

11,12-EET increases porto-sinusoidal resistance and may play a role in endothelial dysfunction of portal hypertension

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ABSTRACT

CYP450-dependent epoxyeicosatrienoic acids (EETs) are potent arterial vasodilators, while 20-hydroxyeicosatetraenoic acid (20-HETE) is a vasoconstrictor. We evaluated their role in the control of portal circulation in normal and cirrhotic (CCl₄ induced) isolated perfused rat liver. Phenylephrine (PE) and endothelin-1 (ET-1) increased portal perfusion pressure, as did arachidonic acid (AA), 20-HETE, and 11,12-EET. Inhibition of 20-HETE with 12,12-dibromododecenoic acid (DBDD) did not affect basal pressure nor the responses to PE, ET-1, or AA. However, inhibition of epoxygenase with miconazole caused a significant reduction in the response to ET-1 and to AA, without affecting neither basal pressure nor the response to PE. Hepatic vein EETs concentration increased in response to ET-1, and was increased in cirrhotic, compared to control, livers. 20HETE levels were non-measurable. Miconazole decreased portal perfusion pressure in cirrhotic livers. In conclusion, 20HETE and EETs increase portal resistance; EETs, but not 20-HETE, mediate in part the pressure response to ET-1 in the portal circulation and may be involved in pathophysiology of portal hypertension.

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1. Introduction

Increased resistance to portal flow plays a major role in the pathogenesis of portal hypertension of cirrhosis [1]. Resistance to portal flow is increased not only because of anatomical alterations, but also because of increased vascular tone [2]. Various vasoconstrictors, like norepinephrine, angiotensin II [3], endothelin-1 (ET-1), are increased in cirrhosis, and upregulation of both ET-A and ET-B receptors has also been shown in hepatic stellate cells [4]. Furthermore, endothelium-dependent vasodilators are probably deficient in the liver, since endothelial NO production is decreased because of post-transcriptional down-regulation of NOS [5]. Among vasoactive systems, cytochrome P450-dependent (CYP450) arachidonic acid (AA) metabolites play an important role in the control of different vascular beds, like the kidney, the heart, the brain, and the mesenteric circulations [6–10]. These compounds have been shown to affect vascular tone directly and indirectly, interacting with other vasoactive systems, like endothelins [11] and nitric oxide [12]. Increased urinary excretion of the potent vaso-

constrictor, 20-hydroxyeicosatetraenoic acid (20-HETE), has been demonstrated in patients with cirrhosis [13]. The liver is the richest organ in cytochrome P450 [14], and the highest production is that of the potent arterial vasodilators epoxyeicosatrienoic acids (EETs) [15]. Recent data have shown that these substances are the most powerful known vasodilators in the coronary circulation [6,7]. No information is available on the role of P450-dependent AA metabolites in the control of porto-sinusoidal circulation and in the pathophysiology of portal hypertension. We evaluated the role of P450-dependent AA metabolites, 20-HETE and the EETs, in the control of isolated perfused porto-hepatic circulation from normal and cirrhotic rats.

2. Materials and methods

Male Sprague–Dawley rats were used. All the animal experiments followed an institutionally approved protocol in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

2.1. Cirrhosis induction

Cirrhosis was induced in animals (approx. 150 g b.w.) by combined treatment with CCl₄ and phenobarbital, the latter given in drinking water (1.75 g/l) [16]. After 1 week of phenobarbital administration, CCl₄ was given by gavage, starting with a dose of 30 µl,

Abbreviations: EET, epoxyeicosatrienoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; AA, arachidonic acid; CYP450, cytochrome P450; PE, phenylephrine; ET, endothelin; DBDD, dibromododecenoic acid; CCl₄, carbon-tetrachloride; COX, cyclooxygenase.

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and then increasing doses were administered according to changes in body weight as described by Proctor et al. [17]. CCl₄ was administered once a week and then twice a week for 8–10 weeks. Treatment was terminated 1 week prior to conducting experiments. Rats on phenobarbital during the time of treatment were used as controls. Free access to standard chow was allowed throughout the study. In all the CCl₄-treated rats used for study, portal pressure was measured by a Satham transducer after direct cannulation of the portal vein before the experiments; rats with portal pressure <8 mmHg were excluded from the study.

2.2. Isolated perfused liver preparation

Rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). After midline and transverse subcostal incisions, the portal vein was cannulated with an 18-gauge cannula and connected to a Satham transducer to determine portal pressure. Once portal pressure was recorded, the cannula was connected to a roller pump. The hepatic artery was tied, as was the inferior vena cava, just above the renal veins. The hepatic veins were cannulated through the right atrium and the inferior vena cava to collect the effluent. The portal vein was perfused with oxygenated (95% O₂, 5% CO₂) Krebs–Henseleit solution, pH 7.4 at 37 °C and a flow rate of 8 ml/100 g b.w./min. In a pilot study, the bile was collected via PE10 tubing and a flow rate >8 μl/min/100 g b.w. was always present. Pressure was measured by a Satham transducer and continuously recorded with a computerized system.

2.3. Responses to agonists and inhibitors

After an initial 30-min basal period, concentration response curves were constructed with PE, -8 to -5 log mol; and ET-1, -12 to -10 log mol, in the presence and absence of 20-HETE synthesis inhibition with 12,12-dibromododec-11-enoic acid (DBDD), 2 μM, or of epoxygenase inhibition with miconazole, 1.0 μM. The doses used were chosen based on our experience and according to Chu et al. [18] and Wang et al. [19]. The maximum response was obtained within 60 s; perfusion pressure returned to baseline values in 2–3 min after PE and 10–15 min after ET-1. Increasing doses were added after return to the baseline pressure. Experiments performed to determine the response to 20-HETE, 11,12-EET were done using three doses: 10–50–100 μmol in 30 s, with and without inhibition of cyclooxygenase (COX) with indomethacin (2.8 μM). Experiments performed to determine the effect of DBDD, miconazole, and indomethacin on the response to arachidonic acid (AA) were also done using the same doses.

2.4. Generation of CYP-dependent AA metabolites by the liver

Levels of cytochrome P450-dependent AA metabolites, 20HETE and EETs (8,9-, 11,12-, and 14,15-EET) were measured in the liver effluent by gas-chromatography–mass-spectrometry, after extraction with ethyl acetate and reverse phase HPLC, as previously reported [13,20]. After addition of deuterated (d2) 20-HETE, 8-9EET, 11-12-EET, 14-15-EET as internal standards, 22–28 ml samples from liver effluents were acidified to pH 3.5, eicosanoids were extracted with ethyl acetate, purified by reverse phase HPLC, using a linear gradient from acetonitrile:water:acetic acid (62.5:37.5:0.05%) to acetonitrile (100%) over 20 min at a flow rate of 1 ml/min, and quantitated by negative chemical GC/MS after derivatization. Pentafluorobenzyl (PFB) esters were prepared by the addition of α-bromo-2,3,4,5,6-penta-fluorotoluene (5 μl) and N,N-diisopropylethylamine (5 μl) to a sample dissolved in acetonitrile (100 μl), and the derivatization was continued at room temperature for 30 min. Trimethylsilyl (TMS) ethers of hydroxyls were prepared by dissolving the sample in

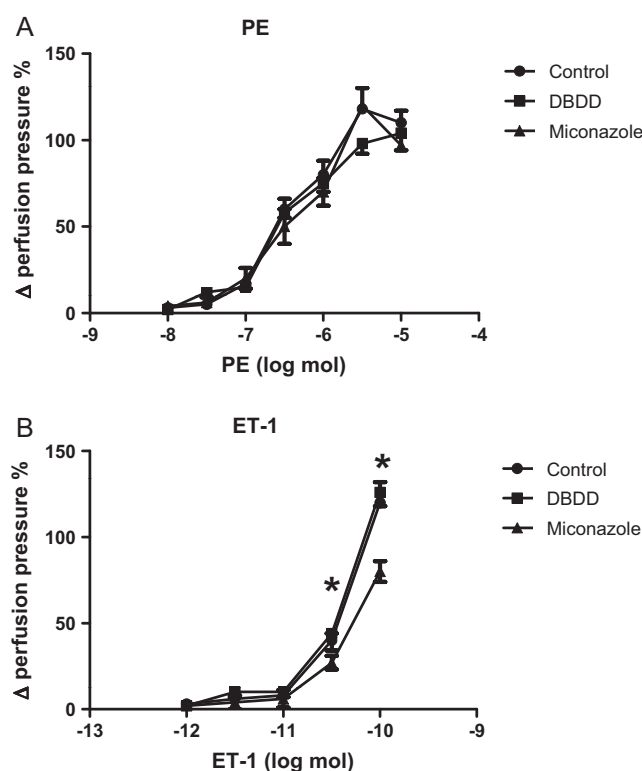


Fig. 1. Pressure response to bolus injections of phenylephrine (PE) (A) and endothelin-1 (ET-1) (B) in isolated perfused livers from normal ($n = 12$) rats, before and after inhibition of 20-HETE synthesis with DBDD (2 μM) and of epoxygenase with miconazole (1 μM). * $p < 0.01$.

N,O-bis(trimethylsilyl)trifluoroacetamide (80 μl) and the reaction was continued at room temperature for 30 min. To separate subterminal P450-HETEs and -EETs, samples were dissolved in iso-octane and 1 μl aliquots were injected into GC column using a temperature program ranging from 150 °C to 300 °C at a rate of 10 °C/min. Methane was used as a reagent gas at a flow resulting in a source pressure of 1.3 torr and the MS was operated in electron capture chemical ionization mode. The endogenous P450-HETEs and -EETs were identified (ion m/z 391) by comparison of GC retention times with authentic P450-HETE standards and quantitated by calculating the ratio of abundance with D2-20-HETE (m/z 393) and d2-EETs.

2.5. Statistical analysis

Results were expressed as means ± S.E.M. Concentration-response data were analyzed by two-way analysis of variance. Differences between groups were evaluated by unpaired Student's t -test. The effects of the inhibitors on the action of vasoconstrictors were evaluated by paired t -test. Statistical significance was set at $p < 0.05$.

3. Results

In the isolated perfused normal liver the vasoconstrictive effect of PE and ET-1 on portal circulation was not influenced by inhibition of 20-HETE synthesis with DBDD (Fig. 1A and B). Unexpectedly, inhibition of EET synthesis with miconazole significantly reduced vasoconstriction to ET-1, but not to PE (Fig. 1A and B).

As expected, 20-HETE caused vasoconstriction of the portal circulation (Fig. 2), which was COX-dependent, as it was inhibited by indomethacin. Surprisingly, also 11,12-EET caused vasoconstriction in the porto-hepatic circulation (Fig. 2). The effect of

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