ABSTRACT

A discussion of the role of endogenous estradiol metabolites in mediating important biological actions of estradiol is essentially nonexistent in standard textbooks of pharmacology and endocrinology. Indeed, the prevailing view is that all biological effects of estradiol are initiated by binding of estradiol per se to estrogen receptors and that estradiol metabolites are more or less irrelevant. This orthodox view, which is most likely incorrect, is the fundamental premise (an estrogen is an estrogen is an estrogen) underlying the design of important clinical trials such as the Heart and Estrogen/Progestin Replacement Study and the Women's Health Initiative Study. Accumulating data provide convincing evidence that some metabolites of estradiol, the major estrogen secreted by human ovaries, are biologically active and mediate multiple effects on the cardiovascular and renal systems that are largely independent of estrogen receptors. More specifically, metabolites of estradiol, particularly catecholestradiols and methoxyestradiols, induce multiple estrogen receptor-independent actions that protect the heart, blood vessels, and kidneys from disease. These protective effects are mediated in part by the inhibition of the ability of vascular smooth muscle cells, cardiac fibroblasts, and glomerular mesangial cells to migrate, proliferate, and secrete extracellular matrix proteins, as well as by an improvement in vascular endothelial cell function. The purpose of this review is to highlight the cardiovascular and renal pharmacology of catecholestradiols and methoxyestradiols. The take home message is simple: that when it comes to cardiovascular and renal protection, the concept that all estrogenic compounds are created equal may not be true.

The focus of this review is to summarize recent evidence supporting an important role for estradiol metabolites, specifically catecholestradiols and methoxyestradiols, in protecting against cardiovascular and renal diseases.

Estradiol Synthesis (Fig. 1)

Human beings make three estrogenic steroids, namely estradiol, estriol, and estrone. Of these, estradiol is the most important and estriol and estrone contribute only marginally to the total estrogenic activity in premenopausal women. The biosynthetic pathways leading to estradiol are well described: Aromatase converts androstenedione to estrone, and 17β-hydroxysteroid dehydrogenase converts estrone to estradiol. In addition, aromatase can also metabolize testosterone directly to estradiol. In premenopausal women, circulating estradiol is synthesized mostly in steroidogenic cells and tissues such as ovarian granulosa cells and placenta. However, several other organs and tissues make estradiol including adipose tissue, skin, endometrium, vaginal mucosa, breast, liver, blood vessels, and heart (Zhu and Conney, 1998; Dubey and Jackson, 2001a,b). Enzymes responsible for estradiol synthesis are also expressed in vascular smooth muscle cells and endothelial cells (Harada et al., 1999) and in cardiac fibroblasts and myocytes (Park 2000), suggesting that local synthesis of estradiol may be important in the cardiovascular system.

Estradiol Metabolism (Fig. 1)

Diverse pathways extensively convert estradiol to multiple nonestrogenic metabolites. Elimination of estradiol is largely

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**ABBREVIATIONS.** CYP450, cytochrome P450; COMT, catechol-O-methyltransferase; ER, estrogen receptor; SHBG, serum hormone binding globulin; VSMC, vascular smooth muscle cell; LDL, low-density lipoprotein; NO, nitric oxide; ERK, extracellular-signal regulated kinase; MAP kinase, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; IGF-1, insulin-like growth factor-1; ICI 182,780, fulvestrant.
mediated via metabolism to water-soluble compounds that are eliminated by the kidneys (into urine) and liver (into feces). In this regard, estradiol undergoes enzymatic oxidation to multiple hydroxylated species (Lee et al., 2003). Estradiol and its hydroxylated metabolites may then undergo glucuronidation, sulfation, esterification, or O-methylation (for details, see reviews by Dubey and Jackson, 2001a,b and Zhu and Conney, 1998).

Several CYP450 isoforms mediate NADPH-dependent oxidative metabolism of estradiol to various hydroxylated metabolites (Lee et al., 2003). Estradiol contains multiple hydroxylation sites, and the type of hydroxylated estradiol metabolite formed is defined by the position of hydroxylation (C-2, C-4, C-6, C-7, C-11, C-14, C-15, C-16, and C-17). Hydroxylation of estradiol at C-2 and C-4, by CYP1A1 and CYP1B1, respectively, results in the formation of catecholestradiols, i.e., 2-hydroxyestradiol and 4-hydroxyestradiol. Following hydroxylation, 2- and 4-hydroxyestradiol are rapidly methylated via enzymatic O-methylation to methoxyestradiols, i.e., 2-methoxyestradiol and 4-methoxyestradiol (Zhu and Conney, 1998). The O-methylation of catecholestradiols is largely catalyzed by the enzyme catechol-O-methyltransferase (COMT), a ubiquitous cytosolic enzyme that is expressed in most tissues (Männisto and Kaakkola, 1999) and present in a highly active form in vascular cells (endothelial cells, smooth muscle cells; Zacharia et al., 2001) and cardiac cells (cardiac fibroblast; Dubey et al., 2002b). As described below, catecholestradiols and methoxyestradiols have biological activity that is independent of estrogen receptors (ERs).

**General Pharmacology of Catecholestradiols and Methoxyestradiols**

With regard to affinity for ERs, catecholestradiols have approximately one-fourth the binding affinity of estradiol, and methoxyestradiols do not bind significantly to ERs (Dubey et al., 2000a). Low micromolar concentrations of 2-methoxyestradiol (IC$_{50}$ of approximately 2 μM) inhibit tubulin polymerization, but estradiol, catecholestradiols and 4-methoxyestradiol are much less potent in this regard (D’Amato et al., 1994). Although 2-hydroxyestradiol has little affinity for steroid hormone binding globulin (SHBG), 2-methoxyestradiol has more than twice the affinity for SHBG compared with estradiol (Dunn et al., 1980). On the other hand, neither 4-hydroxyestradiol nor 4-methoxyestradiol has significant affinity for SHBG.

**Effects of Catecholestradiols and Methoxyestradiols on Key Cell Types in the Cardiovascular and Renal Systems**

**Effects of Catecholestradiols and Methoxyestradiols on Vascular Smooth Muscle Cells (VSMCs).** Migration, proliferation, and extracellular matrix production by VSMCs contribute importantly to the pathophysiology of vascular diseases such as atherosclerosis, restenosis, and neointimal hyperplasia. Thus it is important to investigate the ability of catecholestradiols and methoxyestradiols to alter these aspects of VSMC biology.

In cultured human and rat aortic VSMCs, catecholestradiols and methoxyestradiols differentially inhibit migration, proliferation, and collagen synthesis and in the following order of potency: 2-methoxyestradiol > 2-hydroxyestradiol > 4-methoxyestradiol (Dubey et al., 2000a,b). Estradiol also inhibits these same processes but is less potent than 2-hydroxyestradiol or 2-methoxyestradiol. In contrast to catecholestradiols and methoxyestradiols, estrone, estriol, 16α-hydroxyestrone, 2-hydroxyestrone, and 4-hydroxyestrone are significantly less potent inhibitors and only marginally attenuate these processes and then only at high concentrations (>1 μM) not attained physiologically (Dubey et al., 2000b). The ability of catecholestradiols and methoxyestradiols to inhibit VSMC migration, proliferation, and extracellular matrix production is observed regardless of whether the stimulus for these processes is serum, platelet-derived growth factor, endothelin-1, angiotensin II, or IGF-1 (Dubey et al., 2000a; Barchiesi et al., 2002). Importantly, 2-hydroxyestra-
dio also inhibits free radical (peroxyl radical)-induced VSMC migration, proliferation, and extracellular matrix production (Dubey et al., 1999).

The inhibitory effects of catecholestradiols and methoxyestradiols on VSMCs are apparently not mediated by ERs. For example, ICI 182,780, an ER-receptor antagonist, does not attenuate the ability of either 2-hydroxyestradiol or 2-methoxyestradiol to inhibit VSMC migration, proliferation, and extracellular matrix production (Dubey et al., 2000a; Barchiesi et al., 2002), suggesting that the inhibitory effects of 2-hydroxyestradiol and 2-methoxyestradiol are mediated via an ER-independent mechanism.

**Effects of Catecholestradiols and Methoxyestradiols on Vascular Endothelial Cells.** Vascular endothelial cells determine in part the ability of healthy arteries to resist pathological processes. Consequently, it is critical to examine the effects of catecholestradiols and methoxyestradiols on this pivotal cell type.

In vascular endothelial cells, both 2-hydroxyestradiol and 2-methoxyestradiol stimulate the generation of the potent vasodilatory prostacyclin (Seeger et al., 1999). In this regard, 2-methoxyestradiol and 2-hydroxyestradiol are more potent than estradiol (Seeger et al., 1999).

Findings from our laboratory suggest that 2-hydroxyestradiol improves endothelium-dependent relaxation. In obese ZSF1 rats (an animal model of the metabolic syndrome that expresses genetic obesity, diabetes, hypertension, hyperlipidemia, left ventricular dysfunction, and renal disease) treated chronically (6 months) with 2-hydroxyestradiol, the vasodilatory effects of acetylcholine (endothelium-dependent vasodilator), but not sodium nitroprusside (endothelial-independent vasodilator) in mesenteric vascular beds preconstricted with angiotsensin II and methoxamine are significantly enhanced compared with untreated ZSF1 rats (Tofovic et al., 2001). These findings imply that 2-hydroxyestradiol augments acetylcholine-induced vasodilation largely by increasing the release of endothelium-dependent relaxing factor.

Because 2-hydroxyestradiol and 2-methoxyestradiol are potent anti-oxidants (more potent than vitamin E and estradiol; Seeger et al., 1997; Dubey et al., 1999), they may potentiate the vasodilatory activity of NO release under basal conditions by preventing oxidation of NO. Indeed, estradiol-induced endothelium-derived relaxing factor has been shown to be associated with decreased levels of superoxide anion, and this may account for the enhanced NO bioactivity and decreased peroxynitrite release (Dubey and Jackson, 2001b).

2-Hydroxyestradiol and 2-methoxyestradiol also inhibit endothelin-1 synthesis by coronary artery endothelial cells (Dubey et al., 2001), and this may contribute to their ability to improve endothelial function.

Doubtless, catecholestradiols and methoxyestradiols interact with endothelial cells and influence their growth and function. The growth regulatory effects of catecholestradiols and methoxyestradiols on endothelial cells are concentration-dependent. In this regard, low concentrations (physiological range: 10–100 nM) of 2- and 4-hydroxyestradiol and 2- and 4-methoxyestradiol significantly induce proliferation of cultured vascular endothelial cells (Lippert et al., 2000), whereas, higher concentrations (≥100 nM, pharmacological concentrations) of 2-hydroxyestradiol and 2-methoxyestradiol inhibit endothelial cell proliferation and are anti-angiogenic.

Importantly, the biochemical mechanisms by which 2-methoxyestradiol inhibits endothelial cell growth are partially defined. 2-Methoxyestradiol inhibits endothelial cell growth and induces apoptosis in actively growing, but not confluent, endothelial cells (Fotsis et al., 1994; Yue et al., 1997). The apoptotic effects of 2-methoxyestradiol on vascular endothelial cells are mediated via the stress-activated protein kinase pathway and Fas expression (Yue et al., 1997). In contrast to proliferating endothelial cells, 2-methoxyestradiol fails to induce apoptosis in aortic smooth muscle cells (unpublished findings), suggesting that such effects may differ between cell lines.

**Effects of Catecholestradiols and Methoxyestradiols on Cardiac Fibroblasts.** Abnormal growth of cardiac fibroblasts importantly contributes to pathologic cardiac remodeling associated with hypertension, myocardial infarction, and reperfusion injury. Cardiac fibroblasts comprise 60% of the total heart cells and contribute to pathological structural changes in the heart by undergoing proliferation, depositing extracellular matrix proteins and replacing myocytes with fibrotic scar tissue (Dubey and Jackson, 2001b). Therefore, any discussion of the cardiovascular effects of catecholestradiols and methoxyestradiols should address the actions of these compounds on cardiac fibroblasts.

Our studies show that 2-hydroxyestradiol and 2-methoxyestradiol are more potent than estradiol in inhibiting serum-induced proliferation and collagen synthesis in rat cardiac fibroblasts (Dubey et al., 1998, 2002b). In contrast to 2-hydroxyestradiol and 2-methoxyestradiol, other endogenous estrogens, such as estrone, estriol, and estrogen sulfate, are ineffective and inhibit cardiac fibroblast proliferation and collagen synthesis only marginally at extremely high concentrations (Dubey et al., 1998). We also observe that the inhibitory effects of catecholestradiols and methoxyestradiols on cardiac fibroblasts are enhanced, rather than inhibited, by the partial ER antagonist 4-hydroxytamoxifen, suggesting that these effects are mediated via ER-independent mechanisms (Dubey et al., 1998). This conclusion is further supported by our recent observation that the inhibitory effects of 2-hydroxyestradiol and 2-methoxyestradiol on cardiac fibroblasts are not affected by the specific ER antagonists ICI 182,780 (Dubey et al., 2002b).

**Effects Catecholestradiols and Methoxyestradiols on Glomerular Mesangial Cells.** Similar to the vasculature, abnormal growth of glomerular mesangial cells, a cell phenotypically similar to VSMCs, is associated with the pathogenesis of renal diseases, for example glomerulosclerosis. Pathological glomerular remodeling importantly contributes to the progression of renal diseases and mainly involves increased mesangial cell proliferation, migration, and extracellular matrix production (Dubey et al., 1997). Therefore, it is important to consider the effects of catecholestradiols and methoxyestradiols on the biology of glomerular mesangial cells.

In cultured human and rat glomerular mesangial cells, 2-hydroxyestradiol and 2-methoxyestradiol inhibit mitogen-induced proliferation and collagen synthesis more potently than estradiol (Dubey et al., 2002a). The inhibitory effects of catecholestradiols and methoxyestradiols on glomerular mesangial cells are not blocked by pharmacological antagonism of ERs (Dubey et al., 2003b), suggesting that the inhibitory effects of catecholestradiols and methoxyestradiols on mes-
angial cells are ER-independent. Importantly, administration of 2-hydroxyestradiol prevents puromycin-induced glomerulosclerosis and lowers blood pressure (Tofovic et al., 2002), which suggests that the inhibitory effects of catecholestradiols and methoxyestradiols translates into renal protection.

**Effects of Catecholestradiols and Methoxyestradiols on Other Cells Involved in Vascular Disease.** Activation and adhesion of and invasion by circulating platelets and macrophages play a key role in vascular disease. Therefore, it is important to examine the effects of estradiol metabolites on these processes. As mentioned above, both 2-hydroxyestradiol and 2-methoxyestradiol induce the synthesis of prostacyclin, a local hormone that inhibits platelet aggregation and adhesion to endothelial cells. Moreover, 2-methoxyestradiol inhibits the motility, migration, and adhesion to fibronectin of circulating BCR-ABL transformed cells (Sattler et al., 2003), suggesting that 2-methoxyestradiol may inhibit the ability of circulating inflammatory cells to adhere to and infiltrate vascular lesions. Along these lines, both 2-hydroxyestradiol and 2-methoxyestradiol prevent the oxidation of LDL to oxidized-LDL (Seeger et al., 1997), suggesting that they may protect endothelial cells against free radicals and oxidized LDL-induced injury.

**Mechanisms Mediating the Inhibitory Actions of Catecholestradiols and Methoxyestradiols on Cardiovascular Cells.** As discussed above, catecholestradiols and methoxyestradiols inhibit the growth of VSMCs, cardiac fibroblasts, and glomerular mesangial cells. Rapid progress is being made regarding the mechanisms of these important effects of catecholestradiols and methoxyestradiols. Both 2-hydroxyestradiol and 2-methoxyestradiol are potent anti-oxidants (Seeger et al., 1997) and block free radical (peroxyl-radical)-induced proliferation and migration of VSMCs (Dubey et al., 1999). The fact that free radicals mediate the proliferative effects of several mitogens, including angiotensin II, suggests that this anti-oxidant mechanism may importantly contribute to the inhibitory effects of estradiol metabolites. In this context, the inhibitory effects of 2-hydroxyestradiol on VSMCs are also associated with its ability to attenuate peroxidation of acidic membrane phospholipids (phosphatidylinositol and phosphatidyleserine) (Dubey et al., 1999). Because oxidation of membrane phospholipids is known to trigger cell migration and to activate cellular proliferative pathways such as ERK1/ERK2 and c-fos/c-jun oncogenes, the ability of catecholestradiols and methoxyestradiols to block oxidation of membrane phospholipids may be critical to the inhibitory effects of these compounds on VSMCs, cardiac fibroblasts, and mesangial cells.

As noted above, catecholestradiols and methoxyestradiols bind to and prevent the polymerization of tubulin (D’Amato et al., 1994). Importantly, 2-methoxyestradiol has the highest affinity for tubulin and also is the most potent estradiol metabolite with regard to inhibiting growth of VSMCs, cardiac fibroblasts, and mesangial cells. It is conceivable, therefore, that many of the inhibitory effects of 2-methoxyestradiol are mediated by blockade of tubulin polymerization with subsequent interference with the proper assembly of the cellular machinery needed for migration, proliferation, and collagen secretion.

The initial events leading to the inhibitory effects of estradiol metabolites on VSMCs, cardiac fibroblasts, and mesangial cells probably involve at least an anti-oxidant mechanism as well as an anti-tubulin mechanism. However, other cellular targets may also participate. In this regard, both 2-hydroxyestradiol and 2-methoxyestradiol attenuate PDGF-BB-induced MAP kinase activity in both rat and human aortic VSMCs (Dubey et al., 2000a; Barchiesi et al., 2002) and in rat cardiac fibroblasts (Dubey et al., 1998). Because activation of the MAP kinase cascade is a major pathway via which multiple external stimuli induce cellular proliferation, the inhibitory effects of catecholestradiols and methoxyestradiols on this pathway may also play a pivotal role in mediating their inhibitory effects on VSMC, cardiac fibroblast, and mesangial cell activity. The fact that ICI 182,780 does not attenuate the ability of estradiol metabolites to inhibit MAP kinase activity (Dubey et al., 2000a) suggests that these actions are ER-independent; however the exact mechanisms remain undefined.

Recent unpublished data from our laboratory provide evidence that 2-methoxyestradiol inhibits VSMC proliferation by arresting cells in both G0/G1 and G2/M phases of the cell cycle. This dual blockade of cell cycle progression is accompanied by an inhibition of the phosphorylation of Akt and retinoblastoma protein, as well as by a down-regulation of cyclin D1 and cyclin B1 and by an up-regulation of the cdk inhibitor p27. Presently it is not clear whether these effects represent primary cellular targets for estradiol metabolites or are secondary to anti-oxidant, anti-tubulin, and/or anti-MAP kinase effects.

Apart from the direct inhibitory effects of catecholestradiols and methoxyestradiols on cellular activity, both 2-hydroxyestradiol and 2-methoxyestradiol may indirectly influence VSMCs, cardiac fibroblasts, and mesangial cells by increasing the synthesis of endogenous compounds that inhibit activity (such as NO, cAMP, and prostacyclin) and decreasing the synthesis of endogenous compounds that stimulate activity (such as endothelin-1 and catecholamines). Table 1 summarizes some of the vasoactive molecules and signal transduction pathways known to be affected by 2-hydroxyestradiol and 2-methoxyestradiol.

**Effects of Catecholestradiols and Methoxyestradiols on Plasma Lipids**

As indicated above, catecholestradiols and methoxyestradiols inhibit VSMC, cardiac fibroblast, and mesangial cell migration, proliferation, and extracellular matrix production, and these effects may protect the heart, blood vessels, and kidneys from disease. In addition to these protective effects, catecholestradiols and methoxyestradiols may influence plasma levels of lipids in a beneficial manner. In this regard, administration of 2-hydroxyestradiol, 2-methoxyestradiol, and 4-hydroxyestradiol to ovariectomized rats significantly reduces circulating cholesterol levels (Liu and Bachmann, 1998). Moreover, treatment of genetically obese ZSF1 rats with 2-hydroxyestradiol reduces hypercholesterolemia (from 399 ± 24 mg/dl cholesterol to 247 ± 28 mg/dl cholesterol at 25 weeks into the treatment) (Tofovic et al., 2001). The mechanisms by which catecholestradiols and methoxyestradiols decrease plasma cholesterol levels are unknown.
Evidence That Local Conversion of Estradiol and Catecholestradiols to Methoxyestradiols Mediates the Inhibitory Effects of Estradiol and Catecholestradiols on Cardiovascular Cells

Expression of CYP450s and COMT in Cardiovascular Cells. It is now clear that many cardiovascular cells are enzymatically equipped to metabolize estradiol to catecholestradiols and to convert catecholestradiols to methoxyestradiols. Among the CYP450 isozymes (CYP1A1, CYP1A2, CYP1B1, and CYP3A4) known to metabolize estradiol to catecholestradiols, CYP1A1 and CYP1B1 are expressed in VSMCs, vascular endothelial cells, cardiomyocytes, and cardiac fibroblasts (Park 2000; Dubey et al., 2003a). Moreover, these same cells also contain COMT and metabolize catecholestradiols to methoxyestradiols (Barchiesi et al., 2002; Dubey et al., 2002b, 2003a). Recent studies also provide evidence that VSMCs as well as cardiac myocytes and fibroblasts contain aromatase activity (Harada et al., 1999) and, hence, are capable of synthesizing estradiol and its metabolites locally.

Effects of Manipulating CYP450 and COMT Activity with Pharmacological Agents on the Inhibitory Effects of Estradiol and Catecholestradiols. There is strong pharmacological evidence that methoxyestradiols mediate some of the cellular effects of estradiol and catecholestradiols (Dubey et al., 2000a, 2002a,b, 2003a; Barchiesi et al., 2002). In VSMCs and cardiac fibroblasts, the inhibitory effects of estradiol (but not 2-hydroxyestradiol or 2-methoxyestradiol) on DNA synthesis, cell proliferation, collagen synthesis, and MAP kinase activity are enhanced by CYP450 inducers (2-methylolethanone, phenobarbital, β-naphthoflavone) and blocked by a CYP450 inhibitor (1-aminobenzotriazole). Moreover, VSMCs and cardiac fibroblasts express CYP1A1 and CYP1B1, and the inhibitory effects of estradiol (but not 2-hydroxyestradiol or 2-methoxyestradiol) on VSMC and cardiac fibroblast activity are blocked by ellipticine and pyrene at concentrations that specifically inhibit CYP1A1 and CYP1B1 activity. In contrast to inhibitors of CYP1A1 and CYP1B1, inhibitors of CYP3A4 and CYP1A2 do not block the antimitogenic effects of estradiol, suggesting that CYP1A1- and CYP1B1-derived metabolites play a prominent role in mediating the inhibitory effects of estradiol. The inhibitory effects of both estradiol and 2-hydroxyestradiol, but not 2-methoxyestradiol, are completely blocked by competitive inhibitors of COMT, for example quercetin and OR486.

Although our findings provide evidence that the inhibitory effects of estradiol and catecholestradiols on cardiovascular cells are mediated via methoxyestradiols, which methoxyestradiols (i.e., 2- or 4- or both) mediate these effects remains undefined. As mentioned, the antimitogenic effects of estradiol are attenuated by specific inhibitors of CYP1A1 and CYP1B1, but not by inhibitors of CYP1A2 and CYP3A4. Because CYP1A1 converts estradiol mostly to 2-hydroxyestradiol and CYP1B1 converts estradiol mostly to 4-hydroxyestradiol, it is likely that both 2- and 4-methoxyestradiols are involved. Indeed, we have shown that VSMCs metabolize estradiol to both 2- and 4-hydroxyestradiol and to 2- and 4-methoxyestradiol (Dubey et al., 2003a). The possibility that the conversion of estradiol to estrone and then to methoxyestrone is responsible for the inhibitory effects of estradiol can be ruled out because direct addition of estrone to VSMCs does not inhibit cellular activity (Dubey et al., 2000b).

Studies in COMT Knockout Mice. In a recent publication, we report the effects of estradiol, 2-hydroxyestradiol, and 2-methoxyestradiol on cellular proliferation in VSMCs.
obtained from COMT knockout mice versus VSMCs obtained from wild-type mice (Zacharia et al., 2003). In wild-type VSMCs, estradiol, 2-hydroxyestradiol, and 2-methoxyestradiol inhibit cellular proliferation. In contrast, in COMT knockout VSMCs, both estradiol and 2-hydroxyestradiol are inactive, but 2-methoxyestradiol inhibits cellular proliferation to the same extent as it does in wild-type VSMCs. These data strongly support the conclusion that the cellular inhibitory effects of estradiol and catecholestradiols are mediated by methoxyestradiols.

**Evidence that the Cellular Inhibitory Effects of Estradiol and Catecholestradiols Are ER-Independent.** If the inhibitory effects of estradiol and catecholestradiols are mediated via the nonestrogenic methoxyestradiols, then the inhibitory effects of estradiol and catecholestradiols should be ER-independent. Estradiol inhibits the proliferation of VSMCs in injury-induced vascular lesions in mice lacking ERα (Iafriti et al., 1997), ERβ (Karas et al., 1999), and both ERα and β (Karas et al., 2001), suggesting that the inhibitory effects of estradiol are ER-independent or involve an unidentified ER. Participation of an ER-independent mechanism is also supported by the observations that estradiol inhibits injury-induced neointima formation in gonadectomized, but not intact, male rats, even though VSMCs from both models express ERs (Oparil et al., 1997). Our unpublished studies demonstrate that both 2-hydroxyestradiol and 2-methoxyestradiol inhibit neointimal hyperplasia induced by vascular injury in the rat.

Pharmacological evidence for a role of ERs in mediating the inhibitory effects of estradiol in VSMCs is inconclusive. ICI 182,780, an ER antagonist, attenuated the ability of estradiol to reduce injury-induced neointima formation in one study (Bakir et al., 2000) but not another (Finking et al., 2002). Because ICI 182,780 has a structure similar to estradiol, it not only binds to ERs but also blocks the metabolism of estradiol to hydroxyestradiols by competing for CYP450s that hydroxylate estradiol (Dubey et al., 2000a, 2003a). In VSMCs ICI 182,780 blocks the inhibitory effects of estradiol only at concentrations that block the metabolism of estradiol to hydroxyestradiol (Dubey et al., 2000a; Barchiesi et al., 2002). However, ICI 182,780 does not attenuate the inhibitory effects of either catecholestradiols or methoxyestradiols. Because the inhibitory effects of ICI 182,780 on estradiol metabolism are concentration-dependent, the abrogatory effects of ICI 182,780 in one study may be due to the inhibition of estradiol metabolism by high concentrations of ICI 182,780. This conclusion is supported by the fact that the levels of estradiol are increased by more than 2-fold in rats given ICI 182,780 (Bakir et al., 2000).

**Clinical Implications**

Based on the evidence presented, it is likely that the inhibitory effects of estradiol on VSMC, cardiac fibroblast, and mesangial cell migration, proliferation, and extracellular matrix production are mediated by the hydroxylation of estradiol to catecholestradiols—mediated by CYP450s, followed by methylation of catecholestradiols to methoxyestradiols—mediated by COMT. Under this hypothesis, methoxyestradiols are ultimately responsible for the inhibitor effects of estradiol, as well as the inhibitory effects of catecholestradiols. This conclusion has important clinical implications.

Although premenopausal women are protected against cardiovascular and renal disease, the results of the Heart and Estrogen/Progestin Replacement Study (secondary prevention study; Hulley et al., 1998) and the Women's Health Initiative Study (primary prevention study; Writing Group for the Women's Health Initiative Investigators, 2002) indicate that estrogen replacement therapy does not provide cardiovascular protection to postmenopausal women, and in fact increases the risk of stroke, myocardial infarction, and thromboembolic disease. In this regard, there is a striking disconnect between studies in animals, which uniformly demonstrate cardiovascular and renal protection by estrogens, versus controlled, randomized, prospective studies in human beings, which uniformly demonstrate either no protection or adverse cardiovascular effects. The hypothesis that catecholestradiols and methoxyestradiols, not activation of ERs, confer cardiovascular and renal protection may explain the discrepancies between animal studies, which used estradiol, versus large clinical outcome trials, which employed conjugated equine estrogens that contain little estradiol. The estradiol metabolite hypothesis also explains why premenopausal women are protected from cardiovascular and renal disease, yet administration of conjugated equine estrogens does not restore protection in postmenopausal women. In short, our studies suggest that hormone replacement therapy is best achieved by replacing the hormone that is missing (estradiol) rather than by administering an extract of horse urine (conjugated equine estrogens).

The possibility that metabolism of estradiol by CYP450s and COMT defines the cardiovascular and renal protective effects of estradiol suggests that differences in the activity of these enzymes, whether genetic or acquired, may determine the overall protective effects a woman is afforded by endogenous estradiol. In this regard, factors such as diet and drugs could interfere with the conversion of estradiol to methoxyestradiol and hence abrogate the protective effects of endogenous estradiol. Also, stress may elevate local and circulating levels of catecholamines, which would compete with catecholestradiols for methylation by COMT. Finally, genetic polymorphisms that influence the level of expression or intrinsic activity of CYP450s or COMTs may determine in part the rate of conversion of estradiol to methoxyestradiols. Because the ability to convert estradiol to methoxyestradiols may vary enormously among individuals, this variability may explain why some women do not develop cardiovascular disease whereas others do.

Finally, it is conceivable that methoxyestradiols (or appropriate analogs) could be used clinically to prevent or treat cardiovascular and renal diseases in women without increasing the risk of cancer, stroke, myocardial infarction, or thromboembolic disease. Also, because methoxyestradiols are nonfeminizing, there is no reason why methoxyestradiols could not be used in men.

**Conclusion**

In conclusion, the evidence is increasing that estradiol, via hydroxylation to catecholestradiols with subsequent methylation to methoxyestradiols, protects the heart, blood vessels, and kidneys from pathological processes, particular those involving enhanced cellular migration, proliferation, and extracellular matrix production. Although the biological effects
of catecholestradiols and methoxyestradiols in the cardiovascular and renal systems involve numerous actions, presently it is not possible given the current level of development in the field to specify the hierarchy of the many actions of these agents with regard to their relative importance in mediating the protective effects of estradiol metabolites. Nonetheless, this hypothesis necessitates a serious rethinking of how best to implement hormone replacement therapy in postmenopausal women and offers new avenues for preventing and treating cardiovascular and renal diseases in women and men.

References


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