Characterization of blood pressure and renal function in chromosome 5 congenic strains of Dahl S rats

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Submitted 1 September 2005; accepted in final form 2 January 2006

Romans, Richard J., Kimberly M. Hoagland, Bernardo Lopez, Anne E. Kwitek, Michael R. Garrett, John P. Rapp, Josef Lazar, Howard J. Jacob, and Albert Sarkis. Characterization of blood pressure and renal function in chromosome 5 congenic strains of Dahl S rats. Am J Physiol Renal Physiol 290: F1463–F1471, 2006. First published January 5, 2006; doi:10.1152/ajprenal.00360.2005.—The present study examined whether transfer of overlapping regions of chromosome 5 that include (4A+) or exclude the cytochrome P-450 (CYP) 4A genes from the Lewis rat alters the renal production of 20-hydroxyeicosatetraenoic acid (20-HETE) and/or the development of hypertension in congenic strains of Dahl salt-sensitive (S) rats. The expression of CYP4A protein and the production of 20-HETE in the renal outer medulla was greater in the 4A+ congenic strain than the levels seen in S rats or in overlapping control congenic strains that exclude the CYP4A region. Mean arterial pressure (MAP) rose from 122 ± 2 to 190 ± 7 mmHg in S rats and from 119 ± 2 and 123 ± 2 to 189 ± 7 and 187 ± 3 mmHg in the two control congenic strains fed an 8.0% NaCl diet for 3 wk. In contrast, MAP only increased from 112 ± 2 to 150 ± 5 mmHg in the 4A+ congenic strain. Chronic blockade of the formation of 20-HETE with N-(3-chloro-4-morpholin-4-yl) phenyl-N'-hydroxyimido formamide (TS-011; 1 mg/kg bid) restored the salt-sensitive phenotype in the 4A+ congenic strain and MAP rose to 181 ± 6 mmHg after an 8.0% NaCl dietary challenge. TS-011 had no effect on the development of hypertension in S rats or the two control congenic strains. The pressure-natriuretic and diuretic responses were fivefold greater in the 4A+ congenic strain than in S rats. These results indicate that transfer of the region of chromosome 5 between markers D5Rat108 to D5Rat31 from the Lewis rat into the Dahl S genetic background increases the renal production of 20-HETE, improves pressure-natriuresis and opposes the development of salt-induced hypertension.

cytochrome P-450; kidney; 20-HETE

RENAAL TRANSPLANTATION STUDIES have indicated that some form of renal dysfunction underlies the development of hypertension in humans and in experimental animal models (4, 9, 10, 21). However, the factors responsible for altering kidney function and the genes involved remain to be determined. Several lines of evidence suggest that a deficiency in the renal formation of 20-hydroxyeicosatetraenoic acid (20-HETE) contributes to the development of hypertension in Dahl salt-sensitive (S) rats. In this regard, 1) the formation of 20-HETE and the expression of cytochrome P-450 (CYP) enzymes of the 4A family is reduced in the outer medulla and the thick ascending limb of the loop of Henle (TALH) of S rats (13, 17); 2) 20-HETE is an endogenously-formed inhibitor of Cl− transport in the TALH and Na+ and Cl− reabsorption is elevated in this segment of the nephron in S rats (13, 15, 31); 3) 20-HETE modulates tubuloglomerular feedback (TGF) responses (32) and glomerular hemodynamics is altered in S rats (14, 26, 28); 4) increasing the renal formation of 20-HETE with fibrates or the SOD mimetic Tempol, lowers blood pressure (BP) and improves renal function in S rats (12, 29); and 5) inhibitors of the formation of 20-HETE blunts the pressure-natriuretic response (5) and promotes the development of salt-sensitive hypertension in normotensive strains of rats (11, 25).

These studies suggest that the renal formation of 20-HETE is reduced in S rats and that this abnormality contributes to the sodium retention and the resetting of the pressure-natriuretic relationship in this strain. However, it remains to be determined whether the diminished formation of 20-HETE in S rats is due to a sequence variant in one of the CYP4A genes, which catalyze the formation of 20-HETE from arachidonic acid. On the other hand, the renal expression of CYP4A protein in S rats may be secondary altered by the development of hypertension and renal injury or in response to the actions of other genes causally related to the development of hypertension. The results of previous studies indicating that CYP4A genes map to a region of rat chromosome 5 (RNO5, Rattus norvegicus 5) that cosegregates with hypertension in an F2 cross of S and Lewis rats are consistent with the view that a mutation in one of the CYP4A genes may play a causal role in the development of hypertension in this strain (24). A more recent study suggested that the CYP4A genes should not be considered as candidate genes for the development of hypertension since they could be excluded from the region of RNO5 from the Lewis rat that lowers blood pressure in a congenic strain of S rats (8). However, this study did not address the possibility that the causal gene on chromosome 5 may influence the renal expression of CYP4A enzymes and that upregulation of the formation of the 20-HETE still contributes to the fall in BP in the congenic strain as an effector system.

Thus the present study examined in more detail the role of 20-HETE in the development of hypertension in S rats. We studied whether transfer of overlapping segments of RNO5 (from markers D5Rat130 to D5Rat31) that include (4A+ strain) or exclude (4A− and control congenic strains) the region containing the CYP4A1, 2, 3, and 8 genes from the Lewis rat into the S genetic background alters the renal expression of CYP4A enzymes and the production of 20-HETE, the pressure-natriuretic relationship and/or the development of salt-sensitive hypertension.
Experiments were performed on 9-wk-old male S rats and age-matched rats from three overlapping RNO5 congenic strains. The rats were housed in temperature controlled (21 ± 1°C) environment with 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 except that they were fasted the night before a clearance experiment. All protocols involving animals were approved by the Animal Care Committee of the Medical College of Wisconsin.

The S rats used in the present study were obtained from a colony derived from breeder pairs of SS/Jr rats provided by Dr. J. P. Rapp of the Medical University of Ohio, Toledo, Ohio. This colony has been maintained by strict brother-sister mating at the Medical College of Wisconsin since 1997. The congenic strains, in which overlapping segments of RNO5 from the Lewis rat that include or exclude the CYP4A region were introgressed into the S genetic background, were originally developed by Drs. Rapp and M. R. Garrett at the Medical University of Ohio as previously described (7, 8). These strains have also been maintained by brother-sister mating at the Medical College of Wisconsin for more than 20 generations.

Genotyping. Genomic DNA was extracted from an ear punch tissue sample (16). Genotyping was performed using fluorescently labeled primers as previously described (20). The congenic strains were genotyped with 70 markers (DSMit9, 5, 13, 14; DSGot24, 128, 29, 131, 31, 27, 36, 34, 38, 39, 40, 133, 37, 60, 70, 64, 66, 73, 88, 89; DSWox12; DS rat248, 123, 210, 127, 130, 1, 222, 254, 139, 84, 16, 68, 81, 21, 88, 98, 151, 160, 78, 72, 162, 54, 163, 95, 65, 108, 29, 167, 87, 32, 90, 31, 34, 170, 36, 39, 69, 42, 94, 45, 46, 49; and DSMgh2, 27, 18, 16) spaced at approximately a 2.5-Mbp distance to fine map the introgressed regions on RNO5. In addition, the strains were also genotyped with 150 additional microsatellite markers equally spaced at a 10- to 20-cM resolution across the entire genome to ensure that the genetic background was fixed for S alleles.

Expression of CYP4A protein. These experiments were performed in S rats and the RNO5 congenic strains fed a purified AIN-76A diet containing 0.4% NaCl from weaning until the rats were 9 wk old. The rats were then divided into two groups. One group was fed with a purified AIN-76A diet containing 8.0% NaCl for 3 wk, while the other group remained on the 0.4% NaCl diet. The purified AIN-76A rodent diet was purchased from Dyets (Bethlehem, PA; www.dyets.com/100000.htm) and was formulated as defined by the American Institute of Nutrition in 1977 (2). The mineral and vitamin content of this diet, as well as the percentages of calories derived from carbohydrates, protein and fat, is very similar to that found in standard rodent diets. The protein concentration of the sample was measured using the Bradford method (3) using bovine gamma globulin (Bio-Rad Laboratories, Hercules, CA) as a standard.

The expression of CYP4A protein in the kidneys of S rats and the congenic strains was determined by the Western blot technique. A sample of microsomal protein isolated from the renal cortex (10 μg protein) or the outer medulla (25 μg protein) of S rats and the RNO5 congenic strains was denatured at 94°C for 5 min in a denaturing sample buffer containing 5% mercaptoethanol and separated by electrophoresis on 7.5% SDS-polyacrylamide gel (Bio-Rad-Hercules) for 1 h at 200 V. The proteins were transferred to nitrocellulose membranes, and the membranes were blocked overnight in a buffer containing 10 mM Tris·HCl, 150 mM NaCl, 0.08% Tween 20, and 10% nonfat dry milk. The membranes were rinsed and incubated for 2 h with a 1:2,000 dilution of a CYP4A primary antibody (cat. no. 299230, Daiichi Pure Chemicals, Tokyo, Japan). They were then rinsed several times and incubated with a 1:4,000 dilution of a horseradish peroxidase-coupled secondary antibody (cat. no. SC 2020, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. The blots were developed using an enhanced chemiluminescent kit (West Pico, Pierce, Rockford, IL). Exposure to X-ray film reflected the relative intensities of the bands in the 50- to 52-kDa range were determined using an Eagle eye imaging system (Stratagene, La Jolla, CA) and Un-Scan It software (Silk Scientific, Orem, UT).

Measurement of renal CYP4A activity. The metabolism of arachidonic acid (AA) was determined by incubating microsomes prepared from the renal cortex or outer medulla (0.5 mg protein) with a saturating concentration of [14C]AA (0.2 μCi/ml, 42 μM, Amersham Life Science, Arlington Height, 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 a NADPH-regenerating system (10 mM isocitrate and 0.4 U/ml isocitrate dehydrogenase) at 37°C for 30 min. The reactions were terminated by acidification to pH 3.5 with 1 M formic acid, extracted twice with 3 ml of ethyl acetate and the organic phase dried under N2. The metabolites were separated by HPLC by means of a 2 × 250-mm C18 reverse-phase column (Supelcosil LC18, cat. no. 57935, Supelco, Belfonte, PA) at a flow rate of 0.3 ml/min using a linear gradient ranging from acetonitrile/water/acetic acid (50/50/0.1, vol/vol/vol) to acetonitrile/acetic acid (100/0.1, vol/vol) over a 40-min period. The products were monitored using a radioactivity flow detector (Packard 500TR Series, Packard BioScience, Meriden, CT), and the production rates of the various metabolites were expressed as pico moles of product formed per minute per milligram of microsomal protein in the reactions.

Phenotyping of blood pressure. S rats and the congenic strains were maintained on the purified AIN-76A diet containing 0.4% NaCl from weaning until they were 9 wk of age. The rats were then switched to the diet containing 8.0% NaCl. After 2 wk on this diet, the rats were anesthetized with an im injection of ketamine (40 mg/kg, Phoenix Pharmaceutical, St. Joseph, MO), xylazine (2.5 mg/kg), and acepromazine (0.6 mg/kg). A microrenathane catheter was implanted into the femoral artery using an aseptic technique. The catheter was tunneled subcutaneously, exteriorized at the scapula and advanced through a flexible spring that was secured to a Dacron mesh button (Instech, Plymouth, MA) sutured beneath the skin. The other end of the catheter was tunneled subcutaneously, exteriorized at the scapula and advanced through a flexible spring that was secured to a Dacron mesh button (Instech, Plymouth, MA) sutured beneath the skin. The other end of the spring was attached to a swivel anchored above the cage so that BP could be monitored from rats while they were conscious in their home cages. After surgery, the rats received enrofloxacin (10 mg/kg im, Bayer HealthCare) and buprenorphine (0.1 mg/kg sc, Reckitt Benckiser Health Care) to prevent infection and relieve pain. The catheters were flushed daily with isotonic saline and refilled with a heparinized saline solution (500 U/ml) to maintain the patency of the catheter.

The rats were housed individually in metabolic cages. BP was measured after a 5-day recovery period. The arterial catheters were connected to solid-state transducers (Argon Medical Technologies-Atlanta, TX) that were interfaced with a computerized data-acquisition system. After a 1-h equilibration period, heart rate, systolic BP, diastolic BP, and mean arterial pressure (MAP) were measured during the study.
a 5-h recording session. The digitized signals were processed to 1-min averages that were converted to a single mean value for the recording session. BP was measured on 4 consecutive days and the mean value of the four daily averages is reported. At the end of day 4, a 24-h sample of urine was also collected for measurement of proteinuria.

**Assessment of the time course of the development of hypertension measured by telemetry.** These experiments were performed on S rats and the congenic strains maintained from weaning on the purified AIN-76A diet containing 0.4% NaCl. When the rats were 8 wk old, they were anesthetized with an im injection of ketamine (40 mg/kg), xylazine (2.5 mg/kg), and acepromazine (0.6 mg/kg). A gel-filled catheter attached to a radiotelemetry transmitter (model TA11PA-C40, Data Sciences International, St. Paul, MN) was implanted in the femoral artery. The catheter was tunneled subcutaneously and the transmitter was placed under the skin on the back of the rats. The rats were given enrofloxacin (10 mg/kg im) and buprenorphine (0.1 mg/kg sc) to prevent infection and relieve pain. The rats were housed individually in plastic cages and given 6 days to recover from surgery. During this period, they were maintained on the diet containing 0.4% NaCl. Control MAP was recorded between 1 and 4 PM on 3 consecutive days. The rats were then switched to the purified AIN-76A diet containing 8% NaCl, and MAP was recorded 3, 7, 14, and 21 days later.

**Pharmacological rescue experiments.** Eight-week-old S rats and age-matched rats from the congenic strains maintained from weaning on a 0.4% NaCl diet were prepared for measurement of MAP by telemetry as described above. After surgery, the rats were allowed a 7-day recovery period. Thereafter, MAP was measured for 3 consecutive control days and the average was taken as baseline MAP (day 0). The rats in each strain were then divided into two groups. One group was fed the purified AIN-76A diet containing 8% NaCl and were treated twice a day with a sc injection of a selective inhibitor of the synthesis of 20-HETE N-(3-chloro-4-morpholin-4-yl) phenyl-N'-hydroxyimido formamide (TS-011, 1 mg/kg), (19, 27), for 2 wk. The other group was fed the diet containing 0.8% NaCl and was treated twice a day with vehicle. MAP was recorded on days 3, 7, 10, and 14 during this period.

**Assessment of the pressure-natriuretic relationship.** These experiments were performed on S rats (n = 10) and the 4A- (n = 8) congenic strain maintained on the 0.4% NaCl diet. The rats were prepared for study of the pressure-natriuresis as previously described (22). The rats were anesthetized with thiobutabarbital (Inactin, 50 mg/kg body wt ip, Sigma) and ketamine (25 mg/kg body wt im), and were placed on a heating table to maintain the body temperature at 37°C. A tracheotomy was performed to facilitate breathing. The left jugular vein was cannulated for the infusion of solutions. The left carotid and femoral arteries were catheterized to record MAP above and below the renal artery and for the collection of blood samples. An adjustable clamp was placed around the aorta between the renal arteries, and ligatures were placed around the mesenteric and renal arteries so that the renal perfusion pressure (RPP) could be controlled. The left ureter was catheterized for collection of urine. The left kidney was denervated and an ultrasonic flow probe (1RB, Transonic Systems, Ithaca, NY) placed around the left renal artery for measurement of renal blood flow (RBF). The rats received an iv infusion of 2% bovine serum albumin in 0.9% NaCl solution at a rate of 100 μl/min throughout the experiment. Vasopressin (52 pg/min), aldosterone (20 ng/min), norepinephrine (100 ng/min) and hydrocortisol (20 μg/min) were included in the infusion solution to minimize any potential differences in the circulating levels of these hormones between the strains (22). [3H]ulin (2 μCi/ml) was added to the infusion solution to allow for measurement of glomerular filtration rate (GFR). After a 60-min equilibration period, RPP was sequentially adjusted to 125, 150 and 175 mmHg. At each level of RPP, urine was collected over a 20-min period and a 250-μl sample of blood was collected. Urine flow, sodium excretion, GFR and RBF were measured at each incre-
Fig. 1. Schematic representation of the regions of chromosome 5 of the Lewis rat that were introgressed into the Dahl S genetic background in various congenic strains.

Fig. 2. Expression of cytochrome P-450 (CYP)4A protein in the renal outer medulla of Dahl S rats and 4A\(^+\), 4A\(^-\), and control congenic strains fed a purified AIN-76A diet containing 0.4% or 8.0% NaCl. A: 0.4% NaCl diet: Lanes 2–5 were loaded with microsomes prepared from the renal outer medulla of rats from the 4A\(^+\) congenic strain. Lanes 6–9 were loaded with microsomes prepared from the renal outer medulla of rats from the control congenic strain. Lanes 10–13 were loaded with microsomes prepared from the renal outer medulla of rats from the 4A\(^-\) congenic strain. Lanes 14–17 were loaded with microsomes prepared from the renal outer medulla of Dahl S rats. Lane 1 was a positive control and was loaded with microsomes prepared from the liver of a clofibrate-treated Sprague-Dawley rat. Numbers in parentheses indicate the number of rats studied per group. *Significant difference from the corresponding value in Dahl S rats.

B: 8.0% NaCl diet: Lanes 1–4 were loaded with microsomes prepared from the renal outer medulla of rats from the 4A\(^+\) congenic strain. Lanes 5–8 were loaded with microsomes prepared from the renal outer medulla of rats from the control congenic strain. Lanes 9–12 were loaded with microsomes prepared from the renal outer medulla of rats from the 4A\(^-\) congenic strain. Lanes 13–16 were loaded with microsomes prepared from the renal outer medulla of Dahl S rats. Lane 17 was a positive control loaded with microsomes prepared from the liver of a clofibrate-treated Sprague-Dawley rat. Numbers in parentheses indicate the number of rats studied per group. *Significant difference from the corresponding value in Dahl S rats.
MAP was significantly lower in the 4A⁺ congenic strain and averaged only 159 ± 3 mmHg (n = 32).

The time course of the development of hypertension was characterized using telemetry in S rats and the congenic strains (Fig. 5). Baseline MAP in the 4A⁺ congenic strain maintained from weaning on a diet containing 0.4% NaCl (day 0) was 10 mmHg lower than the corresponding values measured in S rats or the 4A⁻ and control congenic strains. MAP increased by 15 mmHg during the first wk the S rats and the control congenic strains fed an 8.0% NaCl diet. The initial increase in MAP seen during this period was about 10 mmHg less in the 4A⁺ congenic strain. MAP continued to rise to more than 180 mmHg over the next 2 wk in the S rats and the 4A⁻ and control congenic strains fed an 8.0% NaCl diet. In contrast, the development of hypertension was attenuated and MAP averaged only 150 ± 5 mmHg in the 4A⁺ congenic strain fed the same diet for 3 wk.

**Pharmacological rescue experiments.** The effects of chronic blockade of the renal formation of 20-HETE with TS-011 on the development of hypertension in S rats and the congenic strains are summarized in Fig. 6. MAP measured by telemetry increased similarly from 122 ± 4 to 185 ± 6 mmHg (n = 8) in S rats and from 119 ± 2 to 175 ± 4 (n = 6) and 138 ± 2 to 195 ± 4 mmHg (n = 6) in the 4A⁻ and control congenic strains fed an 8.0% NaCl diet for 2 wk. TS-011 had no effect on the development of hypertension in these groups of rats. The results obtained from the S rats and the 4A⁻ and control congenic strains were not significantly different; therefore, the data from these 3 groups were pooled and presented together in Fig. 6 to simplify the presentation of the data. The

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**Fig. 3.** Comparison of the production of CYP metabolites of arachidonic acid by cortical and outer medullary (OM) microsomes prepared from the kidneys of Dahl S rats and 4A⁺, 4A⁻, and control congenic strains fed either a 0.4% (A) or an 8.0% NaCl diet (B) for 3 wk. Numbers in parentheses indicate the number of rats studied per group. *Significant difference from the corresponding value in Dahl S rats.

**Fig. 4.** Mean arterial pressure (MAP) measured in 12-wk-old male Dahl S rats and 4A⁺, 4A⁻, and control congenic strains fed a purified AIN-76A diet containing 8.0% NaCl for 3 wk. Pressures were measured via a chronically implanted arterial catheter on 4 consecutive days as described in MATERIALS AND METHODS. Numbers in parentheses indicate the number of rats studied in each group. *Significant difference from the corresponding value measured in Dahl S rats.

**Fig. 5.** Time course of the development of hypertension in Dahl S rats and 4A⁺, 4A⁻, and control congenic strains fed a purified AIN-76A diet containing 8.0% NaCl for 3 wk. Pressures were recorded from conscious rats using chronically implanted radiotelemetry devices. Numbers in parentheses indicate number of rats studied in each group. *Significant difference from the corresponding value in Dahl S rats.
development of hypertension was significantly attenuated in the 4A<sup>+</sup> congenic strain relative to these control strains and averaged only 145 ± 2 mmHg (n = 9) after the rats were fed a diet containing 8.0% NaCl for 14 days. Chronic blockade of the renal production of 20-HETE with TS-011 rescued the hypertensive phenotype and MAP increased to the same level in the 4A<sup>+</sup> congenic strain fed an 8.0% NaCl diet as that seen in the S rats or the 4A<sup>−</sup> or control congenic strains fed the same diet.

Assessment of the pressure-natriuretic relationship. The effects of transferring a region of RNOS (from markers D5Rat130 to D5Rat31) that includes the CYP4A1, 2, 3 and 8 genes from Lewis rats into the S genetic background on the pressure diuretic and natriuretic responses are presented in Fig. 7. Urine flow and Na<sup>+</sup> excretion increased from 6.9 ± 0.7 to 13.7 ± 2.1 µl·min<sup>−1</sup>·g<sup>−1</sup> and from 0.52 ± 0.12 to 2.58 ± 0.68 meq·min<sup>−1</sup>·g<sup>−1</sup>, respectively, in S rats when RPP was increased from 130 to 170 mmHg (Fig. 7A). The pressure-diuretic and natriuretic responses were fivefold greater in the 4A<sup>+</sup> congenic strain. Similarly, the fractional excretion of sodium (FENa<sup>+</sup>) rose from 1.3 ± 0.37 to 3.91 ± 0.85% in the S rats when RPP was increased over this range of RPP. The magnitude of the increase in the FENa<sup>+</sup> (from 1.29 ± 0.47 to 7.84 ± 0.82%) was significantly greater in the 4A<sup>+</sup> congenic strain.

Renal hemodynamics were also improved in the 4A<sup>+</sup> congenic strain compared with that measured in S rats (Fig. 7B). Baseline RBF averaged 6.6 ± 0.5 ml·min<sup>−1</sup>·g<sup>−1</sup> in the 4A<sup>+</sup> congenic strain, and it remained well autoregulated when RPP was increased from 130 to 170 mmHg. In contrast, RBF was significantly lower in S rats and averaged only 4.7 ± 0.2 ml·min<sup>−1</sup>·g<sup>−1</sup>. GFR increased from 0.30 ± 0.02 to 0.44 ± 0.04 ml·min<sup>−1</sup>·g<sup>−1</sup> when RPP was increased from 130 to 170 mmHg in S rats. Baseline GFR was significantly higher in the 4A<sup>+</sup> congenic strain, averaging 0.56 ± 0.07 ml·min<sup>−1</sup>·g<sup>−1</sup> at 130 mmHg, and it increased to 0.78 ± 0.07 ml·min<sup>−1</sup>·g<sup>−1</sup> kidney wt<sup>−1</sup> when RPP was increased to 170 mmHg.

DISCUSSION

Previous studies have indicated that the expression of CYP4A protein and the renal formation of 20-HETE are

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**Fig. 6.** Effects of chronic blockade of the formation of 20-HETE with TS-011 on MAP in Dahl S rats and 4A<sup>−</sup>, 4A<sup>+</sup> and control congenic strains fed a purified AIN-76A diet containing 8.0% NaCl. Mean arterial pressure was recorded from conscious Dahl S rats and the 4A<sup>−</sup>, 4A<sup>+</sup> and control congenic strains treated with either TS-011 (1 mg/kg, twice a day) to selectively block the formation of 20-HETE or vehicle for 14 days. As the results obtained from the Dahl S rats and the 4A<sup>−</sup> and the control congenic strains were not significantly different, the data from these 3 groups were pooled and presented together to simplify the figure. Numbers in parentheses indicate number of rats studied in each group. ∗Significant difference from the corresponding value in the pooled control group.

**Fig. 7.** Effects of increasing renal perfusion pressure (RPP) on urine flow (UV) and sodium excretion (UNa<sub>V</sub>; A), and renal blood flow (RBF) and glomerular filtration rate (GFR; B) in 10-wk-old male Dahl S rats and the 4A<sup>+</sup> congenic strain. Numbers in parentheses indicate number of rats studied in each group. ∗Significant difference from the corresponding value measured in Dahl S at a similar level of RPP. †Significant difference from control values at RPP of 100 mmHg within a group.
reduced in S rats and that this is associated with an elevation in sodium transport in the TALH (13, 17, 31), resetting of the pressure-natriuretic relationship (1) and the development of hypertension (23). Two questions that remain to be answered, however, are whether the fall in the renal production of 20-HETE in S rats is due to a sequence variant in one of the CYP4A genes that alters the expression of this protein and whether this mutation plays a causal role in the development of hypertension. Alternatively, the fall in the renal expression of CYP4A protein in S rats may be due to changes in the expression of other genes more causally related to the development of hypertension and 20-HETE may only play a secondary role as a downstream effector pathway in this response.

To address these questions, the present study examined whether transfer of regions of RNO5 that include (4A+) or exclude the CYP4A1, 2, 3 and 8 genes from Lewis rats alters the renal production of 20-HETE, the pressure-natriuretic response and/or the development of hypertension in congenic strains of S rats. The results indicate that transferring a region that includes the CYP4A1, 2, 3 and 8 genes from the Lewis rat into the S genetic background lowers MAP by 30–40 mmHg in a 4A+ congenic strain fed an 8.0% NaCl diet for 3 wk compared with the pressures measured in S rats or control congenic strains in which the transferred segment excludes the CYP4A region. The antihypertensive effect in the 4A+ congenic strain was associated with an increase in the expression of CYP4A protein and the production of 20-HETE in the outer medulla of the kidney. In contrast, transfer of other segments of RNO5 that exclude the CYP4A region had no effect on the expression of CYP4A protein or the formation of 20-HETE.

These results are consistent with the view that there may be a functionally significant sequence variant in the regulatory region of one of the CYP4A genes or that the expression of this gene family is regulated by another gene in the interval between markers D5Rat130 to D5Rat31 that differs in S and Lewis rats. The reason why the upregulation of the expression of CYP4A protein in the 4A+ congenic strain is restricted to the outer medulla remains to be determined. One possibility is that the mutation may be in one of the CYP4A isoforms that is preferentially expressed in the TALH rather than in the proximal tubule of rats (13).

The upregulation of the renal formation of the 20-HETE in the 4A+ congenic strain was also associated with an improvement in the pressure-natriuretic response compared with that seen in S rats. This finding is consistent with previous observations that 20-HETE is an endogenous inhibitor of sodium transport in the TALH and the proximal tubule (13, 31), and that inhibition of the formation of 20-HETE blunts the pressure-natriuretic response (6) and promotes the development of salt-induced hypertension in normally salt-resistant strains of rats (11, 25). Further evidence for an important role for 20-HETE in opposing the development of hypertension in the 4A+ congenic strain was derived from the pharmacological rescue experiments. The results of these studies indicate that chronic blockade of the formation of 20-HETE with TS-011, a highly selective inhibitor of the formation of 20-HETE (19, 27), rescues the hypertensive phenotype in the 4A+ congenic strain fed a diet containing 8.0% NaCl. Chronic administration of TS-011 had no effect on the development of hypertension in S rats or the 4A− and control congenic strains in which the expression of CYP4A protein and the baseline production of 20-HETE in the outer medulla of the kidney were lower than that seen in the 4A+ congenic strain.

The present findings suggesting that the transfer of a segment of RNO5 that includes the CYP4A region from Lewis rats improves pressure-natriuresis and attenuates the development of hypertension in the 4A+ congenic strain are consistent with previous results indicating that there is a quantitative trait locus for BP in this region of RNO5 in an F2 cross of S and Lewis rats (5, 25, 30). The present findings using both telemetry and direct measurement of arterial pressure with chronic arterial catheters in the 4A− congenic strain also confirm and extend the results of a more recent study (8) indicating that transfer of this same region of RNO5 from Lewis rats into the S genetic background reduced systolic pressure as measured by tail-cuff in a S.Lew(5)X4 congenic strain by 20 mmHg. The main difference in the results of the present study vs. those of Garrett and Rapp (8) is they also reported that transfer of the region from markers D5Rat130 to D5Rat108 that excludes the CYP4A4 genes in a subsequent subcongenic strain (S.Lew(5)X6) also reduced systolic pressure. In contrast, we found no effect on MAP when we studied our 4A− congenic strain that are direct descendents of the original S.Lew(5)X6 strain. Thus the previous study concluded that the CYP4A4 genes can be excluded as candidate genes for the development of hypertension in S rats, whereas the results of the present study suggest that they remain as viable candidate genes. The reason for the difference in the results between the two studies remains to be determined. One difference is the method used to measure BP. Garrett and Rapp (8) measured systolic pressure using the tail-cuff technique on 9 wk old rats, whereas MAP was directly measured by telemetry and chronic femoral artery catheters in 12-wk-old animals in the present study. Another difference is that the rats were fed a purified AIN-76A diet containing a high NaCl concentration (8.0%) for 3 wk in the present study, while the rats in the previous study were fed a grain-based diet containing 2% NaCl for 4 wk. Indeed, we have recently reported that the degree of hypertension and renal injury is attenuated in S rats fed a grain-based diet vs. the results obtained in rats fed a purified diet (18). Thus we suspect that the differences in the two studies are related to the salt content and compositions of the diets which likely generate different phenotypes. When S rats are challenged with an 8% NaCl diet, they rapidly develop a very severe form of hypertension (MAP>180 mmHg) that is first triggered by salt retention. The 8% NaCl diet also induces severe proteinuria and progressive glomerulosclerosis. On the other hand, the degree of hypertension that develops following administration of a 2% NaCl diet is less severe and is associated with much less renal damage. Thus it is possible that there are multiple quantitative trait loci that can influence BP in this region on RNO5. The present study appears to have uncovered the impact of CYP4A genes from Lewis rats that increase the formation of 20-HETE and oppose salt retention and the development of hypertension in rats challenged with a very high NaCl intake. In contrast, Garrett and Rapp (8) may have detected the presence of another antihypertensive gene in the same region that can influence the level of systolic pressure when the rats were challenged with a more modest salt load.

Previous studies have indicated that a deficiency in the renal formation of 20-HETE contributes to an elevation of Na+ and Cl− transport in the TALH of S rats (13, 31) and that this is
associated with a resetting of the pressure-natriuretic relationship to a higher level of pressure (1). Indeed, we have shown that induction of the renal formation of 20-HETE with fibrates improves the pressure-natriuresis relationship (1) and attenuates the development of hypertension in S rats (23, 29). Therefore, we examined whether the increase in the renal formation of 20-HETE following transfer of the region of RNO5 containing the CYP4A alleles from Lewis rats into the 4A\(^+\) congenic strain was associated with an improvement of the pressure-natriuresis relationship. The results indicate that the pressure-natriuresis relationship is shifted to a lower level of arterial pressure in the 4A\(^+\) congenic strain. This was due in part to a greater inhibition of the tubular reabsorption of sodium in the 4A\(^+\) congenic strain as reflected by a significantly greater increase in \(FE_{\text{Na}}\) than that seen in S rats. It was also due to a significantly higher baseline GFR and greater increase in GFR in the 4A\(^+\) congenic strain than that seen in the S rats. Taken together, these results along with the results of other studies (1, 6, 23, 25, 29) indicate that changes in the renal formation of 20-HETE influences renal function, the pressure-natriuresis relationship and the long-term control of arterial pressure.

The present results indicate that the expression of CYP4A protein and the formation of 20-HETE are increased in a 4A\(^+\) congenic strain in which a region of RNO5 that contains the CYP4A genes from the Lewis rat was introgressed into the S genetic background. These results are consistent with the view that there may be a sequence difference in one of the CYP4A isoforms or in some other gene in this region that regulates the expression of the CYP4A genes in the outer medulla of the kidney. However, validation that CYP4A plays a causal role in the development of hypertension still requires identification of a functionally significant sequence variant in one of the CYP4A isoforms that affects enzyme activity or in the promoter or nearby conserved noncoding regions that affect the expression of one of these isoforms. The importance of the present findings is they provide the rationale for sequencing this region of the genome in S and Lewis rats. Regardless of whether a causal mutation is identified in one of the CYP4A genes, the present results using congenic strains and pharmacological rescue strongly support the view that an elevation in the renal formation of 20-HETE plays at least a permissive role in the antihypertensive effect of transfer of the genes in CYP4A region from Lewis rat into the S genetic background.

GRANTS

This work was supported in part by National Institutes of Health Grants HL-29587, HL-36279, and HL-69321. A. Sarkis was supported by a postdoctoral fellowship award from the American Heart Association. B. Lopez was supported as a postdoctoral Fulbright Scholar awarded by the Fulbright Commission/Spanish Ministry of Education, Culture and Sports (MECD) award FU2003–0973.

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