Gender Differences in Development of Hypertension in Spontaneously Hypertensive Rats
Role of the Renin-Angiotensin System

Jane F. Reckelhoff, Huimin Zhang, Kumud Srivastava

Abstract—Previous data strongly support a role for androgens in promoting the gender difference in hypertension in the spontaneously hypertensive rat(s) (SHR), but the mechanism is not clear. Because males develop higher blood pressures than do females, we hypothesize that androgens may affect the renin-angiotensin system to promote the development of hypertension in male SHR. The present study was performed to determine the effect of converting enzyme inhibition (CEI) on the development of hypertension in SHR. Male, female, castrated male, and ovariectomized (ovx) female SHR (n = 10 per gender per treatment group) received enalapril (250 mg/L) in drinking water for 8 to 10 weeks. Some ovx females were also given testosterone chronically. At 17 to 19 weeks of age, 24-hour protein excretion and mean arterial pressure were measured. By 13 weeks of age, male rats had higher systolic blood pressures by tail plethysmography than did the other rats, and CEI reduced blood pressures to similar levels in all groups. At 17 to 19 weeks, the same trend was found by direct measurement of mean arterial pressure. The ovx females treated with testosterone had serum testosterone and blood pressure levels similar to those found in males. CEI reduced mean arterial pressure to similar levels in all gender groups. Untreated males and ovx females given testosterone had significantly higher levels of urinary protein excretion than did the other groups, and CEI had no effect on proteinuria in any of the rats. These data suggest that the development of hypertension in SHR regardless of sex steroids is mediated by the renin-angiotensin system. However, the data further suggest that androgens promote the exacerbation of hypertension in male SHR via a mechanism involving the renin-angiotensin system. (Hypertension. 2000;35[part 2]:480-483.)

Key Words: gender ■ renin-angiotensin system ■ angiotensin-converting enzyme ■ testosterone

Recent studies using the technique of 24-hour ambulatory blood pressure monitoring have confirmed that blood pressure is higher in men than in premenopausal women at similar ages.1–3 Gender differences in blood pressure are also present in hypertensive rat models: males have higher blood pressures than do females.4–7 For example, the male spontaneously hypertensive rat(s) (SHR) has higher blood pressures than does the female SHR at a similar age.4

Although the mechanism(s) responsible for higher blood pressures in men and male SHR is not clear, androgens have been shown to promote hypertension in rats, in view of the fact that castration of male SHR decreases blood pressure, and chronic testosterone treatment of ovariectomized (ovx) female SHR increases blood pressure.4 Ovariectomy alone does not affect hypertension in females, suggesting that it is not estrogen that protects female SHR from the development of hypertension but a lack of androgens. In humans, the link between androgens and blood pressure has been further strengthened by studies using ambulatory blood pressure monitoring in children; these studies have shown that boys have higher blood pressures than do age-matched girls and that there is a significantly greater increase in blood pressure at puberty in males than in females.8,9

All forms of hypertension studied to date have been shown to be caused by an intrinsic defect of the kidneys that allows an abnormal reabsorption of sodium and water.10,11 We have shown that there is a shift in the renal pressure-natriuresis relationship in male and female SHR, with males excreting less sodium than females at similar renal perfusion pressures.4 Castration of the male restores pressure-natriuresis to the same curve as generated for the female.4 Conversely, testosterone treatment of ovx females also blunts the pressure-natriuresis relationship, again suggesting a role for androgens.

One of the most important mechanisms for control of sodium handling by the kidney is the renin-angiotensin system (RAS).12 Thus, in the present study, our hypothesis was that differences in the RAS are largely responsible for the gender differences in the development of hypertension in the SHR. The questions addressed were as follows: (1) Do male...
SHR have a greater depressor response to angiotensin-converting enzyme inhibition (CEI) than do females? (2) What is the effect of CEI on blood pressure in the absence of sex steroids? (3) Is the depressor response to CEI seen in males similar to the response in ovx females given androgen supplementation?

Methods

Rats
Male and female SHR (n=100), aged 9 weeks, were obtained from Taconic Farms (Germantown, NY). Some rats were castrated or ovariec-tomized by the vendor at 8 weeks of age and received at 9 weeks of age. Before initiation of the study, rats were maintained on standard rat chow (Teklad, Harlan Sprague Dawley) and tap water. The protocols complied with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Protocol for Chronic Testosterone Treatment
Ovx female SHR (n=20) were implanted subcutaneously in the shoulder or back with 19-mm-length Silastic tubing (0.062 in inner diameter, 0.125 in outer diameter; Dow Corning) containing 10 mg testosterone beginning at 11 weeks of age to mimic the time in males when serum testosterone levels reach a maximum.6 Silastic tubing was replaced every 3 weeks for 6 to 8 weeks. At the end of the study, serum was obtained from ovx rats for measurement of testosterone and compared with serum levels in intact male SHR.

Protocol for Chronic Enalapril Treatment
Rats were divided into 10 groups (n=10 in each group). Groups 1, 3, 5, 7, and 9 were intact males, intact females, castrated males, ovx females, and ovx females, respectively, that were treated with testosterone and given tap water throughout the study. Groups 2, 4, 6, 8, and 10 were similar genders treated chronically with enalapril (250 mg/L) in drinking water. Rats were treated for 8 to 10 weeks from 9 to 19 weeks of age. At 17 to 19 weeks of age, blood pressure was measured directly with a cannula inserted in the femoral artery in rats anesthetized by the thiobarbiturate Inactin (RBI; 7 per group). The week before systemic blood pressure measurement, rats were placed in metabolism cages for 24-hour collection of urine for measurement of protein.

Urinary Protein Excretion
Urinary protein excretion was measured by the method of Bradford13 with the use of a commercially available reagent (Bio-Rad). Bovine serum albumin was used as the standard.

Measurement of Arterial Blood Pressure
On the day of study, rats (n=7 in each group) were anesthetized with Inactin (120 mg/kg, RBI) and placed on a temperature-regulated surgery table. A left femoral arterial catheter was placed, and blood pressure was monitored for 60 minutes. A tracheostomy was also performed to facilitate the breathing of the rat. The blood pressure recorded in the last 15 minutes of monitoring after a 45-minute equilibration period was used as the value. At the end of the blood pressure recording, the kidneys were removed and weighed.

Statistical Analyses
Statistical differences for all data were determined by ANOVA with the use of Statview 512 and the Dunnett test.14 Data are expressed as mean±SEM.

Results
Serum testosterone was similar in intact males and ovx females treated chronically with testosterone (male control, 177±28 ng/dL; male+enalapril, 158±34 ng/dL; ovx control, 169±35 ng/dL; and ovx+enalapril, 170±30 ng/dL). Urinary protein excretion in SHR, aged 17 to 19 weeks, was significantly higher in males than in females, castrated males, or ovx females (see [tbc]Table). Testosterone treatment of ovx females increased protein excretion to levels similar to those found in intact males. Castrated males excreted more protein than did intact or ovx females. Enalapril treatment did not change protein excretion rates in any of the groups, except the intact females, in which proteinuria was reduced with enalapril.

As shown in the Table, at 17 to 19 weeks of age, body weights and kidney weights were higher in intact males than in females. Castrated males had body weights similar to those of intact males but lower kidney weights. Ovx females had higher body weights than did intact females, but they had lower body weights than did intact or castrated males, whereas kidney weights were similar to those of intact females. Testosterone treatment significantly increased both body weights and kidney weights in ovx females compared with intact females. Body weights and kidney weights tended to be 5% to 9% lower with enalapril in all groups except ovx females.

At 17 to 19 weeks of age, mean arterial pressure was ~30 mm Hg higher in male SHR than in female, ovx, or castrated male SHR (Figure). Testosterone treatment of ovx females increased mean arterial pressure to levels similar to those in intact males. Enalapril treatment for 6 to 8 weeks decreased blood pressure in all groups. In intact males and ovx females treated with testosterone, blood pressure decreased by 63% with enalapril treatment. In contrast, treatment with enalapril alone decreased blood pressure by 40% to 45% in females, castrated males, and untreated ovx females. However, the reductions in blood pressure with enalapril
Effect of chronic enalapril on mean arterial pressure (MAP) in SHR. MAP is shown for 17- to 19-week-old SHR in the following groups: male, female, castrated male (cast), ovariectomized (ovx) female, and ovx female treated chronically with testosterone (ovx+T) starting at 11 weeks of age. Open bars indicate control rats; solid bars, enalapril-treated rats. *P<0.01 compared with intact males; †P<0.01 compared with females (cast or ovx); ‡P<0.01 compared with untreated rats of a similar gender.

eliminated any gender difference in the level of blood pressure among the groups.

Discussion
As we have previously shown, male SHR at 17 to 19 weeks of age have higher blood pressures than do females. Castration is associated with a reduction in the blood pressure to levels similar to those in females, whereas ovariectomy has no effect on the blood pressure. Androgen receptor antagonism also attenuates hypertension in male SHR.15 In the present study, we have found that the elevated blood pressure found in all genders of SHR is reduced by enalapril treatment, implicating the RAS in the development of hypertension of all SHR regardless of gender. However, and most important, we have also found for the first time that the higher blood pressures in male SHR and ovx female SHR treated with testosterone were normalized by chronic enalapril treatment, further implicating the RAS in androgen-mediated hypertension.

It is well known that abnormalities in the RAS are present in SHR. Giudicelli et al16 also found that CEI in male SHR from 6 to 20 weeks of age protected against the development of hypertension, and Morton et al17 found similar effects with chronic Ang II receptor antagonism. Vyas and Jackson18 found that SHR, aged 6 to 7 weeks, were more responsive to physiological doses of Ang II than were Wistar-Kyoto rats (WKY). Furthermore, the number of Ang II type I (AT₁) receptors in mesenteric vasculature, preglomerular vessels, and proximal tubules has been found to be higher in SHR males than in WKY males.19–21

Several studies have shown that sex hormones affect the RAS. In humans, plasma renin activity is higher in men than in aged-matched women.22–23 It has also been shown that plasma renin activity is higher in postmenopausal women than in premenopausal women or postmenopausal women receiving hormone replacement therapy.24 In animals, 2 studies have shown that renal angiotensinogen mRNA levels in normotensive rats are affected by gender, with angiotensinogen mRNA being higher in kidneys from males than from females.25,26 Castration decreases and testosterone treatment of ovx females increases angiotensinogen mRNA.25,26 Estrogen treatment of ovx female rats has also been shown to reduce angiotensin-converting enzyme activity and mRNA.27 However, in the present study, there was no effect of ovariectomy on hypertension when ovx female SHR were compared with intact female SHR. Furthermore, recent studies have shown that ovariectomy of normotensive female rats results in an increase in mRNA for AT₁ receptors in the kidney but does not affect binding of Ang II to the receptor.28 In those studies, there were no kidneys from male rats examined, so the effect of androgens on AT₁ receptor number or binding is not known. Further studies will be required to determine the role that both androgens and estrogens play in control of the expression of AT₁ receptors.

Typically, a decrease in blood pressure, as we found with enalapril, is associated with a reduction in proteinuria. However, in the present study, enalapril, which normalized the blood pressure in all groups, had no effect in any of the groups on the level of protein excreted by the kidney. These data may reflect little effect of enalapril on glomerular capillary pressure (P_GC). In support of this hypothesis are studies by Arendshorst et al,29 who found that there was no difference in P_GC between 4- to 6-week-old male SHR and age-matched WKY. This group also found that the preglo- merular resistance vessels were significantly more vasoconstricted in SHR than in WKY.30 This finding was later verified by in vitro studies of Ito et al,31 who found that the afferent arterioles of SHR are hyperresponsive to elevations of pressure compared with the afferent arterioles of WKY. In contrast, short-term and acute treatment with CEI in older SHR males has been shown to be effective in reducing P_GC.32,33 Kvam et al32 found that the acute effect of enalapril on P_GC was mainly due to a reduction in efferent arteriolar resistance. From our present study, it is not clear whether P_GC was affected by CEI and whether this was the mechanism by which proteinuria was not affected by CEI.

Our data strongly support a role for the RAS in the development of hypertension in SHR and in the further exacerbation of hypertension in male SHR. However, CEI has been shown to have effects on other systems that affect blood pressure. For example, CEI has been shown to cause an increase in circulating bradykinin levels, in view of the fact that CEI also inhibits the activity of kininase II (kallikrein).34 However, a study by Majima et al34 found that the levels of bradykinin needed to reduce blood pressure in SHR males was 20 to 100 times higher than the level produced by captorpril34; in another study, inhibition of bradykinin with the antagonist Bkant (HOE 140) was shown to have no effect on basal blood pressure in SHR or WKY.35 Importantly, estrogen has been shown to increase kidney tissue kallikrein in rats, and bradykinin receptor (B₂) levels in the kidney decrease in response to ovariectomy.36 Whether bradykinin plays a role in the reduction in blood pressure with CEI was not addressed in the present study. However, with the previous data in mind, one might expect that intact females would have lower blood pressure than ovx females because estrogen should both increase bradykinin and B₂ receptor number, and this difference may be even further exacerbated with enalapril, which should increase bradykinin levels further. However, because there was no difference in blood pressure among the groups, it is not clear why we did not see a similar effect with CEI.
pressure levels in intact and ovariectomized females regardless of whether they were treated or untreated with enalapril, it is doubtful that the bradykinin system played much of a role in the hypotensive effect of enalapril.

In summary, we have found that the gender difference in the development of hypertension in SHR can be abolished by chronic treatment of rats with CEI. These data strongly suggest that the development of hypertension in all SHR, regardless of gender, is mediated by the RAS. The data also support a role for the RAS in the exacerbation of hypertension found in intact males and ovariectomized females treated chronically with testosterone. Further studies will be necessary to determine the mechanism(s) by which androgens promote hypertension in male SHR via the RAS.

Acknowledgment
This study was supported by an Established Investigator Award from the American Heart Association.

References
Gender Differences in Development of Hypertension in Spontaneously Hypertensive Rats: Role of the Renin-Angiotensin System

Jane F. Reckelhoff, Huimin Zhang and Kumud Srivastava

*Hypertension.* 2000;35:480-483
doi: 10.1161/01.HYP.35.1.480

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231 Copyright © 2000 American Heart Association, Inc. All rights reserved. Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/35/1/480

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

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

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