Endothelium-Derived Hyperpolarizing Factor
Characterization as a Cytochrome P450 1A-Linked Metabolite of Arachidonic Acid in Perfused Rat Mesenteric Prearteriolar Bed
Ayotunde S.O. Adeagbo

The isolated perfused rat mesenteric bed releases endothelium-derived hyperpolarizing factor (EDHF) in response to acetylcholine (ACh) or histamine. I propose that EDHF released in the mesenteric vascular bed is a cytochrome P450 (CYP)-linked, arachidonate metabolite. In the presence of nitro-L-arginine methyl ester (L-NAME) and indomethacin, injections of ACh (0.001 to 10 nmol) or histamine (0.1 to 1,000 nmol) elicited transient, dose-dependent dilation of cirazoline (an \(\alpha_1\)-adrenoceptor selective agonist) preconstricted mesenteric beds. The L-NAME-resistant responses to ACh or histamine were insensitive to tetrodotoxin (1 \(\mu\)mol/L), thus negating its neuronal origin, but were profoundly attenuated by a K⁺ channel inhibitor tetrabutylammonium (0.5 mmol/L). 7-Ethoxyresorufin (a selective and competitive blocker of CYP 1A isozyme) blunted ACh and histamine mediated EDHF responses but did not alter vasodilation initiated through K⁺ channel activation by either cromakalim or NS-1619, or through the nitric oxide-cGMP pathway (sodium nitroprusside). Clofibrate, an imidazole that inhibits CYP by binding to the heme moiety, attenuated ACh, histamine, and cromakalim but not sodium nitroprusside-mediated vasodilator responses. Other CYP isozyme selective inhibitors, such as metyrapone (CYP 2B), 7-pentoxysresorufin (CYP 2B1), sulfaphenazole (CYP 2C3A), and 17-octadecynoic acid (4A-linked \(\omega\)-hydroxylase inhibitor), did not alter ACh or histamine-induced EDHF response. The EDHF-mediated dilations initiated by ACh and histamine, as well as \(K_{ATP}\) activation by cromakalim, were blocked by mepacrine, a nonselective phospholipase \(A_2\) inhibitor. Mepacrine did not alter \(K_{Ca}\) activation by compound NS-1619. I conclude that 1) the isolated perfused rat mesenteric prearteriolar bed releases in response to ACh and histamine, an EDHF that causes vasodilation through K⁺ channel activation; 2) the EDHF is most likely a CYP-derived arachidonate product; 3) CYP 1A is well suited as the isozyme responsible for EDHF production in this vascular bed; and 4) \(PLA_2\) appears to mediate the release of the precursor arachidonic acid. Am J Hypertens 1997;10:763–771 © 1997 American Journal of Hypertension, Ltd.

KEY WORDS: Perfused rat mesenteric bed, endothelium-derived hyperpolarizing factor, nitro-L-arginine methyl ester (L-NAME), cytochrome P450 inhibitors.
Considerable evidence now exist that, in addition to prostacyclin (PGI2) and endothelium-derived nitric oxide (EDNO), other mechanisms contribute to endothelium-dependent vasodilation of perfused organs.1-3 One candidate is the so-called endothelium-derived hyperpolarizing factor (EDHF), which causes vascular smooth muscle hyperpolarization and, subsequently, relaxation via an increase in K+ conductance. The production of EDHF prevails during a combined inhibition of cyclooxygenase and nitric oxide synthase with indomethacin and nitro-L-arginine methyl ester (L-NAME), respectively.2 EDHF can be released by acetycholine and a variety of other endothelium-dependent vasodilators. Endothelium-dependent hyperpolarization exists in bovine, porcine, and human epicardial coronary arteries in vitro4,5 and plays an important role in bradykinin-mediated coronary vasodilation in the rat heart.3,6,7 The chemical nature of EDHF is unclear, but there is general agreement that the putative factor initiates vascular relaxation by facilitating potassium efflux. The type of K+ channel involved in EDHF response depends on the tissue, species, and the endothelial stimulants. In the perfused rat mesenteric prearteriolar bed for instance, it has been previously demonstrated2 that the L-NAME-resistant vasodilation is mediated through the activation of Ca2+-, rather than ATP- or voltage-dependent K+ channels. However, perivascular calcitonin gene-related peptide transmitting neurones have been shown to contribute to acetycholine-induced endothelium-dependent vasodilation.8 These authors suggest that the L-NAME-resistant component of acetycholine vasodilation may well be mediated by a neuronal, rather than an endothelial, factor. This assertion was assessed by testing the sensitivity of L-NAME-resistant acetycholine or histamine vasodilation to the sodium channel blocker tetrodotoxin (TTX).

An early demonstration by Furchgott and Zawadzki9 that mepacrine, a phospholipase A2 inhibitor, and eicosatetraynoic acid (ETYA), an analog inhibitor of arachidonic acid metabolism, inhibited endothelium-mediated relaxation to acetycholine suggested that arachidonic acid metabolism might be involved in this phenomenon. More recently, relaxation of the coronary vasculature that was insensitive to the combined inhibition of cyclooxygenase and NO synthase was characterized as resulting from release of a hyperpolarizing factor derived from CYP-mediated metabolism of arachidonic acid.3,7 Both CYP enzyme system and arachidonic acid are ubiquitous in mammalian vascular tissue10, CYP is particularly localized in the endothelium11-13 and can metabolize arachidonic acid to a variety of vasoactive products that might function as an endogenous EDHF. Thus, in this study, I propose that EDHF, released in response to acetycholine or histamine, is a CYP metabolite of arachidonic acid, and that PLA2-mediated release of arachidonic acid is essential for the production of EDHF in this vascular bed. The role of the CYP system on the L-NAME-resistant vasodilation was studied with five chemically distinct, and relatively isoyme-specific inhibitors of the enzyme system, including 7-ethoxyresorufin (7-ER, a specific substrate, and competitive inhibitor of CYP 1A1/2 isozyme), metyrapone (CYP 2B inhibitor), sulfaphenazole (CYP 2C/3A inhibitor), 17-octadecynoic acid (17-ODYA, a CYP 4A inhibitor), and clotrimazole, a commonly used imidazole derivative that binds to the heme moiety of CYP enzymes. The role of PLA2 in generating the precursor arachidonic acid for EDHF production was investigated with the use of mepacrine, a 4-aminoquinoline and nonselective PLA2 inhibitor.14

METHODS
Male Sprague-Dawley rats (200 to 300 g) were used in these studies. Following pentobarbitone anesthesia (60 mg/kg intraperitoneally), the abdominal cavity of individual rats was opened and the superior mesenteric artery was cannulated through an incision at its confluence with the dorsal aorta and then isolated as previously described by McGregor.15 The mesenteric vascular bed was flushed with heparinized physiologic salt solution (PSS), trimmed off the intestinal borders, and then transferred to a warmed chamber where it was perfused with PSS (maintained at 37°C and gassed with 95% O2/5% CO2) at a constant flow rate of 5 mL/min using a peristaltic pump (Masterflex, Cole-Palmer Instruments Co.). The PSS that was used had the following composition (in mmol/L): NaCl, 118; KCl, 4.7; CaCl2, 2.5; KH2PO4, 1.2; MgSO4, 1.2; NaHCO3, 12.5; and glucose, 11.1. Indomethacin (1 μmol/L) was routinely added to all solutions; the pH of the various solutions, after saturation with 95% O2/5% CO2 gas mixture, was 7.4. Changes in perfusion pressure were recorded with a pressure transducer (Statham, Valley View, OH, model P23XL) coupled to a polygraph recorder (Grass, Quincy, MA, model 7H). Tissues were routinely allowed to equilibrate for 1 h before the start of the experiments.

Experimental Protocol Adeagbo and Triggle have previously established that ACh-, and histamine-induced endothelium-dependent vasodilation in the rat mesenteric vascular bed is resistant to inhibition by L-NAME. Thus, all experiments in this study were performed in vascular beds perfused with PSS containing a combination of 100 μmol/L L-NAME and 1 μmol/L indomethacin to block NO synthase and cyclooxygenase, respectively. The experiments were routinely conducted following the elevation of mesenteric vascular tone with an infusion of the α1-adreno-
ceptor agonist, cirazoline (0.2 to 0.5 μmol/L). Time-controlled dose-response relations were usually established for acetylcholine or histamine to verify possible changes in the reactivity of the perfused vascular beds to these agonists over time for a particular protocol of this study. Such control experiments followed the same time frame with protocols involving drug treatment.

Series 1 The first series of experiments was performed to establish the nature of transmembrane channels (K+ or Na+) that mediate L-NAME-insensitive vasodilation of the mesenteric bed to bolus injections of acetylcholine and histamine. Thus, after increasing mesenteric vascular tone with an infusion of cirazoline (an α1-adrenoceptor agonist), dose-response curves to ACh and histamine were established in the absence, and in the presence of either 0.5 mmol/L tetrabutylammonium (TBA, a K+ channel blocker) or 1 μmol/L tetrodotoxin (TTX, a Na+ channel blocker) added to the perfusion solution.

Series 2 The purpose of this series was to assess effects of a variety of CYP inhibitors on L-NAME-resistant vasodilator responses initiated by acetylcholine and histamine. Dose-response curves to ACh and histamine were established before and 30 min after treatment with each of the following CYP inhibitors: 7-alkoxyresorufin (7-ER, 1 and 3 μmol/L; 7-PR, 5 and 10 μmol/L), clotrimazole (5 and 10 μmol/L), 17-octadecynoic acid (17-ODYA, 10 and 20 μmol/L), and metyrapone or sulfaphenazole (each at 50 and 100 μmol/L). Concentrations of inhibitors were selected based on available information in the literature.6,16 Inhibitors found to be effective against L-NAME-resistant vasodilation in response to acetylcholine and histamine were assessed for their influence on vasodilation initiated by a K+ channel activation initiated by cromakalim and NS-1619 (KATP and KCa, respectively), or by a nitric oxide donor, sodium nitroprusside, to determine their direct effect on vascular tone.

Series 3 Experiments in this series were conducted to determine the role of phospholipase A2 in the production of the putative factor that mediates L-NAME-resistant vasodilation initiated by acetylcholine and histamine. Thus, dose-response curves were established for these vasodilators in the absence, and 30 min after the addition of PLA2 inhibitor mepacrine at 1 or 3 μmol/L, to the perfusion solution. As was the case in series 2 for CYP inhibitors, the PLA2 inhibitor was also assessed for its possible direct influence on vasodilation initiated by K+ channel activation initiated by cromakalim and NS-1619 (KATP and KCa, respectively), or by a nitric oxide donor, sodium nitroprusside, to determine their direct effect on vascular tone.

Drugs Acetylcholine bromide, clotrimazole, 7-ethoxyresorufin, 7-pentoxyresorufin, histamine dihydrochloride, indomethacin, metyrapone [2-methyl-1,2-di-3-pyridyl-1-propanone], Nω-nitro-L-arginine methyl ester, sodium nitroprusside, tetrabutylammonium iodide, tetrodotoxin, and mepacrine (quinacrine) dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). 17-Octadecynoic acid (17-ODYA) and compound NS-1619 [1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one] were purchased from Cayman Chemical Co. (Ann Arbor, MI) and Research Biochemicals (Natick, MA), respectively. Cirazoline dihydrochloride was a generous gift from Synthelabo Laboratories, Paris, France. The stock solution of indomethacin was made in 50% ethanol. 17-ODYA was supplied as a solution in ethanol. Clotrimazole, 7-ER, 7-PR, and NS-1619 were dissolved in DMSO. All other compounds dissolved freely in distilled water.

Data and Statistical Analysis Vasodilator responses were measured as percent inhibition of cirazoline-induced tone. Data are presented as means ± SEM and differences between means were statistically tested using Students’ t test for paired data, and were considered significant at P < .05.

RESULTS

Response to ACh and Histamine During Infusion of L-NAME and Indomethacin The basal perfusion pressure (BPP) of mesenteric vascular beds perfused with PSS containing indomethacin (1 μmol/L) and L-NAME (100 μmol/L) was 20.4 ± 0.8 mm Hg (n = 48). Infusion of cirazoline (0.3 μmol/L) initiated a sustained increase (137.5 ± 2.1 mm Hg, n = 22) in the perfusion pressure. Under such conditions, ACh (0.001 to 10 nmol) or histamine (0.1 to 100 nmol) initiated a dose-dependent dilation of the vascular beds (Figure 1). The ED50 (0.24 ± 0.01, 0.21 ± 0.05, and 0.24 ± 0.03 nmol) obtained from successive dose-response curves established to acetylcholine at 1-h intervals were not statistically different. ACh and histamine responses that were insensitive to a combined treatment with L-NAME and indomethacin were attenuated by a K+ channel inhibitor tetrabutylammonium (TBA, 0.5 mmol/L). TBA reduced the vasodilation initiated by 3 nmol cromakalim (a KATP opener), from 88.1 ± 1.8% to 4.6 ± 0.5% (n = 7) and abolished the dilator responses to 30 nmol NS-1619 (a KCa opener), but did not alter the vasodilation initiated by 0.1 nmol sodium nitroprusside (SNP).

Effect of CYP Inhibitors on Vascular Tone and on EDHF-Mediated Vasodilation A suitable CYP inhibitor for purposes of this study is one that preserves the vascular tone (initiated and maintained with continuous infusion of cirazoline) and allows relaxation to be assessed. Infusion of clotrimazole (5 or 10 μmol/L)
caused a transient reduction of the perfusion pressure. The vascular tone recovered to preapplication levels without necessitating an increase in the concentration of vasoconstrictor. Neither 7-ER at 3 μmol/L, metyrapone in concentration up to 100 μmol/L nor 17-ODYA at 20 μmol/L altered the vascular tone (Figure 2). Sulfaphenazole (100 μmol/L) was also without significant effects on the vascular tone.

EDHF-mediated vasodilation in response to acetylcholine and histamine were blunted by 7-ER and clotrimazole (Figure 3). At the concentration that blunted EDHF response, clotrimazole also altered Kᵢₗ channel activation initiated by cromakalim. However, the compound, as well as 7-ER, had no effect on SNP-mediated vasodilation (Figure 4). 7-Pentoxyresorufin, sulfaphenazole, and 17-ODYA had no effect on ACh (Figure 5), cromakalim, or SNP-mediated vasodilation (data not shown). Metyrapone also did not alter the vasodilation initiated by any of the dilator agents.

Effects of Mepacrine on EDHF-Mediated Responses and on Kᵦₜ and Kᵥᵪ Channel Activation Mepacrine was used to assess the role of phospholipase A₂ in the production of EDHF in the rat mesenteric vascular bed. The compound attenuated acetylcholine- and histamine-mediated EDHF dilator responses (Table 1). It also significantly blocked vasodilation initiated by cromakalim (a Kᵦₜ channel activator), but failed to alter vasodilation initiated by either NS-1619 (a Kᵥᵪ channel activator) or SNP, a nitric oxide donor (Figure 6). Mepacrine (3 μmol/L) did not alter the cirazoline-induced tone.

FIGURE 1. Effect of tetrabutylammonium (TBA, 0.5 mmol/L; top panel) and tetrodotoxin (TTX, 1 μmol/L; bottom panel) on vasodilator responses initiated by acetylcholine (left) and histamine (right) in a rat-isolated, perfused mesenteric prearteriolar bed. Vascular beds were perfused with physiologic salt solution containing 1 μmol/L indomethacin and 100 μmol/L L-NAME to block cyclooxygenase and nitric oxide synthase, respectively. Sustained vascular tone was maintained with a continuous infusion of 0.3 μmol/L cirazoline. Each data point represents the mean ± SEM of six to eight experiments. *P < .05.

FIGURE 2. Representative tracings of the effects of clotrimazole, 7-ethoxyresorufin, metyrapone, and 17-octadecynoic acid (17-ODYA) on mesenteric vascular tone maintained with continuous infusion of cirazoline.

DISCUSSION

A significant proportion of endothelium-dependent vasodilation in the rat isolated, perfused mesenteric prearteriolar bed is resistant to blockade by known inhibitors of nitric oxide-cyclic guanosine monophosphate (GMP) pathway alone or in combination with prostanoid synthase inhibitors. The L-NAME-resistant vasodilation was due to an endothelium-derived hy-
perpolarizing factor (EDHF) as it was attenuated by apamin, a small conductance Ca\(^{2+}\)-activated K\(^+\) channel antagonist.\(^1\)\(^2\) Because the vasodilation was also inhibited by 7-ethoxyresorufin, a CYP 1A isozyme inhibitor, and by the PLA\(_2\) inhibitor mepacrine, it appears that the L-NAME-resistant vasodilation of rat mesenteric vascular bed is mediated by a CYP-linked product of arachidonic acid metabolism. Thus, as a consequence of acetylcholine or histamine receptor activation, arachidonic acid is released from the endothelial phospholipid membrane, and is metabolized by CYP to presumably a diffusable product that hypoperpolarizes the underlying vascular smooth muscle (VSM) cells through Ca\(^{2+}\)-activated K\(^+\) channel activity.

The chemical identity of EDHF is not known. Two postulates regarding its nature were explored in this study. First, Scott and Chafe\(^8\) proposed that perivascular calcitonin gene-related peptide (CGRP)-transmitting neurones are involved in acetylcholine-induced endothelium-dependent vasodilation of the perfused rat mesenteric bed. Perivascular sensory neurones transmitting CGRP in the mesenteric vascular bed of the rat are sensitive to the sodium channel blocker tetrodotoxin.\(^7\)\(^7\) Tetrodotoxin would be expected to inhibit acetylcholine or histamine-induced, endothelium-dependent vasodilation. Our data show that tetrodotoxin did not alter the dilator effects of these agonists (Figure 1), therefore negating a neuronal origin for these responses. Second, the postulate that EDHF might be a labile CYP-arachidonate metabolite\(^18\) appears logical in view of observations that novel arachidonate metabolites of this enzyme (CYP) play important roles in intestinal blood flow, and also promote \(^{86}\)Rb (marker for K) efflux\(^19\)\(^20\) and induce VSM cell hyperpolarization.

The phenomenon of L-NAME-resistant (also referred to as nonprostanoid, non-NO) vasodilation of perfused vascular beds is well documented for acetylcholine and bradykinin in perfused rat kidney\(^21\) and heart preparations,\(^7\)\(^6\) and in bovine and porcine cor-

**FIGURE 3.** Effect of 7-ethoxyresorufin (7-ER, top panel) and clotrimazole (CTZ, bottom panel) on EDHF-mediated vasodilator responses initiated by acetylcholine (left) and histamine (right) in PSS-perfused rat mesenteric beds. The perfusion medium contained indomethacin (1 \(\mu\)mol/L) and L-NAME (100 \(\mu\)mol/L). Each data point on the graphs represents the mean ± SEM, \(n = 8\), * or **\(P < .05\).

**FIGURE 4.** Effect of 7-ethoxyresorufin (7-ER, top panel) and clotrimazole (CTZ, bottom panel) on the vasodilator responses initiated by 0.1 nmol nitroprusside (left panel), 1 nmol cromakalim (middle panel), or by 10 nmol NS-1619 (right panel). Solid bars: control responses; hatched and open bars represent responses in the presence of 7-ER (1 and 3 \(\mu\)mol/L, top), and CTZ (5 and 10 \(\mu\)mol/L, bottom panel) respectively. Each data point on the bar graphs represents the mean ± SEM, \(n = 5\), * or **\(P < .05\).
In all these studies, various CYP inhibitors were found to be effective blockers, thus supporting the idea that such vasodilator responses were mediated by a CYP-linked product. There are many isozymes of CYP with broad and often overlapping substrate specificities and susceptibilities to inhibitors. CYP 1A isozymes are ubiquitous, strongly inducible in vertebrate endothelium in vivo and in vitro, and represent the subfamily for which relatively selective inhibitors are available. 7-Ethoxyresorufin (7-ER) is a selective substrate and competitive inhibitor for CYP 1A. 7-ER-inhibited acetylcholine and histamine-induced vasodilation, in a concentration-dependent fashion, may have blunted these responses by serving as a false substrate for the CYP isozyme involved in the L-NAME-resistant, endothelium-dependent vasodilation. The ineffectiveness of 7-pentoxyresorufin (7-PR), an alkoxyresorufin with specificity for the 2B1 subfamily, enhances our conclusion that ACh- or histamine-mediated EDHF effect in the rat mesenteric vascular bed is specifically related to CYP 1A isozyme activity. That 7-ER is relatively specific for endothelium-mediated hyperpolarization is further supported by its lack of effect on endothelium-independent hyperpolarization in response to cromakalim, a K<sub>ATP</sub> channel activator, or compound NS-1619, a selective K<sub>ca</sub> channel activator. 7-ER also did not alter nitroprusside (NO donor)-mediated vasodilation. Other relatively selective isozyme inhibitors—metyrapone (CYP 2B), sulfaphenazole (CYP 2C/3A inhibitor), and 17-octadecynoic acid (17-ODYA)—were also tested during these experiments. The ineffectiveness of these inhibitors further supports the idea that the vasodilator responses are mediated by CYP 1A isozyme activity.

**FIGURE 5.** Effects of 7-pentoxyresorufin (7-PR, top), sulfaphenazole (SPZ, middle), and 17-octadecynoic acid (17-ODYA, bottom) on L-NAME-resistant vasodilator responses initiated by acetylcholine in cirazoline preconstricted vascular beds. Each data point on the graphs represents the mean ± SEM of six experiments.

**FIGURE 6.** Effect of mepacrine (MEPA) on the vasodilation initiated by 1 nmol cromakalim (top panel), 10 nmol NS-1619 (middle panel), or by 0.1 nmol sodium nitroprusside (SNP, lower right panel) in cirazoline preconstricted, rat mesenteric vascular bed. Each data point represents the mean ± SEM (n = 6), *P < .05.
TABLE 1. THE EFFECT OF MEPACRINE (A NONSPECIFIC PLA$_2$ INHIBITOR) ON VASODILATOR RESPONSES OF ISOLATED, PERFUSED RAT MESENTERIC PREARTERIOLAR BEDS

<table>
<thead>
<tr>
<th>Vasodilators</th>
<th>Control</th>
<th>Mepacrine (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent Decrease in Cirazoline-Induced Tone</td>
<td>1</td>
</tr>
<tr>
<td>Acetylcholine (0.1 nmol)</td>
<td>$-61.9 \pm 2.7$ ($n = 6$)</td>
<td>$-50.8 \pm 13.0$ ($n = 6$)</td>
</tr>
<tr>
<td>Histamine (10 nmol)</td>
<td>$-75.1 \pm 11.2$ ($n = 5$)</td>
<td>$-70.2 \pm 13.1$ ($n = 5$)</td>
</tr>
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The PSS used for arterial perfusion contained indomethacin (1 μmol/L) and L-NAME (100 μmol/L).

* P < .05 from control responses.

ODYA (CYP 4A isozyme inhibitor)$^{17}$—were not effective in blocking L-NAME-resistant vasodilation of the perfused rat mesenteric bed. 17-ODYA is a suicide-substrate inhibitor of P450 fatty acid $\alpha$-hydroxylase in rat renal tissues.$^{16}$

Although the chemical identity of EDHF has not yet been established, the notion that this putative factor is a labile CYP-linked metabolite of arachidonic acid$^{18}$ continues to receive a principal focus. As noted earlier, CYP is abundant in the endothelium$^{11–13}$ and mediates the formation of a variety of vasoactive products including epoxygenosatrienoic acids (EETs) in endothelial cells.$^{32}$ CYP-linked metabolites of arachidonic acid such as 5,6-, 8,9-, 11,12-, and 14,15-EETs can hyperpolarize blood vessels and activate K$^+$ channels in vascular smooth muscle cells.$^{33–35}$ Thus, EETs have been proposed as EDHF in the coronary vasculature.$^{35}$ However, two recent studies in isolated guinea pig carotid$^{36,37}$ and rat hepatic$^{37}$ arteries do not support this proposition.

Arachidonic acid (AA) is one of the fatty acids metabolized by CYP to a variety of vasoactive products and is considered, in this study, as the prime EDHF precursor. In mammalian tissues, AA can be liberated from membrane phospholipids by the action of PLA$_2$. If PLA$_2$ activation mediates the generation of precursor AA for EDHF production, its inhibition should result in blockade of the relaxation mediated by EDHF. L-NAME-resistant vasodilation in response to acetylcholine and histamine was attenuated by the nonselective PLA$_2$ inhibitor, mepacrine (this study) and by another chemically distinct PLA$_2$ inhibitor, arachidonyl trifluoromethyl ketone (AACOCF$_3$).$^{38}$ These results suggest that PLA$_2$ mediates the generation of AA used for the production of this putative factor. Mepacrine has been shown to inhibit EDHF-mediated responses in bovine and porcine coronary arteries.$^{3,22,26}$

Two additional observations warrant comment. First, CYP inhibitors are not absolutely specific for the enzyme system. Findings that the various inhibitors employed in the this study (except clotrimazole) did not alter vascular tone maintained with continuous infusion of cirazoline suggest their relative ‘‘purity’’ as investigational tools. Nonspecificity of action is a major problem with the use of this class of compounds to elucidate physiologic function.$^{32,39}$ Clotrimazole, an imidazole antifungal agent and one of the most commonly studied CYP inhibitors, attenuated acetylcholine and histamine-induced, L-NAME-resistant vasodilation of the mesenteric vascular bed. However, unlike 7-ER, this compound also attenuated the dilator effect of cromakalim and NS-1619. It can thus be argued that the attenuating effect of clotrimazole on acetylcholine and histamine dilation in the mesenteric vascular bed most likely reflects inhibition of ion channels in the smooth muscle, rather than CYP-mediated EDHF production in the endothelium. Imidazole derivatives such as clotrimazole, known to block CYPs, have been shown to inhibit plasma membrane Ca$^{2+}$ channels in GH$_3$ pituitary cells and bovine chromaffin cells$^{40}$ and in red blood cells.$^{41}$ The compounds have also been reported to block K$^+$ channel activity in red blood cells.$^{42,43}$ The blockade of cromakalim-induced K$_{ATP}$ channel activation by clotrimazole as observed in this study disagrees with the perfused rat heart data of Fulton et al.$^9$ The reason for this discrepancy is unclear. Second, data from this study show that mepacrine antagonizes mesenteric dilation initiated by cromakalim but not by compound NS-1619. This observation suggests that mepacrine selectively interacts with K$_{ATP}$ channels. Because EDHF-mediated vasodilation in this vascular bed is initiated through K$_{Ca}$ channels, this action of mepacrine is unrelated to its ability to modulate EDHF production through precursor arachidonate generation. Furthermore, although mepacrine is known to exhibit antimuscarinic action,$^14$ such an action would not explain its blockade of EDHF-mediated histamine vasodilation as observed in this study.

In conclusion, this study has demonstrated clearly that: 1) the isolated perfused rat mesenteric prearterio-
lar bed releases an EDHF that causes vasodilation through Ca\(^{2+}\)-activated K\(^+\) channels in response to acetylcholine and histamine; 2) the EDHF is most likely a cytochrome P450-derived product of arachidonic acid metabolism; 3) the P4501A isozyme is well suited for EDHF production in this vascular bed; and 4) PLA\(_2\) appears to mediate the release of the precursor arachidonic acid.

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