Differential Effects of 20-Hydroxyeicosatetraenoic Acid on Intrarenal Blood Flow in the Rat

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ABSTRACT

We recently demonstrated that endothelin-1-induced medullary vasodilation despite a potent cortical vasoconstriction in the rat kidney may be accounted for by 20-hydroxyeicosatetraenoic acid (20-HETE) production. This study characterized the effects of 20-HETE and its metabolites, 20-hydroxy prostaglandin \( \text{E}_2 \) (20-OH PGE\(_2\)) and 20-hydroxy prostaglandin \( \text{F}_2\alpha \) (20-OH PGF\(_{2\alpha}\)), and the contribution of nitric oxide (NO) and prostanoids to the changes evoked in cortical blood flow (CBF) and medullary blood flow (MBF). We tested the hypothesis that 20-HETE produces qualitatively different regional hemodynamic effects in the kidney with 20-OH PGF\(_{2\alpha}\) or 20-OH PGE\(_2\), accounting for the vasoconstriction or vasodilation, respectively, in the cortex and medulla. Renal intra-arterial infusion of 1, 2.5, 5, and 10 ng/min 20-HETE decreased CBF by 10% respectively, in the cortex and medulla. Renal intra-arterial infusion of NO donor 

20-Hydroxyeicosatetraenoic acid (20-HETE), the major eicosanoid in the kidney, is widely known as a constrictor hormone eliciting potent vasoconstriction of the peripheral arteries of the rabbit, the renal afferent arteriole of the rat, dog, and rabbit (Escalante et al., 1989, 1993; Arima et al., 1996; Imig et al., 1996) and the cat cerebral arteriole (Harder et al., 1994). However, some studies have also demonstrated that 20-HETE relaxes bronchial smooth muscle (Jacobs et al., 1996, 1999) and produced vasodilation in certain vascular tissues, including the rabbit perfused kidney (Carroll et al., 1996), human pulmonary artery (Birks et al., 1997), and the bovine coronary artery (Pratt et al., 1998). Further evaluation of its effects demonstrated that both the constrictor and dilator effects of 20-HETE depend not only on an intact endothelium but also on cyclooxygenase (COX) (Escalante et al., 1989, 1993; Schwartzman et al., 1989; Jacobs et al., 1999), suggesting that 20-HETE is metabolized by COX to a mixture of vasoconstrictor and vasodilator prostanoids or that 20-HETE stimulates the release of vasoconstrictor and vasodilator prostanoids from the endothelium. The latter possibility is supported by the study that demonstrated that 20-HETE stimulates the release of prostacyclin (PGI\(_2\)) in the bovine coronary artery (Pratt et al., 1998).

20-HETE and other CYP450 products are good substrates for COX, and their production occurs throughout the kidney. Although the specific metabolic pathways for 20-HETE in the kidney has not been fully identified, a priori, the presence of COX-1 and COX-2 in the kidney (see Imig, 2003) suggests a potential for its metabolism to COX-derived metabolites. Thus, 20-HETE can undergo peroxidation by COX-2 to 20-OH PGD\(_2\), subsequently giving rise to 20-OH PGF\(_{2\alpha}\), which mimics the effects of 20-HETE, as did PGF\(_{2\alpha}\). However, 20-OH PGE\(_2\) increased both CBF and MBF, as did PGF\(_{2\alpha}\). Indomethacin (5 mg/kg) blunted the effects of 20-HETE but not that of 20-OH PGE\(_2\) and 20-OH PGF\(_{2\alpha}\). However, SQ29548 ([1S-[1α,2α(5Z),3α,4α]-7-3Z[(2-phénylamino)carboxy]hydrazino[methyl]]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) (0.1 mg/kg), a prostaglandin \( \text{H}_2 \)/thromboxane \( \text{A}_2 \) receptor antagonist, blunted the cortical and medullary hemodynamic effects elicited by 20-HETE, 20-OH PGE\(_2\), 20-OH PGF\(_{2\alpha}\), but not PGF\(_{2\alpha}\). N\(^{\text{ω}}\)-nitro arginine methyl ester (5 mg/kg), the inhibitor of NO synthase, exacerbated the cortical constrictor effects of 20-HETE and 20-OH PGF\(_{2\alpha}\), without affecting the medullary perfusion produced by 20-HETE or its metabolites. These findings suggest that 20-HETE, through its hydroxyl metabolites, produced differential effects in the kidney. The medullary perfusion appears to be independent of NO.

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ABBREVIATIONS:

- 20-HETE, 20-hydroxyeicosatetraenoic acid
- COX, cyclooxygenase
- PGL\(_2\), prostacyclin
- OH, hydroxy
- 20-OH PGH\(_2\), 20-hydroxy prostaglandin \( \text{H}_2 \)
- 20-OH PGE\(_2\), 20-hydroxy prostaglandin \( \text{E}_2 \)
- PG, prostaglandin
- 20-OH PGF\(_{2\alpha}\), 20-hydroxy prostaglandin \( \text{F}_2\alpha \)
- U46619, 9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin \( \text{F}_2\alpha \)
- COX-2, cyclooxygenase-2
- PGE\(_2\), prostaglandin \( \text{E}_2 \)
- PGF\(_{2\alpha}\), prostaglandin \( \text{F}_2\alpha \)
- PGH\(_{2}\), prostaglandin \( \text{H}_2 \)
- TXA\(_2\), thromboxane \( \text{A}_2 \)
- TP, PGH\(_2\)/TXA\(_2\)
- NO, nitric oxide
- ET-1, endothelin-1
- LD, laser-Doppler
- PU, perfusion unit(s)
- PGH\(_2\)/TXA\(_2\)
- GSH, L-glutathione
- L-NAME, N\(^{\text{ω}}\)-l-nitro arginine methyl ester
- CBF, cortical blood flow
- MBF, medullary blood flow
- LNZ, L-nitro arginine
- COX, cyclooxygenase
- COX-2, cyclooxygenase-2
- TXA\(_2\), thromboxane \( \text{A}_2 \)
- PGL\(_2\), prostacyclin
- COX, cyclooxygenase

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20-OH PGE$_2$, and 20-OH F$_2$- (product literature; Cayman Chemical, Ann Arbor, MI). As an alternative, microsomal CYP450 $\omega$-oxidation of PGF$_{2\alpha}$, or PGE$_2$ can produce 20-OH PGF$_{2\alpha}$, or 20-OH PGE$_{2\alpha}$, respectively. The biologic responses of metabolites of arachidonic acid and by extension, 20-HETE, are a function of their direct effects on the target tissue as well as those of any COX metabolites formed that augment or oppose the actions of the parent compound. Moreover, experimental conditions can alter the expression of COX in various tissues, leading to changes in the types and amounts of COX metabolites of arachidonic acid or 20-HETE formed and, therefore, a modification of the overall vascular response to these compounds. The blockade by indomethacin and endoperoxide receptor antagonists of the vasomotor response to 20-HETE in peripheral arteries of rats and rabbits (Escalante et al., 1989; Schwartzman et al., 1989) suggests the contribution of prostaglandin (PG) endoperoxide analogs. In our previous study (Oyekan and McGiff, 1998), we surmised that COX-dependent effects of 20-HETE could potentially be ascribed to one or more of the endoperoxide (20-OH PG) metabolites, namely 20-OH PGH$_2$, 20-OH TxA$_2$, 20-OH PGE$_2$, and 20-OH PGI$_2$. Therefore, it appears that metabolism by PG isomerases of 20-HETE follows separate pathways, i.e., 20-OH PGH$_2$ $\rightarrow$ 20-OH TxA$_2$/20-OH PGF$_{2\alpha}$, which may constitute the basis for the vasoconstriction, versus 20-OH PGH$_2$ $\rightarrow$ 20-OH PGE$_2$/20-OH PGI$_2$, which may constitute the basis for the vasodilation elicited by 20-HETE in different tissues/organs or species. However, this is purely conjectural.

Before now, it has not been possible to definitively delineate the effects of the component metabolites of 20-HETE in any tissue, including the kidney, due to a lack of specific and selective PG isomerase antagonists and lack of availability of authentic 20-HETE metabolites. With the commercial availability of 20-OH PGF$_{2\alpha}$ and 20-OH PGE$_{2\alpha}$, it is now possible, for the first time, to evaluate the effects of the individual and stable COX metabolites of 20-HETE as a first step to analyzing the specific effects of 20-HETE as they relate to its individual metabolites. Thus, considering that PGF$_{2\alpha}$ generally contracts and PGE$_{2\alpha}$ generally relaxes smooth muscles, we tested the hypothesis that 20-HETE produces qualitatively different regional hemodynamic effects in the kidney with 20-OH PGF$_{2\alpha}$ eliciting the vasoconstriction, whereas 20-OH PGE$_{2\alpha}$ elicits vasodilation in the cortex and medulla of the kidney.

### Materials and Methods

20-HETE, 20-OH PGF$_{2\alpha}$, 20-OH PGE$_2$, PGF$_{2\alpha}$, or PGE$_2$ (Cayman Chemical), supplied in methyl acetate, were stored at $\sim$70°C in 100 $\mu$g/ml ethanol aliquots before use. U46619 (Cayman Chemical) was initially dissolved in 1 mg/ml absolute ethanol, diluted in normal saline to 50 $\mu$g/ml, and stored frozen in aliquots at $\sim$70°C. COX-2 (human recombinant; Cayman Chemical) was reconstituted in 0.1 M Tris buffer, pH 7.6. Indomethacin or 1-tryptophan (Sigma-Aldrich, St. Louis, MO) was prepared fresh dissolved in 0.1 M NaHCO$_3$ and the pH was adjusted to 7.6. SQ29549 (Bristol-Myers Squibb Co., Stamford, CT), PIBMB, L-glutathione (GSH), N-$\omega$-nitro arginine methyl ester (L-NAME) (Sigma-Aldrich), or arachidonic acid (Nuchek Prep Inc., Elysian, MN) was prepared was prepared in 0.9% NaCl. Stannous chloride (Sigma-Aldrich) was prepared in ethanol.

### Animals

Adult male Sprague-Dawley rats (272–365 g) were purchased from Harlan (Indianapolis, IN). The animals were placed in a room with lighting that was adjusted to produce a normal day-night cycle. They were maintained on a standard diet of Purina chow and allowed ad libitum access to water and food. They were allowed at least 3 days to become acclimated to the housing conditions before use in experiments. All protocols were approved by the Institutional Animal Care and Use Committee of the Texas Southern University.

### Chromatographic Analysis of 20-HETE Metabolites

20-HETE metabolites were analyzed by thin-layer chromatography using the method described by Schwartzman et al. (1989). Briefly, COX-2 (1 unit) or homogenate of medullary tissue (5 mg) was preincubated with PHMB (1 mM), an inhibitor of endoperoxide isomerase, for 10 min at room temperature. Thereafter, L-tryptophan (10 mM), hematin (10 mM), and 20-HETE (25 $\mu$g) or arachidonic acid (25 $\mu$g) were added in a reaction volume of 500 $\mu$L, and the mixture was incubated at room temperature for 2 to 3 min. In some experiments, PHMB was omitted but with the addition of GSH (1 mM) or stannous chloride (1 mM) to maximize the production of PGE$_2$ or PGF$_{2\alpha}$-like metabolites, respectively (Hamberg et al., 1974). As a control, 20-HETE was heated to 70°C for 5 min before being added to the incubation mixture. Indomethacin (10 $\mu$M) was added in other experiments to validate COX-derived metabolites. In every case, the reaction was terminated with 10% formic acid, and ethyl acetate was added to extract 20-HETE metabolites. The organic solvent layer was evaporated to dryness, reconstituted in methanol, and subjected to thin-layer chromatography at $\sim$20°C using a solvent system consisting of diethyl ether/petroleum ether (80:20). To assist in identifying the metabolites, 2 $\mu$g of 20-HETE, 20-OH PGE$_2$, 20-OH PGF$_{2\alpha}$ or PGA$_2$ was spotted at the same time, and their migration profiles were determined using phosphomolybdic acid spray reagent (Sigma-Aldrich).

### Hemodynamic Experiments

Rats were anesthetized with inactin (100 mg/kg i.p.) and placed on a heated platform to maintain body temperature at 37°C. A tracheostomy (PE 250) was performed for spontaneous ventilation, and a tail vein was cannulated with a 23-gauge butterfly needle (Abbott Diagnostics, Abbott Park, IL) for drug administration. 20-HETE and its analogs were administered by renal intra-arterial infusion using a 30-gauge needle that was placed in the left renal artery through a left laparotomy. Cortical blood flow (CBF) and medullary blood flow (MBF) were measured simultaneously by laser-Doppler (LD) flowmeter (Periflux System 5000 version 1.20; Perimed, Stockholm, Sweden) via surface probe (PF 407) to measure CBF or an optic fiber LD probe (PF 402) fixed to a micromanipulator and placed in the medulla (5 mm below the kidney surface) to measure MBF. CBF and MBF, measured by laser-Doppler flowmeter (Periflux System 5000 version 1.20), were obtained as volts and expressed as perfusion units (PU) (100 U corresponding to 1 V). The flowmeter was calibrated using a colloidal suspension of latex particles (Perimed Mottility Standard), which, at room temperature, gives a signal of 250 U (2.5 V, $\pm$5%). At the end of the experiment, the renal artery was completely occluded to obtain a zero flow reading in the LD flowmeter, and this value, 30 U (0.03 V) for the cortex or 14 U (0.014 V) for the medulla, was subtracted from the signal recorded during the experiment.

### Experimental Protocol

After surgery and placing of probes for recording CBF and MBF, a 30- to 45-min equilibration period was allowed after which dose-response relationships were established to 20-HETE (1, 2.5, 5, and 10 ng/min), 20-OH PGE$_2$ (5 and 10 ng/min), 20-OH PGF$_{2\alpha}$ (5 and 10 ng/min), PGE$_2$ (5 and 10 ng/min), or PGF$_{2\alpha}$ (5 and 10 ng/min). Each dose of the agent was dried down under nitrogen and reconstituted in methanol, 0.1 M NaHCO$_3$, and stored at $\sim$70°C. At the end of the experiment, the renal artery was completely occluded to obtain a zero flow reading in the LD flowmeter, and this value, 30 U (0.03 V) for the cortex or 14 U (0.014 V) for the medulla, was subtracted from the signal recorded during the experiment.
10% ethanol just before use. Infusion rate was kept at 2.5 μl/min for all agonists. Responses were monitored continuously for 15 min. Responses to vehicle (10% ethanol at 2.5 μl/min; vehicle control) or bolus doses of phenylephrine (10 μg/kg i.v.; negative control) or U46619 (3 μg/kg i.v.; positive control) were also evaluated. U46619 was demonstrated in our previous study to produce cortical vasoconstriction but medullary vasodilation (Hantz et al., 2001). 20-HETE or its analogs were given randomly in a cumulative fashion by renal intra-arterial injection. In some experiments, the effects of inhibitors or antagonists were evaluated on the renal hemodynamic responses to 20-HETE or its analogs. Except in experiments in which indomethacin and SQ29548 were administered, no more than one inhibitor/antagonist was administered in any experiment. In all cases, changes in CBF and MBF were continuously monitored.

**Experimental Groups**

Experiments were performed on five groups of rats (n = 4–6 per group) as follows.

**Group I.** Responses to 20-HETE (1, 2.5, 5, and 10 ng/min), 20-OH PGE\(_2\), (5 and 10 ng/min), 20-OH PGF\(_{2\alpha}\) (5 and 10 ng/min), or PGF\(_{2\alpha}\) (5 and 10 ng/min), given renal intra-arterially in a cumulative fashion, were recorded. The administration of each of these agents was preceded by the administration of 10% ethanol (vehicle) for 15 min to record the baseline value. Responses to U46619 (5 μg/kg) or phenylephrine (10 μg/kg) were also recorded as positive and negative controls, respectively.

**Group II.** This serves as the time control group for 20-HETE and its analogs (n = 4). In this group, baseline measurements of CBF and MBF as well as 20-HETE (5 ng/min)-induced changes in CBF and MBF were recorded (0–15 min) after the equilibration period and repeated during an experimental period (4 h) following the administration of 10% ethanol (vehicle; 2.5 μl/min).

**Groups III and IV.** In these rats, the COX enzyme was inhibited by indomethacin (5 mg/kg i.v.; n = 6), and SQ29548 (0.1 mg/kg i.v.; n = 5), a PGH\(_2\)/TXA\(_2\) (TP) receptor antagonist, was administered. Ten minutes was allowed after administration of indomethacin or SQ29548 before determining responses to 20-HETE, its analogs, PGE\(_2\), or PGF\(_{2\alpha}\). These times and doses are based on our previous data indicating that they are optimal for inhibition of COX or TP receptor antagonism (Hantz et al., 2001).

**Group V.** Renal response to L-NAME was determined in rats that received a bolus dose of L-NAME (5 mg/kg i.v. given slowly over 1 min; n = 5) after the equilibration period. Ten minutes after the injection, responses to 20-HETE or its analogs were evaluated. In some experiments (n = 4), responses to 20-HETE were determined in rats treated with L-NAME and SQ29548 to determine whether the vasoconstrictor effect of 20-HETE in the medulla in the absence of NO is due to TP receptor activation.

Independent effects of the inhibitors/antagonists were evaluated by comparing renal hemodynamics during the baseline period between vehicle- and inhibitor-treated groups. The effects of the inhibitors/antagonists on intrarenal hemodynamics were evaluated by comparing changes in CBF and MBF in response to 20-HETE or its analogs in the presence of an inhibitor or its vehicle. The doses of indomethacin, SQ29548, and L-NAME used in this study were those that we used in our previous studies to effectively diminish prostanoid- or NO-induced renal hemodynamic responses (Herculé and Oyekan, 2000; Hantz et al., 2001).

**Data Analysis**

All hemodynamic responses were recorded as changes (Δ) relative to preinjection (baseline) values, and data were expressed as mean ± S.E. Analysis of variance was used to compare dose-response curves between controls (vehicle-treated) and treated groups followed by Newman-Keuls test. In all cases, p < 0.05 was considered significant.

**Results**

**Identification of 20-HETE Metabolites.** R\(_f\) values identified for authentic 20-OH PGE\(_2\), 20-OH PGF\(_{2\alpha}\), and PGH\(_2\) were 0.19, 0.31, 0.37, and 0.68, respectively. 20-HETE, when incubated with COX-2, generated distinct metabolites with R\(_f\) values of 0.18 and 0.29 that correspond to the migration profiles of 20-OH PGE\(_2\) and 20-OH PGF\(_{2\alpha}\), respectively. Two other metabolites appear, with R\(_f\) values of 0.56 and 0.66. These probably correspond to PG endoperoxides 20-OH PGG\(_2\) and PGH\(_2\), respectively, because they are similar in profile to those generated when arachidonic acid (25 μg) was substituted for 20-HETE as a substrate. In the presence of 10 μM indomethacin or in reactions in which we used boiled 20-HETE, metabolites corresponding to 20-OH PGG\(_2\) and PGH\(_2\) were not generated.

In experiments in which renal medullary tissue was substituted for COX-2, 20-HETE was metabolized to products with a profile that corresponds to 20-OH PGG\(_2\), 20-OH PGH\(_2\), 20-OH PGE\(_2\), and 20-OH PGF\(_{2\alpha}\). In samples incubated with 1 mM stannous chloride, there were two spots, one of which appears to be 20-OH PGE\(_2\) (R\(_f\) value, 0.20), and the other (R\(_f\) value, 0.37) did not correspond to any of the standards we used. In samples incubated with 1 mM GSH, the predominant spot corresponds to 20-OH PGF\(_{2\alpha}\) (R\(_f\) value, 0.31).

**Hemodynamic Experiments.** Basal CBF and MBF in the rats used for this study (n = 36) was 334 ± 21 and 136 ± 10 PU, respectively. In time controls, changes in basal CBF and MBF were −7 ± 10 and 5 ± 3 PU, respectively, after 4 h of renal intra-arterial infusion of 10% ethanol. Moreover, changes in CBF and MBF in response to 20-HETE were not significantly different when evaluated at the beginning of the study and 4 h later.

**Intrarenal Hemodynamic Effects of 20-HETE, 20-OH PGE\(_{2\alpha}\), or 20-OH PGF\(_{2\alpha}\).** 20-HETE (1–10 ng/min) elicited a dose-dependent cortical vasoconstriction but medullary perfusion in the rat kidney. Thus, 20-HETE evoked reductions in CBF by 10 ± 3, 24 ± 4, 40 ± 7, and 58 ± 9 PU at 1, 2.5, 5, and 10 ng/min, respectively (Fig. 1a). However, at the same doses, 20-HETE produced increases in MBF that were 4 ± 2, 16 ± 4, 27 ± 3, and 41 ± 10 PU, respectively. Comparing the hemodynamic effects of 20-HETE in the cortex versus the medulla, the effect was relatively greater in increasing MBF than the reduction in CBF. For example, at the highest dose of 20-HETE (10 ng/min), cortical vasoconstriction was 14 ± 5% in contrast to a value of 24 ± 4% in MBF.

Figure 1b illustrates that 20-OH PGF\(_{2\alpha}\), (5 and 10 ng/min) elicited dose-related cortical vasoconstriction but medullary perfusion in the same qualitative fashion as 20-HETE. The cortical vasoconstriction produced was of equal potency, but the increase in medullary flow was weaker than that produced by 20-HETE. At doses of 5 and 10 ng/min, 20-OH PGF\(_{2\alpha}\) elicited reductions in CBF (Δ = −31 ± 13 and −57 ± 15 PU, respectively) but increases in MBF (Δ = 9 ± 3 and 17 ± 4 PU, respectively). In contrast, 20-OH PGE\(_2\) produced cortical and medullary perfusion (Fig. 1c). Thus, at doses of 5 and 10 ng/min, 20-OH PGE\(_2\) increased CBF by 28 ± 8 and 50 ± 3 PU, respectively, and increased MBF by 13 ± 2 and 22 ± 4 PU, respectively.

Similarly, U46619 (3 μg/kg), a TP receptor agonist, produced cortical vasoconstriction (Δ = −78 ± 12 PU) but medullary perfusion (Δ = 32 ± 6 PU) (data not shown). In con-
Contrast, phenylephrine (10 μg/kg) elicited reductions in CBF (Δ = −64 ± 7 PU) and MBF (Δ = −15 ± 4 PU) (data not shown).

Effect of Indomethacin and SQ29548 on 20-HETE-Induced Changes in Intrarenal Hemodynamics. Indomethacin (5 mg/kg, i.v.) did not significantly affect basal CBF (Δ = −8 ± 4 PU) but decreased basal MBF (Δ = −17 ± 3 PU). Figure 2 illustrates that at the same dose, indomethacin blunted 20-HETE-induced cortical vasoconstriction, uncovering a weak cortical vasodilator effect at low (2.5 and 5 ng/min) doses. Similarly, indomethacin did not affect the intrarenal hemodynamic effects produced by 20-OH PGE2 or 20-OH PGF2α (Figs. 3 and 4). Following the administration of SQ29548 (0.1 mg/kg), a TP receptor antagonist, in indomethacin-treated rats, there was an increase in CBF (Δ = 20 ± 3 PU) but a surprising reduction in MBF (Δ = −11 ± 3 PU). However, there was no further change in the inhibition produced by indomethacin alone of 20-HETE effects (Fig. 2, a and b). In contrast, SQ29548 markedly blunted or abolished the effects on CBF and MBF produced by 20-OH PGE2 or 20-OH PGF2α, (Figs. 3 and 4). Similarly, SQ29548 attenuated PGF2α responses. Thus, reductions by PGF2α in CBF, −16 ± 6 and −25 ± 10 PU, and increases in MBF, 6 ± 3 and 15 ± 6 PU, at 5 and 10 ng/min, respectively, were blunted by SQ29548 (CBF, 72 ± 6%; MBF, 79 ± 4%; p < 0.05) (data not shown). However, SQ29548 did not affect the increases by PGE2 in CBF, 18 ± 5 and 32 ± 13 PU, or the increase in MBF, 8 ± 4 and 13 ± 3 PU, at 5 and 10 ng/min, respectively (data not shown).

Role of NO in 20-HETE-Induced Changes in Intrarenal Hemodynamics. L-NAME (5 mg/kg) reduced CBF (Δ = −63 ± 13 PU) and MBF (Δ = −26 ± 5 PU) and enhanced 20-HETE-induced reduction in CBF by 41 ± 5% (p < 0.05) (Fig. 5a) but did not affect the increase in MBF at low doses of 20-HETE. However, L-NAME reversed the medullary va-
The results of the present study demonstrate that 20-HETE produced a differential effect on intrarenal hemodynamics in the rat, eliciting a cortical vasoconstriction but a marked medullary vasodilation. Contrary to our hypothesis, 20-OH PGF\(_{2\alpha}\) mimics the effects of 20-HETE, whereas 20-OH PGE\(_{2}\) produced vasodilation of both the cortical and medullary regions. The medullary vasodilation produced by 20-HETE or its analogs appears to be mediated by prostanooids, not NO, inasmuch as indomethacin but not L-NAME blunted the effect.

NO is a major player in renal hemodynamics in most animals, and the renal vasculature is extremely sensitive to NO because stimulation of endogenous NO potently increased the diameter of large preglomerular vessels (Gulbins et al., 1993), the afferent and efferent arterioles (Deng and Baylis, 1993), and vasa recta (Pflueger et al., 1999), leading to decreases in renal vascular resistance. Thus, NO modulates renal vascular tone, providing counterregulating renoprotective mechanisms in response to pressor hormones, including angiotensin II and norepinephrine (Parekh et al., 1996; Navar et al., 2000) and ET-1 (Gurbanov et al., 1996; Hercule and Oyekan, 2000). However, NO production and/or activity is subject to regional variation, and this may determine the degree of effect produced by vasoactive hormones in the medulla versus the cortex. Thus, NO production is greater in the medulla compared with the cortex (Zou and Cowley, 1997) and will be expected to mediate increased renal perfusion. However, despite the established mediator role of NO in renal hemodynamics, it appears to play no role in 20-HETE-induced medullary perfusion in these experiments inasmuch as L-NAME did not affect the medullary vasodilation produced by 20-HETE or its analogs.

PGs, the other major players in the control of renal hemodynamics, are released in response to shear stress and administration of many vasoactive agents, including U46619 (Mehta et al., 1984) and ET-1 (Hercule and Oyekan, 2000), and produce vascular effects that differ according to the family they belong to, the specific tissue or organ, and the mammalian species in which they are examined. For example, PGE\(_{2}\) and/or PGI\(_{2}\), either released by hormones or ad-
ministered exogenously, produce vasodilation in most vascular beds (see Imig, 2003). However, in the rat, PGE$_2$ and PGA$_2$ produced vasoconstriction in the rat isolated perfused kidney (Malik and McGiff, 1975). In the kidney, the effects of PGs vary according to the region of the kidney, cortex versus the medulla, and the renal medulla is increasingly being recognized as an important region for overall regulation of hemodynamics. Thus, although prostanooids are important regulators of renal blood flow in the kidney, their major site is the medulla, where, besides the endothelium of vasa recta, interstitial cells, and collecting ducts, a large quantity of dilator PGs is produced, antagonizing the effects of vasoconstrictor hormones in an agonist-specific manner (Parekh and Zou, 1996). However, PGs also produce vasoconstriction in the kidney. For example, indomethacin inhibited the renal vasoconstrictor effect of ET-1 and U46619 in our previous studies (Oyekan and McGiff, 1998; Hercule and Oyekan, 2000; Hantz et al., 2001). However, the reduction by indomethacin of basal MBF in this study suggests that vasodilator PGs contribute to regulation of basal MBF. In the vascular tissue, PG endoperoxides, upon activation of TP receptors, evoke vasodilation due to release of PGI$_2$ (Nicholson et al., 1984), an observation that was independently confirmed with the demonstration that U46619, a TP receptor agonist, released PGI$_2$ in vivo (Mehta et al., 1984; Hui and Ogle, 1993) or in cultured endothelial cells (Nicholson et al., 1984). Based on our earlier studies and others, ET-1 acts by stimulating TP receptors (Asano et al., 1994) and produces contrasting effects on intrarenal hemodynamics (Gurbanov et al., 1996; Hercule and Oyekan, 2000) just as U46619 does (Hantz et al., 2001). Therefore, it appears that activation of TP receptors may yield a constrictor or dilator effect depending on the region of the kidney and the preponderant PG isomerase and, hence, the particular prostanooid formed. Data from this study demonstrate that activation of TP receptors by 20-HETE yields metabolites with dilator and constrictor effects inasmuch as SQ29548 antagonized these effects. Analyzing the effects of 20-HETE further, our data support that 20-HETE yields metabolites with dilator effects in the medulla and constrictor or dilator effects in the cortex. 20-OH PGF$_{2\alpha}$ appears to account for its cortical vasoconstriction, whereas both 20-OH PGF$_{2\alpha}$ and 20-OH PGE$_2$ contribute to the medullary vasodilation. Indeed, under optimal conditions for extraction of unstable endoperoxides (Schwartzman et al., 1989), incubation of 20-HETE with medullary tissue yielded metabolites that we identified as 20-OH PGG$_{2\alpha}$/H$_2$ that yielded 20-OH PGE$_2$ and 20-OH PGF$_{2\alpha}$ in the presence of GSH or stannous chloride, respectively, similar to that described for arachidonic acid (Hamberg et al., 1974). These metabolites are similar in chromatographic migration profile to that obtained following incubation of 20-HETE or arachidonic acid with recombinant COX-2.

Given the central role of 20-HETE as a second messenger in the regulation of renal vascular tone, its contribution to renal hemodynamics induced by various hormones, namely ET-1, angiotensin II, and NO, has been the subject of recent interest. Because we previously demonstrated that 20-HETE is the putative mediator of the renal effects of ET-1 (Oyekan et al., 1997; Oyekan and McGiff, 1998) and that inhibition of 20-HETE production blunted the intrarenal hemodynamic effects of ET-1 in the rat (Hercule and Oyekan, 2000), it stands to reason that the cortical vasoconstriction and the medullary vasodilation by ET-1 may be accounted for by 20-HETE release. However, the present finding challenges the studies that showed that inhibition of 20-HETE production with 17-octadecynoic acid led to increase in MBF (Zou et al., 1994). The present finding also challenges our earlier explanation for the role of 20-HETE in ET-1-induced medullary vasodilation (Hercule and Oyekan, 2000). Additional studies are clearly required to further evaluate the mechanisms involved in the intrarenal hemodynamic effect of 20-HETE. Nonetheless, considering that the renal medulla is increasingly recognized as an important region for overall regulation of renal hemodynamics with a capacity for formation of prostanoids and CYP450 metabolites, the present study demonstrates that 20-HETE may share a similar property with NO and/or PGs in providing counterregulating renoprotective effect in response to pressor hormones in the medulla. Thus, our studies and others that showed that ET-1 and angiotensin II enhanced the formation of 20-HETE in the kidney (Carroll et al., 1996, 1997; Oyekan et al., 1997; Oyekan and McGiff, 1998; Croft et al., 2000) need to be reevaluated because 20-HETE appears to provide a classic counterregulatory effect to these pressor hormones, especially in the medulla.

Fig. 5. Dose-dependent effects of 20-HETE (2.5, 5, and 10 ng/min) on cortical (a) or medullary (b) blood flow in vehicle-treated (Control) rats or rats treated with l-NAME (5 mg/kg i.v.; n = 5). *, p < 0.05 compared with control (n = 6).
References


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