Androgen-mediated induction of the kidney arachidonate hydroxylases is associated with the development of hypertension

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Nakagawa, Kiyoshi, Jackleen S. Marji, Michal L. Schwartzman, Michael R. Waterman, and Jorge H. Capdevila. Androgen-mediated induction of the kidney arachidonate hydroxylases is associated with the development of hypertension. Am J Physiol Regul Integr Comp Physiol 284: R1055–R1062, 2003. First published January 16, 2003; 10.1152/ajpregu.00459.2002.—Hypertension is a leading cause of cardiovascular, cerebral, and renal disease morbidity and mortality, and epidemiological evidence suggests a role for sex-dependent mechanisms in the pathophysiology of hypertension. We show here that treatment of rats with 5α-dihydrotestosterone increases the activity of the kidney arachidonate ω-1 hydroxylase and the biosynthesis of 20-HETE (165 and 177% of control untreated male and female rats, respectively) and raises the systolic blood pressures of male and females rats by 46 and 57 mmHg, respectively. These androgen effects are associated with an upregulation in the kidney levels of CYP 4A8 mRNA and a decrease in CYP 4A1 transcripts. Dissected renal microvessels, the target tissue for most of the prohypertensive actions of 20-HETE, show an androgen-dependent upregulation of vascular CYP 4A8 mRNA and a fourfold increase in 20-HETE synthase activity. We propose that androgens regulate renal function and systemic blood pressure through a combination of transcriptional and hemodynamic mechanisms that are ultimately responsible for the regulation of renovascular tone and function.

P-450 eicosanoids; androgens; CYP 4A8

A now considerable body of evidence shows that microsomal P-450s (CYPs) participate in the in vivo metabolism of arachidonic acid (AA) and that products of this P-450-catalyzed pathway are in vitro modulators of renal ion transport and vascular reactivity and may be involved in the regulation of systemic blood pressure (3, 4, 22, 29). During catalytic turnover, the P-450 monooxygenase(s) metabolize AA via ω-1 hydroxylation (AA ω-1 hydroxylase) to 19- and 20-HETE and/or epoxidation (AA epoxide) to 5,6-, 8,9-, 11,12-, or 14,15-epoxyeicosatrienoic acids (EETs) (4, 5). Recombinant CYPs 4A1, 4A2, 4A8, and to a lesser extent 4A3, support the hydroxylation of AA to either 20-HETE or to mixtures of 19- and 20-HETE (4, 16, 19, 31). All four CYP 4A isoforms are expressed in the male rat kidney (4, 16, 19, 31), with CYPs 4A1 and 4A2/4A3 characterized as the predominant microsomal AA ω-1 hydroxylases (16). The transcriptional regulation of rat CYPs P-450 4A1 and 4A2/4A3 by the peroxisomal proliferator activated receptor-α (45), as well as the sexually dimorphic, testosterone-sensitive expression of rat CYPs 4A2 and 4A8 and of murine Cyp 4a12, has been reported (18, 20, 40, 42).

Studies with the spontaneously hypertensive-Wistar Kyoto (SHR/WKY) rat model of spontaneous hypertension, an extensively characterized model of genetic hypertension (29), suggest a role for renal CYP 4As in the pathophysiology of the disease (3, 29). Thus based on 1) temporal associations between the increases in blood pressure, renal CYP 4A expression and 20-HETE formation (3, 29, 2) experimental manipulations of CYP 4A activity and/or expression (29, 32, 43), and 3) the renovascular effects of 20-HETE (3, 22, 29), a prohypertensive role was proposed for this eicosanoid and for the CYP 4A AA ω-1 hydroxylases (29). More recently, the characterization of Cyp 4a14(−/−) mice showed the animals’ hypertensive phenotype was sexually dimorphic and associated with an increased kidney Cyp 4a12 AA ω-1 hydroxylase activity (20).

Prevalence, complexity, and multiple medical and socioeconomic consequences make hypertension a major health challenge, and, notwithstanding extensive research, its early diagnosis and treatment remains mostly symptomatic and empirical. The study of the molecular causes of hypertension is complicated by a variety of etiologies and the frequent coexistence of additional pathological conditions. Nonetheless, extensive genetic segregation and linkage analyses indicate multifaceted factors as responsible for a significant component of human essential hypertension (13–15, 25, 33). Furthermore, gender differences in incidence and severity have also suggested the involvement of a sex-dependent mechanism in the pathogenesis of human hypertension (1, 10, 14, 25, 27, 34, 35). We report here that treatment of rats with 5α-dihydrotestosterone...
(DHT), a metabolic stable androgen, induces the expression of kidney CYP 4A8, increases the capacity of the renal microcirculation to synthesize prohypertensive 20-HETE (3, 4, 22, 29), and causes systemic hypertension.

MATERIALS AND METHODS

All animal experiments were done following the American Physiological Society’s guiding principles and in accordance with the Institute for Laboratory Animal Research’s Guide for Care and Use of Laboratory Animals. Sprague-Dawley rats (280–350 g) (Harlan Sprague Dawley) were treated by daily injections (≤50 μl) of either corn oil or a suspension of testosterone (TST) or DHT in corn oil (40–120 mg/kg body wt) and allowed free access to water and standard rat chow (Ralston Purina Mills, St. Louis, MO). Blood pressures were determined by the tail cuff method at an ambient temperature of 30°C. Trained animals were allowed to become familiar with the environment and were maintained in the measuring chamber for at least 1 h before measurements. Blood pressures were obtained after four consecutive readings with values within ±5% of their mean. Measurements were done using a blood pressure analyzer instrument (model 178, ITLC, Life Sciences, Woodlands Hills, CA) following the manufacturer’s instructions.

Isolation of microsomal fractions and renal microvessels. Anesthetized (Nembutal, 5 mg/kg ip) rats were killed and their kidneys were removed and freed of connective tissue; microsomal fractions were isolated by differential centrifugation (6). Renal microvessels were dissected exactly as described (28, 41). Briefly, the abdominal aorta (below the renal arteries) of pentobarbital sodium-anesthetized rats was cannulated, the superior mesenteric and aorta above the renal arteries were ligated, and the kidneys were perfused first with ice-cold Tyrode buffer and then with Tyrode buffer containing 6% albumin and 1% Evan’s blue solution. Kidneys were removed, sliced into hemisections, and pushed through a 180-μm mesh screen. The vascular trees trapped on the screen were digested in Tyrode buffer containing 1 mg/ml collagenase, 1 mg/ml soybean trypsin inhibitor, 1 mg/ml DTT, 1 mg/ml albumin, and the following: 10 μM Na-succinate, 10 μM l-alanine, 6 mM sodium lactate, 2 mM sodium l-glutamate. Vessels were incubated 60 min at 37°C with shaking and superfusion with O2 gas. The tissue suspension was filtered (75 μm, nylon filter), and the digested tissue was resuspended in Tyrode buffer and rinsed several times. Microvessels, primarily preglomerular arteries, were collected, free of tubular components, by microdissection under a stereomicroscope (Fig. 1).

Enzymatic studies. Microsomal incubations with [1-14C]arachidonic acid (2–8 μCi/μmol, 70–100 μM, final concentration) were performed exactly as described (5, 6). Timed aliquots were extracted with acidified ethyl ether and analyzed by reversed-phase HPLC with online β-detection (6). Initial velocities (in pmol of product formed·mg of protein−1·min−1) were calculated from the linear portions of product concentration vs. incubation time plots.

Nucleic acid hybridizations and immunoelectrophoresis. Total kidney RNAs were isolated by the guanidinium isothiocyanate/CsCl method (16), electrophoresed in 1.2% agarose gels containing 0.2 M formaldehyde, transferred to nitrocellulose membranes, and hybridized to the following [32P]-labeled sequence specific probes: 1) P-450 4A1: 542 bp extending from nucleotide 1558 to 2100 (30), 2) P-450s 4A2/4A3: 486 bp extending from nucleotide 1514 to 2000 (30), and 3) P-450 4A8: 636 bp, extending from nucleotide 1564 to 2200 (30). Probes were labeled with [32P]dCTP using a Nick Translation kit (Pharmacia Biotechnology, Uppsala, Sweden) following the manufacturer’s instructions. Hybridizations were done at 42° in Hybrisol (Oncor, Gaithersburg, MD), and, after several washes in SSC/SDS mixtures, the membranes were exposed to X-ray film.

Immunoelectrophoresis (10% wt/vol acrylamide, 100 × 60 × 1 mm slabs; PVDF membranes) were done as published (16). Antigen-antibody reactions were detected using a SuperSignal Substrate Western Blotting kit (Pierce, Rockford, IL) and the manufacturer’s instructions. Polyclonal anti-CYP 4A1 and 4A2 antibodies were raised and purified as described (16). CYPs 4A1 and 4A2 were expressed and purified as reported (16).

RESULTS AND DISCUSSION

The prohypertensive properties of 20-HETE were deduced from studies in isolated renal cells and perfused kidney preparations (3, 22, 26, 29) showing that the eicosanoid blocked Na+ and K+ transport in medullary thick ascending limb of Henle cells, inhibited medullary K+ channels, and caused the vasocostriction of renal afferent arterioles (3, 22, 26, 29). These studies indicated a role for 20-HETE in pressure natriuresis and urinary Na+ excretion (22, 26, 29). Chemical or antisense nucleotide inhibition of kidney CYP 4A activity or biosynthesis reduced 20-HETE formation and normalized the blood pressures of hypertensive SHR (32, 43). Moreover, kidney microsomes from hypertensive Cyp 4a14(−/−) mice generated 20-HETE at rates higher than microsomes from Cyp 4a14(+/+) mice, a response associated with the androgen-mediated induction of Cyp 4a12 in Cyp 4a14(−/−) mice (20).

To characterize the role of androgens in the regulation of the microsomal AA monooxygenase activity and 20-HETE biosynthesis, kidney microsomes from control and DHT-treated rats were incubated with AA in the presence of NADPH. As reported (5), microsomes
from control male and female rats metabolize AA to 19-and 20-HETE as the predominant products and to mixtures of 5,6-, 8,9-, 11,12-, and 14,15-EETs (Table 1). Regardless of gender, 20-HETE is the principal product of renal AA ω ω-1 hydroxylase (Table 1) (5, 6). Treatment with DHT had only minimal effects on the overall rates of AA metabolism by microsomes isolated from 2 (controls) or 3 (DHT-treated) male or female rats (Table 2) (4). Importantly, androgen-mediated increases in renal 20-HETE synthase activity (Table 1) occurred in the presence of DHT-induced reductions in renal CYP 4A1 mRNA levels (Fig. 2), 2 moderate decreases (males) or increases (females) in renal CYP 4a2/4a3 transcripts (Fig. 2), and 3 increases in kidney CYP 4A8 transcripts that are gender independent (Fig. 2). Taken together, the results of the Northern analysis suggested that the up-regulated expression of CYP4A8 was responsible for the increased AA ω-1-hydroxylase activity of microsomes isolated from DHT-treated rats. The sexually dimorphic, TST-sensitive expression of kidney CYP 4A2 and 4A8 mRNAs was reported to be both testosterone/gender dependent and independent (18, 40, 42). High degrees of CYP 4A sequence homology, limited selectivity of the DNA probes (18, 40, 42), and differences in rat strain and/or age (16, 18, 40, 42) are likely responsible for these apparently conflicting results. Nonethe-

**Table 1. Metabolism of AA by microsomes isolated from the kidneys of control and 5-α-DHT-treated rats**

<table>
<thead>
<tr>
<th>Source of Microsomes</th>
<th>ω-1 Hydroxylase</th>
<th>Epoxigenase</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control males</td>
<td>16 ± 2</td>
<td>91 ± 2</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>DHT-treated males</td>
<td>17 ± 5</td>
<td>150 ± 5</td>
<td>73 ± 8</td>
</tr>
<tr>
<td>Control females</td>
<td>23 ± 2</td>
<td>131 ± 2</td>
<td>116 ± 3</td>
</tr>
<tr>
<td>DHT-treated females</td>
<td>23 ± 4</td>
<td>229 ± 2</td>
<td>22 ± 1</td>
</tr>
</tbody>
</table>

Values shown (nmol of product·min⁻¹·mg of protein⁻¹) are averaged initial velocities ± SE calculated from at least 6 different experiments performed using microsomes isolated from at least 3 different groups of rats. Animals were injected daily (ip) with a suspension of 5α-DHT in corn oil (DHT treated) or corn oil (control). After 2 wk, kidney microsomes were isolated, incubated with [1-¹⁴C]arachidonic acid (AA), and the reaction products were extracted and quantified as described in MATERIALS AND METHODS.

To explore the CYP P-450 isoform specificity of the DHT-induced changes in enzyme activity, total kidney RNA was hybridized to probes specific for CYPs 4A1 and 4A8 and to a probe that recognizes both CYP 4A2 and 4A3. The high degree of nucleotide sequence homology displayed by the CYP 4A2 and 4A3 mRNAs (97% sequence identity) (16, 30) precludes their individualization by nucleic hybridization techniques. As shown in Fig. 2, the adult male rat kidney expresses CYPs 4A2/3, 4A8, and 4A1 transcripts (16, 19, 31), whereas in females, CYP 4A1 and 4A8 appear to be the predominant kidney CYP 4A mRNA, and CYP 4A2/4A3 is nearly undetectable (Fig. 2) (16). In males and females, DHT caused a marked decrease in the concentration of CYP 4A1 transcripts and the upregulation of the organ CYP 4A8 mRNA levels (Fig. 2). Conversely, the kidney levels of CYP 4A2/4A3 transcripts are regulated by DHT in a gender-specific fashion, i.e., slightly downregulated in males and upregulated in females (Fig. 2). In summary, DHT shows similar qualitative effects on the male and female CYP P-450 4A8 and 4A1 genes, and its effects in CYP 4A2/4A3 appear to be gender specific (Fig. 2). Furthermore, the androgen-mediated increase in microsomal 20-HETE synthase shown in Table 1 occurred in the presence of DHT-induced reductions in renal CYP 4A1 mRNA levels (Fig. 2). Moderate decreases (males) or increases (females) in renal CYP 4a2/4a3 transcripts (Fig. 2), and 3 increases in kidney CYP 4A8 transcripts that are gender independent (Fig. 2). Taken together, the results of the Northern analysis suggested that the up-regulated expression of CYP4A8 was responsible for the increased AA ω-1-hydroxylase activity of microsomes isolated from DHT-treated rats. The sexually dimorphic, TST-sensitive expression of kidney CYP 4A2 and 4A8 mRNAs was reported to be both testosterone/gender dependent and independent (18, 40, 42). High degrees of CYP 4A sequence homology, limited selectivity of the DNA probes (18, 40, 42), and differences in rat strain and/or age (16, 18, 40, 42) are likely responsible for these apparently conflicting results. Nonethe-

**Table 2. Metabolism of AA by microsomes isolated from the livers of control and 5-α-DHT-treated rats**

<table>
<thead>
<tr>
<th>Source of Microsomes</th>
<th>ω-1 Hydroxylase</th>
<th>Epoxigenase</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control males</td>
<td>0.30 ± 0.01</td>
<td>0.95 ± 0.02</td>
<td>1.25 ± 0.06</td>
</tr>
<tr>
<td>DHT-treated males</td>
<td>0.30 ± 0.01</td>
<td>0.80 ± 0.01</td>
<td>1.10 ± 0.08</td>
</tr>
<tr>
<td>Control females</td>
<td>0.31 ± 0.03</td>
<td>0.58 ± 0.05</td>
<td>0.89 ± 0.08</td>
</tr>
<tr>
<td>DHT-treated females</td>
<td>0.37 ± 0.06</td>
<td>0.63 ± 0.10</td>
<td>1.01 ± 0.04</td>
</tr>
</tbody>
</table>

Values shown (nmol of product·min⁻¹·mg of protein⁻¹) are averaged initial velocities ± SE calculated from at least 6 different experiments, using microsomes isolated from 2 (controls) or 3 (DHT-treated) different groups of animals. Animal treatment, incubation conditions, and product extraction, resolution, and quantification were done as described in the legend to Table 1 and in MATERIALS AND METHODS.

**Fig. 2. Effects of androgen administration on the regulation of the renal CYP 4A isoforms: equal amounts of total kidney RNA, extracted from 3 different male and female control (C) and DHT-treated (D) rats were mixed, loaded into formaldehyde containing 1.2% agar gels (10 μg each), fractionated by electrophoresis, transferred to nitrocellulose membranes, and hybridized to CYP 4A isoform specific 32P-labeled DNA probes. After several high-stringency washes, the membranes were exposed to X-ray films for 13, 2, and 3 h for CYP 4A1, 4A2, and 4A8, respectively. RNA loadings were normalized using a rat β-actin probe.**
that of vehicle-treated controls. The administration of androgens (40 mg/animal) caused marked increases in the systemic blood pressure of male rats (Fig. 4). Compared with controls, the systolic, diastolic, and mean arterial blood pressures (MABP) of TST- and DHT-treated rats increased by ~17 and 29; 19 and 46; and 15 and 21 mmHg, respectively (Fig. 4). Similarly, the administration of DHT to female rats led to substantial increases in arterial systolic, and MABP (57 and 33 mmHg, respectively) (Fig. 5). As it was observed in mice (20), the MABP of control and hypertensive DHT-treated females is significantly lower than those of comparable males (Figs. 4 and 5). It is of interest that sexual dimorphism in the incidence and severity of hypertension is a frequent feature of the most common forms of human hypertension, with premenopausal females exhibiting systemic blood pressures that are significantly lower than males (1, 11, 27, 34, 35). The pressure effects of DHT were J time dependent in that blood pressures began to increase after the first week of treatment, reached a maximum at the end of the second week, and remained constant for at least one additional week (not shown) and 2) dose dependent in that they become apparent with daily doses of DHT ≥20 mg and reached a maximum between 40 and 50 mg·animal⁻¹·day⁻¹ (not shown). Substantial increases in plasma DHT concentrations were required to increase the activity of the renal 20-HETE synthase and to raise blood pressures to the levels shown in Figs. 4 and 5 (Table 3). Regardless of gender, maximal increases in enzyme activity and blood pressure were observed when the plasma DHT levels were between 150 to 250 ng DHT/ml plasma (Table 3). Whereas similar androgen-mediated pressure and CYP 4A met-

less, our results confirm those of Stromstead et al. (40) and are in agreement with data obtained with mice, where Cyp 4a12, the murine homologue of CYP 4A8, is expressed in the male but not in the female kidney, is androgen responsive in males and females, and is up-regulated in hypertensive Cyp 4a14(−/−) null mice (20).

Immunoelectrophoresis of kidney microsomes from DHT-treated and untreated rats using a CYP 4A1 antibody unreactive toward CYP 4A2/4A3 (16) and a CYP4A2 antibody cross-reactive toward CYP 4A3 and 4A1 (16) showed that DHT caused a decrease in CYP 4A1 levels in males and females, and of 4A2/4A3 in males, confirming their downregulation by the androgen (Fig. 3). Also, these studies confirmed published results (16) and showed that female kidney microsomes contain nearly undetectable amounts of CYP 4A2/4A3-immunoreactive proteins, even after the apparent transcriptional upregulation of the CYP 4A2 gene by DHT (Figs. 2 and 3). This dissociation between CYP 4A2 mRNA expression and microsomal CYP 4A2/4A3 protein levels was reported a few years ago and was attributed to a posttranscriptional control of CYP 4A2/4A3 biosynthesis in the female kidney (16). The androgen-mediated increase in microsomal metabolism (Table 1) and kidney expression of CYP 4A8 mRNAs (Fig. 2), in conjunction with decreases in the levels of microsomal CYP 4A1- and 4A2/4A3-immunoreactive proteins (Fig. 3), provides further support to the proposition that the DHT-mediated induction of the kidney microsomal CYP 4A8 is responsible for most of the increased 20-HETE biosynthetic capacity of the organ (Table 1). However, since the immunoreactivity of CYP 4A8 toward the CYP 4A1 and 4A2 antibodies is unknown, its contribution to renal AA hydroxylation is yet to be defined unequivocally. Finally, and as reported (19), purified recombinant CYP 4A8 catalyzed the metabolism of AA to generate 19- and 20-HETE in 1:10 molar ratio and at a rate of 0.40 ± 0.08 min⁻¹ (n = 3).

To analyze the functional consequences of the androgen-induced upregulation in renal 20-HETE synthase expression and activity, adult male rats were treated with TST or its metabolically stable analog DHT, and, after 2 wk, their blood pressures were compared with...
DHT-treated rats were not significantly different from controls (P > 0.09). Evident at androgen plasma levels that are substantially different from the from controls: values in mmHg and are averages of DHT-treated rats, respectively. Diastolic pressures of DHT-treated rats were not significantly different form controls (P < 0.0001 and < 0.005, for the systolic and mean arterial blood pressures of DHT-treated rats, respectively). Diastolic pressures of 0.04 0.5 0.04 0.5 0.0001 and 1 wk after, were injected daily (ip) with a suspension of DHT in corn oil (DHT), and the levels of plasma androgens in samples were collected before treatment and 1 and 2 wk after, were determined using a commercially available RIA kit (Research Diagnostics Inc. Pleasant Hills, NJ). 39). In situ hybridization data showed abundant and selective expression of 4a12 transcripts in the renal cortex and medullary rays of hypertensive Cyp 4a14(−/−) null mice (20). The presence of this 20-HETE synthase in close physical contact with the glomerular microcirculation suggested that paracrine or endocrine mechanisms could be responsible for the proposed vasoconstrictor functions of 20-HETE, an issue that, for technical reasons, could not be addressed in mice. To establish whether the target tissue of the proposed 20-HETE prohypertensive effects, i.e., the renal vasculature expresses an androgen-sensitive AA ω-hydroxylase activity, we dissected vessels from the kidneys of control and DHT-treated rats and confirmed their vascular purity by phase contrast microscopy (Fig. 1). Although the microdissection technique used yields mostly preglomerular arteries (28, 41), the relative contents of afferent, interlobular, or arcuate arterioles was not determined, and efforts were directed toward a complete separation of the vascular and renal tissues. Microvessels from control rats metabolize AA to mixtures of ω-ω-alcohols and EETs, with 20-HETE as their major product (Fig. 6). Animal treatment with more direct effects of the androgen in gene transcription. Finally, the reasons for the high androgen requirement and the delayed response of the kidney androgen receptor are unknown, but they could serve to provide effective protection against acute variation in the plasma androgen concentrations.

The known biochemical and functional segmentation of the nephron facilitates associations between functional roles and protein expression patterns. The distribution of rat CYP 4A isoforms in dissected segments of the rat nephron has been inferred from measurements of enzymatic activity or PCR amplification (23, 39). In situ hybridization data showed abundant and selective expression of 4a12 transcripts in the renal cortex and medullary rays of hypertensive Cyp 4a14(−/−) null mice (20). The presence of this 20-HETE synthase in close physical contact with the glomerular microcirculation suggested that paracrine or endocrine mechanisms could be responsible for the proposed vasoconstrictor functions of 20-HETE, an issue that, for technical reasons, could not be addressed in mice. To establish whether the target tissue of the proposed 20-HETE prohypertensive effects, i.e., the renal vasculature expresses an androgen-sensitive AA ω-hydroxylase activity, we dissected vessels from the kidneys of control and DHT-treated rats and confirmed their vascular purity by phase contrast microscopy (Fig. 1). Although the microdissection technique used yields mostly preglomerular arteries (28, 41), the relative contents of afferent, interlobular, or arcuate arterioles was not determined, and efforts were directed toward a complete separation of the vascular and renal tissues. Microvessels from control rats metabolize AA to mixtures of ω-ω-alcohols and EETs, with 20-HETE as their major product (Fig. 6). Animal treatment with

<table>
<thead>
<tr>
<th>Time</th>
<th>Testosterone</th>
<th>5α-Dihydrotestosterone</th>
<th>5α-Dihydrotestosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Control</td>
<td>2.5 ± 0.4</td>
<td>≤0.04</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>1 wk</td>
<td>4.0 ± 0.8</td>
<td>3.9 ± 2.0</td>
<td>165 ± 32</td>
</tr>
<tr>
<td>2 wk</td>
<td>6.4 ± 3.0</td>
<td>3.8 ± 1.7</td>
<td>236 ± 47</td>
</tr>
</tbody>
</table>

Values (in ng/ml of plasma) are averages ± SE obtained from at least 5 different animals. Rats were injected daily (ip) with a suspension of DHT in corn oil (DHT), and the levels of plasma androgens in samples were collected before treatment and 1 and 2 wk after, were determined using a commercially available RIA kit (Research Diagnostics Inc. Pleasant Hills, NJ).

Fig. 5. Effects of androgen treatment on the systemic blood pressure of female rats: female rats (280–320 g body wt) were treated daily with intraperitoneal injections of either corn oil (control) or a suspension of DHT in corn oil. After 2 wk, the animals systemic blood pressures were measured as described in MATERIALS AND METHODS. Values are in mmHg and are averages ± SE obtained from 4 control or 6 DHT-treated rats. Significantly different from the from controls: P < 0.0001 and < 0.005, for the systolic and mean arterial blood pressures of DHT-treated rats, respectively. Diastolic pressures of DHT-treated rats were not significantly different form controls (P < 0.09).

Fig. 6. Metabolism of arachidonic acid (AA) by microvessels isolated from the kidneys of control (B) and DHT-treated (A) male rats. Renal microvessels (20 mm each) were isolated from control and DHT-treated rats, and incubated with [1-14C]AA (50 μCi/μmol, 30 μM final concentration) in the presence of indomethacin (10 μM, final concentration). After 20 min at 35°C, organic soluble products were extracted and resolved by reversed-phase HPLC with online β-detection. Shown in the radiochromatograms are the HPLC retention times of synthetic 20-HETE, DHETs, and EETs. Shown are the results of 1 of 2 experiments performed using different animal groups and which yielded comparable results.
DHT increased the 20-HETE biosynthetic capacity of the vessels by approximately fourfold and reduced the AA epoxyenase activity to a minimum (Fig. 6). Nucleic acid hybridization of total RNA from these microvessels using a CYP 4A8 specific probe showed that DHT caused the upregulation of the CYP 4A8 gene (Fig. 7). Finally, semiquantitative RT-PCR analysis confirmed the hybridization data (Fig. 7) and provided further support to the idea that renal microvasculature expresses an active, androgen-sensitive CYP 4A8 20-HETE synthase.

Extensive functional studies have documented the vasoconstrictor effects of synthetic 20-HETE in the renal microcirculation (3, 4, 22, 26, 29), and the presence of an AA ω/ω-1 hydroxylase in isolated rat kidney microvessels has been reported (28, 41). Some of the prohypertensive effects of 20-HETE have been attributed to increased afferent glomerular arteriolar resistance and the associated changes in pressure-natriuresis, resulting from increases in its local production (22, 26, 29). Moreover, hypertensive Cyp 4a14(−/−) mice showed increased renal vascular resistance and impaired autoregulatory efficiency (20). We conclude that the androgen treatment caused an increase in the formation of vasoconstrictor, prohypertensive 20-HETE in its target tissue, the renal microcirculation, and that vasoconstriction, and attendant changes in renal resistance are responsible for the observed increases in systemic blood pressures. Whereas the proposed mechanism is in agreement with the known functional properties of 20-HETE, as well as with its proposed roles in renal hemodynamics (3, 4, 22, 26, 29), alternate, P-450-independent effects of the androgens in the regulation of systemic blood pressure cannot be ruled out.

Similarities between these results and components of the hypertensive phenotype of SHR rats and Cyp 4a14 knockout mice strongly suggest that, as proposed earlier (29), kidney P-450 4A isoforms participate in the control of systemic blood pressure (20). For example, blood pressures in hypertensive female SHR are significantly lower than in males (10, 36–38), castration reduces the MABPs of hypertensive SHR by 30–40 mmHg (11, 36–38), and the normotensive effects of castration in the male SHR are reversed by TST replacement (11, 34–38). Furthermore, androgen-induced changes in the pressure-natriuresis relationship of SHR have been reported (36). We now extend those studies and show that the androgen-sensitive component of the SHR hypertensive phenotype is in fact associated with the male hormone-mediated regulation of CYP 4A8 and with an increased biosynthesis of prohypertensive 20-HETE in the renal microcirculation.

The biochemical and functional data presented here, in conjunction with the phenotypic characterization of Cyp 4a14(−/−) mice (20), provide a conceptually different and innovative approach to the study of systemic blood pressure regulation. Figure 8 summarizes our view of the mechanism involved in the pressure response to changes in androgen plasma levels and introduces a combination of transcriptional and hemodynamic elements as the key components of the pressure response. We postulate that unique CYP 4A isoforms (Cyp 4a14 in mice) catalyze the formation of a yet to be characterized mediator that controls circulating androgen levels (20) (Fig. 8). Studies with the Cyp 4a14(−/−) mouse showed that 1) the disruption of this gene does not alter the microsomal metabolism of TST (20) and 2) none of the murine Cyp 4a isoforms metabolizes TST (20). Increased plasma androgen levels induce CYP 4A8 (Cyp 4a12 in mice) gene expression (by acting either directly at the gene loci or through a signaling pathway) and cause attendant increases in renovascular 20-HETE biosynthesis (Fig. 8). Systemic hypertension results from alterations in nephron hemodynamics, including afferent arteriole autoregulation and renal blood flow (22), caused by increased levels of vasoconstrictor 20-HETE (22, 26, 29) (Fig. 8). Additionally, the observed androgen-mediated reductions in kidney epoxyenase activity (Table 1) could further accentuate the hypertensive response by decreasing the biosynthesis of antihypertensive EETs (3–5, 7, 22, 29). This interpretation is in agreement with the known renal effects of these eicosanoids and their
proposed pathophysiological roles (3–5, 7, 22, 26, 29) and suggest transcriptional events as additional targets for future therapeutic approaches.

Inasmuch as epidemiological data suggest associations between gender, sex hormones, and the pathophysiology of human hypertension, these studies may have important clinical implications. CYP 4A11, the human homologue of rat and mouse CYP 4A8 and 4A12, respectively, is an AA ω/ω-1 hydroxylase expressed in the human kidney (21) and, therefore, a novel and attractive candidate gene for future studies of the genetic and molecular basis of human hypertension. It is now widely accepted that the identification and characterization of genes involved in the pathogenesis of hypertension will advance our understanding of the disease and its causes, but, more importantly will lead to the development of novel and rational targets for clinical diagnosis and intervention.

The authors are grateful to National Institute of Diabetes and Digestive and Kidney Diseases Grants 28366 (to J. Capdevila) and 28350 (to M. Waterman) and National Heart, Lung, and Blood Institute Grant 34300 (to M. Schwartzman) for generous support of the studies. Blood pressure measurements were carried out at the Vanderbilt University Small Animal Physiology Core Laboratory, a shared facility supported, in part, by an NCI Center Grant (CA 68485).

REFERENCES