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.—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 indomethacin (2.8 μM), the HO inhibitor chromium mesoporphyrin (CrMP) (15 μM), chromium mesoporphyrin (CrMP) (15 μM), the soluble guanylyl cyclase (GC) inhibitor ODQ (10 μM), and the calcium-activated potassium channel inhibitor ibotenic acid (25 nM). The vasodilator response to 11,12-EET was abolished by CrMP and ibotenic acid, 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.

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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. 1A) 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. 1A), 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. 1B). 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. 1D) but did not affect the response to 11,12-EET (Fig. 1C). To evaluate whether the mesenteric vasodilator action of 11,12-EET was dependent on activation of K\(_{\text{ca}}\) channels, we determined the ability of the K\(_{\text{ca}}\) channel inhibitor, iberiotoxin, to affect the vascular response to 11,12-EET. Iberiotoxin (25 nM) abolished the vasodilation produced by 11,12-EET (Fig. 1E).

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. 2B) and partially inhibited the effect of 14,15-EET (Fig. 2C), but it did not affect vasodilatation to 5,6-EET (Fig. 2A). Furthermore, ODQ did not affect vasodilatation to 5,6-EET (Fig. 2A) and 8,9-EET (Fig. 2B), whereas it decreased the effect of 14,15-EET (Fig. 2C).

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).

**RESULTS**

**Fig. 1.** Dose-response effects of 11,12-epoxyeicosatrienoic acid (EET; A) and acetylcholine (ACh; B) on mesenteric arterial microvessels preconstricted with phenylephrine (PE) before and after inhibition of heme oxygenase (HO) with chromium mesoporphyrin (CrMP; 15 μM; C and D), and inhibition of calcium-activated potassium (K\(_{\text{ca}}\)) channel with iberiotoxin (25 nM; E). Results (means ± SE) are expressed as percent decrease in vasoconstriction produced by PE; n = 6. *P < 0.05.
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 KCa 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 KCa channels with iberiotoxin, charybdotoxin, or tetraethylammonium prevented the vasodilator response to EETs provide strong support for a primary role of KCa channels in mediating the vasodilator response to EETs (5, 8). Several investigators found that EETs have no effect on the activity of the KCa channel in inside-out detached membrane patches excised from VSM cells (26), suggesting that EETs do not directly activate the KCa channel in VSM.

CO directly activates KCa channels in vascular smooth muscle cells by altering the apparent calcium dependence of KCa 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 KCa 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 KCa channels. Because vasodilation 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. 1C). The vascular responses to both 11,12-EET and CO were inhibited by blockade of KCa channels (20, 26). Dependency of the vascular action on HO activity was evident for 8,9-EET (Fig. 2B) and accounted partially for the mesenteric vasodilator effect of 14,15-EET (Fig. 2C) but not for 5,6-EET (Fig. 2A). 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...
arteries; namely, rat mesenteric vasodilation produced by 11,12-EET is related to activation of the HO system.

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