Androgens augment renal vascular responses to ANG II in New Zealand genetically hypertensive rats

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Song, Jin, Curtis K. Kost Jr., and Douglas S. Martin. Androgens augment renal vascular responses to ANG II in New Zealand genetically hypertensive rats. *Am J Physiol Regul Integr Comp Physiol* 290: R1608–R1615, 2006. First published February 9, 2006; doi:10.1152/ajpregu.00364.2005.—Males develop higher blood pressure than do females. This study tested the hypothesis that androgens enhance responsiveness to ANG II during the development of hypertension in New Zealand genetically hypertensive (NZGH) rats. Male NZGH rats were obtained at 5 wk of age and subjected to sham operation (Sham) or castration (Cas) then studied at three age groups: 6–7, 11–12, and 16–17 wk. Mean arterial blood pressure (MAP), heart rate (HR), and renal blood flow (RBF) measurements were recorded under Inactin anesthesia. These variables were measured after enalapril (1 mg/kg) treatment and during intravenous ANG II infusion (20, 40, and 80 ng/kg/min). Plasma testosterone was measured by ELISA. Angiotensin type 1 (AT1) receptor expression was assessed by Western blot analysis and RT-PCR. ANG II-induced MAP responses were significantly attenuated in Cas NZGH rats. At the highest ANG II dose, MAP increased by 40 ± 4% in Sham vs. 22 ± 1% in Cas NZGH rats of 16–17 wk of age. Similarly, renal vascular resistance (RVR) responses to ANG II were reduced by castration (209 ± 20% in Sham vs. 168 ± 10% in Cas NZGH rats at 16–17 wk of age). Castration also reduced MAP recorded in conscious NZGH rats of this age group. Testosterone replacement restored baseline MAP and the pressor and RVR responses to ANG II. Castration reduced testosterone concentrations markedly. Testosterone treatment restored these concentrations. Neither castration nor castration + testosterone treatment affected AT1 receptor mRNA or protein expression. Collectively, these data suggest that androgens modulate renal and systemic vascular responsiveness to ANG II, which may contribute to androgen-induced facilitation of NZGH rat hypertension.

angiotensin; sex steroids; kidney; hypertension

MALES DEVELOP HIGHER BLOOD pressure than do age-matched, premenopausal females. Gender-associated differences in blood pressure regulation have been demonstrated in both human and animal studies (31). The relative contribution of estrogens and androgens to these gender differences remains unclear (11, 20, 31). Nevertheless, several observations suggest that androgens play an important role in gender differences of blood pressure regulation. For example, castration attenuated the development of hypertension in several animal models (2, 15), whereas androgen treatment restored hypertension in castrated males (21, 36). The mechanism(s) of this prohypertensive effect of androgens remain(s) to be fully established.

The kidneys are critical in regulating sodium and water balance and play an important role in controlling the long-term level of arterial pressure (17). Evidence from the spontaneously hypertensive rat (SHR) model suggested that aging males showed exacerbated glomerular injury, increased proteinuria, and decreased glomerular filtration rate. Castration at an early stage, however, can improve renal function and attenuate hypertension development (14). Furthermore, current evidence supports an important but complex interaction between sex steroids and the renin angiotensin system (RAS) (24, 32). Some studies have reported that plasma renin levels and renin activity are higher in male than in female rodents (6, 44), consistent with higher expression of mRNA for renin in male compared to female mice (44). Similarly, aldosterone levels are reportedly elevated in male vs. female New Zealand genetically hypertensive rats (2). Both estrogens and androgens appear to contribute to these gender differences in the RAS (24, 32). In the case of androgens, treatment with testosterone increased plasma renin activity in rats (31) and renal angiotensinogen expression in mice (44). Similarly, dihydrotestosterone treatment of Sprague-Dawley rats increased renin expression of angiotensinogen (30). Conversely, castration of male Wistar-Kyoto reduced angiotensinogen expression (12). In the SHR, orchidectomy reduced plasma renin levels and expression of angiotensinogen mRNA in the kidney, whereas testosterone treatment reversed these changes (6). Stimulation of androgen receptors increased expression of mRNA for renin and angiotensinogen (6, 44). Collectively, these data suggest that androgens may play an important role in modulating the function of the RAS. Studies from SHR suggested that the development of hypertension requires the vasoactive peptide ANG II and that hypertension is due, in part, to enhanced effects of ANG II on the renal vasculature (1, 19, 26, 31). Whether sex steroids modulate the effect of ANG II in the renal vasculature remains an open question.

Accordingly, we tested the general hypothesis that androgens affect renal function and participate in hypertension development, at least in part, by increasing the renal vascular responses to ANG II. To date, the SHR is the most widely used genetic rat model of hypertension. As mentioned above, the SHR shows exaggerated renal vascular responses to ANG II. It is of interest whether this effect is generalized to other models of spontaneous hypertension. Although there are a number of models of spontaneous hypertension in rats, the New Zealand genetically hypertensive (NZGH) rat was derived from the same Wistar background as the SHR. The NZGH rat exhibits some parallels to the SHR, such as the time course and sexual dimorphism in the development of hypertension, but there are also some differences between these strains, for example, in...
hemodynamics and vascular reactivity (2, 28, 42). Therefore, the NZGH rat was chosen for these studies primarily to determine whether blood pressure and renal vascular function were affected by androgens in a manner similar to that which had been established in the SHR. Rapid acceleration of the blood pressure rise in NZGH rats occurs primarily between the 8th and 12th wk of age (42). On the basis of the time course of arterial pressure development of NZGH rat model, we chose three different age groups for study: prehypertensive stage (6–7 wk of age), hypertension development stage (11–12 wk of age), and established hypertension stage (16–17 wk of age). Collectively, we focused on the effect of androgens on the renal vascular responses to ANG II with or without androgens.

METHODS

Animals

Male NZGH rats (Charles River Laboratory, Wilmington, MA) were obtained at 4 wk of age and maintained on commercially available rat chow (Harland Teklad 2016) and tap water in an environment with a 12:12-h light-dark cycle. The rats were studied at three age groups: 6–7, 11–12, and 16–17 wk. All experiments and protocols were performed in accordance with the regulations set forth by the National Institutes of Health Council on Animal Care and were approved by the Institutional Animal Care and Use Committee at the University of South Dakota.

Castration and Chronic Testosterone Treatment

At 5 wk of age, rats were subjected to sham operation or castration. Briefly, the testes were exposed via a midline scrotal incision, the vascular supply to the testes was ligated, and the testes were removed. The scrotal sac was sutured closed. Sham operation consisted of exposing, but not removing, the testes. In addition, one group of castrated rats, designated for acute study at 16–17 wk of age only, was provided testosterone replacement. In these rats, a testosterone pellet (100 mg, 90-day release, Innovative Research of America, Windham, NH) was implanted subcutaneously immediately following the castration procedure. Plasma testosterone levels in 16- to 17-wk-old NZGH rats were assayed by ELISA (Alpco Diagnostics, Windham, NH).

Renal Responses to ANG II in Anesthetized NZGH Rats

Rats were anesthetized with an injection of Inactin (100 mg/kg ip) and placed on a temperature-regulated surgery table. A catheter was inserted into the femoral artery for measuring mean arterial blood pressure (MAP). Catheters were also placed in the left femoral and jugular vein for the infusions of ANG II and saline (4.2 ml/h), respectively. The abdomen was opened, and an ultrasonic flow probe (Transonic, Ithaca, NY) was placed on the left renal artery to measure the renal blood flow (RBF). A catheter [polyethylene-10 (PE-10)] was inserted into the left ureter for urine collection. A tracheotomy was also performed to facilitate the breathing of the rat. After 60 min of recovery, MAP and heart rate (HR) were measured via the femoral arterial catheter for a period of 60 min. Average MAP and HR were derived from the final 30 min of baseline recording from an average of three periods when the animals were calm.

Assessment of AT1 Receptor Expression

Expression of angiotensin type 1 (AT1) receptor mRNA and protein in rat renal cortex was assessed by real-time RT-PCR and Western blot analysis procedures, respectively, as described previously (38) with some modifications. A separate cohort of NZGH rats (16–17 wk of age) was euthanized, and the kidneys were harvested. One kidney was preserved with RNALater (Ambion, Austin, TX) for RT-PCR analysis and frozen, while the other was fast frozen for Western blot analysis. Analysis was performed on renal cortex punches.

For real-time RT-PCR, RNA was extracted from kidney cortex, using an RNaseasy Mini Kit (Qiagen, Valencia, CA). Prevalidated primers and probes directed toward the AT1a receptor (Rn00578456_m1) were obtained via Assays-on-Demand (Applied Biosystems, Foster City, CA). An RNA concentration-response curve was run to determine the amount of sample RNA to be used. A one-step RT-PCR reaction was then performed using the Taqman Gold RT-PCR kit (Applied Biosystems), which contained a reverse transcriptase, thermal stable DNA polymerase, RNase inhibitor, nucleotides, and buffer. The RNA template and gene-specific primers and probe were added to the kit reagents in a total volume of 25 μl. Samples were run in duplicate. The threshold cycle (Ct) was determined for the AT1a and cyclophilin genes. These raw values were then analyzed with a modified delta Ct method using a PCR data analysis program, qBase (version 1.2.2) (http://mbedgen.ugent.be/qbase/) to obtain relative quantification values.

For Western blot analysis, the renal cortex tissue samples were homogenized under nonreducing conditions in RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 2 mM EDTA, 10 mM NaF, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 20 μg/ml aprotinin). Equal amounts of protein (50 μg/well) were loaded onto a 10% acrylamide gel and subjected to SDS-PAGE electrophoresis under nonreducing conditions. After transfer to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA), the membranes were blocked in 1:1 blocking buffer (Li-Cor Biosciences, Lincoln, NE) overnight and incubated for 3 h at room temperature with a monoclonal anti-AT1 receptor antibody (TONI-1 clone), which recognizes AT1 receptors expressed in Chinese hamster ovary cells (1:800, Novus Biologicals, Littleton, CO). The membranes were then washed four times in PBS containing 0.05% Tween 20 solution and incubated at room temperature for 1 h with a fluorescent-labeled anti-mouse secondary antibody (1:800; Rockland, Gilbertsville, PA). After this incubation, the membranes were washed four times with PBS containing 0.05% Tween 20 solution, washed two times with PBS, and imaged with an Odyssey Infrared Imager (Li-Cor Biosciences). The protein bands were quantified using densitometric analysis software on the Odyssey Imager. Actin served as a loading control. Data were expressed as the ratio of AT1 band density to actin band density.

Statistical Analysis

Data are expressed as means ± SE. Baseline values for MAP, RVR, and urine output after enalapril treatment were compared within age groups only using unpaired Student’s t-test for the 6- to 7- and 11- to 12-wk old age groups and one-way ANOVA for the 16- to 17-wk-old age group. Responses to angiotensin were compared using two-way ANOVA (GraphPad Prism 4, San Diego, CA). Plasma testosterone concentrations and AT1 receptor expression levels were analyzed with one-way ANOVA (GraphPad Prism 4). Post hoc testing
was performed using Student-Newman-Keuls multiple comparisons test. Differences were reported as significant when \( P < 0.05 \).

**RESULTS**

**Baseline Values**

Baseline values for MAP, RVR, and urine output after endogenous ANG II inhibition by enalapril are shown in the figure legends for the graph of each variable. No significant differences in baseline values were observed among different surgery groups within each age period, except that RVR was higher in castrated males compared with sham-operated males at 11–12 wk of age. These baseline values for MAP, RVR, and 30-min urine output after enalapril treatment were the starting levels for the pressor, renal vascular, and urine output responses to ANG II.

As expected, there were significant age-related increases in body weight in both sham-operated and castrated NZGH rats. Castration per se did not significantly affect body weight within any age group (6–7 wk group: sham 210 ± 13 g vs. castrated 201 ± 18 g; 11–12 wk group: sham 296 ± 7 g vs. castrated 294 ± 6 g; 16–17 wk group: sham 340 ± 6 g vs. castrated 340 ± 4 g). Testosterone-treated castrated NZGH rats were heavier (399 ± 4 g) than age-matched sham-operated or castrated NZGH rats. Kidney weight also increased with age but was not different between sham and castrated NZGH rats in the 6- to 7-wk and 11- to 12-wk-old groups. In the 16- to 17-wk-old group, castrated NZGH kidney weight (0.96 ± 0.04 g) was slightly reduced compared with sham-operated rats (1.13 ± 0.02 g). Kidney weight in the castrated + testosterone treatment group (1.07 ± 0.02 g) was not different from sham-operated NZGH rats.

**Pressor Responses to ANG II in Anesthetized Male NZGH Rats**

Dose-response curves were constructed to cumulative infusions of ANG II (20, 40, and 80 ng·kg\(^{-1}\)·min\(^{-1}\)). As shown in Fig. 1, ANG II infusion caused overall dose-dependent increases in MAP in all age groups. In sham-operated NZGH rats, these pressor responses ranged between ~10 and 40% of baseline. In contrast, in castrated rats, the pressor responses to ANG II ranged between 8 and 22% of baseline. Thus castration attenuated the pressor responses to ANG II. This effect appeared to be the greatest in rats of the 16–17 wk age group.

**Renal Vascular Responses to ANG II in Anesthetized Male NZGH Rats**

As illustrated in Fig. 2, ANG II infusion caused overall dose-dependent increases in RVR in all groups. No difference in RVR responses to ANG II was observed between sham-operated and castrated male NZGH rats in both 6–7 and 11–12 wk age groups (Fig. 2, A and B). However, castration significantly attenuated the RVR responses to ANG II in the established stage of hypertension (16–17 wk of age) (Fig. 2C).

**Urine Output Responses to ANG II in Anesthetized Male NZGH Rats**

As shown in Fig. 3, ANG II infusions were associated with dose-dependent increases in urine flow. There were no significant effects of castration on urine output responses to ANG II in both 6–7 and 11–12 wk age groups (Fig. 3, A and B). On the other hand, castration significantly attenuated the urine output responses to ANG II at 16–17 wk of age (Fig. 3C).

**Effect of Testosterone Replacement on Pressor, Renal Vascular, and Urine Output Responses to ANG II in Anesthetized Male NZGH Rats**

To further assess the role of androgens in the castration-induced effects, a separate group of male rats slated for study...
at 16–17 wk of age was subjected to castration with testosterone replacement. Plasma testosterone levels were assayed by ELISA. The concentrations measured in each of the groups are as follows: sham, 1.29 ± 0.26 ng/ml; castrated, 0.13 ± 0.01 ng/ml; and castrated+testosterone replacement, 1.45 ± 0.30 ng/ml. Plasma testosterone concentration was significantly higher in the sham group compared with the castrated group.

The testosterone replacement protocol restored plasma testosterone concentrations to values comparable to the sham-operated group. As shown in Fig. 1C, exogenous androgens restored the pressor responses to ANG II, which were similar to the values found in sham-operated male rats. Similar effects were also observed with the renal vascular responses (Fig. 2C) and urine output responses to ANG II (Fig. 3C). Thus testosterone replacement reversed the castration-induced reduction in pressor, renal vascular, and urine output responses to ANG II.

Fig. 2. Renal vascular responses to ANG II (20, 40, and 80 ng·kg⁻¹·min⁻¹) in anesthetized NZGH. A: 6–7 wk of age (n = 5, Sham, baseline 11.6 ± 1.5 mmHg·ml⁻¹·min⁻¹·g kidney⁻¹; n = 5, Cas, baseline 15.7 ± 2.1 mmHg/ ml⁻¹·min⁻¹·g kidney⁻¹). B: 11–12 wk of age (n = 5, Sham, baseline 17.7 ± 2.0 mmHg·ml⁻¹·min⁻¹·g kidney⁻¹; n = 5, Cas, baseline 22.9 ± 0.8 mmHg·ml⁻¹·min⁻¹·g kidney⁻¹). C: 16–17 wk of age (n = 8, Sham, baseline 15.9 ± 2.0 mmHg·ml⁻¹·min⁻¹·g kidney⁻¹; n = 8, Cas, baseline 11.6 ± 1.0 mmHg·ml⁻¹·min⁻¹·g kidney⁻¹; n = 6, Cas+T, baseline 15.0 ± 2.0 mmHg·ml⁻¹·min⁻¹·g kidney⁻¹). Values are expressed as means ± SE. *P < 0.05 Sham vs. Cas. #P < 0.05 Cas+T vs. Cas.

Fig. 3. Urine output responses to ANG II (20, 40, and 80 ng·kg⁻¹·min⁻¹) in anesthetized NZGH. A: 6–7 wk of age (n = 5, Sham, baseline 0.88 ± 0.22 ml·30 min⁻¹·g kidney⁻¹; n = 5, Cas, baseline 0.75 ± 0.13 ml·30 min⁻¹·g kidney⁻¹). B: 11–12 wk of age (n = 5, Sham, baseline 0.25 ± 0.06 ml·30 min⁻¹·g kidney⁻¹; n = 5, Cas baseline 0.51 ± 0.16 ml·30 min⁻¹·g kidney⁻¹). C: 16–17 wk of age (n = 8, Sham, baseline 0.40 ± 0.10 ml·30 min⁻¹·g kidney⁻¹; n = 8, Cas, baseline 0.48 ± 0.11 ml·30 min⁻¹·g kidney⁻¹; n = 6, Cas+T, baseline 0.30 ± 0.02 ml·30 min⁻¹·g kidney⁻¹). Values are expressed as the means ± SE. *P < 0.05 Sham vs. Cas. #P < 0.05 Cas+T vs. Cas.
Effect of Androgens on Blood Pressure and Heart Rate in Conscious Male NZGH Rats

Castration and testosterone replacement-induced changes of pressor and renal vascular responses to ANG II were observed in the anesthetized 16- to 17-wk-old rats. To ascertain whether these treatments also might have an impact on blood pressure in conscious rats, MAP and HR were measured via indwelling catheters in 16- to 17-wk-old conscious male NZGH rats subjected to sham operation, castration, or castration with testosterone replacement. As shown in Fig. 4, castration significantly reduced the final level of MAP achieved at 16–17 wk of age by ~25 ± 1 mmHg. Testosterone replacement with subcutaneous pellets in the castrated group resulted in MAP that was not significantly different from sham-operated NZGH rats. Thus androgens appear to contribute significantly to hypertension in the NZGH model. In contrast, neither castration nor testosterone supplementation had an effect on HR (data not shown).

AT1 Receptor Expression

To determine whether changes in ANG II responses might be due to androgen modulation of AT1 receptor expression, AT1 receptor expression was assessed by RT-PCR and Western blot analysis approaches. Figure 5A shows the relative quantification values for AT1a mRNA expression obtained from the three treatment groups. There was no significant difference between these values. Figure 5B top shows an example of Western blot analysis obtained, whereas Figure 5B, bottom shows the summary data for this experiment. AT1 receptor signal was detectable in the renal cortical homogenates taken from all three groups. However, there were no statistically significant differences in blot density among the sham-operated, castrated, and castrated+testosterone-treated NZGH rats.

DISCUSSION

Males are at higher risk for hypertension than age-matched, premenopausal females. The gender-associated differences in blood pressure regulation have been clearly demonstrated in both human and animal studies (11, 31). Although the exact mechanisms are not clear, several observations indicate that androgens play an important role in gender differences of blood pressure regulation. In the current study, we show that castration attenuates the hypertension and moderates the MAP and renal vascular responses to ANG II in the NZGH rat, an animal model of spontaneous hypertension. Conversely, chronic testosterone treatment restored hypertension, as well as the pressor and renal vascular responses to ANG II in castrated male NZGH rats. Collectively, these findings suggest that androgens play an important role in modulating the development of spontaneous hypertension in the NZGH rat, possibly via effects on RVR and renal sensitivity to ANG II.

One of the most frequently used animal models of spontaneous hypertension is the SHR, and a number of previous studies have reported that androgens play an important role in facilitating the development of hypertension in this model (5, 7, 13, 15, 35, 36). Ganten et al. (15) showed that castration at ~3 wk of age reduced the final level of systolic blood pressure observed at 25 wk of age in male SHR by ~40 mmHg. Similarly, in a series of studies, Reckelhoff and colleagues (31, 34) reported that castration of male SHR at 7 wk of age reduced MAP levels recorded in mature SHR by 20–25 mmHg. In addition, the effects of surgical castration could be
mimicked by treatment with androgen receptor antagonists (15, 35). The NZGH represents another model of spontaneous hypertension that was developed independently of the SHR (42). Although the time course and sexual dimorphic pattern of hypertension development in the NZGH rats are similar to that in the SHR, the NZGH rat exhibits differences from the SHR in other cardiovascular parameters (28, 41, 42). However, to our knowledge, no previous studies had examined whether androgens influence the development of hypertension in the NZGH rat. Thus a primary goal of the present study was to determine whether manipulation of androgens could affect hypertension development in the NZGH model. In conscious NZGH rats, we observed that castrated male NZGH rats had MAP values 25 ± 1 mmHg lower than sham-operated NZGH rats. The magnitude of this change is comparable to that which has been reported previously by some investigators for the SHR. Conversely, testosterone treatment of castrated NZGH rats restored blood pressure to the values found in sham-operated groups. Thus our findings expand previous work by demonstrating that androgens contribute significantly to hypertension in a Wistar-derived spontaneous model of hypertension other than the SHR.

The mechanism(s) underlying the prohypertensive effect of androgens remain to be fully delineated. Nevertheless, the renal mechanisms remain an attractive target because of their central role in blood pressure control (18). Indeed, renal transplantation studies in experimental hypertensive animal models provided convincing evidence that the kidneys are critical in regulating sodium and water balance and play an important role in controlling the long-term level of arterial pressure (37, 39). Despite considerable interest in the role of androgens on blood pressure control, relatively few studies have explored the relationship between androgens and renal function. Androgens appeared to promote renal disease in aging normotensive and hypertensive rats (4, 14, 22). In the SHR model of spontaneous hypertension, elegant studies by Reckelhoff and colleagues (31, 34–36), demonstrated that androgens potentiate the development of hypertension via an interaction with the RAS. In addition, aging male SHR showed increased proteinuria, RVR and glomerular injury, and decreased glomerular filtration, which could be largely prevented by castration (13), suggesting important actions of androgens on the kidney. Indeed, castrated SHR showed enhanced urinary sodium excretion compared with sham-operated SHR (36). Interestingly, Ashton et al. (2) also reported a gender difference in sodium excretion in the NZGH rat model, with males excreting less sodium than females.

The mechanisms underlying the renal effect of androgens are not currently fully understood. One possibility is that androgens may affect renal vascular function. No differences in baseline renal plasma flow or RVR were reported between 19-wk-old male sham-operated SHR or castrated SHR, although female ovariectomized SHR treated with testosterone exhibited a significant increase in renal vascular resistance (36). On the other hand, castration of male SHR has been reported to attenuate the age-related increase in RVR observed in this strain (13, 33). In the present study, we also did not observe any consistent differences in baseline renal vascular resistance between sham-operated and castrated NZGH rats. It is important to note that these values were obtained after angiotensin-converting enzyme inhibition, which may have masked an effect of endogenous ANG II on renal vascular resistance. In fact, renal vasoconstrictor reactivity was increased in male compared with female dogs (25) and rats (25, 45), and enhanced renal vascular reactivity to ANG II has been previously reported in the SHR model of hypertension (26). Accordingly, we tested the effect of ANG II on the pressor and RVR responses in sham-operated and castrated NZGH rats. The acute pressor responses to ANG II were significantly greater in androgen-replete rats compared with castrated rats. In addition, we observed that the dose-RVR relationship for ANG II was displaced to the right in castrated compared with sham-operated NZGH rats. Thus removal of endogenous androgens blunted the renal vascular responses to ANG II. Conversely, testosterone replacement restored renal vascular responses to ANG II toward those observed in the sham-operated NZGH rats. Collectively, these data suggest that androgens play an important role in modulating renal vascular reactivity. This effect may represent another mechanism by which androgens potentiate hypertension in the NZGH model. Interestingly, recent studies have reported that there were sex differences in the development of ANG II-induced hypertension in conscious mice and rats with males developing higher blood pressure than females in response to chronic administration of ANG II (43, 46).

Several mechanisms may participate in the increased renal vascular responses to ANG II observed in the androgen-replete rats. First, androgens may increase the expression of the AT1 receptor in the renal vasculature. The effect of sex steroids on AT1 receptor expression remains controversial. A recent study reported that AT1 mRNA expression is greater in the kidney of male compared with female SHRs (40). However, these gender differences may be ascribed, at least in part, to an estrogenic influence in females since estrogen treatment of ovariectomized SHR or Dahl salt-sensitive rats reduced AT1 mRNA expression and ligand binding in the kidney (20, 40). In contrast, others have reported that estrogen treatment of ovariectomized rats did not affect AT1 mRNA expression but enhanced AT1 receptor binding in the kidney (3). The effect of androgens on AT1 receptor expression has been relatively unexplored. Leung et al. (27) reported that AT1 receptor protein in rat epididymis was nearly absent after castration, whereas its expression was restored to the control level when the castrated rats were treated with testosterone. On the other hand in the present study, we observed that neither castration nor castration + testosterone replacement significantly influenced AT1 receptor mRNA or protein expression in homogenates of renal cortex punches. Admittedly, our approach cannot rule out potential regional changes in AT receptor expression (3) nor does our approach assess potential androgen-induced changes in receptor affinity or binding capacity as has been reported for estrogen (20, 40). Nevertheless, these data suggest that functional differences in response to ANG II infusion among the three groups of NZGH rats are not related to overall changes in expression of the receptor protein per se. Alternatively, androgen modulation may also occur at the level of postreceptor signaling. Consistent with the latter possibility, signaling transduction pathways downstream from the AT1 receptor, such as the PKC and Rho kinase pathways, exhibit gender-associated differences in expression or activity in rats (8, 23). Third, androgens may potentiate the interaction between the RAS and the sympathetic nervous system. Increased
activities of these two systems contribute to the development of hypertension in both human and animal models (6, 10). As described by DiBona (9), there are both peripheral and central interactions between the RAS and the renal sympathetic nerves in control of renal function. Each of these possibilities represents avenues for future investigation. In any case, the potentiation of vascular responses to ANG II appears to be a generalized effect of androgens, because we found that the acute pressor responses to ANG II, as well as the RVR responses were significantly attenuated in castrated males compared with sham-operated males. In addition, chronic testosterone treatment in castrated males restored the pressor responses to ANG II in NZGH of 16–17 wk of age. The increased systemic pressor responses to ANG II mediated by androgens may reflect the overall direct effects on systemic vascular reactivity, increased cardiac contractility, facilitation of sympathetic tone, and/or amplified noradrenergic neurotransmitter release, which may contribute to the androgen-induced hypertension development in the NZGH rat model.

We also found that ANG II caused a dose-dependent increase of urine output in all age groups. At first glance, this response may appear somewhat paradoxical, because, at low concentrations, ANG II stimulates sodium reabsorption by sodium-hydrogen exchange in the proximal tubule. Nevertheless, others have also reported an increase in urine volume after administration of ANG II in anesthetized dogs (29). ANG II is known to be a potent vasoconstrictor in the kidney, and the sensitivity to ANG II was reported to be much higher in the efferent arterioles compared with the afferent arterioles. Thus ANG II will have the potential to increase glomerular capillary pressure, even though there is a reduction in RBF. In this setting, glomerular filtration rate (GFR) and urine output would be predicted to be increased. Indeed, intravenous infusion of ANG II into conscious dogs reduced RBF, but increased GFR and urine output (16), which also affirms our results. In addition we demonstrated that androgens affect the urine output responses to ANG II. Castration attenuated urine output responses to ANG II in the 16- to 17-wk-old NZGH rats, while testosterone replacement in castrated males restored these responses. Insofar as the urine output response may be an index of renal efferent arteriolar responses to ANG II, these findings suggest that androgens augment efferent arteriolar responsiveness to ANG II. This possibility is consistent with recent reports that androgens increase hypertension-induced glomerular damage (13, 22).

Perspectives

The present study showed that endogenous androgens modulate blood pressure development and renal vascular and excretory function in the NZGH rat. These studies expand previous work by demonstrating that androgen modulation of blood pressure and renal function are present in a model of spontaneous hypertension in addition to the SHR. The parallels between the SHR and NZGH, spontaneous hypertensive models that were developed independently, suggest that the interaction between androgens and angiotensin may represent a fundamental mechanism involved in hypertension development and/or maintenance. Future studies are needed to ascertain the point(s) of interaction between these two hormonal systems.

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REFERENCES


