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20-Hydroxyeicosatetraenoic acid and renal function in Lyon hypertensive rats

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Abstract

To evaluate the contribution of cytochrome *P*450 (CYP450) metabolites of arachidonic acid in the increased renal vascular resistance and blunted pressure–natriuresis response exhibited by Lyon hypertensive (LH) rats, the effects of an intrarenal infusion of 17-octadecynoic acid (3 μM), an inhibitor of the formation of epoxyeicosatrienoic and 20-hydroxyeicosatetraenoic acids, were compared in 8-week-old LH and low blood pressure (LL) control rats. 17-Octadecynoic acid failed to affect renal function in LL rats. In contrast, it reduced renal vascular resistance and shifted the pressure–natriuresis relationship to lower pressures in LH rats. Blockade of thromboxane–endoperoxide (TP) receptors with GR 32191B prevented the renal vasodilator response to 17-octadecynoic acid but not its natriuretic action. Miconazole (1 μM), an inhibitor of epoxygenase activity, had no effect on renal function in LH rats. These results indicate that CYP450 metabolites of arachidonic acid, likely 20-hydroxyeicosatetraenoic acid, contribute to the resetting of the pressure–natriuresis relation in LH rats and that the renal vasoconstrictor effects of 20-hydroxyeicosatetraenoic acid in LH rats may be related to activation of TP receptors.

Keywords: Lyon hypertensive, rat; Hydroxyeicosatetraenoic acid; Epoxyeicosatrienoic acid; 17-Octadecynoic acid

1. Introduction

Arachidonic acid is metabolized via the cytochrome *P*450 (CYP450) epoxygenases and hydroxylases into epoxyeicosatrienoic and hydroxyeicosatetraenoic acids, respectively. 11,12-Epoxyeicosatrienoic acid, 14,15-epoxyeicosatrienoic acid and 20-hydroxyeicosatetraenoic acid are the primary metabolites of arachidonic acid produced by renal arterioles, proximal tubules, and glomeruli (Yoshimoto et al., 1986). Epoxyeicosatrienoic acids dilate renal arterioles in vitro (Carroll et al., 1990; Campbell et al., 1996; Zou et al., 1996b; Li and Campbell, 1997; Li et al., 1997) and are thought to promote Na^+ excretion following an increase in salt intake (Makita et al., 1994). In contrast, 20-hydroxyeicosatetraenoic acid is a potent vasoconstrictor that has a direct effect on the open state probability of potassium channels in renal vascular smooth muscle cells (Imig et al., 1996). 20-Hydroxyeicosatetraenoic acid can also be metabolized by cyclooxygenase to form thromboxane and vasoconstrictor endoperoxide (Schwartzman et al., 1989). At the level of the renal tubule, 20-hydroxyeicosatetraenoic acid inhibits Na^+ reabsorption in the proximal tubule by attenuating Na^+–K^+-ATPase activity (Nowicki et al., 1997). It also inhibits Na^+ reabsorption in the thick ascending loop of Henle by blocking the Na^+–K^+–$2Cl^-$ cotransporter (Zou et al., 1996a).

Alterations in the renal metabolism of CYP450 metabolites of arachidonic acid have been reported in different models of hypertension. In spontaneously hypertensive rats (SHR), the renal production of 20-hydroxyeicosatetraenoic acid is significantly higher than that seen in Wistar-Kyoto (WKY) (Omata et al., 1992) or Brown Norway (BN) rats (Stec et al., 1996b). Moreover, inhibition of the renal production of 20-hydroxyeicosatetraenoic acid lowers blood pressure in SHR and can prevent or delay the development of hypertension (Escalante et al., 1991; Da Silva et al., 1994). Nevertheless, the CYP4504A gene, which encodes for the enzyme that produces 20-hydroxyeicosatetraenoic acid, does not cosegregate with blood

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pressure in an F2 population of rats derived by crossing of SHR and BN rats (Stec et al., 1996b). These studies indicate that changes in the formation of 20-hydroxyeicosatetraenoic acid may contribute to the alterations in renal function in SHR but it is not a primary genetic cause of hypertension in this strain.

In Dahl salt-sensitive rats (DS), the production of 20-hydroxyeicosatetraenoic acid is reduced in the outer medulla as compared to that seen in Dahl salt-resistant (DR) rats (Ma et al., 1994) and Lewis Wistar rats (Stec et al., 1996a). The CYP4504A genotype strongly cosegregated with blood pressure in an F2 population derived from an F2 cross of DS and Lewis rats (Stec et al., 1996a). Since 20-hydroxyeicosatetraenoic acid acts as an endogenous inhibitor of the Na^+–K^+–$2Cl^-$ transporter, these results suggest that a diminished production of 20-hydroxyeicosatetraenoic acid in the outer medulla of DS rats may contribute to the elevation in loop Cl^- transport and to the development of hypertension in this strain (Zou et al., 1996a).

We have reported that the pressure–natriuresis relationship is shifted to higher pressures in LH rats and that this is associated with a marked elevation in renal vascular resistance. The finding that cyclooxygenase inhibitors and thromboxane/endoperoxide receptor antagonist improve the renal function in Lyon hypertensive (LH) rats indicates that a defect in the renal metabolism of arachidonic acid may be involved in the resetting of kidney function in this model (Liu et al., 1994). Since recent studies have indicated a major role for CYP450 metabolites of arachidonic acid in the control of renal vascular tone, the purpose of the present study was to examine the role of the CYP450 metabolites of arachidonic acid in altering renal function in LH rats. To this end, we compared the effects of intrarenal infusion of an inhibitor of the production of epoxyeicosatrienoic acids and 20-hydroxyeicosatetraenoic acid, 17-octadecynoic acid, vs. miconazole, an inhibitor of epoxygenases, on renal hemodynamics and Na^+ excretion in LH and Lyon low blood pressure (LL) control rats. We also compared the renal metabolism of arachidonic acid in microsomes prepared from the kidneys of LH and LL rats. Finally, we examined the interaction between thromboxane receptors and CYP450 metabolites of arachidonic acid in altering renal function in LH rats since cyclooxygenase and thromboxane/endoperoxide receptor antagonists improve renal function in LH rats and there is evidence that the renal vasoconstrictor effects of 20-hydroxyeicosatetraenoic acid are cyclooxygenase-dependent.

2. Materials and methods

2.1. Animals

Experiments were performed, according to our institutional guidelines for animal care, in 8-week-old LL and LH male rats weighing 250 to 280 g. They were housed under controlled conditions of temperature (21 ± 1°C), humidity (60 ± 10%) and lighting (8–20 h). They were fed a standard rat chow containing 0.3% Na^+ (Elevage UAR, Villemoisson sur Orge, France) and had free access to tap water. Systolic blood pressure was measured by a plethysmographic method (Narco Biosystems, Houston, TX) in unrestrained conscious rats on the day before the experiment.

2.2. Surgical preparation

The left kidney was surgically prepared for in vivo study of the pressure–natriuresis response with neural and hormonal influences controlled as previously described (Roman and Cowley, 1985). Seven to 10 days before the experiment the right kidney and adrenal gland were removed. On the day of the experiments, the rats were anesthetized with ketamine (60 mg kg^{-1} i.p. Sigma, St. Louis, MO) and thiobutabarbital (Inactin®, 80 mg kg^{-1} i.p., Byk-Gulden, Constance, Germany) and placed on a heating blanket (model 50-6980, Harvard Apparatus, Edenbridge, Kent, UK) to maintain the body temperature at 37°C. After tracheotomy, the left jugular vein was cannulated for infusions. Catheters were placed in the left carotid artery and in the abdominal aorta via the femoral artery to sample blood and to record the mean arterial blood pressure using a pressure transducer (Model P23 ID, Statham Instrument Division, Gould, Cleveland, OH). The kidney was denervated by stripping all of the visible renal nerves and coating the renal artery with a 10% solution of phenol in ethanol (Merck, Darmstadt, Germany). The remaining adrenal gland was removed and the ureter was cannulated for urine collection. Two adjustable silastic balloon cuffs were placed around the aorta, above and below the left renal artery, and ligatures were placed around the superior mesenteric and celiac arteries so that renal perfusion pressure could be fixed at different levels. A 30-gauge needle was inserted into the abdominal aorta above the left renal artery for intrarenal infusions. Finally, a flow probe (1RB, Transonic Systems, Ithaca, NY) was placed around the left renal artery for measurement of renal blood flow, using a Transonic transit-time flowmeter (model T106, Transonic Systems).

During the experiments, pulsatile arterial pressure and renal blood flow were continuously monitored, using a computerized recording system (LabVIEW 4.0.1; Software, National Instruments, Austin, TX). Data was sampled every 2 ms and stored on CD-ROM. Average mean arterial pressure and renal blood flow were computed off line.

The animals received a bolus i.v. dose (250 mg kg^{-1}) of polyfructosan (Inutest®, Laevosan, Linz, Austria) followed by a constant infusion (4.2 mg kg^{-1} min^{-1}) for measurement of glomerular filtration rate. A hormone

cocktail, containing D-aldosterone (66 ng kg^{-1} min^{-1}), hydrocortisone (33 ng kg^{-1} min^{-1}), norepinephrine (333 ng kg^{-1} min^{-1}) and [Arg^8]vasopressin acetate (0.17 ng kg^{-1} min^{-1}), was continuously infused at a rate of 0.33 ml kg^{-1} min^{-1} (pump model 2400-001-Harvard Apparatus, South Natick, MA) to minimize possible interstrain differences in circulating levels of these Na^+ and water retaining hormones. All of the drugs were obtained from Sigma and were dissolved in a 0.9% NaCl solution containing 1% bovine serum albumin (fraction V, Sigma). At the end of the experiment, the kidney was decapsulated, removed, cut in half, blotted and weighed. Renal perfusion pressure was measured by means of a catheter in the femoral artery and in the carotid artery when the proximal or the distal aortic cuffs were inflated, respectively. Glomerular filtration rate (ml min^{-1} g^{-1}) was estimated by the clearance of polyfructosan. The concentration of polyfructosan in urine and plasma samples was measured using a commercially available spectrophotometric assay with inulinase (Boehringer-Mannheim, Germany). Urine flow rate (μl min^{-1} g^{-1}) was determined gravimetrically. Na^+ concentration was measured by flame photometry (IL meter, model 243, Lexington, MA) and urinary Na^+ excretion (μmol min^{-1} g^{-1}) was calculated. All the renal function parameters were normalized per gram of kidney weight.

2.3. Experimental protocols

2.3.1. Protocol 1: Effects of 17-octadecynoic acid on renal hemodynamics and the pressure–natriuresis relation in LH and LL rats

After a 1-h equilibration period, 17-octadecynoic acid (Sigma), an inhibitor of both epoxyeicosatrienoic and hydroxyeicosatetraenoic acids synthesis, or vehicle (ethanol 0.02% in 0.9% NaCl solution containing 6% bovine serum albumin) was infused at a rate of approximately 30 nmol min^{-1} directly into the renal artery of two groups of eight LH rats and two groups of eight LL rats. The rate of infusion of 17-octadecynoic acid was adjusted to the level of renal blood flow to achieve a final concentration of 3 μM in the blood reaching the kidney. This concentration has been previously described to inhibit the formation of 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids by renal cortical microsomes of rats by 84 and 89%, respectively (Zou et al., 1994a). Thirty minutes after beginning the infusion of 17-octadecynoic acid, renal perfusion pressure was lowered to 100 mmHg for 30 min, then progressively increased to 120 mmHg for 25 min and finally to 140 mmHg for 20 min. A 10-min equilibration period was allowed after each change in renal perfusion pressure before urine samples were collected to minimize dead space errors. An arterial blood sample (300 μl) was collected at the end of each period and was replaced by an equal volume of 0.9% NaCl.

2.3.2. Protocol 2: Effects of 17-octadecynoic acid on renal function after blockade of thromboxane A_2-prostaglandin H_2 (TP) receptors in LH rats

The purpose of these experiments was to evaluate the possibility that CYP450 derived epoxygenase and/or hydroxylase metabolites of arachidonic acid might serve as substrates for cyclooxygenase and constrict the kidney of LH rats through activation of the TP receptors. In these experiments, the effects of 17-octadecynoic acid on renal function were compared to those of its vehicle (ethanol 0.02% in 0.9% NaCl solution containing 6% bovine serum albumin) in two groups of 7 LH rats pretreated with a TP receptor antagonist. The experimental protocol was identical to that described above, except that the TP receptor antagonist, GR 32191B (0.1 mg kg^{-1} min^{-1}) (Glaxo Group research, Ware, Hertfordshire, UK), was infused intravenously throughout the experiment. This dose of GR 32191B was chosen because our previous experiments indicated that this dose completely blocks the vasoconstrictor response to a bolus i.v. injection of the thromboxane receptor agonist U46619 (Liu et al., 1994).

2.3.3. Protocol 3: Effects of miconazole on renal hemodynamics and the pressure–natriuresis relation in LH rats

The purpose of these experiments was to determine the contribution of epoxyeicosatrienoic acids to the renal responses to 17-octadecynoic acid in LH rats. The experimental protocol was identical to that described above except that after surgery and a 1-h equilibration period miconazole (400 μM, Sigma), an epoxygenase inhibitor, or vehicle (ethanol 0.02% in 0.9% NaCl solution containing 6% bovine serum albumin) was infused directly into the renal artery of two groups of eight LH rats. The rate of miconazole infusion was adjusted to the level of renal blood flow to achieve a final concentration of 1 μM in the blood reaching the kidney. This concentration has been previously described to selectively reduce the production of epoxyeicosatrienoic acids by renal cortical microsomes of the rat by 86% without having an effect on the formation of 20-hydroxyeicosatetraenoic acid (Zou et al., 1994a).

2.3.4. Protocol 4: Renal metabolism of arachidonic acid

At the end of the experiments, the kidneys of LH and LL rats infused with vehicle were removed, frozen in liquid nitrogen and kept at −80°C until assayed for the metabolism of arachidonic acid. Renal cortical tissue was homogenized in 10 mM potassium phosphate buffer (pH 7.7) containing 250 mM sucrose, 1 mM EDTA and 10 mM $MgCl_2$. The homogenate was centrifuged for 5 min at $3000 \times g$ to remove tissue chunks and at $11{,}000 \times g$ for 15 min to remove nuclei and mitochondria. Microsomal protein was isolated from the supernatant by centrifugation at $100{,}000 \times g$ for 60 min and resuspended in a 0.1-M potassium phosphate buffer (pH 7.25) containing 1 mM EDTA, 1 mM 1,4-dithiothreitol, 0.1 μM phenylmethyl-

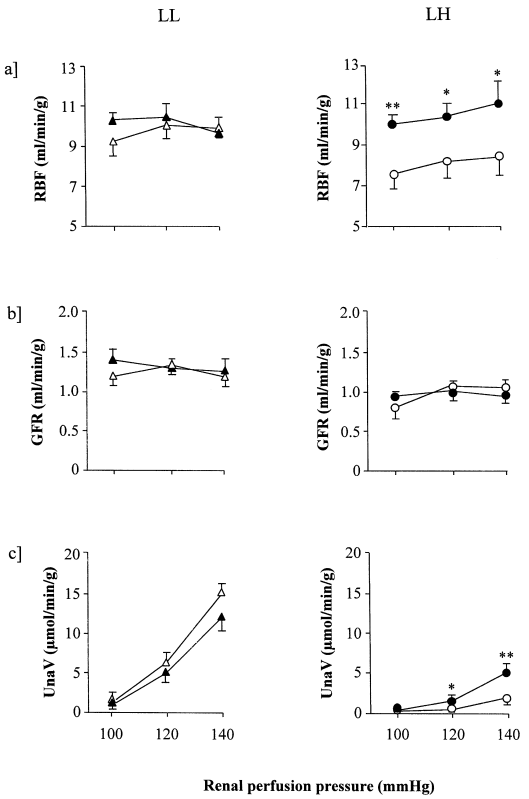

Fig. 1. Effects of 17-octadecynoic acid, an inhibitor of the renal metabolism of arachidonic acid by CYP450 enzymes, on renal blood flow (RBF) (a), glomerular filtration rate (GFR) (b) and the pressure–natriuresis relation (c) in 8-week-old Lyon low blood pressure (LL) and hypertensive (LH) rats. Mean values ± S.E.M. from the two groups of eight LL rats treated with vehicle (△) or 17-octadecynoic acid (▲) and the two groups of eight LH rats treated with vehicle (○) or 17-octadecynoic acid (●), respectively, are presented. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. vehicle within a strain.

sulfonyl fluoride and 30% glycerol. Microsomes (0.5 mg protein) were incubated for 30 min at 37°C with [1-^{14}C]-arachidonic acid (0.1 μCi ml^{-1}, 10 μM) in 1 ml of 100 mM potassium phosphate buffer (pH 7.4) containing 5 mM $MgCl_2$, 1 mM EDTA, 1 mM NADPH and a NADPH-regenerating system consisting of 10 mM isocitrate and 0.4 units ml^{-1} isocitrate dehydrogenase. The reactions were terminated by acidification to pH 3.5 with 1 M formic acid and the metabolites were extracted twice with 3 ml of ethylacetate. The ethylacetate was back extracted with 1 ml of water to remove excess acid and the samples were dried under nitrogen. Metabolites were separated on a 2 mm × 25 cm C18-reverse phase high performance liquid chromatography column with a linear elution gradient ranging from acetonitrile:water:acetic acid (50:50:0.1) to acetonitrile:acetic acid (100:0.1) over 40 min. The radioactive products were monitored using a radioactive flow detector (Model 120, Radiomatic Instruments, Tampa, FL). Product formation is expressed as pmol min^{-1} mg^{-1} microsomal protein.

2.4. Statistics

Values are expressed as means ± S.E.M. The statistical significance of the differences between experimental groups was assessed using a two-way analysis of variance followed by a Fisher multiple range test. $P < 0.05$ was considered to be statistically significant.

3. Results

Systolic blood pressure was significantly higher in LH than in LL rats and averaged 136 ± 2 vs. 103 ± 1 mmHg, respectively. The kidney weight/body weight ratios were similar in the two strains (0.60 ± 0.02 and 0.59 ± 0.02 per 100 g body weight in LH and LL rats, respectively). The baseline hematocrit was not significantly different in LH and LL rats (39 ± 1 and 41 ± 1%) and did not change during the experiment in either group of rats.

As shown in Fig. 1a and c, LH kidneys, perfused at a renal perfusion pressure level of 100 mmHg, differed from

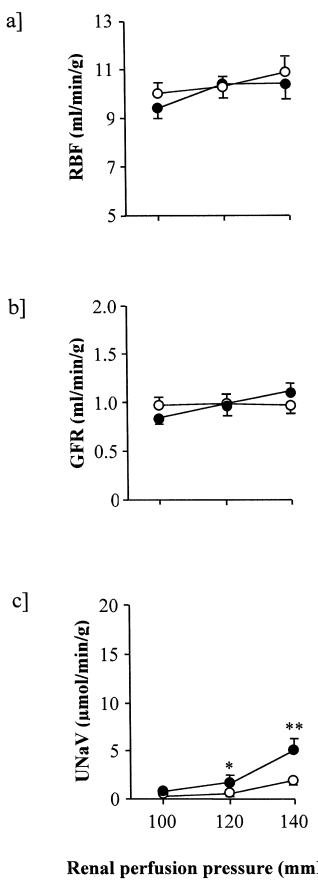

Fig. 2. Effects of 17-octadecynoic acid, an inhibitor of the renal metabolism of arachidonic acid by CYP450 enzymes, on RBF (a), GFR (b) and the pressure–natriuresis relation (c) in 8-week-old LH rats pretreated with a thromboxane receptor antagonist. Mean values ± S.E.M. from the two groups of seven LH rats treated with vehicle (○) or 17-octadecynoic acid (●), respectively, are presented. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. the corresponding value in vehicle-treated rats.

LL ones by having a lower basal renal blood flow (7.4 ± 0.6 vs. 9.2 ± 0.4 ml min^{-1} g^{-1}; $P < 0.05$) and Na^+ excretion (0.2 ± 0.1 vs. 1.6 ± 0.6 μmol min^{-1} g^{-1}; $P < 0.05$). Basal glomerular filtration rate was not significantly different between the strains although it tended to be slightly lower in LH than in LL rats (0.8 ± 0.1 vs. 1.2 ± 0.1 ml min^{-1} g^{-1}, respectively).

In response to stepwise increases in renal perfusion pressure from 100 to 140 mmHg, renal blood flow and glomerular filtration rate were well autoregulated in both strains (Fig. 1a and b). However, the pressure–natriuresis relation was significantly blunted in LH rats in comparison to that seen in LL rats (Fig. 1c). Since, at a renal perfusion pressure level of 140 mmHg, glomerular filtration rate was similar in both strains (1.1 ± 0.1 vs. 1.2 ± 0.1 in LH and LL rats, respectively), the reduced Na^+ excretion in LH rats was due to a significant increase in tubular Na^+ reabsorption (98.7 ± 0.3 vs. 90.1 ± 0.9% of the filtered load of Na^+ in LH and LL rats, respectively).

Intrarenal infusion of 17-octadecynoic acid (3 μM) had no effect on renal blood flow, glomerular filtration rate or the pressure–natriuresis relation in LL rats (Fig. 1a,b and c). In contrast, renal blood flow increased in LH rats given 17-octadecynoic acid to a level which was similar to that found in LL rats (9.9 ± 0.5 and 10.3 ± 0.6 ml min^{-1} g^{-1} in LH and LL rats, respectively) (Fig. 1a). In addition, 17-octadecynoic acid increased urinary Na^+ excretion and partially corrected the impaired pressure–natriuresis response in LH rats (Fig. 1c).

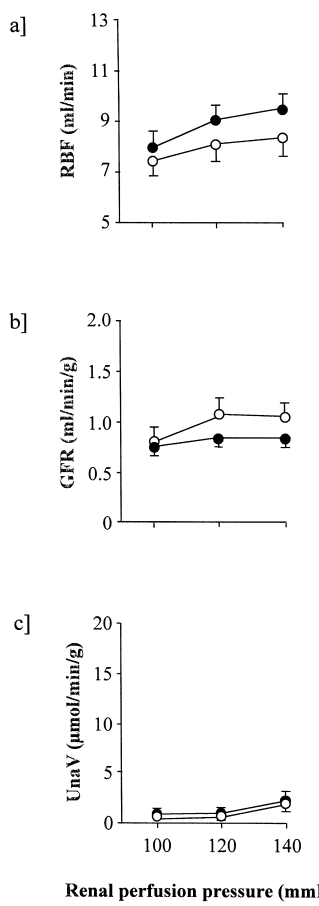

Fig. 3. Effects of miconazole, an inhibitor of renal epoxygenase activity, on RBF (a), GFR (b) and the pressure–natriuresis relation (c) in 8-week-old LH rats. Mean values ± S.E.M. from the two groups of eight LH rats treated with vehicle (○) or miconazole (●), respectively, are presented.

Table 1
Conversion of radiolabelled arachidonic acid to epoxyeicosatrienoic acids and 20-hydroxyeicosatetraenoic acid in renal cortical microsomes of LL and LH rats. LL, Lyon low blood pressure rat; LH, Lyon hypertensive rat; EETs, epoxyeicosatrienoic acids (11,12- and 14,15-EET); 20-HETE, 20-hydroxyeicosatetraenoic acid

	LL ($n = 4$)	LH ($n = 4$)
EETs (pmol min^{-1} mg^{-1})	4.2 ± 0.8	1.7 ± 0.3[a]
20-HETE (pmol min^{-1} mg^{-1})	65.5 ± 3.1	42.7 ± 4.2[a]

[a] $P < 0.05$ vs. LL.

The effects of blockade of TP receptors on the renal response to 17-octadecynoic acid in LH rats are presented in Fig. 2. Blockade of TP receptors with GR 32191B (0.1 mg kg^{-1} min^{-1}) increased baseline renal blood flow (from 7.4 ± 0.6 to 10.0 ± 0.4 ml min^{-1} g^{-1}, $P < 0.01$) but it had no effect on glomerular filtration rate or Na^+ excretion. In LH rats pretreated with the TP receptor antagonist, 17-octadecynoic acid had no effect on renal blood flow or glomerular filtration rate (Fig. 2a and b). However, it still increased Na^+ excretion and potentiated the pressure–natriuretic response.

The effects of selective blockade of renal epoxygenase activity with miconazole on the renal function of LH rats are presented in Fig. 3. In contrast to the effects seen when the formation of 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids was inhibited with 17-octadecynoic acid, intrarenal infusion of miconazole (1 μM) had no significant effect on renal blood flow, glomerular filtration rate or the pressure–natriuresis relation in LH rats (Fig. 3a,b and c).

A comparison of the production of epoxyeicosatrienoic acids and 20-hydroxyeicosatetraenoic acid by microsomes prepared from the kidneys of LH and LL rats is presented in Table 1. Microsomes prepared from the kidneys of both strains of rats produced primarily 20-hydroxyeicosatetraenoic acid when incubated with arachidonic acid. The production of epoxyeicosatrienoic acids was 20-fold lower than that of 20-hydroxyeicosatetraenoic acid. The synthesis of 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids was significantly lower by 35 and 60%, respectively, in kidneys obtained from LH rats in comparison to that seen in LL rats.

4. Discussion

The present study evaluated the possible role of CYP450 metabolites of arachidonic acid in altering renal function in LH rats. The results indicate that the inhibition of the

metabolism of arachidonic acid by CYP450 enzymes (primarily the production of 20-hydroxyeicosatetraenoic acid) normalized renal blood flow in LH rats and improved the pressure–natriuresis response. In addition, they suggest that the renal hemodynamic effects of these metabolites may result from activation of TP receptors.

The experiments were conducted in young LH rats to determine whether the changes in the control of renal vascular tone and the pressure–natriuresis relationship by CYP450 metabolites of arachidonic acid preceded the full development of hypertension and to avoid renal damage resulting from a long-standing hypertension in older animals. The results confirm our previous findings that renal blood flow is reduced, renal vascular resistance is elevated and the pressure–natriuresis relation is shifted toward higher pressures in LH rats in comparison to the levels seen in LL rats (Liu et al., 1994). Infusion of 17-octadecynoic acid, an inhibitor of the formation of epoxyeicosatrienoic acids and HETEs, had no effect on renal function in LL rats but it markedly increased renal blood flow and improved the pressure–natriuresis relation in LH rats. These studies indicate that CYP450 metabolites of arachidonic acid contribute to the elevation in renal vascular resistance and blunted pressure–natriuresis relation seen in LH rats.

In additional studies, we found that intrarenal infusion of miconazole had no effect on renal hemodynamics and the pressure–natriuresis relation in LH rats. This lack of effect is consistent with the very low production of epoxyeicosatrienoic acids in renal cortical microsomes of LL and LH rats, which was 20-fold lower than the production of 20-hydroxyeicosatetraenoic acid. These findings suggest that the renal vasodilator response to 17-octadecynoic acid in LH rats is probably due to blockade of the production of the potent vasoconstrictor 20-hydroxyeicosatetraenoic acid (Imig et al., 1996) rather than to the formation of epoxyeicosatrienoic acids.

To determine whether the vasodilator response to 17-octadecynoic acid was due to blockade of the direct renal vasoconstrictor actions of 20-hydroxyeicosatetraenoic acid or to an interaction of this compound with TP receptors, the effects of 17-octadecynoic acid were re-evaluated in LH rats pretreated with a TP receptor antagonist. Blockade of TP receptors with GR 32191B increased baseline renal blood flow but it had no effect on the pressure–natriuresis response. These findings are consistent with our previous results showing that activation of TP receptors contributes to the abnormalities in renal hemodynamics but not to the resetting of the pressure–natriuresis relation in LH rats (Liu et al., 1994). Pretreatment of LH rats with GR 32191B prevented the renal vasodilator response to 17-octadecynoic acid in LH rats, but not its action to improve the pressure–natriuretic relation. These results suggest that CYP450 metabolites of arachidonic acid, likely 20-hydroxyeicosatetraenoic acid, interact with TP receptors in some way to alter renal hemodynamics in LH rats. The nature of this interaction is unknown but there are several possibilities. Previous studies have indicated that 20-hydroxyeicosatetraenoic acid can serve as a substrate for cyclooxygenase and in some studies, the renal vasoconstrictor response to 20-hydroxyeicosatetraenoic acid and other CYP450 metabolites of arachidonic acid was dependent on the formation of a cyclooxygenase metabolite that interacted with TP receptors (Schwartzman et al., 1989). Since previous studies indicate that cyclooxygenase activity may be elevated in the kidneys of LH rats (Geoffroy et al., 1986), the lower production of 20-hydroxyeicosatetraenoic acid observed in this strain could reflect a greater metabolism of 20-hydroxyeicosatetraenoic acid via the cyclooxygenase pathway to more polar metabolites. An argument against this possibility, however, is that we were unable to detect any difference in the production of cyclooxygenase or thromboxane metabolites of arachidonic acid in microsomes prepared from the kidneys of LH and LL rats. However, a limitation of the present study that may have prevented us from detecting an important difference in the formation of these products is that we studied the metabolism of arachidonic acid in renal microsomes that consisted primarily of proximal tubules, which exhibit little cyclooxygenase activity. It is possible that if we had compared the metabolism of arachidonic acid by renal microvessels or isolated glomeruli from LH and LL rats, we may have had a better chance of detecting significant strain differences in the production of cyclooxygenase metabolites of 20-hydroxyeicosatetraenoic acid and other CYP450 metabolites of arachidonic acid.

Another possibility is that 20-hydroxyeicosatetraenoic acid, by blocking potassium channels and depolarizing vascular smooth muscle cells, may sensitize the renal vasculature to the vasoconstrictor effects of thromboxane and endoperoxides formed in the renal vasculature and glomerulus. Thus, although the production of 20-hydroxyeicosatetraenoic acid is lower in the kidney of LH rats, it could still contribute to the elevated renal vascular tone in this strain if cyclooxygenase activity is elevated in the kidneys of LH rats.

In contrast to the effects of 17-octadecynoic acid on renal hemodynamics, its effects on the pressure–natriuresis relation in LH rats were not dependent on an interaction with TP receptors. The mechanism by which blockade of the renal production of 20-hydroxyeicosatetraenoic acid could promote natriuresis and normalize the pressure–natriuresis relation is not immediately obvious. 20-Hydroxyeicosatetraenoic acid inhibits Na^+ transport in the proximal tubule by blocking Na^+–K^+-ATPase activity (Nowicki et al., 1997). It also inhibits Na^+–K^+–$2Cl^-$ cotransport in the medullary thick ascending limb (Amlal et al., 1996). Blockade of the formation of this substance by 17-octadecynoic acid should increase Na^+ reabsorption. Nevertheless, intrarenal infusion has been reported to increase Na^+ excretion in both Sprague–Dawley rats and one-kidney, one-clip, Goldblatt hypertensive rats by in-

creasing papillary blood flow and renal hydrostatic interstitial pressure (Zou et al., 1994b, 1995). Since the pressure–natriuresis response has been repeatedly shown to be associated with changes in renal medullary blood flow and renal interstitial pressure, the most likely explanation for the partial normalization of the pressure–natriuresis relation in LH rats given 17-octadecynoic acid is that this drug increases medullary blood flow and renal interstitial pressure in this strain.

In conclusion, the results of the present study suggest that CYP450 metabolites of arachidonic acid, likely 20-hydroxyeicosatetraenoic acid, contribute to the alterations in renal function seen in LH rats given a normal salt diet. Moreover, they suggest that 20-hydroxyeicosatetraenoic acid may serve as a substrate for the formation of vasoconstrictor endoperoxides that interact with TP receptors in the kidney of LH rats and that these metabolites contribute to the enhanced renal vascular tone but not to the blunted pressure–natriuresis response in this strain.

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