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Leukotrienes and cyclooxygenase products mediate anaphylactic venoconstriction in ovalbumin sensitized rat livers

Sen Cui, Toshishige Shibamoto *, Hiromichi Takano, Wei Zhang, Yasutaka Kurata

Department of Physiology 2, Kanazawa Medical University, Uchinada, Ishikawa, 920-0293, Japan

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Abstract

Hepatic anaphylactic venoconstriction is partly involved in anaphylactic hypotension. We determined the chemical mediators responsible for anaphylaxis-induced segmental venoconstriction in perfused livers isolated from ovalbumin-sensitized rats. Livers were perfused portally and recirculatingly at constant flow with diluted blood. The portal venous pressure (Ppv), hepatic venous pressure (Phv), liver weight and hepatic oxygen consumption were continuously measured. The sinusoidal pressure was measured by the double occlusion pressure (Pdo), and was used to determine the pre-sinusoidal (Rpre) and post-sinusoidal (Rpost) resistances. After antigen injection, both Ppv and Pdo increased, resulting in 5.6- and 1.6-fold increases in Rpre and Rpost, respectively. Liver weight showed a biphasic change of an initial decrease followed by an increase. Hepatic oxygen consumption significantly decreased after antigen. Anaphylaxis-induced increase in Rpre was most extensively inhibited by 38.6% by pretreatment with ONO-1078 (100 μM, a cysteinyl leukotriene receptor-1 antagonist), among all antagonists or inhibitors administrated individually including TCV-309 (20 μM), AA-2414 (10 μM), ketanserin (10 μM) and indomethacin (10 μM). Combined pretreatment with indomethacin and ONO-1078 exerted additive inhibitory effects and attenuated Rpre by 65.8%. However, TCV-309, a platelet activating factor (PAF) receptor antagonist, did not affect the anaphylactic response. In contrast, anaphylaxis-induced increase in Rpost was attenuated only by ONO-1078 combined pretreatment. The antigen-induced changes in liver weight and hepatic oxygen consumption were attenuated significantly when hepatic venoconstriction was attenuated. It is concluded that cysteinyl leukotrienes and cyclooxygenase products, but not PAF, are mainly involved in anaphylaxis-induced pre-sinusoidal constriction in isolated perfused rat livers.

Keywords: Leukotriene; Cyclooxygenase; Double occlusion pressure; Hepatic vascular resistance

1. Introduction

Anaphylactic hypotension is sometimes life-threatening, and is caused by a decrease in effective circulating blood volume (Brown, 1995). It is reported that livers are partly involved in anaphylactic hypotension (Pavek et al., 1979; Shibamoto et al., 2005a). Indeed, anaphylaxis causes hepatic venoconstriction in rats (Hines and Fisher, 1992; Shibamoto et al., 2005a), guinea pigs (Ruan et al., 2004b), rabbits (Karasawa et al., 2007) and dogs (Yamaguchi et al., 1994). This anaphylactic hepatic venoconstriction results in portal hypertension which then causes congestion of the upstream splanchnic organs, with resultant decrease in venous return and effective circulating blood volume, and finally augmentation of anaphylactic hypotension. Furthermore, if anaphylactic venoconstriction occurs substantially in the hepatic post-sinusoidal veins, hepatic congestion, i.e., pooling of blood in liver itself develops, which could contribute to anaphylactic hypotension, as observed in canine anaphylaxis (Yamaguchi et al., 1994). Measurement of the sinusoidal pressure with the vascular occlusion method in guinea pig livers revealed that hepatic congestion was also caused by significant post-sinusoidal constriction, although the pre-sinusoidal constriction was predominant (Ruan et al., 2004b). On the contrary, in rats (Shibamoto et al., 2005a) and rabbits

Abbreviations: PAF, platelet-activating factor; Tx, thromboxane; Ppv, portal venous pressure; Phv, hepatic venous pressure; Pdo, double occlusion pressure; Q, portal blood flow rate; Rt, total portal-hepatic venous resistance; Rpre, pre-sinusoidal resistance; Rpost, post-sinusoidal resistance.

* Corresponding author. Tel.: +81 76 218 8104; fax: +81 76 286 8010.
E-mail address: shibamo@kanazawa-med.ac.jp (T. Shibamoto).

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(Karasawa et al., 2007), pre-sinusoidal veins almost selectively contract with a resultant reduction of liver blood volume.

Anaphylaxis is the classic well-established immunoglobulin E dependent reaction (Montanaro and Bardana, 2002). Various inflammatory mediators, including histamine, serotonin, platelet-activating factor (PAF), and eicosanoids such as cysteinyl leukotrienes, thromboxane (Tx) A_2, and prostaglandin D_2, are involved in the initiation and propagation of the anaphylactic responses (Hagmann et al., 1992; Lieberman, 1996; Morel et al., 1991; Selig et al., 1993; Terashita et al., 1990). However, the primary mediators responsible for the local anaphylactic response may differ among species, organs and tissues involved. Concerning hepatic anaphylaxis, histamine, which is stored in mast cells and released during degranulation, caused hepatic venoconstriction in guinea pigs, but not in rats (Shibamoto et al., 2004). We have further reported that PAF and cysteinyl leukotrienes, but not histamine, serotonin or cyclooxygenase products, were mainly involved in anaphylaxis-induced hepatic venoconstriction of guinea pig livers (Shibamoto et al., 2005b). But the mediators responsible for hepatic anaphylaxis in other species including rats have not been determined. The products of the arachidonic acid cascade, such as LTD_4 (Bilzer and Lauterburg, 1993; Cui et al., 2006a; Haussinger et al., 1988; Shibamoto et al., 2005b), TxA_2 (Cui et al., 2006a; Ruan et al., 2004a) and prostaglandins (Miwa et al., 1997), and the membrane phospholipid product of PAF (Cui et al., 2006a; Ruan et al., 2004a; Shibamoto et al., 2005b), all can induce hepatic venoconstriction. Serotonin, the receptor of which was expressed on hepatic stellate cells, is involved in portal hypertension via induction of portal venoconstriction (Datte et al., 2005; Li et al., 2006). However, it is not known which mediator is mainly involved in the anaphylactic pre- or post-sinusoidal constriction and hepatic liver weight change in the rat. Thus, the purpose of the present study was to determine the roles of PAF, TxA_2, cysteinyl leukotrienes, serotonin and prostaglandins in the pre- and post-sinusoidal constriction and liver weight change during rat hepatic anaphylaxis by using the corresponding receptor antagonists or enzyme-synthesis inhibitors.

2. Materials and methods

2.1. Animals

Sixty-six male Sprague–Dawley rats (Japan SLC, Shizuoka, Japan) weighing 353 ± 2 (S.E.M.) g were used in this study. Rats were maintained at 23 °C and under pathogen-free conditions on a 12/12-hours dark/light cycle, and received food and water ad libitum. The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University.

2.2. Antigen sensitization

Rats were actively sensitized by the subcutaneous injection of an emulsion made by mixing complete Freund's adjuvant (0.5 ml) with 1 mg ovalbumin (grade V, Sigma) dissolved in physiological saline (0.5 ml) (Cui et al., 2006b,c; Shibamoto et al., 2005a).

2.3. Isolated liver preparation

Two weeks after sensitization, the animals were anesthetized with pentobarbital sodium (60 mg/kg, ip) and mechanically ventilated with room air. The methods of isolation and perfusion of rat livers were previously described (Cui et al., 2006c). In brief, a polyethylene tube (0.5 mm ID, 0.8 mm OD) was placed in the right carotid artery. After laparotomy, the hepatic artery was ligated. At 5 min after intraarterial heparinization (500 U kg^{-1}), 8–9 ml of blood was withdrawn through the carotid arterial catheter. The intra-abdominal inferior vena cava above the renal veins was ligated, and the portal vein was cannulated with a stainless steel cannula (1.3 mm ID, 2.1 mm OD). After thoracotomy, the supradiaphragmatic inferior vena cava was cannulated through a right atrium incision with a larger stainless steel cannula (2.1 mm ID, 3.0 mm OD), then portal perfusion was begun with the heparinized autologous blood diluted with Krebs solution containing 5% bovine albumin at a Hct of 12%. The liver was rapidly excised, suspended from an isometric transducer (TB-652T, Nihon-Kohden, Japan) and weighed continuously throughout the experimental period.

The livers were perfused at a constant flow rate in a recirculating manner via the portal vein with blood that was pumped using a Masterflex roller pump from the venous reservoir through a heat exchanger (37 °C). The recirculating blood volume was 40 ml. The perfused blood was oxygenated in the venous reservoir by continuous bubbling with 95% O_2 and 5% CO_2 (perfusate PO_2 = 300 mmHg).

2.4. Measurement of hepatic vascular pressures and vascular resistances

The portal venous (Ppv) and hepatic venous (Phv) pressures were measured using pressure transducers (TP-400T, Nihon-Kohden, Japan) attached by sidearm to the appropriate cannulas with the reference points at the hepatic hilus. Portal blood flow rate (Q) was measured with an electromagnetic flow meter (MFV 1200, Nihon-Kohden, Japan), and the flow probe was positioned in the inflow line. The hepatic sinusoidal pressure was measured by the double occlusion pressure (Pdo) (Ruan et al., 2004b; Yamaguchi et al., 1994). Both the inflow and outflow lines were simultaneously and instantaneously occluded for 13 s using the solenoid valves, after which Ppv and Phv rapidly equilibrated to a nearly or identical pressure, which was Pdo. The principle of the double occlusion method to estimate the sinusoidal pressure (Yamaguchi et al., 1994) is derived from the concept of the mean circulating filling pressure (Pmcf) of the systemic circulation (Rothe, 1993). The Pmcf in the systemic circulation represents the pressure of the most compliant vascular segments, and therefore the Pdo, (i.e., the Pmcf in the isolated perfused livers) represents the pressure of the most compliant vessels in the liver, that is the sinusoids. Actually, Pdo values were obtained from the digitized data of Ppv and Phv