Role of NO and cytochrome P-450-derived eicosanoids in ET-1-induced changes in intrarenal hemodynamics in rats

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Hercule, H. C., and A. O. Oyekan. Role of NO and cytochrome P-450-derived eicosanoids in ET-1-induced changes in intrarenal hemodynamics in the rat. Am J Physiol Regulatory Integrative Comp Physiol 279: R2132–R2141, 2000.—Endothelin-1 (ET-1) produces potent renal effects that we have previously shown to be dependent on cytochrome P-450 (CYP450) metabolites of arachidonic acid (24). This study evaluated the role of these metabolites in the effects produced by ET-1 on renal blood flow (RBF), cortical blood flow (CBF), medullary blood flow (MBF), and mean arterial blood pressure (MAP). ET-1 (20–200 pmol/kg) increased MBF, renal vascular resistance (RVR), and MBF but reduced CBF and RBF in a dose-dependent manner. The decreases in CBF and RBF, and increases in MBP and RVR were blunted by BMS-182874, an ET A receptor antagonist or BQ-788, an ET B receptor antagonist. Similarly, indomethacin, an inhibitor of cyclooxygenase activity, or 12,12-dibromo-dodecenoic acid (DBDD), a CYP450-dependent inhibitor of production of 20-hydroxyeicosatetraenoic acid (20-HETE), blunted these effects. ET-3 elicited dose-related reduction in CBF and increase in MBF. Indomethacin accentuated the reduction in CBF and attenuated the increase in MBF, as did DBDD. ET-1-induced increase in MBF was attenuated by BQ-788, Nω-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO) synthesis, indomethacin, or DBDD. DBDD inhibited the hemodynamic effects of L-NAME. Miconazole, the inhibitor of CYP450-dependent epoxygenase activity, was without effect. These results indicate that hemodynamic changes produced by ET-1 are mediated by vasoconstrictor prostanooids and/or prostanooid-like substances, possibly, 20-HETE via activation of ET A and ET B receptors. However, the increase in MBF is mediated by vasodilator prostanooids or by NO via ET B receptor activation.

20-hydroxyeicosatetraenoic acid; cortical blood flow; medullary blood flow; mean arterial blood pressure; endothelin-1; nitric oxide

ENDOTHELIN (ET)-1 is a 21-amino acid peptide product of endothelial cells and the most potent renal vasoconstrictor known. Extensive work has been directed at the renal effects of ET because the kidney can synthesize the peptide and it possesses ET receptors. ET has a number of renal effects, which include a sustained increase in renal vascular resistance (RVR) associated with a marked reduction in renal blood flow (RBF) (15). Two ET receptors (ET A and ET B) have been identified by cloning and sequencing from a wide variety of species, including humans, and are associated with different functions of the peptide. Reverse transcription and polymerase chain reaction assays on the localization of ET receptor mRNA in the rat nephron indicated that ET A mRNA predominates in the collecting tubules and glomeruli, whereas ET B receptor mRNA is found only in the vascular system (35). Many studies indicate that the ET A receptor mediates the vasoconstrictor and mitogenic effects, whereas the ET B receptor mediates the vasodilator and possibly some transport effects of ET-1 (40). However, other studies observed that mesangial cells, vasa recta, and arcuate arteries express both ET A and ET B receptors (35) so the vascular smooth muscle cell where ET is released into the subendothelial space binds with either receptor to cause vasoconstriction (18, 33). There is also evidence that ET B receptors are located on the vascular endothelium and, when stimulated, evoke vasodilation by inducing the release of nitric oxide (NO), prostaglandin (PG) PGL 2 (prostacyclin), or both (8, 9). Indeed, the initial vasodepressor action of ET-1, when injected intravenously, was ascribed to ET B-mediated release of NO or PGL 2 (9). In the medulla, where there is a pronounced presence of ET B receptors, ET increased the production of PGE 2 (38) and increased medullary perfusion while causing reductions in cortical blood flow (CBF) (12). Thus PGs and NO are involved in ET-1 effects. NO not only opposed the vasoconstrictor effects of ET but also suppressed ET gene expression (7, 17) and facilitated the termination of the action of the peptide (11).

Eicosanoid products of the cytochrome P-450 (CYP450) monoxygenase system are important paracrine hormones in the kidney that make important contributions to the regulation of vasomotor tone as well as the regulation of salt and water excretion (19). NO interferes with this enzyme system as it does with the ET system. NO therefore inhibits CYP450 enzymes of the 1A, 2B1 (39), 3C (14), and 4A (2) families, probably by forming iron-nitrosyl complexes at the catalytic heme binding sites of these enzymes. In ad-
dition, NO suppresses CYP450-dependent oxygenation reactions in renal microsomes (2), decreases CYP450 activity and content (14), and inhibits CYP450-dependent production of arachidonic acid (AA) metabolites that dilate the rat renal vasculature (22). Inhibition of NO formation with L-arginine analogs such as Nω-nitro-L-arginine methyl ester (L-NAME), which competitively inhibit NO synthase, resulted in increased plasma levels of ET-1 (29), the renal hemodynamic effects of which we demonstrated could be accounted for by peptide-induced release of 20-hydroxyicosatetraenoic acid (20-HETE), a major CYP450 product (24). In a recent study, we demonstrated that the renal functional response to administration of L-NAME in anesthetized rats expressed a CYP450-dependent component that is linked to ET1 receptor (25), from which we concluded that 20-HETE is a mediator of the response of the kidney to increased ET-1 levels resulting from withdrawal of NO in the rat. The present study was designed to evaluate the role of 20-HETE in the differential effects of ET-1 in the kidney. As NO and PGs modulate or mediate the biological effects of ET-1 and 20-HETE, we also evaluated their roles in the regulation of renal and systemic hemodynamic responses to ET-1.

METHODS

L-NAME (Sigma, St. Louis, MO) was dissolved in normal saline, indomethacin (Sigma) was dissolved in 0.1 M NaHCO3, miconazole (Sigma) in DMSO (25% final concentration, Sigma), BMS-182874 ([5-dimethylamino]-N-(3,4-dimethyl-5-isoxazolyl)-1-naphthalene sulfonamide) a gift from Dr. C. T. Stiers of our department was dissolved in 0.1 M NaHCO3 and pH was adjusted to 7.6. ET-1 and ET-3 (Peninsula Laboratories, Belmont, CA) were dissolved in 0.1% acetic acid and BQ-788 in 25% DMSO. 12,12-Dibromododecenoic acid (DBDD) was a gift from Dr. Camille Falck (University of Texas Southwestern Medical Center) and was stored in ethanol at −20°C.

The experiments were performed on male Sprague-Dawley rats (body wt 309 ± 7 g; Charles River Laboratory, Wilmington, MA). The animals were maintained on standard rat food (Purina Chow) and were allowed ad libitum access to water and food until the beginning of the experiments. Experimental protocols for these studies in rats were approved by the Institutional Animal Care and Use Committee.

Rats were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg; Research Biochemicals International, Natick, MA) and placed on a heated platform to maintain body temperature at 37°C. A tracheostomy (PE-250) was performed for spontaneous ventilation, and a cannula (PE-50) was placed in the right carotid artery to monitor blood pressure. Two tail veins were cannulated with 23-G butterfly needles (Abbott Hospitals, Chicago, IL) for infusion or administration of drugs. A left laparotomy was performed, and electromagnetic flow probes (Carolina Medical Electronics) were placed over the left renal artery to measure RBF. Mineral oil (37°C) was poured on the kidney surface to prevent drying. Subsequently, CBF and medullary blood flow (MBF) were measured simultaneously by laser-Doppler flowmeter (Periflux System 5000 ver 1.20, Stockholm, Sweden) via surface probe (PF-407) to measure CBF or an optic fiber laser-Doppler probe (PF-402) fixed to a micromanipulator and placed in the medulla (5 mm below the kidney surface) to measure MBF. CBF and MBF were obtained as perfusion units (PU) and expressed as volts (100 U corresponding to 1 V). The flowmeter was calibrated using a colloidal suspension of latex particles (Perimed Motility Standard), which at room temperature gives a signal of 250 U (2.5 V ± 5%). At the end of the experiment the renal artery was completely occluded to obtain a zero flow reading in the laser-Doppler 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. Mean arterial blood pressure (MBP) was measured with a pressure transducer (Statham model P231 D; Statham, Oxnard, CA). All recordings were made using the Windaq Acquisition Data DI-150RS waveform multichannel recording system (DataQ Instruments, Akron, OH) connected to an IBM PC (Acer Pentium CTX model 707P). RVR was estimated from the MBP and RBF values using the formula (MBP-5)/RBF; 5 mmHg was used as an estimate of renal venous pressure.

Experimental protocol. After surgery and placement of probes for recording blood flows, a 30- to 45-min equilibration period was allowed after which a dose-response relationship was established to ET-1 (20, 40, 100, and 200 pmol/kg) or ET-3 (40 and 100 pmol/kg) in the presence of an inhibitor or antagonist or its vehicle. In the first experimental period, starting immediately after the equilibration period, vehicle was infused for ~15 min, after which different doses of ET-1 or ET-3 were given randomly by bolus injection. The rat was allowed to recover fully from the effect of one dose before another dose was given. After the last dose of ET, an inhibitor or antagonist was administered and a period of 30 min was allowed for the inhibitor to exert an effect. Responses to ET were reestablished (2nd experimental period). In time controls (n = 4) responses to ET-1 (20–200 pmol/kg) were obtained 1 h after the equilibration period. These doses were repeated 30 min later. It took about 1 h to complete a full dose-response study, and each experiment typically lasted about 2.5 h after the equilibration period.

The effects of ET-1 or ET-3 on blood pressure, total RBF, or regional blood flows were studied in the presence of DBDD (2.5 mg·kg−1·h−1 iv, n = 6), a suicide substrate CYP-dependent selective inhibitor of ω1-hydroxylation production of 20-HETE (37); miconazole, an inhibitor of epoxigenase activity (16.5 nmol/min, n = 7); BMS-182874, an ET, receptor antagonist (40 mg/kg iv, n = 5); BQ-788, an ET1, receptor antagonist (0.5 or 1 mg/kg iv, n = 5–8); indomethacin (5 mg/kg iv, n = 6), an inhibitor of cyclooxygenase (COX); L-NAME (5 mg/kg iv, n = 7), an inhibitor of NO production, or their respective vehicles: 0.1 M NaHCO3 for indomethacin and BMS-182874, 50% ethanol for DBDD, or 25% DMSO for BQ-788 and miconazole. In some experiments, responses to ET-1 (40 and 100 pmol/kg) were evaluated in the presence of combined administration of BMS-182874 and BQ-788 (n = 5) or L-NAME and DBDD (n = 5). Except for L-NAME (ET responses were determined after 10 min of administration), a 30-min period was allowed (after the 1st experimental period) following administration of an inhibitor or antagonist or its respective vehicle before redetermining responses to ET. In all cases, changes in MBP, RBF, CBF, and MBF were continuously monitored. Indepedent effects of the inhibitors or antagonists were evaluated by comparing hemodynamic responses before and after the administration of a particular inhibitor. The effects of the inhibitors or antagonists on ET-induced changes in hemodynamics were evaluated by comparing renal effects to ET between the first and second experimental periods.
Data analysis. All responses were recorded as changes (Δ) relative to preinjection values, and data are expressed as means ± SE. Analysis of variance was used to compare dose response curves between controls (vehicle treated) and treated groups followed by the Newman-Keuls test. In all cases \( P < 0.05 \) was considered significant.
RESULTS

Renal hemodynamic response to ET-1. Baseline values of MBP, RBF, CBF, MBF, and RVR in rats used for these experiments were 118 ± 6 mmHg, 9.7 ± 0.5 ml/min, 422 ± 21 PU, 162 ± 15 PU, and 11.7 ± 0.8 mmHg·ml⁻¹·min, respectively (n = 22). Administration of ET-1 (20, 40, 100, and 200 pmol/kg iv) resulted in dose-dependent increases (above basal) in MBP (Δ = 7–25 mmHg); reductions in RBF (Δ = −0.7 to −2.2 ml/min) and CBF (Δ = −52 to −175 PU); and increases in MBF (Δ = 19 to 54 PU) and RVR (Δ = 1.9 to 4.2 mmHg·ml⁻¹·min). At doses <20 pmol/kg there were no observable increases in MBP and MBF despite a 5–10% reduction in CBF and RBF. These changes were instantaneous and sustained except for the increase in MBF, which was short-lived, lasting about 2–8 min. Full recovery occurred within 10 min for the lower doses of ET-1 (≤40 pmol/kg), whereas recovery took up to 20 min at higher doses (≥100 pmol/kg). At higher doses, effects of ET-1 manifested more as increased duration of action than as an increase in peak response. For CBF and MBF, the percent changes in magnitude of response to ET-1 are similar. However, the duration of the response to ET-1 was greater on CBF (5–20 min) than MBF (2–8 min). In time controls (n = 4) responses to ET-1 were not diminished; if anything, they were ~6% greater during the second experimental period when a dose-response relationship was repeated for ET-1. In rats that received the vehicles for DBDD (50% ethanol, n = 4), indomethacin and BMS-182874 (0.1 M NaHCO₃, n = 6), and BQ-788 or miconazole (25% DMSO, n = 7), basal renal hemodynamic responses were not significantly different from those that received normal saline (0.9% NaCl, n = 5).

Effect of ET₄ and/or ET₆ receptor antagonism on ET-1-induced changes in hemodynamics. BMS-182874 (40 µg/kg iv, n = 7), the ET₄ receptor antagonist did not significantly alter basal MBP (control 118 ± 6 mmHg vs. experimental 109 ± 5 mmHg). Relative to basal values BMS-182874 did not significantly alter MBP (Δ = 14 ± 8 µHg, P > 0.05) but increased CBF (Δ = 38 ± 9 µHg, P < 0.05) and RBF (Δ = 0.6 ± 0.2 ml/min, P < 0.05) thereby reducing basal RVR (Δ = −1.4 ± 0.3 mmHg·ml⁻¹·min, P < 0.05). In addition, BMS-182874 blunted ET-1-induced reduction in CBF (64 ± 8%, P < 0.05) without affecting the increase in MBF (Fig. 1A). BMS-182874 also blunted the increases in MBP (36 ± 6%, P < 0.05; Fig. 1B) and RVR (46 ± 7%, P < 0.05; Fig. 1C) and attenuated the reduction in RBF (24 ± 6%, P < 0.05; Fig. 1C), suggesting the involvement of ET₄ receptors in the renal hemodynamic response to ET-1 in the cortex but not the medulla. In contrast to BMS-182874, BQ-788 (0.5 mg/kg iv, n = 5), the potent, selective ET₆ receptor antagonist, did not produce significant changes in the hemodynamic parameters evaluated. Thus in the presence of BQ-788, basal values recorded were CBF 438 ± 12 µHg, MBF 148 ± 10 PU, RBF 10.3 ± 0.3 ml/min, and MBP 122 ± 4 mmHg, values not significantly different from those in vehicle-treated (n = 22) rats. When ET-1 responses were evaluated in the presence of BQ-788, the reductions by ET-1 in CBF and MBF were inhibited (Fig. 2A), a relatively greater inhibitory effect being observed on MBF where BQ-788 blunted ET-1-induced increase in MBF by 84 ± 15% (P < 0.05) vs. 43 ± 10% (P < 0.05) of the reduction by ET-1 of CBF (Fig. 2A). In two of five experiments, ET-1 elicited a medullary vasoconstrictor response, suggesting greater involvement of ET₆ receptors in ET-1-induced increase in medullary perfusion. BQ-788 markedly attenuated the initial vasodepressor effect of ET-1 on MBP without significantly affecting the sustained increase in MBP (Fig. 2B). For example, the hypertensive response to 200 pmol/kg ET-1, 18 ± 3 mmHg was reduced to 5 ± 2 mmHg (P < 0.05) in the presence of BQ-788. BQ-788 also attenuated ET-1-induced reduction in RBF (P < 0.05) and the increase in RVR (P < 0.05; Fig. 2C). In rats (n = 5) treated with the combination of BMS-182874 and BQ-788, basal MBP did not change (4 ± 4 mmHg) but basal CBF and MBF increased by 22 ± 9 PU (P < 0.05) and 14 ± 2 PU (P < 0.05), respectively. Moreover, ET-1-induced reduction in CBF was reduced by 75 ± 5% (P < 0.05), whereas the increase in MBF was reduced by 67 ± 19% (Table 1). The reduction in CBF but not MBF was greater than that obtained with BMS-182874 or BQ-788 alone (P < 0.05). In addition, BMS-182874 and BQ-788 attenuated the increases by ET-1 on MBP (44 ± 11%, P < 0.05; Table 1). The combination of

Table 1. Effects of combined administration of BMS-182874 and BQ-788 on the actions of ET-1 on changes in cortical, medullary, and total renal blood flow, and on mean arterial blood pressure in Inactin-anesthetized rats

<table>
<thead>
<tr>
<th>ET-1, pmol/kg</th>
<th>Cortical Blood Flow, PU</th>
<th>Medullary Blood Flow, PU</th>
<th>Mean Arterial Blood Pressure, mmHg</th>
<th>Renal Blood Flow, ml/min</th>
<th>Renal Vascular Resistance, mmHg·ml⁻¹·min</th>
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<tbody>
<tr>
<td>40</td>
<td>−73 ± 12</td>
<td>−20 ± 14*</td>
<td>16 ± 3</td>
<td>6 ± 6*</td>
<td>16 ± 8</td>
</tr>
<tr>
<td>80</td>
<td>−87 ± 9</td>
<td>−7 ± 5*</td>
<td>13 ± 3</td>
<td>2 ± 4*</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>100</td>
<td>−130 ± 17</td>
<td>−36 ± 5*</td>
<td>25 ± 7</td>
<td>12 ± 4*</td>
<td>21 ± 5</td>
</tr>
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</table>

Values are means ± SE; n = 5 rats. BMS-182874, 40 µg/kg; BQ-788, 0.5 or 1 mg/kg; ET-1, endothelin-1; PU, perfusion unit. Control, responses to ET-1 in the presence of 1 ml/kg bolus administration of the mixture of 25% DMSO and 0.1 M NaHCO₃ (vehicle) 30 min before testing the responses to ET-1. Data given in bold refer to responses in the presence of 1 mg/kg BQ-788 (n = 8) or its vehicle (control). *P < 0.05 vs. control.
BMS-182874 and a higher dose of BQ-788 (1 mg/kg) further inhibited the effects of ET-1 on all the hemodynamic parameters. However, this higher dose did not abolish the effects of ET-1 (Table 1).

**Effects of COX inhibition on ET-1- and ET-3-induced changes in hemodynamics.** The COX pathway is implicated in the biological effects induced by ET-1 (3, 20) and ET-3 (34). A functional COX is required for the expression of the renal vasoconstrictor effects of 20-HETE (4), a mediator of the renal effects of ET-1 in the rat (23, 24). These studies were conducted to evaluate the role of COX and the receptors involved in the changes elicited by ET-1 on blood flow in the kidney. Indomethacin (5 mg/kg iv, n = 6) selectively reduced basal MBF (Δ = -32 ± 8 PU, P < 0.05) and marginally reduced CBF (Δ = -28 ± 5 PU, P > 0.05) but was without effect on basal MBP, RBF, and RVR. In the presence of indomethacin, the reductions in CBF and MBF by ET-1 were blunted by 53 ± 5% (P < 0.05) and 44 ± 6% (P < 0.05), respectively (Fig. 3A). Indomethacin did not affect the increase in MBP by ET-1 (Fig. 3B) but attenuated the reduction in RBF (22 ± 4%, P < 0.05) and the increase in RVR (Fig. 3C). On the other hand, ET-3 at doses of 40 and 100 pmol/kg reduced CBF and increased MBF in a dose-related manner without affecting MBP. However, indomethacin accentuated ET-3-induced reduction in CBF by 110 ± 20% (P < 0.05) but attenuated the reductions in MBF and MBP (P < 0.05), converting medullary vasodilation to vasoconstriction and uncovering a hypotensive effect of ET-3 (Table 2).

**Effects of acute inhibition of NO synthesis on ET-induced response.** Because NO release by ET-1 accounted for the initial vasodepressor effect of ET-1 in the rat (8), these experiments evaluated the role of NO in ET-1 hemodynamic responses, using L-NAME at a dose (5 mg/kg iv) that did not elicit profound changes on renal and systemic hemodynamics. Thus L-NAME (5 mg/kg iv, n = 7) produced a modest increase in basal MBP of 31 ± 6 mmHg (P < 0.05) and reduced basal CBF (Δ = -62 ± 12 PU), MBF (Δ = -29 ± 8 PU), and RBF (Δ = -2.6 ± 0.3 ml/min). L-NAME also enhanced ET-1-induced reductions in CBF (47 ± 7%, P < 0.05) but markedly blunted ET-1-induced increase in MBF (73 ± 9%, P < 0.05; Fig. 4A), indicating that NO is a mediator of ET-1-induced medullary perfusion. In addition, L-NAME exacerbated the reduction by ET-1 in RBF (73 ± 10%, P < 0.05; Fig. 4C) and enhanced the increases in MBP (77 ± 16%, P < 0.05; Fig. 4B) and RVR (199 ± 46%, P < 0.05; Fig. 4C).

**Effect of inhibition of 20-HETE production on ET-induced hemodynamic responses.** Products of the ω/ω-1-hydroxylase pathway of CYP450-dependent AA metabolites are synthesized in the kidney (13) and influence renal vascular responses by themselves and in response to ET-1 (23). DBDD, a suicide substrate inhibitor of CYP450 fatty acid ω/ω-1-hydroxylase, which produces 20-HETE, was used to evaluate the role of 20-HETE in the hemodynamic response to ET-1 and ET-3. DBDD (2.5 mg·kg⁻¹·h⁻¹, n = 6) increased basal CBF (Δ = 37 ± 4 PU, P < 0.05), MBF (Δ = 29 ± 4 PU,
P < 0.05) and RBF (Δ = 0.9 ± 0.1 ml/min, P < 0.05) above basal values, and reduced RVR (Δ = −1.2 ± 0.3 mmHg·ml⁻¹·min, P < 0.05), suggesting that vasoconstrictor products of the ω/ω-1-hydroxylase pathway contribute to maintenance of regional and total blood flow in the kidney. DBDD attenuated ET-1-induced reductions in CBF (P < 0.05), and although unexpected DBDD also blunted ET-1-induced increase in MBF (P < 0.05; Fig. 5A), uncovering a vasoconstrictor effect in three of six experiments. The increase in MBP by ET-1 was blunted (P < 0.05; Fig. 5B), as were the reduction in RBF (P < 0.05; Fig. 5C) and the increase in RVR (P < 0.05; Fig. 5C). DBDD was without effect on the hemodynamic effects of phenylephrine (10 μg/kg) (CBF Δ = −53 ± 19; vehicle vs. −43 ± 8 PU experimental), but it attenuated the hemodynamic effects of ET-3. Thus ET-3 at doses of 40 and 100 pmol/kg reduced CBF by 49 ± 11 and 61 ± 18 PU in vehicle-treated (n = 5) rats (control) compared with 29 ± 8 and 46 ± 10 PU (P < 0.05), respectively, in the presence of DBDD. DBDD also attenuated the increases by 40 and 100 pmol/kg ET-3 in MBF from 9 ± 3 and 16 ± 3 PU to −5 ± 6 and 9 ± 4 PU, respectively (P < 0.05). DBDD also modified the effect of ET-3 on MBP, abolishing the hypotensive effect of ET-3 at the 40 pmol/kg dose and uncovering a hypotensive effect at the 100 pmol/kg dose (Table 2).

Effect of combined inhibition of production of NO and 20-HETE on ET-1 responses. In rats (n = 5) pre-treated with DBDD to inhibit 20-HETE production, the increase in L-NAME in basal MBP was reduced by 32 ± 4% (P < 0.05) and the reductions in basal CBF and MBF were attenuated by 35 ± 3 and 37 ± 6% (P < 0.05), respectively (Table 3). DBDD also attenuated the enhancement by L-NAME of ET-1-induced reductions in CBF (P < 0.05) and MBF (P < 0.05). In addition, DBDD reduced the enhancement by L-NAME of ET-1 increase on MBP (P < 0.05; Table 3).

Effect of inhibition of the CYP450 epoxygenase pathway on ET-1 responses. As epoxygenase metabolites of CYP450-dependent AA metabolism also contribute to renal vasomotor activity (19), we evaluated the role of the epoxygenase pathway on the renal hemodynamic effects of ET-1. In rats treated with miconazole (16.5 nmol/min, n = 7), a selective inhibitor of epoxygenase, there were no changes in renal hemodynamic responses to ET-1. Moreover, miconazole was without effect on ET-1-induced changes in CBF, MBF, MBP, RBF, and RVR as there were no significant differences in these parameters in response to ET-1 before and after administration of miconazole.

DISCUSSION

The precise effects of ET-1 on the kidney are not clearly defined, but the consensus is that ET-1 evokes a potent renal vasoconstriction in the rat and increases systemic blood pressure (15, 16). Interestingly, the effects of ET-1 are qualitatively different. Thus despite its potent vasoconstrictor effect, intravenous administration of the peptide in the rat elicited an initial vasodepressor effect that was associated with the release of NO or PGL₂ (8, 9). The rationale for the present study was based on our published findings and reports from other laboratories that showed that the renal vasoconstrictor action of ET-1 was influenced by a number of paracrine hormones in the kidney; viz, NO (8), prostanoids (8, 36), and CYP450-derived AA metabolites (24). The results of the present study demonstrate that administration of ET-1 at a dose that caused a profound reduction in CBF was associated with an increase rather than a decline in medullary perfusion, findings that are in agreement with the study of Gurbanov et al. (12). A differential effect in blood flow also has been observed for adenosine A₂ receptor agonists (1) and in such pathological conditions as renal failure (5), congestive heart failure (6), and cirrhosis (10), conditions characterized by intense cortical vasoconstriction and increased ET-1 production. These observations indicate that blood flow within the kidney is independently regulated. This operates so that an accompanying increase in medullary perfusion following a strong cortical vasoconstriction is necessary to avoid a deterioration in the oxygen supply to the medulla, which normally operates at a hypoxic level. In this study, the magnitude of these changes is not equal because the increase in medullary perfusion is not matched by the reduction in cortical perfusion. Thus the increase in MBF in response to maximal dose of ET-1 does not rise to the level that changes the hypoxic milieu of the medulla but may just
be sufficient to offset reductions in perfusion in the cortex and thereby preserve medullary perfusion.

The present study also demonstrates that the disparate effects of ET-1 on regional blood flow are mediated via different ET receptors coupled to different signaling mechanisms. The reduction in CBF is mediated through ET\textsubscript{A} and ET\textsubscript{B} receptors inasmuch as BMS-182874, the ET\textsubscript{A} receptor antagonist, or BQ-788, the ET\textsubscript{B} receptor antagonist, each diminished cortical vasoconstriction by ET-1. These observations are in agreement with the studies that demonstrated that unlike in other species renal vasoconstriction to ET-1 in the rat was mediated by ET\textsubscript{A} (8, 27) and/or ET\textsubscript{B} receptors (28), especially the ET\textsubscript{B2} subtype. In the present study, attenuation of medullary vasodilation by BQ-788 demonstrates that in the rat, activation of ET\textsubscript{B} receptors is involved not only in ET-1-induced cortical vasoconstriction but also in medullary vasodilation. Unlike BMS-182874, BQ-788 did not affect basal RVR, suggesting that the role of ET\textsubscript{B} receptors in renal vasodilation only assumes significance when RBF is profoundly reduced as may occur in disease or following administration of agonists. After combined blockade of ET\textsubscript{A} and ET\textsubscript{B} receptors with permissible doses of BMS-182874 and BQ-788, the effects of ET-1 on blood pressure and renal hemodynamics were not completely abolished, suggesting incomplete blockade of ET receptors or the presence of another receptor. However, this notion is difficult to resolve as the resultant net effect of antagonism of ET\textsubscript{B1} receptor-mediated vasodilation or ET\textsubscript{B2} receptor-mediated vasoconstriction by BQ-788 is unknown. Moreover, the selectivity of BQ-788 or other available ET\textsubscript{B} antagonists for the ET\textsubscript{B1} vs. ET\textsubscript{B2} receptor is unknown.

With respect to the signaling mechanisms associated with the renal hemodynamic effects of ET-1, NO is a major mediator. Thus L-NAME, an inhibitor of NO synthesis, enhanced the effects of ET-1 on MBP, CBF as well as total RBF, thus amplifying ET-1-induced increase in RVR. The enhancement by L-NAME of the cortical vasoconstriction and reduction in total RBF to ET-1 is consistent with removal of the tonic inhibitory influence of endogenous NO. In contrast, L-NAME inhibited ET-1-induced medullary perfusion, suggesting that the increase in medullary perfusion is due to an ET\textsubscript{B}-linked increase in NO production.

ET-1 activates phospholipases and expresses a COX component causing the release of PGH\textsubscript{2} from vascular tissues (3) and PGE\textsubscript{2} in the medulla (38). These observations corroborate the data from our previous study that indomethacin inhibited ET-1-induced renal vasoconstriction (23). In the present study, indomethacin inhibited both total renal and cortical vasoconstriction as well as medullary vasodilation by ET-1, suggesting that vasoconstrictor prostanoids, e.g., PGH\textsubscript{2}, contribute to total renal and cortical vasoconstriction, whereas vasodilator prostanoids, e.g., PGE\textsubscript{2} and PGI\textsubscript{2}, contribute to medullary vasodilation. These observations are consistent with data from other laboratories that the medullary circulation is under tonic vasodilator influence of PGs (26, 30). However, the effects of
indomethacin on MBP and CBF are not definitive because we observed no change in MBP and only a slight decrease in CBF, whereas others reported that MBP and CBF were unchanged (26) or increased (30). The reduction by indomethacin of the effects of ET-1 on CBF and RBF in the present study suggests that vasoconstrictor prostanoids and/or prostanoid-like compounds contribute to cortical and whole kidney perfusion. The net attenuation of ET-1-induced increase in RVR therefore suggests that regional increase in vasodilator PGs in the medulla by ET-1 is not enough to override the increase in vasoconstrictor PGs elsewhere in the kidney. In assessing whether COX-derived eicosanoids contribute to ET-1 actions via ETₐ or ETₐ receptors, our data were surprising in that indomethacin enhanced the hemodynamic effects of ET-3, an ETₐ-selective agonist, in contrast to its inhibition of the hemodynamic effects of ET-1, a nonselective agonist. We interpret this observation to mean that there is a differential involvement of vasoconstrictor vs. vasodilator prostanoids in the contrictor effects of ET-1 vs. ET-3. Taken together, the different observations with indomethacin support the notion by Walder and associates (36) that the role of prostanoids on ET-1 in vivo is complex, being “more complex than one of simple physiological antagonism or potentiation at the level of the vascular smooth muscle.”

Apart from prostanoids, ET-1 is also capable of metabolizing AA to CYP450-derived metabolites, which exert profound effects on the kidney (19). These metabolites, notably, 20-HETE, a ω/ω-1-hydroxylase product, and 5,6-epoxyeicosatrienoic acid, an epoxygenase product, interact with COX to yield PG endoperoxide analogs that may account for their vascular effects (21, 22).

Table 3. Effects of L-NAME, DBDD, or the combination on changes in cortical blood flow, medullary blood flow, and mean arterial blood pressure alone or in rats that received bolus intravenous injection of ET-1

<table>
<thead>
<tr>
<th>ET-1, pmol/kg</th>
<th>L-NAME, 5 mg/kg iv</th>
<th>DBDD, 2.5 mg/kg 0.1h iv</th>
<th>L-NAME/DBDD, n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical blood flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10 ± 4</td>
<td>-62 ± 12*</td>
<td>37 ± 4*</td>
</tr>
<tr>
<td>40</td>
<td>-82 ± 10</td>
<td>-113 ± 11*</td>
<td>-48 ± 15*</td>
</tr>
<tr>
<td>100</td>
<td>-146 ± 35</td>
<td>-199 ± 29*</td>
<td>-83 ± 15*</td>
</tr>
</tbody>
</table>

Medullary blood flow

| 0 | 4 ± 2 | -29 ± 8* | 29 ± 8* | -18 ± 4†|
| 40 | 12 ± 4 | -6 ± 4* | -9 ± 8* | 1 ± 3† |
| 100 | 20 ± 6 | -19 ± 5* | -12 ± 9* | -8 ± 5† |

Mean arterial blood pressure

| 0 | 2 ± 2 | 31 ± 6* | -4 ± 4 | 21 ± 4† |
| 40 | 13 ± 4 | 25 ± 3* | 7 ± 3* | 2 ± 5† |
| 100 | 20 ± 8 | 33 ± 4* | 11 ± 4 | 5 ± 8† |

Values are means ± SE; n = no. rats studied. The effects of the agents alone (pmol/kg) are also presented. Control rats received 50% ethanol (vehicle). L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05 vs. control †P < 0.05 L-NAME/DBDD vs. DBDD.
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In our previous study, we used DBDD and CoCl₂, which inhibited P450 enzymes by different mechanisms to demonstrate that 20-HETE is a major contributor to the renal effects of ET-1 (24). In the same study, we demonstrated that in the euvolemic anesthetized rat, DBDD inhibited 20-HETE production by about 70%. Moreover, DBDD was 25 times less potent on renal microsomal conversion of [14C]AA to epoxides (37). In an earlier study (23), we demonstrated that ET-1-induced renal vasoconstriction was accompanied by a fourfold increase in the release of 20-HETE and inhibition of 20-HETE production blunted ET-1-induced vasoconstriction and 20-HETE release. The present study demonstrates that miconazole, an inhibitor of CYP450-dependent epoxygenase activity, which generates epoxyeicosatrienoic acids, was without effect on the changes in hemodynamics by ET-1. However, DBDD, an inhibitor of 20-HETE production, attenuated ET-1-induced reductions in CBF and RBF, effects that are consistent with the established role of 20-HETE as a vasoconstrictor of renal vasculature. However, we did not expect DBDD treatment to attenuate the increase in medullary perfusion by ET-1. Because ET-1-induced increase in NO was suggested to account for the increase by ET-1 in MBF, this observation suggests a unique interaction between NO and CYP450 enzymes. In a recent study on NO-CYP450 interaction in the kidney, we presented evidence that NO exerts a tonic inhibitory influence on CYP450 enzymes as inhibition of NO synthase uncovered a major interaction in the kidney, we presented evidence that 20-HETE is a major contributor to renal effects consequent to inhibition of NO donors were blunted by inhibition of 20-HETE. This provocative concept must be subjected to rigorous testing in order for future studies to be validated. However, this study and others indicate that there is a unique interaction between NO and 20-HETE. This is further supported by the observation that in rats treated with l-NAME and DBDD, basal changes in blood pressure and renal hemodynamics were less pronounced than that obtained in rats treated with l-NAME alone. These observations further confirm our previous observation that CYP450-derived mediator(s) contribute to renal effects consequent to inhibition of NO production (25). In addition, hemodynamic responses to ET-1 in rats treated with l-NAME and DBDD showed that DBDD attenuated the effects of l-NAME on ET-1. The situation is more complex in the medullary circulation in which one would have expected an additive inhibition of ET-1-induced medullary vasodilation by l-NAME and DBDD. Further studies are clearly needed to evaluate this unique interaction.

In conclusion, we have presented evidence that ET-1 activation of ET₂ and ET₃ receptors elicited disparate effects on the kidney causing ET₂- and ET₃-mediated vasoconstriction in the cortex and the whole kidney and ET₃-mediated increase in MBF. ET-1-induced release of NO and vasodilator prostanooids in the medulla contributed to vasodilation, whereas 20-HETE, an eicosanoid generated via CYP450 monoxygenase, contributed to the renal hemodynamic effects of ET-1 in the cortex and medulla, mediating vasoconstriction in the cortex and preventing full vasodilator expression to NO in the medulla.

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