Transfer of the CYP4A region of chromosome 5 from Lewis to Dahl S rats attenuates renal injury


DAHL SALT-SENSITIVE (Dahl S) rats rapidly develop severe hypertension and proteinuria, glomerulosclerosis, and renal interstitial fibrosis when fed a high-salt (HS) diet (3–5, 23, 25, 27, 29, 38, 41). The renal injury resembles that seen in patients with hypertension- and diabetes-induced nephropathy (20, 30, 37). However, the factors and genes that contribute to the pathogenesis of hypertension-induced glomerulosclerosis and renal interstitial fibrosis in Dahl S rats remain to be identified.

Previous studies have indicated that the expression of cytochrome P-450 4A (CYP4A) protein is reduced in the thick ascending limb of Henle of Dahl S rats and that this contributes to an increase in loop Cl⁻ transport, blunted pressure natriuresis, and the development of hypertension (9, 15, 35). More recently, our group (28) reported that transfer of a region of chromosome 5 (Rattus norvegicus, RNO5) containing the CYP4A alleles of Lewis rats into the Dahl S genetic background increases the renal production of 20-hydroxyecosatetraenoic acid (20-HETE), improves pressure natriuresis, and attenuates the development of hypertension. However, the effects of transfer of this region of RNO5 on the development of hypertension-induced renal injury were not examined previously. Indeed, there is evidence that suggests that 20-HETE might have renoprotective actions beyond blunting of the development of hypertension. For example, upregulation of the renal expression of CYP4A protein and the formation of 20-HETE with fibrates reduces glomerulosclerosis in Dahl S rats maintained on a low-salt (LS) diet in the absence of any changes in arterial pressure (41). There also is evidence that the glomerulus avidly synthesizes 20-HETE and that reducing the endogenous formation of 20-HETE following administration of transforming growth factor (TGF)-β (5) or a 20-HETE synthesis inhibitor increases glomerular permeability to albumin (P₂₀H) (40). Preventing the fall in glomerular 20-HETE levels by adding a 20-HETE mimetic attenuates the effects of TGF-β or a 20-HETE inhibitor on P₂₀H (5, 40). These observations suggest that the endogenous production of 20-HETE in the glomerulus plays at least a permissive role in maintaining the glomerular permeability barrier to albumin.

In other studies, 20-HETE has been reported to alter renal vascular tone, autoregulation of renal blood flow (RBF), and tubuloglomerular feedback responses (14, 42, 43). In the present study, we hypothesized that a deficiency in the renal formation of 20-HETE may enhance the transmission of systemic pressure to the renal circulation and increase glomerular capillary pressure (Pgc) during development of hypertension in Dahl S rats. The rise in Pgc may trigger an increase in the glomerular expression of TGF-β, which promotes the development of proteinuria by increasing P₂₀H and the production of extracellular matrix, leading to glomerulosclerosis and renal interstitial fibrosis. Therefore, the present study examined whether the renal production of 20-HETE is deficient in Dahl
S rats compared with other strains of rats and whether transfer of overlapping segments of RNO5 that include or exclude the region containing the CYP4A genes from the Lewis rat onto the Dahl S genetic background increases the renal formation of 20-HETE and opposes the elevation in Pgc, Pabs, and the development of hypertension-induced proteinuria and renal disease in RNO5 congenic strains fed a HS diet.

METHODS

General

Experiments were performed using a total of 726 9- to 12-wk-old male rats. Sprague-Dawley (SD) and Brown Norway (BN) rats were purchased from Harlan Laboratories (Indianapolis, IN); spontaneously hypertensive rats (SHR) were purchased from Taconic Farms (Germantown, NY), and Lewis rats were purchased from Charles River Laboratories (Wilmington, MA). Studies also were performed on Dahl SS/Jr rats and two overlapping RNO5 congenic strains in which a portion of rat chromosome 5 (RNO5) that includes (4A⁺ congenic) or excludes (4A⁻ congenic) the CYP4A alleles from the Lewis rat were introgressed onto the Dahl S rat genetic background (28). The SS/Jr and RNO5 congenic strains were obtained from in-house colonies maintained at the Medical College of Wisconsin since 1997 that were derived from breeder pairs originally developed and provided by John Rapp (Medical College of Ohio) (7, 8, 28). All the rats in the present studies were housed in a temperature-controlled (21 ± 1°C) environment with a 12:12-h light-dark cycle in the Animal Care Facility at the Medical College of Wisconsin, which is approved by the American Association for the Accreditation of Laboratory Animal Care. The rats had free access to food and water throughout the study. All protocols received approval by the Animal Care Committee of the Medical College of Wisconsin.

Protocol 1: Comparison of the Expression of CYP4A Protein and the Metabolism of Arachidonic Acid in Dahl S and Other Rat Strains

Previous studies have indicated that the renal metabolism of arachidonic acid (AA) to 20-HETE and/or epoxyeicosatrienoic acids (EETs) are reduced in Dahl S rats relative to other strains and that the production of these metabolites may be differentially regulated by changes in salt intake among various strains of rats (13). However, the results from these previous studies are not consistent, since the studies were done in different laboratories using different diets and ages of rats. Thus it is still unclear whether the renal metabolism of AA is uniquely altered in Dahl S rats. In this protocol, we compared the expression of CYP4A protein and the renal metabolism of AA in microsomes prepared from the renal cortex and outer medulla of 12-wk-old Lewis, Dahl S, SD, BN, and SHR rats that were maintained from weaning on a purified AIN-76 diet (Dyets, Bethlehem, PA) containing 0.4% NaCl (LS) or switched to a AIN-76 diet containing 8.0% NaCl (HS) for 7 days before the experiment. The reason why these rats were challenged with the HS diet for only 7 days was to minimize the potential influence that hypertension-induced renal damage and tubular necrosis would have on the expression of CYP4A protein and eicosanoid production, especially in Dahl S rats.

Metabolism of AA in renal microsomes. Microsomes were prepared from the renal cortex and outer medulla as previously described (2). Briefly, the renal cortex (0.5 g) or outer medulla (0.3 g) was homogenized in 3 ml of a 10 mM potassium buffer (pH 7.7) containing 250 mM sucrose, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). The homogenates were centrifuged at 3,000 g for 5 min and at 9,000 g for 15 min. The supernatant was centrifuged at 100,000 g for 1 h to obtain the microsomal fraction. The pellets were resuspended in the 100 mM potassium buffer (pH 7.25) containing 20% glycerol, 1 mM dithiothreitol, and 0.1 mM PMSF, frozen in liquid N₂, and stored at −80°C until assayed.

The microsomes were incubated with a saturating concentration of [14C]AA (0.2 μCi/ml; Amersham Life Science, Arlington Heights, IL) in 1 ml of a 100 mM potassium phosphate buffer (pH 7.4) containing 10 mM MgCl₂, 1 mM EDTA, 1 mM NADPH, and an NADPH-regenerating system (10 mM isocitrate and 0.4 U/ml isocitrate dehydrogenase) at 37°C for 30 min. The incubations were stopped by acidification to pH 3.5 with formic acid and extracted twice with 3 ml of methyl acetate. The organic phase was collected and dried under N₂. The metabolites were separated by HPLC using a 250-×-2.0-mm C18 reverse-phase column (Supelcosil LC18, catalog no. 57935; Supelco, Bellefonte, PA) at a flow rate of 0.3 ml/min with a linear gradient ranging from acetoniitrile-water-acetic acid (50:50:0.1 vol/vol/vol) to acetoniitrile-acetic acid (100:0.1 vol/vol) over a 40-min period. The radioactive products were monitored using a radioactivity flow detector (model 500 TR series; Radiomatic Instrument, Tampa, FL), and the production rates of the various metabolites were expressed as picomoles of product formed per minute per milligram of protein.

Expression of CYP4A protein. The expression of CYP4A protein in renal cortical and outer medullary microsomes was compared in the various strains maintained on a LS diet from weaning or challenged with a HS diet for 7 days. A sample of microsomal protein was separated by electrophoresis on 7.5% SDS-polyacrylamide gel (Bio-Rad, Hercules, CA). The proteins were transferred to nitrocellulose membranes and blocked overnight in a buffer containing 10% nonfat dry milk. The membranes were incubated for 2 h with a 1:4,000 dilution of CYP4A primary antibody (catalog no. 299230; Daichi Pure Chemicals, Tokyo, Japan), followed by a 1:4,000 dilution of secondary antibody (sc2020; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. The blots were developed using an enhanced chemiluminescent kit and exposed to X-ray film, and the relative intensities of the bands in the bands in the 50- to 52-kDa range for CYP4A were expressed as picomoles of product per minute per milligram of protein.

Protocol 2: Comparison of the Metabolism of AA in Dahl S and the RNO5 Congenic Strains

These experiments were performed in Dahl S rats and the RNO5 congenic strains fed a LS diet from weaning until the rats were 9 wk of age. The rats were then divided into two groups. One group was fed with a HS diet (8.0% NaCl) for 7 days, while the other group remained on the LS diet. Microsomes were prepared from the liver of a fenofibrate-treated rat were used as the CYP4A standard (1 μg loaded). Equal loading of the microsomal protein was assessed by the staining of the membrane with Ponceau S solution (Sigma, St. Louis, MO).

Glomeruli were also isolated from the kidneys of Dahl S and 4A⁺ congenic rats by differential sieving, as previously described (5, 32, 33, 40). Homogenates of the isolated glomeruli were prepared, and the expression of CYP4A protein was determined by Western blot analysis. In a previous study, we observed that the expression of CYP4A protein in the renal cortex of these strains was not significantly different (28), so we wanted to determine whether the transfer of the CYP4A genes from the Lewis rat increases CYP4A expression in glomeruli isolated from these strains. The metabolism of AA was determined by incubating homogenates of the isolated glomeruli (0.5 mg of protein) in 1 ml of RPMI 1640 medium (Invitrogen, Grand Island, NY) in the presence of a saturating concentration of AA (42 μM; Amersham Biosciences, Piscataway, NJ) and 1 mM NADPH for 30 min at 37°C. The incubations were stopped by acidification to pH 3.5 with formic acid and extracted twice with 3 ml of ethyl acetate after the addition of 2 ng of an internal standard, d₁₀-20-HETE (Cayman Chemicals, Ann Arbor, MI). The organic phase was collected and dried under N₂. The samples were reconstituted with 50%
methanol and water, and the metabolites were separated by HPLC on a Betabasic C18 column (150 × 2.1 mm, 3 μm; Thermo Hypersil-Keystone, Belletonte, PA) at a flow rate of 0.2 ml/min using an isocratic elution starting from a 51:9:40:0.01 mixture of acetonitrile-methanol-water-acetic acid for 30 min, followed by a step change to 68:13:19:0.01 acetonitrile-methanol-water-acetic acid-water for 15 min. The eluent was ionized using a negative ion electrospray, and the peaks eluting with a mass-to-charge ratio of 319 > 245 (20-HETE), 319 > 301 (HETEs and EETs), 337 > 319 (dihydroxyecosatrienoic acids, DHETEs), or 325 > 251 (internal standard) were monitored using an Applied Biosystems 3000 triple-quadrupole mass spectrometer (Foster City, CA). The ratios of ion abundances in the peaks of interest versus those seen with the internal standard were determined and compared with standard curves generated over a range from 0.5 to 10 ng for the various eicosanoids. Values are expressed as determined and compared with standard curves generated over a range of the primers used are as follows: 4A1, 5'-AAT GAG ATG TGA GCA GAT GGA GT-3'; 4A2, 5'-CAG CCT TGG TGT AGG ACC T3-3'; 4A3, 5'-CTC TTA CTT CGG AGA ATG GAG AA-3'; 4A4, 5'-AGA GCC AAT CCA CCC GAA GCC TTA TCA TTA ACT-3'; 4A8, 5'-ATC CAG AGG TGT ACC CTT CTG GAA TTT AT-3'; 4A9, 5'-CAG CTT GGG ACC AGG ACC TTA TCA TTA ACT-3'; 5'-AAU ACT GTA GTA GTA GTA GAT GTA GTT GAT GTA GT-3'; and β-actin, 5'-CTA TGA TGC AGT CTA AGG GGA GC-3', 5'-GAT AGA GCC ACC AAT CCA CAC AG-3'.

Protocol 3: Comparison of the Degree of Proteinuria and Renal Injury in Dahl S Rats and the RNO5 Congenic Strains

Experiments were performed on 9-wk-old Dahl S and the RNO5 congenic strains maintained on a LS diet from weaning. The rats were then switched to a HS diet for 21 days. After 14 days on the HS diet, the rats were anesthetized and a micronephathic catheter was implanted in the femoral artery as previously described (38). After a 5-day recovery period, an overnight urine sample was collected for measurement of protein and creatinine excretion, and mean arterial pressure (MAP) was recorded for 5 h per day for 3 consecutive days between the hours of 10:00 AM to 3:00 PM while the rat was free moving in its home cage. At the end of the experiments, a terminal blood sample was collected for the measurement of plasma creatinine concentration. The kidneys were collected and weighed to assess the degree of hypertrophy and fixed in a 10% buffered formalin solution. Thin paraffin sections (3 μm) were prepared and stained with Mason’s trichrome. The degree of glomerular injury was accessed on ~30 glomeruli per section. The percentage of the glomerular capillary area filled with matrix material was scored according to the method of Raji et al (26) on a 0 to 4 scale with 0 representing no injury, 2 indicating loss of 50% of glomerular capillary area, and 4 representing the complete loss of capillaries.

Protocol 4: Comparison of the Time Course of the Development of Hypertension and Proteinuria in Dahl S Rats and the RNO5 Congenic Strains

These experiments were performed on 9-wk-old SD, Dahl S, and the RNO5 congenic strain rats maintained from weaning on the AIN-76 diet containing 0.4% NaCl. A gel-filled catheter attached to a radiotelemetry transmitter (model TA11PA-C40; Data Sciences International, St. Paul, MN) was implanted in the femoral artery, and the transmitter was placed under the skin. After a 5-day recovery period, MAP was recorded after 1:00 and 4:00 PM during a 3-day control period. The rats were then switched to a diet containing 8% NaCl, and MAP was recorded on days 7, 14, and 21 of the HS diet. At each time point, urine was collected overnight to determine protein excretion. A subset of rats in each strain were chronically treated once daily with subcutaneous injections with vehicle (1% sulfobutyl ether β-cyclo-dextrin; CyDex, Lenexa, KS) or a selective inhibitor of the synthesis of 20-HETE, N-hydroxy-4-(4-butyl-2-methylphenyl)formamidine (HET0016; 10 mg/kg sc). At the end of the experiment, plasma and the kidneys were collected, and the kidneys were weighed and fixed in a 10% buffered formalin solution. Paraffin sections (3 μm) were prepared and stained with Mason’s trichrome to determine the degree of glomerular injury.

Protocol 5: Measurement of Pgc

Pgc was measured in 12-wk-old 4A- congenic and Dahl S rats fed either a LS or HS diet for 10 days. A third group of rats in each strain was fed a HS diet and treated daily with HET0016 (10 mg/kg sc) to chronically block the synthesis of 20-HETE. On the day of the acute experiment, the rats were anesthetized with ketamine (30 mg/kg im) and Inactin (50 mg/kg ip), and catheters were placed in the femoral artery for the measurement of arterial pressure. The rats received an intravenous infusion of 1% BSA in a 0.9% NaCl solution via the jugular vein at a rate of 1.2 ml/h. Pgc was directly measured using a Servomull micropressure device (model 900; WPI, Sarasota, FL) as previously described (39). This was possible because the Dahl S rats and the 4A- congenic strain used in this study have surface glomeruli. Pgc was also estimated indirectly in the absence of tubuloglomerular feedback by measuring plasma oncotic pressure and stop flow pressure in four to six early proximal tubules after blocking the tubular lumen with bone wax.

Protocol 6: Measurement of Glomerular P_g

Glomeruli were isolated using the sieving method in a medium containing 5 g/dl albumin, and P_g was determined by measuring the change in glomerular volume (ΔV) after exchange of the bath with fresh medium containing 1% albumin as previously described (5, 32, 33, 40). P_g was calculated as 1 − (ΔVexperimental/ΔVcontrol), where ΔVcontrol was taken as the mean change in volume measured in all glomeruli isolated from 6-wk-old control SD rats, which are assumed to have no preexisting renal damage. Experiments also were performed using glomeruli isolated from 9-wk-old SD, Dahl S, and RNO5 congenic strain rats maintained from weaning on LS or challenged with HS diet for 7 days. Other rats were chronically treated daily with the inhibitor of the synthesis of 20-HETE, HET0016 (10 mg·kg⁻¹·day⁻¹·sc). A minimum of 10 glomeruli were studied from each rat, and these experiments were performed using a minimum of 6 rats per strain.
Protocol 7: Expression of TGF-β2 in the Kidney of 4A+ Congenic and Dahl S Strains

The expression of TGF-β2 in homogenates of isolated glomeruli was compared in male 4A+ congenic and Dahl S rats fed either a LS or HS diet for 7 and 21 days. Glomeruli were isolated as previously described in 200 μl of homogenization buffer and centrifuged at 4,000 g, and the supernatant was collected and prepared for Western blot analysis as described above using a 1:500 dilution of TGF-β2 primary antibody (sc90; Santa Cruz Biotechnology) and a 1:4,000 dilution of a horseradish peroxidase-coupled secondary antibody (sc2004; Santa Cruz Biotechnology) for 1 h. This antibody recognizes the mature form of TGF-β2 associated with a latent protein (TGF-β2/LAP, ~75 kDa) and the active form of TGF-β2 (~12 kDa). Equal protein loading of homogenates was assessed by stripping and reprobing the membranes using a 1:8,000 dilution of β-actin primary antibody (Sigma) and a 1:8,000 dilution of horseradish peroxidase-coupled secondary antibody (Santa Cruz Biotechnology) for 1 h. In a previous study, we used a TGF-β primary antibody that detected all three isoforms, TGF-β1, -β2, and -β3 (5). In the present study, we used isoform-specific antibodies and found that the TGF-β2 antibody was the best. However, we did observe similar results with TGF-β1.

The kidneys of other animals were perfusion fixed with 4% paraformaldehyde solution for 15 min at 100 mmHg for the localization of TGF-β2 by immunohistochemistry. The kidneys were postfixed overnight in a 30% sucrose solution, imbedded in OCT compound, and frozen in liquid nitrogen. Frozen sections (5 μm) were cut, postfixed in acetone, and dried. The sections were immunostained by exposure to 1:100 dilution of primary antibodies to TGF-β2 antibody was followed by a 1:100 dilution of an Alexa Fluor 488-labeled secondary antibody (Invitrogen). The slides were counterstained with 0.01% Evans blue to quench endogenous fluorescence. Images were obtained using a fluorescence microscope equipped with a high-sensitivity camera (Q-Imaging digital camera; W. Nuhsbaum, McHenry, IL), and overlaid images were prepared using Image-Pro Plus software.

Statistical Analysis

Values are means ± SE. The significance of differences in control and experimental values in the same animal was determined using an unpaired t-test. The significance of differences in the mean values between groups was determined using one-way ANOVA followed by the Holm-Sidak test. P < 0.05 was considered to be significant.

RESULTS

Protocol 1: Renal CYP4A Protein Expression and 20-HETE Formation in Different Strains

Previous studies have indicated that Dahl S rats display a reduction in the renal metabolism of AA to 20-HETE and/or EETs compared with other strains in response to various HS treatments (13). Thus it is still unclear whether the renal metabolism of AA is uniquely altered in Dahl S rats compared with other strains given the same diet. In this protocol, we compared the expression of CYP4A protein and renal metabolism of AA in microsomes prepared from the renal cortex and outer medulla. A comparison of the expression of CYP4A protein and the formation of 20-HETE in the kidneys of Dahl S rats versus other strains fed either a LS diet from birth or challenged with a HS diet for 7 days is presented in Figs. 1 and 2. The expression of CYP4A protein was significantly lower in the renal cortex of Dahl S rats compared with that in any other strains when rats were fed a HS diet (Fig. 1A). In the outer medulla, we observed a reduction in the expression in CYP4A protein in Dahl S rats compared with that in the other strains fed either a LS or HS diet (Fig. 1B). The production of 20-HETE was significantly lower in microsomes prepared from the renal cortex or outer medulla of Dahl S rats fed a LS or HS diet compared with that in other strains (Fig. 2, A and B).
total epoxygenase activity in Dahl S and the other strains of rats is presented in Table 1. Baseline renal epoxygenase activity on a LS diet is lower in Dahl S rats than in Lewis or BN rats, but not in SD or SHR rats. Epoxygenase activity did not increase in Dahl S, SHR, or SD rats fed a HS diet for 7 days, and it decreased significantly in Lewis and BN rats. The DiHETE/EET formation (an index of epoxide hydrolase activity) is highest in Lewis rats, intermediate in SHR and BN rats, and lowest in Dahl S and SD rats.

Extent of the introgressed region in the congenic strains. A genetic map summarizing the regions of RNO5 introgressed from the Lewis rat into the Dahl S genetic background in the RNO5 congenic strains is presented in Fig. 3. The 4A+ congenic strain carries an ~100-Mbp segment of RNO5 from the Lewis rat between markers D5Rat130 and D5Rat31 that includes the region containing the CYP4A1, 2, 3, and 8 genes located between markers D5Rat108 and D5Rat31 (RNO5: 134.8–139.9 Mbp). The control congenic, the 4A− strain, contains an overlapping region of Lewis RNO5 between markers D5Rat130 and D5Rat72 that excludes the CYP4A region.

Protocol 2: Expression of CYP4A and Metabolism of AA in Dahl S and RNO5 Congenic Rats

A comparison of the production of CYP-derived metabolites of AA in microsomes prepared from the renal cortex and the

---

Table 1. Formation of EETs and DiHETEs and total epoxygenase activity in the renal cortex in SHR, Dahl S, BN, Lewis, and SD rats fed a low-salt diet or challenged with a high-salt diet for 7 days

<table>
<thead>
<tr>
<th></th>
<th>LS Diet</th>
<th>HS Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EETs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>43±4 (12)</td>
<td>41±2† (12)</td>
</tr>
<tr>
<td>Dahl S</td>
<td>51±7 (22)</td>
<td>84±5* (19)</td>
</tr>
<tr>
<td>BN</td>
<td>77±6† (16)</td>
<td>40±3† (21)</td>
</tr>
<tr>
<td>Lewis</td>
<td>48±5 (23)</td>
<td>32±5† (28)</td>
</tr>
<tr>
<td>SD</td>
<td>102±7† (11)</td>
<td>69±6* (12)</td>
</tr>
<tr>
<td><strong>DiHETEs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>65±8 (12)</td>
<td>54±4† (9)</td>
</tr>
<tr>
<td>Dahl S</td>
<td>26±5 (30)</td>
<td>12±1* (19)</td>
</tr>
<tr>
<td>BN</td>
<td>65±7 (16)</td>
<td>70±6† (21)</td>
</tr>
<tr>
<td>Lewis</td>
<td>173±23† (24)</td>
<td>147±5† (23)</td>
</tr>
<tr>
<td>SD</td>
<td>18±4 (11)</td>
<td>14±2 (12)</td>
</tr>
<tr>
<td><strong>Epoxygenase activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>108±11 (12)</td>
<td>95±5† (9)</td>
</tr>
<tr>
<td>Dahl S</td>
<td>82±8 (25)</td>
<td>96±6 (19)</td>
</tr>
<tr>
<td>BN</td>
<td>141±12† (16)</td>
<td>109±7* (21)</td>
</tr>
<tr>
<td>Lewis</td>
<td>225±25† (24)</td>
<td>179±20† (23)</td>
</tr>
<tr>
<td>SD</td>
<td>120±10 (11)</td>
<td>83±7 (12)</td>
</tr>
</tbody>
</table>

Values are means ± SE determined in spontaneously hypertensive (SHR), Dahl salt-sensitive (Dahl S), Brown Norway (BN), Lewis, and Sprague-Dawley (SD) rats fed a low-salt (LS) or high-salt (HS) diet for 7 days. Formation of epoxyeicosatrienoic acids (EETs) and dihydroxyeicosatrienoic acids (DiHETEs) and epoxygenase activity in the renal cortex are expressed as picomoles of product formed per minute per milligram of protein. Numbers in parentheses indicate the number of rats studied per group. *P < 0.05 vs. the corresponding values on a LS diet within the same strain. †P < 0.05 vs. the corresponding values in the Dahl S strain.

---

Fig. 2. Comparison of the formation of 20-hydroxyeicosatetraenoic acid (20-HETE) in the renal cortex (**A**) and outer medulla (**B**) of SHR, Dahl S, BN, Lewis, and SD rats fed either a LS or HS diet for 7 days. Numbers in bars indicate the number of rats studied per group. Values are means ± SE. 

Fig. 3. Schematic map illustrating the region of chromosome 5 (RNO5) of the Lewis rat that was introgressed into the Dahl S genetic background in the Dahl S RNO5 Lewis congenic strains (4A+ and 4A−).
outer medulla of Dahl S and the RNO5 congenic strains is presented in Fig. 4. In the renal cortex, the production of 20-HETE and EETs was relatively similar (<20% difference) in Dahl S rats and the congenic strains fed a LS or HS diet (Fig. 4, A and B). In contrast, the formation of 20-HETE in the renal outer medulla was twofold higher in the 4A− congenic strain fed either a LS or HS diet for 7 days compared with the corresponding values observed in Dahl S or the control 4A+ congenic strain.

Real-time PCR of CYP4A isoforms in 4A+ and 4A− congenic rats. A comparison of the expression of CYP4A isoforms in the renal cortex and outer medulla of the 4A+ and control 4A− congenic strains maintained on LS or after a HS diet for 7 days is presented in Fig. 5. There were no differences in the expression of CYP4A isoforms in the renal cortex of 4A+ and 4A− congenic strains fed either a LS or HS diet. However, the expression of CYP4A1, 2, and 3, was two- to threefold higher in the outer medulla of the 4A+ rats fed a LS diet than the corresponding value in the 4A− congenic strain (Fig. 5C).

Expression of CYP4A protein and the glomerular formation of 20-HETE in 4A+ congenic and Dahl S rats. The basal expression of CYP4A protein and formation of 20-HETE in glomeruli isolated from 4A+ congenic and Dahl S rats fed a LS diet was not significantly different. The production of 20-HETE decreased significantly in glomeruli of Dahl S rats fed a HS diet for 7 days but not in the 4A− congenic strain (Fig. 6B). CYP4A expression and 20-HETE formation in glomeruli isolated from Dahl S and 4A− congenic rats were similar (data not shown).

Protocol 3: Proteinuria and Renal Injury in Dahl S Rats and the RNO5 Congenic Strains

A comparison of the severity of renal injury in the Dahl S and the RNO5 congenic strains challenged with a HS diet for 3 wk is presented in Table 2. MAP, protein excretion, kidney weight, and glomerular size were all significantly lower in the 4A− congenic rats compared with Dahl S rats.

Protocol 4: Time Course of the Development of Hypertension and Proteinuria in Dahl S Rats and the RNO5 Congenic Strains

The time course of the development of hypertension and proteinuria in 4A+ and control congenic and Dahl S strains fed a HS diet for 21 days is presented in Fig. 7. Baseline MAP in the Dahl S and 4A− rats maintained on a LS diet (day 0) was ~8 mmHg higher than the corresponding value measured in the 4A+ congenic rats (Fig. 7A). After 7 days on a HS diet, MAP increased similarly by ~15 mmHg in Dahl S and 4A− congenic strains but only increased by 8 mmHg in the 4A+ congenic strain. Over the next 14 days, MAP rose to 185 ± 8 and 189 ± 3 mmHg in Dahl S and 4A− congenic rats, respectively. However, the development of hypertension was attenuated in the 4A+ congenic strain, and MAP only reached 148 ± 4 mmHg after 21 days on the HS diet.

Baseline proteinuria was similar in all three strains fed a LS diet (Fig. 7B). After 7 days of HS, Dahl S and 4A− congenic rats excreted twice as much protein (118 ± 7 and 114 ± 15 mg/day, respectively) than their 4A+ congenic counterparts (56 ± 7 mg/day). The degree of proteinuria was about fourfold higher in Dahl S (423 ± 40 mg/day) and 4A− congenic rats (481 ± 37 mg/day) fed a HS diet for 21 days than in 4A+ congenic rats (125 ± 15 mg/day).

Chronic blockade of the formation of 20-HETE. The effects of chronic treatment of the rats with HET0016, an inhibitor of the formation of 20-HETE, on the development of hypertension and proteinuria in SD, Dahl S, and the RNO5 congenic strain rats are presented in Fig. 8. After 3 wk on a HS diet, MAP rose to 190 ± 7 and 185 ± 3 mmHg, respectively, in Dahl S and 4A− congenic rats treated with vehicle (Fig. 8A). The increase in MAP in the vehicle-treated 4A− rats was much less and averaged only 150 ± 5 mmHg. Exposure to the HS diet for 21 days had no effect on MAP in SD rats and averaged only 130 ± 5 mmHg. Chronic blockade of the formation of 20-HETE with HET0016 increased MAP in the 4A+ congenic rats to the same levels as that seen in Dahl S and 4A− congenic strain. In contrast, treatment with HET0016 had no effect on MAP in SD or Dahl S rats or in the 4A− congenic strain rats fed a HS diet for 21 days.
Protein excretion was significantly lower in SD and 4A/H11001 congenic rats fed a HS diet for 3 wk than the levels observed in Dahl S and 4A/H11002 congenic rats (Fig. 8B). Chronic administration of HET0016 significantly increased protein excretion in the 4A/H11001 congenic strain. In contrast, HET0016 had no effect on proteinuria in SD, Dahl S, or the 4A/H11002 congenic rats.

Measurement of glomerular injury. The effect of inhibition of the formation of 20-HETE on the degree of glomerular injury in SD, Dahl S, 4A/H11001, and 4A/H11002 congenic rats fed a HS diet for 21 days is summarized in Fig. 9. Histological examination of glomeruli indicated that there was more expansion of mesangial matrix in the glomeruli of Dahl S and 4A/H11002 congenic rats than in 4A/H11001 rats (Fig. 9A). The glomerular injury score was significantly lower in SD and 4A/H11001 congenic rats than the corresponding values in Dahl S and 4A/H11002 congenic rats (Fig. 9B). Treatment with HET0016 significantly increased glomerular injury in the 4A/H11001 congenic strain to levels similar to those observed in Dahl S and 4A/H11002 congenic rats. It had no significant effect on glomerular injury in SD rats.

Protocol 5: Measurement of Pgc

We also examined whether changes in renal hemodynamics might trigger the development of renal injury in Dahl S rats fed a HS diet and mediate the renoprotective effect seen in the 4A/H11001 congenic strain. The results of these experiments are presented in Fig. 10. MAP measured during the micropuncture experiments under ketamine and Inactin anesthesia were ~10 mmHg higher in the Dahl S rats than in the 4A/H11001 congenic strain fed either a LS or HS diet (Fig. 10A). Directly measured Pgc was significantly lower in 4A/H11001 congenic rats fed a LS diet than in Dahl S rats. Pgc increased by 8 mmHg in Dahl S rats fed a HS diet 10 days, whereas it remained unchanged in 4A/H11002 congenic rats (Fig. 10B). Treatment with HET0016 increased Pgc in the 4A/H11001 congenic rats fed a HS diet to the same level as that seen in Dahl S rats. Pgc increased by 8 mmHg in Dahl S rats fed a HS diet 10 days, whereas it remained unchanged in 4A/H11002 congenic rats (Fig. 10B). Treatment with HET0016 had no effect on Pgc in Dahl S rats. In each animal, Pgc was also estimated in the absence of tubular glomerular feedback by measuring proximal tubular stop flow pressure (Fig. 10C). Estimated Pgc was much higher than Pgc measured directly in both Dahl S and 4A/H11001 congenic strains. There was no difference in Pgc estimated from the stop flow pressures in Dahl S and 4A/H11001 congenic strain fed either a LS or HS diet or after chronic treatment with HET0016.

Protocol 6: Measurement of Glomerular P_{alb}

Baseline P_{alb} was significantly higher in Dahl S and the control 4A/H11001 congenic strains fed a LS diet than the values measured in SD or 4A/H11002 congenic rats (Fig. 11). P_{alb} increased significantly in Dahl S and 4A/H11002 congenic rats fed a HS diet for 7 days, but it did not increase in SD or 4A/H11001 congenic rats.
in Fig. 13. TGF-β2 was expressed primarily in proximal tubules in both Dahl S and 4A⁺ rats fed a LS diet. The expression of TGF-β2 increased markedly in glomeruli and proximal tubules of Dahl S rats fed a HS diet for 7 or 21 days and to a lesser extent in 4A⁺ congenic rats.

**DISCUSSION**

The present study examined whether the renal expression of CYP4A protein and the formation of 20-HETE is reduced in Dahl S rats compared with other strains of rats and whether transfer of a region of RNO5 containing the CYP4A genes from the Lewis rat onto the Dahl S genetic background upregulates the renal production of 20-HETE and opposes the development of renal injury in SS,5Lew congenic strain rats fed a HS diet for 21 days. There are four primary findings. First, the expression of CYP4A protein and the formation of 20-HETE is reduced in the renal cortex and outer medulla of Dahl S rats fed either a LS or HS diet compared with four other strains of rats. Second, the development of proteinuria and renal injury in Dahl S rats is accompanied by elevations in Pgc, P₂⁺, and the renal expression of TGF-β. Third, transfer of the region of RNO5 containing CYP4A alleles from the Lewis rat onto the Dahl S genetic background corrects the deficiency in the renal formation of 20-HETE, prevents the increase in Pgc, TGF-β, and P₂⁺, and attenuates the development of hypertension, proteinuria, and glomerulosclerosis. Fourth, chronic administration of an inhibitor of the synthesis of 20-HETE reverses the reduction in MAP and renoprotection seen in 4A⁺ congenic rats.

It is now widely accepted that some form of renal dysfunction underlies the development of hypertension in humans and experimental animals, although genes and factors responsible for altering renal function have not been clearly identified. There have been several reports that the renal production of 20-HETE is reduced in Dahl S rats and that induction of this epoxygenase activity relative to SD rats when fed a HS diet increases P₂⁺, proteinuria, and attenuates the development of hypertension (13). In the present study, we confirmed that the formation of EETs does not increase in Dahl S rats fed a HS diet. However, we did not find that the renal formation of epoxygenase metabolites of AA increased in SD, Lewis, SHR, or BN rats fed a HS diet for 7 days. The reason for the differences in the results may be that we used a shorter period of exposure to the elevated sodium intake of the high-salt diet.

**Table 2.** **MAP and renal function data in Dahl S and RNO5 congenic rats fed a HS diet for 21 days**

<table>
<thead>
<tr>
<th></th>
<th>4A⁺ Congenic</th>
<th>4A⁺ Congenic</th>
<th>Dahl S</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>25</td>
<td>20</td>
<td>51</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>159±4†</td>
<td>192±2†</td>
<td>185±2</td>
</tr>
<tr>
<td>Protein excretion, mg/day</td>
<td>139±8†</td>
<td>334±15</td>
<td>326±12</td>
</tr>
<tr>
<td>P₂⁺, mg/dl</td>
<td>0.46±0.02†</td>
<td>1.05±0.09</td>
<td>1.05±0.05</td>
</tr>
<tr>
<td>Ccr, M/L/min</td>
<td>1.55±0.10†</td>
<td>0.93±0.05†</td>
<td>0.57±0.04</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>1.66±0.05†</td>
<td>2.09±0.06</td>
<td>1.98±0.03</td>
</tr>
<tr>
<td>Glomerular diameter, μm</td>
<td>114±2†</td>
<td>176±4†</td>
<td>164±2</td>
</tr>
</tbody>
</table>

Values are means ± SE determined in Dahl S and rat chromosome 5 (RNO5) congenic strains. MAP, mean arterial pressure; P₂⁺, plasma creatinine; Ccr, creatinine clearance. †P < 0.05 vs. the corresponding values in Dahl S rats.

**Fig. 6.** Comparisons of the expression of CYP4A protein (A) in glomeruli isolated from 4A⁺ and Dahl S rats fed either a LS or HS diet for 7 days. Intensities in various strains were compared with that seen in the first lane loaded with 1 μg of the CYP4A protein standard (STD). Numbers in bars indicate the number of rats studied per group. Values are means ± SE. *P < 0.05 vs. the corresponding value in rats fed a LS diet within the same strain. †P < 0.05 vs. the corresponding value in the Dahl S strain within same diet.

Chronic administration of the inhibitor of the formation of 20-HETE increased P₂⁺ in both SD and 4A⁺ congenic rats fed a HS diet for 7 days, but it had no effect on P₂⁺ in Dahl S and 4A⁻ congenic rats.

**Protocol 7: Expression and Localization of TGF-β**

The comparison of the expression of TGF-β2 in glomeruli of 4A⁺ congenic and Dahl S rats is summarized in Figs. 12 and 13. This antibody detected free TGF-β2 (~12 kDa; data not shown) and mature TGF-β2 dimer bound with its latent associated protein (TGF-β2/LAP, ~75 kDa). The basal expression of the mature TGF-β2/LAP (75 kDa) was greater in glomeruli isolated from Dahl S rats than in 4A⁺ congenic rats fed a LS diet (Fig. 12). The expression of TGF-β2/LAP increased in Dahl S rats fed a HS diet for 7 days, but it did not increase significantly in the 4A⁻ rats. A representative immunohistochemical localization of the expression of TGF-β2 is presented
intake to reduce the impact of chronic hypertension on the genes contributing to the formation of epoxides. Overall, the results of the present study are consistent with previous findings in our laboratory (11, 15, 17, 28) that a deficiency in the renal formation of epoxides does not appear to contribute to the development of hypertension, at least not in Dahl rats of the SS/Jr strain.

On the other hand, the results of the present study confirm previous findings that the expression of CYP4A protein and the production 20-HETE are reduced in the outer medulla of Dahl S rats, relative to Dahl salt-resistant controls (15, 17), and we now have extended this finding to show that this deficiency in Dahl S rats is unique among four other strains that were not studied previously. We also observed that the production of 20-HETE in the renal cortex and outer medulla is either unchanged or decreases in some of the strains fed a HS diet. This finding fits with previous reports that the renal expression of CYP4A may be regulated by circulating levels of angiotensin II (2, 21, 24). The present findings suggesting that a deficiency in the renal formation of 20-HETE is associated with the development of hypertension in Dahl S rats are also in agreement with previous findings that induction of the renal formation of 20-HETE with fibrates lowers blood pressure and improves pressure natriuresis in Dahl S rats (1, 29, 34) and that the chronic inhibition of 20-HETE produces salt-sensitive hypertension in salt-resistant strains (10, 15, 35). These results are also consistent with reports that targeted disruption of the CYP4A10 and CYP4A14 genes leads to the development of salt-sensitive forms of hypertension in mice (12, 22) and with more recent findings that a point mutation in the CYP4A11 gene that decreases the formation of 20-HETE is associated with an increased incidence of hypertension in at least four different human association studies (6, 16, 18, 19).

In further studies, we found that transfer of a region containing the CYP4A rats of the Lewis rat increased the expression of CYP4A protein and the formation of 20-HETE in the renal outer medulla of the 4A* congenic strain compared with the 4A− control strain, and this was associated with a twofold increase in the expression of CYP4A 1, 2, and 3 mRNA. We also observed that 4A* congenic rats exhibited a reduction in MAP of nearly 40 mmHg compared with 4A− congenic and

![Fig. 7. Time course of the development of hypertension (A) and proteinuria (B) in Dahl S rats and the 4A* and 4A− congenic strains fed a HS diet for 21 days. Numbers in parentheses indicate the number of rats studied per group. Values are means ± SE. †P < 0.05 vs. the corresponding value in the Dahl S rats. MAP, mean arterial pressure.](http://ajprenal.physiology.org/)

![Fig. 8. Effects of the chronic administration of an inhibitor of the formation of 20-HETE, HET0016 (10 mg·kg−1·day−1 sc), on MAP (A) and protein excretion (B) measured in 4A* and Dahl S rats fed a HS for 21 days. Numbers in bars indicate the number of rats studied per group. Values are means ± SE. *P < 0.05 vs. the corresponding value in rats treated with vehicle within a strain. †P < 0.05 vs. the corresponding value in Dahl S rats.](http://ajprenal.physiology.org/)
Dahl S rats fed a HS diet for 21 days. This finding is consistent with our previous observations that the increase in the renal production of 20-HETE improves the pressure natriuretic response and attenuates the development of hypertension in this strain (28). We also found that the development of proteinuria and glomerular injury was markedly reduced in 4A congeneric rats fed a HS diet compared with Dahl S and 4A congeneric rats. These findings also are consistent with previous results indicating that induction of the renal formation of 20-HETE with fibrates reduces the degree of renal injury and proteinuria during the development of hypertension in Dahl S rats (34, 41).

Further studies were performed to determine whether this is simply due to the reduction in MAP in the 4A congeneric strain or whether the upregulation of 20-HETE has some additional renoprotective effects. We found that arterial pressure was similar among all three strains following 7 days on a HS diet, but protein excretion was twofold higher in the Dahl S and the 4A control strain than in 4A congeneric rats. To better understand the mechanisms behind the accelerated development of proteinuria in Dahl S rats, we measured Pgc and P in these strains. Baseline P was significantly higher in Dahl S and 4A congeneric rats compared with 4A congeneric rats fed a LS diet. Moreover, Dahl S and 4A congeneric rats fed a HS diet for 7 days exhibited a marked increase in P that paralleled the rise in Pgc and proteinuria. In contrast, P did not increase in the 4A congeneric strain. Chronic treatment with HET0016 significantly raised P in the 4A congeneric rats fed a HS diet.
the corresponding values observed in the Dahl S and 4A\(^+\) congeneric rats. These results indicate that the upregulation of renal CYP4A expression and the formation of 20-HETE protect the glomerulus from injury even before the development of hypertension, because arterial pressures in all strains were similar when fed a LS diet. They further suggest that the elevation in renal 20-HETE formation in the 4A\(^+\) congeneric strain may oppose the development of proteinuria and glomerulosclerosis not only by preventing transmission of pressure to the glomerular capillaries but also, perhaps, by opposing the subsequent increase in \(P_{\text{alb}}\). Additional studies also were performed in SD rats that were chronically treated with HET0016 and fed a HS diet for 7 days. MAP remained unchanged in these rats; however, \(P_{\text{alb}}\) increased significantly to the same levels as those seen in the Dahl S and control congenic strains. These results are consistent with our recent finding that even acute inhibition of the synthesis of 20-HETE in the glomerulus disrupts the glomerular permeability barrier and increases \(P_{\text{alb}}\) (5, 40). Surprisingly, protein excretion remained unaltered after HET0016 treatment. This apparent discrepancy probably reflects the large capacity of SD rats to reabsorb all of the filtered load of protein in the proximal tubule, at least for some time, even in situations in which the glomerular filtration barrier is damaged (31).

The current study also explored the possibility that the reduction in protein excretion in 4A\(^+\) congeneric rats was due to elevations in the glomerular formation of 20-HETE. In a previous study, we observed that TGF-\(\beta\) increases \(P_{\text{alb}}\) in part by inhibiting the formation of 20-HETE in the glomerulus and that preventing the fall of 20-HETE with a 20-HETE agonist attenuated this effect (5). Interestingly, transfer of CYP4A genes from the Lewis rat onto Dahl S genetic background did not alter expression of CYP4A protein or the production of 20-HETE in glomeruli of the 4A\(^+\) congeneric rats compared with Dahl S rats fed a LS diet. However, the Dahl S strain displayed a marked downregulation in the formation of 20-HETE in the glomerulus when fed a HS diet for 7 days, whereas the glomerular production of 20-HETE in 4A\(^+\) congeneric rats remained unchanged. We also observed that the expression of TGF-\(\beta\) increased to a greater extent in the proximal tubules and glomeruli of Dahl S rats than in 4A\(^+\) congeneric rats after 7 days on a HS diet. The elevated 20-HETE production may help explain why the 4A\(^+\) congeneric rats have lower values of \(P_{\text{alb}}\) compared with Dahl S rats when fed a HS diet. The present finding that treatment of the 4A\(^+\) congeneric rats fed a HS diet with a 20-HETE inhibitor increased Pgc, \(P_{\text{alb}}\), and protein excretion to the same levels seen in the Dahl S and the control 4A\(^-\) congeneric strain supports this view.

![Figure 10](http://ajprenal.physiology.org/)

**Fig. 10.** Effects of the chronic administration of HET0016 (10 mg·kg\(^{-1}\)·day\(^{-1}\)·sc) on MAP (A), directly measured glomerular capillary pressures (Pgc; B), and Pgc estimated from stop flow pressures (C) in 4A\(^+\) and Dahl S rats fed a LS or HS diet for 10 days. Numbers in bars or parentheses indicate the number of glomeruli and/or rats studied per group. Values are means ± SE. *\(P < 0.05\) vs. the corresponding value in rats fed a LS diet within a strain. †\(P < 0.05\) vs. the corresponding value in the Dahl S rats.

![Figure 11](http://ajprenal.physiology.org/)

**Fig. 11.** Comparison of effects of the chronic administration of HET0016 (10 mg·kg\(^{-1}\)·day\(^{-1}\)·sc) on glomerular permeability to albumin (\(P_{\text{alb}}\)) measured in SD, 4A\(^-\), 4A\(^+\), and Dahl S rats fed either a LS or HS diet for 7 days. Numbers in bars indicate the number of glomeruli and rats studied per group. Values are means ± SE. *\(P < 0.05\) vs. the corresponding value in rats fed a LS diet within a strain. †\(P < 0.05\) vs. the corresponding value in the Dahl S rats.
The mechanism by which a HS decreases the glomerular production of 20-HETE has yet to be determined. One possibility is that HS has been shown to increase Pgc and TGF-β expression in the renal cortex of Dahl S rats (4, 5, 36). Dahly-Vernon et al. (5) showed that even acute elevations in TGF-β levels inhibit the formation of 20-HETE in the glomerulus of SD rats and increase Pgc. Collectively, these data support the view that elevations in Pgc that increase the levels of TGF-β may trigger the pronounced fall in the formation of 20-HETE in the glomerulus of Dahl S rats fed a HS diet and

![Image]

Fig. 12. Comparison of expression of transforming growth factor-β dimer bound with its latent associated protein (TGF-β2/LAP) in glomeruli of 4A⁺ and Dahl S rats fed either a LS or HS diet for 7 days. Numbers in bars indicate the number of rats studied per group. Values are means ± SE. *P < 0.05 vs. the corresponding value in rats fed a LS diet within a strain. †P < 0.05 vs. the corresponding value in the Dahl S rats.

![Image]

Fig. 13. Immunohistochemical localization of TGF-β2 expression in the renal cortex of 4A⁺ and Dahl S rats fed a LS or HS diet for 7 and 21 days.
that this contributes to the elevation in $P_{\text{abl}}$ and the development of proteinuria and renal disease.

In summary, the results indicate that the introgression of a region of RNO5 containing the CYP4A genes from the Lewis rat onto the Dahl S genetic background increases the renal formation of 20-HETE and opposes the rise in $P_{\text{cr}}$, $P_{\text{abl}}$, and TGF-β and the development of hypertension-induced proteinuria and glomerular disease. Overall, these findings indicate that the upregulation of the renal formation of 20-HETE is renoprotective and that 20-HETE mimetics or agonists may have therapeutic potential in reducing MAP, proteinuria, and glomerular disease in salt-sensitive hypertension.

GRANTS
This work was supported by National Heart, Lung, and Blood Institute Grants HL29587 and HL36279 and by a United Negro College Fund/Merck Postdoctoral Science Research Fellowship (to J. Williams).

REFERENCES


35. Stec DE, Mattson DL, Roman RJ. Inhibition of renal outer medullary
20-HETE production produces hypertension in Lewis rats. *Hypertension*
36. Tamaki K, Okuda S, Nakayama M, Yanagida T, Fujishima M.
Transforming growth factor-beta 1 in hypertensive renal injury in Dahl
37. Tierney WM, McDonald CJ, Luft FC. Renal disease in hypertensive
adults: effect of race and type II diabetes mellitus. *Am J Kidney Dis*
38. Tolins JP, Raij L. Comparison of converting enzyme inhibitor and
calcium channel blocker in hypertensive glomerular injury. *Hypertension*
39. Van Dokkum RP, Sun CW, Provoost AP, Jacob HJ, Roman RJ.
Altered renal hemodynamics and impaired myogenic responses in the fawn-hooded
40. Williams JM, Sharma M, Anjaiah S, Falck JR, Roman RJ. Role of
endogenous CYP450 metabolites of arachidonic acid in maintaining the
glomerular protein permeability barrier. *Am J Physiol Renal Physiol* 293:
41. Wilson TW, Alonso-Galicia M, Roman RJ. Effects of lipid-lowering
agents in the Dahl salt-sensitive rat. *Hypertension* 31: 225–231,
1998.
42. Zou AP, Imig JD, Kaldunski M, Ortiz de Montellano PR, Sui Z,
Roman RJ. Inhibition of renal vascular 20-HETE production impairs
autoregulation of renal blood flow. *Am J Physiol Renal Fluid Electrolyte
43. Zou AP, Imig JD, Ortiz de Montellano PR, Sui Z, Falck JR, Roman
RJ. Effect of P-450 omega-hydroxylase metabolites of arachidonic acid
on tubuloglomerular feedback. *Am J Physiol Renal Fluid Electrolyte