Renin-Angiotensin-Aldosterone System

Postmenopausal Hypertension
Role of the Renin-Angiotensin System

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Abstract—After menopause, blood pressure increases in women. However, the underlying mechanisms responsible for postmenopausal hypertension are not completely understood. This study was conducted to determine the role that the renin-angiotensin system (RAS) plays in post-menopausal hypertension. Post-estrous cycling (postmenopausal) spontaneously hypertensive rats or young female controls were treated with losartan, an angiotensin (Ang) II type 1 receptor blocker, for 25 days. Mean arterial pressure was recorded continuously by radiotelemetry. Losartan significantly decreased blood pressure in postmenopausal rats and young female controls but failed to normalize blood pressure in postmenopausal rats to levels found in young controls. Plasma renin activity and plasma angiotensinogen were significantly elevated, and intrarenal Ang II type 1 receptor and renin mRNA expression were significantly downregulated in postmenopausal rats. Therefore, RAS only partially contributes to hypertension in postcycling spontaneously hypertensive rats, whereas hypertension in young females is mediated mainly by the RAS. The data suggest that other mechanisms besides activation of the RAS are likely involved in postmenopausal hypertension. (Hypertension. 2010;56:359-363.)

Key Words: postmenopausal hypertension ■ aging ■ kidney ■ blood pressure

Men are at greater risk for cardiovascular and renal disease than are women of similar age. Men also have higher blood pressure (BP) than women.1,2 However, these sex differences change following menopause, when the risk for cardiovascular disease and prevalence of hypertension increases in women.3

There are few clinical data regarding effectiveness of different antihypertensive therapy in controlling BP in postmenopausal hypertensive women. The Women’s Health Initiative report was based on nearly 100,000 women, ages 50 to 79 years, and was the largest and best-characterized cohort of postmenopausal women in the United States. This report showed that although older hypertensive women (aged 70 to 79 years) were as likely to be on treatment for hypertension (63.2%) as the younger women (64.2%), a substantially smaller percentage of them had their BPs under control (29.3 versus 41.3% for the older versus younger women, respectively).4 Similar findings were observed when the results from the National Health and Nutrition Estimation Survey (NHANES) III dataset (ending 1994) were compared with the NHANES IV dataset (ending 2004).5 Thus, the best therapeutic options for treatment of postmenopausal hypertension are unclear.

A key system for modulating BP and body fluid volume is the renin-angiotensin system (RAS).6 Angiotensin (Ang) II causes vasoconstriction though activation of Ang II type 1 receptor (AT1R). Increases in Ang II and dysregulation (upregulation or activation) of the vasoconstrictor arm of the RAS have been implicated as adverse factors in cardiovascular pathophysiology.

Postmenopausal women exhibit an increase in plasma renin activity (PRA),7,8 but the use of RAS blockers, Ang-converting enzyme inhibitors, or Ang receptor blockers (ARBs), are not frequently used to treat postmenopausal hypertension.9 Perhaps one reason for this is that one study showed that monotherapy with an ARB only controlled BP to normal levels in 30% of the patients treated.9

We have previously characterized the aging female spontaneously hypertensive rat (SHR) (postmenopausal rat; PMR) as a suitable model to study postmenopausal hypertension.10 In this experimental animal model, the estrous cycle stops at 12 and 16 months of age; BP is significantly higher than in young cycling female SHR. We showed previously that a portion of the hypertension in PMR is mediated by activation of the endothelin system, because the endothelin type A receptor blocker ABT627 decreases BP.11 However, the use of ABT627 only reduced BP = 20 mm Hg, and BP remained significantly higher in the PMR than in young female rats.

Young male SHR have higher BP than aged-matched females. We found previously that in young SHRs, the sex difference in BP is abolished by Ang I–converting enzyme (ACE) inhibition.12 Furthermore, we showed recently that AT1R blockade with losartan in 16-month-old male SHR

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359
normalized BP, suggesting that the RAS via the AT$_1$R is the dominant mechanisms that mediates hypertension in the aging male SHR. However, the role that the AT$_1$R plays in mediating postmenopausal hypertension is unclear.

Thus, the present study was performed to analyze the role of the RAS in hypertension in PMR and young female SHRs. We tested the hypothesis that as in male SHRs, activation of the RAS is the sole mediator for hypertension in YFs, whereas in PMR, the RAS is only one of the mechanisms responsible for the hypertension. If this hypothesis were true, it would explain in part why BP is difficult to control in postmenopausal women. To address this hypothesis, components of the systemic and intrarenal RAS were measured in PMR and young females, and the rats were given losartan, an ARB, for 25 days, and BP was measured in conscious freely moving rats by radiotelemetry.

### Methods

#### Rats

Female SHRs, 8 months of age, were obtained from Taconic Farms and were aged in the Laboratory Animal Facility of the University of Mississippi Medical Center, until they were 16 months of age (PMR). Young females, aged 4 months, were obtained from the same vendor. All rats were maintained on standard rat chow (Teklad, Harlan SD) and tap water in an environment with 12-hour/12-hour light/dark cycle. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center, and studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health.

#### Depressor Response to Losartan

To determine whether the RAS plays a role in postmenopausal hypertension, the ARB, losartan, was used. Young females and PMR (n=7 per group) were given losartan (40 mg/kg per day; a generous gift from Merck Laboratories) in the drinking water for 25 days. We showed previously that at this dose, the hypertensinogenic effect of Ang II is completely blocked in conscious old female SHR.

Controls received tap water, and water consumption was monitored without losartan in young females and PMR. The reduction in MAP with losartan was similar in PMR and young females (YF), whereas in PMR, the RAS is only one of the mechanisms responsible for the hypertension. If this hypothesis were true, it would explain in part why BP is difficult to control in postmenopausal women. To address this hypothesis, components of the systemic and intrarenal RAS were measured in PMR and young females, and the rats were given losartan, an ARB, for 25 days, and BP was measured in conscious freely moving rats by radiotelemetry.

#### Measurement of Mean Arterial Pressure in Conscious Rats by Radiotelemetry

Radiotelemetry BP was monitored, as we have previously reported, throughout baseline and losartan treatment. At the end of the experimental period, blood was withdrawn from the abdominal aorta to measure PRA and angiotensinogen, as we previously described.

#### Study of Components of the Intrarenal RAS

PMR, aged 16 months, and young females SHR, aged 4 months (n=8 each group), were anesthetized with isoflurane anesthesia, and their kidneys were perfused with 2% heparin in saline, removed and separated into cortex and medulla, and snap frozen in liquid nitrogen.

#### Measurement of Intrarenal Angiotensinogen, Renin, Ang-Converting Enzyme, AT$_1$R, and AT$_2$R mRNA Expression With Real-Time RT-PCR

mRNA was isolated from cortex and medulla of kidneys and real-time RT-PCR was performed, as we previously reported. Elongation factor I primers were used as controls. Results are expressed as arbitrary units and standardized against elongation factor I mRNA expression.

### Results

#### Antihypertensive Response to ARB in PMR and Young Female SHR

As shown in Figure 1, mean arterial pressure (MAP) was measured in conscious rats instrumented with radiotelemetry probes and was recorded 24 hours per day. MAP was measured during 5 days of baseline, and then losartan was given in their drinking water for 25 days. MAP was significantly reduced to normotensive levels in young females treated with losartan compared with baseline period but not in treated PMR. The reduction in MAP with losartan was similar in young females and PMR (PMR, 31±3 versus 33±5 mm Hg; P<0.05). In addition, at the end of the experimental protocol, losartan treatment significantly decreased MAP after 1 day of losartan administration in PMR. After 1 week, MAP was significantly reduced to normotensive levels in young females treated with losartan compared with baseline period but not in treated PMR. The reduction in MAP with losartan was similar in young females and PMR (PMR, 31±3 versus 33±5 mm Hg; P<0.05). In addition, at the end of the experimental protocol, MAP was still significantly elevated above normotensive levels in PMR but was normalized in young female SHR (7-day average, 138±1 versus 102±2 mm Hg; P<0.05).

#### Characterization of Systemic RAS Components

As shown in Figure 2A, PRA was significantly higher in PMR compared with their young female counterparts, and as expected, losartan treatment significantly increased PRA in both PMR and young females. However, PRA was increased more with losartan in young females than PMR. Plasma angiotensinogen levels were approximately 3-fold higher in PMR than in young females (Figure 2B).
Characterization of Intrarenal RAS Components

Expression of mRNA for components of the RAS in kidneys harvested from old female SHR, aged 16 months, and young females, age 4 months, were measured by real-time RT-PCR. There was no significant difference in the intrarenal levels of angiotensinogen or ACE mRNA in cortex or medulla between PMR and young females (Figure 3A and 3C). Intrarenal renin mRNA was significantly reduced in both cortex and medulla of PMR compared with young females (Figure 3B). In addition, intrarenal mRNA expression of AT1R (Figure 3D) was significantly downregulated in PMR compared with young female SHR. Intrarenal mRNA expression of AT2R was not different between the groups (data not shown).

Discussion

The main findings of this article are: (1) the RAS through AT1R is one mechanism involved in the pathophysiology of postmenopausal hypertension in PMR; (2) activation of the RAS through AT1R is likely the most important regulator of BP in young females; (3) PRA and plasma angiotensinogen levels are significantly higher in PMR; (4) intrarenal mRNA expression of renin and AT1R are significantly downregulated in PMR; and (5) ARB treatment with losartan failed to normalize MAP in PMR, suggesting that other factors may contribute to the higher MAP observed in PMR compared with young female SHR.

The Women’s Health Initiative report stated that although 64% of the hypertensive women in the study were treated with antihypertensive medication, BP was only controlled in about a third of them.4 Similarly, the NHANES III (1988 to 1991) data indicate that although 81% of hypertensive women, aged 18 to 74 years, were aware of their condition, and 65% were undergoing treatment, only 38% had their hypertension under control.1 These data are surprising, because women typically see their physicians more frequently than age-matched men and they are often more compliant with medications than are men. These data strongly indicate, then, that the mechanism(s) responsible for postmenopausal hypertension is (are) poorly understood.

Our present study supports the notion that postmenopausal hypertension is mediated in part by activation of the RAS, because the ARB, losartan, significantly decreased MAP in PMR. The data also suggest that the RAS is not more activated with age in PMR than in young females, since losartan reduced the BP by the same amount in both groups. Unlike in young females, MAP was not normalized in PMR with losartan. In young SHRs, MAP is higher in males than in females, and inhibition of the RAS with enalapril, an ACE inhibitor, decreased MAP in young male rats but not in females. Yet, losartan normalized MAP in young female SHR.5 These data further support the notion that the mechanism(s) responsible for postmenopausal hypertension is (are) different from those in young females.

Figure 2. A, PRA in losartan (Los)-treated and untreated PMR and young females (YF). PMR has significantly higher levels of PRA than YF SHR. Losartan increased PRA to similar levels in both YF and PMR. Data are expressed as mean±SEM. *P<0.05 compared with young females SHR; #P<0.05, compared with untreated rats. B, Plasma angiotensinogen level in PMR and YF. PMR had higher levels of angiotensinogen than YF. Data are expressed as mean±SEM. *P<0.05 compared with young females.

Figure 3. mRNA levels of kidney RAS components from PMR and young females (YF) by real-time RT-PCR. A, Angiotensinogen mRNA level was the same in cortex and medulla in PMR and YF. B, Renin mRNA expression was significantly downregulated in PMR compared with young rats. C, ACE mRNA level was the same in cortex and medulla in PMR and YF. D, AT1R mRNA expression was significantly downregulated in PMR compared with young rats. Data are expressed as mean±SEM. *P<0.05 compared with YF.
inhibitor, eliminates this sex difference. In other studies, we found that treatment of old male SHR and PMR with an ARB, normalized BP in old male SHR but not old females. Based on these data, we concluded that hypertension in both young and old male SHR is predominantly mediated by the RAS. In contrast, taking these previous data with our current findings, our data suggest that another mechanism(s) besides the RAS is (are) involved in postmenopausal hypertension in PMR.

The RAS is the most important regulator of BP and fluid homeostasis. PRA is higher in postmenopausal women than in premenopausal ones. Recently, it was shown that monotherapy with candesartan, an ARB, only controlled BP in 36% of women after 1 month of treatment. These data are also consistent with our present findings. PRA was also higher in our PMR model, and why this is the case for both women and PMR is not clear. We hypothesize that an increase in androgens could increase angiotensinogen, the substrate for renin and thus increase PRA. We could also speculate that sympathetic activity may be increased with aging in PMR and women, which could increase renin levels. Sympathetic activity has been shown to play a role in mediating the hypertension in SHR. Because our data also show that AT,R mRNA expression is downregulated in PMR compared with young females, it is possible that intrarenal Ang II levels may be increased, because downregulation of the AT,R is known to occur when Ang II levels are increased and Ang II has been shown to destabilize AT,R mRNA. However, the fact that ACE expression was not different between PMR and young female controls does not support an increase in Ang II, although the levels of ACE are not rate limiting in the synthesis of Ang II. Future studies will be necessary to determine whether Ang II levels are indeed increased in kidneys of PMR.

Many investigators have found either by direct measurement of expression or as suggested by binding studies and response to antagonists that AT,R expression increases with aging in humans and most rat models. Most studies that were performed in male animals or the human studies did not segregate data in women from data in men. However, Hinojosa-Laborde et al found increased AT,R binding (Bmax) in aging female Dahl salt sensitive (DS) rats. This is in contrast to our present findings that AT,R mRNA expression was downregulated in PMR. The reasons for the inconsistencies between these studies are not readily apparent. BP in DS females is responsive to the presence of estrogens, i.e., ovarioctomized rats exhibit increased BP. Estrogens have been shown to downregulate AT,R expression. Therefore, with aging and reduction in estrogens, AT,R expression may increase in DS rats. Estrogens do not play a role in mediating hypertension in female SHR, because ovariectomy has no effect on their BP, thus they may not exhibit increases in AT,R expression with age that usually accompanies estrogen depletion. One caveat for our present study is that AT,R mRNA expression may not adequately represent the protein expression or receptor binding capacities.

What other mechanisms may contribute then to hypertension in postmenopausal women and rats? Previously, we showed that endothelin receptor (ET,T) blockers decrease BP in PMR but have no effect on BP in young females. Despite the effect of endothelin receptor antagonists on MAP in PMR, the BP in PMR was still ~170 mm Hg. These data support a role for endothelin in mediating a portion of the postmenopausal hypertension in PMR. Taken with the observation that Ang II can stimulate synthesis of preproendothelin, our data suggest that both the RAS and endothelin may be involved in postmenopausal hypertension in our PMR and may play a role in postmenopausal hypertension in women as well. As mentioned previously, androgens have been shown to increase angiotensinogen in male rats and PMR exhibits a 4-fold increase in plasma testosterone compared with young females. Thus, it is possible that the increase in testosterone may contribute to the increased angiotensinogen that we found in PMR compared with young females. It is also possible that reductions in the vasodilator arm of the RAS, the Ang 1-7 pathway, could play a role in the hypertension in PMR, although we did not find differences in intrarenal mRNA expression of AT,R. Future studies will be necessary to thoroughly investigate this alternative.

In summary, we found that the ARB, losartan, reduces MAP in both PMR and young females, but normalized BP only in young females and not in PMR. We also found that AT,R and intrarenal renin mRNA expression is reduced in PMR despite higher PRA. Plasma angiotensinogen levels were also higher in PMR. Taken together, these data suggest that increased angiotensinogen may lead to increased Ang II despite lower intrarenal renin expression and thus plays a role in hypertension in PMR. However, because losartan did not normalize MAP in PMR as it did in young females, it is likely that other factors also contribute to hypertension in PMR and perhaps in postmenopausal women as well.

Perspectives

Postmenopausal hypertension is a complex disease with multiple contributing mechanisms involved. The Joint National Committee VI and Joint National Committee VII Guidelines recommend the use of diuretics and β-blockers in uncomplicated hypertensive patients. Postmenopausal hypertension is often associated with more cardiovascular risk factors than found in essential hypertensive patients. At the present, many postmenopausal hypertensive women are treated with calcium channel blockers, diuretics, or a combination of the two. Treatment with calcium channel blockers is associated with increased morbidity and mortality in some cohorts and also may increase the transmission of the systemic pressure to the glomeruli, exacerbating renal injury, and thus further complicate hypertension. In addition, diuretics can worsen metabolic alterations often found in postmenopausal hypertension. Unfortunately, ACE inhibitors and ARBs are rarely used to treated postmenopausal hypertension. Our previous data with endothelin receptor antagonists and our current data with ARBs reinforce the notion that postmenopausal hypertension is a complex disease that needs to be treated with blockers specific for the systems that mediate the hypertension rather than nonspecific treatment with calcium channel blockers and diuretics that may cause more harm than good. Our data also reinforce the fact that additional studies are necessary in postmenopausal women.
to determine the exact mechanisms by which their BP is elevated.

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**Disclosures**

None.

**References**

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