Androgens augment proximal tubule transport

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Quan, Albert, Sumana Chakravarty, Jian-Kang Chen, Jian-Chun Chen, Samer Loleh, Neel Saini, Ray C. Harris, Jorge Capdevila, and Raymond Quigley. Androgens augment proximal tubule transport. Am J Physiol Renal Physiol 287: F452–F459, 2004.—The proximal tubule contains an autonomous renin-angiotensin system that can synthesize and secrete angiotensin II (AII). Intraluminal AII is present in proximal tubular fluid at levels 100-fold higher than that found in plasma, thus indicating its robust local production within the proximal tubule (4, 5, 29, 36, 40). The components of the renin-angiotensin system found within the proximal tubule include angiotensinogen and its mRNA, renin activity, renin mRNA, brush-border membrane angiotensin-converting enzyme, and angiotensin II receptors (6, 7, 11, 12, 20, 40). Intraluminal angiotensin II is present in proximal tubular fluid at levels 100-fold higher than that found in plasma, thus indicating its robust local production within the proximal tubule (4, 5, 29, 36, 40).

Using in vivo microperfusion in the hydropenic rat, it has been shown that this intraluminal angiotensin II regulates proximal tubule transport (30). Luminal perfusion of an angiotensin-receptor antagonist (losartan) or of an angiotensin-converting enzyme inhibitor (enalaprilat) decreased the rate of proximal tubule volume reabsorption by 35–40% (30). The decrease in transport observed with luminal enalaprilat is completely reversed with addition of luminal 10−8 M angiotensin II (27). These studies are consistent with a role for intraluminal angiotensin II to directly stimulate proximal tubule transport.

Androgens have been found to augment the proximal tubule renin-angiotensin system. Castration of male rats lowers renal angiotensinogen mRNA levels by 60% (15). Renal angiotensinogen mRNA levels rise to normal in castrated weanling male rats when given replacement testosterone (15). In addition, castration of male mice lowers activity of the Na/H exchanger, a brush-border membrane transporter regulated by angiotensin II (23). Subsequent testosterone replacement raises and normalizes Na/H activity levels (23). These data indicate that androgens might stimulate the proximal tubule renin-angiotensin system and, thereby, proximal tubule transport under angiotensin II regulation. However, the effect of androgens on proximal tubule transport remains unknown. The purpose of the present study was to examine the effect of androgens on proximal tubule transport and the role of intraluminal angiotensin II as a mediator of androgen regulation of proximal tubule transport.

MATERIALS AND METHODS

Preparation of Animals

Male Sprague-Dawley rats weighing between 200 and 250 g were used for this study. Rat preparation and the in vivo microperfusion described below have been previously described in detail (30, 31). Briefly, all animals were allowed free access to food and water before anesthesia with intraperitoneal Inactin (100 mg/kg). Rats were placed on a servo-controlled heated table set to maintain body temperature at 37°C. The jugular vein was cannulated for infusion of normal saline (0.9% NaCl) at 2.8 ml/h. A flank incision was used to expose the left kidney, which was then immobilized in a Lucite cup. The kidney was then bathed with water-equilibrated light mineral oil heated to 37°C that was previously bubbled with 95% O2-5% CO2. The ureter was cannulated with polyethylene tubing (PE-50) to ensure a free flow of urine. Microperfusion experiments commenced 45 min after placement of the ureteral cannula.

The research reported in this manuscript adheres to APS’s Guiding Principles in the Care and Use of Animals and was approved by the Institutional Animal Care and Use Committee.

Sprague-Dawley rats injected with dihydrotestosterone (DHT; Sigma, St. Louis, MO) were given intraperitoneal injections at a dose of 50 mg/day for 10 days before microperfusion experiments. DHT (250 mg/ml of corn oil) was dissolved in corn oil using sonication and
vortexing before intraperitoneal injection. Control rats were injected with corn oil vehicle daily for 10 days before microperfusion experiments.

Blood pressure in all rats was measured using a tail cuff (model 179, IITC, Woodlands, CA), where the body of the rat was restrained in an enclosed acrylic tube contained within a Lucite box. The temperature within the box was maintained at ~30°C to vasodilate the tail vessels for accurate blood pressure measurement. All animals were initially trained daily for 5 days before the actual measurement. Systolic blood pressure (SYS BP) and mean arterial pressure (MAP) are expressed in millimeters mercury. The use of the tail cuff to measure blood pressure is well substantiated with liquid Microtip (Model 709C, Portex, UK).

Glomerular Filtration Rate Determination

The glomerular filtration rate (GFR) was measured in rats from both the DHT- and vehicle-injected groups. Briefly, rats were prepared surgically as described above. They were subsequently given an intravenous injection of [3H]inulin (~60 μCi) followed by an infusion of [3H]inulin in normal saline at a rate of 0.45 ml/h (20 μCi/h). After a 60-min period of equilibration, four separate 30-min collections of blood and urine were made and a 100-μl aliquot was subsequently placed into the scintillation fluid and counted in a scintillation counter (Packard Tri-Carb). The GFR (ml/min) was calculated by

\[
\text{GFR} = \frac{U \times V}{P}
\]

where U is the counts per minute (cpm) obtained in the urine, V is the urine flow rate in ml/min, and P is the counts per minute obtained in the plasma specimen.

In Vivo Microperfusion

Proximal tubule segments on the surface of the kidney were initially mapped with an injection of a small droplet of oil, and early and late loops were identified as previously described (30, 31). These tubule segments represent the S2 segment of the proximal tubule. A wax block was inserted into the lumen of an early loop by a hydraulic system or with the solution containing 10 M losartan. Intraluminal angiotensin II production within the proximal tubule, and also from flow into the tubule segments distal to the block. Subsequently, a microperfusion pipette was inserted into the lumen immediately distal to the wax block, and an ultrafiltrate-like solution was perfused at 30 ml/min using a microperfusion pump system (Effenberg K; Vestavia Scientific, Birmingham, AL). In a late proximal tubule loop distal to the perfusion pipette, a collection pipette was inserted, and the perfused ultrafiltrate-like solution was collected after an oil block was placed distally. Fluid collections were made over a 2- to 3-min period. The composition of the ultrafiltrate-like solution was (in mM) 120 NaCl, 25 NaHCO3, 5 KCl, 1 MgSO4, 1.8 CaCl2, 1 NaPO4, 5 glucose, 5 alanine, and 5 urea as well as FD & C green dye no. 3. Exhaustively dialyzed methoxy-[3H]inulin was added as a volume marker.

Proximal tubules were perfused with the above ultrafiltrate-like solution or with the solution containing 10 M enalaprilat or 10 M losartan. Enalaprilat (10-4 M) has been previously shown to inhibit intraluminal angiotensin II production within the proximal tubule, and 10 M losartan has been shown to block the effects of angiotensin in the proximal tubule (30, 31). These studies were performed in an unpaired fashion. Several tubules from each rat were studied with at least one tubule in the control group to ensure that there were no technical problems.

After all collections were performed, the entire tubule was injected with liquid Microfil (Flow-Tech, Carver, MA) and allowed to harden overnight in the refrigerator. The kidney was later placed in 6 N HCl at 37°C for 1 h. The Microfil tubule casts were dissected and photographed, and the tubular length between the perfusion and collection sites was measured. The length of the tubule between the perfusion and collection sites averaged 1.5 ± 0.2 mm. All collected tubular fluid was transferred to constant-bore capillary tubing for measurement of volume with a microinert (Mitutoyo, City of Industry, CA) and then microinert with scintillation fluid for radioactivity counting. The rate of fluid reabsorption was calculated as the difference between perfused and collected volumes divided by the time of collection and by the measured tubule length and is expressed as nanoliters per minute per millimeter.

Plasma DHT Assay

Plasma DHT levels were measured via a radioimmunoassay kit for testosterone/DHT (Amersham Pharmacia Biotech, Piscataway, N1). Briefly, the androgenic hormones within the plasma samples from the rats were first extracted from the aqueous phase with diethyl ether. The ether phase was then dried under a nitrogen stream, the residue was dissolved in assay buffer, and the reconstituted solution was oxidized. The oxidation of the androgenic hormones allows assay of DHT without prior chromatography separation of DHT from testosterone. The oxidized and ether-extracted plasma sample was then subjected to a radioimmunoassay with tritiated DHT (5α-dihydro[1,2,4,5,6,7-3H]testosterone) as the tracer using the supplied antibody to testosterone. Standards were prepared with each assay, and the unknown plasma samples were determined from the constructed standard curve.

Plasma Angiotensin II Assay

Plasma samples were collected from nonsedated rats that were decapitated and therefore not subjected to anesthesia or surgery. Plasma samples were collected into a solution containing EDTA and enalaprilat with final concentrations of 5 mM and 20 μM, respectively (5). EDTA and enalaprilat have both been previously shown to prevent further degradation or generation of angiotensin II within the sample (5). Plasma samples were extracted on a phenyl-bonded sodium phosphate-EDTA column (Bond-Elut; Analytichem, Harbor City, CA). Before sample application, the column was preswashed with 3 ml of 90% methanol in water and 6 ml of distilled deionized water. After the sample was applied, the column was washed with 3 ml of distilled deionized water, followed by 1.5 ml hexane, and, finally, 1.5 ml chloroform. The angiotensin peptides retained on the column were then eluted with 2 ml of 90% methanol in water, dried under vacuum in a Speed Vac, and subsequently stored at ~80°C until the assay. Measurement of plasma angiotensin II levels prepared as described above has been previously validated and shown to be accurate and correlate with acute and chronic changes in extracellular volume (16, 30).

The angiotensin II assay was performed with an enzyme immunoassay kit (Peninsula Laboratories, Belmont, CA) as previously described by our laboratory (31). Briefly, samples and standards were added to microtiter wells containing antibody to angiotensin II bound to the walls of the well. Next, biotinylated “tracer angiotensin II” was added to the well, which competes with angiotensin II in the sample or standard for binding to the angiotensin II antibody. The amount of biotinylated angiotensin II bound to the angiotensin II antibody is inversely proportional to the amount of angiotensin II in the sample or standard. After incubation, avidin-horseradish peroxidase conjugate is added, which binds only to biotinylated angiotensin II. In the last step, a colorimetric agent (3,3',5,5'-tetramethyl benzidine dihydrochloride) is added to each well and allowed to react with bound horseradish peroxidase. The intensity of color depends on the amount of horseradish peroxidase bound to biotinylated angiotensin II. All microtiter wells are read in a colorimetric microtiter plate reader (Titertek

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Multiscan, McLean, VA), a standard curve is constructed from the optical density readings, and sample angiotensin II concentrations are read from this curve. A standard curve is constructed each time the assay was performed. This assay has been previously demonstrated to accurately and reproducibly measure angiotensin II levels (31).

Angiotensinogen Message Abundance

Renal cortical mRNA samples were then denatured and separated on a 1% agarose-formaldehyde gel (3 µg/µl) and transferred to GeneScreen Plus hybridization transfer Membrane (New England Nuclear, Boston, MA) by the capillary method overnight at room temperature. The samples were then crosslinked in UV Stratalinker. Overnight hybridization was done by using ULTRAnhyb (Ambion). Radiolabeled, denatured DNA probe for angiotensinogen (a gift from O. Moe, Univ. of Texas Southwestern Medical Center, Dallas, TX) was used after prehybridization of 1 h at 42°C in a roller bottle hybridization oven. Then, the blot was washed twice (5 min each) with low-stringency wash solution (2× SSC, 0.1% SDS), followed by two 15-min washes with high-stringency wash solution (0.1× SSC, 0.1% SDS). Finally, the blot was exposed to X-ray film for autoradiography with intensifying screens. After stripping of the blot, the same procedure was repeated for probing 18S RNA (Ambion) to show the equal loadings for each lane.

Brush-Border Membrane Vesicle Preparation

The animals were killed, and the kidneys were rapidly removed and immediately placed in ice-cold phosphate-buffered saline (in mM: 137 NaCl, 2.7 KCl, 10.1 Na2HPO4, 1.7 KH2PO4, pH 7.4). The capsule was removed, and the cortex was dissected, minced, and placed in 15 ml of an ice-cold isolation buffer (in mM: 300 mannitol, 16 HEPES, 5 EGTA, titrated to pH 7.4 with Tris containing protease inhibitors aprotonin (2 µg/ml), leupeptin (2 µg/ml), and phenylmethylsulfonyl fluoride (100 µg/ml)). The minced cortex was then homogenized with Power Gen 125 (Fisher Scientific) homogenizer at 4°C. After addition of 230 µl of 1.0 M MgCl2 to precipitate cell debris, the homogenate was shaken vigorously for 10 s every 5 min for 20 min, as previously described (2). Subsequently, the homogenate was centrifuged at 2,500 g for 15 min at 4°C. The supernatant was decanted, added to 230 µl of 1.0 M MgCl2, shaken vigorously for 10 s every 5 min for 20 min, and centrifuged for 15 min at 2,500 g at 4°C. The supernatant was then centrifuged at 48,400 g for 30 min at 4°C. The pellets were resuspended in 1.5 ml of ice-cold resuspension buffer (5 mM HEPES, pH 7.4; osmolality adjusted to 80 mosmol/kg H2O with d-mannitol) using 22- and 25-gauge needles. Protein was determined in the crude homogenate and brush-border membrane vesicles (BBMV) using a BCA protein assay (Pierce, Rockford, IL). Alkaline phosphatase activity was used to determine the enrichment as described previously (27).

Protein Abundance of Na/H Exchanger in Brush-Border Membranes

BBMV protein was denatured and separated on a 7.5% polyacrylamide gel as previously described (50 µg/lanne) (2, 27). The separated proteins within the gel were then transferred to poly-vinylidene difluoride membrane overnight at 140 mA at 4°C. The blot was blocked with Blotto (5% nonfat milk, 0.05% Tween 20, and PBS, pH 7.4) for 1 h, and then a primary antibody to rat NHE3 (a gift from O. Moe, Univ. of Texas Southwestern Medical Center) was added at 1:1,200 dilution and incubated for 2 h at room temperature on a shaker. β-Actin antibody (Sigma) was added at 1:15,000 dilution. The blot was washed with PBS containing 1% Tween, and then the secondary horseradish peroxidase-conjugated anti-rabbit immunoglobulin [for Na/H exchanger (NHE3) antibody] and anti-mouse immunoglobulin (for β-actin antibody) were added at 1:10,000 dilution for 1 h in Blotto at room temperature. The blot was subsequently washed with PBS containing 1% Tween, and enhanced chemiluminescence was used to detect bound horseradish peroxidase-conjugated antibody (Amer sham Life Sciences, Arlington Heights, IL).

Angiotensin Binding Assay

Preparation of renal proximal tubule membranes. The kidneys from vehicle- and DHT-treated rats were removed, and renal proximal tubules were isolated as previously reported (18), using a modified method of Vinay et al. (42). Briefly, renal cortices were minced and digested for 45 min with 0.03% collagenase in the presence of 0.01% soybean trypsin inhibitor prepared in an isotonic buffer containing (in mM) 105 NaCl, 24 NaHCO3, 5 KCl, 1.5 CaCl2, 1 MgSO4, 2.0 NaH2PO4, 10 HEPES, 8.3 glucose, and 1 alanine as well as 0.2% bovine serum albumin, while being gassed with 95% O2-5% CO2 at 37°C. The cortical suspension was strained through a 250-µm-pore sieve, washed three times with the isotonic buffer above, resuspended in 50 ml of a 40% Percoll solution in the oxygenated isotonic buffer described above, and centrifuged at 12,200 g for 30 min at 4°C. After centrifugation, the tissue was separated into four distinct bands. Proximal tubules enriched in the bottom-most band were collected, washed three times with ice-cold Dulbecco’s phosphate-buffered saline, and homogenized to isolate membranes as described (38). Membrane protein concentration was measured with a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) according to the manufacturer’s instructions.

125I-labeled angiotensin II binding studies of renal proximal tubule membranes. Proximal tubule membrane binding assays were performed in duplicate as previously described (9). Briefly, membranes (100 µg) were incubated at room temperature in a binding solution containing (in mM) 100 NaCl, 5 EDTA, 10 HEPES (pH 7.5), 100 mannitol, 0.1 MgSO4, 4 DTT, and 0.1 phenylmethylsulfonyl fluoride as well as 0.5% bovine serum albumin, 0.5% trypsin inhibitor, 0.005% aprotinin, and 0.1 nM 125I-labeled angiotensin II (specific activity: 2,200 Ci/mmol, PerkinElmer Life Sciences, Boston, MA), along with binding buffer without membranes as a background control. After rapid filtration through presoaked 0.65-µm-pore filters (DWP, Millipore, Bedford, MA) and four washes with wash buffer (150 mM NaCl, 50 mM Tris, and 0.1% bovine serum albumin), radioactivity bound to the filters was counted in a gamma counter. Data are presented as means ± SD (n = 4) of specific binding in counts per minute per 100 micrograms of membrane protein. Specific binding was determined by subtracting the nonspecific binding component measured in the presence of excess unlabeled angiotensin II (10–6 M).

Analyses

Analysis of variance and Student’s t-test were used to determine statistical significance depending on the number of groups being compared. Tukey’s multiple comparison test was used as the posttest...
with ANOVA. The Mann-Whitney rank sum test was used to compare the plasma angiotensin II concentrations because they were not normally distributed, as indicated by Sigma-Stat software (Jandel). All data are expressed as means ± SE; n represents the number of tubules.

RESULTS

Blood Pressure and GFR

Blood pressure in rats was obtained via a tail cuff after a 5-day period of training as described above. SYS BP and MAP in DHT-treated rats were higher than in control rats (137 ± 4 vs. 124 ± 4 mmHg, P < 0.05 and 104 ± 2 vs. 88 ± 4 mmHg, P < 0.005, respectively) as seen in Fig. 1. The whole animal GFR in DHT-injected rats was similar to that in vehicle-injected control rats as seen in Fig. 2 (2.34 ± 0.23 vs. 2.82 ± 0.15 ml/min).

Effect of DHT Injections on Blood DHT, Angiotensin II Levels, and Weight Gain

Blood from rats who received either DHT injections or vehicle injections was assayed for DHT levels. DHT levels were higher in rats that received the DHT injections vs. the corn oil vehicle injections (432.14 ± 104.07 vs. 114.63 ± 41.93 ng/ml, P < 0.02, n = 5/group) (Table 1). Serum angiotensin II levels were also measured in DHT- and vehicle-injected animals (n = 18). As seen in Table 1, serum angiotensin II levels were lower in animals receiving DHT vs. vehicle injections (60.45 ± 10.27 vs. 86.15 ± 9.06 pM, Mann-Whitney rank sum test, P < 0.05). Weight gain in these same animals receiving DHT was greater than control rats over the 10-day period of injections (Table 1) (75 ± 13 vs. 37 ± 12 g/10 days, P < 0.05).

Effect of DHT on Proximal Tubule Volume Reabsorption

The effect of DHT on proximal tubule transport is shown in Fig. 3. As seen, the volume reabsorptive rate rose from 3.31 ± 0.23 nl·min⁻¹·mm⁻¹ in control rats to 4.57 ± 0.31 nl·min⁻¹·mm⁻¹ in DHT-injected rats (P < 0.01).

Effect of DHT on mRNA Abundance of Angiotensinogen in Renal Tissue

To determine the effect of DHT on the intrarenal renin-angiotensin system, renal cortical angiotensinogen was measured using Northern blot analysis. These results are shown in

Table 1. Comparison of control vehicle-treated rats and dihydrotestosterone-injected rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain over 10 days, g</td>
<td>37±12</td>
<td>75±13*</td>
</tr>
<tr>
<td>DHT concentration, ng/ml</td>
<td>114.6±4.19</td>
<td>432.1±104.1†</td>
</tr>
<tr>
<td>Angiotensin II concentraion, pM</td>
<td>86.15±9.06</td>
<td>60.45±10.27*</td>
</tr>
</tbody>
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Values are means ± SE. DHT, dihydrotestosterone. *P < 0.05 vs. control. †P < 0.02 vs. control.
Fig. 4. Densitometry showed that the angiotensinogen/18S ratio was increased by DHT treatment by twofold (control ratio: 0.18 ± 0.02 vs. DHT ratio: 0.41 ± 0.08; \( P < 0.05 \), \( n = 6 \)/group). Thus DHT treatment increased the expression of angiotensinogen, the substrate for renin and precursor for angiotensin II.

Effect of DHT on Protein Abundance of NHE3 in Brush-Border Membranes

The protein abundance of NHE3 was measured in BBMV extracted from kidneys of both DHT-injected and control vehicle-injected rats using the Western blotting technique. Protein abundance of NHE3 was expressed as the ratio of NHE3 protein to \( \beta \)-actin protein (NHE3/\( \beta \)-actin) (Fig. 5). As seen in Fig. 5, the amount of NHE3 protein relative to \( \beta \)-actin protein within the brush-border membrane was higher in kidneys from DHT-treated rats than from control rats (1.33 ± 0.11 vs. 0.86 ± 0.11, \( P < 0.05 \)). These results are consistent with a stimulatory role for androgens to augment expression of NHE3 protein within the brush-border membrane.

Effect of DHT on Binding of Angiotensin II to Renal Membranes

To determine whether the effects of DHT on proximal tubule transport could have been mediated by changes in receptors for angiotensin II, binding studies were performed in proximal tubule membranes from control and DHT-treated rats. These results are presented in Fig. 6 and demonstrate that there was no difference in the specific binding of angiotensin II after treatment with DHT. Specific binding in the control group was 2,734 ± 220 cpm/100 \( \mu \)g tissue and was 2,710 ± 37 cpm/100 \( \mu \)g tissue in the DHT-treated rats (\( P = \text{NS} \), \( n = 4 \) in each group). Thus treatment with DHT did not alter the expression of angiotensin receptors.

DISCUSSION

Hypertension significantly contributes to the development of cardiovascular and renal disease in the United States (14). The prevalence and incidence of hypertension, in both animal and human studies, are more common in males than in females (1, 14, 17). Male spontaneously hypertensive rats (SHR), DOC salt-sensitive rats, and Dahl salt-sensitive rats all have higher blood pressure readings than their female counterparts (13, 17, 24, 37). Similarly, in the Framingham study of cardiovascular disease and hypertension in the United States, the prevalence of hypertension in men was higher than in women (33 vs. 27%) (14). However, the etiology of this difference is largely un-
known. Animal models of hypertension suggest that androgens may play an important role in the pathogenesis of higher blood pressure in males (32).

In the SHR model of hypertension, castration of male rats at 4 wk of age attenuates the development of hypertension in adulthood, whereas testosterone replacement after castration restored hypertension (10). Male SHR given flutamide, an androgen-receptor antagonist, also demonstrated lower MAP, similar to castrated male rats (33). Ovariectomy had no effect on blood pressure in female SHR (10). However, normotensive female SHR become hypertensive when given testosterone treatment as neonates. More recent studies using mice with a Cyp 4a14 gene (arachidonic acid hydroxylase) knockout corroborate the above studies (19). Mice with the Cyp 4a14 gene knockout are hypertensive, but the blood pressure in male mice is higher than in female mice (19). Plasma androgens levels in male Cyp 4a14 knockout mice are twofold higher than in their wild-type male counterparts. When male knockout mice were castrated, their blood pressure decreased, comparable to the wild-type, whereas DHT replacement restored hypertension (19). These studies indicate that androgens, through interactions with the androgen receptor, are important in the development of hypertension in males.

The mechanism by which androgens play a role in the development of hypertension remains unknown. Receptors for DHT have been found on the peripheral arterial walls and in brain areas thought to be important in the regulation of blood pressure and heart rate (1), i.e., the area postrema and the preoptic region. Thus androgens may participate in the regulation of blood pressure both centrally and in the peripheral vasculature. Androgen receptors and their mRNA have also been found in the kidney via immunohistochemistry and Northern blot analysis and have been located predominantly in the proximal and distal tubules as well as the glomerulus (21, 41, 43). Androgens have also been found to affect the activity of the Na/H exchanger, one of the key brush-border membrane transporters in the kidney for solute reabsorption (23). These findings provide support for the possibility that androgens may have a direct effect on proximal tubule transport.

Recently, androgens have been found to alter the metabolism of arachidonic acid in the renal cortex (28). The authors demonstrated that administration of DHT to rats increased their blood pressure and altered the expression and activity of the renal microsomal cytochrome P-450 enzymes (28). The exact mechanism by which cytochrome P-450 metabolites of arachidonic acid affect regulation of blood pressure and renal function remains elusive; however, there are abundant data that these metabolites play a role in these processes (35).

Our data reveal that the proximal tubule renin-angiotensin system may play an important role in the development of androgen-mediated hypertension. The proximal tubule contains all components of the synthetic machinery of the renin-angiotensin system, including angiotensinogen, renin, angiotensin-converting enzyme, angiotensin receptors, and angiotensin II (4–7, 11, 12, 20, 25, 26, 34, 36, 39, 40). Angiotensin II has been detected in the luminal fluid of the proximal tubule at a 100-fold higher level than blood, thus indicating its robust “local” intraluminal production (4, 5, 29, 40). It has been previously demonstrated that luminal angiotensin II stimulates proximal tubule transport independently of the systemic renin-angiotensin system (30, 31). Inhibition of proximal tubule intraluminal angiotensin II production with $10^{-4}$ M luminal enalaprilat (angiotensin-converting enzyme inhibitor) reduced the volume reabsorptive rate by 40% (30). Subsequent addition of luminal angiotensin II, in the presence of enalaprilat, raised the volume reabsorptive rate to control levels, consistent with a stimulatory role for luminal angiotensin II in the regulation of proximal tubule transport (30). Furthermore, it was demonstrated that luminal angiotensin II augments proximal tubule transport to a greater degree during extracellular volume contraction than during extracellular volume expansion (31). In contrast, the effect of basolateral angiotensin II on proximal tubule transport is less clear. Some studies have demonstrated a stimulatory effect, whereas other studies have demonstrated no effect (3, 39). However, the stimulatory effect of intraluminal angiotensin II on proximal tubule transport occurs independently of any changes in the peritubular or basolateral milieu, as demonstrated in an in vitro microperfusion study (3).

The present study demonstrates that DHT augments proximal tubule reabsorption (Fig. 3). Proximal tubule reabsorption can also rise as a result of a rise in the GFR, a phenomenon known as glomerulotubular balance. However, the rise in proximal tubule reabsorption in DHT-treated animals occurred without any change in the GFR (Fig. 2), consistent with a direct tubular effect. Our findings are consistent with those of a previous study in SHR, where androgen receptor blockade did not change the GFR (33). These results are consistent with an effect of androgens on proximal tubule transport independently of changes in the GFR.

To investigate the potential role of intraluminal angiotensin II in the augmentation of volume reabsorption in the DHT-treated animal, enalaprilat or losartan was perfused into the lumen of the proximal tubule. As seen in Fig. 3, the decrease in the volume reabsorptive rate after inhibition of luminal angiotensin II production is consistent with a stimulatory role for angiotensin II in proximal tubule transport in the DHT-treated animals. Similar results were obtained with the perfusion of losartan in the tubule lumen. The decrease in transport...
observed with inhibition of angiotensin II production (luminal enalaprilat) was greater in the DHT-treated animals than in control animals. This larger decrement in transport suggests that luminal angiotensin II may be responsible for the augmentation in proximal tubule volume reabsorption resulting from androgen administration. This is supported by the fact that the transport rate in the DHT-treated animals after the addition of enalaprilat or losartan is identical to that of the control animals after addition of luminal enalaprilat (Fig. 3).

Androgenic stimulation of the proximal tubule renin-angiotensin system is supported by the finding that whole kidney angiotensinogen mRNA levels fall by >60% after castration in male Wistar-Kyoto rats (15). Furthermore, when female rats or male rats castrated as weanlings were implanted with testosterone pellets, kidney angiotensinogen mRNA levels rose to levels comparable to those in normal male rats (15). In the present study, we also demonstrated that administration of DHT to rats increased the expression of angiotensinogen in the renal cortex. An increased level of kidney angiotensinogen mRNA is consistent with increased expression of the renal renin-angiotensin system through an increase in the production of angiotensinogen, a precursor of angiotensin II. Similar effects of castration and testosterone replacement on renal angiotensinogen mRNA were found in SHR (10).

Androgenic stimulation of the proximal tubule renin-angiotensin system is also supported by our data indicating that brush-border membrane protein abundance of the Na/H exchanger, a membrane transport protein under the regulation of the Na/H activity is known to be under regulation by angiotensin II, is higher in DHT-treated vs. control animals (Fig. 4) (22). Our findings are corroborated by a previous study in which BBMV Na/H exchanger activity was stimulated by the presence of androgens (23). In these studies, BBMV Na/H exchanger activity was higher in male than in female mice and castration of male mice decreased exchanger activity, whereas testosterone replacement increased the rate of exchange by >100% (23). The higher Na/H activity after testosterone replacement is consistent with our results indicating greater Na/H protein abundance in the brush-border membrane of the DHT-treated animals (Fig. 4).

Na/H activity is known to be under regulation by angiotensin II (3, 22). Administration of luminal angiotensin II has been shown to augment bicarbonate transport in the in vitro microperfused rabbit proximal tubule (3). The rise in transport was inhibited by luminal losartan, an angiotensin II-receptor antagonist (3). Given that the Na/H exchanger is under regulation by androgens and angiotensin II, it is intriguing to consider that androgens may regulate the Na/H exchanger via modulation of the proximal tubule renin-angiotensin system.

In contrast to androgenic stimulation of the proximal tubule renin-angiotensin system, the systemic renin-angiotensin system may be suppressed. As seen in Table 1, the serum angiotensin II levels in the DHT-treated animals were lower than those found in the control animals. It is conceivable that the increased proximal tubule volume reabsorption would increase extracellular volume and suppress systemic angiotensin II levels. A rise in extracellular volume in the DHT-treated animals would be consistent with three of our findings, including hypertension, increased weight gain, and lower serum angiotensin II levels. Weight gain over the 10-day period of DHT/vehicle injections was twofold higher in the androgen-treated animals (75 ± 13 vs. 37 ± 12 g, P < 0.05), and MAP in androgen-treated animals was ~18% higher, as shown in Fig. 1 (104 ± 2 vs. 88 ± 4 mmHg, P < 0.005). Androgens may augment proximal tubule volume reabsorption via the proximal tubule renin-angiotensin system and raise extracellular volume and blood pressure.

In summary, we demonstrate that DHT administration in rats increases weight gain, blood pressure, and proximal tubule volume reabsorption and lowers serum angiotensin II levels. The DHT-associated increase in proximal tubule transport results from upregulation of the effect of intraluminal angiotensin II and subsequent upregulation of NHE3. These results suggest that androgens can augment proximal transport by stimulation of the proximal tubule renin-angiotensin system and may contribute to higher blood pressure.

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REFERENCES

ANDROGENS AUGMENT PROXIMAL TUBULE TRANSPORT


