Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms

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Dubey, Raghvendra K., and Edwin K. Jackson. Estrogen-induced cardiorenal protection: potential cellular, biochemical, and molecular mechanisms. Am J Physiol Renal Physiol 280: F365–F388, 2001.—A number of cellular and biochemical processes are involved in the pathophysiology of glomerular and vascular remodeling, leading to renal and vascular disorders, respectively. Although estradiol protects the renal and cardiovascular systems, the mechanisms involved remain unclear. In this review we provide a discussion of the cellular, biochemical, and molecular mechanisms by which estradiol may exert protective effects on the kidneys and vascular wall. In this regard, we consider the possible role of genomic vs. nongenomic mechanisms and estrogen receptor-dependent vs. estrogen receptor-independent mechanisms in mediating the protective effects of estradiol on the renal and cardiovascular systems.

estradiol; metabolism; methoxyestradiol; hyperplasia; growth factors; antimitogenic; menopause; smooth muscle cells; mesangial cells; endothelial cells

Premenopausal women have a decreased incidence of cardiovascular disease and a decreased rate of progression of renal disease. With the onset of menopause, however, decreased synthesis of 17β-estradiol (estradiol) is accompanied by an increased incidence of cardiovascular disorders and accelerated progression of renal diseases.

The glomerulus and the vascular wall are not static, and components of these structures dynamically increase, decrease, or reorganize in response to physiological and pathological stimuli. Although multiple cellular and biochemical processes are involved in glomerular and vascular remodeling, glomerular mesangial cells (GMCs) in the kidney and smooth muscle cells (SMCs) in the vasculature are the final common pathway for dynamic changes in glomerular and vascular wall structure. In glomerular and vascular remodeling, GMCs and SMCs undergo one or more of four basic processes: cell growth involving hypertrophy or hyperplasia; cell migration involving the immigration of GMCs or SMCs from one locale to another in the glomerular tuft or vascular wall, respectively; modulation of the amount and types of extracellular matrix (ECM); and apoptosis, which provides an important means of population control for GMCs and SMCs. Glomerular and vascular endothelial cells also play a critical role in maintaining homeostasis by generating a battery of both growth-inhibitory and growth-stimulatory factors, as well as relaxing and contracting factors. Consequently, endothelial damage or dysfunction often leads to increased SMC and GMC migration, proliferation, and ECM synthesis.

Estradiol may induce protective effects on the renal and cardiovascular system by altering SMC, GMC, or endothelial cell biology so as to prevent glomerular and vascular remodeling, and the main purpose of this review is to provide an overview of the participating mechanisms in this regard. A prerequisite for comprehending these mechanisms is an understanding of the
processes by which estradiol induces its cellular, biochemical, and molecular effects.

CELLULAR AND BIOCHEMICAL EFFECTS OF ESTRADIOL

Estradiol influences cell growth and differentiation of the male and female reproductive tissues. For example, estradiol regulates the development of mammary glands, uterus, vagina, ovary, testes, epididymis, and prostate (130) and also plays an important role in the vascular system, a system that is essential to reproductive processes. (107, 130, 173, 243). Findings in the last decade indicate that estradiol induces its biological effects via genomic and nongenomic mechanisms and that the effects of estradiol are triggered by estrogen receptor-dependent as well as estrogen receptor-independent mechanisms.

Role of Estrogen Receptors

Estradiol diffuses through the plasma membrane of cells and binds to specific, high-affinity intracellular estrogen receptors (ERs) within the target cell. The nuclear hormone receptor complex binds to chromatin at specific regions of the DNA estrogen response elements (EREs), and this interaction of activated ERs with EREs stimulates or inhibits specific gene expression and protein synthesis.

Changes in the generation of intracellular proteins triggers a cascade of events that influences metabolic processes and translates into cell growth and differentiation. It is well documented that estradiol induces uterine growth (hypertrophy and hyperplasia) and the expression of protooncogenes, which may play a role in estradiol-induced cell growth and proliferation (230). In uterine cells, treatment with estradiol rapidly increases the steady-state expression of N-myc, c-myc, c-ras, and c-fos mRNA (230). In addition, ERs increase the synthesis of growth factors such as epidermal growth factor (EGF) and insulin-like growth factor (IGF) and stimulate the levels of growth-promoting peptides that can act in an autocrine fashion to induce cell growth (see reviews in Refs. 230 and 237).

In MCF-7 cell lines, estradiol increases EGF-induced activator protein-1 (AP-1), suggesting that there may be signaling connections between ERs and EGF-induced AP-1 activity (216). Thus estradiol not only induces growth factor synthesis and growth factor receptor synthesis (230) but also facilitates growth factor receptor signal transduction mechanisms. Estradiol, by inducing c-fos, synergizes with low concentrations of insulin, which is able to induce c-jun but not c-fos (282). Even when c-fos and c-jun synthesis and AP-1 activity are maximally induced by growth factors, estradiol can still enhance AP-1-dependent transcriptional activity (248). ER mutants lacking the DNA binding domain interact with fos and jun to increase transcription (75). This demonstrates that the ER, a classic DNA binding protein, can act through protein-protein interactions without binding directly to DNA. Consequently, anything that alters the ER to fos or jun ratio will alter the rate of transcription of specific target genes. This observation provides the basis for crosstalk between multiple pathways. Via the above mechanism the AP-1 DNA binding sequence in the chicken ovalbumin gene promoter can be a target for both estradiol activation and cooperation for AP-1 (75). Moreover, in vivo studies in ovariectomized rats show that EGF enhances the nuclear localization of ERs, suggesting that EGF activates the ER to bind to ERE sequences (108).

Recent studies by Kuiper et al. (134) demonstrate that, in addition to classic ER receptors cloned more than a decade ago (84) and now classified as ER-α, cells from rats, mice, and humans also express another ER, termed ER-β (59, 134, 178, 277). Rat ER-β cDNA encodes a protein of 485 amino acid residues with a molecular mass of 54,200. In the DNA binding domain, this ER protein is highly homologous to rat ER-α, with 95% amino acid identity, whereas in the COOH-terminal ligand binding domain, it has 55% homology. Similar homologies between ER-α and ER-β have been found in ERs from mice (215, 277) and humans (59). ER-β is expressed in prostate, ovary, epididymis, testis, bladder, uterus, kidney, lung, thymus, colon, small intestine, blood vessels, pituitary, hypothalamus, cerebellum, and brain cortex (59, 107, 133, 215, 277).

Whether ER-α and ER-β play a similar or different role in mediating the physiological effects of estradiol is unclear. However, differential expression of ER-α and ER-β is observed in some tissues (131, 133, 134), which suggests different physiological roles for these receptors. In this regard, compared with ER-α, high amounts of ER-β mRNA are in fetal ovaries, testes, adrenals, and spleen of the midgestational human fetus (24). Moreover, differential activation by liganded ER-α vs. ER-β occurs at the AP-1 site (208), and xenestrogens differentially activate ERs when liganded to ER-α vs. ER-β (213).

In addition to the classic ERs, another ER, termed type II ER, exists (160). Some ligands for type II ER, such as bioflavonoids, have no affinity for ER-α or ER-β yet abrogate the effects of estradiol on cell growth (161), suggesting that, in addition to ER-α and ER-β, the biological effects of estradiol may be modulated via type II ER. The type II ER may also be involved in inducing the effects of estradiol in the vasculature and the kidney.

Apart from the cytosolic/nuclear ERs, estradiol also binds with high affinity to membrane fractions prepared from isolated pituitary and uterine cells (209, 217, 230). The functional role of the membrane receptors for estradiol is evident from the findings that estradiol stimulates adenyl cyclase activity in membranes prepared from secretory human endometrium (230). Moreover, estradiol induces rapid changes in intracellular calcium levels/flux, K⁺ conductance, and cAMP levels (9, 183, 217).

Role of Estradiol Metabolism

Several lines of evidence suggest that some of the effects of estradiol may be mediated via its metabolites. Estradiol is eliminated from the body by metabolic
conversion by cytochrome P-450 enzymes (CYP450s; 164). Many isoforms of CYP450s exist; however, the isoforms that metabolize steroids such as estradiol are CYP1, CYP2, and CYP3 (164, 307). Although most of the metabolites of estradiol are hormonally less active, water soluble, and excreted in the urine, some of the metabolites have significant growth regulatory effects (see review in Ref. 307). The importance of estradiol metabolism is illustrated by the findings that inhibition of CYP450 enzymes by cimetidine increases estradiol levels and high doses of cimetidine may cause gynecomastia (72).

Estradiol is largely metabolized within the liver via oxidative metabolism (to form hydroxylated metabolites such as 2- and 4-hydroxyestradiol) (164); glucuronidation (to form glucuronide conjugates) (307); sulfatase action (to form sulfates) (307); esterase action (to form fatty acid esters) (307); and O-methylation of catechol estradiols (to form O-methylated catechols; 12; for details, see reviews in Refs. 230 and 307).

Even though estradiol is largely metabolized within the liver, cells in several other tissues, including the kidney and the vasculature, contain CYP450 and metabolize estradiol and its metabolites (307). The metabolism of estradiol locally within a tissue may be of immense importance in mediating several of its physiological as well as pathophysiological effects. Several studies provide evidence that the catechol estradiols can induce biological effects, and 2-hydroxyestradiol is a weak ligand for ER and may regulate multiple mechanisms in reproductive tissues, including growth of cancer cells and generation of prostaglandins in the uterus during pregnancy (12, 230, 307). 2-Hydroxyestradiol attenuates catabolism of catecholamines by inhibiting catechol O-methyltransferase activity, and this may modulate the neurophysiological and pharmacological effects of catecholamines within the kidneys and vasculature (12, 230, 307). 2-Hydroxyestradiol also modulates the interaction of dopamine with its receptors (230, 307). Additionally, 2-hydroxyestradiol is a potent antioxidant and thereby protects membrane phospholipids and cells against peroxidation (56).

Similar to 2-hydroxyestradiol, 4-hydroxyestradiol induces several important biological effects. Even though it is not the dominant metabolite formed by the liver, 4-hydroxyestradiol is a major metabolite formed in some extrahepatic tissues such as rat pituitary and human myometrial, myoma, and breast tissue, as well as kidney and vasculature tissue (307). In contrast to estradiol, 4-hydroxyestradiol binds with low affinity to ER; however, its dissociation rate from the receptor is much lower than that observed for estradiol (230). Within the renal system, 4-hydroxyestradiol has been shown to stimulate tumor growth in Syrian hamsters (307) and uterotrophic effects in rats (164, 225). 4-Hydroxyestradiol is more efficacious than estradiol in inducing progesterone receptor expression in the rat pituitary (230). Similar to 2-hydroxyestradiol, 4-hydroxyestradiol also acts as a cooxidant and increases the formation of prostaglandins from arachidonic acid within the uterus during pregnancy (230). 4-Hydroxyestradiol prevents inactivation of catecholamines by inhibiting catechol O-methyltransferase activity and thereby regulates the neuropsychological/pharmacological effects of catecholamines on the central nervous system (230, 307).

In contrast to 2-hydroxyestradiol, 4-hydroxyestradiol induces carcinogenic effects (230, 307). In fact, recent studies provide evidence for reduced 2-hydroxylation and increased 4-hydroxylation of estradiol in subjects with cancer, suggesting that 2-hydroxyestradiol or its methylated metabolite (2-methoxyestradiol) may be anticarcinogenic, whereas 4-hydroxyestradiol and its methylate 4-methoxyestradiol may be carcinogenic (148, 230, 307). Because abnormal growth of cells is a hallmark for both atherosclerosis, glomerulosclerosis, and cancer, it is feasible that the catechol metabolites of estradiol may also play an important role in regulating cell growth within the vessel wall and the glomeruli.

Role of Binding Proteins

The effects of estradiol can also be influenced by factors regulating its transport to target tissues. In this regard, estradiol is known to bind to serum hormone-binding globulin (SHBG), the synthesis of which is influenced by other hormones (103). The unbound fraction of estradiol is important for inducing its biological activity; moreover, the extent of binding also influences the rate of estradiol metabolism and its elimination from the body, thereby influencing its half-life and pharmacological effects. Hence, any factors that influence the levels of SHBG will subsequently influence the biological effects/activity of estradiol. Within the circulation, 40% of estradiol is bound to SHBG, and the levels of SHBG are influenced by estrogens (increased; 103, 230); androgens (decreased; 103); progestins (decreased; 103); and pathological conditions such as polycystic ovarian syndrome (103). Apart from SHBG, estradiol can also bind to albumin and other membrane proteins (103), which may also influence its biological effects.

Some metabolites of estradiol, e.g., 2- and 4-methoxyestradiol, have low binding affinity to classic ERs but have a higher binding affinity to SHBG than estradiol (230, 307). This suggests that the methoxy metabolites of estradiol may have a longer half-life and may be present within the circulation at much higher levels.

Role of Aryl Hydrocarbon Receptors in Regulating the Biological Effects of Estradiol

The aryl hydrocarbon receptor (AhR) may play a critical role in influencing the effects of estradiol. The unoccupied AhR is a helix-loop-helix (HLH) protein localized within the cytosolic compartment of cells and is a member of the HLH superfamily of proteins, which includes AhR nuclear translocator protein (ARNT); the Drosophila proteins, single minded and period; as well as hypoxia-inducible factor-1α (230). In addition to the lung, pancreas, brain, heart, liver, reproductive or-
gans, tonsils, and B lymphocytes, AhRs are found in the kidney and the vasculature (230, 231, 237), and vascular abnormalities have been observed in AhR-knockout mice (158).

The inactive cytosolic AhR is found complexed with two heat shock protein 90 (hsp90) molecules and a 43-kDa protein (p43). Binding of halogenated aromatic hydrocarbons and environmental estrogens to the AhR initiates disassociation of the hsp90 and p43 molecules and formation of an AhR-ARNT dimer (231). The liganded AhR-ARNT complex is active, translocates to the nucleus, binds to DNA at xenobiotic regulatory elements, and induces the expression of several genes including CYP450 (231, 242). Because CYP450 is involved in estradiol metabolism, the activation of AhR results in increased metabolism of estradiol and influences its biological activity. Moreover, the profile of estradiol metabolites is influenced depending on the types of CYP450 isozymes induced. In MCF-7 cell lines, ligand-activated AhR induces CYP1A1 activity and estradiol metabolism via increases in oxidative metabolism by activating 2- and 4-hydroxylation as well as the 15α- and 16α-hydroxylases (230), suggesting that AhR may influence the biological effects of estradiol by increasing its metabolism.

Crosstalk between ERs and AhRs may also play a role in modulating the effects of estradiol (230, 237). In both MCF-7 and T47D cell lines, ligand-induced activation of AhR inhibits several ER-induced responses. In this regard, AhR decreases ER-induced secretion/production of tissue plasminogen activator (230, 237), postconfluent focus proteins (230, 237), 52- and 160-kDa proteins (237), cathepsin D mRNA, cathepsin D protein (237), pS2 mRNA (237), progesterone receptors and progesterone receptor mRNA levels (230). Moreover, AhR activation downregulates ER mRNA and protein. Safe (237) proposes additional mechanisms via which ligand-activated AhR may induce antiestrogenic effects including interactions between AhR and the ER-induced pathways via generation of intermediary metabolites; direct interactions between the nuclear AhR and the cis-acting genomic sequences in the promoter regions of ER-regulated or growth factor-regulated genes; and induction of trans-acting factors or proteins that facilitate degradation of the nuclear ER (for details, see reviews in Refs. 230, 231, 237).

VASOPROTECTIVE EFFECTS OF ESTRADIOL: ROLE OF GENOMIC VS. NONGENOMIC AND RECEPTOR VS. NONRECEPTOR MECHANISMS

Estradiol most likely induces vasoprotective effects; however, the mechanisms by which these effects are induced remain unclear. Alterations in plasma concentrations of lipoproteins [decrease in low-density lipoprotein (LDL) levels, decrease in oxidized LDL formation, increase in high-density lipoprotein (HDL) levels], hemostatic factors, glucose, insulin, and endothelium-derived factors (decrease in endothelin, increase in nitric oxide and prostaglandins) and inhibition of smooth muscle cell migration and proliferation induced by various mitogens [i.e., platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), insulin-like growth factor I (IGF-I), and ANG II] are hypothesized to contribute to the vasoprotective effects of estradiol (62, 67, 166, 203, 229). Regardless of the mechanisms, the two most important effects of estradiol in the cardiovascular system are modulation of vascular tone and inhibition of vascular growth.

Effects on Vascular Tone

Estradiol induces vasodilatory effects on the vasculature within minutes, suggesting that its vasodilatory effects are nongenomic in nature. In this regard, acute administration of estradiol ex vivo and in vivo induces a rapid vasodilatation in coronary arteries of cholesterol-fed ovariectomized primates and other animals (62, 166, 267, 298). In humans, estradiol dilates coronary and brachial arteries in postmenopausal women and in men (38, 80, 87, 222). Moreover, in vitro studies with isolated vessels provide convincing evidence that estradiol can acutely induce vasodilatory effects (295, 297).

The acute vasodilatory effect of estradiol is largely mediated via generation of nitric oxide (NO) because the vasodilatory effect of estradiol is blocked by NO synthesis inhibitors (31, 35, 139). Estradiol also enhances NO synthesis in endothelium denuded rat aorta (20). Evidence for the role of ERs in mediating the effect of estradiol on NO synthesis comes from the findings that the vasodilatory effect of estradiol and the effect of estradiol on NO synthesis by endothelial cells are blocked by ICI-182780 (31, 35), an ER antagonist. With regard to the role of ERα and ERβ in regulating NO synthesis, studies show that compared with wild-type mice, estradiol-induced NO synthesis and relaxation of the aorta are reduced in mice lacking ERα (232). Moreover, increased endothelial-derived nitric oxide synthase (eNOS) activation in response to estradiol is observed in endothelial cells overexpressing ERα (35). Additionally, a significant association between the number of ERs and basal release of NO is observed in ERα-knockout mice (232); however, there was no correlation between NO release and estradiol levels. These findings suggest that the effects on NO synthesis are ERα mediated. The fact that these effects are triggered within 5 min suggests that the effects are nongenomic (262) and that NO plays a major role in mediating the acute vasodilatory effects of estradiol. This notion is further supported by the observation that estradiol-induced activation of eNOS is not abrogated by actinomycin D (35).

The mechanisms by which estradiol induces the rapid increase in NO release from endothelial cells are unclear and under debate. Recent findings suggest the involvement of calcium-dependent translocation of eNOS from cell membrane to the nucleus (81). NO release by estradiol may involve a plasma membrane rather than a nuclear ER (123). Plasmalemmal caveolae possess ER (123), eNOS, and caveolin; plasma
membrane-impermeable BSA-conjugated estradiol stimulates NO release; and the effects of estradiol on NO release are blocked by agents that decrease intracellular calcium and by the ER antagonist ICI-182780 (123). Recent studies demonstrate ER-α in endothelial cell membranes, and this membrane receptor induces acute effects on NO synthesis (235). Although, the above findings and vast majority of studies suggest a role of intracellular calcium in regulating the rapid release of NO in response to estradiol, this conclusion is not supported by one study (31). Other mechanisms that may be involved are activation of tyrosine kinase and mitogen-activated protein kinase pathways because inhibitors for these pathways block the effects of estradiol on NO production (35, 168). It is proposed that the nongenomic stimulation of NO synthesis may involve intracellular proteins, such as hsp90, which is known to bind to and activate eNOS and may interact with ERs (168). Estradiol upregulates the expression of hsp25 in endothelial cells (45). Alternatively, the antioxidant effects of estradiol may potentiate the activity of NO released under basal conditions. In this regard, estradiol increases rat aorta endothelium-dependent relaxing factor (EDRF) activity in the absence of changes in eNOS gene expression and this increase in EDRF is associated with decreased generation of O₂⁻ in response to estradiol and could account for the enhanced EDRF-NO bioactivity and decreased peroxynitrite release (13, 55).

The role of NO in mediating the acute, nongenomic vasodilatory effects of estradiol is well established. Even so, other mechanisms may also mediate estradiol-induced vasodilation. In this regard, membrane fluidity and ion-channel activity (e.g., Maxi-K and voltage-dependent L-type calcium channels) may play an important role. Application of estradiol stimulates rapid discharge of transient outward currents and induces an increase in the intracellular free calcium concentration in endothelial cells (234). Estradiol also causes coronary vasodilation by opening calcium-activated K⁺ channels (200) and relaxes endothelium-denuded porcine coronary arteries by opening large-conductance (BKCa), calcium-activated, and voltage-activated K⁺ channels via NO and cGMP (44). Estradiol inhibits voltage-dependent L-type calcium currents in SMCs (186) and induces potent stimulatory effects on large-conductance, calcium-activated, and voltage-activated K⁺ channels in coronary artery SMCs. Because the vasodilatory effects of estradiol in intact arteries is abrogated by BKCa channel blockers and inhibitors of cGMP-dependent protein kinase, it is feasible that estradiol induces its vasodilatory effects by opening BKCa channels via NO and cGMP-dependent pathways (295, 297).

Although the role of ERs in mediating the effects of estradiol on ion channels has not been intensively investigated, findings from some studies provide evidence that they may not participate in the process. The inhibitory effects of supraphysiological concentrations (>100 nM) of estradiol on L-type calcium channels are mimicked by the ER antagonist ICI-182780 (233). Moreover, in endothelium-denuded vessels, attenuation of high-K⁺-induced force development and myosin light chain phosphorylation are not blocked by an ER antagonist and are mimicked by estradiol analogs with negligible affinity for ERs (126). Interestingly, recent studies demonstrate that at concentrations of >100 nM (pharmacological concentration) estradiol binds to the β-subunit of Maxi-K channels in vascular SMCs and induces Maxi-K channel activation. This finding provides the first evidence that the direct interaction of estradiol with a voltage-gated channel subunit may be responsible for the direct and acute relaxing effects of estradiol on SMCs (280).

Estradiol rapidly activates adenyl cyclase activity and increases cAMP production (52, 61). Moreover, via the cAMP-adenosine pathway, estradiol induces the production of adenosine in vascular SMCs (52). The fact that the effects of estradiol on cAMP and adenosine synthesis are blocked by an ER antagonist, but not by cyclohexamide, suggests that these effects are mediated via nongenomic, but ER-linked, mechanisms. Because adenylyl cyclase is a membrane-bound enzyme, it is feasible that estradiol may directly interact with adenylyl cyclase. Alternatively, activation of ion channels (as discussed above) may be responsible for the stimulatory effects of estradiol on adenylyl cyclase. Additional studies are required to elucidate the mechanisms by which estradiol induces cAMP and adenosine synthesis.

Although the above findings provide evidence that estradiol induces cAMP production, whether these effects are induced by physiological concentrations of estradiol remains unresolved. In this regard, some studies (37) show that physiological concentrations of estradiol are unable to induce significant increases in cAMP production. However, studies from our laboratory provide evidence that the effects of physiological concentrations of estradiol are significantly blocked by the adenylyl cyclase inhibitor dideoxyadenosine, suggesting that physiological concentrations of estradiol are capable of stimulating cAMP production (52).

Finally, in addition to the above pathways, other mechanisms may also be responsible for the acute nongenomic effects of estradiol on the vasculature. For instance, because estradiol is a phenol, it may induce antioxidant effects and protect the vasculature from free radical-induced deleterious effects.

The rapid-onset, nongenomic effects of estradiol may play an important role in regulating vascular tone; however, the long-term genomic effects of estradiol also importantly contribute to the vascular protective effects of estradiol. Long-term treatment with estradiol induces eNOS (294) and abrogates vasoconstrictor effects on vascular tissues (39, 122, 232). Compared with premenopausal women, NO synthesis is decreased in postmenopausal women and is normalized by estradiol replacement therapy (110, 228). This effect of estradiol replacement therapy, however, is diminished by coadministration of synthetic progesterin, medroxyprogesterone, and cyproterone acetate (110). Vascular NO synthesis is decreased in mice lacking ER-α (232), and
long-term administration of estradiol increases acetylcholine-mediated coronary vasodilation in nonhuman primates (298, 299), male-to-female transsexuals (197), postmenopausal women (95), and in postmenopausal women with angina and normal coronary arteries (224). The role of genomic effects of estradiol on vascular tone is further supported by the recent observation that impaired endothelium-dependent vasorelaxation (267) and early coronary calcification (266) are observed in a subject lacking functional ER-α. However, the vasodilatory response to estradiol was adequate, suggesting a nongenomic response and that a lack of ER-α may not be as deleterious as previously hypothesized (266, 267). Indeed, estradiol prevents neointima formation in ER-α-knockout mice (107). Estradiol also increases the synthesis of prostacyclin by inducing the expression of prostacyclin synthase (33, 169) and cyclooxygenase (115).

Estradiol reduces blood pressure in various animal models (26, 43, 142, 249), and the modulatory effects of estradiol on vascular tone may be responsible for estradiol-induced effects on blood pressure. Multiple clinical studies show that both long-term and short-term administration of estradiol replacement therapy is associated with either the lowering of blood pressure (92) or blood pressure-neutral effects (275) in most postmenopausal women. However, estradiol replacement therapy infrequently increases blood pressure in postmenopausal women (152). The blood pressure-lowering effect of estradiol may be mediated by direct effects of estradiol on ion channel activity or increased synthesis of vasodilatory substances such as NO, cGMP, cAMP, adenosine, and prostacyclin. Alternatively, estradiol may lower blood pressure by reducing the synthesis of ANG II and endothelin-1 (ET-1) or by interfering with the synthesis of and decreasing the plasma levels of catecholamines (150, 212, 244).

Effects on Vascular Growth

In vivo studies conducted in several animal species and using various models [balloon injury-induced neointima formation, allograft-induced dysplasia, cholesterol/lipid-induced atherosclerosis, and vascular narrowing-induced neointima formation (23, 34, 36, 65, 66, 68, 88, 107, 144, 153, 179, 204, 238, 270)] provide evidence that estradiol prevents pathological vascular remodeling processes and neointima formation. Yet, the exact mechanisms involved remain unclear. Evidence suggests that the inhibitory effects of estradiol on vascular remodeling processes leading to occlusive disorders are mediated via multiple pathways, involving interactions with a variety of growth factors, cell types, and biochemical/molecular mechanisms (62, 67, 166, 203). These mechanisms are discussed below.

Interactions with SMCs. Abnormal activity of SMCs is one of the key processes responsible for vascular pathology. In this regard, estradiol inhibits SMC proliferation, migration and extracellular matrix (collagen) synthesis induced by serum, PDGF, ANG II, ET-1, fibronectin, free radicals, IGF-1, bFGF, oxidized-LDL, and mechanical pulsatile stretch (122, 132, 156, 203, 205, 229).

Our recent study provides evidence that estradiol inhibits PDGF-BB-induced growth and mitogen-activated protein (MAP) kinase activity in human aortic SMCs (49, 230). Estradiol also inhibits FCS, ANG II, and ET-1-induced MAP kinase and MAP kinase activity in vascular SMCs (176) and downregulates mitogen-induced expression of both c-myc and c-fos (176), which are known to be activated downstream from MAP kinase.

Although estradiol induces its antimitogenic effects on SMCs in part by inhibiting the MAP kinase pathway, other mechanisms are operative. For instance, estradiol inhibits the mitogenic effects of IGF-1 (156), which acts by stimulating phosphatidylinositol turnover, diacylglycerol formation, intracellular calcium flux (22), and protein kinase C (PKC) activity (55). Moreover, estradiol downregulates the expression of IGF-1 receptors in SMCs (156), thus providing another mechanism by which estradiol abrogates the mitogenic effects of IGF. Also, estradiol inhibits transplant arteriosclerosis in the rat aorta accelerated by topical exposure to IGF-1 (179). Because the mitogenic effects of IGF-1 and bFGF on SMC are associated with activation of PKC (55, 290), it is feasible that the inhibitory effects of estradiol are in part mediated via downregulation of PKC activity; however, direct evidence in this regard is lacking.

Estradiol stimulates the synthesis of growth inhibitory molecules in SMCs such as cAMP (52, 61). Data from our laboratory provide evidence that estradiol enhances the synthesis of adenosine in SMCs via the cAMP-adenosine pathway, which involves the conversion of cAMP to adenosine via sequential action of ectophosphodiesterase and ecto-5’-nucleotidase at the surface of SMC membranes (52, 55). Because the cAMP-adenosine pathway induces inhibitory effects on SMC growth (55), the antimitogenic effects of estradiol may be mediated in part via this mechanism. Indeed, our studies show that the inhibitory effects of estradiol on serum-induced growth of aortic SMCs are significantly reversed by the adenyl cyclase inhibitor dideoxyadenosine and by the A3 adenosine-receptor antagonists 1,3-dipropyl-8-p-sulfophenylxanthine and KF-17837 but not by the cAMP-dependent protein kinase inhibitor Rp-cAMPS (52). Moreover, the inhibitory effects of estradiol are associated with increased production of cAMP and adenosine, an effect that is blocked by the ER antagonist ICI-182780 but not by cyclohexamide (52). These findings provide evidence that the inhibitory effects of estradiol mediated via the cAMP-adenosine pathway involve the participation of a nongenomic pathway linked to ERs.

Estradiol also upregulates NO synthesis, and NO inhibits SMC proliferation and migration (49, 52, 74). Hence, it is feasible that increased production of NO may also contribute to the inhibitory effects of estradiol on SMC growth. A significant reduction in the atherogenic effect of estradiol was observed after long-term inhibition of NO synthesis in cholesterol-clamped...
rabbits (100). However, inhibition of NO synthesis did not abrogate the protective effects of estradiol in apolipoprotein-deficient mice (58) or cholesterol-induced atherosclerosis in rabbits (189). Thus, although estradiol-induced NO synthesis may participate in mediating inhibition of vascular SMC biology, other mechanisms are also involved.

Estradiol also inhibits migration of SMCs induced by fibronectin via ER-linked genomic pathways (129). Moreover, via ER-dependent mechanism estradiol enhances the release of matrix-metalloproteinase-2 (a regulator of cell migration) in SMCs (300). Estradiol inhibits mitogen-induced synthesis of elastin and various types of collagen, including types I and III (17, 64). Because collagen is known to induce SMC migration, this mechanism may contribute to estradiol-induced inhibition of SMC migration. The influence of estradiol on generation of inhibitory extracellular molecules or downregulation of the generation of stimulatory molecules from SMCs is supported by the recent findings of Li et al. (145). These investigators demonstrate that, in contrast to conditioned medium obtained from untreated SMCs, conditioned medium from estradiol-treated SMCs inhibits mitogen- as well as SMC-induced migration of adventitial fibroblasts (145). Importantly, conditioned medium obtained from SMCs treated with estradiol plus ICI-182780 does not inhibit fibroblast migration. Because adventitial fibroblasts participate in the vascular remodeling process associated with coronary artery disease, it is feasible that estradiol may directly prevent neointimal thickening by interacting with SMCs to stimulate or inhibit the synthesis of factors that affect the migration of adventitial fibroblasts.

Interactions with endothelial cell, macrophages, and monocytes. Interactions of estradiol with endothelial cells, macrophages, and monocytes may contribute to the vasoprotective effects of estradiol. Under normal circumstances, the endothelium is thought to induce a net inhibitory effect on SMC growth (55), and damage or dysfunction of the endothelium by balloon catheters, vascular cuffs, and immune reactions results in abnormal proliferation of SMCs and neointima formation, effects that are abrogated by estradiol (23, 34, 36, 65, 66, 68, 88, 107, 144, 153, 179, 204, 238, 270).

Estradiol regulates the angiogenesis process by inducing endothelial cell growth in multiple reproductive organs (173); moreover, estradiol accelerates functional endothelial recovery after arterial injury (132). Although the growth-promoting effects of estradiol on endothelial cells are regulated by multiple factors, vascular endothelial growth factor (VEGF) and bFGF appear to play a key role in mediating the mitogenic effects of estradiol on endothelial cells. In aortic endothelial cells estradiol increases bFGF production via a PKC- and ER-dependent mechanism that is non-genomic (5). Moreover, studies show that estradiol induces delayed mitogenesis in human umbilical vein endothelial cells via activation of extracellular signal-regulated kinase 1/2 and that these effects are blocked by antibodies to bFGF (124). These findings suggest that the mitogenic effects of estradiol on endothelial cells may be bFGF mediated.

Aortic intimal hyperplasia in ovariectomized sheep is associated with a twofold increase in bFGF levels, and this effect is abrogated in sheep receiving estradiol replacement (247). The fact that bFGF is a mitogen for SMCs and neointima formation occurs in ovariectomized sheep without endothelial damage suggests that the antimitogenic effects of estradiol may rely more on blocking bFGF-induced SMC growth than on stimulating bFGF-induced endothelial cell growth. Additionally, in endothelium injury- or transplant-induced intimal dysplasia, the effects of bFGF on SMC growth may be enhanced as cell injury results in the release of bFGF and NO (generated in response to cytokines via inducible nitric oxide synthase (iNOS); see review in Ref. 55), and in combination with NO the mitogenic effects of bFGF are known to be enhanced severalfold (55). Because estradiol inhibits iNOS activity (121), this would block the synergistic interaction between NO and bFGF. Estradiol may differentially regulate the growth effects of bFGF in SMC and EC (55). Indeed, estradiol inhibits mitogen-induced MAP kinase and MAP kinase activity in SMCs (176) and induces MAP kinase activity in ECs (177, 196). A possible cause for these different effects may be due to the expression of heterogeneous forms of the cognate receptors (97). Alternatively, differential generation of coactivators or coactivator proteins, which bind to steroid hormone receptors and silence the transcription process (172, 251), may contribute to the observed differential effects.

VEGF is another important factor that induces endothelial cell growth. Both ECs and SMCs synthesize VEGF, and estradiol induces the synthesis of VEGF in endothelial cells (272). In vivo studies provide evidence that infusion of VEGF after balloon injury results in rapid recovery of the endothelium and inhibition of neointima formation (10, 293), suggesting that VEGF may protect against vasoocclusive disorders. This notion is further supported by the fact that transfer of the VEGF gene reduces intimal thickening in cuff-induced neointima formation in the presence of intact endothelium (138). The mechanism by which VEGF prevents neointima formation is unclear; however, studies show that inhibition of neointima formation in animals with VEGF gene transfer is reversed by the NO synthesis inhibitor nitro-l-arginine methyl ester, suggesting that NO may be the ultimate mediator of the antimitogenic effects of VEGF on SMCs (138). Although estradiol may induce antimitogenic effects on SMCs via VEGF-induced NO synthesis, the role of NO in mediating the inhibitory effects on neointima formation is not supported by all studies. In this regard, protective effects of estradiol on cuff- as well as diet-induced vascular remodeling and neointima formation are not abolished by NO synthesis inhibitors in some studies (58, 100, 189). Moreover, the role of prostacyclin can be ruled out as indomethacin does not abrogate the antimitogenic effects of estradiol (4). Hence, other mechanisms than endothelium-derived NO and prostacyclin generation...
may be involved in mediating the antimitogenic effects of estradiol on SMCs.

VEGF induces its mitogenic effects on ECs via activation of a Raf-MEK-MAP kinase-PKC-dependent pathway (273). Because estradiol induces MAP kinase activity in ECs, it is feasible that the effects of estradiol on EC growth and VEGF's synthesis are MAP kinase mediated. Estradiol not only induces the synthesis of VEGF but also increases the synthesis and expression of VEGF receptor-2 in endothelial cells (272). Although ER and VEGF mediate the mitogenic effects of estrogen on ECs, however, the role of ER-α and ER-β in mediating these effects remains controversial (7, 113).

Apart from inducing endothelial cell growth, estradiol protects endothelial cells against apoptosis, an effect that is ER mediated (258). This provides yet another mechanism by which estradiol may enhance the functional recovery of endothelial cells. This may be particularly important in dysplasia and remodeling associated with balloon injury or chronic allograft rejection, where the endothelium is damaged as a result of initial inflammation, infiltration with lymphocytes, macrophages, and thrombocytes, and generation of cytokines such as TNF-α and IL-1β (227). Indeed, TNF-α-induced apoptosis is prevented by estradiol in cultured endothelial cells (258). Estradiol also improves endothelial cell survival and inhibits apoptosis by improving endothelial cell interactions with the substructure and increasing tyrosine phosphorylation of p125 focal adhesion kinase (8). Because free radicals are generated at sites of cell injury and in response to cytokines, it is feasible that estradiol, being an antioxidant, prevents apoptosis by scavenging free radicals. Indeed, estradiol protects neuronal cells against free radical-induced apoptosis (16).

A key process in the cascade of events after endothelial cell injury and inflammation is the recruitment and activation of leukocytes, macrophages, and monocytes at the site of injury. This process is facilitated by expression of adhesion molecules, followed by subsequent adhesion of macrophages and monocytes. Adhesion leads to activation, transendothelial migration, and localization of inflammatory cells within the subendothelial space. Once in the subendothelial space, macrophages take up LDL, become foam cells, and generate growth factors that subsequently result in SMC growth and vascular remodeling. Because increases in the expression of adhesion molecules such as intercellular cell adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E-selectin are associated with cardiovascular disease (105), it is feasible that estradiol induces vasoprotective effects in part by downregulating the expression of these adhesion molecules. In this regard, several studies demonstrate the effects of estradiol on the levels and expression of adhesion molecules in the circulation and on endothelial cells. Overall, most studies, but not all, provide evidence that in endothelial cells estradiol attenuates TNF-α-induced expression of extracellular cell adhesion molecule-1, ICAM-1, and VCAM-1 (301), IL-1β-induced expression of E-selectin, VCAM-1, and ICAM-1 (32, 185), lysophosphatidylcholine-induced expression of VCAM-1, and lipopolysaccharide (LPS)-induced VCAM-1 expression (256). The inhibitory effects of estradiol on stimulated expression of adhesion molecules at the protein and mRNA level are blocked by the ER antagonists ICI-182780 or ICI-164384 (256), suggesting that these effects are mediated via ER-linked genomic mechanisms. Similar to the effects on endothelial expression of adhesion molecules, estradiol lowers serum levels of circulating adhesion molecules E-selectin, ICAM-1, and VCAM-1 (127).

The effects of estradiol on the expression of adhesion molecules is thought to be mediated through a classic direct regulatory action on the 5′-flanking region of the VCAM-1 gene; however, there are no EREs on the VCAM-1 gene promoter (106). Endothelial activation and expression of adhesion molecules by cytokines are critically regulated through the interplay of various transcription factors such as NF-κB and AP-1. Hence, it is feasible that interaction of estradiol with ERs interferes with one or more transcription factors induced via this mechanism and as already described in other biological systems (263). Alternatively, estradiol may directly interact with AP-1 or NF-κB (40).

In addition to the above-mentioned adhesion molecules, estradiol inhibits the expression of P-selectin (112) and monocyte chemotactic protein-1 (214) and inhibits monocyte adhesion to endothelial cells in response to cytokines and oxidized LDL (271). In vivo studies provide evidence that estradiol blocks macrophage infiltration in aortic allograft-induced atherosclerosis and intimal hyperplasia (36). Moreover, in hypercholesterolemic rabbits estradiol prevents the transendothelial migration of leukocytes into the subendothelial space (190).

Estradiol prevents allograft transplant-induced arteriosclerosis and hyperplasia in the absence of immunosuppressants, inhibits allograft-inducible major histocompatibility complex class II antigen expression (153), and protects against transplant arteriosclerosis even in the presence of upregulation of IFN-γ ligand and receptor (239). Increased synthesis of NO is observed in allograft transplant-induced arteriosclerosis and at sites of vascular injury and neointima formation. It is postulated that excessive NO generation via iNOS could lead to formation of peroxynitrite, a cytotoxic molecule that damages cells and releases bFGF (55). Because bFGF is a potent mitogen in conjunction with NO (55), this would provide a potent growth stimulus for SMCs. In rat allografts, estradiol inhibits iNOS while preserving eNOS activity, and this is accompanied by the inhibition of neointima formation (238). Interestingly, estradiol also inhibits IFN-γ and LPS-induced NO synthesis by macrophages, which also participate in the vascular remodeling process (93). Together, these findings suggest that the inhibitory effects of estradiol on cytokine-inducible iNOS activity play a critical role in preventing injury as well as immune-induced dysplasia. Although the inhibitory effects of estradiol on iNOS activity are well documented, this contention is not supported by all studies.
as cytokine-induced NO synthesis was not inhibited by estradiol in murine macrophages and osteoclasts (171, 281).

Modulation of circulating growth factors. Estradiol also induces antivasoocclusive effects by modulating the synthesis of circulating factors that are mitogenic for SMCs and damaging to ECs. In this regard, estradiol downregulates the expression of angiotensin-converting enzyme (ACE) in serum as well as in the aorta and reduces ANG II formation (71). ACE expression is increased at sites of balloon injury and angioplasty (63), ANG II is a potent mitogen for SMCs (49, 54), and ANG II is known to induce vascular remodeling processes associated with cardiovascular disease. The finding that estradiol downregulates ACE and reduces ANG II synthesis suggests that estradiol may abrogate SMC growth in part by decreasing ANG II biosynthesis. Estradiol replacement therapy decreases ANG II levels and ACE activity in postmenopausal women (218) and suppresses renin levels (244). Estradiol also downregulates the expression of AT1 receptors in SMCs (198). Because these receptors mediate the mitogenic effects of ANG II, estradiol may abrogate the effects of ANG II on growth. Indeed, our studies show that estradiol inhibits ANG II-induced growth of human SMCs in vitro (229). Additionally, estradiol induces the synthesis of ANG 1–7, a vasodilator and SMC growth inhibitor (55).

Another important molecule associated with vasoocclusive disorders is homocysteine. It is well documented that homocysteine induces endothelial cell damage (287), inhibits endothelial cell growth, and induces SMC growth (278). Clinical studies provide evidence that estradiol reduces circulating levels of homocysteine in postmenopausal women. In female-to-male transsexuals, homocysteine levels increase with androgen treatment, whereas in male-to-female transsexuals homocysteine levels decrease with estradiol substitution (283). Compared with women and male-to-female transsexuals, an increased incidence of cardiovascular disease is observed in men as well as in female-to-male transsexuals (79). It is feasible that by lowering homocysteine levels, estradiol protects the vascular endothelium and SMCs from damage and growth, respectively, and this inhibits the remodeling process and protects against vasoocclusive disorders.

Another mechanism by which estradiol may induce antivasoocclusive actions is via upregulation of leukemia inhibitory factor (LIF), a factor that inhibits c-fos injury-induced neointima formation (175) and hypercholesterolemia-induced fatty streak formation (174) as well as upregulates LDL receptors and lowers serum cholesterol levels (174). This notion is supported by our finding that estradiol can upregulate LIF synthesis in reproductive tissue (221). However, further studies are required to determine whether estradiol induces LIF synthesis in vascular tissue.

Estradiol inhibits serum and ANG II-stimulated synthesis and mRNA expression of ET-1 (177) in endothelial cells via ER receptor-dependent mechanisms (3). Estradiol also blocks the mitogenic effects of ET-1 on SMCs and inhibits ET-1-induced MAP kinase activation (176). Compared with premenopausal women, ET-1 levels are increased in postmenopausal women not taking estradiol, and ET-1 levels are reduced in postmenopausal women after estradiol substitution (304).

Estradiol also influences the synthesis of factors associated with coagulation, atherogenesis, and neointima formation. In this regard, estradiol decreases plasma concentrations of procoagulant factors such as clotable fibrinogen (77, 128), soluble thrombomodulin, plasminogen activator inhibitor 1 (128), antithrombin III, and protein S (78, 184, 275). Moreover, most (91), but not all (184, 241), studies report that estradiol decreases levels of von Willebrand factor. It is important to note that the effects of estradiol on coagulation and fibrinolytic factors depend on the type of estrogen used. For example, compared with estradiol, ethinyl estradiol, an oral contraceptive, has different effects on factors involved in regulating coagulation (94). Finally, estradiol stimulates NO (35, 139) and prostacyclin synthesis (33), factors well known to prevent platelet aggregation and adhesion and to induce antimitogenic effects on SMCs. Indeed, the antiaggregatory effects of estradiol can be blocked by NO synthesis inhibitors (187), suggesting that the antiaggregatory effects are NO-mediated. In contrast, Zoja et al. (308) demonstrated that estradiol corrects platelet dysfunction in uremia by inhibiting NO and that estradiol has a procoagulant role in patients with chronic renal disease (308).

Antioxidant effects. The antioxidant effects of estradiol and its metabolites may play a critical role in the antivasoocclusive effects of estradiol. Indeed, physiological and supraphysiological concentrations of estradiol induce antioxidant activity in vitro and ex vivo in vivo, respectively (245, 252), and estradiol inhibits oxidized-LDL-induced endothelial damage (191) and superoxide anion (30)- and cholesterol-induced SMC growth (99). We recently demonstrated that physiological concentrations of 2-hydroxyestradiol, a prominent estradiol metabolite, is a potent antioxidant that prevents peroxyl radical-induced peroxidation of vascular SMC membrane phospholipids and inhibits peroxyl radical-induced proliferation and migration of SMCs (56). 2-Hydroxyestradiol prevents the oxidation of acidic membrane phospholipids (phosphatidyllysinsitol and phosphatidylserine), which are known to activate PKC activity and play a critical role in regulating SMC growth (56). Makides et al. (162) demonstrate that methoxy metabolites of estradiol are also potent antioxidants. Because SMCs rapidly convert 2-hydroxyestradiol to 2-methoxyestradiols (53a), it is feasible that the antioxidant effects of 2-hydroxyestradiol on membrane lipids are mediated by methoxyestradiol. Estradiol also reduces glycooxidative damage in the artery wall (288), suggesting that the antioxidant effects of estradiol and its metabolites at the cell membrane level may play a critical role in mediating the growth regulatory and antimitogenic effects of estradiol. Finally however, even though multiple in vitro and ex vivo studies support the notion
that estrogen increases the resistance of LDL to oxidation, ample studies in postmenopausal women receiving estrogen show no difference (see review in Ref. 245). Taken together, the evidence for in vivo antioxidant effects of physiological concentrations of estrogen remains controversial.

**Interaction with lipids.** Estradiol influences the vascular effects of LDL cholesterol. Estradiol, which is a phenol with antioxidant properties, prevents the oxidation of LDL and very-low-density lipoprotein (VLDL) to oxidized LDL and oxidized VLDL, both in vivo and in vitro (163, 223, 236) and protects the vasculature against the deleterious effects of oxidized lipids. Estradiol also prevents compromise of the endothelial barrier mediated by LDL and attenuates the accumulation of LDL and oxidized LDL in the artery wall (73). Also, TNF-α-mediated oxidation and accumulation of LDL in the artery wall are prevented by estradiol (288). Moreover, estradiol increases the catabolism of LDL, LDL apolipoprotein (B), and β-VLDL via LDL receptor-dependent and -independent mechanisms (41, 46). In this regard, estradiol has been shown to increase the expression of LDL receptors (46). Pharmacological levels of estradiol increase hepatic mRNA for the LDL receptor (18, 96, 259) and increase the synthesis of LDL receptor protein out of proportion to the increase in hepatic LDL receptor mRNA, indicating both transcriptional and posttranscriptional regulation of the LDL receptor by estradiol (18). Estradiol also improves the clearance of VLDL, decreases LDL production (302), reduces LDL particle size, increases the clearance of both the light and dense LDL (27), increases expression of VLDL and LDL receptors in the left ventricles of the heart (48, 165), and induces sterol-27-hydroxylase activity, which decreases LDL production (136). Estradiol-induced removal of VLDL is associated with increased activities of hepatic lipase, lipoprotein lipase, and expression of LDL receptors (46). There is also evidence for crosstalk between ERs and LDL receptors and for upregulation of ER associated with an increase in LDL receptor expression, and these effects can be blocked by the ER antagonist tamoxifen (210).

Foam cell and fatty streak formation is a key step in plaque formation and involves the interaction of LDL, macrophages, and SMCs. Several lines of evidence suggest that estradiol can interfere with key biochemical pathways associated with these processes. In this regard, incubation of a human THP-1 macrophage cell line with estradiol reduces the uptake and metabolism of 125I-labeled human LDL (268), suggesting the possibility that estradiol may reduce degradation of oxidized-LDL via scavenger receptors (211). In vivo studies in cynomolgus macaques show that the rate of LDL degradation is strikingly decreased in arteries in response to estradiol (286). Moreover, estradiol reduces cholesterol accumulation and esterification (268, 285), a key step for foam cell formation. Pharmacological concentrations of estradiol inhibit migration of monocyte cells stimulated with oxidized-LDL by inhibiting secretion of MCP-1 by monocyte cells (201). Simultaneous treatment with estradiol inhibits oxidized-LDL-induced adhesion of monocytes to endothelial cells (271). Because estradiol downregulates the expression of adhesion molecules, it is feasible that estradiol inhibits the adhesion process by preventing oxidized LDL-induced expression of VCAM-1 and ICAM-1 (42).

In contrast to LDL, increases in HDL are associated with cardioprotection. Apolipoprotein A-I accounts for most of the protein in HDL, and plasma concentrations of apolipoprotein A-I are increased in premenopausal women and in postmenopausal women treated with estradiol (219, 289). Clinical studies provide evidence for estradiol-induced increases in the rate of production of HDL subfractions (289) as well as reduced plasma clearance of HDL (219). Studies in rats and mice show that estradiol increases hepatic apolipoprotein A-I mRNA levels and increases hepatic rates of apoprotein AI transcription (259, 219). In a hepatoblastoma cell line, pharmacological concentrations of estradiol increase apolipoprotein A-I secretion and apolipoprotein A-I mRNA levels by increasing the rate of apoprotein AI mRNA transcription without effecting apoprotein AI mRNA stability (274). Estradiol also induces the synthesis of apolipoprotein E (260), and this may confer vasoprotection. However, in apolipoprotein E-deficient mice, estradiol prevents both fatty streak formation and atherosclerotic lesions (58). Therefore, mechanisms other than changes in apolipoprotein E synthesis are also involved in mediating the vasoprotective effects of estradiol.

Although alterations in LDL and HDL levels by estradiol may protect the cardiovascular system, the stimulatory effects of estradiol on triglyceride levels (276) may be deleterious to the cardiovascular system. However, the association between triglycerides and cardiovascular disease has been shown to be weak (70). Although the increases in triglyceride levels may not be important for cardiovascular disease, increases in triglyceride levels may have deleterious effects on the progression of renal diseases (discussed in the renal section).

**Evidence for non-receptor-mediated antimitogenic effects of estradiol.** Vascular ECs and SMCs express the functional ERs, ER-α and ER-β (98, 262). However, whether the cardiovascular effects of estradiol are ER mediated is still unresolved and under debate. Recent studies by Isfrati et al. (107) show that estradiol induces vasoprotective effects in mice lacking ER-α, suggesting that the effects may be mediated via ER-β. The notion that ER-β mediates antivasoocclusive effects of estradiol is further supported by the finding that, similar to estradiol, low concentrations (25 μg·kg^-1·day^-1) of genistein, a phytoestrogen that binds exclusively to ER-β and has negligible affinity for ER-α binding (135), inhibits balloon injury-induced neointima formation (157). However, this study does not provide any evidence regarding whether the effects of genistein are blocked by ER antagonists such as ICI-182780; moreover, inhibitory effects of genistein on SMC growth are observed at concentrations of >2.5 μM. Hence it is feasible that the effects of genistein are mediated via some alternative pathway not involving ERs. Indeed, at mi-
cromolar concentrations genistein is a tyrosine kinase inhibitor and inhibits growth of cancer cells containing or lacking ERs (292). Moreover, our previous study demonstrates that the inhibitory effects of genistein on human aortic SMCs are not reversed by ICI-182780 (50). Also, Karas et al. (118) provide evidence that estradiol protects against vascular injury in mice in which ER-β is genetically disrupted. Together, these finding provide evidence that estradiol can induce vasoprotective effects via mechanisms independent of ERs. However, the possibility that the ER-α may compensate for ER-β in ER-β-knockout mice, and vice versa, remains an unresolved issue. In a recent study, Bakir et al. (11) demonstrated that the inhibitory effects of estradiol on neointima formation are blocked by ICI-1827980, suggesting that the effects are ER mediated. However, the authors also observed that concentrations of ICI-182780 that blocked the effects of estradiol also induced circulating levels of estradiol (11). Because estradiol metabolites are more potent than estradiol in inhibiting SMC growth and ICI-182780 inhibits estradiol metabolism (53a), it is feasible that the effects of ICI-182780 may be due to inhibition of metabolism of estradiol and not to the antagonistic effects on ERs.

Several lines of evidence support the hypothesis that estradiol may induce cardiovascular-protective effects via mechanisms not involving ERs. In this regard, increased expression of both ER subtypes occurs in the vasculature of male rats after allograft and balloon injury (149, 154). However, estradiol is unable to prevent neointima formation in nongonadectomized male rats after balloon injury (204), suggesting that mechanisms other than ERs are responsible for mediating the antivasoocclusive effects. Indeed, we and others report that pharmacological agents such as tamoxifen and 4-hydroxytamoxifen, which compete with estradiol for ERs, do not abrogate the antimitogenic effects of estradiol. Moreover, in vitro studies from our laboratory provide evidence that the estradiol metabolites, 2-hydroxyestradiol and 2-methoxyestradiol, which have minimal affinity for ERs are several times more potent than estradiol in inhibiting SMC as well as cardiac fibroblast growth (51, 56, 199). Also, the inhibitory effects of estradiol, but not estradiol metabolites, are reversed by the pure ER antagonist ICI-182780 (53a). The above findings, together with the fact that SMCs and endothelial cells are capable of metabolizing estradiol (15), suggest that the protective effects of estradiol may be mediated via local generation of estradiol metabolites and independent of ERs. Finally, the upregulation of ERs after balloon injury may be merely a consequence of the increased generation of growth factors at the site of injury and not related to the protective effects of estradiol. In this regard, metabolites induce the synthesis of ERs in vascular cells, and this effect is mediated via a MAP kinase-independent pathway (117).

Interestingly, even though the binding affinity of ICI-182780 to ERs is similar to estradiol (134, 135), 50 times higher concentrations of ICI-182780 are required to block the effects of estradiol on SMC growth (49). Moreover, preliminary studies from our laboratory provide evidence that ICI-182780, which is structurally similar to estradiol, inhibits estradiol metabolism (53a). Concentrations of ICI-182780 lower than the inhibition constant value are ineffective in blocking the inhibitory effects of estradiol, even when the estradiol-to-ICI ratio is 1:1,000. Together, these findings provide evidence that metabolism of estradiol may play a key role in mediating the inhibitory effects of estradiol. This notion is supported by the fact that 2-methoxyestradiol, an estradiol metabolite, is a potent antiangiogenic factor that inhibits growth of SMCs and cardiac fibroblasts and does not bind to ERs (51, 69, 199, 279). The role of estradiol metabolites is further strengthened by the findings that 2-hydroxyestradiol and 2-methoxyestradiol induce prostacyclin and NO synthesis, inhibit lipid peroxidation, and lower cholesterol levels (151, 199, 246, 279).

ENDOGENOUS ESTROGEN: BIOLOGICAL POTENCY AND PATHOPHYSIOLOGICAL IMPORTANCE

In addition to estradiol, numerous other estrogens are present in vivo and are known to possess biological activities that are similar to estradiol as well as activity that is opposite to estradiol. Hence, the effects of estradiol may not reflect the effects of all estrogens. Conversely, the effects of other estrogens may not reflect the biological effects of estradiol. Our recent studies show that the inhibitory effects of various estrogens on mitogen-induced SMC growth and MAP kinase activity vary considerably. Estrone and estriol, which are present in large amounts endogenously, are not effective in inhibiting SMC growth and MAP kinase activity. In fact, they significantly abrogate the inhibitory effects of estradiol (49), suggesting that inactive estrogens may block the biological effects of estradiol. Indeed, this may explain in part why the use of conjugated estrogens apparently do not reduce cardiovascular risk in postmenopausal women (104). In contrast to estrone and estriol, metabolites of estradiol such as 2-hydroxyestradiol, 2-methoxyestradiol, and 4-methoxyestradiol, which show minimal binding to ER, are more potent inhibitors of mitogen-induced SMC growth compared with estradiol.

Apart from the differential potency of various estrogens on ERs, they also have significant differences in their chemical properties. In this regard, the antioxidant potency of estrone and estriol is much less than estradiol, whereas the catechol estriol are more potent antioxidants than estradiol. Together, these findings suggest that the biological effects of estrogens may vary considerably.

The above findings may have clinical implications as several different estrogens are used for hormone replacement therapy. Indeed, recent in vitro studies from our laboratory provide evidence that clinically used estrogens differentially inhibit SMC growth and MAP kinase activity (49). Because direct interactions of estrogens with SMCs may be responsible for the protec-
tive effects of estrogens on the vasculature, it is feasible that estrogens unable to inhibit SMC growth would be inactive against neointima formation or vasoocclusive disorders.

Another important issue is what concentrations of estradiol are relevant to physiology. Although most studies use circulating levels of estradiol \((10^{-9} \text{ mM})\) as a physiological concentration, under in vivo conditions estradiol may exist in several biologically active forms. Thus the levels of estradiol in the plasma may not reflect the true physiological effects of estradiol. For example, the concentrations of 2-hydroxyestradiol range between 0.12 and 0.3 \(\mu \text{M}\) in peripheral blood, and the rate of urinary excretion of 2-hydroxyestradiol is 20–180 \(\mu \text{g}/24\) h in urine \((6, 78)\). Finally, the recent finding that vascular SMCs express aromatase activity and synthesize estradiol \((96)\) suggests that the local levels of estradiol may be higher.

**VASCULAR PROTECTIVE EFFECTS OF ESTRADIOL: EPIDEMIOLOGICAL EVIDENCE**

The evidence for a role of estradiol in regulating vascular structure and function is that ovarian dysfunction and estradiol deficiency are linked to an increased risk of cardiovascular disease in postmenopausal women. Thus compared with men, women within the reproductive age group are protected against cardiovascular disease \((143, 203, 296)\), and these differences decrease with the onset of menopause \((143)\). Also, premenopausal women who undergo premature surgical menopause (i.e., bilateral oophorectomy) have twice the risk of cardiovascular disease than do age-matched premenopausal controls \((103, 225)\).

Because the ovaries are the main source of estradiol production, their dysfunction with age results in a decreased production of estradiol. In this regard, it is important to note that the levels of estradiol, which is the most potent natural estrogen and is largely produced in the ovary, decrease to undetectable levels in postmenopausal women. Compared with estradiol, the levels of other estrogens such as estrone, which can be synthesized in the peripheral compartment, do not fall as dramatically. This suggests that the beneficial effects of ovarian steroids on the cardiovascular system may largely be mediated by estradiol.

Several epidemiological studies demonstrate a reduction in risk of coronary heart disease in postmenopausal women treated with oral estrogen compared with untreated women \((25, 83, 203)\). Three meta-analyses estimate an overall reduction in risk of \(-50\%\) for women who had at any time received estrogen therapy. Four studies examine the association between estrogen use and angiographically defined coronary artery disease in postmenopausal women and demonstrate that the calculated odds ratios for severe coronary stenosis or \(70\%\) occlusion are 0.50 or lower for estrogen users compared with nonusers. A reduction in mortality among women with diagnosed coronary atherosclerosis is reported in relation to estrogen use \((101, 203, 261, 269)\). Although, estrogen replacement therapy may reduce vasoocclusive disorders in postmenopausal women, hormone replacement therapy using premarin (conjugated estrogen) plus medroxyprogesterone (the HERS study) does not reduce the overall incidence of coronary artery disease in postmenopausal women with established coronary heart disease \((104)\). Several lines of evidence suggest that unlike progesterone, synthetic progestin such as medroxyprogesterone can abrogate the protective effects estradiol on vascular cells. In this regard medroxyprogesterone attenuates the inhibitory effects of estradiol on neointima formation \((2, 144)\) and on NO synthesis \((110)\). Hence it is possible that the lack of effects of estrogen replacement therapy in the HERS study is due to the negative effects of medroxyprogesterone. Alternatively, the lack of effects could also be due to the type of estrogen used. In this regard, our studies show that estrone, estradiol, and estrone sulfate, which constitute a major portion of the conjugated estrogen, have little or no inhibitory effects on mitogen-induced SMC growth \((53)\). Because direct interaction of estrogen with SMC in part contributes to the cardioprotective effects of estrogen, it is feasible that the lack of effect of conjugated estrogen is due to the lack of inhibitory estrogen in that specific preparation. Indeed, several studies show that estradiol replacement reduces neointimal thickening in the carotid arteries of postmenopausal women with established coronary artery disease and undergoing percutaneous transdermal coronary angioplasty \((203)\). Taken together, the above findings provide evidence that overall estradiol induces protective antivasoocclusive effects on the cardiovascular system.

Whether estradiol induces antivasoocclusive effects in subjects with established coronary artery disease or has only acute protective effects in patients with minimal occlusive disorders is under intense debate. In this regard, the protective effects of estradiol on the vasculature may depend on the stage or condition of the vascular disease. Indeed, the antiatherogenic effects of estradiol are abolished by balloon catheter injury in cholesterol-clamped rabbits; moreover, the direct antiatherogenic effects of estradiol is present, absent, or reversed depending on the state of the arterial endothelial cells \((99)\). Animal as well as human studies provide convincing evidence that vascular remodeling after balloon injury and percutaneous transluminal coronary angioplasty is significantly inhibited by estradiol. However, estradiol does not prevent neointima formation in a nonhuman primate model with preexisting atherosclerosis \((1, 76)\). Estradiol is able to inhibit the further progression of atherosclerosis in vessel wall with moderate, but not severe, preexisting alterations \((89)\). In contrast, estradiol reduced plaque size and aortic lesion formation in hypercholesterolemic rabbits with severe endothelial dysfunction \((189)\). Future studies should focus on effects of estradiol in vessels with established vasoocclusive disorders.
PROTECTIVE EFFECTS OF ESTRADIOL ON THE RENAL SYSTEM: CELLULAR AND BIOCHEMICAL MECHANISMS

Women are also protected against the progression of renal disease (255); moreover, gender differences in renal hemodynamics (182) and renal response to ANG II (170) are well established. Results from a meta-analysis performed using 68 studies indicate that men with chronic renal disease of various etiologies show a more rapid decline in renal function with time than do women (192). Ample evidence from epidemiological/clinical studies and from experimental models of renal injury suggest that estradiol is responsible for the resistance of kidneys in women to the progression of renal disease (255, 265).

The potential role of estradiol in regulating renal function is also evident from the observation that the kidney expresses both the classic ER-α and the newly discovered ER-β. In human fetal kidneys, ER-β is the prominent renal ER expressed, whereas ER-α is only marginally expressed (24). In contrast, in adult renal tissue, most studies demonstrate the expression of ER-α, but not ER-β, in humans, rats, and mice (134, 178, 277). However, ER-β exists in rat kidneys (202). Because ER-α and ER-β respond differently to estradiol in mediating its transcriptional activity at EREs (120, 178), and respond differentially to ligands at the AP-1 site (208), it is feasible that the differential expression of ER subtypes may have important physiological relevance within the kidney.

Increased generation and deposition of extracellular matrix proteins is an initial step in the development of glomerular obsolescence and progressive loss of renal function (47). Estradiol suppresses collagen synthesis in GMCs (137, 141, 253, 254), suggesting that estradiol may limit the progression of glomerulosclerosis by reducing matrix accumulation after glomerular injury. In contrast to SMCs (49), the inhibitory effects of estradiol on collagen synthesis in GMCs are mediated via activation of MAP kinase and upregulation of transcription factor AP-1 (253), specifically the c-fos component. However, similar to the SMCs, the inhibitory effects of estradiol on collagen synthesis in GMCs is blocked by the ER antagonist ICI-182780, suggesting that the inhibitory effects are receptor mediated (194); however, whether ER-α or ER-β mediates these effects remains unclear.

In addition to inhibiting serum-induced collagen synthesis, estradiol also inhibits collagen synthesis induced by ANG II and TGF-β (194, 254), growth factors implicated in the pathophysiology of progressive renal injury in various experimental models for kidney disease (21, 250). TGF-β is known to mediate the mitogenic effects of ANG II as well as ET-1 on SMC and GMC growth (55, 82). Moreover, estradiol antagonizes the effects of TGF-β on collagen synthesis in mesangial cells (254). Because ET-1 and ANG II induce their mitogenic effects on mesangial cells via TGF-β (116), it is likely that estradiol also attenuates the deleterious effects of ET-1 and ANG II on the kidney. Indeed, Neugarten and Silbiger (195) show that estradiol attenuates the effects of endothelin and ANG II on mesangial cell collagen synthesis. Thus there is strong evidence that estradiol protects the kidneys in part by abrogating the mitogenic effects of multiple growth factors that participate in the pathophysiology of glomerulosclerosis.

Although estradiol prevents mitogen-induced collagen synthesis, its effects on GMC proliferation are less clear. In cultured GMCs that are not growth arrested, estradiol induces DNA synthesis and proliferation at low concentrations, yet inhibits GMC proliferation at concentrations equal to or greater than 1 μM (137). In contrast, results from our laboratory provide evidence that in growth-arrested GMCs, estradiol inhibits serum-induced DNA synthesis and cell proliferation in a concentration-dependent manner (302a). The disparate effects of estradiol in the two studies may be due to the culture conditions. In this regard, estradiol induces MAP kinase activity in mesangial cells that are not growth arrested, whereas in growth-arrested (serum-starved) GMCs estradiol has no effect on basal MAP kinase activity and inhibits PDGF-induced and ANG II-induced MAP kinase activity (194). Because PDGF-BB and ANG II are known to induce cell proliferation via activation of MAP kinase activity (5355, 176) and estradiol inhibits these effects, it is feasible that estradiol may abrogate the promitogenic effects of ANG II and PDGF on GMCs. Because increased proliferation of GMCs plays a key role in glomerulosclerosis, estradiol may protect against glomerular remodeling by inhibiting cell growth. However, future studies are required to investigate this possibility in more detail.

Estradiol may also influence GMC growth indirectly by influencing the synthesis of growth promoters and growth inhibitors. In this regard, estradiol downregulates the synthesis of several molecules that are known to induce GMC growth and glomerulosclerosis. Estradiol lowers circulating and renal levels of ANG II by downregulation of ACE activity (71), suggesting that estradiol may protect the kidney by inhibiting the synthesis of ANG II. Estradiol lowers the production of ET-1 (177, 304), ET-1 is a mitogen for GMCs (55), and increased production of ET-1 by glomerular cells is associated with glomerulosclerosis (55). It is feasible, therefore, that the inhibitory effects of estradiol on ET-1 synthesis would protect the kidney against glomerular remodeling. Estradiol also decreases the levels of homocysteine (79, 283), and homocysteine is known to cause glomerular damage and induce glomerulosclerosis (159).

Several lines of evidence suggest that ANG II stimulates ET-1 synthesis, and ET-1 increases the conversion of ANG I to ANG II, an effect that can be blocked by ACE inhibitors (55). These findings suggest that increased generation of ET-1 within glomeruli may lead to increases in ANG II, synthesis which in turn would upregulate ET-1 synthesis (57, 82). Generation of ANG II and ET-1 through this vicious cycle might importantly modulate renovascular tone and growth of GMCs, thus leading to renal dysfunction (55). The fact...
that estradiol downregulates ACE activity and ET-1 synthesis suggests that estradiol may protect against glomerular remodeling by blocking this vicious cycle of ANG II and ET-1 synthesis. In the same vein, estradiol also downregulates the synthesis of both IGF-1 and IGF-1 receptor (155, 156). Because IGF-1 is a potent mitogen for GMCs (55), it is feasible that estradiol may abrogate the promitogenic effects of IGF-1 on GMCs and protect against glomerular remodeling.

Another important molecule that regulates GMC growth and participates in glomerular remodeling is plasminogen activator inhibitor-1 (PAI-1; 14, 55). It is a key regulator of the plasmin-mediated proteolytic cascade that modulates fibrinolysis, ECM turnover, and degradation and regulation of cell migration (55). PAI-1, via inhibition of proteolytic cascades (plasminogen activator and urokinase plasminogen activator), induces abnormal growth and ECM synthesis in the glomerulus (14, 55). The finding that estradiol lowers PAI-1 levels by ~50% (128) suggests that estradiol may protect against the promitogenic and deleterious effects of PAI-1 on GMCs. PAI-1 activity is regulated by ANG II (55), and inhibition of ACE results in reduced expression of PAI-1 and ECM deposition in glomerulosclerosis (55). Indeed, PAI-1 synthesis is increased in glomerular cells of sclerotic kidneys (14, 55). Moreover, TGF-β, which mediates the mitogenic effects of ANG II on GMCs, is known to induce PAI-1 expression in GMCs (303). The above findings, along with the fact that estradiol inhibits ANG II synthesis and downregulates TGF-β expression, suggest that inhibitory effects of estradiol on these molecules would result in decreased PAI-1 synthesis, and this in turn would protect the GMCs against cell proliferation, collagen synthesis, and glomerular remodeling.

In addition to the above factors, growth within the glomerulus is also stimulated by free radicals (55). Free radicals induce GMC cell growth (55) and contribute to the process of glomerulosclerosis in various renal diseases (86). The mechanism by which free oxygen radicals induce their mitogenic effects on GMCs include induction of ET-1 synthesis, oxidation of LDL to oxidized LDL, oxidation of lipoproteins, and activation of the MAP kinase pathway (55). Because estradiol is a potent antioxidant that scavenges free radicals, estradiol may protect GMCs against the growth effects of free radicals. Indeed, oxidation of LDL to oxidized LDL by mesangial cells is blocked by estradiol (194), and estradiol blocks the effects of free radicals on cell growth and lipid peroxidation in SMCs (30, 56), which are phenotypically similar to GMCs. In hypercholesterolemic female rats, ovariectomy induces glomerular injury, and this effect is reversed by 0.2 mg, but not 0.1 mg, of estradiol (240). These studies provide evidence that antioxidant effects of estradiol may protect against glomerular remodeling. However, further studies are required to conclusively demonstrate whether estradiol blocks free radical-induced mitogenesis in GMCs.

The growth inhibitory and protective effects of estradiol on the kidney are also supported by recent in vivo studies. Mulroney et al.(181) report that after unilateral nephrectomy, male remnant kidneys grow by 117% whereas female remnant kidneys grow by only 57%. Also, compared with control kidneys, glomerular volume of male remnant kidneys increases by 126% whereas in females no changes in glomerular volume are observed. In contrast to that in males, no glomerular or tubular damage is observed in female remnant kidneys. Studies in ovariectomized female rats show that the deleterious effects in males are due to testosterone, whereas estradiol is protective (180, 181).

Abnormal growth within the glomerulus is also a key factor in chronic renal allograft rejection, and estradiol protects the vasculature against allograft-induced dysplasia. Indeed, compared with testosterone, estradiol treatment improves graft function, reduces glomerulosclerosis, and diminishes cellular infiltration (180). Moreover, these changes are accompanied by reduced ICAM-1, fibronectin, laminin, and TGF-β. These findings suggest that estradiol induces protective effects on the kidney and protects against glomerulosclerosis.

One of the major factors involved in regulating renal function is NO. Gender differences in renal NO synthesis are reported by Reckelhoff et al. (220), who demonstrate that the levels of eNOS are 80% higher in women than in men. Although basal synthesis of NO is protective, excessive synthesis of NO may induce deleterious effects on the kidney. Indeed, increased NO synthesis by activated monocytes/macrophages infiltrating the glomeruli causes glomerular injury (119, 188). Increased NO synthesis in acute glomerulonephritis in rats causes lysis of GMCs and accumulation of ECM (188). Moreover, inhibition of NO synthesis by Nω-monomethyl-L-arginine reverses some of these effects, suggesting that inhibition of NOS may protect against the deleterious effects of NO (305). Estradiol upregulates eNOS (NOS I) essential for normal renal function; however, it inhibits iNOS (NOS II), suggesting that estradiol may prevent the deleterious effects of NO by downregulating iNOS-derived NO. Importantly, estradiol inhibits iNOS activity in activated macrophages/macrophages, which play a critical role in inflammatory glomerulonephritis, in experimental models of anti-thy 1 glomerulonephritis and in lupus glomerulonephritis (93, 271). Together, these findings suggest that estradiol may protect the kidney by abrogating the deleterious effects of iNOS-derived NO. In contrast to the above studies, Neugarten et al. (193) report stimulatory effects of estradiol on renal iNOS. Hence the interactive role of NO and estradiol in glomerulosclerosis needs to be further investigated.

Although mesangial cells importantly contribute to the glomerular remodeling process, injury to glomerular endothelial cells (GECs) also participates in this process (111, 140). Growth of GECs participate in capillary repair of glomerulonephritis (206). Because estradiol induces growth of aortic ECs (124, 258), it is feasible that estradiol may also facilitate the glomerular repair process by inducing growth of GECs. In this regard, it is interesting to note that in vascular ECs estradiol induces VEGF synthesis (272), and VEGF is known to repair GEC in-
Table 1. Potential renal and vascular protective mechanisms influenced by estradiol and role of estrogen receptors

<table>
<thead>
<tr>
<th>Vasoactive Molecules</th>
<th>Influence of Estradiol on Synthesis or Effects</th>
<th>Role of ER</th>
<th>Vascular and Renal Effects of the Molecule (Biological, Physiological, and Pathophysiological)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitric oxide</td>
<td>+</td>
<td>+</td>
<td>Vasodilatation; inhibits SMC and GMC growth</td>
</tr>
<tr>
<td>cNOS</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostacyclin</td>
<td>+</td>
<td>+</td>
<td>Vasodilatation; cell (SMC/GMC) damage after injury</td>
</tr>
<tr>
<td>cAMP</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>+</td>
<td>+</td>
<td>Vasodilatation; inhibits SMC and GMC growth</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>-</td>
<td>+</td>
<td>Vasocostriction; induces SMC and GMC growth</td>
</tr>
<tr>
<td>Angiotensin II/AT1 receptors</td>
<td>-</td>
<td>?</td>
<td>Vasocostriction; induces SMC and GMC growth</td>
</tr>
<tr>
<td>Angiotensin 1-7</td>
<td>+</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>ACE</td>
<td>-</td>
<td>?</td>
<td>Vasocostriction; induces SMC and GMC growth via Ang-II</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>-</td>
<td>?</td>
<td>Causes SMC and GMC damage; effects on endothelial cell damage</td>
</tr>
<tr>
<td>VEGF</td>
<td>+</td>
<td>+</td>
<td>Induces EC growth and angiogenesis and glomerular repair</td>
</tr>
<tr>
<td>TGF-β</td>
<td>-</td>
<td>?</td>
<td>Regulates SMC/GMC growth and vascular/glomerular remodeling</td>
</tr>
<tr>
<td>Collagen</td>
<td>-</td>
<td>+</td>
<td>Matrix deposition and vascular/glomerular remodeling</td>
</tr>
<tr>
<td>Adhesion molecules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCAM, ICAM, E-selectin, MCP, P-selectin</td>
<td>-</td>
<td>+</td>
<td>Induce cell adhesion and transendothelial cell migration; associated with atherosclerosis and glomerulonephritis</td>
</tr>
<tr>
<td>Matrix metalloproteinases (MMP-2)</td>
<td>+</td>
<td>+</td>
<td>Regulates matrix deposition/degradation in vascular and glomerular remodeling</td>
</tr>
<tr>
<td>Mitogens/cytokines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF, bFGF, IGF-1, LIF, FCS, insulin, ET-1</td>
<td>-</td>
<td>+</td>
<td>Induce SMC/GMC growth; associated with atherosclerosis and glomerular remodeling; protects from atherosclerosis and glomerulosclerosis</td>
</tr>
<tr>
<td>Free radicals</td>
<td></td>
<td>+</td>
<td>SMC/GMC mitogen; induce atherosclerosis and glomerulosclerosis; induce glomerular/Vascular EC damage, LDL oxidation</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL/oxidized LDL</td>
<td>-</td>
<td>?</td>
<td>SMC/GMC mitogen, EC damage (atherosclerosis/glomerulosclerosis)</td>
</tr>
<tr>
<td>HDL</td>
<td>+</td>
<td>?</td>
<td>Prevents atherosclerosis and glomerulosclerosis</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>+</td>
<td>?</td>
<td>No significant role in atherosclerosis; induces renal injury</td>
</tr>
<tr>
<td>Apolipoprotein E</td>
<td>+</td>
<td>?</td>
<td>Prevents atherosclerosis</td>
</tr>
<tr>
<td>Coagulation/fibrinolysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1</td>
<td>-</td>
<td>?</td>
<td>Antifibrinolytic; associated with glomerulosclerosis/atherosclerosis</td>
</tr>
<tr>
<td>Soluble thrombomodulin/fibrinogen</td>
<td>-</td>
<td>?</td>
<td>Procoagulant</td>
</tr>
<tr>
<td>Tissue plasminogen activator</td>
<td>+</td>
<td>?</td>
<td>Fibrinolysis</td>
</tr>
<tr>
<td>Anti-thrombin III, protein-S</td>
<td>-</td>
<td>?</td>
<td>Anticoagulation</td>
</tr>
<tr>
<td>Ion channels</td>
<td></td>
<td>+</td>
<td>Vasodilatation; associated with vascular remodeling and renal function</td>
</tr>
<tr>
<td>Na⁺, K⁺, Ca²⁺</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

cNOS, constitutive nitric oxide synthase; iNOS, inducible NOS; ACE, angiotensin-converting enzyme; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-β; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; LIF, leukemia inhibitory factor; FCS, fetal calf serum; IGF-1, insulin-like growth factor-1; ET-1, endothelin-1; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VEGF, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; E-selectin, endothelial leukocyte adhesion molecule-1; MCP, monocyte chemotactic protein-1; GMC, glomerular mesangial cells; ECG, endothelial cells; SMC smooth muscle cells; +, induction/positive; -, inhibition; ?, unknown.

jury (206), suggesting that estradiol may protect the glomeruli by inducing VEGF synthesis in GEC. However, this possibility remains to be investigated. In the same vein, estradiol prevents TNF-α and LPS-induced apoptosis of vascular ECs (258). Because TNF-α and LPS induce apoptotic death of GECs (167), it is feasible that estradiol can also prevent the deleterious effects of TNF-α and LPS on GECs.

Other mechanisms that may contribute to the renoprotective effects of estrogen include inhibition of mesangial cell apoptosis (257); upregulation of antiaggregatory pathways by induction of ecto-ADPase in glomeruli and the vessel wall (60); upregulation of renal oxytocin receptor gene expression responsible for regulating renal fluid dynamics (207); prevention of urinary stone formation with inhibition of crystal deposition and calcium content in renal tissue; decrease in urinary excretion of oxalate and downregulation of the expression of osteopontin mRNA in renal tissue (109); upregulation of the synthesis of antimitogenic prostaglandins (33, 115, 169); attenuation of IgA-induced nephropathy (85); and upregulation of adenosine synthesis in SMCs, which is renoprotective (52). Finally, recent studies from our laboratory provide evidence that 2-hydroxyestradiol and 2-methoxyestradiol are more potent than estradiol in inhibiting serum-induced growth of GMCs (302a), suggesting that the antimitogenic and glomeruloprotective effects of estradiol can also be mediated via its metabolites. Because these metabolites have low or negligible affinity for ER, the effects are mediated via an ER-independent mechanism.

Defective metabolism of estradiol induces deleterious effects on the kidneys. For instance, in Syrian
hamsters estradiol induces tumor formation and causes cancer, and these effects are thought to be mediated via the estradiol metabolite 4-hydroxysteradiol (148). Indeed, compared with the 2-hydroxylation pathway, the 4-hydroxylation pathway is more prominent in the kidneys of hamsters (307). Because 4-hydroxysteradiol, but not 2-hydroxysteradiol, is carcinogenic (307), the explanation for the specific carcinogenic effects of estradiol in hamster kidneys may lie in the fact that the reproductive and the urogenital tracts of the Syrian hamsters arise from the same embryonic germinal ridge (125). Because estradiol is known to induce cancer within the reproductive tissue, it is feasible that hamster kidneys may carry the cancer genes that are expressed and responsive to estradiol (102). In hamster kidneys, estradiol upregulates c-myc and c-jun genes that stimulate cell proliferation (19, 146), induces cathepsin D protein (147), and induces lipid peroxidation and DNA adduct formation (291). However, these deleterious effects are specific for the Syrian hamsters as the CYP450 enzymes responsible for the metabolism of estradiol to 4-hydroxysteradiol (a carcinogen) are more prominent, and the enzymes responsible for counteracting these effects via generation of 2-hydroxysteradiol or detoxification via methylation are downregulated (307).

In contrast to the aforementioned renoprotective effects, estradiol enhances gentamicin-induced nephrotoxicity (29); induces glomerulosclerosis in albuminemic rats (114) and fibrosis in corticomedullary junction in Beagle dogs (314); significantly increases the rate of nephropathy in Cohen diabetic rats (226) and rate of glomerulonephrites associated with experimental lupus nephrites (28); and aggravates the progression of renal diseases that are characterized by the nephrotic syndrome via increased levels of serum triglycerides with resultant triglyceride-mediated renal injury (114, 264).

CONCLUSION

There is overwhelming evidence that, in addition to being a reproductive hormone, estradiol induces protective effects on the cardiovascular and renal systems (Table 1). However, these protective effects are modulated by various factors (Table 1). In this regard, drug-drug interactions, binding affinity to specific ER subtypes, expression of specific ER subtypes, and metabolism of estradiol locally within the tissue as well as by the liver all influence the effects of estrogen on the vasculature, heart, and kidneys.

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