional prospective studies of estrogen alone or in combination with other compounds (such as progestins and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors). It is likely that the rapid progress in this field in both basic and clinical sciences will soon lead to the development of more specific hormonal therapies for cardiovascular disease.

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