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# Rat mesenteric arterial dilator response to 11,12-epoxyeicosatrienoic acid is mediated by activating heme oxygenase

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**Sacerdoti, David, Massimo Bolognesi, Marco Di Pascoli, Angelo Gatta, John C. McGiff, Michal Laniado Schwartzman, and Nader G. Abraham.** Rat mesenteric arterial dilator response to 11,12-epoxyeicosatrienoic acid is mediated by activating heme oxygenase. *Am J Physiol Heart Circ Physiol* 291: H1999–H2002, 2006. First published June 30, 2006; doi:10.1152/ajpheart.00082.2006.—11,12-Epoxyeicosatrienoic acid (11,12-EET), a potent vasodilator produced by the endothelium, acts on calcium-activated potassium channels and shares biological activities with the heme oxygenase/carbon monoxide (HO/CO) system. We examined whether activation of HO mediates the dilator action of 11,12-EET, and that of the other EETs, on rat mesenteric arteries. Dose-response curves ($10^{-9}$ to $10^{-6}$ M) to 5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET, and ACh ($10^{-9}$ to $10^{-4}$ M) were evaluated in preconstricted ($10^{-6}$ mol/l phenylephrine) mesenteric arteries (<350 μm diameter) in the presence or absence of *1*) the cyclooxygenase inhibitor indomethacin (2.8 μM), *2*) the HO inhibitor chromium mesoporphyrin (CrMP) (15 μM), *3*) the soluble guanylyl cyclase (GC) inhibitor ODQ (10 μM), and *4*) the calcium-activated potassium channel inhibitor iberiotoxin (25 nM). The vasodilator response to 11,12-EET was abolished by CrMP and iberiotoxin, whereas indomethacin and ODQ had no effect. In contrast, the effect of ACh was attenuated by ODQ but not by CrMP. The vasodilator effect of 8,9-EET, like that of 11,12-EET, was greatly attenuated by HO inhibition. In contrast, the mesenteric vasodilator response to 5,6-EET was independent of both HO and GC, whereas that to 14,15-EET demonstrated two components, an HO and a GC, of equal magnitude. Incubation of mesenteric microvessels with 11,12-EET caused a 30% increase in CO release, an effect abolished by inhibition of HO. We conclude that the rat mesenteric vasodilator action of 11,12-EET is mediated via an increase in HO activity and an activation of calcium-activated potassium channels.

epoxygenase; carbon monoxide; endothelial cell; mesenteric artery

ARACHIDONIC ACID can be metabolized in endothelial cells (21) by epoxygenases to four epoxyeicosatrienoic acids (EETs): 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET (23, 29). The 11,12-EET exhibits diverse biological activities, including dilation of preglomerular microvessels (7, 12), stimulation of angiogenesis (16), and inhibition of sodium transport (27). Additionally, 11,12-EET demonstrates anti-inflammatory properties, e.g., inhibition of adhesion molecule expression in response to inflammatory mediators (19), and contributes to opposing elevation of blood pressure in response to salt loading (15). The 11,12-EET has also been proposed to act as an endothelial-derived hyperpolarizing factor by activating calcium-activated potassium channels ($K_{Ca}$) (3, 5, 9). Carbon monoxide (CO), generated by the heme oxygenase (HO) system, also activates potassium channels (14), dilates arteries (24, 30), and exhibits anti-inflammatory (2, 24) and antihypertensive properties (22, 30). Furthermore, both EETs (28) and the HO/CO system attenuate ischemic injury (25).

Because EETs, particularly 11,12-EET, and the HO system share overlapping biological activities, we examined a possible link between 11,12-EET and HO activity in the regulation of vascular tone in rat mesenteric microvessels. We report here that 11,12-EET dilated rat mesenteric arteries via an HO-dependent mechanism, acting through $K_{Ca}$ channels. The 5,6-EET, 8,9-EET, and 14,15-EET vasodilated mesenteric arteries. However, only 8,9-EET acted through a mechanism similar to that of 11,12-EET, whereas the 5,6-EET vasodilator effect was independent of HO and guanylyl cyclase and the effect of 14,15-EET was only partly reduced by chromium mesoporphyrin (CrMP).

## MATERIALS AND METHODS

*Chemicals.* CrMP was obtained from Porphyrin Products (Logan, UT), and 5,6-, 8,9-, 11,12, and 14,15-EET were from Cayman Chemical (Ann Arbor, MI). All other chemicals were obtained from Sigma (St. Louis, MO). ACh was dissolved in deionized water and diluted with Krebs buffer. CrMP was dissolved in a solution of 50 mM $NaCO_3$. Indomethacin was dissolved in ethanol and diluted with Krebs buffer. CORM-3 was a generous gift from Dr. J. R. Falck and was dissolved in water and then in Krebs buffer.

*Mesenteric microvessels.* The study was performed on adult male Wistar rats (Charles River, Calco, Italy), with body weights of 200–225 g. The third- and fourth-order branches of the superior mesenteric artery (<350 μm in diameter, 1–2 mm in length) were removed from the mesenteric vascular bed (4, 21) and mounted on glass micropipettes in a water-jacketed perfusion chamber (Living Systems Instrumentation, Burlington, VT) in warmed (37°C), oxygenated (95% $O_2$-5% $CO_2$) Krebs-Henseleit buffer solution. The vessels were mounted on a micropipette connected to a pressure servo controller. Subsequently, the lumen of the vessel was flushed to remove residual blood, and the end of the vessel was mounted on a micropipette connected to a three-way stopcock. The vessel was pressurized to 80 mmHg and superfused with Krebs-Henseleit buffer solution (4 ml/min) at 37°C and gassed with 95% $O_2$-5% $CO_2$. Vascular diameters were measured by a video system, which included a microscope with a CCD television camera (Eclipse TS100-F, Nikon, Tokyo, Japan), a television monitor (Ultrak, Lewisville, TX), and a video measuring system (Living Systems Instrumentation). After 45 min of equilibration, the presence of a functional endothelium was confirmed by relaxation to ACh ($10^{-6}$ M) after preconstriction with

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phenylephrine (PE) ($10^{-6}$ mol/l). Arteries with <50% relaxation of PE-induced contraction were discarded. Vasodilation to increasing 11,12-EET (from $10^{-9}$ M to $10^{-6}$ M) and ACh (from $10^{-9}$ M to $10^{-4}$ M) concentrations were investigated in arteries preconstricted with PE ($10^{-6}$ mol/l), before and after inhibition of HO with CrMP (15 μM), of cyclooxygenase with indomethacin (2.8 μM), of guanylyl cyclase with ODQ (10 μM), and of $K_{Ca}$ channels with iberiotoxin (25 nM). Vasodilatation to the CO donor CORM-3 (50–200 μM) was also investigated in arteries preconstricted with PE. CORM-3 was diluted in water and then added to the buffer. All experiments were approved by the Institutional Animal Care and Use Committee and conducted under the *Guidelines for the Care and Use of Laboratory Animals* published by the Office of Science and Health Reports, National Institutes of Health.

*Measurement of HO activity.* The effect of 11,12-EET on HO activity was evaluated by incubating mesenteric microvessels with 11,12-EET and measuring CO release. Vessels were incubated at 37°C for 60 min with and without 11,12-EET ($10^{-6}$ M) and CrMP (15 μM) in amber glass vials (2 ml) containing 1.0 ml of Krebs buffer saturated with 95% $O_2$-5% $CO_2$. The incubations were terminated by placement of the samples in ice. Subsequently, internal standards made of isotopically labeled CO ($^{13}C^{16}O$ and $^{13}C^{18}O$) were injected into samples, and the CO content of the headspace gas (expressed as pmol·mg protein$^{-1}$·60 min$^{-1}$) was determined by gas chromatography/mass spectroscopy analysis as previously reported (1).

*Statistical analyses.* The data are presented as the means ± SE. Statistical significance ($P < 0.05$) among experimental groups was determined by the Fisher method of analysis of multiple comparisons. For comparison among treatment groups, the Null hypothesis was tested by a single-factor ANOVA for multiple groups or unpaired *t*-test for two groups.

## RESULTS

*Differential effects of CrMP, ODQ, and iberiotoxin on 11,12-EET-induced vasodilatation.* The 11,12-EET caused a dose-dependent increase in diameter (Fig. 1*A*) of mesenteric arterial segments preconstricted with PE. Indomethacin did not affect the vascular response to 11,12-EET (data not shown). Inhibition of HO with CrMP abolished the vasodilation produced by 11,12-EET (Fig. 1*A*), suggesting that activation of HO contributes to the mechanism of the vascular action of 11,12-EET. In contrast, the response to ACh was not affected by inhibition of HO with CrMP (Fig. 1*B*). To address a possible interaction with cGMP, we evaluated the mesenteric arterial response to inhibition of soluble guanylyl cyclase with ODQ. ODQ reduced the vasodilator response to ACh by ~50% (Fig. 1*D*) but did not affect the response to 11,12-EET (Fig. 1*C*). To evaluate whether the mesenteric vasodilator action of 11,12-EET was dependent on activation of $K_{Ca}$ channels, we determined the ability of the $K_{Ca}$ channel inhibitor, iberiotoxin, to affect the vascular response to 11,12-EET. Iberiotoxin (25 nM) abolished the vasodilatation produced by 11,12-EET (Fig. 1*E*).

*Effect of different EETs on mesenteric microvessel tone.* We then evaluated the effects of the other EETs on mesenteric microvessels. The 5,6-, 8,9-, and 14–15-EETs caused a dose-dependent increase in diameter of variable magnitude (Fig. 2) of the mesenteric arterial segments preconstricted with PE. CrMP abolished the effect of 8,9-EET (Fig. 2*B*) and partially inhibited the effect of 14,15-EET (Fig. 2*C*), but it did not affect vasodilatation to 5,6-EET (Fig. 2*A*). Furthermore, ODQ did not affect vasodilatation to 5,6-EET

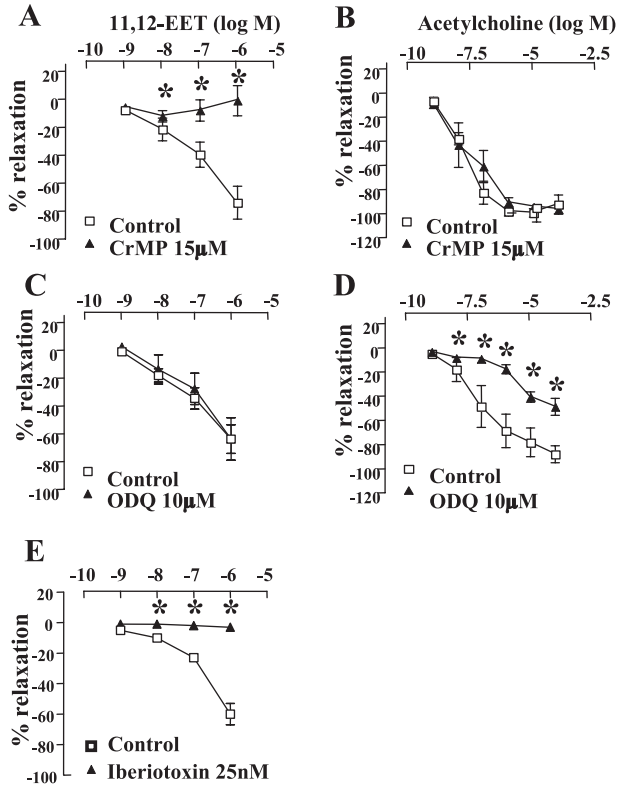


Fig. 1. Dose-response effects of 11,12-epoxyeicosatrienoic acid (EET; *A*) and ACh (*B*) on mesenteric arterial microvessels preconstricted with phenylephrine (PE) before and after inhibition of heme oxygenase (HO) with chromium mesoporphyrin (CrMP; 15 μM) (*A* and *B*), inhibition of guanylyl cyclase with ODQ (10 μM; *C* and *D*), and inhibition of calcium-activated potassium ($K_{Ca}$) channel with iberiotoxin (25 nM; *E*). Results (means ± SE) are expressed as percent decrease in vasoconstriction produced by PE; $n = 6$. *$P < 0.05$.

(Fig. 2*A*) and 8,9-EET (Fig. 2*B*), whereas it decreased the effect of 14,15-EET (Fig. 2*C*).

*Effect of 11,12-EET on HO activity.* To confirm that the effect of 11,12-EET on mesenteric microvessels was mediated by activation of HO, we incubated the microvessels with 11,12-EET and measured CO release. As shown in Fig. 3, 11,12-EET caused a 30% increase in CO release from mesenteric microvessels. To ascertain whether 11,12-EET mediated the increase in HO activity, mesenteric microvessels were treated with 11,12-EET and CrMP. The addition of CrMP prevented the 11,12-EET-mediated increase in HO activity.

*Effect of CORM-3 on mesenteric microvessel tone.* To ascertain whether mesenteric microvessels respond to exogenous CO, a CO-donor (CORM-3) was applied to vessels preconstricted with PE. When added to a buffer with normal pH, CORM-3 releases CO. The CO released by CORM-3 caused vasodilatation of mesenteric microvessels only when pretreated with CrMP (Fig. 4).

## DISCUSSION

Because 11,12-EET shares many of the effects that derive from activation of HO, the possibility that some actions of 11,12-EET are mediated by an HO-dependent mechanism has been examined in the present study and has been answered in the positive for rat mesenteric arterial vessels. Indeed, our results suggest that a key mechanism of action of 11,12-EET

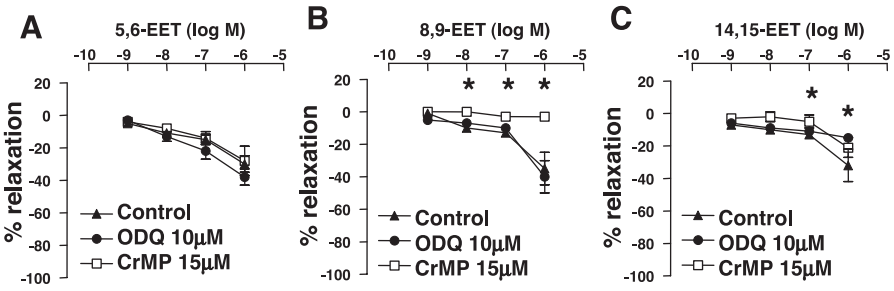


Fig. 2. Dose-response effects of 5,6-EET (*A*), 8,9-EET (*B*), and 14,15-EET (*C*) on mesenteric arterial microvessels preconstricted with PE before and after inhibition of HO with CrMP (15 μM) and inhibition of guanylyl cyclase with ODQ (10 μM). Results (means ± SE) are expressed as percent decrease in vasoconstriction produced by PE; $n = 4$. *$P < 0.05$.

on rat mesenteric arteries is driven by stimulation of HO activity. This novel observation was substantiated by enzymatic activity, indicating that 11,12-EET increased HO activity as measured by increased CO production that was abolished by inhibition of HO with CrMP (Fig. 3). That a product of HO activity mediates the vasodilator action of 11,12-EET in the mesenteric microcirculation is supported by our results showing that vasodilation to 11,12-EET was independent of COX and was unaffected by inhibition of cGMP formation but was abolished by inhibition of HO with CrMP and of $K_{Ca}$ channels with iberiotoxin. These findings are in agreement with those of Naik et al. (18) who demonstrated that CO, a product of HO, dilated the mesenteric circulation. Previous findings that depolarization of vascular smooth muscle (VSM) cells with a high-$K^+$ media and blockade of $K_{Ca}$ channels with iberiotoxin, charybdotoxin, or tetraethylammonium prevented the vasodilator response to EETs provide strong support for a primary role of $K^+$ channels in mediating the vasodilator response to EETs (5, 8). Several investigators found that EETs have no effect on the activity of the $K_{Ca}$ channel in inside-out detached membrane patches excised from VSM cells (26), suggesting that EETs do not directly activate the $K_{Ca}$ channel in VSM.

CO directly activates $K_{Ca}$ channels in vascular smooth muscle cells by altering the apparent calcium dependence of $K_{Ca}$ channel activation (10, 13, 18, 26). The mechanisms of action of endogenous vs. exogenous CO are different. Naik et al. (18) showed that the effect of exogenous CO is sensitive to ODQ and iberiotoxin, whereas the effect of endogenous CO is cGMP independent, via activation of large-conductance $K_{Ca}$ channels. Thus the effects of 11,12-EET and CO share similarities; our study suggests that the most important mechanism of action of 11,12-EET on the mesenteric microcirculation is through stimulation of endogenous CO production acting on $K_{Ca}$ channels. Because vasodilatation to 11,12-EET was not inhibited by ODQ, whereas the effect of ACh was partially inhibited by ODQ, we can also exclude an effect of 11,12-EET through nitric oxide.

In response to 11,12-EET, the rabbit renal vasculature dilated independently of cyclooxygenase (6), as did the rat renal arcuate, interlobular, and afferent arterioles (7). 5,6-EET also dilated the rat renal arcuate artery (7) and the rabbit renal vasculature (6); however, in contrast to 11,12-EET, it exhibited a cyclooxygenase dependency. In our mesenteric microvessels, 5,6-EET caused vasodilatation that could not be blocked by inhibition of HO with CrMP. Additionally, the dilator response of the renal afferent arteriole to the sulfonimide analog of 11,12-EET was unaffected by guanylyl cyclase inhibition (11), as was the case for dilation of superior mesenteric arterial vessels in the present study (Fig. 1*C*). The vascular responses to both 11,12-EET and CO were inhibited by blockade of $K_{Ca}$ channels (20, 26). Dependency of the vascular action on HO activity was evident for 8,9-EET (Fig. 2*B*) and accounted partially for the mesenteric vasodilator effect of 14,15-EET (Fig. 2*C*) but not for 5,6-EET (Fig. 2*A*). Thus the mechanism of the vascular action for each EET demonstrated a degree of specificity. However, species differences, experimental conditions (in vivo vs. in vitro), vascular territories (coronary vs. renal), and different blood vessel sizes (conduit arteries vs. arterioles) are factors that urge caution when interpreting differences noted on comparing studies of vascular responses to eicosanoids.

In conclusion, our results suggest a close interaction involving 11,12-EET and the HO/CO system in rat mesenteric

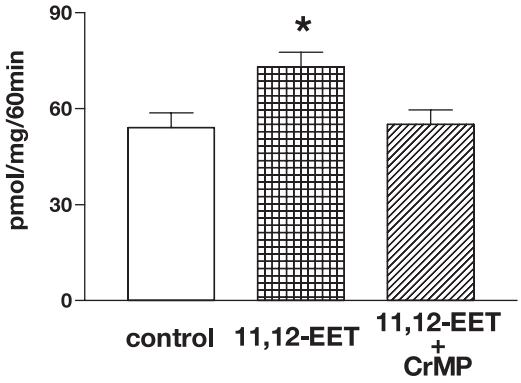


Fig. 3. Effect of 11,12-EET on HO activity in mesenteric microvessels. Microvessels were incubated for 60 min with either 11,12-EET (10 μM) or 11,12-EET (10 μM) + CrMP (15 μM). Carbon monoxide was measured by gas chromatography. Results are expressed as means ± SE of 3 experiments. *$P < 0.05$ vs. nontreated mesenteric microvessels.

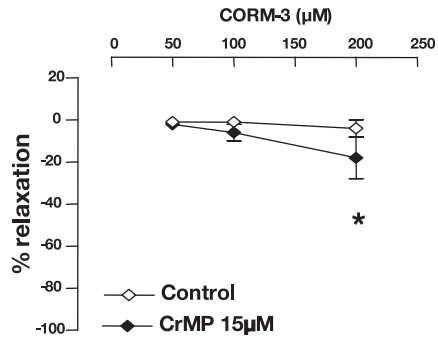


Fig. 4. Dose-response effects of CORM-3 on mesenteric arterial microvessels preconstricted with PE before and after inhibition of HO with CrMP (15 μM). Results (means ± SE) are expressed as percent decrease in vasoconstriction produced by PE; $n = 4$. *$P < 0.05$.

arteries; namely, rat mesenteric vasodilation produced by 11,12-EET is related to activation of the HO system.

## GRANTS

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