Role of 20-hydroxyeicosatetraenoic acid in the renal and vasoconstrictor actions of angiotensin II

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PREVIOUS STUDIES INDICATE that ANG II enhances the activity of phospholipases to release arachidonic acid (AA) (39, 42, 43) and stimulates the formation of cyclooxygenase (15, 27, 28, 37, 38, 40, 41) and lipoxygenase metabolites of AA (5, 40, 48) in various tissues. These products modulate the vasoconstrictor response to ANG II in both the renal and peripheral circulation (27, 30). In this regard, ANG II normally stimulates the formation of prostacyclin, which attenuates the vasoconstrictor response to ANG II (12, 28, 30, 32, 37, 38, 40). ANG II also promotes the formation of 12-hydroxyeicosatetraenoic acid (12-HETE), which potentiates the rise in intracellular calcium concentration and the vasoconstrictor and mitogenic response to ANG II in vascular smooth muscle (VSM) cells (5, 16, 31, 48, 53). In hypertensive animals, the balance between the formation of vasodilator and vasoconstrictor metabolites of AA shifts toward the formation of vasoconstrictor metabolites, i.e., thromboxane A2 and endoperoxides, and these products potentiate the vasoconstrictor response to ANG II (27, 30). More recent studies indicate that ANG II also markedly increases the formation of 20-HETE in rat renal arterioles (8, 10) and promotes the release of 20-HETE from the isolated perfused rabbit kidney (6, 7). 20-HETE is a potent constrictor of renal and cerebral arteries that acts by depolarizing VSM cells secondary to blocking Ca2+ channels (13, 20, 51, 57). 20-HETE plays an important role in autoregulation of renal and cerebral blood flow (11, 17, 19, 58), tubuloglomerular feedback control of glomerular filtration rate (GFR) (58, 59), and in the renal vasoconstrictor response to endothelin (ET) (14, 18, 34) and vasopressin (55). Given the central role of 20-HETE in the regulation of vascular tone, it seems likely that ANG II-induced elevations in 20-HETE production may contribute to its vasoconstrictor actions by facilitating calcium entry secondary to blockade of KCa channels and the hyperpolarization of VSM cells following agonist-induced release of intracellular calcium.

Much less is known about the chronic effects of ANG II on the formation of 20-HETE in the kidney and peripheral vasculature and the influence of 20-HETE in the development of ANG II-dependent forms of hypertension. Several investigators reported that the ex-
pression of cytochrome P-450 4A (CYP4A) proteins and the formation of 20-HETE fall in the kidney (21, 25, 35, 47) and renal vasculature (2) of rats fed a high-salt diet. More recently, Carroll et al. (8) reported that the formation of 20-HETE in renal microvessels increases in rats fed a low-salt diet. These studies suggest that changes in the renal or circulating levels of ANG II that accompany changes in salt intake may regulate the formation of 20-HETE in the kidney and peripheral vasculature. If true, then it is possible that ANG II-induced upregulation of the expression of CYP4A and the formation of 20-HETE in the vasculature may contribute to the slow development of hypertension (slow pressor effects) produced by chronic infusion of subpressor doses of ANG II. However, little is known about the role of 20-HETE in modulating the acute and chronic effects of ANG II on renal function and vascular tone in intact animals. Thus the purpose of the present study was to examine the effects of a chronic infusion of ANG II on the synthesis of 20-HETE and epoxyeicosatrienoic acids (EETs) and CYP4A protein expression in the kidney and to evaluate the effects of inhibitors of the formation of 20-HETE on the acute and chronic pressor actions of ANG II in rats in vivo.

**METHODS**

Experiments were performed in adult male Sprague-Dawley (SD) rats weighing between 275 and 325 g, obtained from Harlan Sprague Dawley (Indianapolis, IN). The rats were housed 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, and had free access to food and water throughout the study. All protocols involving animals received approval by the Animal Care Committee of the Medical College of Wisconsin.

**Contribution of 20-HETE to the renal vasoconstrictor response to ANG II in vitro.** The kidneys from rats were removed and placed in ice-cold physiological saline solution (PSS) containing (in mmol/l): 119 NaCl, 4.7 KCl, 1.6 CaCl₂, 1.17 MgSO₄, 1.18 NaH₂PO₄, 12 NaHCO₃, 10 glucose, and 0.03 EDTA, pH 7.4. Renal interlobular arterioles (>125-μm inner diameter) were removed by microdissection and mounted on glass micropipettes in a water-jacketed perfusion chamber containing PSS that was equilibrated with a 95% O₂-5% CO₂ gas mixture and maintained at 37°C. The vessels were mounted on the micropipettes, the outflow line was clamped off, and intraluminal pressure was set to 90 mmHg. Previous studies indicated that ANG II stimulates the release of prostacyclin (15, 37, 38), nitric oxide (NO) (22, 23, 36, 44–46, 54), and EETs (4, 16, 23) from the endothelium and that these substances attenuate the vasoconstrictor response to ANG II. Therefore, to reveal the contribution of 20-HETE to the vasoconstrictor response to ANG II, we added indomethacin (5 μmol/l), baicalein (0.5 μmol/l), miconazole (1 μmol/l), and N²-nitro-L-arginine methyl ester (L-NAME; 50 μmol/l) to block the contribution of cyclooxygenase, lipooxygenase, epoxygenase, and NO pathways. Cumulative dose-response curves to ANG II (10⁻¹¹ to 10⁻⁵ mol/l) were then generated before and after administration of 17-octadecenoic acid (17-ODYA; 1 μmol/l), which inhibits the formation of 20-HETE and EETs in the kidney and renal microvessels (21, 58, 60). Preliminary studies showed that the vasoconstrictor response to ANG II in our preparation was maximal at 3 min after the addition of ANG II to the bath; thus vascular diameters were measured 3 min after the addition of each dose of ANG II to the bath by using a video system as previously described (1, 3, 50–52).

**Contribution of 20-HETE to the pressor response to ANG II in vivo.** The effects of a selective inhibitor of the synthesis of 20-HETE on the pressor response to ANG II was evaluated in adult male SD rats anesthetized with ketamine (30 mg/kg) and thiobutylbarbitol (50 mg/kg) and maintained at 37°C. Cannulas were inserted into the left femoral artery for the measurement of systemic mean arterial pressure (MAP) and into both femoral veins for intravenous infusion of drug or vehicle. The rats (n = 5) received an intravenous saline solution containing 3% albumin (1.2 ml/h) throughout the experiment to replace fluid losses. After a 30-min equilibration period, baseline MAP was recorded, and the MAP responses to intravenous infusions of ANG II at doses of 5, 10, 25, 50, 100, and 150, and 200 ng/min were sequentially studied. Five minutes were allowed between each dose to allow for equilibration of MAP before testing the effects of the next dose. After the control responses to ANG II were recorded, the rats received a 2-mg intravenous bolus injection of dillromododecyl methyl sulfinamide (DDMS; 6 mg/kg) followed by a maintenance infusion at a rate of 1.2 mg/h. After a 1-h equilibration period, the MAP response to a graded ANG II infusion was redetermined.

**Effect of ANG II on renal formation of 20-HETE.** The effects of a 5-day intravenous infusion of ANG II on the renal formation of CYP metabolites of AA were determined in microsomes prepared from the renal cortex and outer medulla of male SD rats. Rats were maintained on a fixed sodium intake (2.9 meq/day) by being given an intravenous infusion of 18 ml of sterile 0.9% saline per day and fed a low-salt (0.1% NaCl) diet. The control group received a continuous intravenous infusion of vehicle (0.9% saline), and the experimental group received a constant infusion of ANG II in saline at a rate of 50 ng·kg⁻¹·min⁻¹ for 5 days. After 5 days, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg), and the kidneys were rapidly removed. The renal cortex and outer medulla were homogenized in a 10 mmol/l potassium phosphate buffer (pH 7.7) containing 250 mmol/l sucrose, 1 mmol/l EDTA, 10 mmol/l magnesium chloride, 2 μmol/l leupeptin, 1 μmol/l pepstatin, 2 μg/ml aprotinin, and 0.1 μmol/l phenylmethylsulfonyl fluoride. Microsomes were prepared by differential centrifugation as our laboratory previously described (25, 47). CYP-dependent metabolism of AA was determined by incubating microsomal protein (0.5 mg) for 15 min at 37°C with 1-[¹⁴C]AA (0.1 μCi; 40 μmol/l) (Amersham, Arlington Heights, IL). Metabolites of AA were separated with a 25 cm × 2-mm internal diameter (Supelco, Bellefonte, PA) C₁₈ reverse-phase HPLC column and a linear elution gradient ranging from acetonitrile-water-acetic acid (50:50:0.1) to acetonitrile-water-acetic acid (100:0.1) over a 40-min period and detected with a radioactive flow detector (Packard, Tampa, FL).

**Effects of ANG II on the expression of CYP4A protein.** Ten to twenty micrograms of microsomal protein were separated by electrophoresis on an 8 × 10 cm, 7.5% SDS-polyacrylamide gel for 1.5 h at 150 V and transferred to a nitrocellulose membrane. Nonspecific binding was blocked by incubating the membrane overnight at 4°C in Tris-buffered saline (TBS)-T buffer (6 mmol/l Tris-HCl, 4 mmol/l Tris-base, 150 mmol/l NaCl, and 0.08% Tween 20, pH 7.5) containing 10% nonfat dry milk. The membrane was subsequently incubated for 2 h with a polyclonal antibody raised against rat liver CYP4A1 (Gentest, Woburn, MA) at a 1:4,000 dilution in TBS-T buffer containing 2% milk. The membrane was then washed three times with TBS-T buffer and incubated with...
goat anti-rabbit IgG conjugated with horseradish peroxidase (Santa Cruz Biotech, Santa Cruz, CA) at a 1:8,000 dilution in 2% milk for 1 h. Immunoblots were developed with an enhanced chemiluminescence kit (West Pico, Pierce, Rockford, IL).

**Contribution of 20-HETE to the development of ANG II hypertension.** These experiments examined the effects of DDMS, a selective inhibitor of the formation of 20-HETE, and 1-aminobenzotriazole (ABT), which blocks the formation of both EETs and 20-HETE, on the pressor response to a chronic intravenous infusion of ANG II. Male SD rats were anesthetized with an intramuscular injection of ketamine- xylazine-acepromazine (4:2:0.24:0.06 mg/100 g body wt). Catheters were implanted into the left femoral artery for the measurement of MAP and into the right femoral vein for intravenous infusion of drugs or their vehicles. Femoral artery and vein catheters were exteriorized at the back of the neck. The catheters were protected with a stainless steel spring and connected to a dual-channel swivel (Instech, Plymouth Meeting, PA). Sodium intake was fixed at 2.9 meq/day by a continuous intravenous infusion of 18 ml of sterile 0.9% saline per day combined with a low-NaCl (0.1%) diet. After a 7-day recovery period in metabolism cages, MAP was re- measured for 4 control days for 8 h/day by using a computerized recording system. After the control period, the rats received a continuous intravenous infusion of ANG II (50 ng·kg⁻¹·min⁻¹) for 5 days. Group 1 received ANG II plus the vehicle used for DDMS (20 mmol/l Na₂CO₃ in saline + 200 μl ethanol per day). Group 2 received ANG II plus DDMS (1 mg·kg⁻¹·day⁻¹). Group 3 received ANG II plus the vehicle used for ABT (0.9% saline), and group 4 received ANG II plus ABT (50 mg·kg⁻¹·day⁻¹).

**Measurement of urinary excretion of 20-HETE.** Rats were housed in stainless steel metabolism cages, and 24-h urine samples were collected on ice. 20-HETE concentration in the samples was measured by using a fluorescent HPLC assay (26). Briefly, 25 pg of an internal standard 20-5(Z), 14(Z)-hydroxyeicosadienoic acid (WIT-002) were added to the urine samples. The samples were acidified to pH 4 with formic acid and extracted with 1 ml of ethyl acetate, and the organic phase was dried with argon gas. The samples were redissolved in 1 ml of 20% acetonitrile and loaded onto a Sep-Pak Vac column (Waters, Milford, MA). The column was washed twice with 1 ml of 30% acetonitrile, and the fraction containing HETEs and EETs was eluted with 400 μl of 90% acetonitrile. The samples were diluted with 600 μl of water, applied to a Sep-Pak Vac column, eluted with 500 μl of ethyl acetate, and then dried down. The lipid fraction was labeled with 20 μl of acetonitrile containing 36.4 mM 2-(2,3-naphthalimino) ethyl trifluoromethanesulfonate. N,N-diisopropylethylamine (10 μl) was added to catalyze the reaction. The sample was reacted for 30 min at room temperature. Excess dye was removed with a Sep-Pak extraction (26), and the samples were dried under argon, resuspended in 100 μl of methanol, and analyzed by reverse-phase HPLC (Waters) by using a fluorescence detector (model number L-7480, Hitachi, Naperville, IL). The amount of 20-HETE in the sample was determined by comparing the area of the 20-HETE peak to that of the internal standard.

**Statistics.** Data presented are means ± SE. The significance of differences in mean values within and between groups was determined by analysis of variance for repeated measures followed by Duncan’s multiple-range test. A P value of <0.05 using a two-tailed test was considered to be statistically significant.

**Results.** Contribution of 20-HETE to the renal vasoconstrictor actions of ANG II. The contribution of 20-HETE to the vasoconstrictor response to ANG II in isolated renal arterioles is summarized in Fig. 1. In vessels treated with L-NAME, miconazole, baicalein, and indomethacin, ANG II (10⁻¹¹ to 10⁻⁷ mol/l) reduced vascular diameter in a concentration-dependent manner by 81 ± 8%. Blockade of 20-HETE synthesis with 17-ODYA (1 μmol/l) reduced the maximum vasoconstrictor response to ANG II to 61 ± 6% and shifted the ED₅₀ to the right by one order of magnitude.

**Contribution of 20-HETE to the acute pressor response to ANG II.** Control MAP averaged 116 ± 7 mmHg (n = 5 rats) in this group of SD rats anesthetized with thiobutybarbitol. A graded intravenous infusion of ANG II at doses of 5, 10, 25, 50, 100, 150, and 200 ng/min increased MAP by 5 ± 1, 9 ± 2, 15 ± 2, 20 ± 3, 35 ± 8, 37 ± 4, and 39 ± 5 mmHg, respectively (Fig. 2). After administration of DDMS (10 mg/kg), MAP fell to 109 ± 7 mmHg. In the presence of DDMS, the vasoconstrictor response to an intravenous infusion of ANG II...
ANG II (5 to 200 ng/min) was significantly blunted, and MAP only increased by 8 ± 1, 8 ± 2, 9 ± 2, 14 ± 3, 22 ± 8, 21 ± 4, and 21 ± 5 mmHg, respectively.

Effect of chronic infusion of ANG II on the renal metabolism of AA. The effects of a 5-day intravenous infusion of ANG II (50 ng·kg⁻¹·min⁻¹) on the formation of 20-HETE and EETs by microsomes prepared from the renal cortex and outer medulla of SD rats are summarized in Table 1. The formation of 20-HETE by renal cortical microsomes increased by 60% in rats infused with ANG II (n = 4) compared with levels seen in vehicle-treated rats (n = 8). The renal cortical formation of EETs increased fourfold in ANG II-infused compared with vehicle-treated rats. In microsomes prepared from the outer medulla, the production of 20-HETE increased fourfold in ANG II-infused rats, whereas the formation of EETs was undetectable in vehicle-treated and ANG II-infused rats.

Table 1. Effect of a 5-day intravenous infusion of ANG II on the renal metabolism of AA

<table>
<thead>
<tr>
<th></th>
<th>20-HETE, pmol·min⁻¹·mg⁻¹</th>
<th>EETs, pmol·min⁻¹·mg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (8)</td>
<td>156.3 ± 7.4</td>
<td>18.2 ± 6.7</td>
</tr>
<tr>
<td>ANG II (4)</td>
<td>248.8 ± 29.8*</td>
<td>73.0 ± 12.1*</td>
</tr>
<tr>
<td>Outer medulla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (8)</td>
<td>28.1 ± 8.3</td>
<td>undetectable</td>
</tr>
<tr>
<td>ANG II (4)</td>
<td>127.8 ± 37.8*</td>
<td>undetectable</td>
</tr>
</tbody>
</table>

Mean values ± SE are presented. 20-HETE, 20-hydroxyicosatetraenoic acid; EETs, epoxyeicosatrienoic acid; AA, arachidonic acid. The formation of 20-HETE and EETs was measured in microsomes prepared from the renal cortex and outer medulla of adult male Sprague-Dawley rats maintained on a fixed sodium intake (2.9 meq/day) and infused with vehicle (0.9% NaCl) or ANG II (50 ng·kg⁻¹·min⁻¹) intravenously for 5 days. *P < 0.05 vs. vehicle group. Numbers in parentheses indicate number of rats studied per group.

**Fig. 2.** Effect of an intravenous infusion of ANG II (5 to 200 ng/min) on mean arterial pressure (MAP) in thioctylbarbitol-anesthetized male Sprague-Dawley rats (n = 5) before and after blockade of the formation of 20-hydroxyicosatetraenoic acid (20-HETE) with dibromomodocenyl methylsulfimide (DDMS; 10 mg/kg). Results are expressed as the absolute change in MAP in mmHg compared with baseline MAP values. Values are means ± SE. *P < 0.05 vs. corresponding values before administration of DDMS.

**Fig. 3.** Effect of blockade of the formation of 20-HETE with 1-amino-benzotriazole (ABT) or DDMS on the development of hypertension in conscious, chronically catheterized adult male Sprague-Dawley rats infused with ANG II (50 ng·kg⁻¹·min⁻¹). MAP was measured in rats treated with vehicle (0.9% saline or saline containing 20 mmol/l Na₂CO₃), ABT (50 mg·kg⁻¹·day⁻¹), or DDMS (1 mg·kg⁻¹·day⁻¹) administered intravenously before and after starting a chronic infusion of ANG II (50 ng·kg⁻¹·min⁻¹). After a 7-day recovery period, MAP was measured during 4 consecutive control days. MAP measured during 3 control (C1 through C3) and 5 experimental treatment days (E1 through E5) are plotted. Values are means ± SE; n, no. of rats. *P < 0.05 vs. vehicle-treated group.
Chronic treatment of the rats with ABT reduced the formation of 20-HETE and EETs in the renal cortex by 35 and 45%, respectively, compared with the values seen in vehicle-treated rats. Similarly, the production of 20-HETE in microsomes prepared from the outer medulla of DDMS-treated rats was 34% less than that seen in vehicle-treated rats (Fig. 4D). The expression of CYP4A protein was not significantly different in either the renal cortex or outer medulla (Fig. 4, C and D) of rats infused with ANG II and treated with vehicle or DDMS.

Effect of ANG II and CYP4A inhibitors on the urinary excretion of 20-HETE. Baseline urinary excretion of 20-HETE was similar in all of the groups of rats and averaged 350 ± 23 ng/day (Fig. 5). In vehicle-treated rats, 5 days of an intravenous infusion of ANG II (50 ng·kg⁻¹·min⁻¹) increased the urinary excretion of 20-HETE to 1,020 ± 105 ng/day. In contrast, the urinary excretion of 20-HETE in rats chronically infused with ANG II and ABT was significantly less and averaged...
were measured with an HPLC fluorescent assay. Values are means ± SE; n, no. of rats. *P < 0.05 vs. veh-treated group. §P < 0.05 vs. ANG II + veh-treated group.

only 400 ± 40 ng/day. Similarly, the urinary excretion of 20-HETE was also significantly reduced in rats chronically infused with ANG II and DDMS.

**DISCUSSION**

Recent studies indicate that ANG II increases the formation of 20-HETE in renal microvessels (8, 10) and the release of 20-HETE from the isolated perfused kidney of rabbits (6, 7). There is also evidence that elevations in dietary salt intake, which suppress the renin-angiotensin system, decrease the expression of CYP4A protein and the production of 20-HETE in renal microvessels (2, 8) and the renal cortex (largely proximal tubules) of the rat (2, 21, 25, 35, 47). However, little is known regarding what role, if any, changes in the production of 20-HETE play in modulating the acute and chronic effects of ANG II on renal tubular ion transport and/or vascular tone in intact animals. Thus the present study examined the effects of inhibitors of the formation of 20-HETE on the acute and chronic pressor actions of ANG II in the rat, both in vivo and in vitro.

We first examined the effects of 17-ODYA on the vasoconstrictor response of rat renal interlobular arteries to ANG II in vitro. Previous studies established that ANG II stimulates release of prostacyclin, EETs, NO, and 12-HETE, all of which influence the vascular response to ANG II (15, 16, 22, 23, 33, 37, 38, 44, 48). Therefore, to reveal the contribution of 20-HETE to the vascular response to ANG II, the vessels were pretreated with L-NAME, indomethacin, baicalein, and miconazole to block NO synthesis and the cyclooxygenase, lipoxygenase, and epoxygenase pathways, respectively. Under these experimental conditions, 17-ODYA shifted the dose-response curve to ANG II to the right by one order of magnitude and reduced the maximal constrictor response to ANG II to 60% of control. Previous studies indicated that 17-ODYA blocks the formation of both EETs and 20-HETE in the kidney (60), glomeruli (21), and renal microvessels (20, 58). However, under the present experimental conditions, the effects of 17-ODYA are likely due to inhibition of the synthesis of 20-HETE, because the vessels were treated with miconazole at a concentration that completely blocks epoxygenase activity in renal microvessels of the rat (58).

Overall, our results are consistent with the view that ANG II stimulates the formation of 20-HETE in renal VSM cells and that 20-HETE mediates, in part, the vasoconstrictor response to ANG II. The mechanism by which 20-HETE contributes to the vasoconstrictor response to ANG II remains to be established, but previous studies indicated that 20-HETE depolarizes renal VSM cells (24) by blocking K<sub>Ca</sub> channels (24, 57). Depolarization of these cells increases the open-state probability of Ca<sup>2+</sup> channels and enhances Ca<sup>2+</sup> influx and the vasoconstrictor response to ANG II and other vasoconstrictor agonists. In this regard, recent studies documented that ET increases the release of 19- and 20-HETE from the isolated perfused kidney of the rat (33). Acute blockade of the formation of 20-HETE with 12,12 dibromododec-11-enamide (DBDD) reduces the renal vasoconstrictor response to ET-1 in the isolated perfused kidney of the rat by 30% (33) and the vasoconstrictor response to ET-1 in renal interlobular arteries by 50% (14). Acute inhibition of the formation of 20-HETE with DBDD also reduces the fall in GFR and the natriuretic response to ET-1 in rats (34).

Recently, Imig et al. (18) explored the mechanism by which 20-HETE contributes to the vasoconstrictor response to ET. They found that blockade of the formation of 20-HETE had no effect on the transient rise in intracellular Ca<sup>2+</sup> concentration in renal VSM cells produced by ET-1. However, it enhanced the sustained rise in intracellular Ca<sup>2+</sup> that is dependent on influx through voltage-sensitive Ca<sup>2+</sup> channels. These findings are consistent with the view that 20-HETE potentiates the vasoconstrictor response to ET-1 and ANG II by preventing activation of K<sub>Ca</sub> channels following receptor-mediated release of intracellular Ca<sup>2+</sup> stores.

Even though we were able to demonstrate a contribution of 20-HETE to the vasoconstrictor response to ANG II in isolated renal vessels, the functional significance of this interaction in the overall control of arterial pressure is unknown. Therefore, the present study examined the contribution of 20-HETE to the acute vasoconstrictor response to ANG II in anesthetized rats. The results of these experiments indicate that acute (1 h) systemic blockade of the formation of 20-HETE with DDMS attenuated the rise in MAP produced by a graded intravenous infusion of ANG II by 40%. These findings suggest that elevations in the formation of 20-HETE in the peripheral vasculature contribute to the acute vasoconstrictor response to ANG II. They are also consistent with the recent report by Chu et al. (9) indicating that acute blockade of 20-HETE formation with DDMS attenuates the vasoconstrictor response of the mesenteric circulation of spontaneously hypertensive rats to ANG II.
We next explored whether chronic elevations in circulating levels of ANG II would alter the renal metabolism of AA or the expression of CYP4A protein in the kidney. Chronic intravenous infusion of ANG II (50 ng·kg⁻¹·min⁻¹) in rats for 5 days increased the formation of 20-HETE by 60% and resulted in a fourfold increase in the formation of EETs in microsomes prepared from the renal cortex. ANG II also produced a fourfold increase in the production of 20-HETE in microsomes prepared from the outer medulla. These studies are consistent with the results of earlier studies indicating that the expression of CYP4A enzymes in the kidney and renal microvessels is diminished by elevations in salt intake (2, 8, 21, 35, 47). They are also consistent with recent findings by Croft et al. (10) indicating that chronic administration of ANG II for 3 or 14 days doubles the formation of 20-HETE by renal microvessels. Overall, our findings and those of Croft et al. (10) indicate that chronic elevations in circulating levels of ANG II increase the expression of CYP4A enzymes in the renal microcirculation and the formation of 20-HETE in both the kidney and renal microcirculation.

Because chronic elevations in circulating levels of ANG II increase the expression of CYP4A enzymes and the formation of 20-HETE in the kidney and because 20-HETE contributes to the acute vasoconstrictor response to ANG II, we next examined the effects of chronic blockade of the formation of 20-HETE with two mechanistically different inhibitors on the development of hypertension in rats infused with ANG II (50 ng·kg⁻¹·min⁻¹). The results indicate that ABT attenuates the development of ANG II-induced hypertension. Our findings are consistent with those of Muthalif et al. (29), who also found that chronic administration of ABT markedly reduced blood pressure of rats infused with a high dose of ANG II (350 ng/min) for 6 days, from 171 to 113 mmHg. However, the magnitude of the antihypertensive effect of ABT was much greater in this previous study. This is probably related to the fact that blood pressure was acutely measured under pentobarbital sodium anesthesia, whereas in the present study, we measured MAP in conscious, chronically catheterized rats.

In the present study, the antihypertensive effects of ABT were associated with complete inhibition of the formation of 20-HETE, subterminal HETEs, Di-HETEs, and EETs in renal microsomes. ABT also reduced the urinary excretion of 20-HETE in rats infused with ANG II to levels not different than control and greatly diminished the expression of CYP4A protein in the kidney. This latter effect may be due to increased turnover of this enzyme following the irreversible binding of ABT. The inhibitory effects of ABT on the formation of EETs and 20-HETE in the present study were not specific to the kidney because ABT also reduced the formation of EETs and 20-HETE in the liver by >50% (data not presented). Thus our results indicate that ABT is a relatively nonspecific inhibitor of CYP enzymes that metabolize AA. Our results are inconsistent with the findings of Su et al. (49), which suggested that ABT might be a selective inhibitor of the formation of 20-HETE in the kidney of rats. The reason for the differences in results is not clear, but it should be noted that Su et al. (49) only studied the effects of a single dose of ABT, whereas the effects of a chronic, constant intravenous infusion of ABT for 5 days were examined in the present study.

Because ABT proved to be a nonselective inhibitor of the formation of 20-HETE in our hands, we repeated the experiment with DDMS, which is a more selective, competitive inhibitor of the formation of 20-HETE in the kidney (1, 56). There have been no previous long-term studies performed with this compound that have attempted to establish an effective in vivo dose in rats. In the present study, we found that continuous intravenous infusion of DDMS at a dose of 1 mg·kg⁻¹·day⁻¹ significantly attenuated the development of hypertension in rats infused with ANG II. This dose selectively reduced the formation of 20-HETE in the kidney by ~50% and had much less effect on the formation of EETs. The degree of inhibition produced in vivo is likely to be much higher than 50%, because one must remember that DDMS is a competitive inhibitor, and the levels of this compound are diluted on the order of 50- to 100-fold as one prepares tissue for the in vitro determination of the metabolism of AA.

Unlike ABT, the effects of DDMS appeared to be selective for the kidney. DDMS had no significant effect on the formation of 20-HETE or EETs in microsomes prepared from the liver of these same animals (data not presented). DDMS also significantly reduced the urinary excretion of 20-HETE in rats infused with ANG II, even though it did not completely prevent the rise in 20-HETE excretion produced by infusion of ANG II. Collectively, these studies are consistent with the view that DDMS reduced the renal formation of 20-HETE and that 20-HETE contributes to the elevation in MAP produced by chronic infusion of ANG II.

The mechanism by which CYP inhibitors attenuated the development of ANG II-induced hypertension in the present study is unknown. CYP450 metabolites of AA clearly have both pro- and antihypertensive actions. At the level of the renal tubule, 20-HETE and EETs inhibit sodium transport, whereas 20-HETE is a potent vasoconstrictor. In the present study, DDMS and ABT reduced the formation of 20-HETE and/or EETs in renal microsomes, which largely represent proximal tubules. This should promote sodium retention. Nevertheless, both drugs reduced blood pressure in rats infused with ANG II. Thus it appears that the effects of ABT and DDMS to inhibit the vascular formation of 20-HETE and reduce its vasoconstrictor effects must predominate over any sodium retention associated with blockade of the renal formation of 20-HETE and EETs.

In conclusion, the results of the present study indicate that chronic elevations in circulating levels of ANG II increase the renal synthesis of 20-HETE and that 20-HETE contributes to both the acute and chronic pressor actions of ANG II.
REFERENCES


