Gender Differences in Hypertension in Spontaneously Hypertensive Rats
Role of Androgens and Androgen Receptor

Jane F. Reckelhoff, Huimin Zhang, Kumud Srivastava, Joey P. Granger

Abstract—Males are at greater risk of cardiovascular and renal disease than are females. For example, male spontaneously hypertensive rats (SHR) have higher blood pressures than females. Androgens have been strongly implicated in the hypertension of male SHR, because castration attenuates the hypertension. This study determined whether the androgen receptor plays a role in hypertension in male SHR and whether testosterone alone can cause the hypertension or whether conversion to dihydrotestosterone is necessary. Male SHR, aged 10 weeks, were given the androgen receptor antagonist flutamide (8 mg/kg SC; n=8) or the 5α-reductase inhibitor finasteride (30 mg · kg⁻¹ · d⁻¹ SC; n=11) daily for 5 to 6 weeks. Control rats (n=10) received vehicle (20% benzyl benzoate or ethanol in castor oil). After 5 to 6 weeks, blood pressure (mean arterial pressure) and glomerular filtration rate were measured. Long-term flutamide treatment caused a reduction in mean arterial pressure (control 178±5 mm Hg; flutamide 159±3 mm Hg; P<0.01), but finasteride had no effect (180±5 mm Hg). There were no differences in glomerular filtration rate among the groups. These data indicate that hypertension in male SHR is mediated via the androgen receptor and does not require conversion of testosterone to dihydrotestosterone. ([Hypertension. 1999;34[part 2]:920-923.])

Key Words: flutamide ■ finasteride ■ blood pressure ■ gender

Males are at greater risk of cardiovascular and renal disease than are females. For example, studies using the technique of ambulatory blood pressure monitoring have shown that blood pressure is higher in men than in women of similar ages.¹ ² In hypertensive rat models, we and other investigators have found that males have higher blood pressures than do females.³ – ⁸ For example, male spontaneously hypertensive rats (SHR) have higher blood pressures than do females of similar ages.³ ⁴ ⁶

Although the incidence of higher blood pressure in men and in male animals has been documented, the mechanisms responsible for the increase in blood pressure in the males are unknown, but androgens have been shown to have a potential role in both humans and rats. For example, studies using ambulatory blood pressure monitoring techniques have also shown that after the onset of puberty, boys have higher blood pressures than do age-matched girls.⁵,¹⁰ In animal studies, castration at a young age (3 to 5 weeks) attenuates the development of hypertension in rat models such as SHR and Dahl salt-sensitive rats.³ ⁴ ⁶ ¹¹ ¹² In contrast, ovariectomy has no effect on the development of hypertension in females.³ These data not only support a role for androgens in mediating the higher blood pressure in male SHR, they also demonstrate that it is not estrogen in SHR females that protects them from developing the higher pressures found in males.

Although our previous studies have implicated androgens in the gender difference in control of blood pressure in SHR, there is little information concerning whether the androgen receptor is involved in hypertension in males. To further investigate the role of androgens in exacerbation of hypertension in male SHR, the present studies addressed whether the androgen receptor plays a role in the development of hypertension in male SHR. Many recent studies¹³ – ¹⁵ have shown that there are nongenomic effects of androgens that are independent of gene transcription and thus do not involve the androgen receptor. Thus, it is possible that hypertension in male SHR may be mediated by androgens independently of the androgen receptor. The present studies also sought to determine whether testosterone alone can cause hypertension in male SHR or whether conversion to dihydrotestosterone is necessary, because many functions of testosterone do require conversion.

Methods

Rats
Male SHR were obtained from Taconic (Germantown, NY) at 9 weeks of age. Rats were maintained on standard rat diet (Teklad, Harlan SD) and tap water in a 12-hour/12-hour light/dark cycle and were allowed to equilibrate for ≥1 week before study. The study protocols were in accordance with the Guidelines for the Care and

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Experimental Design for Flutamide and Finasteride Treatment

SHR were divided into 3 groups. Group 1 consisted of control rats given vehicle (20% benzyl benzoate or ethanol in castor oil; n = 10). Group 2 consisted of SHR given the androgen receptor antagonist flutamide (8 mg/kg SC; n = 8). Flutamide is a nonsteroidal antiandrogen that has no effect on ornithine decarboxylase activity, which shows that it has no androgenic activity. This dose is equivalent to the dose given to men in the treatment of prostatic cancer. Group 3 received the 5α-reductase inhibitor finasteride (30 mg · kg⁻¹ · d⁻¹; n = 11), which prevents conversion of testosterone to dihydrotestosterone. In rats, a comparable dose of 25 mg · kg⁻¹ · d⁻¹ has been shown to cause feminization of the external genitalia and a decrease in prostatic size. SHR were injected with vehicle, flutamide, or finasteride daily for 5 to 6 weeks beginning at 10 weeks of age, because we have previously found that serum testosterone reaches a peak at ~12 weeks of age in the SHR male.

Short-Term Renal-Function Studies

On the day of study, rats were anesthetized by intraperitoneal injection of Inactin (100 to 110 mg/kg body weight; RBI), and placed on a heat-regulated surgery table to maintain rectal temperature at 36°C to 38°C. The following catheters were placed: femoral arterial, for continuous monitoring of blood pressure and for blood sampling; femoral venous, for infusion of isoncotic artificial rat plasma (2.5 g/dL bovine immunoglobulin, 2.5 g/dL bovine serum albumin in Ringer’s solution) at 12.5 mL · kg⁻¹ · h⁻¹ for 45 minutes during the preparatory surgery and thereafter at 1.5 mL · kg⁻¹ · h⁻¹ throughout the experimental period to maintain a euvolemic preparation; left jugular venous, for infusion of 0.9% saline with or without 'H-inulin (15 to 20 μCi/mL; 0.9% saline; New England Nuclear) at 1 mL/h; and left ureteral, for collection of urine samples into oil in graduated glass tubes. A tracheostomy was also performed. In flutamide-treated rats and 6 control rats, the left renal vein was cannulated in the retrograde position with a 23-gauge needle connected to PE-50 tubing to be used for renal venous blood sampling for calculation of renal plasma flow and renal vascular resistance. These parameters were not measured in finasteride-treated rats or 4 of the control rats. After a 50-minute equilibration period for 'H-inulin infusion, two 20- to 30-minute urine collections were obtained, and midpoint arterial and renal venous blood samples were taken. After the experiment, the left kidney was removed and weighed. Urine (1 μL) and plasma (5 μL) samples were counted by liquid scintillation and used to calculate renal hemodynamics, as previously described.

Statistical Analyses

Statistical differences in the data were analyzed by ANOVA with StatView 512 software and Dunnett test. Significance was defined as *P*<0.05. All values are expressed as mean±SEM.

Results

Effect of Long-Term Flutamide Treatment on Systemic and Renal Hemodynamics

Although numerically higher in control rats, body weights were not statistically different between flutamide-treated rats (306.7±9.7 g) and controls (321.8±5.3 g). Left kidney weights were also not different (flutamide 1.00±0.03 g; control 1.07±0.02 g). As shown in Figure 1, flutamide treatment reduced mean arterial pressure in intact male SHR by ~20% compared with controls or finasteride-treated rats. However, as shown in Figure 2, glomerular filtration rate (GFR) was not affected by androgen receptor antagonism.
the presence of estrogens but the lack of androgens in female SHR that affords protection against the higher blood pressure found in male SHR.

In the present study, we further addressed the question of the role androgens may play in hypertension in male SHR by determining the role played by the androgen receptor. In male SHR given a nonsteroidal androgen receptor antagonist, we have found that hypertension was attenuated. These data strongly support a role for the androgen receptor in mediating the hypertensive effect of androgens in male SHR. Recent studies have shown that androgens can exert vasodilatory actions on rat thoracic aorta and canine coronary arteries that are not mediated by the androgen receptor. However, these actions of androgens have been found in short-term studies only, whereas the ability of androgens in male SHR to cause an increase in blood pressure is a long-term effect.

Our present data are consistent with a previous report by Ganten and colleagues, who found that flutamide treatment of neonatal rats for 10 days after birth attenuates hypertension in SHR males when blood pressure was measured at 6 to 13 weeks of age. In other studies, Ely and colleagues investigated the effect of the lack of the androgen receptor on blood pressure in F1 hybrid males produced by crossing female King-Holtzman rats, who carry the gene for testicular feminized males (Tfm), with normal SHR males. The genotype of the Tfm is of an XY male, but the phenotype is that of a female, and internally there is an undescended testis that secretes testosterone. However, these rats lack active androgen receptors and therefore lack secondary androgen characteristics. In those studies, the Tfm-SHR F1 hybrids had lower blood pressure than did the male F1 hybrids with normal androgen receptors. However, castration of the Tfm hybrids resulted in a further decrease in blood pressure, prompting the investigators to propose that in the hybrid males, the hypertension was mediated by both the androgen receptor and an independent testis-derived factor.

The other question we addressed in these studies was whether conversion of testosterone to dihydrotestosterone was necessary for promotion of hypertension in male SHR. This is an important question, because testosterone and dihydrotestosterone can have different biological actions. For example, it is dihydrotestosterone that is the important androgen in mediating prostatic hypertrophy. The use of finasteride to treat men with this condition is well known to prevent and even reduce already developed prostatic hypertrophy. Similarly, it is dihydrotestosterone that is responsible for male pattern baldness, and finasteride is also used as a treatment for this condition. However, because finasteride afforded no protection against the development of hypertension, the data show that conversion of testosterone to dihydrotestosterone is not necessary to promote hypertension in male SHR.

Although the present studies suggest an important role for androgens in hypertension in male SHR, the mechanism by which this occurs is still unclear. Substantial evidence supports the theory that some form of renal dysfunction plays a role in the development and maintenance of all forms of hypertension. There was no effect of androgen receptor antagonism on renal hemodynamics, which suggests that the mechanism by which androgens promote hypertension in male SHR is not renal hemodynamically mediated. However, a common defect that has been characterized in several forms of hypertension is a shift in the pressure-natriuresis relationship. We have previously shown that castration normalizes the pressure-natriuresis relationship in male SHR. In contrast, ovariectomized female SHR given testosterone for 5 weeks demonstrated the same blunted pressure-natriuresis relationship as the intact male SHR. Whether the increase in blood pressure in female SHR given testosterone was mediated via the androgen receptor was not determined in those studies and remains to be investigated.

In summary, we found that androgen receptor antagonism, using flutamide, attenuates hypertension in male SHR. In addition, conversion of testosterone to dihydrotestosterone is not necessary for development of hypertension. Taken together, these data lend additional support to our hypothesis that androgens play an important role in the regulation of hypertension in male SHR.

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References

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