

Review Article

Gender Differences in the Regulation of Blood Pressure

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Abstract—Men are at greater risk for cardiovascular and renal disease than are age-matched, premenopausal women. Recent studies using the technique of 24-hour ambulatory blood pressure monitoring have shown that blood pressure is higher in men than in women at similar ages. After menopause, however, blood pressure increases in women to levels even higher than in men. Hormone replacement therapy in most cases does not significantly reduce blood pressure in postmenopausal women, suggesting that the loss of estrogens may not be the only component involved in the higher blood pressure in women after menopause. In contrast, androgens may decrease only slightly, if at all, in postmenopausal women. In this review the possible mechanisms by which androgens may increase blood pressure are discussed. Findings in animal studies show that there is a blunting of the pressure-natriuresis relationship in male spontaneously hypertensive rats and in ovariectomized female spontaneously hypertensive rats treated chronically with testosterone. The key factor in controlling the pressure-natriuresis relationship is the renin-angiotensin system (RAS). The possibility that androgens increase blood pressure via the RAS is explored, and the possibility that the RAS also promotes oxidative stress leading to production of vasoconstrictor substances and reduction in nitric oxide availability is proposed. (*Hypertension*. 2001;37:1199-1208.)

Key Words: sex characteristics ■ hypertension ■ angiotensin II ■ nitric oxide ■ oxidative stress

In this review gender differences in blood pressure control are explored, including possible mechanisms by which androgens may increase blood pressure.

Gender Differences in Blood Pressure Regulation in Humans

Men are generally at greater risk for cardiovascular and renal disease than are age-matched, premenopausal women. Recent studies using the technique of 24-hour ambulatory blood pressure monitoring have shown that blood pressure is higher in men than in women at similar ages. As shown in Figure 1, Wiinber and colleagues¹ studied 352 normotensive (for age) Danish men and women, aged 20 to 79 years, and found that blood pressure increased with aging in both men and women, but that men had higher 24-hour mean blood pressure, by approximately 6 to 10 mm Hg, than did women, until the age of 70 to 79 years, when blood pressure was similar for men and women. Khoury and colleagues² performed ambulatory blood pressure monitoring on 131 men and women, aged 50 to 60 years, and found that men had higher blood pressure than did women. Findings were similar in a meta-analysis study performed by Staessen et al.³ In addition, the Third National Health and Nutrition Evaluation Survey (NHANES III) showed that, in general, men had higher blood pressure than women through middle age.⁴ Furthermore, the incidence of uncontrolled hypertension is also greater in men than in women.⁵

After menopause, however, blood pressure increases in women as well. The data from NHANES III, shown in Figure 2, confirmed that by 60 to 69 years of age, non-Hispanic black and Hispanic women developed higher blood pressure than men of similar ethnic background.⁴ However, the mechanisms responsible for the hypertension in these populations are complicated by comorbid conditions of obesity and type II diabetes, both of which lead to increases in blood pressure.⁴ In the non-Hispanic white population, in which the incidence of obesity and type II diabetes with aging is not as high, blood pressure also increased after the average age of menopause (51.4 years). Therefore, by 60 to 69 years of age, non-Hispanic white women had blood pressure similar to that of men, and by 70 to 79 years of age, this population of women had higher blood pressure than did men.⁴

Gender Differences in Blood Pressure Regulation in Animals

The gender-associated differences in blood pressure observed in humans have also been documented in various animal models. In hypertensive rat models, many investigators have found that males have higher blood pressure than do females. For example, as shown in Figure 3, male spontaneously hypertensive rats (SHR) have higher blood pressure than do females of similar ages.^{6–9} Similar gender differences in development of hypertension are also found in Dahl salt-sensitive (DS) rats,^{10,11} deoxycorticosterone-salt hypertensive

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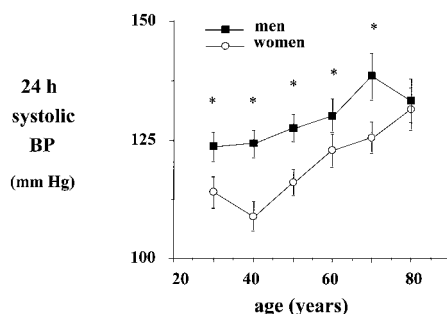


Figure 1. Effect of aging and gender on blood pressure (BP) measured by 24-hour ambulatory technique in a Danish cohort. Data are presented as mean \pm SEM. * $P < 0.05$ compared with women of similar age. Data presented with permission from *Am J Hypertens*.¹

rats,¹² and the New Zealand genetically hypertensive rat.¹³ Therefore, as found in humans and hypertensive rat models, males have higher blood pressure than age-matched females.

To date there have been no studies in which a consistent gender difference in blood pressure in normotensive animals has been documented. Contrary to data in humans, Calhoun and colleagues¹⁴ reported that 24-hour blood pressure measured at 12 weeks of age in male Wistar-Kyoto rats (WKY) was lower than in female WKY by approximately 9 mm Hg (males, 96 ± 3 ; females, 105 ± 1 mm Hg). However, by 14 weeks of age there was no difference in blood pressure over 24 hours between the genders (males, 101 ± 3 ; females, 106 ± 1 mm Hg).¹⁴ It is possible that averaging blood pressure over 24 hours would diminish gender differences that would be exposed when blood pressure is evaluated during the day or night individually. In any case, from the small differences in blood pressure found in normotensive human subjects, it is clear that blood pressure measurement in conscious rats during acute studies is not sufficient to be able to detect the small differences one would expect to find between normotensive male and female rats. Thus, it will be necessary to measure telemetric blood pressure in normotensive rats over a prolonged (months) period of time to determine whether there are in fact gender differences with increasing age in normotensive rats, as found in normotensive humans.

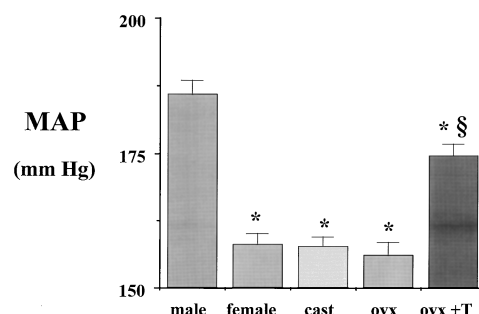


Figure 3. Mean arterial blood pressure (MAP) measured in anesthetized SHR, aged 17 to 19 weeks, during the established phase of hypertension. Some males and females were castrated (cast) or ovariectomized (ovx) at 7 weeks of age. Some ovariectomized females were given testosterone (T) by implantation of silicone elastomer pellets for the last 5 to 6 weeks before blood pressure was measured. * $P < 0.01$ compared with males and/or testosterone-treated ovariectomized females; § $P < 0.01$ compared with females, castrated males, or ovariectomized females. Data presented with permission from *Hypertension*.²⁷

Mechanisms for Gender Differences in Blood Pressure Control: Role of Testosterone

Although the mechanisms responsible for the gender differences in blood pressure control are not clear, there is significant evidence that androgens, such as testosterone, play an important role in gender-associated differences in blood pressure regulation. For example, studies using ambulatory blood pressure monitoring techniques in children have shown that with increasing age, blood pressure increases in both boys and girls. However, after the onset of puberty, boys have higher blood pressure than do age-matched girls.^{15,16} At ages 13 to 15 years, systolic blood pressure was approximately 4 mm Hg higher in boys than girls, and at ages 16 to 18 years, boys had higher systolic blood pressures than girls by 10 to 14 mm Hg.¹⁶ The blood pressure in postpubescent boys also does not dip as low at night as in girls.^{15,16} A reduction in nocturnal dipping is recognized as a hallmark of early dysfunction in blood pressure regulation.^{15,16} These data clearly show that in adolescence and puberty, when androgen levels are increasing, blood pressure is higher in boys than in girls.

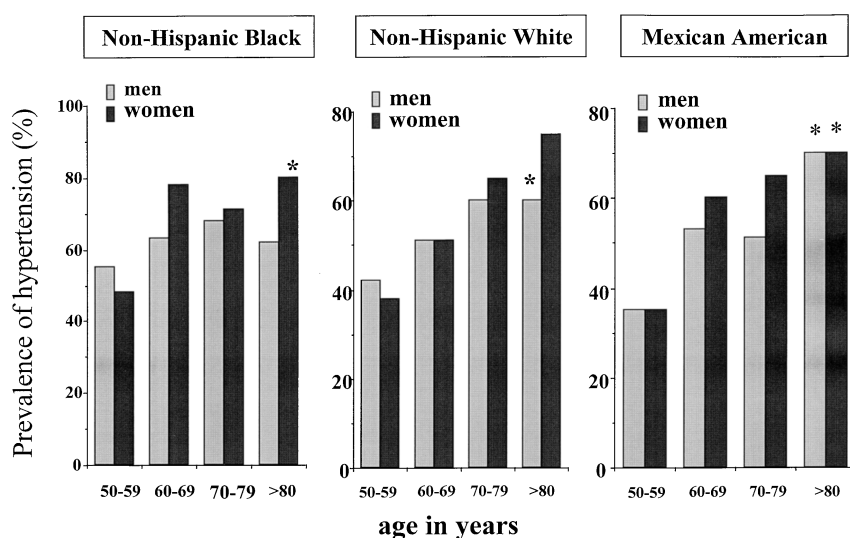


Figure 2. Effect of aging and gender on prevalence of hypertension (in percentage) in non-Hispanic blacks, non-Hispanic whites, and Mexican Americans compiled from the NHANES III cohort. With advancing age (postmenopause), the prevalence of hypertension in women increases to levels higher than in age-matched men. *Too few individuals available for statistical evaluation. Data presented with permission from *Hypertension*.⁴

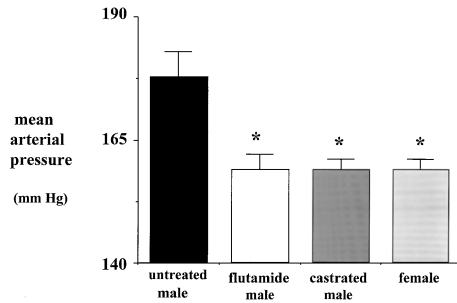


Figure 4. Androgen receptor antagonism in intact male SHR reduced mean arterial blood pressure at 14 to 16 weeks of age to the same level as found in castrated males and intact female SHR of similar ages. Male SHR were given daily injections of vehicle (control) or flutamide (8 mg/kg per day) for 5 to 6 weeks. For purposes of illustration, data from untreated intact female and castrated SHR of similar ages are also presented. * $P < 0.01$ compared with untreated males. Data presented with permission from *Hypertension*.²¹

Another line of evidence that testosterone may play an important role in higher blood pressure in males is castration studies in male rats. Castration at a young age (3 to 5 weeks) attenuates the development of hypertension in SHR (Figure 3), in DS male rats, in male rats subjected to 2-kidney, 1 clip (Goldblatt) maneuver,^{6,7,9–11,17,18} and in male rats subjected to reduced renal mass.^{19,20} Furthermore, as shown in Figure 4, we have found that chronic blockade of the androgen receptor with the antagonist flutamide attenuates blood pressure in male SHR to the level found in female SHR.²¹ Both testosterone and dihydrotestosterone (DHT) bind to the androgen receptor, and DHT rather than testosterone is the androgen involved in such conditions as male pattern baldness and benign prostatic hypertrophy.^{22,23} When treated with finasteride, the inhibitor of the conversion of testosterone to DHT, baldness of this type is attenuated, and the prostatic hypertrophy is reversed.^{22,23} However, conversion to DHT was not found to be important in promoting hypertension in male SHR because chronic treatment with finasteride did not have an effect on the hypertension.²¹

On the other hand, increases in androgens in humans and animals increase blood pressure. Women with polycystic ovary syndrome or adrenal virilizing tumors, which are characterized by elevated testosterone levels, experience hypertension.^{24–26} In animal studies testosterone treatment increases blood pressure in ovariectomized female and castrated male SHR (Figure 3).^{9,27} Furthermore, chronic testosterone treatment of normotensive, uninephrectomized female rats increases arterial blood pressure that was found not to be reversible, depending on the length of time the testosterone was given.^{28,29} Thus, increases in androgens in humans and in normotensive and hypertensive rats lead to higher blood pressure.

Mechanisms for Gender Differences in Blood Pressure Control: Role of Estrogens

Because men and male rats have higher blood pressures than do females, it is possible that female hormones may play a role in protecting females from developing higher blood pressures. In women menopause is characterized by increases

in blood pressure, as determined by the NHANES III study and others (Figure 2).^{4,30,31} Interestingly, the blood pressure does not increase during the transitional phase from perimenopause to menopause,³² but rather the increase in blood pressure after menopause takes an average of 5 to 20 years to develop,⁴ suggesting that lack of female hormones may not be the only contributing factor for the elevated blood pressure.

Hormone Replacement Therapy in Postmenopausal Women

The possibility that lack of female hormones may not be the only factor contributing to the increase in blood pressure after menopause is supported by the numerous studies in postmenopausal women given hormone replacement therapy (HRT), in whom blood pressure was measured by ambulatory blood pressure monitoring techniques. In these studies, blood pressure was not affected by HRT,^{33–35} was only minimally affected by HRT,^{36–38} or the reduction in blood pressure with HRT was evident only at night³⁸ or only in normotensive individuals.³⁹ Furthermore, the route of delivery was important in whether HRT was effective in lowering blood pressure, with transdermal HRT being more effective than oral preparations.^{34,38} Importantly, the Heart and Estrogen/Progestin Replacement Study (HERS) also found that there was no overall beneficial effect on secondary prevention of coronary heart disease in postmenopausal women during the 4.1 years of study.⁴⁰

Estrogen has been shown to stimulate nitric oxide (NO) production.^{41,42} Thus, loss of estrogen with menopause could play a role in the increased blood pressure in women after menopause. However, since estrogen replacement therapy has not been shown to decrease blood pressure, it is doubtful that the effect of estrogen on NO is the protective mechanism by which blood pressure is lower in premenopausal women. We have shown in previous studies that aging in rats is associated with a reduction in NO substrate (L-arginine) and excretion of NO metabolites.⁴³ Thus, it is also possible that the effect of advanced age on other components of the NO overwhelms the effect of estrogen on NO production in postmenopausal women.

Androgens in Postmenopausal Women

With regard to androgen levels after menopause, there is some controversy since studies have shown that serum testosterone levels in postmenopausal women may decrease slightly, may not change at all, or may actually increase.^{44–47} A recent report from the Rancho Bernardo Study, a community-living, population-based study in Rancho Bernardo, Calif, emphasized that in 685 women, aged 50 to 89 years, the status of whether or not the women had undergone hysterectomy with or without oophorectomy affected serum testosterone levels.⁴⁸ These investigators found that in intact women serum testosterone levels decreased immediately after menopause but increased with aging to premenopausal levels by 70 to 79 years of age.⁴⁸ In women who had undergone hysterectomy with bilateral oophorectomy, both total and bioavailable testosterone levels were reduced by 40%. Postmenopausal women who had undergone hysterectomy without oophorectomy had intermediate serum testosterone lev-

els.⁴⁸ These data show that the ovary is a very important source of androgens after menopause in women. Since increased androgen levels have been shown to increase BP in women with polycystic ovary syndrome and in animal models, it is possible that with the loss of estrogens at menopause, the unopposed effect of androgens in postmenopausal women may contribute to their elevated BP. This hypothesis remains to be tested in both women and female animal models.

Role of Female Hormones in Blood Pressure Control in Animal Models

Studies in female SHR have supported the notion that female hormones do not cause protection against the higher blood pressure found in male SHR. As shown in Figure 3, ovariectomy of female SHR at 4 to 5 weeks of age does not result in higher blood pressures than in intact females at 18 to 20 weeks of age.^{27,49} However, androgen treatment of ovariectomized female SHR causes an increase in blood pressure that is dose dependent.^{27,49} These data suggest that it is not female hormones but rather lack of testosterone that may protect female SHR from the higher blood pressure found in males.

There are differences in rat models of hypertension with regard to the role that ovariectomy plays in the control of blood pressure in female rats. Hinojosa-Laborde and colleagues⁵⁰ found that ovariectomy of DS rats resulted in higher blood pressure than in either males or females. When rats were maintained on a high salt diet, blood pressure increased in all rats, but to a greater extent in males and ovariectomized females than in intact females. Surprisingly, reversal of the diet to low salt in these animals reversed the hypertension in intact male and female DS rats but not in ovariectomized DS rats.⁵⁰ Similar effects of ovariectomy in causing an increase in blood pressure compared with intact females have also been found in females in the model of deoxycorticosterone-salt hypertension.⁵¹ It is not clear why loss of female sex hormones results in elevation of blood pressure in these models.

Abnormal Pressure-Natriuresis in Hypertension: Role Played by Testosterone

Substantial evidence supports the theory that some form of renal dysfunction plays a role in the development and maintenance of hypertension.^{52,53} Providing the strongest support for this theory are observations that transplantation of prehypertensive kidneys from SHR to WKY produces hypertension.⁵³ Similar results have been obtained in renal transplantation studies between DS and Dahl salt-resistant rats.⁵³ Of particular relevance to human hypertension is the study by Curtis et al,⁵⁴ which demonstrated that blood pressure returns to normal in hypertensive patients who receive kidneys from normotensive donors. The results indicate that a defect within the kidney may play a crucial role in the pathogenesis of hypertension. A common defect that has been characterized in several forms of hypertension is a shift in the pressure-natriuresis relationship.⁵³ The pressure-natriuresis relationship refers to the fact that increased arterial pressure elicits a marked increase in sodium excretion.^{55,56} According to the renal body fluid feedback concept, a long-term increase in arterial pressure or hypertension occurs as a result of reduc-

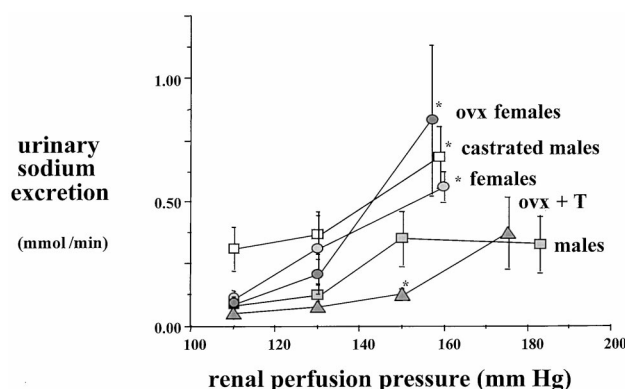


Figure 5. Acute pressure-natriuresis relationship comparison in male, female, castrated male, untreated ovariectomized (ovx) female, and ovariectomized female SHR treated chronically for 5 to 6 weeks with testosterone (ovx + T). Acute pressure-natriuresis studies were conducted in anesthetized SHR, aged 17 to 19 weeks. Renal perfusion pressure was reduced by tightening a snare around the aorta above the renal arteries in a stepwise fashion. * $P < 0.05$ compared with males. Data presented with permission from *Hypertension*.²⁷

tion in renal excretory function or a rightward shift in the pressure-natriuresis relationship.^{55,56}

As shown in Figure 5, we have recently reported that the pressure-natriuresis relationship is blunted in male SHR compared with females.²⁷ Castration of the male SHR restored the pressure-natriuresis relationship, whereas ovariectomy of female SHR had no effect.²⁷ Testosterone treatment of ovariectomized female SHR resulted in an increase in blood pressure and a concomitant blunting of the pressure-natriuresis relationship.²⁷ Preliminary data have shown that the androgen receptor is located predominantly in proximal tubule segments of the nephron.⁵⁷ These data provide initial support for the notion that androgens may have a direct effect on sodium reabsorption in the proximal nephron.

As mentioned above, many studies have demonstrated that "hypertension follows the kidney"; accordingly, when the kidney of SHR is transplanted into a normotensive rat, the blood pressure in the previously normotensive rat increases.⁵³ However, Harrap and colleagues⁵⁸ reported that when the kidney from male SHR was transplanted into female SHR, this maneuver did not result in a significant rise in blood pressure such that female SHR with male kidneys had blood pressure similar to that in female SHR with female kidneys. However, when the kidney from female SHR was transplanted into male SHR, blood pressure was not attenuated in the male with female kidneys compared with blood pressure in a male SHR with male kidneys.⁵⁸ These data indicate that the 25 to 30 mm Hg higher blood pressure in the male SHR compared with the female is not due to an intrinsic defect of the male kidney but rather is due to some external factor in the male that further increases blood pressure, perhaps because of a reduction in pressure-natriuresis. We hypothesize that androgens are the factor in males by which the pressure-natriuresis relationship is blunted and higher blood pressure results.

Testosterone-Induced Reduction in Pressure-Natriuresis: Role of the Renin-Angiotensin System

The key system for controlling blood pressure and body fluid volume (ie, pressure-natriuresis) is the renin-angiotensin system (RAS).^{55,56} For example, under normal conditions, any perturbation that increases arterial pressure will also provoke an increase in sodium and water excretion via pressure-natriuresis. This will lead to a decrease in extracellular fluid volume, venous return, and cardiac output, and blood pressure will return to normal. Long-term pressure-natriuresis is modulated by the RAS. Angiotensin II (Ang II) increases proximal sodium reabsorption by the kidney by stimulating epithelial transport.⁵⁶ In the event of abnormal Ang II levels for the level of volume in the body, the blood pressure will increase with abnormal sodium and water reabsorption, leading to blunting of the pressure-natriuresis relationship. Similarly, if total body fluid volume levels are "perceived" incorrectly, and thus Ang II levels do not respond appropriately, increases in blood pressure will also occur.⁵⁹

Gender differences in components of the RAS have been shown to exist that may play a role in the control of blood pressure. James and colleagues⁶⁰ measured plasma renin activity (PRA) in men and women over a 9-year period and documented that in this normotensive population, PRA was 27% higher in men than in women regardless of age and ethnic heritage. Kaplan and associates⁶¹ reported similar findings. Other studies in older individuals have shown that PRA is higher in postmenopausal women than in premenopausal women but that PRA is still higher in men than in women of similar age.⁶² Thus, renin activity is greater in men than in women. The cause of this gender difference is unclear. However, these data lend credence to the hypothesis that the RAS may play a role in mediating the gender difference in blood pressure regulation.

In animal studies, male SHR have higher PRA than do females,⁶³ testosterone treatment of ovariectomized female rats causes increases in PRA,^{64,65} and PRA decreases with castration in male rats.^{64,65} Furthermore, as presented in Figure 6, we have found that there is a linear correlation ($r=0.904$) between the level of serum testosterone and PRA in Sprague-Dawley rats treated chronically (2 weeks) with increasing doses of testosterone. Blood pressure also increases with chronic testosterone in normotensive rats. Therefore, these data suggest that testosterone stimulates the RAS. The mechanism by which androgens increase PRA is not clear, but data from 2 groups have independently shown in SHR and normotensive WKY that castration decreases renal angiotensinogen mRNA and chronic testosterone increases renal angiotensinogen mRNA.^{64,66} Chronically increased renal angiotensinogen could increase renal tissue Ang II if renin enzyme is not working at maximal velocity, which has been reported in both humans and rats.⁶⁷ In support of this hypothesis, studies in mice have demonstrated that an increase in angiotensinogen gene copy numbers causes increases in blood pressure.⁶⁸ Alternatively, if testosterone plays a role in directly increasing proximal sodium reabsorption, as hypothesized above, the reduction in tubular sodium

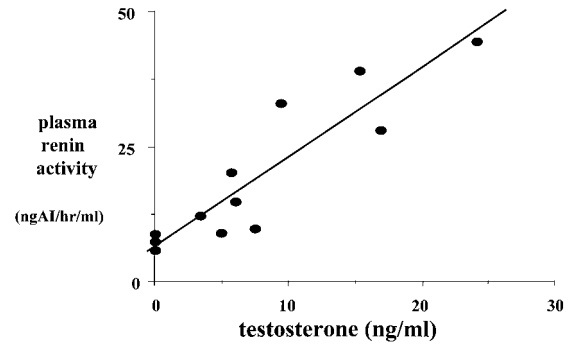


Figure 6. In normotensive rats, increasing serum testosterone causes an increase in PRA. Castrated male Sprague-Dawley rats ($n=9$) were implanted with testosterone pellets of increasing concentration (Innovative Research). After 2 weeks, rats were anesthetized, and plasma was taken for measurement of renin activity and testosterone by radioimmunoassays, as previously described.²⁷ Each point represents the data from an individual rat. Rats with 0 testosterone ($n=3$) were placebo-implanted castrated males. Statistical analyses were performed with Origin software (Microcal). $R=0.904$. AI indicates angiotensin I.

would be perceived by the macula densa and would therefore result in renin release, causing an increase in PRA. Future studies will be necessary to determine the exact mechanism by which androgens increase PRA.

To test the hypothesis that the RAS plays a role in mediating the gender difference in blood pressure in SHR, we found that chronic blockade with the Ang II-converting enzyme inhibitor enalapril resulted in normalization of the blood pressure regardless of gender,⁴⁹ thus removing the gender-induced difference in blood pressure in SHR (Figure 7). In male SHR and ovariectomized female SHR treated with testosterone, in which blood pressure was elevated by 30 mm Hg, blood pressure was reduced by 65% with enalapril, whereas in female, castrated male, and untreated ovariectomized female SHR, blood pressure was only reduced by 40%.⁴⁹ These

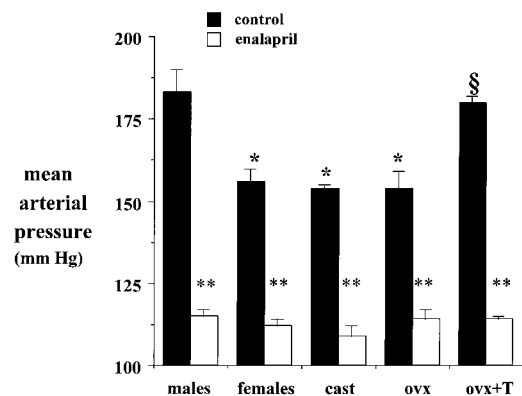


Figure 7. Chronic treatment of SHR with angiotensin-converting enzyme inhibitor removes the gender difference in blood pressure but decreases blood pressure more in male and ovariectomized female SHR treated chronically (6 weeks) with testosterone (ovx+T) than in rats in the other 3 groups. SHR were treated for 6 to 8 weeks with the Ang II-converting enzyme inhibitor enalapril (250 mg/L) in the drinking water. * $P<0.05$ compared with control males; ** $P<0.05$ compared with control rats of same sex; § $P<0.05$ compared with control females, castrated males (cast), and ovariectomized females (ovx). Data presented with permission from *Hypertension*.⁴⁹

data suggest that the RAS plays an important role in mediating the hypertension in SHR regardless of gender, but, more importantly, that the androgen-promoted exacerbation of the blood pressure in male and testosterone-treated ovariectomized female SHR is also mediated by the RAS.

Mechanism(s) by Which Ang II May Increase Blood Pressure in Males: Role of Oxidative Stress

Both supraphysiological and physiological doses of Ang II can cause oxidative stress. For example, Rajagopalan and colleagues⁶⁹ found that pharmacological doses of Ang II (0.7 mg/kg per day SC by minipump) increased blood pressure and superoxide levels in aortic segments of rats, while infusion of norepinephrine, which resulted in an increase in blood pressure similar to that of Ang II, had no effect on superoxide levels. These data suggested that infusion of Ang II at pharmacological doses was capable of inducing oxidative stress independent of elevated blood pressure. In addition, these investigators found that increased superoxide levels could be normalized with losartan, the Ang II receptor antagonist, or with liposomes containing superoxide dismutase.⁶⁹ In additional experiments they also reported that Ang II increases superoxide production via increased NAD(P)H oxidase activity.⁷⁰

Superoxide is known to interact with NO to produce peroxynitrite, one of the most potent oxidative compounds known.^{71,72} Thermodynamically speaking, the reaction of NO and superoxide is preferential since the rate of reaction is more rapid than the reaction rate of superoxide and its scavenger, superoxide dismutase.⁷³ Although peroxynitrite itself is a vasodilator, Villa and colleagues⁷⁴ demonstrated that tachyphylaxis occurs at peroxynitrite concentrations of 3 $\mu\text{mol/L}$, which is subthreshold as a vasodilator in coronary circulation; this not only prevents further response to its own vasodilator actions but also causes long-lasting impairment of the response to other vasodilators. In support of this notion, Benkusky and colleagues⁷⁵ found that the development of tachyphylaxis to peroxynitrite attenuated the hemodynamic vasodilator effect produced by systemic administration of acetylcholine and prostacyclin in hindquarter, renal, and mesenteric circulation. Furthermore, Kooy and Lewis⁷⁶ reported that after tachyphylaxis to peroxynitrite infusion, blood pressure in rats increased by 20% and renal vascular resistance increased by 93%, along with increases in hindquarter and mesenteric vascular resistances. Therefore, the vasodilator action of peroxynitrite will play only a minimal role in control of vascular tone, if at all. However, not only will quenching of NO by superoxide increase the vascular tone, but the increase in peroxynitrite could potentiate this effect by causing tachyphylaxis to residual NO.

It may not be surprising that high doses of Ang II could cause oxidative stress since Ang II is a powerful vasoconstrictor; however, we have recently shown that chronic infusion of suppressor doses (ie, doses that do not elicit an immediate blood pressure response) of Ang II (10 ng/kg per minute) for 14 days to normotensive rats that were given enalapril to block endogenous Ang II formation resulted in the slow-onset development of hypertension and an increase

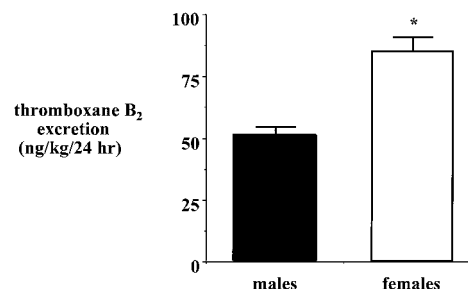


Figure 8. Male SHR excrete less thromboxane B₂ than do females. Male and female SHR, aged 3 to 4 months, were maintained for 24 hours in individual metabolism cages, and urine was collected on ice for thromboxane B₂, as measured by radioimmunoassay. Data are expressed as mean \pm SEM. Statistical differences were determined by Student's *t* test. **P* < 0.01 compared with males.

in plasma F₂-isoprostanes, an indicator of oxidative stress.⁷⁷ Two factors suggest that the increase in blood pressure in this model may require a secondary mechanism in addition to Ang II itself. The first is the time delay required for the increase in blood pressure to develop (5 to 10 hours, typically reaching a maximum in 4 to 5 days),^{77,78} and the second is lack of a significant increase in plasma Ang II levels accompanying the increase in blood pressure.^{59,79} Peroxynitrite, by virtue of its potent oxidative ability, can produce oxidation of lipids and produce other products that have vasoconstrictive actions. One such group of compounds are the isoprostanes, which are prostaglandin-like compounds produced by nonenzymatic, free radical-induced peroxidation of arachidonic acid.⁸⁰ One of the F-ring isoprostanes (8-iso-prostaglandin F_{2 α} or F₂-isoprostanes) has been shown to be a very potent renal vasoconstrictor, mainly by increasing afferent resistance, and can also raise blood pressure at higher doses.^{80,81} In addition, Sametz and colleagues⁸² recently reported that coinfusion of F₂-isoprostane and Ang II resulted in significant potentiation of the vasoconstrictor effect of Ang II. Furthermore, F₂-isoprostanes have been shown to increase endothelin,⁸³ which would also contribute to renal vasoconstriction. We hypothesize that androgens stimulate the RAS and increase Ang II, which causes oxidative stress with increased superoxide production, quenching of NO (leading to a further increase in blood pressure), and production of peroxynitrite, which causes a reduction in the renal vascular response to vasodilators, including residual NO, and production of vasoconstrictor F₂-isoprostanes, which will in turn potentiate the vasoconstrictor effects of Ang II and stimulate endothelin production to increase blood pressure even further. To extend this hypothesis, thromboxane receptor number has been shown to increase with testosterone treatment in aortic vascular smooth muscle cells.⁸⁴ Thromboxane receptors have been shown to mediate at least in part the biological action of F₂-isoprostanes.⁸⁵ Thus, androgens could also increase the number of thromboxane receptors by which the F₂-isoprostanes cause vasoconstriction. It is doubtful that thromboxanes themselves play any role in mediating the higher blood pressure in male SHR since, as shown in Figure 8, we have found that male SHR excrete less

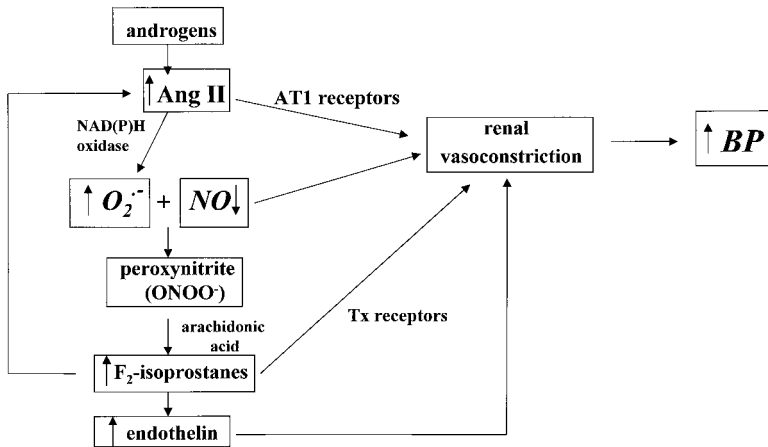


Figure 9. Schematic diagram describing the speculation of the possible mechanisms by which androgens may increase oxidative stress and thus renal vasoconstriction and lead to increases in blood pressure (BP). $O_2^{\cdot -}$ indicates superoxide; Tx, thromboxane.

thromboxane B_2 , the stable metabolite of thromboxane A_2 , than do females.

In support of the hypothesis that oxidative stress, and more directly superoxide, plays a role in the hypertension in male SHR, we have preliminary data in which SHR were chronically treated with the chemical scavenger of superoxide, TEMPOL, for 6 weeks.⁶³ With TEMPOL treatment, the mean arterial pressure of SHR males was attenuated to the level found in untreated female SHR. Chronic TEMPOL also decreased PRA in male SHR to levels found in untreated female SHR. In contrast, there was no effect of TEMPOL on blood pressure or PRA in female SHR.⁶³ Together with the data from our enalapril studies in SHR discussed above, these preliminary data provide strong evidence that Ang II and oxidative stress play important roles in the higher blood pressure in male SHR.

Figure 9 serves to illustrate the possible mechanisms by which oxidative stress could play a role in at least partially mediating androgen-induced increases in blood pressure. Androgens could stimulate superoxide production either directly or via the effect of Ang II on NAD(P)H oxidases. Superoxide production would quench NO, leading to vasoconstriction. The combination of superoxide and NO produces peroxynitrite, which would oxidize arachidonic acid to produce F_2 -isoprostanes. F_2 -isoprostanes, mediated by thromboxane receptors, which are upregulated by androgens, would cause renal vasoconstriction directly and indirectly by potentiating the vasoconstrictor actions of Ang II and stimulating endothelin production, which in turn would cause further renal vasoconstriction. These hypotheses remain to be tested.

Other Mechanism(s) by Which Androgens May Influence Blood Pressure: Role of Ang II Receptors

One mechanism by which androgens may affect the sensitivity to Ang II is by exerting an effect on Ang II receptors in the kidney. In contrast to female SHR and female rats subjected to reduced renal mass, female DS rats on a high salt diet exhibit an increase in blood pressure after ovariectomy.^{86,87} Nickenig and colleagues⁸⁸ reported that ovariectomy of normotensive rats results in an increase in angiotensin type 1 (AT_1) receptor number in the aorta. In preliminary studies by

Harrison-Bernard and Raij,⁸⁶ AT_1 receptor concentration in the kidney was found to be higher in ovariectomized female DS rats. It is possible that the increase in blood pressure in DS rats after ovariectomy may result from the increase in renal AT_1 receptor number. Angiotensin type 2 (AT_2) receptors are thought to be associated with the vasodilatory actions of Ang II, which may be mediated by NO,⁸⁹ and it is possible that male SHR also have lower AT_2 receptor numbers than females, which could contribute to the higher blood pressure in males than in females. To date there have been no studies to determine whether androgens affect the Ang II receptor subtypes, numbers, or affinity.

Other Mechanism(s) by Which Androgens May Influence Blood Pressure: Role of Aldosterone

Ang II stimulates the production of aldosterone, which is responsible for increasing sodium reabsorption in the distal nephron. It is possible that androgens could increase sodium reabsorption via Ang II-mediated or androgen-mediated increases in aldosterone. There is evidence to suggest that this may be the case, since Miller and colleagues⁹⁰ found higher blood pressure and aldosterone levels in men than in women, and Schunkert et al⁹¹ found a positive correlation between dehydroepiandrosterone sulfate (a metabolite of testosterone), aldosterone levels, and blood pressure in a population of hypertensive men. However, Kau and colleagues⁹² reported that testosterone replacement in castrated male rats decreased corticotropin-stimulated aldosterone release.

Mechanistic Scheme

This review has attempted to critically examine large numbers of fragmentary observations to create a coherent set of hypotheses (albeit complicated) by which gender differences in blood pressure control may be explained. The speculative hypotheses described in Figure 9 represent the possible mechanisms by which androgens, mediated via Ang II, could induce oxidative stress to potentiate renal vasoconstriction. In Figure 10 are illustrated the mechanisms by which androgen-mediated increases in Ang II could lead to a shift in the pressure-natriuresis relationship and renal vasoconstriction, both of which are known to affect blood pressure. As we have shown in animal studies, androgens could promote an in-

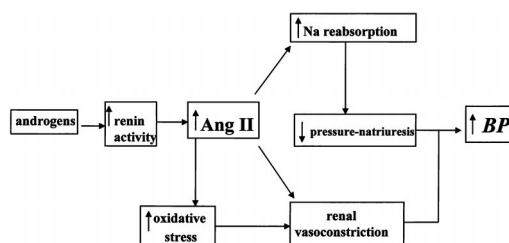


Figure 10. Schematic diagram by which androgens could affect the RAS to cause an increase in blood pressure (BP).

crease in blood pressure in males by stimulating renin activity and Ang II formation, either by stimulating renin release and/or by increasing renal renin activity. Androgens may stimulate renin release by reducing glomerular filtration rate, directly stimulating sodium reabsorption, and thus decreasing delivery of sodium to the macula densa. Alternatively, renin activity (and thus Ang II) could also be increased if androgens cause a chronic increase in renal angiotensinogen and renin enzyme is working below its maximal velocity. Androgens may affect the number and affinity of receptors for Ang II, thereby affecting sodium reabsorption and/or renal vasoconstriction. Ang II via AT₁ receptors may directly cause renal vasoconstriction and may also stimulate proximal tubule sodium reabsorption and/or stimulate aldosterone-mediated distal tubule sodium reabsorption, blunt pressure-natriuresis, and increase blood pressure. The combination of increased sodium reabsorption and renal vasoconstriction would lead to the increase in blood pressure.

If androgen levels are similar in normotensive and hypertensive rats and yet blood pressure differences are difficult to detect in normotensive rats but are very obvious in hypertensive rat strains, this may suggest that hypertensive rats may exhibit an exaggerated response to androgens that normotensive rats do not. This is intriguing because increasing responsiveness to androgens may be an important factor in why postmenopausal women experience increases in blood pressure, if in fact androgen levels are not significantly reduced with aging in women^{44–47} and are left unopposed because of lack of estrogen. The increasing response to androgens could be mediated by changes in Ang II receptors, aldosterone, and/or oxidative stress. Future studies will be necessary to investigate these possibilities.

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