Role of endothelin in mediating postmenopausal hypertension in a rat model

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Yanes, Licy L., Damian G. Romero, Valeria E. Cucchiarelli, Lourdes A. Fortepiani, Celso E. Gomez-Sanchez, Francisco Santacruz, and Jane F. Reckelhoff. Role of endothelin in mediating postmenopausal hypertension in a rat model. Am J Physiol Regul Integr Comp Physiol 288: R229–R233, 2005. First published August 19, 2004; doi:10.1152/ajpregu.00697.2003.—Cardiovascular disease is the leading cause of death in women after menopause. Hyper tension, a major cardiovascular risk factor, becomes more prevalent after menopause. The mechanisms responsible for the increase in blood pressure (BP) in postmenopausal women are unknown. We have recently characterized the aged, postestrous-cycling (PMR) spontaneously hypertensive rats (SHR) as a model of postmenopausal hypertension. The purpose of the present study was to determine whether endothelin plays a role in the increased BP in PMR. Premenopausal female SHR, aged 4–5 mo (YF), and PMR, aged 16 mo, were studied. Expression of preproendothelin-1 mRNA was not different in either renal cortex or medulla between PMR and YF (n = 7–8/group). In contrast, ET-1 peptide expression was significantly higher in renal cortex of PMR than in renal cortex of YF, but there was no difference in medullary ET-1. Expression of endothelin ETA receptor (ETA,R) mRNA was lower in renal cortex and medulla of PMR than of YF. Additional groups of rats (n = 6–7/group) were treated for 3 wk with the ETA,R antagonist ABT-627 (5 mg·kg−1·day−1). BP was significantly higher in PMR than in YF. ETA,R antagonist reduced BP in PMR by 20% to the level found in control YF. ETA,R antagonist had no effect on BP in YF. These data support the hypothesis that the increase in BP in PMR is mediated in part by endothelin and the ETA,R.

ETA receptor; ETB receptor; kidney

AFTER MENOPAUSE, blood pressure (BP) increases in women such that the prevalence of hypertension is higher in postmenopausal women than in age-matched men (5, 24). The reasons why BP increases in women after menopause are not known. However, endothelin has been suggested to play a role in the increase in BP in postmenopausal women because plasma levels of endothelin have been shown to be increased after menopause (15), and hypertensive postmenopausal women have higher endothelin levels than normotensive ones (25).

Recently, we characterized a model of postmenopausal hypertension in the aging female spontaneously hypertensive rat (SHR). Throughout their reproductive lives, female SHR have lower BPs than males (17). However, the female SHR stops cycling at 10–12 mo of age, and, by 16–18 mo of age, BP levels are similar to or higher than those in age-matched male SHR (11). This loss of the sexual dimorphism in BP is due to an increase in BP in the females rather than any change in BP in the males after 8 mo of age (11). Postcycling female SHR (PMR) also exhibit increases in plasma renin activity, renal vasoconstriction, and renal injury when compared with premenopausal females (11), suggesting that a vasoconstrictor may be involved in the increase in BP in PMR.

In the present study, we determined whether endothelin plays a role in mediating postmenopausal hypertension in PMR. Because the kidney is the major long-term controller of BP, we tested the hypothesis of whether there was an increase in the expression of endothelin-1 (ET-1) in the kidneys of PMR compared with young female rats and whether a selective ETA receptor (ETA,R) antagonism would lower BP in PMR but not in young female rats. We also determined whether ETAR or ETB receptor (ETB,R) expression in the kidney was altered in PMR.

METHODS

Rats. Female SHR, 8 mo old (n = 6–8 each group), were obtained from Taconic Farms (Germantown, NY) and were aged to 16 mo (PMR). Female SHR, obtained at 12 wk and studied at 5 mo of age (n = 5–7 per group), were used as premenopausal controls. Rats were maintained on standard rat chow (Teklad, Harlan, Indianapolis, IN) and tap water in an environment with 12:12-h light-dark cycles. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of tissue ET-1 peptide. We conducted studies with young and old female SHR (n = 7–8 rats per group) according to the methods of Ref. 21. Rats were anesthetized with isoflurane, and kidneys were perfused free of blood with saline containing 2% heparin. Kidneys were removed and separated into cortex and medulla. Renal cortical and medullary sections were homogenized in 10 vol of 1 M acetic acid containing heparin A (10 μg/ml), heated to 100°C for 10 min, chilled, and centrifuged at 23,000 g, 4°C, for 30 min. We measured ET-1 in the supernatant using a commercially available enzyme-linked chemiluminescent assay (R&D Systems, Minneapolis, MN), according to the manufacturer’s directions. Protein content in the supernatant was determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA), using bovine serum albumin as standard (4). Data are expressed as picograms of ET-1 per milligram of protein.

Measurement of renal preproendothelin, ETA, and ETB mRNA. Total RNA was extracted with Tri-Reagent (MRC, Cincinnati, OH), resuspended in diethyl pyrocarbonate-H2O, DNase treated with DNA-free kit (Ambion, Austin, TX), and quantified by spectrophotometry.

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Five micrograms of RNA were reverse transcribed (RT) with 0.5 μg of T2,V primer and Superscript III (Invitrogen, Carlsbad, CA) in a final volume of 20 μl. The reaction was carried out for 60 min at 50°C and terminated by incubation at 75°C for 15 min. Primers for preproendothelin-1 (sense: 5′-CGTCGCGTGAGAATGGAGGAA-3′, antisense: 5′-GTGTTGCTTGGTGTTGCGTG-3′, product size: 87 bp), ETAR (sense: 5′-GCAACAGGGAAGATGACTGGA-3′, antisense: 5′-GCAACAGGGAAGATGACTGGA-3′, product size: 93 bp), and ETBR (sense: 5′-CGATTGTATCATGCCTCGTG-3′, antisense: 5′-GGGACCATTTCTCATGCACT-3′, product size: 87 bp) were designed with Primer3 software (19) and checked for absence of cross-reactivity by BLAST search. Elongation factor 1 (EF-1) primers have already been described (14). Real-time polymerase chain reaction (PCR) contained 1 μl of RT product, 0.1 μM each of primer, 0.2 mM dNTPs, SYBR green I (1:20,000 final concentration; Molecular Probes, Eugene, OR), and 1 μl of titanium Taq DNA polymerase (Clontech, Palo Alto, CA). Amplifications were performed in a real-time thermal cycler (iCycler, Bio-Rad Laboratories). Cycling conditions were 1 min at 95°C, followed by 50 cycles of 15 s at 95°C; then 15 s at 67.5°C for ET-1, ETAR, and ETBR or 60°C for EF-1 (control); and then 60 s at 72°C. Fluorescence data were collected during the elongation step. After PCR amplification, the specificity of the PCR was confirmed by melting temperature determination of the PCR product and electrophoretic analysis in 2% agarose gels. Standard curves were performed with serial dilutions of pooled RT samples. Results are expressed as arbitrary units and normalized against EF-1 mRNA expression.

**ETAR antagonist treatment in PMR and premenopausal rats.** Rats (n = 6–7/group) were treated with ABT-627 (5 mg·kg⁻¹·day⁻¹), a selective ETAR antagonist (a generous gift from Abbott Laboratories, East Chicago, IL) (26) or vehicle (0.02% sodium hydroxide) in drinking water for 3 wk. Water consumption was monitored daily. The dose of ABT-627 that was given was adjusted according to daily water consumption to make certain that the rats received the same dose of drug. This dose has been shown to cause complete blockade of pressor responses to endothelin bolus injections and to reduce BP in endothelin-dependent hypertension (2, 3).

**Measurement of BP.** Before the end of the treatment, rats were anesthetized by isoflurane gas anesthesia, and a catheter was placed in the femoral artery for blood sampling and BP monitoring. The catheter was exteriorized at the back of the neck, as previously described (18). The next day, conscious BP recordings were made in animals that were placed in restraining cages. Rats had been habituated to the restraining cages before catheter placement. Mean arterial pressure was monitored in conscious rats with a pressure transducer connected to a Grass recorder (model 7B-chart, Grass Instrument). After a 60-min stabilization period, recordings were made for two periods of 30 min each, and the data were averaged.

**Statistics.** Data are presented as means ± SE. Comparisons among groups were analyzed by one-way ANOVA, followed by Dunnett’s test (9). A value of P < 0.05 was considered statistically significant.

**RESULTS**

**Characterization of the endothelin system in the kidney: expression of preproendothelin-1 mRNA.** Real-time RT-PCR was performed to determine the level of expression of preproendothelin mRNA in renal cortex and medulla from premenopausal and PMR rats. As shown in Fig. 1, there were no differences in expression of preproendothelin-1 mRNA in...
cortex or medulla from PMR compared with premenopausal females.

Expression of ET-1 peptide in kidney. As shown in Fig. 2, ET-1 peptide content in the renal cortex was significantly higher in PMR than in premenopausal rats. ET-1 peptide expression was 10-fold higher in medulla of both pre- and postmenopausal rats compared with that in cortex. There was a tendency for ET-1 to be also higher in the medulla of PMR than in the medulla of premenopausal rats, although this did not reach statistical significance ($P = 0.11$).

Expression of ET$_A$R and ET$_B$R mRNA in kidney. As shown in Fig. 3, ET$_A$R mRNA expression was significantly lower in renal cortex and medulla of PMR than in renal cortex and medulla of premenopausal rats. ET$_B$R mRNA was also lower in cortex of PMR than in cortex of premenopausal rats; in medulla, however, there was a tendency for ET$_B$ mRNA levels to be lower in PMR, but these results did not reach statistical significance ($P = 0.065$).

Effect of ET$_A$R antagonist. Body, kidney, and heart weights are shown in Table 1. Body and kidney weights were higher in PMR than in premenopausal rats. ET$_A$R antagonism had no effect on body, kidney, or heart weights in premenopausal females, but both heart and kidney weights were decreased in PMR compared with untreated controls.

Figure 4 shows mean arterial pressure in conscious, chronically catheterized control rats or rats treated with ABT-627, an ET$_A$R antagonist. Control PMR had a 20% higher BP than premenopausal rats. Treatment with ABT-627 for 3 wk had no

Table 1. Effect of endothelin ET$_A$ receptor antagonist, ABT-627, on body, kidney, and heart weights in young and old female SHR

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Kidney Weight, g</th>
<th>KW/BW ($\times 10^{-3}$)</th>
<th>Heart Weight, g</th>
<th>HW/BW ($\times 10^{-3}$)</th>
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<tbody>
<tr>
<td><strong>Young female rats</strong></td>
<td></td>
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<tr>
<td>Control (n = 6)</td>
<td>201.2 ± 3.6</td>
<td>1.49 ± 0.10</td>
<td>7.52 ± 0.58</td>
<td>0.875 ± 0.031</td>
<td>4.40 ± 0.16</td>
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<tr>
<td>ABT (n = 6)</td>
<td>205.6 ± 3.0</td>
<td>1.51 ± 0.05</td>
<td>7.28 ± 0.23</td>
<td>0.883 ± 0.023</td>
<td>4.24 ± 0.09</td>
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<tr>
<td><strong>Postmenopausal rats</strong></td>
<td></td>
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<tr>
<td>Control (n = 6)</td>
<td>252.6 ± 4.1*</td>
<td>1.77 ± 0.05*</td>
<td>7.66 ± 0.28</td>
<td>1.307 ± 0.158*</td>
<td>5.54 ± 0.28*</td>
</tr>
<tr>
<td>ABT (n = 7)</td>
<td>245.6 ± 2.7*</td>
<td>1.48 ± 0.06†</td>
<td>6.21 ± 0.45†</td>
<td>1.067 ± 0.066†</td>
<td>4.65 ± 0.23†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. ABT, ET$_A$ receptor antagonist ABT-627; KW/BW, kidney weight-to-body weight ratio; HW/BW, heart weight-to-body weight ratio; SHR, spontaneously hypertensive rats. *$P < 0.05$ compared with young females, similar treatment; †$P < 0.05$ compared with controls.
that were not different from those shown in control premenopausal females. In contrast, SHR, comparing with YF rats; ‡

development of hypertension in male SHR, since endothelin had previously shown that endothelin plays very little role, if any, in the renin-angiotensin pathway in SHR. Our present studies do not allow for speculation on which of these hypotheses is correct, and future investigations will need to be performed.

Alternatively, there may be differences in preproendothelin-1 mRNA in cortex or medulla from kidneys of PMR. This is a novel finding; to our knowledge, there have been no other studies that have investigated the effect of endothelin receptor antagonism in female SHR at any age.

Fig. 4. Effects of the chronic ETAR antagonist (ABT-627) on mean arterial blood pressure (MAP) in conscious, chronically catheterized YF and PMR SHR. Rats were treated with 5 mg·kg−1·day−1 ABT-627 for 3 wk, as described in METHODS. Data are expressed as means ± SE. *P < 0.01, compared with YF rats; ‡ P < 0.01, compared with control PMR.

effect on mean arterial pressure in premenopausal SHR; however, BP levels were reduced by ~38 mmHg in PMR, to levels that were not different from those shown in control premenopausal females.

DISCUSSION

The aged female SHR (PMR) model demonstrates increasing BP after cessation of estrous cycling, exhibiting many of the same characteristics as postmenopausal women (11). PMR exhibit reductions in plasma estradiol, increases in plasma renin activity, and oxidative stress (11). Furthermore, just as in postmenopausal women, although BP is usually lower in female SHR than in male rats (17), BP levels in the PMR increase to equal or higher levels than in the old males (11). In the present study, we show for the first time that renal cortical ET-1 peptide is higher in PMR than in premenopausal rats and that selective ETAR antagonism reduces BP in PMR but has no effect in premenopausal rats. These data support our hypothesis that endothelin, mediated by ETAR, plays a role in the increase in BP in PMR. ETAR antagonism also reduced kidney and heart weights in PMR but not in premenopausal females. These data suggest that either endothelin plays a role in renal and cardiac hypertrophy in PMR or that, and most likely, the reduction in BP with ETAR antagonism also reduced the renal and cardiac hypertrophy associated with chronic hypertension in PMR.

In the present study, we also found that expression of preproendothelin-1 mRNA in cortex or medulla from kidneys of PMR was not different from that of premenopausal rats. Because ET-1 peptide expression was higher in PMR than in premenopausal females, these data suggest the possibility that the ET-1 may be coming from a source other than the kidney. Alternatively, there may be differences in preproendothelin mRNA stability or in translational regulation of ET-1 expression between premenopausal SHR and PMR. Our present studies do not allow for speculation on which of these hypotheses is correct, and future investigations will need to be performed.

Endothelin is a potent vasoconstrictor that, when given chronically, causes increases in sodium reabsorption by the kidney and increases BP (26). Schiffert et al. (22) reported previously that endothelin plays very little role, if any, in the development of hypertension in male SHR, since endothelin receptor antagonists had no effect on BP. In the present study, we also found no effect of ETAR antagonism on BP in premenopausal females. This is a novel finding; to our knowledge, there have been no other studies that have investigated the effect of endothelin receptor antagonism in female SHR at any age.

However, there is evidence that female sex hormones may affect endothelin expression. For example, Webb et al. (23) found that 17ß-estradiol decreased endothelin in coronary circulation of postmenopausal women. In addition, Wilcox et al. (25) found that estrogen supplements decreased plasma endothelin levels in postmenopausal women. Using rats, David et al. (7) reported that ovariectomy caused an increase in endothelin mRNA and that treatment with estradiol and estrogen plus progesterone reversed the upregulation of ET-1 in ovariectomized rats with DOCA-salt hypertension. In bovine aortic endothelial cells in culture, Morey et al. (16) found that the angiotensin II-stimulated increase in ET-1 was inhibited by 60–70% with estradiol and progesterone. Thus the reduction in estradiol that our laboratory (11) found previously may explain why ET-1 may be increased in kidneys of PMR.

Factors other than changes in sex hormones may affect ET-1 expression in PMR. For example, as mentioned above in cell culture studies and in animals, angiotensin II stimulates endothelin production (2, 16). Furthermore, blockade of the ETAR protects against angiotensin II hypertension (3), suggesting that angiotensin II hypertension is mediated by endothelin. Because plasma renin activity is significantly increased in PMR (11), it is possible that angiotensin II could be increasing ET-1 in kidneys of PMR. Another possible reason for the increase in ET-1 could be that oxidative stress is stimulating endothelin production in PMR (14). Just as in postmenopausal women (13), PMR also exhibit increased oxidative stress and their BP can be lowered by chronic treatment with antioxidants (11). Alternatively, both endothelin and oxidative stress could be produced by angiotensin II, as has been previously shown (2, 3).

ET-1 is secreted by the endothelium and acts in a paracrine or autocrine fashion on smooth muscle cells of the vasculature by interacting with ETAR to cause contraction (1, 20). ETBR are linked to vasorelaxants such as nitric oxide and prostacyclin (8). In the kidney, ETAR are present in the vasculature and in tubular cells, mainly in the cortex, whereas ETBR are mainly present in the medulla (12). Our data suggest that there are differences in expression of the ETAR and ETBR in kidneys of female SHR with age. Along with increases in ET-1 in the kidney, we also found that mRNA expression of ETAR and ETBR were reduced in cortex of PMR compared with premenopausal rats. ETAR, but not ETBR, mRNA was also reduced in the medulla. The downregulation of the receptors in the cortex of the kidney may reflect a compensatory reduction in expression due to the increase in ET-1 that we found in the cortex. This hypothesis is supported by the studies of Clozel et al. (6) who found that an increase in ET-1 secreted from glomerular mesangial cells or endothelial cells in culture caused a reduction in 125I-labeled ET-1 binding either by downregulation or by changes in binding of endothelin receptors. In their study, however, the authors did not discriminate between ETAR and ETBR. We have not measured endothelin receptor protein levels, and future studies will be necessary to verify whether protein expression of the receptors is also decreased in the kidney.

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To our knowledge, there have been no previous studies in which endothelin receptor expression has been studied in intact and ovariectomized rats. However, there have been studies that have shown that there are sex differences in endothelin receptor expression. For example, in human saphenous veins, Ergul et al. (10) found that ETAR and ETBR expressions were significantly lower in women than in men. As such, the contractile response to endothelin was twofold higher in men than in women. In contrast, DOCA and salt treatment of rats increased expression of endothelin (ET-1) and ETBR and decreased ETAR expression in aortas and mesenteric arteries. For example, in human saphenous veins, Ergul et al. (10) found that ETAR and ETBR expressions were significantly lower in women than in men. In contrast, DOCA and salt treatment of rats increased expression of endothelin (ET-1) and ETBR and decreased ETAR expression in aortas and mesenteric arteries from males but had no effect in females (7). Future studies will be needed to determine whether sex steroids, male or female, modulate endothelin receptor expression.

In summary, in the present study, the role that endothelin may play in the increase in BP in postcyling SHR was evaluated. Expression of preproendothelin-1 mRNA in the cortex and medulla of kidneys of PMR was not different from that shown in premenopausal SHR. In contrast, ET-1 peptide levels were significantly higher in cortex of PMR vs. that shown in premenopausal rats, and ETAR and ETBR expressions were reduced in cortex of PMR. Blockade of the ETAR effectively lowered the BP in PMR but had no effect in premenopausal rats. These data support a role for endothelin and ETAR in mediating postmenopausal hypertension in SHR.

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