Cytochrome P450 and Cyclooxygenase Metabolites Contribute to the Endothelin-1 Afferent Arteriolar Vasoconstrictor and Calcium Responses

John D. Imig, Bao Thang Pham, Elizabeth A. LeBlanc, K. Malla Reddy, John R. Falck, Edward W. Inscho

Abstract—Arachidonic acid metabolites contribute to the endothelin-1 (ET-1)–induced decrease in renal blood flow, but the vascular sites of action remain unknown. Experiments performed in vitro used the isolated juxtamedullary nephron preparation combined with videomicroscopy. The response of afferent arterioles to ET-1 was determined before and after cytochrome P450 (CYP450) or cyclooxygenase (COX) inhibition. Afferent arteriolar diameter averaged 20 ± 1 μm (n = 17) at a renal perfusion pressure of 100 mm Hg. Superfusion with 0.001 to 10 nmol/L ET-1 caused a graded decrease in diameter of the afferent arteriole. Vessel diameter decreased by 30 ± 2% and 41 ± 2% in response to 1 and 10 nmol/L ET-1, respectively. The afferent arteriolar response to ET-1 was significantly attenuated during administration of the CYP450 hydroxylase inhibitor N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS), such that afferent arteriolar diameter decreased by 19 ± 3% and 22 ± 3% in response to 1 and 10 nmol/L ET-1, respectively. COX inhibition also greatly attenuated the vasoconstriction elicited by ET-1, whereas the CYP450 epoxygenase inhibitor N-methylsulfonyl-6-(2-proparglyoxyphenyl) hexanamide enhanced the ET-1–mediated vascular response. Additional studies were performed using freshly isolated smooth muscle cells prepared from preglomerular microvessels. Renal microvascular smooth muscle cells were loaded with the calcium-sensitive dye fura 2 and studied by use of single-cell fluorescence microscopy. Basal renal microvascular smooth muscle cell [Ca^{2+}]i averaged 95 ± 3 nmol/L (n = 42). ET-1 (10 nmol/L) increased microvascular smooth muscle cell [Ca^{2+}]i to a peak value of 731 ± 75 nmol/L before stabilizing at 136 ± 8 nmol/L. Administration of DDMS or the COX inhibitor indomethacin significantly attenuated the renal microvascular smooth muscle cell calcium response to ET-1. These data demonstrate that CYP450 hydroxylase and COX arachidonic acid metabolites contribute importantly to the afferent arteriolar diameter and renal microvascular smooth muscle cell calcium responses elicited by ET-1. (Hypertension. 2000;35[part 2]:307-312.)

Key Words: endothelin-1 ■ renal hemodynamics ■ cytochrome P450 ■ cyclooxygenase ■ cytosolic calcium ■ microcirculation

Endothelin-1 (ET-1) is a 21-amino-acid peptide synthesized by endothelial cells that acts as a potent vasoconstrictor on vascular smooth muscle cells.1–3 Elevations in circulating and tissue ET-1 levels have been implicated in a number of pathological states, including acute renal failure, cyclosporine nephrotoxicity, and hypertension.4–6 The ET-1 effects on the renal circulation are consistent with the view that the peptide plays a crucial role in maintaining fluid and electrolyte homeostasis. Intrarenal infusion of ET-1 decreases renal blood flow and glomerular filtration rate while increasing sodium excretion.5,7 Previous studies have demonstrated that ET-1 constricts the afferent arteriole9–13; however, the preglomerular vascular smooth muscle cellular signaling mechanisms responsible are not well understood. ET-1 activates G proteins that in turn stimulate phospholipase C and subsequent release of intracellular calcium ions ([Ca^{2+}]), leading to vascular smooth muscle cell contraction.14 In addition, ET-1 binding to ETα and ETβ receptors stimulates phospholipase A₂ activation, arachidonic acid release, and production of cytochrome P450 (CYP450) and cyclooxygenase (COX) metabolites from the kidney, endothelial cells, and vascular smooth muscle cells.1,14–16 CYP450 and COX metabolites contribute to the ET-1–induced decrease in renal blood flow, but the vascular sites of action and involvement in cellular signaling mechanisms remain undefined.

The present studies determined the contribution of CYP450 and COX enzymatic pathways in mediating the effects of ET-1 on afferent arteriolar diameter using the in vitro perfused juxtamedullary nephron preparation. Experi-
ments were also performed using pregglomerular smooth muscle cells freshly isolated from interlobular arteries and afferent arterioles. The relative contributions of the CYP450 and COX pathways to the ET-1–mediated increase in renal microvascular smooth muscle [Ca\(^{2+}\)], were determined. The results of these studies indicate that CYP450 and COX metabolites are importantly involved as second messengers in the afferent arteriolar vasoconstriction and elevation in [Ca\(^{2+}\)], elicited by ET-1.

**Methods**

**Vascular Preparation**

Experiments were performed on male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass) weighing an average of 350 to 450 g. All experiments were approved by the Tulane University Animal Care and Use Committee. The rats were anesthetized with sodium pentobarbital (40 mg/kg body wt IP), the right carotid artery was cannulated, and a midline abdominal incision was made. The right renal artery of the kidney was cannulated via the superior mesenteric artery, and the kidney was immediately perfused with Tyrode’s solution containing 6% albumin and a mixture of L- amino acids.\(^{17}\)

Blood was collected through the carotid artery cannula into a heparinized syringe (2000 U). Erythrocytes were separated from plasma and leukocytes by centrifugation, as previously described.\(^{17}\) The erythrocytes were resuspended in Tyrode’s solution containing 6% albumin to yield a hematocrit of 20%. The reconstituted blood solution was filtered and stirred continuously in a closed reservoir that was pressurized by a 95% O\(_2\)/5% CO\(_2\) tank. The kidney was removed and maintained in an organ chamber at room temperature throughout the isolation and dissection procedure. The juxtamедullary microvasculature was isolated for study as previously described.\(^{17}\) The Tyrode’s solution was then replaced with reconstituted blood, and renal artery perfusion pressure, measured at the tip of the cannula, was set to 100 mm Hg. The organ chamber was warmed, and the tissue surface was continuously superfused with Tyrode’s solution containing 1% albumin at 37°C. After a 20-minute equilibration period, an afferent arteriole was chosen for study, and baseline diameter was measured with videomicroscopy techniques as previously described.\(^{17}\) Vessel diameter was measured with a calibrated image-shearing monitor, which yielded reproducible measurements within 0.5 \(\mu m\).

**Involvement of CYP450 and COX Pathways in the Afferent Arteriolar Vasoconstrictor Response to ET-1**

After the equilibration period, baseline diameter measurements of the afferent arteriole were made. The arteriole was subsequently exposed to increasing concentrations of ET-1 (0.001 to 10 nmol/L), and diameter changes were monitored for 3 minutes at each concentration. After the control concentration-response profile to ET-1 was obtained, selective metabolic inhibitors were added to the perfusion and superfusion solutions. The concentration-response profile to ET-1 was repeated in the presence of the CYP450 hydroxylase inhibitor \(N\)-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS, 25 \(\mu mol/L\)),\(^{18,19}\) the CYP450 epoxygenase inhibitor \(N\)-methylsulfonyl-6-(2-proparglyoxyphenyl) hexanamide (MS-PPOH, 50 \(\mu mol/L\)),\(^{18,19}\) or the COX inhibitor indomethacin (10 \(\mu mol/L\)).\(^{17}\) The kidney was exposed to the inhibitors for a minimum of 20 minutes. Inhibitors were administered only if vessel diameter returned to within 5% of control diameter after removal of ET-1. Steady-state diameter was attained by the end of the second minute, and the average diameter of the third minute of each treatment period was used for statistical analysis. No differences in the repeat dose-response to ET-1 (n=4) were observed in time-control experiments.

**Renal Microvascular Smooth Muscle Cell Isolation**

Male Sprague-Dawley rats were anesthetized with sodium pentobarbital (40 mg/kg body wt IP), and the abdominal cavity was exposed to permit cannulation of the abdominal aorta via the superior mesenteric artery. Ligatures were placed around the abdominal aorta at sites proximal and distal to the left and right renal arteries, respectively. The kidneys were cleared of blood by perfusion of the isolated aortic segment with an ice-cold, low-calcium physiological salt solution (low-calcium PSS; pH 7.35) of the following composition (in mmol/L): NaCl 125, KCl 5.0, MgCl\(_2\) 1.0, glucose 10.0, HEPES 20.0, and CaCl\(_2\) 0.1, plus 6% BSA. After the kidneys were rinsed of blood, the perfusate was changed to a similar solution containing 1% Evans blue dye in low-calcium PSS.

The kidneys were resected from the animal and decapsulated, and the renal medullary tissue was removed. The cortical tissue was washed several times with ice-cold low-calcium PSS and enzymatically digested to obtain renal microvascular smooth muscle cells as previously described.\(^{20}\) The dispersed renal microvascular smooth muscle cells were gently resuspended in 1.0 mL Dulbecco’s minimum essential medium supplemented with 20% FCS, 100 U/mL penicillin, and 200 \(\mu g/mL\) streptomycin. Renal microvascular cell suspensions were stored on ice until use.

**Calcium Measurements in Single Renal Microvascular Smooth Muscle Cells**

Experiments were performed with a standard microscope-based fluorescence spectrophotometry system. The excitation wavelengths were set at 340 and 380 nm, and the emitted light was collected at 510±20 nm. Measurements of fluorescence intensity were collected at 5 data points per second, and the data were collected and analyzed with the aid of Photon Technology International software. The fluorescence data were calibrated as previously described.\(^{21}\)

Measurement of [Ca\(^{2+}\)]\(_i\) in single microvascular smooth muscle cells was performed as previously described.\(^{20}\) Suspensions of freshly isolated renal microvascular cells loaded with the calcium-sensitive fluorescent probe fura 2 acetoxymethyl ester (fura 2-AM; 4.0 \(\mu mol/L\)). An aliquot of cell suspension was transferred to the perfusion chamber and mounted to the stage of a Nikon Diaphot inverted microscope. The cells were continuously superfused (1.3 mL/min) with a 1.8-mmol/L-calcium PSS of the following composition (mmol/L): NaCl 125, KCl 5.0, MgCl\(_2\) 1.0, glucose 10.0, HEPES 20.0, and CaCl\(_2\) 1.8, plus 0.111 g/L BSA. For each experiment, a single microvascular cell was isolated in the optical field by positioning the adjustable sampling window directly over the cell of interest. All fluorescence measurements were obtained with background subtraction, and a new coverslip of cells was used for each experiment.

**Involvement of CYP450 and COX Pathways in the Renal Microvascular Smooth Muscle Cell Calcium Response to ET-1**

The effects of ET-1 on [Ca\(^{2+}\)]\(_i\) were determined by exposing single cells to PSS containing ET-1 concentrations of 1 to 100 nmol/L. ET-1–mediated responses at each concentration were evaluated by determining the average magnitude of the peak and steady-state [Ca\(^{2+}\)]\(_i\) achieved. Peak responses were defined as the maximum agonist-induced [Ca\(^{2+}\)]\(_i\) attained during the 200 seconds of agonist administration. Steady-state responses were obtained by calculating the average [Ca\(^{2+}\)]\(_i\), over the last 50 seconds of agonist administration. The role of CYP450 and COX pathways in the ET-1–mediated [Ca\(^{2+}\)]\(_i\) response was assessed by adding the CYP450 hydroxylase inhibitor DDMS (25 \(\mu mol/L\)), the CYP450 epoxygenase inhibitor MS-PPOH (50 \(\mu mol/L\)), or the COX inhibitor indomethacin (10 \(\mu mol/L\)) to the PSS solution. Time-control experiments were performed in which the ET-1 vascular smooth muscle cell [Ca\(^{2+}\)]\(_i\) response was determined after addition of the vehicle (0.01% ethanol) to the superfusion solution. Addition of vehicle to the superfusate had no effect on the [Ca\(^{2+}\)]\(_i\) response elicited by ET-1 (n=10 cells from 3 dispersions).
Statistics
Data are presented as mean±SEM. The significance of differences in mean afferent arteriolar diameter values between groups was evaluated with a 2-way ANOVA for repeated measures followed by Duncan’s multiple-range test. Differences within groups of renal microvascular smooth muscle cell [Ca^{2+}] values were analyzed by ANOVA for repeated measures. Differences between groups of cell [Ca^{2+}] values were analyzed by 1-way ANOVA followed by Newman-Keuls multiple-range test. A value of \( p < 0.05 \) was considered statistically significant.

Results
Involvement of CYP450 and COX Pathways in the Afferent Arteriolar Vasoconstrictor Response to ET-1
Figure 1 depicts the effect of CYP450 hydroxylase inhibition on the afferent arteriolar vasoconstrictor response to ET-1. Afferent arteriolar diameter decreased after superfusion with ET-1 and reached a steady-state diameter by the end of the second minute. The CYP450 hydroxylase inhibitor DDMS attenuated the afferent arteriolar diameter decrease to ET-1. Administration of 0.1, 1, and 10 nmol/L ET-1 decreased afferent arteriolar diameter by 20±4%, 35±5%, and 43±4%, respectively, under control conditions and by 12±2%, 19±3%, and 22±3%, respectively, in the presence of CYP450 hydroxylase inhibition. Alternatively, the afferent arteriolar vasoconstrictor response to ET-1 was significantly (\( p < 0.05 \)) enhanced by the CYP450 epoxygenase inhibitor MS-PPOH. Afferent arteriolar diameter averaged 19.6±0.2 \( \mu m \) (n=4) and decreased by 4±3%, 8±3%, 17±3%, 23±5%, and 37±3% in response to 0.001, 0.01, 0.1, 1, and 10 nmol/L ET-1, respectively. In the presence of 50 \( \mu mol/L \) MS-PPOH, afferent arteriolar diameter averaged 18.5±0.4 \( \mu m \) and decreased by 13±4%, 20±3%, 26±2%, 28±3%, and 35±4% in response to 0.001 to 10 nmol/L ET-1, respectively.

The effects of the COX inhibitor indomethacin on ET-1–induced afferent arteriolar vasoconstriction is depicted in Figure 2. Control afferent arteriolar diameter averaged 21.0±1.6 \( \mu m \) and decreased by 39±4% to 12.7±1.3 \( \mu m \) during superfusion with 10 nmol/L ET-1. COX inhibition significantly attenuated the afferent arteriolar vasoconstrictor response to ET-1. In the presence of indomethacin, afferent arteriolar diameter averaged 20.5±1.8 \( \mu m \) and decreased by 10±2% to 18.4±1.7 \( \mu m \) during superfusion with 10 nmol/L ET-1.

Involvement of CYP450 and COX Pathways in the Renal Microvascular Smooth Muscle Cell Calcium Response to ET-1
A total of 144 single renal microvascular smooth muscle cells prepared from 45 tissue dispersions were examined in the present study. The baseline [Ca^{2+}] averaged 89±2 nmol/L (n=144) and was not significantly altered by administration of DDMS (95±4 nmol/L; n=34 cells) or indomethacin (95±6 nmol/L; n=39 cells) for 300 seconds.

Figure 3 depicts representative traces demonstrating the effect of the CYP450 hydroxylase inhibitor DDMS and the COX inhibitor indomethacin on the renal microvascular smooth muscle cell [Ca^{2+}], elicited by 100 nmol/L ET-1. As shown in Figure 3, ET-1 caused a rapid increase in [Ca^{2+}], resulting in an initial peak value that was followed by a recovery to a steady-state concentration. The steady-state...
elevation in \([\text{Ca}^{2+}]\), remained for several hundred seconds after the removal of ET-1 from the superfusion solution. Unlike other vasoconstrictor agents that we have shown to initiate the rise in \([\text{Ca}^{2+}]\) after a very predictable period of exposure, the time between the start of ET-1 superfusion and the initial rise in \([\text{Ca}^{2+}]\) was quite variable. Figure 3, top, demonstrates the effect of DDMS administration on the \([\text{Ca}^{2+}]\) response to 100 nmol/L ET-1 superfusion. In the presence of DDMS, ET-1 elicited a peak \([\text{Ca}^{2+}]\) response similar to that in control conditions, but the steady-state response was completely eliminated. Likewise, Figure 3, bottom, demonstrates that the COX inhibitor indomethacin did not alter the peak response and greatly attenuated the steady-state response to 100 nmol/L ET-1.

ET-1 concentration-response experiments were performed in the absence and presence of CYP450 or COX inhibition. As shown in Figure 4, the renal microvascular smooth muscle cell \([\text{Ca}^{2+}]\) response to ET-1 demonstrates a steep dose-response effect. ET-1 concentrations of 1 (n=10 cells from 5 dispersions), 10 (29 cells from 16 dispersions), and 100 (42 cells from 20 dispersions) nmol/L elicited peak elevations in \([\text{Ca}^{2+}]\), of 91±60, 604±58, and 636±75 nmol/L, respectively (top). After administration of the CYP450 inhibitor DDMS, the peak response to 1 and 10 nmol/L ET-1 was attenuated by 88% and 75%, respectively, but the response to 100 nmol/L ET-1 was not significantly altered. In contrast, COX inhibition with indomethacin had no effect on the peak \([\text{Ca}^{2+}]\) response elicited by ET-1.

The effect of CYP450 and COX inhibition on the steady-state renal microvascular smooth muscle cell \([\text{Ca}^{2+}]\), response to ET-1 is presented in Figure 4, bottom. Superfusion of renal microvascular smooth muscle cells with 1, 10, and 100 nmol/L ET-1 resulted in sustained elevations in \([\text{Ca}^{2+}]\), of 5±2, 40±10, and 41±7 nmol/L, respectively. The CYP450 hydroxylase inhibitor DDMS significantly attenuated the steady-state response to 10 and 100 nmol/L ET-1 by 58% and 88%, respectively. Administration of the COX inhibitor indomethacin had no effect on the plateau response to 1 and 10 nmol/L ET-1 but greatly attenuated the plateau response to 100 nmol/L ET-1, by 93%.

Additional experiments were performed to determine whether the CYP450 epoxygenase inhibitor MS-PPOH had an effect on the renal microvascular smooth muscle calcium response elicited by ET-1. ET-1 (100 nmol/L) increased \([\text{Ca}^{2+}]\), from a control value of 65±4 to a peak of 494±80 nmol/L, followed by a decrease to a steady-state average of 97±6 nmol/L (n=8 cells from 3 dispersions). MS-PPOH did not significantly alter the baseline \([\text{Ca}^{2+}]\), which averaged 61±5 nmol/L before and 64±8 nmol/L after administration for 300 seconds (n=7 cells from 3 dispersions). CYP450 epoxygenase inhibition had no effect on the \([\text{Ca}^{2+}]\) response to 100 nmol/L ET-1. ET-1 increased \([\text{Ca}^{2+}]\), to a peak value of 465±52, followed by a decline to a steady state of 94±8 nmol/L in the presence of MS-PPOH.

**Discussion**

The contribution of metabolites of the COX and CYP450 pathways to the afferent arteriolar diameter and calcium...
responses to ET-1 were determined. Inhibition of CYP450 hydroxylase or COX arachidonic acid metabolic pathways attenuated the afferent arteriolar vasoconstriction to ET-1. Conversely, ET-1–mediated vasoconstriction was enhanced in the presence of the CYP450 epoxygenase inhibitor MS-PPOH. In other experiments, the renal microvascular smooth muscle [Ca\(^{2+}\)] response to ET-1 was determined in the presence of CYP450 or COX inhibitors. ET-1 resulted in a biphasic increase in [Ca\(^{2+}\)], which had an initial peak response that gradually decreased to a sustained elevation during the late phase of the response. The renal microvascular smooth muscle [Ca\(^{2+}\)], response evoked by ET-1 was attenuated by COX or CYP450 hydroxylase inhibition but was not altered by CYP450 epoxygenase inhibition. These data suggest that activation and production of COX and CYP450 hydroxylase metabolites by renal microvascular smooth muscle contributes to the elevation in [Ca\(^{2+}\)], and afferent arteriolar vasoconstriction in response to ET-1.

ET-1 is an endothelium-derived peptide that exerts a powerful vascular influence on the renal circulation, resulting in decreases in renal blood flow and glomerular filtration.\(^3,7,9\) Actions of ET-1, via ETA and ETB receptors, include phospholipase A\(_2\) stimulation and the resultant increase in arachidonic acid–derived mediators.\(^1,7,15,21\) Previous studies have investigated the involvement of CYP450 hydroxylase and COX metabolites in the renal vasoconstrictor response to ET-1.\(^7,9,10,16,22\) Oyekan et al, using in vitro and in vivo techniques, have shown that the CYP450 hydroxylase pathway contributes to the ET-1–evoked decrease in renal blood flow, but the role of the COX pathway appears to be complex.\(^7,16,23\) In the isolated perfused kidney, COX inhibition attenuated the renal vasoconstrictor response to ET-1,\(^16\) but when studied in vivo, the COX inhibitor indomethacin potentiated the decrease in glomerular filtration rate elicited by ET-1.\(^1\) These paradoxical findings demonstrating COX metabolites as positive and negative contributors to the ET-1–mediated vasoconstriction are not limited to the renal vasculature and may reflect experimental conditions or species variation.\(^3,14,24\) In the present in vitro study, the CYP450 hydroxylase inhibitor DDMS and the COX inhibitor indomethacin attenuated the renal microvascular vasoconstrictor response to ET-1.

The possible involvement of the CYP450 epoxygenase pathway in the ET-1–mediated afferent arteriolar vasoconstrictor and vascular smooth muscle [Ca\(^{2+}\)] responses were also evaluated. Inhibition of the CYP450 epoxygenase pathway with MS-PPOH enhanced the vasoconstrictor response to ET-1. Because MS-PPOH selectively inhibits the epoxygenase pathway, these studies indicate that vasoconstrictor actions of ET-1. This finding conflicts with an in vivo study that showed that CYP450 epoxygenase inhibition with clotrimazole had no effect on the decrease in renal blood flow and glomerular filtration rate induced by ET-1.\(^7\) The opposing action of the CYP450 pathway does not involve calcium regulation at the level of the renal microvascular smooth muscle cell, because MS-PPOH did not alter the [Ca\(^{2+}\)] response to ET-1. Thus, the results of the present study suggest that ET-1 increases the production of an endothelium-derived EET that opposes the preglomerular vasoconstrictor activity of the peptide.

ET-1 vasoconstriction is biphasic and is associated with a rapid decrease in vessel caliber that is sustained for prolonged periods. Second messengers responsible for the ET-1–mediated vascular response include phospholipase C activation and subsequent calcium mobilization from intracellular stores, as well as phospholipase A\(_2\) stimulation, resulting in production of arachidonic acid metabolites.\(^1,14,21,23\) In freshly isolated smooth muscle cells from large-caliber renal vessels, Gordinien et al\(^25\) demonstrated that ET-1–induced mobilization of intracellular calcium was accompanied by membrane depolarization. ET-1 also stimulated Ca\(^{2+}\)–activated K\(^+\) channels in renal vascular smooth muscle cells.\(^25\) Activation of these cellular signaling messengers has been demonstrated in smooth muscle cells isolated from a number of vascular beds.\(^1,14\) In the present study, CYP450 hydroxylase inhibition attenuated the initial elevation in [Ca\(^{2+}\)], in response to 1 and 10 nmol/L ET-1. In addition, the sustained increase in [Ca\(^{2+}\)], evoked by 100 nmol/L ET-1 was blunted by the presence of DDMS or indomethacin. These data demonstrate that vascular smooth muscle–derived arachidonic acid metabolites contribute importantly to the ET-1–mediated elevation in [Ca\(^{2+}\)].

The ability of CYP450 and COX inhibition to attenuate the increase in renal microvascular smooth muscle [Ca\(^{2+}\)], is consistent with the actions of metabolites produced by these pathways. A major product of the CYP450 hydroxylase pathway is the endogenous renal microvascular vasoconstrictor metabolite 20-HETE.\(^26,27\) 20-HETE vasoconstriction of the afferent arteriole is the result of Ca\(^{2+}\)–activated K\(^+\) channel inhibition, resulting in membrane depolarization and subsequent activation of L-type Ca\(^{2+}\) channels.\(^27,28\) Afferent arteriolar vasoconstriction in response to the COX metabolite thromboxane (TX) is also dependent on L-type Ca\(^{2+}\) channel activation.\(^29\) Experiments conducted in our laboratory have determined that ET-1 increases [Ca\(^{2+}\)], in freshly isolated renal microvascular smooth muscle cells primarily by activation of ETA receptors.\(^30\) In addition, the peak responses to ET-1 are generated primarily by mobilization of Ca\(^{2+}\) from intracellular stores, and the sustained elevations in [Ca\(^{2+}\)], are the result of calcium influx.\(^30\) CYP450 hydroxylase inhibition significantly attenuated the peak response at the lower doses and at higher doses of ET-1 diminished the sustained [Ca\(^{2+}\)] phase, which supports the concept that 20-HETE produced by the vascular smooth muscle cell contributes to ET-1–evoked preglomerular vasoconstriction. In contrast, indomethacin did not alter the peak response but significantly attenuated the sustained [Ca\(^{2+}\)] response to 100 nmol/L ET-1. These results suggest that an endothelium-derived and a vascular smooth muscle–derived COX metabolite contribute to the ET-1–mediated afferent arteriolar response, because the ability of COX inhibition to attenuate the response was greater in the in vitro juxtaglomerular preparation with an intact endothelium than in the freshly isolated vascular smooth muscle cells.

Although the results of the present study clearly demonstrate contributions of CYP450 hydroxylase and COX metabolites in the renal microvascular response to ET-1, the identification of the metabolites involved remains unresolved. In the isolated perfused kidney, ET-1 increases the release of 20-HETE into the
renal effluent. ET-1 also has the capacity to increase production of vasodilator prostaglandins (PGs), PGH2, and PGE2, as well as a COX metabolite, TXA2.1,10,14,21 Interestingly, the CYP450 hydroxylase metabolite 20-HETE can be transformed by COX to a vasoconstrictor PGH2 analogue, 20-OH PGH2.15,32 Consistent with the hypothesis that 20-HETE conversion to a TX/PGH2 (TP) receptor agonist contributes to its action, the aortic vasoconstriction in response to 20-HETE can be blocked by COX inhibition or TP receptor antagonism.24 In contrast, ET-1–mediated vasoconstriction in the kidney has been demonstrated to be inhibited by the COX inhibitor indomethacin but was not altered by TP receptor antagonism.10 The exact nature of the COX and CYP450 arachidonic acid metabolites involved in the afferent arteriolar diameter and calcium response to ET-1 remains to be defined. Nevertheless, the results of the present study demonstrate that the COX and CYP450 hydroxylase metabolites that contribute to the ET-1–evoked vasoconstriction are to a significant degree generated by the renal microvascular smooth muscle cells, because either COX or CYP450 inhibition attenuated the [Ca2+]i response elicited by ET-1.

In summary, the CYP450 hydroxylase inhibitor DDMS and the COX inhibitor indomethacin attenuated the decrease in afferent arteriolar diameter to ET-1. The afferent arteriolar vasoconstrictor response to ET-1 was enhanced by CYP450 epoxygenase inhibition. In addition, inhibition of CYP450 hydroxylase or COX pathways attenuated the ET-1–stimulated increase in [Ca2+]i, in freshly isolated preglomerular smooth muscle cells. These data demonstrate that arachidonic acid metabolites of the CYP450 and COX pathways contribute importantly to the afferent arteriolar vasoconstrictor and smooth muscle calcium response to ET-1.

Acknowledgments
This work was supported by grants DK-38226, DK-44628, and HL-59699 from the National Institutes of Health and AHA 95001370 from the American Heart Association. Dr Inscho is an Established Investigator of the American Heart Association. The authors thank Anthony Cook, Paul Deichmann, and Shi Shen Yiu for excellent technical assistance with these experiments.

References
Cytochrome P450 and Cyclooxygenase Metabolites Contribute to the Endothelin-1 Afferent Arteriolar Vasoconstrictor and Calcium Responses

John D. Imig, Bao Thang Pham, Elizabeth A. LeBlanc, K. Malla Reddy, John R. Falck and Edward W. Inscho

Hypertension. 2000;35:307-312
doi: 10.1161/01.HYP.35.1.307

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/35/1/307

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org//subscriptions/