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Effect of sex hormones on renal estrogen and angiotensin type 1 receptors in female and male rats

Jennifer L. Rogers,1* Adam R. Mitchell,1* Christine Marie,1,2,3 Kathryn Sandberg,1,2,3 Adam Myers,1,3 and Susan E. Mulroney1,3

Departments of 1Physiology and Biophysics and 2Medicine and 3Center for the Study of Sex Differences in Health, Aging and Disease, Georgetown University School of Medicine, Washington, DC

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Rogers, JL, Mitchell AR, Marie C, Sandberg K, Myers A, Mulroney SE. Effect of sex hormones on renal estrogen and angiotensin type 1 receptors in female and male rats. Am J Physiol Regul Integr Comp Physiol 292: R794 –R799, 2007. First published September 21, 2006; doi:10.1152/ajpregu.00424.2006.—Although the mechanisms are not understood, evidence suggests that 17β-estradiol (E2) confers protection from cardiovascular and renal complications in many diseases. We have reported that E2 decreases angiotensin type 1 receptors (AT1Rs) in different tissues and hypothesize that E2 exerts tonic inhibition on AT1Rs, reducing effects of ANG II. This study determined the effects of E2 and dihydrotestosterone (DHT) on cortical estrogen receptors (ERs) and glomerular AT1R binding in rats. Animals underwent sham operation, ovariectomy (Ovx) or orchidectomy (Cas) and were treated (Ovx ± E2; Cas ± DHT) for 3 wk. Cortical ERα protein was 2.5 times greater, and ERβ was 80% less in females vs. males (P < 0.01). Glomerular AT1R binding was lower in females than males [4,657 ± 838 vs. 7,457 ± 467 counts per minute (cpm), P < 0.01]. Ovx reduced ERα protein by 50%, whereas E2 increased ERα expression after Ovx. The decrease in cortical ERα in Ovx rats was associated with a significant increase in AT1R binding (6,908 ± 609 cpm), and E2 prevented this increase. There was no change in ERα or AT1R binding following Cas ± DHT (25 mg) treatment, although Cas did elevate cortical ERβ (P < 0.01). Interestingly, the high dose DHT (200 mg) elevated ERα 150% above intact levels and profoundly decreased AT1R binding (1,824 ± 705 cpm, P < 0.001 vs. intact male). This indicates that under normal conditions, glomerular AT1R binding is significantly greater in male than female animals, which may be important in development of cardiovascular and renal disease in males. Furthermore, E2 regulates ERα and is inversely associated with glomerular AT1R binding, supporting our hypothesis that E2 tonically suppresses AT1Rs and suggesting a potential mechanism for the protective effects of estrogen.

Premenopausal women have significantly lower mean blood pressures (BP) compared with men, and BP in postmenopausal women rises above that of same-aged men (28, 36), leading to the hypothesis that estrogen confers protection against the development of hypertension. Animal studies (28) support this concept, but clinical trials using estrogen and progesterone do not (20, 27). These discrepancies could arise from the dosing method or hormone preparation (8, 32) and suggest that additional studies are needed. It is also noteworthy that in diabetic patients, renal disease progresses more rapidly in women than in men (1, 2, 9, 14, 43), and the “female advantage” of lower blood pressure is lost (44). This may be due to decreased 17β-estradiol (E2) levels in diabetic women, a finding that has been observed in diabetic female animals (16, 39, 42). 17β-Estradiol administration has also been shown to attenuate the renal damage in streptozotocin-diabetic animals (29). In addition, the protective effects of E2 on the vasculature are also lost in diabetes (5). Taken together, these findings support the concept that E2 normally confers protection against development of hypertension and diabetes-associated complications and highlight the impact that sex differences have on health and disease.

ANG II has been implicated in many cardiovascular and renal disease states, such as hypertension and diabetic nephropathy (41). Indeed, both angiotensin-converting enzyme (ACE) inhibitors and AT1R antagonists (ARB) are clearly effective in lowering blood pressure and attenuating the progression of diabetic nephropathy in humans (7, 30, 35). In the kidney, the major actions of ANG II are mediated through the AT1R. Stimulation of angiotensin type 1 receptors (AT1Rs) has been shown to increase intrarenal cell proliferation and matrix remodeling, as well as disrupt the medullary interstitial concentrating system (10, 21). Furthermore, ANG II has been shown to induce TGF-β gene expression in cultured mesangial cells, and a subsequent increase in matrix deposition, a step that is integral to the development and progression of renal disease. Recent findings suggest that sex differences exist in the regulation of the AT1R, and the development of renal disease (13, 22). We hypothesize that the interaction between sex steroids and ANG II function may contribute to observed sex differences in development and progression of renal and cardiovascular diseases.

In support of this concept, we and others have shown that E2 decreases tissue levels of ANG II (34, 47) and ACE expression...
and activity in female rats (6, 33, 38). We have also shown that ovariectomy increases AT1R levels in adrenal, pituitary, and uterine tissues compared with intact females, while E2 in overiectomized (Ovx) rats prevents the Ovx-induced increase in AT1R levels in those tissues (47, 48). We have also observed this effect of E2 in canine adrenal, myocardium, liver, and glomeruli using dynamic PET measurements (25). In vitro, E2 has also been found to decrease the growth-promoting actions of ANG II on vascular smooth muscle cells by inducing phosphatases, which may serve to reduce the response to vascular injury (37). These findings suggest that under normal conditions, E2 tonically suppresses the effects of ANG II. This action could contribute to the “protective” effects of E2 against the development of cardiovascular and renal disease. In sharp contrast to E2, Leung et al. (17) found that testosterone increases AT1R binding in epididymal cells, but whether this represents a tissue-specific response to the testosterone is not clear. Thus, while the effects of gonadal steroids on renal AT1R activity are not completely understood, there is compelling evidence that the interactions of these important hormonal systems on the kidney are important in both health and disease and may account for the observed sex differences in susceptibility and rate of progression of vascular and kidney disease.

The purpose of the present study was to determine the effects of 17β-estradiol and dihydrotestosterone on cortical renal ERs. In addition, the changes in ER protein expression were correlated with glomerular AT1R binding in female and male rats.

METHODS

Hormone replacement. All procedures were approved by the Georgetown University Animal Care and Use Committee. Female Sprague-Dawley (S-D) rats (Harlan, Madison, WI) were obtained at 12–14 wk of age and randomly divided into treatment groups: sham (intact control, n = 6); ovariectomized (Ovx, n = 6), and ovariectomized with E2 replacement (Ovx + E2, n = 6). Animals were anesthetized (12% tribromoethanol), the ovaries were exposed via bilateral flank incision and excised. Sham-operated animals underwent flank incision and manipulation of the ovaries before the wound was closed. Immediately after surgery, the animals were given daily injections of E2 (5 μg/day sc) dissolved in 100 μl corn oil or corn oil vehicle (injections were used as they produced end-organ results comparable to intact female rats). The animals were fed a phytoestrogen-free diet (Harlan) and given tap water ad libitum. After 21 days of treatment, the animals were weighed and anesthetized, and blood was collected via cardiac puncture for measurement of plasma DHT levels. Kidneys were snap frozen in liquid nitrogen for Western blot analysis and binding assays.

Western blot analysis. The renal cortex was dissected from the frozen tissue, homogenized, and prepared for Western blot analysis on the Bio-Rad Criterion system using 10% gels. Protein concentration was determined by the Bradford method using bovine serum albumin as the standard (Bio-Rad Laboratories, Hercules, CA). Thirty milligrams of total protein was loaded in each lane, and after electrophoresis, the proteins were transferred to a nitrocellulose membrane (45, 46). The blots were then blocked in 5% nonfat milk in PBS/tris (PBST) at 4°C and then exposed to primary antibody [ERα (1:1,000 dil) or ERβ (1:500 dil), Santa Cruz Laboratories, Santa Cruz, CA] for 2 h at room temperature on a shaking platform. Blots were washed in 1% PBS and then incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:1,000, KPL, Gaithersburg, MD). Proteins were then detected by chemiluminescent peroxidase (Amersham, Buckinghamshire, UK) reaction and recorded on X-ray film. Relative density of bands was determined, normalized against a Coomassie blue protein band, and reported as arbitrary density units. Quantitative comparisons of male and female ER were made on the data from the same gels. At least three Western blot analyses were performed on each group of samples for both ERα and ERβ. To compare male and female samples (and confirm measurements), we used four samples in each group per gel, and multiple gels were used to analyze all of the samples from each group.

AT1R binding. Kidney glomeruli were isolated for AT1R binding studies, as previously described (22). Protein concentrations of isolated glomeruli were determined by the Bradford method using bovine serum albumin (Bio-Rad). These proteins were then run on a 4% to 12% SDS-PAGE gel, transblotted to nitrocellulose (45, 46), and then probed with a rabbit polyclonal antibody against AT1R (1:500 dil), Santa Cruz Laboratories, Santa Cruz, CA. proteins were visualized with chemiluminescent detection (Amersham, Piscataway, NJ). Blots were scanned, and relative density was determined with ImageJ software (National Institutes of Health). Quantitative comparisons of male and female AT1R binding were made on data from the same binding studies. Statistical analysis between groups was performed using two-way ANOVA, with Student-Neuman-Keul’s post hoc tests. Analysis within groups was performed by Student’s t-tests or one-way ANOVA with Student-Neuman-Keul’s post hoc tests. All data is presented as means ± SE; significance was designated at P < 0.05.

RESULTS

Kidney, heart, and body weights are given in Table 1. Although the loss of estrogen with Ovx increased whole body weight, organ weights were not different from intact female controls. In contrast, the loss of testosterone in the male was associated with significantly lower kidney and heart weights. Kidney and heart weights in DHT-supplemented rats were not different from those in intact males.

Plasma estradiol levels were reduced following Ovx (54 ± 3 vs. 91 ± 27 pg/ml in intact female controls, P = 0.12) but did not reach significance because of typical variability in the intact animals. Replacing E2 in the Ovx rats elevated circulating levels to a physiological range (157 ± 27 pg/ml, P < 0.05 vs. Ovx). E2 levels in intact and Cas male rats were comparable to the Ovx female levels (45 ± 6 and 51 ± 4 pg/ml in intact and Cas male rats, respectively, not significant vs. Ovx). Interestingly, the high DHT dose in the male rats produced extremely high E2 levels (>1,000 pg/ml), despite the fact that DHT is not supposed to be acted on by aromatase.
Table 1. Effect of hormone replacement on organ and body weights in male and female rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial BW, g</th>
<th>Final BW, g</th>
<th>KW, g</th>
<th>KW/BW, %</th>
<th>HW, g</th>
<th>HW/BW, %</th>
</tr>
</thead>
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<tr>
<td>Sham female</td>
<td>213.3±6.5</td>
<td>236.8±5.2</td>
<td>1.42±0.03</td>
<td>0.60±0.01</td>
<td>0.91±0.05</td>
<td>0.38±0.01</td>
</tr>
<tr>
<td>Ovx</td>
<td>223.8±4.6</td>
<td>268.3±17.7</td>
<td>1.48±0.09</td>
<td>0.55±0.01*</td>
<td>0.97±0.04</td>
<td>0.36±0.02</td>
</tr>
<tr>
<td>Ovx + E2</td>
<td>224.8±2.1</td>
<td>241.5±5.3</td>
<td>1.48±0.02</td>
<td>0.61±0.02</td>
<td>0.89±0.02</td>
<td>0.37±0.02</td>
</tr>
<tr>
<td>Sham male</td>
<td>368.5±2.0</td>
<td>400.8±3.4</td>
<td>2.58±0.05</td>
<td>0.65±0.01</td>
<td>1.66±0.08</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>Cas</td>
<td>359.5±3.5</td>
<td>364.0±2.0*</td>
<td>2.30±0.02*</td>
<td>0.63±0.01</td>
<td>1.43±0.05*</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>Cas + DHT (25)</td>
<td>370.0±8.1</td>
<td>383.3±10.3*</td>
<td>2.50±0.23</td>
<td>0.65±0.02</td>
<td>1.68±0.13</td>
<td>0.44±0.01</td>
</tr>
<tr>
<td>Cas + DHT (200)</td>
<td>374.5±5.2</td>
<td>392.1±4.1*</td>
<td>2.54±0.07</td>
<td>0.65±0.02</td>
<td>1.67±0.07</td>
<td>0.43±0.02</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. Ovx, overiectomized; E2, 17β-estradiol; Cas, castrated; DHT, dihydrotestosterone; BW, body weight; KW, kidney weight; HW, heart weight. *P < 0.05 vs. sham same sex.

DHT levels were also determined in male and female rats. DHT was significantly reduced in Cas compared with intact male rats (44 ± 5 vs. 2,025 ± 238 pg/ml in intact males, P < 0.05), and DHT replacement in Cas males increased circulating DHT compared with Cas male rats (321 ± 163 and 3,363 ± 1,106 pg/ml in 25 mg and 200 mg/21 day doses, respectively, P < 0.05 vs. Cas).

Sex differences in renal estrogen receptor regulation. Figure 1, A and B illustrate renal cortical ERα and ERβ proteins in intact male and female rats (For comparisons between sexes made on same gels, see representative gel strips in Fig. 1C). Renal cortical ERα protein expression was ~2.5 times greater in female, compared with male rats. In contrast, ERβ expression was over four times greater in male compared with female cortex. Following Ovx, there was a 50% reduction (P < 0.01) in cortical ERα expression compared with intact female rats, whereas there was no change in ERα protein expression following Cas in male rats (Fig. 2A). Interestingly, while Ovx did not affect cortical ERβ protein expression, Cas significantly increased cortical ERβ in male kidneys by 30% (Fig. 2B).

E2 replacement in Ovx rats significantly increased cortical ERα toward levels observed in intact female controls (Fig. 3A). Interestingly, while replacement of Cas male rats with DHT (low dose 25 mg/21 days) had no significant effect on cortical ERα, the high DHT dose (200 mg/21 days) had profound effects on ERα expression: there were significant increases in cortical ERα compared with intact or Cas kidneys (Fig. 3B).

Sex differences in renal AT1R binding. Renal glomerular AT1R binding is illustrated in Fig. 4. Specific AT1R binding was ~40% lower in glomeruli from female, compared with male rats (P < 0.05). Compared with intact female rats, Ovx

Fig. 1. Sex differences in renal cortical estrogen receptor (ER) protein abundance in adult rats. A: cortical ERα protein expression was significantly greater in female than male kidneys. B: cortical ERβ protein was significantly greater in male than female kidneys. C: representative gels for ERα and ERβ, showing representative male and female comparisons (4 samples for each group). Protein from the same animals (n = 6, each sex) is analyzed in A and B. *P < 0.05 between male and female animals. Cas, castrated; Ovx, overiectomized.
increased glomerular AT1R binding by \( \sim 50\% \) (\( P < 0.01 \)) in Ovx animals. E2 replacement prevented this Ovx-induced increase in glomerular AT1R binding; no differences in binding were observed between intact and Ovx + E2 animals. Interestingly, AT1R binding in the Ovx female was not different from binding in intact or castrated males. The presence of normal testosterone (in intact males) or low-dose DHT did not significantly alter AT1R binding. However, high-dose DHT significantly suppressed AT1R binding by 80\% (Fig. 4).

**DISCUSSION**

The potential interactions between sex hormones and AT1Rs have become a focus of intense investigation, in great part because of the observed sex differences in the development and progression of ANG II-associated cardiovascular and renal diseases. Although recent reports have linked the presence of estrogen with downregulation of AT1R activity, the effects of estrogen on its own receptors and AT1R binding in both male and female kidneys are not well understood. The present findings extend previous reports showing that E2 attenuates AT1R binding in various tissues (47) by demonstrating that E2 (17\(^\beta\)-estradiol) prevents the Ovx-induced decrease in ER\( \alpha \) protein expression in the renal cortex (Fig. 3A) under conditions in which the Ovx-induced increase in glomerular AT1R binding is also prevented (Fig. 4). The effects suggest an association with ER\( \alpha \), since the unique change in ER\( \beta \) protein observed in the cortex of Cas male rats had no effect on AT1R expression (Figs. 2B and 4). Furthermore, this study compared AT1R binding in both sexes and found that AT1R binding is \( \sim 40\% \) lower in intact female compared with intact male glomeruli (Fig. 4). Thus this study suggests that the E2-mediated attenuation of AT1R binding in the kidney is one potential mechanism by which E2 exerts “protection” from vascular and renal disease in the estrogen-replete female.

![Fig. 2](image2.png)  
*Fig. 2. Effect of gonadectomy on renal cortical ER protein abundance. A: Ovx reduced cortical ER\( \alpha \) protein abundance by 50\%; Cas did not change ER\( \alpha \) protein expression. B: Ovx had no effect on cortical ER\( \beta \). In contrast, Cas increased ER\( \beta \) protein expression in male animals. Representative blots of two samples from the Western blot analysis are shown over the corresponding group in the graph. \( *P < 0.01 \) vs. intact same sex; \( n = 6 \) analyzed in each group.*

![Fig. 3](image3.png)  
*Fig. 3. Effect of E2 and dihydrotestosterone (DHT) replacement on renal cortical ER protein abundance. A: E2 replacement at 5 \( \mu \)g/day significantly increased ER\( \alpha \) protein abundance in Ovx female animals (\( *P < 0.01 \)). B: DHT at a dose of 25 mg/21 days did not affect ER\( \alpha \) protein expression in Cas male animals; however, there was a 150\% increase in ER\( \alpha \) protein expression in the renal cortex from animals given a high dose of DHT (200 mg/21 days). \( n = 6 \) analyzed in each group; \( *P < 0.01 \) vs intact male.*

![Fig. 4](image4.png)  
*Fig. 4. AT1R binding in female and male glomeruli. Ovx increased glomerular ANG type 1 receptor (AT1R) binding by \( \sim 50\% \), while E2 replacement prevented this increase. There was no change in AT1R binding in castrated rats treated with or without DHT (25 mg/21 days) compared with intact males. In sharp contrast, the high dose of DHT significantly reduced AT1R binding to levels lower than observed in intact female glomeruli. Glomerular samples are from the same animals as shown in Fig. 3; \( *P < 0.01 \) vs. intact female, \#\( P < 0.001 \) vs. intact male and intact female; \( n = 6 \) analyzed in each group.*
is, circulating E2 ionically inhibits AT1R binding in the renal cortex; when this inhibition is lifted, such as when circulating E2 is reduced following menopause or in diabetes, AT1R binding is increased. This would result in enhanced ANG II signaling, which increases the degree of susceptibility to vascular and renal disease, as well as increases the rate of existing disease progression.

The current study found that the level of AT1R binding was inversely related to ERα protein expression (Figs. 3, A and B, and 4), and this was true even with the high dose of DHT (200 mg pellet), which increased ERα and profoundly decreased AT1R binding compared with intact controls. This finding was unexpected, as neither parameter was altered by castration or the lower dose of DHT. The finding is especially intriguing, as plasma estradiol levels appeared to be very high (which supported our findings of high cortical ERα), despite the fact that DHT is not a substrate for aromatase. This suggests that very high circulating DHT may affect other hormones or systems (which could alter estradiol and ERα). Also, in the brain, DHT has also been shown to act through ERβ receptors to affect stress responses (19); this opens the possibility that under certain conditions, DHT might act through these receptors in peripheral organs; however, the potential physiological relevance is unclear and warrants further study.

Our studies also clearly demonstrate the sex differences in AT1R binding in glomeruli from intact female and male rats. Although higher AT1R binding was consistently observed in male kidneys in the presence or absence of physiological levels of DHT, intact and E2-supplemented female rats had significantly lower AT1R binding (Fig. 4) compared with males; Ovx brought binding up to levels observed in male glomeruli. This strongly supports the idea that E2 limits the development of hypertension and renal disease through tonic inhibition of the AT1R. Investigators have reported that Ovx significantly increases the density of glomerular (11, 12), and adrenal (12) AT1Rs, as well as blood pressure in the Dahl salt-sensitive rat, compared with E2-supplemented Ovx or intact rats. Harrison-Bernard et al. (11) have also demonstrated that AT1R blockade prevents the increase in blood pressure in salt-sensitive Ovx rats. These findings further support the concept that loss of E2 (as with age) increases AT1R activity and contributes to the development of hypertension. This interpretation fits well with the finding that tissue responsiveness to ANG II is reduced in the presence of E2 (31).

Whether the actions of E2 and testosterone are tissue and/or disease specific or are more widespread remains to be determined; however, studies by our lab and others suggest that the degree of protection or damage may depend on the underlying physiological state. For example, in uninephrectomized rats, male but not female kidneys exhibit overt testosterone-mediated glomerular hypertrophy, tubular damage, and proteinuria (3, 4, 23), which is associated with increases in glomerular AT1R density, and is prevented by ARB treatment (22). Li et al. (18) have also reported that testosterone contributes to the sex-related differences in cardiac hypertrophy observed in male guanylyl cyclase-A knockout mice. These findings suggest that under certain circumstances, testosterone upregulates renal AT1Rs, as it has been shown to do in epididymal fat cultures (17). Thus, depending on the specific tissue and disease process, testosterone may promote disease. Interestingly, while the progression of disease in many forms of chronic renal failure (including membranous nephropathy, IgA nephropathy, and polycystic kidney disease) is clearly accelerated in men compared with women (24), this is not true for diabetes. In fact, in diabetes, the progression of hypertension and nephropathy is accelerated in women (14, 26, 40). Evidence is building that the sex differences in diabetic disease are related to estrogen and ER status. 17β-estradiol levels are decreased in diabetic female rodents (16, 39, 42), and recent findings have also shown that ERα are significantly decreased in mesangial cells from female db/db mice (Type 2 diabetes model) compared with wild type (apparently by local IGF-I) (15), again supporting the notion that ER expression is altered in diabetes. Together with our findings, this further strengthens the concept that the loss of E2 with menopause or in diseases, such as diabetes, predisposes women to a higher risk of developing ANG II-associated pathologies. In contrast, testosterone has been associated with increased disease progression.

In conclusion, these studies support and extend previous work by demonstrating that the E2-replete rat has higher ERα expression and lower AT1R binding in the renal cortex compared with the intact male or the Ovx female. The inverse correlation between ERα expression and glomerular AT1R at a key site for development of renal pathology strongly supports the concept that renoprotection in the female occurs, in part, by the E2-mediated attenuation of AT1R activity in the kidney.

GRANTS

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REFERENCES

SEX DIFFERENCES IN RENAL ESTROGEN AND AT1 RECEPTORS


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