Catecholamines Block the Antimitogenic Effect of Estradiol on Human Glomerular Mesangial Cells

Raghvendra K. Dubey, Lefteris C. Zacharia, Delbert G. Gillespie, Bruno Imthurn, Edwin K. Jackson

Abstract—Local sequential conversion of estradiol to hydroxyestradiols and methoxyestradiols by CYP450 and catechol-O-methyltransferase, respectively, contributes to the antimitogenic effects of estradiol on glomerular mesangial cell growth via estrogen receptor–independent mechanisms. Catecholamines are also substrates for catechol-O-methyltransferase and therefore, might abrogate the renoprotective effects of estradiol by inhibiting formation of methoxyestradiols. To test this hypothesis, we investigated the antimitogenic effects of estradiol on human glomerular mesangial cell proliferation and collagen synthesis in the presence and absence of catecholamines. Norepinephrine, epinephrine, and isoproterenol abrogated the inhibitory effects of estradiol on cell number, DNA synthesis, and collagen synthesis. For example, serum-induced DNA synthesis was inhibited from 100% to 62±1.9% by 0.1 μmol/L estradiol, and these inhibitory effects were reversed to 91±1.9% by 1 μmol/L epinephrine, 90.7±3.3% by 1 μmol/L isoproterenol, 87.5±2.8% by 10 μmol/L norepinephrine, and 92±1% by 10 μmol/L OR486 (catechol-O-methyltransferase inhibitor). The interaction of catecholamines with estradiol was not affected by phenolamine or propanolol, α- and β-adrenoceptor antagonists, respectively. Similar to estradiol, the antimitogenic effects of 2-hydroxyestradiol were abrogated by epinephrine, isoproterenol, and OR486. In contrast to estradiol and 2-hydroxyestradiol, the antimitogenic effects of 2-methoxyestradiol were not attenuated by epinephrine, isoproterenol, or OR486. Norepinephrine, epinephrine, and isoproterenol inhibited the conversion of both estradiol and 2-hydroxyestradiol to 2-methoxyestradiol. Our findings suggest that catecholamines within the glomeruli might abrogate the antimitogenic effects of estradiol by blocking the conversion of 2-hydroxyestradiol to 2-methoxyestradiol. (Hypertension. 2003;42:349-355.)

Key Words: estrogen ■ catecholamines ■ peptides ■ metabolism ■ renal disease ■ glomerulosclerosis

Evidence indicates that 17β-estradiol (estradiol) is renoprotective. The rate of progression of renal disease is decreased in premenopausal women compared with age-matched men. With the onset of menopause and the reduction in estradiol synthesis, the progression of renal disease accelerates.1–3 Moreover, premenopausal diabetic women have a lower risk of developing end-stage renal disease than do age-matched male diabetics,3 and in postmenopausal women this protection is lost.3 The mechanisms by which estradiol affords renoprotection are unknown. However, it is known that sequential conversion of estradiol to hydroxyestradiols and methoxyestradiols by cytochrome P450 (CYP450) and catechol-O-methyltransferase (COMT), respectively, is responsible for inhibiting the pathologic responses (cell proliferation and extracellular matrix production) to vascular injury.1,4–6 In this regard, our results suggest that methoxyestradiols importantly contribute to estradiol-induced vascular protection by inhibiting smooth muscle cell growth via estrogen receptor–independent mechanisms.4–6 Analogous to the vascular remodeling process in atherosclerosis, abnormal growth and increased extracellular matrix protein (eg, collagen) production by glomerular mesangial cells (GMCs) after glomerular injury contribute to the glomerular remodeling process associated with glomerulosclerosis.1 Because GMCs are phenotypically similar to vascular smooth muscle cells, estradiol might protect against glomerulosclerosis in part via sequential conversion to hydroxyestradiols and methoxyestradiols in GMCs. In support of this hypothesis, our recent studies demonstrate that estradiol inhibits growth of GMCs via conversion to hydroxyestradiols and methoxyestradiols and via estrogen receptor–independent mechanisms.7

It is conceivable that increased levels of catecholamines might abrogate the renoprotective effects of estradiol by competing for COMT and inhibiting the conversion of hydroxyestradiols to methoxyestradiols. This hypothesis is supported by the observations that patients with the nephrotic syndrome have higher levels of catecholamines,8 that in-
increased sympathetic activity accelerates the process of glomerulosclerosis in animal models,9,10 and that increased synthesis of catecholamines under pathologic conditions induces vaso-occlusive disorders.11 Indeed, our previous studies demonstrate that catecholamines, which are physiologic substrates/competitive inhibitors of COMT,11 abrogate the inhibitory effects of 2-hydroxyestradiol on vascular smooth muscle cell growth by blocking the metabolism of 2-hydroxyestradiol to 2-methoxyestradiol.6

The goals of the present study were to test the hypothesis that catecholamines can reduce the antigrowth effects of estradiol on GMCs via a mechanism that involves the competitive inhibition of methoxyestradiol formation by COMT. To test this hypothesis, we determined whether catecholamines abrogate the antimitogenic effects of estradiol and whether the abrogatory effects of catecholamines were due to inhibitory effects on the metabolism of estradiol to methoxyestradiol or due to direct, growth-stimulatory effects of catecholamines via α- or β-adrenoceptors.

Methods

Culture of Human GMCs

GMCs cultured from normal female donors in the third passage were obtained from Clonetics Corp (Walkersville, Md). All chemicals for cell growth studies ([3H]thymidine incorporation, [3H]proline incorporation, cell number) were purchased from suppliers as described before.7 GMCs in the fourth or fifth passage were used for all experiments. In brief, GMCs were grown under standard tissue-culture conditions in phenol red-free Dulbecco’s modified Eagle’s medium/Ham’s F12 medium supplemented with 10% steroid-free fetal calf serum (FCS) and antibiotics. Confluent GMCs were made quiescent by culture in medium containing 0.25% FCS for 48 hours and treated every 24 hours for 4 days. On day 5, the cells were dislodged and counted (cell proliferation, Figure 1C). Treatment with 100 nmol/L estradiol alone had a slight, but significant, stimulatory effect on [3H]thymidine incorporation (Figure 1D) and cell number (cell proliferation, Figure 1D), and whether the abrogatory effects of catecholamines were due to inhibitory effects on the metabolism of estradiol to methoxyestradiol or due to direct, growth-stimulatory effects of catecholamines via α- or β-adrenoceptors.

Growth Studies

[3H]Thymidine incorporation (index of DNA synthesis), [3H]proline incorporation (index of collagen synthesis), and cell proliferation were conducted as previously described.4,5 For [3H]thymidine incorporation, GMCs grown to subconfluence were growth-arrested by incubation with 2-hydroxyestradiol for 1 hour in the presence or absence of catecholamines (isoproterenol, epinephrine, or norepinephrine) and whether the abrogatory effects of catecholamines were due to inhibitory effects on the metabolism of estradiol to methoxyestradiol or due to direct, growth-stimulatory effects of catecholamines via α- or β-adrenoceptors.

Results

Treatment with 2.5% FCS stimulated DNA synthesis ([3H]thymidine incorporation) and collagen synthesis ([3H]proline incorporation) by ≈7- and 6-fold (P<.001 vs 0.25% albumin), respectively. Treatment with 1 to 100 nmol/L estradiol concentration-dependently inhibited FCS-induced [3H]thymidine incorporation (Figure 1A), [3H]proline incorporation (Figure 1B), and cell number (cell proliferation, Figure 1C). Treatment with 100 nmol/L estradiol inhibited FCS-induced [3H]thymidine incorporation (Figure 1D) and [3H]proline (Figure 1B) incorporation by ≈50%. Epinephrine, norepinephrine, and isoproterenol abrogated the effects of 100 nmol/L estradiol on FCS-induced [3H]thymidine incorporation (DNA synthesis) in a concentration-dependent manner (Figure 1D). Treatment with the catecholamines alone had a slight, but significant, stimulatory effect on FCS-induced [3H]thymidine incorporation (Figures 2A and 2B). At a concentration of 1 μmol/L isoproterenol, norepinephrine and epinephrine induced thymidine incorporation by
panels A, C, and E) and isoproterenol (ISO, 1 μmol/L; right panels B, D, and F) on the inhibitory effects of estradiol (E2, 0.1 μmol/L) on 2.5% FCS-induced DNA synthesis ([3H]thymidine incorporation, A and B), cell number (cell proliferation; C and D), and collagen synthesis ([3H]proline incorporation; E and F) in the presence and absence of α- and β-adrenoceptor blockers—phentolamine (PHE, 3 μmol/L) and propranolol (PRO, 3 μmol/L), respectively. $P<0.05$ vs control (FCS); $\dagger P<0.05$ vs cells treated with estradiol (significant reversal of inhibitory effects); $\ddagger P<0.05$ vs cells treated with epinephrine alone (significant reversal of mitogenic effects of EPI).

13±3.4%, 12.6±2.4%, and 9.1±3%, respectively. Isoproterenol at concentrations of 0.1, 1, and 10 μmol/L reversed the inhibitory effect of estradiol on FCS-induced [3H]thymidine incorporation from 46% to 19±1.6%, 8±0.72%, and 5±0.06% (Figure 1D), respectively; epinephrine at concentrations of 0.1, 1, and 10 μmol/L reversed the inhibitory effect from 46% to 28±1.3%, 11±0.7%, and 8±0.54% (Figure 1D), respectively; and norepinephrine at concentrations of 0.1, 1, and 10 μmol/L reversed the inhibitory effect from 46% to 42±1.4%, 31±0.8%, and 12.5±0.4% (Figure 1D), respectively. OR486, a selective inhibitor of COMT, at a concentration of 1 μmol/L, reversed estradiol-mediated inhibition of FCS-induced [3H]thymidine incorporation, from 46% to 9±1.1% (Figure 1D).

FCS increased cell number in growth-arrested GMCs by ~8-fold (data not shown). Estradiol at 10 nmol/L inhibited FCS-induced increases in cell number by 45%, and the catecholamines reversed this inhibitory effect (Figure 1C). At a concentration of 10 μmol/L isoproterenol, epinephrine and norepinephrine reversed the inhibitory effects of estradiol from 45±0.4% to 9±0.7%, 7±0.27%, and 11±1.3%, respectively. OR-486 at a concentration of 10 μmol/L reversed the inhibitory effect from 45±0.4% to 7±0.46%. Treatment with catecholamines alone induced cell proliferation marginally but significantly (6% to 12%; Figures 2C and 2D). Treatment with OR486 alone had no effect on GMC proliferation (data not shown).

Similar to the effects on FCS-induced [3H]thymidine incorporation, the catecholamines abrogated the inhibitory effects of estradiol on collagen synthesis ([3H]proline incorporation; Figure 1B). At concentrations of 10 μmol/L, proterenol, epinephrine, and norepinephrine dramatically reversed the inhibitory effects of 100 nmol/L estradiol on [3H]proline incorporation, from 43% to 10.2±0.6%, 8±0.3%, and 11±1.2%, respectively (Figure 1B). The catecholamines alone significantly stimulated proline incorporation (collagen synthesis) by 13% to 17% (Figures 2E and 2F). At a concentration of 10 μmol/L, OR486 completely reversed the inhibitory effect of estradiol (1 to 100 nmol/L) on [3H]proline incorporation (Figure 1B).

Catecholamines mediate their growth effects via α-adrenoceptors and β-adrenoceptors.12 Norepinephrine and epinephrine activate α-adrenoceptors (both subtypes 1 and 2) and β-adrenoceptors (subtype 1 for norepinephrine and both subtypes 1 and 2 for epinephrine), whereas isoproterenol activates primarily β-adrenoceptors (both subtypes 1 and 2), with lesser effects on α-adrenoceptors.12 In the present study, the stimulatory effects of epinephrine on DNA synthesis, cell proliferation, and collagen synthesis were blocked by phentolamine, an α-adrenoceptor antagonist (Figures 2A, 2C, and 2E). Similar to epinephrine, norepinephrine caused mitogenesis in GMCs, and these effects were also blocked by phentolamine (data not shown). These findings suggest that α-adrenoceptors mediate the mitogenic effects of epinephrine and norepinephrine. The mitogenic effects of isoproterenol were not blocked by propranolol, a β-adrenoceptor antagonist (Figures 2B, 2D, and 2F). Thus, the mitogenic effects of isoproterenol are independent of β-adrenoceptors and might involve spillover effects on α-adrenoceptors or on a propranolol-insensitive β-adrenoceptor pathway, as has been demonstrated in smooth muscle cells and cardiac fibroblasts.13

To investigate whether the abrogatory effects of catecholamines were mediated via adrenoceptors, we investigated their capability to block the antimitogenic effects of estradiol in the presence and absence of the α- and β-adrenoceptor antagonists phentolamine and propranolol, respectively. As shown in Figure 2F, 1 μmol/L epinephrine blocked the inhibitory effects of 100 nmol/L estradiol on FCS-induced DNA synthesis (Figure 2A), cell proliferation (Figure 2C), and collagen synthesis (Figure 2E), and the abrogatory effects of epinephrine were not diminished by 3 μmol/L phentolamine. Similarly, the abrogatory effects of isoproterenol on estradiol-mediated inhibition of FCS-induced DNA synthesis, collagen synthesis, and cell proliferation were not prevented by propanolol (Figures 2B, 2D, and 2F).

To determine whether catecholamines block the antimitogenic effects of estradiol by preventing the conversion of 2-hydroxyestradiol to 2-methoxyestradiol, we investigated the growth-inhibitory effects of 2-hydroxyestradiol and 2-methoxyestradiol in the presence and absence of epinephrine and isoproterenol. As shown in Figure 3, the inhibitory effects of 2-hydroxyestradiol on FCS-induced DNA synthesis (A, left), cell proliferation (B, left), and collagen synthesis (C, left) were abrogated by epinephrine and isoproterenol and also by OR486 (data not shown). Moreover, the abrogatory effects of epinephrine and isoproterenol were not reversed by phentolamine or propranolol, suggesting that the effects were independent of adrenoceptors. Similar to 2-hydroxyestradiol, 2-methoxyestradiol also inhibited FCS-induced GMC growth and collagen synthesis (Figures 3A to 3C, right). However, in
contrast to estradiol and 2-hydroxyestradiol, the inhibitory effects of 2-methoxyestradiol were not blocked by catecholamines (Figures 3A to 3C, right) or by OR486 (data not shown), suggesting that catecholamines abrogate the effects of estradiol by blocking the conversion of 2-hydroxyestradiol to 2-methoxyestradiol.

To determine whether catecholamines can block the conversion of estradiol to 2-methoxyestradiol, we first evaluated the effects of isoproterenol, epinephrine, and norepinephrine on the conversion of 2-hydroxyestradiol to 2-methoxyestradiol. GMCs efficiently metabolized 2-hydroxyestradiol to 2-methoxyestradiol. Isoproterenol, epinephrine, and norepinephrine inhibited methylation of 2-hydroxyestradiol (Figure 4A). At a concentration of 10 μmol/L, norepinephrine, epinephrine, and isoproterenol inhibited methylation of 2-hydroxyestradiol (0.25 μmol/L) by 9 ± 0.7%, 14 ± 1.3%, and 26 ± 3%, respectively (P < 0.05). OR486 at a concentration of 1 μmol/L inhibited 2-hydroxyestradiol methylation by 83 ± 3.6%. Compared with norepinephrine and epinephrine, isoproterenol more effectively inhibited 2-hydroxyestradiol metabolism.

In GMCs treated for 1 hour with supernatants of microsomes incubated with estradiol, significant amounts (6.16 pmol · min · 10⁶ cells) of 2-methoxyestradiol were formed. The formation of 2-methoxyestradiol from estradiol was inhibited by isoproterenol in a concentration-dependent manner (Figure 4B, top). Moreover, the formation of 2-methoxyestradiol was significantly inhibited by catecholamines (Figure 4B, bottom). At a concentration of 50 μmol/L isoproterenol, epinephrine and norepinephrine inhibited 2-methoxyestradiol formation by 43 ± 2.4%, 28 ± 1.7%, and 18 ± 0.8%, respectively (Figure 4B, bottom). Estradiol is metabolized to hydroxyestradiols by CYP450. In this regard, the CYP450 isoforms CYP1A1 and CYP1B1 are responsible for converting estradiol to 2- and 4-hydroxyestradiol.1 Importantly, we detected by Western blot analysis the presence of the CYP450 isoforms CYP1A1 and CYP1B1 in GMCs (Figure 4C). As shown in Figure 4D, both 2- and 4-methoxyestradiol inhibited FCS-induced DNA synthesis in a concentration-dependent manner; moreover, 2-methoxyestradiol was more potent than 4-methoxyestradiol in inhibiting GMC growth (Figure 4D).

**Discussion**

In the present study, physiologic and pharmacologic concentrations of estradiol inhibited GMC growth, and these effects were attenuated by catecholamines. The abrogatory effects of catecholamines were not reduced by α- and β-adrenoceptor blockers, and catecholamines blocked the inhibitory effects of 2-hydroxyestradiol, but not of 2-methoxyestradiol, on GMC growth.

6.16 ± 0.6 pmol · min · 10⁶ cells (represents 100%). Bottom shows inhibitory effects of 50 μmol/L isoproterenol (ISO), epinephrine (EPI), and norepinephrine (NE) on 2-methoxyestradiol formation in GMCs incubated with microsomal extracts. *P < 0.05 vs 2-methoxyestradiol formation in absence of inhibitors. C. Western blots showing expression of CYP450 isoforms CYP1A1 and CYP1B1 in human GMCs. D. Line graph comparing the concentration-dependent inhibitory effects of 2-methoxyestradiol (2-ME) and 4-methoxyestradiol (4-ME) on FCS-induced DNA synthesis ([³H]thymidine incorporation) in GMCs. *P < 0.05 vs cells treated with FCS alone; **P < 0.05 vs cells treated with 2-ME. Values represent mean ± SEM from at least 3 independent experiments. Each experiment was conducted at least in triplicate.
growth. The abrogatory effects of catecholamines were mimicked by the selective COMT inhibitor OR486. GMCs efficiently metabolize 2-hydroxysteradiol to 2-methoxysteradiol and expressed CYP450 enzymes responsible for converting estradiol to 2-hydroxysteradiol. Moreover, both catecholamines and OR486 inhibited the conversion of estradiol and 2-hydroxysteradiol to 2-methoxysteradiol by GMCs. Taken together, our findings provide evidence that catecholamines block the antimitogenic effects of estradiol in GMCs by inhibiting 2-methoxysteradiol formation.

Although the conventional view is that the biologic effects of estradiol on vascular smooth muscle cell growth are estrogen receptor mediated, the recent findings that estradiol attenuates the proliferation of vascular smooth muscle cells in injury-induced lesions of mice lacking estrogen receptor (ER)-α, ER-β, or both ER-α and β suggests that estrogen receptor–independent mechanisms might also participate in mediating the protective effects of estradiol on the vessel wall. Indeed, our recent findings in rat aortic vascular smooth muscle cells indicate that the vascular protective effects of estradiol are mediated in part by conversion of estradiol to 2-hydroxysteradiol, which in turn is methylated by COMT to yield the highly antiproliferative estradiol metabolite 2-methoxysteradiol. We hypothesized that a similar mechanism might apply for the renoprotective effects of estradiol, and indeed, our recent studies demonstrate that estradiol inhibits the growth of GMCs via its CYP450- and COMT-derived metabolites 2-hydroxysteradiol and 2-methoxysteradiol, respectively, and via estrogen receptor–independent mechanisms.

Inasmuch as 2-methoxysteradiol formation is catalyzed by COMT, it is conceivable that endogenous substrates for COMT would abrogate the renoprotective effects of estradiol and 2-hydroxysteradiol. In this regard, catecholamines are well-known substrates for COMT and are implicated in glomerulosclerosis. It is possible, therefore, that catecholamines might worsen renal injury by decreasing the conversion of 2-hydroxysteradiol to 2-methoxysteradiol by GMCs. In support of this hypothesis, in the present study we show that (1) the antimitogenic effects of estradiol and 2-hydroxysteradiol, but not 2-methoxysteradiol, are blocked by catecholamines; (2) catecholamines inhibit the metabolism of estradiol-derived 2-hydroxysteradiol to 2-methoxysteradiol by GMCs; and (3) the abrogatory effects of catecholamines are mimicked by OR486, a specific inhibitor of COMT. Our findings provide strong evidence that catecholamines can abrogate the renoprotective effects of estradiol by inhibiting COMT activity and thereby reducing the biosynthesis of 2-methoxysteradiol. Our results also imply that the renoprotective effects of estradiol in any individual might depend in part on the levels of catecholamines in the glomeruli.

Catecholamines are known to affect cell growth, and it is possible that the reversal of the effects of estradiol and 2-hydroxysteradiol by catecholamines is mediated by adrenergic receptors rather than by inhibition of COMT. In this regard, in vascular smooth muscle cells, catecholamines induce and inhibit growth via α-adrenoceptors and β2-adrenoceptors, respectively. Indeed, in the present study, the mitogenic effects of catecholamines were blocked by the α-adrenoceptor antagonist phentolamine but not by the β-adrenoceptor antagonist propranolol, suggesting that the mitogenic effects in GMCs are mediated by α-adrenoceptors. Our finding that the abrogatory effects of catecholamines are not blocked by phentolamine or propranolol provides strong evidence that catecholamines abrogate the effects of estradiol by inhibiting COMT and not by activating adrenergic receptors. A direct effect of catecholamines on GMC growth is also ruled out by the observation that catecholamines only marginally affect GMC growth in the absence of estradiol and 2-hydroxysteradiol.

We have previously shown that the antimitogenic effects of estradiol on GMCs are blocked by CYP450 inhibitors and enhanced by CYP450 inducers. Because CYP450 isozymes are responsible for metabolizing estradiol to 2-hydroxysteradiol, a precursor of 2-methoxysteradiol, we postulated that the sequential conversion of estradiol to 2-hydroxysteradiol and 2-methoxysteradiol is responsible for mediating the antimitogenic effects of estradiol. Our finding that GMCs expressed CYP1A1 and CYP1B1 and that catecholamines block the conversion of estradiol and 2-hydroxysteradiol to 2-methoxysteradiol provides strong evidence that in GMCs, estradiol can be locally converted to 2-hydroxysteradiol and 2-methoxysteradiol. Our contention that local conversion of estradiol to methoxyestradiols is responsible for its estrogen receptor–independent antimitogenic effects on GMCs is also supported by our recent findings that local metabolism of estradiol to methoxyestradiol inhibits growth of vascular smooth muscle cells, which are phenotypically similar to GMCs, as well as cardiac fibroblasts.

In the present study, concentrations (10 μmol/L) of catecholamines that inhibited 2-methoxysteradiol formation by 5% to 20% were able to completely reverse the inhibitory effects of estradiol on GMC growth. A potential explanation for the difference in the inhibitory effects of catecholamines on metabolism versus growth might be the difference in experimental conditions (cell number, cell density, time of treatment, concentration of estradiol). In this context, it is important to note that the inhibitory effects of catecholamines on the metabolism of estradiol and the antimitogenic effects of estradiol are time dependent. Importantly, in the metabolism studies, the cells were incubated for 1 hour, whereas in the growth studies, the cells were treated for 24 hours or 4 days.

Our findings suggest that catecholamines block the antimitogenic effects of estradiol by blocking the formation of 2- and 4-methoxyestradiols; however, additional studies are required to elucidate the exact contribution of 2- and 4-methoxyestradiol in mediating the antimitogenic effects of estradiol. It is well established that 2-methoxyestradiol is the major endogenous metabolite of estradiol, whereas 4-methoxyestradiol is a minor metabolite. Moreover, CYP1B1 metabolizes estradiol largely to 4-hydroxysteradiol and, to a lesser extent, to 2-hydroxysteradiol. In contrast, CYP1A1 metabolizes estradiol largely to 2-hydroxysteradiol, whereas 4-hydroxysteradiol is a minor product. Because GMCs express both CYP1A1 and CYP1B1, the continuous formation of hydroxysteradiols might play an important role in mediating the antimitogenic effects of estradiol on GMCs. This notion is further supported by our finding that both 2-methoxyestradiol and 4-methoxyestradiol inhibited FCS-induced GMC growth. Because, CYP1B1 is constitutively expressed, whereas CYP1A1 is inducible, it is feasible that...
under basal conditions, the effects of 4-methoxyestradiol might be more important.

In vivo metabolism of estradiol to 2-hydroxyestradiol accounts for 50% of the estradiol metabolites formed, and the levels of catecholestradiols range between 0.12 and 0.3 μmol/L in peripheral blood. Substantial amounts of 2-hydroxyestradiol are thus available to be converted to 2-methoxyestradiol. Owing to the rapid conversion of 2-hydroxyestradiol to 2-methoxyestradiol, accurate data on the levels of 2-hydroxyestradiol are not available. Nonetheless, serum levels of 2-methoxyestradiol in pregnant women are 30 nmol/L, and rough estimates suggest that 2-methoxyestradiol levels might be several-fold higher than the levels of estradiol. GMCs and intact kidneys are endowed with COMT, ensuring pharmacologically active, steady-state levels of methoxyestradiol.

Circulating levels of norepinephrine and epinephrine are generally 1 to 2 nmol/L, increasing to as high as 12 nmol/L during moderate sympathetic nerve stimulation. However, the concentration of norepinephrine in the average neuroeffector junction is ∼4 times greater than its plasma levels, ie, ∼50 nmol/L. The level of norepinephrine in any given neuroeffector junction during sympathetic activation will depend on such factors as the width of the junction and the efficiency of uptake and metabolism of norepinephrine. Thus, norepinephrine levels in some neuroeffector junctions would be much greater than 50 nmol/L during sympathetic activation. In the present study, concentrations of catecholamines as low as 100 nmol/L significantly attenuated the growth-inhibitory effects of 100 nmol/L 2-hydroxyestradiol. Because the interaction between catecholamines and 2-hydroxyestradiol at the level of COMT is competitive, even lower levels of catecholamines would be expected to attenuate the antimitogenic effects of lower levels of 2-hydroxyestradiol. Moreover, the sequential conversion of estradiol to 2-hydroxyestradiol and 2-methoxyestradiol locally within the GMCs would be more susceptible to inhibition by catecholamines, owing to the rate-limiting step of 2-hydroxyestradiol formation. These considerations imply that increased synthesis of catecholamines under pathologic conditions could effectively attenuate the inhibitory effects of 2-hydroxyestradiol on GMC growth.

Patients with the nephrotic syndrome have higher levels of catecholamines and higher rates of secretion of norepinephrine. These patients have approximately twice the level of plasma norepinephrine than normal and an ∼3 times higher secretion rate than normal. This also implies that at neuroeffector junctions, the levels of norepinephrine in these patients are much higher than normal levels. The higher levels of norepinephrine could potentially occupy COMT to such an extent as to inhibit or limit metabolism of other catechols.

In animal models in which sympathetic activity is pharmacologically inhibited or minimized by denervation, the progression of glomerulosclerosis is substantially reduced compared with untreated animals. This indirect evidence implicates the adverse effects of catecholamines in glomerulosclerosis. Our findings suggest that increased catecholamines might have an adverse effect on progression of glomerulosclerosis by inhibiting COMT and thereby reducing the methylation of 2-hydroxyestradiol. We have recently shown that in a model of obese rats (ZSF1) with the nephrotic syndrome, 2-hydroxyestradiol reduces glomerulosclerosis as indicated by histopathology and reduced proteinuria. This further supports the conclusion that inhibiting methylation of estradiol-derived 2-hydroxyestradiol might result in accelerating the progression of renal disease.

Perspectives
Here we provide evidence that GMCs metabolize estradiol-derived 2-hydroxyestradiol to 2-methoxyestradiol and that COMT-mediated conversion of 2-hydroxyestradiol to 2-methoxyestradiol is essential for the inhibitory effects of estradiol on GMC growth. Our findings suggest that interactions between catecholamines and endogenous catecholestradiols might play an important role in defining the overall protective effects of estradiol in the kidney. These findings imply that estradiol metabolism might be an important determinant of the renal protective effects of estradiol. Thus, interindividual differences, either genetic or acquired, in estradiol metabolism might define a given female’s risk of renal disease and influence the renal benefit she receives from estradiol replacement therapy in the postmenopausal state. These findings also imply that nonfeminizing estradiol metabolites might confer renal protection in both women and men.

Acknowledgments
This work was supported by Swiss National Science Foundation grant 32-64040.00 and National Institutes of Health grant 55314.

References


Catecholamines Block the Antimitogenic Effect of Estradiol on Human Glomerular Mesangial Cells
Raghvendra K. Dubey, Lefteris C. Zacharia, Delbert G. Gillespie, Bruno Imthurn and Edwin K. Jackson

Hypertension. 2003;42:349-355; originally published online August 11, 2003; doi: 10.1161/01.HYP.0000088320.81260.26
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/42/3/349

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/