Cytochrome P-450–Dependent Vasodilator Responses to Arachidonic Acid in the Isolated, Perfused Kidney of the Rat

A.O. Oyekan, J.C. McGiff, and J. Quilley

Pretreatment of phenylephrine (0.5 μM)-preconstricted, isolated perfused kidneys of the male rat with indomethacin (2.8 μM) or BM 13.177 (20 μM) abolished the vasconstrictor response to arachidonic acid (AA), uncovering a vasodilator response. BW 755C (25 μM), a dual cyclooxygenase/lipoxygenase inhibitor, did not modify the vasodilator effect of AA, whereas 5,8,11,14-eicosatetraynoic acid (10 μM), which blocks all pathways of AA metabolism, abolished AA-induced vasodilation, thus suggesting the involvement of nonlipoxygenase AA metabolites. Clotrimazole (0.7 μM) and 7-ethoxyresorufin (1 μM), both considered to be specific inhibitors of the cytochrome P-450 monoxygenase enzymes, inhibited the vasodilator effect, suggesting that AA-induced renal vasodilation is mediated by one or more cytochrome P-450–derived AA metabolites. None of these interventions affected the vasodilator responses to acetylcholine (100 ng) and nitroprusside (1 μg). Denudation of the endothelium with CHAPS (10 mg/l) reduced the vasodilator responses to AA, suggesting a requirement of an intact endothelium, whereas inhibition of guanylate cyclase with methylene blue (10⁻⁴ M) was without effect, suggesting that cGMP was not involved in the vasodilator response to AA. The AA-induced renal vasodilation was accompanied by the generation of biologically active material or materials released into the renal effluent, which relaxed endothelium-intact and endothelium-denuded rings of isolated aorta and mesenteric and celiac arteries of the rabbit. These results suggest that in the rat kidney, AA is metabolized by endothelial cytochrome P-450–dependent enzymes to vasodilator metabolites. (Circulation Research 1991;68:958–965)

In the renal vasculature, arachidonic acid (AA) elicits species-dependent effects, producing vasodilation in rabbit and dog kidneys1,2 but vasoconstriction in the rat kidney.3 In the dog and rabbit kidney, the vasodilation produced by AA is dependent on cyclooxygenase activity. In the rat kidney, the vasoconstrictor effect of AA is also mediated by cyclooxygenase metabolites,4,5 most likely the prostaglandin endoperoxides as the response was blocked by inhibitors of cyclooxygenase and antagonists of thromboxane A2/prostaglandin H2 receptors but not by thromboxane synthetase inhibitors. In our studies with AA in the rat kidney, we observed that after blockade of cyclooxygenase or thromboxane/ prostaglandin H2 receptors, a vasodilator response (which was blocked by SKF 525A) was uncovered.

In peripheral arteries, various vasoactive substances (acetylcholine, ATP, bradykinin, thrombin, AA, and A23187) cause relaxations that are dependent on the presence or formation of certain endothelium-derived chemical factors, possibly noncyclooxygenase AA metabolites.6–9 Some other studies implicated lipoxygenase products of AA metabolism in AA-induced relaxation of blood vessels.7,10 Evidence was later presented that the most recently discovered pathway of AA metabolism, the cytochrome (cyt) P-450–dependent mixed-function oxidase system, is localized primarily in the endothelium11 and is involved in the endothelium-dependent relaxations to AA in the pulmonary arteries of the rabbit12 and the canine coronary artery.13

The new pathway of AA metabolism, via the cyt P-450 monoxygenase system, metabolizes AA to several metabolites, namely, 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acids and the corresponding dihydroxyeicosatrienoic acids, as well as several hydroxyeicosatrienoic acids, ω- and ω-1 hydroxylation products (see Reference 13). Cyt P-450–depen-

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dent activity has been found in the cortex and medulla of the rat kidney, yielding AA metabolites with potential effects on renal function.\textsuperscript{14} These metabolites have been shown to exhibit diverse biological activities, including vasodilatation of arteries,\textsuperscript{15} vasodilatation of intestinal microcirculation,\textsuperscript{16} inhibition of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, modulation of hormone secretion, and mobilization of microsomal Ca\textsuperscript{2+} from aortic smooth muscle cells.\textsuperscript{13}

This investigation evaluates the role of the cyt
P-450 pathway in the renal vasodilator response to AA in the rat isolated kidney using several inhibitors of AA metabolism, including those of the cyt P-450 monooxygenase system.

Materials and Methods

Sodium arachidonate (Nuchek, Elysian, Minn.) was dissolved in distilled water in a stock solution (1 mg/ml) and stored under nitrogen at \(-70^\circ\text{C}\). Aliquots were thawed for each experiment and kept on ice and shielded from light during the experiment. 5,8,11,14-Eicosatetraynoic acid (ETYA) (Calbiochem Corp., San Diego, Calif.) and clotrimazole (Sigma Chemical Co., St. Louis) were stored in ethanol at \(-70^\circ\text{C}\) until use. Indomethacin (Sigma) was always prepared daily using 4.2% NaHCO\textsubscript{3} as the diluent. BW 755C (a gift from Burroughs-Wellcome, Beckenham, UK) was freshly prepared in distilled water. 7-Ethoxyresorufin (7-ER; Fierce Chemical Co., Rockford, Ill.) was stored in methanol at \(-20^\circ\text{C}\) until use. Because of its light-sensitive nature, the beaker containing the perfusate was wrapped in aluminum foil. Methylene blue (Sigma) was dissolved in distilled water as a stock concentration of \(10^{-2}\) M and stored at 4°C until use. The other drugs—phenylephrine, acetycholine, nitroprusside, scopolamine, mepyramine, methysgeride, and propranolol—were obtained from Sigma. Stock solutions were prepared using normal saline (0.9% NaCl) and kept at \(-4^\circ\text{C}\). These were appropriately diluted at the point of use in physiological salt solution (PSS). (3-[[3-Cholamidopropyl]-dimethylammonio]-1-propanesulphonate) dihydrate (CHAPS) (Aldrich Chemical Co., Milwaukee, Wis.) was always freshly prepared in PSS.

Male Wistar rats (Charles River Laboratories, Inc., Wilmington, Mass.) 10–12 weeks old (289±7 g body wt) and male New Zealand White rabbits (1.5–2.1 kg body wt) were used in this study.

Isolated Perfused Kidney In Situ

The isolated perfused kidney in situ has been described elsewhere.\textsuperscript{5} Briefly, after pentobarbital anesthesia (60 mg/kg i.p.), the right kidney was exposed by midline ventral laparotomy, the right renal artery was cannulated via the mesenteric artery, and the kidney was perfused by means of a pump (model 502S, Watson-Marlow, New Brunswick Scientific, Edison, N.J.) with warmed (37°C), oxygenated (95% O\textsubscript{2}–5% CO\textsubscript{2}) Krebs-Henseleit buffer (PSS; millimolar composition: NaCl 118, NaHCO\textsubscript{3} 25, CaCl\textsubscript{2} 1.9, MgSO\textsubscript{4} 1.19, KCl 4.75, KH\textsubscript{2}PO\textsubscript{4} 1.19, and dextrose 11.1, pH 7.2). Indomethacin (final concentration, 2.8 \muM) was routinely added to the perfusate to inhibit cyclooxgenase. In some of the studies, the thromboxane receptor antagonist BM 13.177 (20 \muM; Boehringer Mannheim, Mannheim, FRG) was added. The flow of perfusate was adjusted in each kidney to obtain a perfusion pressure of 80–120 mm Hg. The inferior vena cava was cut to allow the exit of perfusate, and animals were killed by an intracardiac injection of 10 mg pentobarbital. The perfusion pressure was monitored constantly by means of a pressure transducer (Harvard Apparatus, South Natick, Mass.) connected to a recorder (model 1246, Soltex Corp., San Fernando, Calif.). Drugs were kept on ice throughout the experiments and injected close— intra-arterially in volumes of 20 \muL. All agents used to examine the responses of AA were included in the perfusate from the start of the perfusion. Mean basal perfusion pressures among the various groups were 91–110 mm Hg for mean flow rates of 10.9–12.8 ml/min. Elevated mean perfusion pressures were 162–182 mm Hg among the various groups except the group treated with 2 \muM 7-ER, in which elevated mean perfusion pressure was 154±5 mm Hg.

Experimental Procedure

After a stable perfusion pressure of 80–120 mm Hg was attained, phenylephrine (5×10\textsuperscript{-7} M) was added to the perfusate to elevate vascular tone, resulting in a perfusion pressure of 155–195 mm Hg, and thereby amplifying vasodilator responses. Vasodilator responses to AA (1–10 \mug) were recorded after the injection of bolus doses of AA into the perfusate line just proximal to the kidney. The effects of the inhibitors (see Figure 1) BW 755C (25 \muM), ETYA (10 \muM), clotrimazole (0.7 \muM), and 7-ER (1 \muM) were evaluated by comparing the vasodilator responses to AA with those obtained when appropriate vehicles were included in the perfusate. To ascertain the selectivity of any effects obtained, responses to submaximal doses of acetylcholine (100 ng) and nitroprusside (1 \mug) were evaluated in the presence and absence of the inhibitors. Data obtained from experiments using higher concentrations of inhibitors that significantly affected the vasodilator responses of acetylcholine and nitroprusside were not included. Thus, in preliminary experiments, BW 755C (>50 \muM), clotrimazole (>1.4 \muM), and 7-ER (2 \muM) were found to reduce responses to acetylcholine and nitroprusside as well as those to AA, indicating a lack of selectivity at higher concentrations.

Effect of CHAPS on the Renovascular Response to Arachidonic Acid

CHAPS was infused to determine the endothelium requirement of the vasodilator response to AA. In our hands, the protocol reported by Bhardwaj and Moore\textsuperscript{17} involving a 30-second infusion of 4.7 mg/ml CHAPS lowered the perfusion pressure unacceptably and therefore was not used. Subsequent experiments used low doses of CHAPS (10 mg/l, final concentra-
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Acid–Induced Vasodilation in the Kidney

Methylene blue (10\textsuperscript{-4} M, n=5) was used to inhibit guanylate cyclase activity. However, its inclusion in the perfusate was found to elevate vascular tone to a similar level (162±4 mm Hg) as phenylephrine (5×10\textsuperscript{-7} M). It therefore was used in place of phenylephrine to elevate perfusion pressure. After stabilization of perfusion pressure, vasodilator responses of the kidneys to AA were examined. The effects of acetylcholine (100 ng) and nitroprusside (1 µg) also were tested. The effects of methylene blue were evaluated by comparing responses to AA, acetylcholine, and nitroprusside with those obtained in control kidneys that were phenylephrine constricted.

Superfusion Bioassay of Renal Effluent From Arachidonic Acid–Treated Kidneys

In the superfusion bioassay experiments, the biological activity of the materials generated by the kidney from AA was evaluated on isolated rings of blood vessels of the rabbit. A correlation also was sought between the renal effects of AA and the generation of these materials. For these experiments, the kidney was suspended in an organ bath so that the renal effluent superfused isolated rings of the rabbit aorta and the mesenteric and celiac arteries. Rings (3–4 mm wide) of these vessels from male New Zealand White rabbits were prepared. Care was taken during the dissection to avoid unnecessary stretching or contact of the instruments with the luminal surface of the rings to ensure the integrity of the vascular endothelium. In some experiments, the endothelium was intentionally disrupted by rubbing a cotton-tipped applicator on the luminal surface of the rings. The rings, mounted and superfused with warmed, oxygenated PSS, were equilibrated for 60–90 minutes under a resting tension of 2 g for aorta and 1.5 g for mesenteric and celiac arteries. Changes in tension were measured using model FT03C transducers (Grass Instrument Co., Quincy, Mass.) coupled to a 775BA recorder (Hewlett-Packard Co., Palo Alto, Calif.).

After the equilibration period, the vascular rings were superfused with the renal effluent. The kidney perfusate also provided the indomethacin to inhibit cyclooxygenase, as well as phenylephrine to preconstrict the vessels. In addition, a solution consisting of a mixture of ETYA (10 µM), scopolamine (3 µg/ml), propranolol (2 µg/ml), methysergide (2 µg/ml), and pyrilamine (1 µg/ml) was perfused directly over the isolated vessels from an infusion pump at a rate of 45 ml/hr to inhibit the effects of any unmetabolized AA or of vasoactive materials released from the kidney. Once a stable elevated perfusion pressure and tension were obtained in the kidney and the vessels, respectively, AA (1–10 µg) was injected into the kidney, and corresponding effects were noted on the vessels. With each preparation, AA (10 µg) was injected directly over the assay tissues, and the resultant effect on the assay tissue was used to differentiate the effects of AA injected through the kidney. These effects were evaluated on intact as well as on endothelium-denuded vessels, acetylcholine (100 ng) being used to confirm removal of the endothelium.

Expression of Data and Statistical Analysis

Vasodilation or vasoconstriction in the kidney was expressed as reduction or elevation in perfusion pressure, respectively, and expressed as absolute changes (millimeters of mercury). On the other hand, contractions of isolated rings of blood vessels elicited by phenylephrine or relaxation evoked by AA, acetylcholine, and nitroprusside were expressed in grams of tension. Dose–response curves to AA in untreated kidneys were compared with those in kidneys treated with the various agents using analysis of variance followed by Duncan’s test for multiple comparisons. Results were expressed as mean±SEM, and in all cases, a value of p<0.05 was considered significant.

Results

Renovasodilator Effects of Arachidonic Acid

Under basal perfusion pressure, AA elicited a vasoconstrictor effect that was blocked by indomethacin (included in the perfusate) or a thromboxane A\textsubscript{2}/prostaglandin H\textsubscript{2} receptor antagonist. Elevation of perfusion pressure to 155–195 mm Hg uncovered a vasodilator response to AA (Figure 2). AA dose-dependently reduced the perfusion pressure by 16±2, 29±2, and 36±3 mm Hg for 1, 5, and 10 µg AA, respectively (Figure 3). Doses of AA higher than 10 µg sometimes gave biphasic responses characterized by an initial, brief vasoconstrictor response followed by a pronounced and prolonged vasodilator response. However, to avoid problems of data interpretation, only effects of AA doses up to 10 µg
are included. The perfusate flow rate in this group of kidneys (control) was 12.4±0.3 ml/min, which gave a basal perfusion pressure of 91±3 mm Hg. With the same kidneys, the vasodilator responses to the reference vasodilator agents acetylcholine (100 ng) and nitroprusside (1 μg) were 58±5 and 28±2 mm Hg, respectively. The responses to these doses of acetylcholine and nitroprusside were used as reference responses to determine the vascular responsiveness of the kidney as well as the selectivity of the various inhibitors of AA metabolism on AA-induced renal vasodilation.

Effects of BW 755C and ETYA on Arachidonic Acid Responses in Rat Kidneys

BW 755C (25 μM) did not affect the renovasodilator effects of AA or the vasodilator responses to acetylcholine and nitroprusside (n=5, Figures 3 and 4). At a higher concentration (50 μM), there was inhibition of the responses of the kidney to AA as well as to those of acetylcholine and nitroprusside associated with marked falls in perfusion pressure on the order of 36±2 mm Hg (n=3).

The addition of ETYA (10 μM) significantly inhibited AA-induced vasodilation (n=6, p<0.001) but had no appreciable effect on the vasodilation induced by acetylcholine and nitroprusside (Figures 3 and 4).

**Effects of Clotrimazole and 7-Ethoxycoumarin on Arachidonic Acid–Induced Vasodilation**

Clotrimazole (0.7 μM) produced significant inhibition (p<0.01) of AA-induced vasodilation (n=5, Figure 5). The renovasodilator effect of AA (10 μg) was reduced (p<0.01) from a maximum value of −36±2 to a value of −4±2 mm Hg (n=5). However, the vasodilator responses to acetylcholine (100 ng) and nitroprusside (1 μg) were not significantly altered (p>0.05). Higher concentrations of clotrimazole (1.4 and 2.1 μM), which completely abolished AA-induced vasodilation, only partially inhibited the responses to acetylcholine and nitroprusside (n=4, p<0.05) being 52±6% in both cases. Figure 4 shows the effects of BW 755C (25 μM), ETYA (10 μM), and clotrimazole (0.7 μM) on the vasodilator responses to acetylcholine and nitroprusside. None of these interventions had any appreciable effect on the vasodilator responses to these reference vasodilators, whereas significant effects were produced by ETYA (10 μM) and clotrimazole (0.7 μM) on AA-induced vasodilation.

The effects of 7-ER on AA-induced vasodilation are shown in the top panel of Figure 6. Compared with responses obtained in control (vehicle-treated) rats, 7-ER (0.5 μM, n=6; 1.0 μM, n=6; and 2.0 μM, n=4) inhibited the renovasodilator effects of AA in a dose-dependent manner. Maximal inhibition was achieved with a concentration of 1.0 μM.

At concentrations of 0.5 and 1.0 μM, 7-ER had no effect on the vasodilator responses to acetylcholine and nitroprusside (Figure 6, bottom panel). At 2.0 μM, there were significant inhibitory effects on the vasodilator effects of acetylcholine (p<0.05) and nitroprusside (p<0.05) as well as on the vasoconstrictor effects of phenylephrine (data not shown). Thus, lower con-
centrations of 7-ER (0.5 and 1.0 µM) were selective in inhibiting the vasodilator effects of AA.

Effects of CHAPS and Methylene Blue on Arachidonic Acid–Induced Vasodilation

The effect of the inclusion of CHAPS (10 mg/l) in the renal perfusate is shown in Figure 7. The infusion of CHAPS (4.7 mg/ml; 0.7 ml/min for 30 seconds) as reported by Bhardwaj and Moore17 elicited significant inhibitory effects on AA, acetylcholine, and nitroprusside responses, whereas CHAPS alone had pronounced biphasic effects on elevated perfusion pressure, with an initial vasoconstrictor response followed by a sustained vasodilator response. To circumvent these problems, CHAPS (10 mg/l) was included in the perfusate from the beginning of the perfusion, and a period of at least 15 minutes was allowed before the responses to AA were tested. CHAPS (10 mg/l) significantly inhibited the AA-induced responses (p<0.05). Responses to acetylcholine also were reduced from a control value of −56±3 to −21±3 mm Hg (p<0.01). There was no effect on nitroprusside-induced vasodilation.

On the other hand, methylene blue (10⁻⁴ M) had no significant effect on the responses to AA, whereas responses to acetylcholine (100 ng) were reduced from −52±6 to −25±2 mm Hg (p<0.01), and responses to nitroprusside were reduced from a control value of −30±2 to −18±3 mm Hg (p<0.01).

Vasorelaxant Effects of Renal Effluent on Isolated Ring Preparations

Phenylephrine (5×10⁻⁷ M) resulted in tensions of 2.1±0.4 g (celiac arteries), 2.2±0.2 g (mesenteric arteries), and 2.5±0.3 g (aorta) in isolated rings (Figure 8). These were not modified by the presence or absence of endothelium (n=8 for each). Administration of AA (1–10 µg) to the kidney resulted in dose-dependent relaxation of the vascular rings that was unaffected by the presence or absence of endothelium on the bioassay tissues. AA (10 µg) reduced the tension of intact rings of the rabbit aorta and celiac and mesenteric arteries by 0.7±0.2, 0.6±0.2, and 0.6±0.1 g, respectively. In the endothelium-denuded rings, the corresponding responses were similar to those in endothelium-intact rings. Only responses in aortic and celiac arterial rings are shown in Figure 8.

After the administration of the highest dose of AA (10 µg) directly to the isolated vessels, little or no relaxant effect was produced. The inclusion of ETYA (10 µM) in the perfusate abolished the renal vasodilator effect of AA as well as the generation

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**FIGURE 4.** Effects of clotrimazole (Clotr.) (0.7 µM; n = 5), BW755C (25 µM; n = 5), and 5,8,11,14-eicosatetraynoic acid (ETYA) (10 µM; n = 6) on the vasodilator responses to acetylcholine (Ach) (100 ng) and nitroprusside (NP) (1 µg) in the phenylephrine-constricted, indomethacin-treated perfused rat kidney. Values are mean±SEM.

**FIGURE 5.** Effects of clotrimazole (Clotr.) (0.7 µM) on arachidonic acid (AA)–induced falls in perfusion pressure in phenylephrine-preconstricted, indomethacin-treated kidneys. Data are mean±SEM (n=8 for control; n=5 for clotrimazole).
and release of vasorelaxant material or materials from the kidney.

**Discussion**

This study was designed to address the role of cyt P-450 metabolism in the renovasodilator effect of AA in the isolated perfused kidney of the rat. Several important points were highlighted by this study. First, AA elicited vasodilator effects via the cyt P-450 monoxygenase system. Second, the results obtained suggest that the generation of biologically active cyt P-450 metabolites by the kidney (from exogenous AA) elicited vasorelaxant activity on isolated rings of rabbit aorta and celiac and mesenteric arteries. Third, denudation of the endothelium of the resistance vessels in the kidney blunted the vasodilator effects of AA, consistent with the fact that the intima of blood vessels is heavily invested with cyt P-450 monoxygenases.

In the isolated kidney of the rat, several studies have shown that AA elicited vasoconstriction, an effect dependent on cyclooxygenase. In our recent study, the vasoconstrictor effect was shown to involve an interaction of one or more metabolites of AA by cyclooxygenase with thromboxane A2/prostaglandin H2 vascular receptors. Blockade of the vasoconstrictor response by inhibition of cyclooxygenase uncovered a vasodilator effect of AA. In characterizing this response, we evaluated the effects of BW 755C, a dual cyclooxygenase/lipoxygenase inhibitor. The vasodilator response was not affected by BW 755C and therefore is not mediated by lipoxygenase products of AA. However, the inclusion of ETYA in the renal perfusate, to block all the pathways of AA metabolism, blocked the vasodilator

**Figure 6.** Effects of 7-ethoxyresorufin (7ER) on arachidonic acid (AA)--induced vasodilation (top panel) and on acetylcholine (Ach)–nitroprusside (NP)–induced vasodilation (bottom panel) in the isolated perfused kidney of rat treated with indomethacin and constricted with phenylephrine. Different groups of kidneys were treated with different concentrations of 7ER or the appropriate vehicles (n=6 for all groups except 2.0 μM 7ER, n=4). Data are mean±SEM.

**Figure 7.** Changes in perfusion pressure to arachidonic acid (AA) in indomethacin-treated, phenylephrine-constricted kidneys in the presence (n=6) and absence (control, n=6) of 3-[3-cholamidopropyl]-dimethylammonio]-1-propanesulfonate dihydrate (CHAPS) (10 mg/l). Changes in perfusion pressure in methylene blue (Meth Blue) (10^-4 M)–constricted kidneys (n=5) also are shown. Data are mean±SEM.
responses to AA. This observation suggests that the vasodilator effect of AA was mediated by its metabolites that are neither cyclooxygenase nor lipooxygenase derived but, possibly, are dependent on the cyt P-450 monooxygenase pathway. This possibility therefore was evaluated by using known inhibitors of the cyt P-450 monooxygenase system.

The prototypical inhibitor of the cyt P-450 pathway is SKF 525A. In preliminary studies, SKF 525A inhibited AA-induced vasodilation. These results were regarded with caution as SKF 525A exhibited nonspecific effects (vasodilator responses to acetylcholine and nitroprusside being equally blunted). Moreover, it reversed phenylephrine-induced vasoconstriction. SKF 525A has been reported to inhibit the influx of Ca$^{2+}$ and K$^+$, act as a local anesthetic, and interfere with nicotinic receptor activity, actions independent of an effect on cyt P-450 enzymes. Consequently, we used clotrimazole and 7-ER. Clotrimazole was reported to be a specific inhibitor of epoxygenase, and 7-ER has been shown to be a substrate (competitive inhibitor) of cyt P-450 epoxygenase. Clotrimazole (0.7 μM) selectively blocked the vasodilator effects of AA at low concentrations, while higher concentrations (1.4 and 2.1 μM) also blunted the responses to other vasodilators—acetylcholine and nitroprusside. Similarly, 7-ER (0.5–2.0 μM) exerted dose-dependent inhibitory effects on AA-induced vasodilation. These effects were selective for AA at 0.5 and 1.0 μM (Figure 6) and indicate that the vasodilator effect of AA on the kidney is mediated by cyt P-450–dependent metabolites. These data support previous studies that showed that AA-induced relaxations of isolated blood vessels involved the cyt P-450 pathway.

The importance of the endothelium to the control of vascular responses was first demonstrated by Furchgott and Zawadzki, and endogenous substances like AA were later reported to have a partial requirement for the presence of the endothelium to exert a full relaxant effect (see Reference 8). Moreover, recent studies showed that vascular cyt P-450–dependent activity was localized primarily, but not exclusively, in the endothelium. In view of these observations, we sought to assess the effect of AA after the endothelium had been removed from the renal vessels with CHAPS. Our data showed that AA required intact endothelium for the expression of its vasodilator effect, as removal of the endothelium inhibited its effects. The vasodilator response to the AA metabolite or metabolites was independent of guanylate cyclase activity or release of endothelium-derived relaxing factor, because methylene blue, an agent that inhibits guanylate cyclase, had no significant effect on AA-induced vasodilation. However, acetylcholine-induced vasodilation was abrogated.
A unique observation in this study was the demonstration of a vasorelaxant effect of the renal effluent on rings of isolated vessels. Thus, the renal effluent, superfusing a cascade of rings of the rabbit aorta and celiac and mesenteric arteries, caused dose-dependent relaxation of these vessels concomitant with dose-dependent vasodilator responses in the kidney after the injection of increasing doses of AA. The vasorelaxant effect was produced on endothelium-intact as well as endothelium-denuded rings. This suggests that the vasorelaxant product, having been formed in the endothelium of the kidney, requires no further transformation in the endothelium of the blood vessels to exert an effect. The effects observed were blunted with ETYA, suggesting that the vasorelaxant material is an AA metabolite. This is supported by the observation that in vessels superfused with pharmacological antagonists and ETYA (to inhibit the effects of any unmetabolized AA and of vasoactive materials from the kidney), direct application of AA elicited little or no effect, whereas injection of the same dose of AA through the kidney caused relaxation (Figure 8). Moreover, preliminary data showing that 7-ER also reduces the release of vasorelaxant material from the kidney in response to AA provides evidence that the material results from cyt P-450–dependent metabolism of AA.

In conclusion, the results of this investigation demonstrate that the renovasodilator effects of AA in the rat kidney require endothelial conversion of AA via cyt P-450–dependent enzymes. The vasodilator material or materials released from the kidney are capable of relaxing isolated blood vessels of the rabbit aorta and celiac and mesenteric arteries. Further work continues to identify the product or products released from the kidney.

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References

Key Words • vasodilation • rat isolated kidney • arachidonic acid • cytochrome P-450 monooxygenase
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