Induction of Renal 20-Hydroxyeicosatetraenoic Acid by Clofibrate Attenuates High-Fat Diet-Induced Hypertension in Rats

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ABSTRACT

This study compared renal hemodynamics, the expression of CYP4A isoforms [the enzymes for 20-hydroxyeicosatetraenoic acid (20-HETE) production], and tubular sodium transporters in male rats fed a high-fat (HF) or control diet for 10 weeks. We also studied the effect of treatment with clofibrate, a CYP4A inducer, on sodium retention and renal function and on CYP4A expression in HF rats. HF rats had higher blood pressure (BP), renal plasma flow, and glomerular filtration rate (GFR), but no significant change in renal vascular resistance. Reverse transcription-polymerase chain reaction analysis showed that CYP4A1 and CYP4A8 expression was significantly decreased in the renal cortex of HF rats. Western blot analysis showed up-regulation of expression of the α-subunit of epithelial sodium channel (α-ENaC), the β-subunit of the epithelial sodium channel (β-ENaC), sodium/hydrogen exchanger (NHE)-3, and the renal outer medulla K⁺ channel (ROMK) in HF rats, whereas expression of the γ-subunit of the epithelial sodium channel and the α1-subunit of Na⁺-K⁺-ATPase remained unchanged. Thus, HF treatment caused the reduction of renal CYP4A1 and CYP4A8 expression, whereas the increases in α-ENaC, β-ENaC, NHE-3, and ROMK expression in renal tubules may have contributed sodium retention and hypertension in HF rats. Furthermore, clofibrate treatment (240 mg/kg/day) caused the decrease of BP and GFR and the attenuation of cumulative sodium balance in HF rats. The attenuation of sodium retention by clofibrate treatment is linked to decreased expression of NHE-3 in renal cortex. Clofibrate induction of CYP4A4 expression occurred in proximal tubules and in the thick ascending limb of the loop of Henle but not in renal microvessels. This induction correlated with the expression of peroxisome proliferator-activated receptor (PPARα) in renal tubules. Therefore, these results suggest that the effects of clofibrate on sodium retention and blood pressure regulation in HF rats may be due to the induction of renal tubular 20-HETE production through the PPARα pathway.

Findings from the 1999–2000 National Health and Nutrition Examination Survey showed that 59 million adults or 31% of those in the United States are obese. Obesity is an important factor in essential hypertension (Hall, 2003). We have chosen to study rats fed a HF diet because this model mimics the effects of human HF-food consumption. These rats develop characteristic features of human obesity-induced hypertension such as increased sodium balance, renal tubular reabsorption, and glomerular filtration rate (Dobrian et al., 2000; Hall, 2003).

20-Hydroxyeicosatetraenoic acid (20-HETE) is a major metabolite of arachidonic acid in the rat kidneys; its synthesis and actions have been localized primarily to the microcirculation and renal tubules (Roman, 2002). 20-HETE synthesis is catalyzed primarily by CYP4A isoforms (Roman, 2002). In the rat, four isoforms have been identified: CYP4A1, CYP4A2, CYP4A3, and CYP4A8. Several reports have dem-

ABBREVIATIONS: HF, high fat; HETE, 20-hydroxyeicosatetraenoic acid; TALH, thick ascending limb of the loop of Henle; ROMK, renal outer medulla K⁺ channel; ENaC, epithelial sodium channel; NHE, sodium/hydrogen exchanger; Na⁺-K⁺-ATPase, α₁-subunit of sodium-potassium adenosine triphosphatase; EET, epoxyeicosatrienoic acid; RT-PCR, reverse transcription polymerase chain reaction; MAP, mean arterial pressure; FITC, fluorescein isothiocyanate; RBF, renal blood flow; GFR, glomerular filtration rate; RPF, renal plasma flow; PPARα, peroxisome proliferator-activated receptor; α₁, β₁, and γ-ENaC, α₁-, β₁-, and γ-subunits of the ENaC; PPRE, peroxisome proliferator response element.
onstrated that 20-HETE synthesis is carried out by these CYP4A isoforms (Nguyen et al., 1999; Hoch et al., 2000; Cowart et al., 2002). In the renal vasculature, 20-HETE causes vasoconstriction (McGiff and Quillley, 1999; Imig, 2000). In renal tubular segments, 20-HETE is important in the regulation of tubular sodium transport. For instance, 20-HETE inhibits Na⁺-K⁺-ATPase in the proximal tubules (Schwartzman et al., 1985), inhibits Na⁺-K⁺-2Cl⁻ cotransporter activity in the thick ascending limb of the loop of Henle (TALH) (Escalante et al., 1994), and blocks the renal outer medulla K⁺ channel (ROMK) in the apical membrane of TALH cells (Wang and Lu, 1995).

The present study was designed to determine which CYP4A isoforms are responsible for the down-regulation of 20-HETE synthesis in HF rats and whether sodium retention is associated with changes in the expression of sodium transporters such as ENaCs, NHE-3, ROMK, and Na⁺-K⁺-ATPase α1 in the renal tubules. In addition, we determined the effects of clofibrate on renal function and sodium retention in HF rats. This study provides valuable information regarding the function of renal 20-HETE in the regulation of blood pressure in HF rats.

Materials and Methods

Materials. We obtained [1-14C]arachidonic acid (56 mCi/mmole) from DuPont-New England Nuclear (Boston, MA). We purchased the reagents for Western blot analysis from Amersham Biosciences (Piscataway, NJ). All high-performance liquid chromatography solvents and chemicals for buffers were from Sigma-Aldrich (Milwaukee, WI). We purchased epoxyeicosatrienoic acids (EETs) and 20-HETE standard from Cayman Chemical (Ann Arbor, MI).

Animals. Three-week-old male Sprague-Dawley rats purchased from Harlan (Indianapolis, IN) were divided into two groups, one of HF rats, which were fed a modified chow containing 36% fat (15.2% saturated and 20.8% unsaturated), 35% carbohydrate, 20% protein, and 0.4% salt (Bio-Serv, Frenchtown, NJ), and the other of control rats, which were fed normal rat chow containing 4.4% fat (2.5% saturated and 20.8% unsaturated), 35% carbohydrate, 20% protein, and 0.4% salt (Bio-Serv, Frenchtown, NJ). All high-performance liquid chromatography solvents and chemicals for buffers were from Sigma-Aldrich (Milwaukee, WI). We purchased epoxyeicosatrienoic acids (EETs) and 20-HETE standard from Cayman Chemical (Ann Arbor, MI).

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Protocol to Determine Renal Hemodynamics and Urinary Excretion Parameters in HF and Control Male Rats. We placed 13-week-old male HF rats after treatment with clofibrate (240 mg/kg/day intragastrically) for 2 weeks (n = 8), 13-week-old male HF rats (n = 8), and age-matched male rats (n = 8) in metabolic cages to obtain their basal levels of 24-h urinary sodium excretion and urine volume. We determined urinary sodium concentrations using the Beckman Synchron EL-ISE electrolyte system (Beckman, Brea, CA). We then used these HF rats and age-matched control rats for a renal functional study. After finishing that study, we immediately removed the rats' kidneys for renal cortical and section preparation. These renal tissues were used for RT-PCR, immunohistochemical analysis, Western blot, and arachidonic acid metabolism.

Renal Functional Measurements. We weighed each rat before surgery. We anesthetized each rat with 2% isoflurane delivered by an anesthesia apparatus. We then placed one polyethylene cannula in the trachea (PE-205) to allow free breathing, one in the bladder (PE-240) to collect urine, one in the femoral artery (PE-205) to measure and recording MAP with a pressure transducer, and one (PE-50) in the femoral vein for the infusion of agents. We adminis-tered a priming dose of 0.5 ml of fluorescein isothiocyanate (FITC)-inulin (8 mg/ml in phosphate-buffered saline (Sigma-Aldrich)) over 2 min. We performed a left laparotomy and placed a transonic flow probe (Transonic Systems Inc., Ithaca, NY) over the left renal artery to measure renal blood flow (RBF). Throughout these procedures, each rat's body temperature was maintained at 37°C by a temperature controller (Cole-Parmer Instrument Co., Vernon Hills, IL) connected to a heating mat and a rectal temperature probe. We infused a saline solution containing 0.2% bovine serum albumin equal to 1.25% of body weight for the replacement of volume loss during surgery. We then infused saline (3 ml/h i.v.) and FITC-inulin (12 mg/h i.v.) to maintain isotonic NaCl and a constant concentration of FITC-inulin.

After surgery, we allowed at least 45 min for equilibration before beginning 30-min urine collections. Arterial blood (0.4 ml) was drawn from the femoral artery in the middle of each 30-min clearance period for measurement of GFR. An equal volume of normal saline was infused for volume replacement. We obtained MAP, RBF, RPF, and renal vascular resistance from a computerized data collection and analysis system (EMKA Technologies, Falls Church, VA). We determined the concentration of FITC-inulin in the plasma and urine of our rats using a GENios Plus fluorescent plate reader (Tecan, Research Triangle Park, NC) at 485 nm excitation and 538 nm emission. As previously described (Qi et al., 2004; Huang et al., 2005), we used the concentration of FITC-inulin in the plasma and urine to calculate the GFR.

RT-PCR. A reverse transcription reaction was performed using a first-strand cDNA synthesis kit (Pharmacia Biotech, Milwaukee, WI). Briefly, we added total RNA (0.003–0.09 μg) from the cortex of HF and control male rats to 15 μl of reverse transcription reaction mixture containing 45 mM Tris (pH 8.3); 68 mM KCl; 15 mM dithiothreitol, 9 mM MgCl₂; 0.08 mg/ml bovine serum albumin; 1.8 mM dioxynucleoside-5'-triphosphate; 40 pmol of either CYP4A1, CYP4A2, CYP4A3, CYP4A8, or β-actin backward primer; and Maloney murine leukemia virus reverse transcriptase. The reactions were incubated for 1 h at 37°C. PCR was carried out in 25 μl of PCR SuperMix ( Gibco BRL) containing 22 mM Tris-HCl (pH 8.4); 55 mM KCl; 1.65 mM MgCl₂; 200 μM dGTP; 200 μM dATP; 200 μM dCTP; 22 U of recombinant TaqDNA polymerase per ml; 20 pmol of CYP4A1, CYP4A2, CYP4A3, CYP4A8, and β-actin primer pairs; and 4 μl of first-strand synthesis reaction. Reactions were cycled 30 times through a 5 min denaturing step at 95°C, a 1 min annealing step at 60°C, and a 2 min extension step at 72°C. We then did a final 7 min elongation step at 72°C. As in our previous publications (Wang et al., 2001; Marji et al., 2002), the CYP4A1, CYP4A2, CYP4A3, CYP4A8, and β-actin primers were designed to amplify 351-, 317-, 321-, 349-, and 764-base pair fragments, respectively, from each of the corresponding cDNAs. The specificity of these CYP4A primers was examined by using CYP4A isoform cDNA as a template for the PCR (Wang et al., 2001). The sequences of the primers were as follows: CYP4A1, 5'-CTC TTA CCT CGG AGA ATG GAG AA-3' (forward primer), 5'-GAC TTG GAT ACC CTT GGG TAA AG-3' (backward primer); CYP4A2, 5'-AGA TCC AAA GCC TTA TCA ATC-3' (forward primer), 5'-CAG CCT TGG TGT AGG ACC- T-3' (backward primer); CYP4A3, 5'-CAA AGG CTT CTG GAA TTT ATC-3' (forward primer), 5'-CAG CCT TGG TGT AGG ACC T-3' (backward primer); CYP4A8, 5'-ATC CAG AGG TGG TTG ACC CCT AT-3' (forward primer), 5'-AAT GAG ATG TGA GCA GAT GGT-3' (backward primer); and β-actin, 5'-TGT TAA CCA ACT GGG AGG ATA TGG-3' (forward primer), 5'-GAT CTT GAT CAT TAT GGT GCT AGG-3' (backward primer). An aliquot (10 μl) of each PCR product was separated on a 1.5% agarose gel. PCR products were stained with ethidium bromide.

Immunohistochemical Analysis. We isolated kidneys from HF and control rats (n = 4) and cut them into small slices. A specimen cup containing 2-methyl butane was precooled for 45 min in a Styrofoam cooler containing dry ice and ethanol. We embedded the kidney slices in a specimen mold containing OCT compound (Miles Scientific).
ments were conducted for 15 days. Sodium concentration was measured for sodium balance. The daily measurements included blood pressure and urinary volume for sodium balance study. After treatment, we measured the blood pressure of HF rats and age-matched male rats treated with clofibrate (240 mg/kg/day) for 2 weeks. Clofibrate was dissolved in corn oil and was given intragastrically to HF rats. The age-matched male rats were used as control for sodium balance experiments.

**Western Blot Analysis.** We separated renal samples (10 μg) from HF and control rats by electrophoresis on a 10 × 20-cm, 8% SDS-polyacrylamide gel at 25 mA/gel at 4°C for 18 h. We have previously described the detailed procedures for transfer, blocking, and washing the samples (Wang et al., 2003). The membranes were incubated for 10 h with antibodies for CYP4A (1:2000; Gentest), PPARα (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), Na+/K+-ATPase α1 (1:1000; Upstate, Lake Placid, NY), NHE-3 (1:1000; Chemicon, Temecula, CA), α-ENaC (1:1000; Abcam Inc., Cambridge, MA), β-ENaC (1:1000; Abcam), γ-ENaC (1:1000; Abcam), ROMK (1:500; Alomone Labs, Jerusalem, Israel), or β-actin (1:5000; Sigma Chemical, St. Louis, MO). We washed the membranes several times with Tris-buffered saline and further incubated them with secondary antibodies for these proteins. We developed the immunoblots using an ECL detection kit (Amersham, Arlington Heights, IL). The expression of these proteins was normalized to the expression of β-actin as previously described (Wang et al., 2003).

**Proteins to Evaluate the Effect of Clofibrate on Sodium Retention and Blood Pressure in HF Rats.** To determine the effect of clofibrate in HF rats, we divided HF rats into two groups. We treated 11-week-old HF rats (n = 5) with clofibrate (240 mg/kg/day) or vehicle control (corn oil) for 2 weeks. Clofibrate was dissolved in corn oil and was given intragastrically to HF rats. The age-matched male rats were used as a control for sodium balance experiments. The dosage of clofibrate was based on a previous study (Roman et al., 1993). During the treatment, these HF rats and age-matched male rats were placed in individual metabolic cages for 15 days of sodium balance study. After treatment, we measured the blood pressure of these rats in a conscious condition as described in our previous publication (Wang et al., 1999) and then euthanized them and analyzed samples of their renal cortex, renal microvessels, proximal tubules, and TALH for cytochrome P450 protein expression and enzyme activity using methods described previously (Wang et al., 2002, 2003).

**Sodium Balance.** HF and age-matched male rats were placed in individual metabolic cages. Daily food intake, water intake, and urine volume were measured for sodium balance. The daily measurements were conducted for 15 days. Sodium concentration was measured using the Beckman Synchron EL-ISE electrolyte system at the conclusion of the study. Daily sodium intake, excretion, balance, and cumulative sodium balance were calculated as described previously (Hall et al., 1993).

**Arachidonic Acid Metabolism.** Renal cortical microsomes (150 μg) isolated from HF rats treated with either vehicle or clofibrate were incubated with [1-14C]arachidonic acid (0.4 μCi, 7 nmol) and NADPH (1 mM) in 0.3 ml of potassium phosphate buffer (100 mM, pH 7.4) containing 10 mM MgCl2 for 30 min at 37°C. The reaction was terminated by acidification to pH 3.5 to 4.0 with 2 mol/l formic acid, and arachidonic acid metabolites were extracted with ethyl acetate. The ethyl acetate was evaporated under nitrogen, and the metabolites were resuspended in 50 μl of methanol and subjected to reverse-phase high-performance liquid chromatography using a 5-μm ODS-Hypersil column, 4.6 × 200 mm (Hewlett Packard, Palo Alto, CA) and a linear gradient of acetonitrile-water-acetic acid ranging from 50:50:0.1 to 100:0:0.1 at a flow rate of 1 ml/min for 30 min. The elution profile of the radioactive products was monitored by a flow detector (In/us System Inc., Tampa, FL). The identities of arachidonic acid metabolites (20-HETE, dihydroxyeicosatrienoic acids, and EETs) were confirmed with authentic standards. The activity of the formation of these metabolites was estimated based upon the specific activity of the added [1-14C]arachidonic acid and was expressed as picomoles per milligram of protein per minute.

**Statistical Analysis.** Data are expressed as means ± S.E. All data were analyzed by computer software (SPSS Inc., Chicago, IL). We used the one-way analysis of variance or the Student’s unpaired two-tailed test for statistical analysis. Statistical significance was set at P < 0.05.

**Results**

**Effect of High-Fat Diet on Body Weight, Renal Hemodynamics, and Urinary Excretion Parameters.** After 10 weeks of HF treatment, we examined renal hemodynamics in HF and control rats. We found that MAP, RBF, and GFR were significantly elevated in HF rats compared with control rats (Table 1). The HF rats had significantly greater body weights than did control rats as well as lower urinary volume and urinary sodium excretion (Table 1).

**Effect of High-Fat Diet on CYP4A Isoform Expression in Renal Cortex and Renal Section.** Previously, using Western blot analysis, we showed that the expression of CYP4A in HF rats was significantly decreased (Wang et al., 2003). However, the antibody against CYP4A that we used is polyclonal, cannot distinguish individual CYP4A isoforms, and cannot be used to estimate the expression of individual CYP4A isoforms. To evaluate the effect of the HF diet on relative changes in renal mRNA levels of individual CYP4A isoforms, we performed RT-PCR using specific primers.

**TABLE 1**

Renal hemodynamics and urinary excretion parameters in HF and control male rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clo-HF Rats</th>
<th>HF Rats</th>
<th>Control Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>375 ± 8a</td>
<td>420 ± 5a</td>
<td>364 ± 7</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>103 ± 2b</td>
<td>123 ± 3a</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>11 ± 1</td>
<td>10.3 ± 1b</td>
<td>7.8 ± 1</td>
</tr>
<tr>
<td>Renal vascular resistance (mm Hg/ml/min)</td>
<td>10 ± 1</td>
<td>11.6 ± 1</td>
<td>11.5 ± 1</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>1.1 ± 0.2a</td>
<td>1.4 ± 0.1a</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.42 ± 0.2</td>
<td>0.42 ± 0.01</td>
<td>0.41 ± 0.01</td>
</tr>
<tr>
<td>RPF (ml/min)</td>
<td>6.4 ± 0.3</td>
<td>6.1 ± 0.25a</td>
<td>4.0 ± 0.44</td>
</tr>
<tr>
<td>Urinary volume (ml/24 h)</td>
<td>10 ± 1.2a</td>
<td>6 ± 0.7a</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Urinary sodium (mEq/24 h)</td>
<td>1.7 ± 0.1a</td>
<td>1.4 ± 0.1a</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

a P < 0.05 from HF rats.
b P < 0.05 from control.
ers for CYP4A isofoms on renal cortex isolated from HF and control rats as described previously (Marji et al., 2002). The CYP4A1, CYP4A2, CYP4A3, CYP4A8, and β-actin primers were designed to amplify 351-, 317-, 321-, 349-, and 764-base pair fragments, respectively, from each of the corresponding cDNAs. Expression of each isoform was normalized with β-actin (semiquantitative RT-PCR).

Ten-week administration of the HF diet caused expression of CYP4A1 and CYP4A8 to decrease by 12% (1.20 ± 0.02 versus 1.36 ± 0.04 arbitrary units, n = 4, P < 0.05) and 36% (0.58 ± 0.12 versus 0.9 ± 0.1 arbitrary units, n = 4, P < 0.05), respectively, whereas there was no significant change in CYP4A2 and CYP4A3 mRNA in the kidneys of HF rats (Fig. 1A). To substantiate the results of Western blot analyses in our previous study (Laffer et al., 2003), we performed immunohistochemical analyses using CYP4A antibody. As shown in Fig. 1B, CYP4A proteins were expressed in the renal cortex, with the most intense staining in the renal tubules; glomeruli showed very low immunoreaction to CYP4A proteins. We observed decreasing expression of CYP4A in the renal tubules of renal sections from HF rats. The staining intensity revealed that CYP4A expression was significantly decreased by 43% (7.1 ± 0.4 versus 12.4 ± 0.3 arbitrary units, n = 4, P < 0.05).

**Effect of High-Fat Diet on the Expression of Sodium Transporters in Renal Tissues.** To test whether there were any changes in the expression of sodium transport proteins in the tubules of HF rats, we examined renal tubular Na⁺-K⁺-ATPase, NHE-3, ROMK, and ENaC expression in 13-week-old rats fed the HF diet for 10 weeks and age-matched male control rats. Na⁺-K⁺-ATPase, NHE-3, ROMK, and ENaC are important proteins involved in sodium reabsorption in the proximal tubules, TALH, and collecting duct.

With respect to ENaC, as shown in Fig. 2A, there was significant up-regulation of two of its subunits, α-ENaC and β-ENaC, but no change in the expression of the γ-ENaC subunit in homogenates of whole kidneys from HF rats. Specifically, densitometry analysis normalized with β-actin demonstrated that in HF rats compared with control rats, expression of α-ENaC was increased 60% (0.45 ± 0.05 versus 0.28 ± 0.03 arbitrary units, n = 3, P < 0.05), whereas that of β-ENaC was increased 31% (1.02 ± 0.04 versus 0.78 ± 0.05 arbitrary units, n = 3, P < 0.05). Similarly, the expression of NHE-3 was significantly increased by 112% (0.70 ± 0.1 versus 0.33 ± 0.08 arbitrary units, n = 3, P < 0.05), as was that of ROMK to 40% (4.3 ± 0.3 versus 3.1 ± 0.2 arbitrary units, n = 3, P < 0.05) in homogenates of the renal medulla of HF rats. In homogenates of the renal cortex of HF rats, the expression of NHE-3 was increased by 50% (0.3 ± 0.05 versus 0.2 ± 0.04 arbitrary units, n = 3, P < 0.05). However, there was no significant change in expression of Na⁺-K⁺-ATPase α1 in homogenates of either the medulla or cortex of HF rats.

**Effects of Clofibrate Treatment on Sodium Balance and Renal Function in HF Rats.** To examine whether the HF diet has any effect on sodium retention, we determined the cumulative sodium balance for 15 days in 11-week-old rats fed the HF diet for 8 weeks and in age-matched control rats fed the control diet for 8 weeks. As shown in Fig. 3A, on the 15th day of measurement, cumulative sodium balance was significantly increased (1.16 ± 0.04 mEq) in the HF rats.
compared with that in control rats (−0.7 ± 0.05 mEq, \( P < 0.04, n = 4 \)). Sodium retention in HF rats was associated with a significant increase in MAP (139 ± 1 versus 122 ± 2 mm Hg in conscious HF and control male rats, respectively, \( P < 0.05 \)) (Fig. 3B). To examine whether clofibrate has any effect on sodium retention, we conducted cumulative sodium experiments on 11-week-old HF rats treated with clofibrate (240 mg/kg/day) for 2 weeks. As shown in Fig. 3, clofibrate treatment of HF rats caused the cumulative sodium balance curve and MAP to return to the levels in control male rats. In addition, clofibrate treatment of HF rats caused decreased body weight, MAP, and GFR, whereas it increased urinary volume and urinary sodium excretion (Table 1).

Effects of Clofibrate Treatment on 20-HETE Production and the Expression of CYP4A in Renal Tissues of HF Rats. To examine the selectivity of clofibrate in the induction of renal 20-HETE and EET production, 11-week-old HF rats were orally treated with clofibrate (240 mg/kg/day) or vehicle control for 2 weeks. As shown in Fig. 4A, clofibrate selectively induced renal \( \omega \)-hydroxylase activity (20-HETE production) without having a significant effect on epoxygenase activity (EET production). In addition, clofibrate treatment significantly induced CYP4A expression in the renal cortex, proximal tubules, and TALH (Figs. 4B and 5). However, clofibrate treatment had no induction effect on CYP4A in the renal microvessels (Fig. 5). Densitometry analysis normalized with \( \beta \)-actin showed that compared with values in vehicle-treated control rats, clofibrate treatment of HF rats caused expression of CYP4A to increase 69% (1.47 ± 0.2 versus 0.87 ± 0.1 arbitrary units, \( n = 3, P < 0.05 \)) in the renal cortex, 30% (1.43 ± 0.2 versus 1.1 ± 0.1 arbitrary units, \( n = 3, P < 0.05 \)) in renal proximal tubules, and 47% (5.6 ± 0.2 versus 3.8 ± 0.1 arbitrary units, \( n = 3, P < 0.05 \)) in TALH. To examine whether clofibrate treatment has any effects on NHE-3 expression, we examined the renal medulla NHE-3...
expression in HF rats treated with either clofibrate or vehicle control and age-matched control male rats. As shown in Fig. 6, HF diet treatment caused increased expression of NHE-3, whereas treatment with clofibrate in HF rats resulted in the expression of NHE-3 returning to the levels in control male rats. To further understand the induction effect of clofibrate through the PPARα pathway in the kidneys, we examined the tissue distribution of PPARα expression in renal microvessels, proximal tubules, and TALH. As shown in Fig. 7, we detected a strong signal of PPARα expression in the proximal tubules and TALH but no signal in the renal microvessels.

Discussion

This study demonstrates that obesity-induced hypertension is associated with abnormal renal hemodynamics, including increases in RBF, GFR, RPF, and cumulative sodium balance and decreases in urinary sodium excretion and urinary volume. It also demonstrates that the sodium retention in obese rats is linked to decreased expression of CYP4A1 and CYP4A8 (enzymes for 20-HETE production), whereas there is increased expression of α-ENaC, β-ENaC, NHE-3, and ROMK, major sodium transporters, in the renal tubules. Moreover, the PPARα agonist clofibrate, a selective CYP4A inducer, decreases sodium retention, GFR, and blood pressure in HF rats. The effects of clofibrate are associated with the induction of CYP4A expression in renal tubules, whereas the expression of CYP4A in renal microvessels is unaffected. These results demonstrate that the decreased expression of CYP4A1 and CYP4A8 is responsible for the decreased renal 20-HETE production in HF rats. The decreased expression of CYP4A1 and CYP4A8, together with the increased expression of sodium transporters, contributes to sodium retention in HF rats. However, the overproduction of renal tubular 20-HETE induced by clofibrate treatment through the PPARα pathway can decrease sodium retention and reduce blood pressure in obese HF rats.

Obesity-induced hypertension is characterized by increases in arterial blood pressure, cardiac output, and glomerular filtration rate (Hall, 2003). Because 20-HETE inhibits renal tubular sodium transport and affects blood pressure in many hypertensive animal models (Roman, 2002), we hypothesized that 20-HETE may also be involved in the regulation of blood pressure in obesity. In a previous study (Zhou et al., 2005), we demonstrated that the decreased expression of CYP4A in the proximal tubules is associated with hypertension in HF rats. However, the relative contributions of individual CYP4A isoforms to the decreased 20-HETE production still were not clear. In the present study, we have shown that the expression of CYP4A1 and CYP4A8 is significantly decreased after rats have received the HF diet for 10 weeks. It has been shown that CYP4A enzymes are the major contributors to 20-HETE production in the rat kidney (Roman, 2002). For instance, several reports have demonstrated that recombinant CYP4A1, CYP4A2, CYP4A3, and CYP4A8 can catalyze the η-hydroxylation of arachidonic acid into 20-HETE (Wang et al., 1996; Nguyen et al., 1999; Cowart et al., 2002) and that CYP4A1 is the low K_m form of η-hydroxylase (Nguyen et al., 1999). This finding implies that down-regulation of CYP4A1 and CYP4A8 by the HF diet is responsible for the decreased 20-HETE synthesis in rat kidneys.

In the renal tubules, there are several major sodium transporters: Na^+-K^+-ATPase, NHE-3, ROMK, and ENaCs. These transporters, located in different segments of the nephron, are responsible for sodium transport in the kidneys. For instance, Na^+-K^+-ATPase, a sodium pump, transports sodium ions out of cells and extracellular potassium into cells. For each ATP molecule hydrolyzed, Na^+-K^+-ATPase moves two potassium ions into the cell and three sodium ions out of the cell (Feraille and Doucet, 2001). Na^+-K^+-ATPase contains two subunits, the α- and β-subunits, which are associated in a 1:1 M ratio. In renal tubular cells, Na^+-K^+-ATPase is exclusively located in the basolateral membranes. Na^+-K^+-ATPase is the major pump that catalyzes the transcellular transport of sodium in each tubular segment of the nephron.

NHEs are ubiquitous membrane ion transporters that are responsible for the extrusion of intracellular hydrogen ions in exchange for external sodium (LaPointe et al., 2002). Of the five known isoforms of the NHE family (NHE 1–5), NHE-3 is unequivocally located in the apical membranes of the proximal tubules and TALH. There sodium is actively reabsorbed primarily by the apical NHE-3 and extruded through the basolateral Na^+-K^+-ATPase (Magyar et al., 2000). ROMK, located in the apical membrane of TALH, is responsible for the recycling of K^+ into the lumen of the TALH. The recycling of K^+ by ROMK is necessary to keep the lumen-positive transepithelial potential, which is an important force for sodium reabsorption in the TALH (Wang and Lu, 1995). ENaCs, located in the apical membranes of connecting tubules and the collecting duct, are the major proteins responsible for sodium transport in these segments of the nephron. ENaC is a hetero-oligomer composed of α-, β-, and γ-subunit proteins (Bickel et al., 2001).

We have previously shown that feeding rats a HF diet causes hypertension, sodium retention, and down-regulation of proximal tubular 20-HETE synthesis (Zhou et al., 2005). Accordingly, renal 20-HETE synthesis may have a critical function in sodium retention and subsequently cause hypertension. However, the mechanisms whereby 20-HETE causes sodium retention have not been elucidated. To test whether there are any changes in the expression of sodium transport
proteins in the tubules of HF rats, we examined the expression of several sodium transporters by Western blot analysis in 13-week-old rats that had been fed the HF diet for 10 weeks and age-matched rats fed the control diet for 10 weeks. The present study demonstrated significant up-regulation of α-ENaC, β-ENaC, NHE-3, and ROMK expression, but no significant changes in γ-ENaC and Na+/K+-ATPase α1 expression in the renal tubules of HF rats. In a previous study, Bickel et al. (2001) used up-regulation of the expression of Na+/K+-ATPase α1, α-ENaC, and β-ENaC as an index to correlate sodium retention in obese Zucker rats. Similarly, Hoagland et al. (2004) have correlated the elevated expression of Na+/K+2Cl− cotransporter and ROMK with sodium retention and hypertension in Dahl salt-sensitive rats. These results, taken together, demonstrated that the up-regulation of Na+/K+2Cl− cotransporter and ROMK plays a role in sodium retention in HF rats. PPAREα is a ligand-dependent transcription factor that regulates other genes by binding to specific peroxisome proliferator response elements (PPREs) located in the promoter region of the targeted genes (Simpson, 1997). Once it is activated by peroxisome proliferators, PPAREα forms a heterodimer with the retinoid X receptor and binds to PPREs in the 5′ flanking sequence of the targeted genes. This binding leads to an increase in the transcription rates of genes. PPAREα is the target for fibrate drugs such as clofibrate, which is used to lower triglycerides in dyslipidemic patients. PPAREα has been shown to have a prominent function in regulating CYP4A expression. PPREs are found in the promoter regions of CYP4A genes (Muerhoff et al., 1992; Aldridge et al., 1995). In this study, we have shown that clofibrate treatment selectively induces renal 20-HETE production without exerting significant effects on EET production in HF rats (Fig. 4). These results are consistent with those of studies showing that clofibrate induces renal CYP4A expression and that its induction is correlated with increased 20-HETE production in the rat kidney (Cummings et al., 1999; Ishizuka et al., 2003).

Studies by Roman et al. (1993) and Alonso-Galiccia et al. (1998) showed that clofibrate prevents the development of hypertension and improves pressure-natriuresis in Dahl salt-sensitive rats. However, the location in the kidneys of the induction site for 20-HETE production by clofibrate treatment still was not known. In the present study, we have shown that clofibrate treatment induces the expression of CYP4A in both proximal tubules and TALH, but not in renal microvessels (Fig. 5). The induction effects of CYP4A expression are correlated with the expression of PPAREα in the renal tubules, but no PPAREα expression was found in the renal microvessels (Fig. 7). Similar tissue distribution of PPAREα expression in the renal tubules but not in pregglomerular arterioles has been reported (Ishizuka et al., 2003). More interestingly, the up-regulation of CYP4A in response to clofibrate treatment of HF rats is associated with reduced sodium retention and blood pressure (Fig. 3). The reduced sodium retention by clofibrate treatment in HF rats is linked to decreased expression of NHE-3 (Fig. 6). These results are consistent with the results that 20-HETE may mediate the distribution of NHE-3 in the renal tubules (Dos Santos et al., 2004). Taken together, these results demonstrate that the effects of clofibrate treatment on blood pressure regulation in HF rats are mainly consequences of reduced sodium retention. The reduction of sodium retention in HF rats by clofibrate treatment is, in turn, a consequence of the induction of renal tubular 20-HETE production by the PPAREα pathway.

In summary, this study demonstrates that hypertension induced by the HF diet is associated with abnormal renal hemodynamics and sodium retention. The sodium retention in HF rats is correlated with the decreased expression of CYP4A1 and CYP4A8 and with increased expression of sodium transporters. Our findings also demonstrate that clofibrate, a selective CYP4A inducer, induces renal tubular CYP4A expression, but has no induction effect on CYP4A expression in renal microvessels. The induction of CYP4A expression by clofibrate treatment is associated with reductions in sodium retention and blood pressure in obese HF rats. This study raises the possibility that 20-HETE has an important function in the regulation of renal function and blood pressure in obesity-induced hypertension.

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References


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