



Regular Article

Hepatic microvascular pressure during anaphylactic shock in anesthetized rats[☆]Hiromichi Takano^{a,1}, Toshishige Shibamoto^{a,*}, Wei Zhang^a, Yasutaka Kurata^a, Hirohisa Toga^b^a Department of Physiology II, Kanazawa Medical University, Uchinada 920-0293, Japan^b Department of Respiratory Medicine, Kanazawa Medical University, Uchinada 920-0293, Japan

ARTICLE INFO

Article history:

Received 11 May 2009

Revised 22 June 2009

Accepted 22 June 2009

Available online 30 June 2009

Keywords:

Anaphylaxis

Hepatic circulation

Portal hypertension

Servo-null system

Micropipettes

Microcirculation

ABSTRACT

Objective: Hepatic venoconstriction plays a significant role in anaphylactic hypotension in anesthetized rats. The purpose of this study is to determine whether the primary site of anaphylactic venoconstriction in the liver venous circulation occurs prior to or distal to the sinusoidal capillaries. We also determined whether the hepatic blood volume is increased during anaphylactic hypotension.

Methods: We measured, using a servo-null micropipette pressure-measuring system, the hepatic venular transmural pressure (P_{uhv}) at the liver surface of anesthetized rats sensitized with the antigen of ovalbumin (1 mg). We also measured the liver lobe thickness, using the ultrasonic crystal dimension measuring system. Anaphylactic hypotension was induced by an intravenous injection of 0.6 mg ovalbumin.

Results: When the antigen was injected, the systemic arterial pressure decreased profoundly from 118 ± 9 to 45 ± 4 mm Hg, which was accompanied by an increase in P_{pv} and P_{uhv} : P_{uhv} only transiently increased from 3.1 ± 0.9 to 8.8 ± 1.5 cmH₂O at 1 min and then rapidly returned to the baseline within 2 min, when P_{pv} continued to increase and reached the peak of 36 ± 7 cmH₂O at 3.5 min after antigen. This greater increase in P_{pv} -to- P_{uhv} gradient than that in P_{uhv} -to- P_{cv} gradient after antigen indicated that the constriction of the portal veins and the sinusoids much predominates over that of the hepatic veins. Along with this hepatic pre- and sinusoidal constriction, the liver lobe thickness significantly decreased by 4% after antigen.

Conclusion: Pre-sinusoidal constriction during anaphylactic shock in anaesthetized rats increased the portal venous pressure while the hepatic venular pressure only increased slightly and transiently. This predominant pre-sinusoidal constriction is accompanied by a decrease in liver volume.

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Introduction

Anaphylactic hypotension is in part caused by a decrease in effective circulating blood volume, which could be due to vasodilation with the peripheral pooling and increased vascular permeability with a shift of intravascular fluid to the extravascular space (Brown, 1995). In canine anaphylactic shock, hepatic venoconstriction, especially selective constriction of post-sinusoidal hepatic veins, induces pooling of blood in liver itself, as well as in upstream splanchnic organs (Enjeti et al., 1983; Essex, 1965; Yamaguchi et al., 1994). Likewise, in rat anaphylactic hypotension, hepatic venoconstriction also occurs (Hines and Fisher, 1992) and plays a significant role in the pathogenesis of blood pressure fall because hepatectomy combined with elimination of the splanchnic vascular beds attenuates the antigen-induced systemic hypotension in sensitized anesthetized rats

(Shibamoto et al., 2005). However, it is not known whether systemic anaphylaxis in rats causes constriction of post-sinusoidal hepatic veins, resulting in hepatic congestion, as observed in canine anaphylactic shock. Thus, the purpose of the present study was to determine, by measuring the hepatic venular pressure with a micropipette servo-null pressure measurement technique, whether the primary site of anaphylactic venoconstriction in the liver venous circulation occurs prior to or distal to the sinusoidal capillaries. We also measured the liver lobe thickness, as an index of the hepatic volume, using the ultrasonic crystal dimension measuring system during systemic anaphylaxis.

Methods

Animals

The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University. Twelve male Sprague–Dawley rat (Japan SLC, Shizuoka, Japan) weighing 405 ± 9 g were used in this study. Rats were maintained at 23 °C and under pathogen-free conditions on a 12:12-hour dark/light cycle, and allowed food and water ad libitum.

[☆] The authors declare that there is no conflict of interest.

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Sensitization

Rats were sensitized by the subcutaneous injection of an emulsion made by mixing equal volumes of complete Freund's adjuvant (0.5 mL) with 1 mg ovalbumin (grade V, Sigma) dissolved in physiologic saline (0.5 mL) (Shibamoto et al., 2005). Non-sensitized rats were injected with complete Freund's adjuvant and ovalbumin-free saline.

Surgical preparation of animals

After two weeks, the animals were anesthetized with pentobarbital sodium (50 mg/kg, ip), and placed on a thermostatically controlled heating pad (BWT-100, Bioresearch center inc. Nagoya, Japan) that maintained body temperature at 37 ± 0.5 °C throughout the experiment. The adequacy of anesthesia was monitored by the stability of blood pressure and respiration under control conditions and during tail pinch. Supplemental doses of anesthetic (10% of initial dose) were given intraperitoneally if necessary. Arterial pressure (Psa) was recorded from the right carotid artery. The right external jugular vein was catheterized and the catheter tip was positioned at the confluence of the superior vena cava and the right atrium. This catheter was used for an intravenous injection of antigen and measurement of the central venous pressure (Pcv). After midline incision, a catheter was inserted into the main portal vein via the cecal vein without occlusion of the portal vein for continuous measurement of the portal venous pressure (Ppv). The reference level was set at the level of right atrium. Pressures were measured using transducers (TP-400T, Nihon-Kohden, Japan), and digitally recorded and displayed continuously at 20 Hz (PowerLab, AD Instruments, Australia). Heart rate was determined by triggering the pulsatile Psa waves recorded in Power Lab.

Measurement of the hepatic venular pressure

Respiratory movement of the diaphragm affected hepatic microvascular pressure measurement and the liver thickness measurement using ultrasonic crystal, as described below. Therefore, after the hepatic ligament was severed, a 1 mm thick lead barrier, shaped to conform to the diaphragm, was inserted between the diaphragm and liver lobes. A slot in the shield prevented compression of the vena cava and the aorta.

The left lateral liver lobe was placed on a plastic table to provide a stable support. For establishing the servo-null pipette circuit, the liver surface was bathed with a continuous drip of saline heated to body temperature with a heat exchanger.

Hepatic microvascular transmural pressure was measured with servo-null micropipette pressure-measuring system (900A, World Precision Instruments Inc. Sarasota, FL, USA). We used borosilicate glass micropipettes (G-1.5, Narishige, Tokyo, Japan). The pipettes were pulled to 2–5 μ m OD, sharpened to a 30° bevel. Just before use the pipettes were backfilled with a solution of 2 M NaCl. The micropipette was then hydraulically and electrically connected to the servo-null micropressure-measuring system (900A, World Precision Instruments Inc. Sarasota, FL, USA) and the output from the system was recorded on one of the recording channels of Powerlab system (AD Instruments, Sydney, Australia). The pipette was inserted into a microvessel using a micromanipulator (model MX-2, Narishige, Tokyo, Japan), while the field was viewed with a microscope (SZH, Olympus, Tokyo, Japan). Vessel size was measured with a scale on the microscope lens. A vessel was considered to be a post-sinusoidal hepatic venule if its flow and that of its branches were convergent (Maass-Moreno and Rothe, 1997). Fig. 1 shows the diagram for hepatic venules in which a micropipette was inserted. Sinusoids, portal venules, or hepatic arterioles could not be identified in our rats. At the end of the experiment, the height of the liver with respect to the middle of the right atrium was measured so that all pressures could be referred to

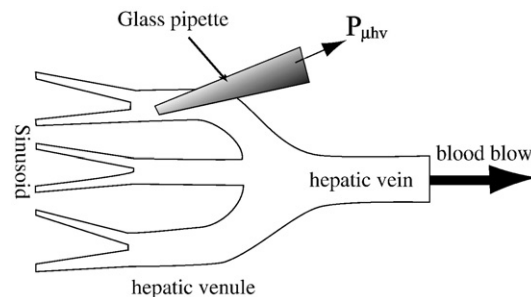


Fig. 1. A diagram showing the hepatic venules in which a micropipette was inserted.

this level. Movement of the liver caused by the inspiratory phase of ventilation induced artifacts in the pressure recording because the pipette tip intermittently touched or came within close proximity to the vessel wall as the tissue moved relative to the pipette tip. The lower segment of each pressure cycle recorded was not influenced by inspiratory pressure changes in that a brief stoppage of the ventilation pump at end expiration had little or no effect on this pressure. Then the average pressures during expiration were used. Liver volume change during anaphylactic hypotension also induced artifacts in the pressure recording. Some artifacts in the pressure recording could be eliminated by a movement of the pipette within the vessel lumen.

Liver thickness measurement

A pair of ultrasonic crystals (1 mm diameter; Sonometrics Co., Canada) was applied to the surface of the middle lobe of the liver; one crystal was attached on the lower surface of the lobe and another on the upper surface. The distance between these two crystals, an index of the liver lobe thickness, was measured with a digital ultrasonic crystal measurement system, sonomicrometer (Sonometrics Co., Canada).

Protocol of the experiment

The sensitized rats were assigned to the anaphylactic shock group ($n = 6$), while the non-sensitized rats to the control group ($n = 6$). Hemodynamic parameters were observed for at least 20 min after surgery until a stable state was obtained. After the baseline measurements, ovalbumin antigen (0.6 mg) was intravenously injected in both the anaphylactic shock and control groups. The Psa, Pcv and Ppv were continuously measured with pressure transducers. The separation of a pair of crystals was also measured simultaneously.

Statistics

All results are expressed as the means $\pm$ SE. For statistical analysis, comparison of individual values within a group was made by repeated measure analysis of variance followed by the Bonferroni post-hoc test correction method. Comparison of individual points between the two groups was made by Student's *t*-test. Differences were considered statistically significant at $P < 0.05$.

Results

The basal hepatic vascular pressure profile

The basal hepatic vascular pressure profile, as obtained in 12 measurements from 12 anesthetized rats, are as follows; Ppv was 11 ± 0.9 cmH₂O, P μ hv 3.1 ± 0.9 cmH₂O, and Pcv 1.2 ± 0.1 cmH₂O. Thus the post-sinusoidal pressure gradient comprised $20 \pm 9\%$ of the total Ppv-to-Pcv gradient in anesthetized rats.

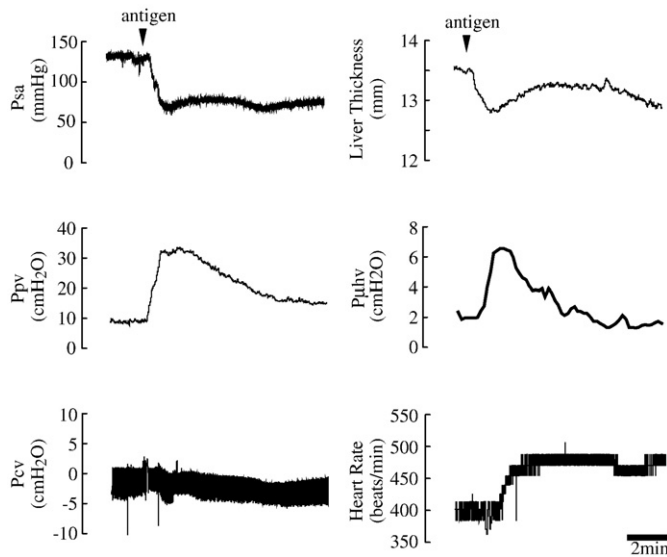


Fig. 2. A representative recording of the response to ovalbumin antigen (0.6 mg) in an anesthetized rat. Psa, systemic arterial pressure; Ppv, portal vein pressure; Pcv, central venous pressure; Puhv, hepatic venular pressure measured by servo-null micropipette.

Anaphylactic hypotension

Fig. 2 shows a representative example of the response to a venous injection of the antigen in an anesthetized rat sensitized with ovalbumin. After an antigen injection in the sensitized group, Psa and Ppv simultaneously began to decrease and increase, respectively. Psa rapidly decreased from the baseline of 118 ± 9 mm Hg to 75 ± 11 mm Hg at 1 min after antigen and then continued to decrease progressively to the nadir of 45 ± 3.9 mm Hg at 5 min, followed by a gradual recovery to 90 ± 4 mm Hg at 60 min, as shown in Fig. 3 (upper panel). Ppv increased from the baseline of 11.0 ± 0.9 cmH₂O to the peak value of 36.0 ± 7.2 cmH₂O at 3.5 min after antigen and then decreased to 15.0 ± 0.3 cmH₂O at 10 min (middle panel of Fig. 3). After that, Ppv remained at this level. In contrast, Puhv, which was measured by the micropipette, increased only transiently from the baseline of 3.1 ± 0.9 cmH₂O to the peak of 8.8 ± 1.5 cmH₂O at 1 min after antigen and then quickly returned to the baseline at 2.5 min, followed by a decrease to 0.3 ± 1.6 cmH₂O at 8 min and finally recovery to 2.5 ± 0.8 cmH₂O at 60 min, as shown in Fig. 3 (middle panel). Pcv significantly decreased from the baseline of 1.2 ± 0.1 cmH₂O to the nadir of -0.15 ± 0.4 cmH₂O at 6 min after antigen and then recovered to 1.0 ± 0.4 cmH₂O at 10 min. Heart rate significantly increased when Psa decreased. In the control groups, these hemodynamic variables did not change significantly throughout the experimental period.

Fig. 3 (lower panel) shows the summary data of time course changes in hepatic vascular pressure gradients among Ppv, Pcv and Puhv. In the sensitized group, the Ppv-to-Puhv gradient increased markedly from a baseline of 8.0 ± 1.3 to 34 ± 8.5 cmH₂O at 3.5 min after antigen. The Puhv-to-Pcv gradient increased minimally, but significantly, from the baseline of 1.9 ± 0.9 to 7.8 ± 2.7 cmH₂O at 1 min, and then decreased to 2.0 ± 0.9 at 3.5 min (Fig. 3). This indicates that an injection of the antigen almost selectively increased the portal vein and sinusoid gradient rather than post-hepatic venule gradient, as reflected by a significant increase in the (Ppv-to-Puhv)/(Ppv-to-Pcv) ratio from 0.81 ± 0.06 to 0.94 ± 0.08 . In the control group, an injection of the antigen did not produce any significant change in the variables of the pressure gradients.

Fig. 4 shows the time course changes in liver lobe thickness. The baseline liver middle lobe thickness as measured by ultrasonic crystals ranged from 8.2 to 13.7 mm. In the sensitized group, concomitant with hepatic venoconstriction, the liver thickness decreased to the nadir values of $95.8 \pm 0.8\%$ of the baseline at 1.5 min after antigen. Thereafter

it returned to $98 \pm 1.3\%$ of baseline at 7 min, followed by a gradual decrease to $94 \pm 2\%$ of baseline at 60 min. The liver thickness in the control group did not change significantly throughout the experimental period.

Discussion

In the present study we measured Puhv, Ppv and Pcv, along with liver thickness during anaphylactic shock in the anesthetized rats. We found that Puhv and Ppv significantly increased after antigen, but the increase in Puhv was not only much shorter in duration, but also much smaller in magnitude than that in Ppv. These findings indicate that the pre-sinusoidal and/or sinusoidal vessels predominantly contract over the post-sinusoidal vessels during rat anaphylactic shock. This predominant pre/sinusoidal constriction was accompanied by a decrease in the middle lobe thickness, indicating that the hepatic blood volume was reduced by a pre-sinusoidal vasoconstriction during anaphylactic hypotension in anesthetized rats. These findings

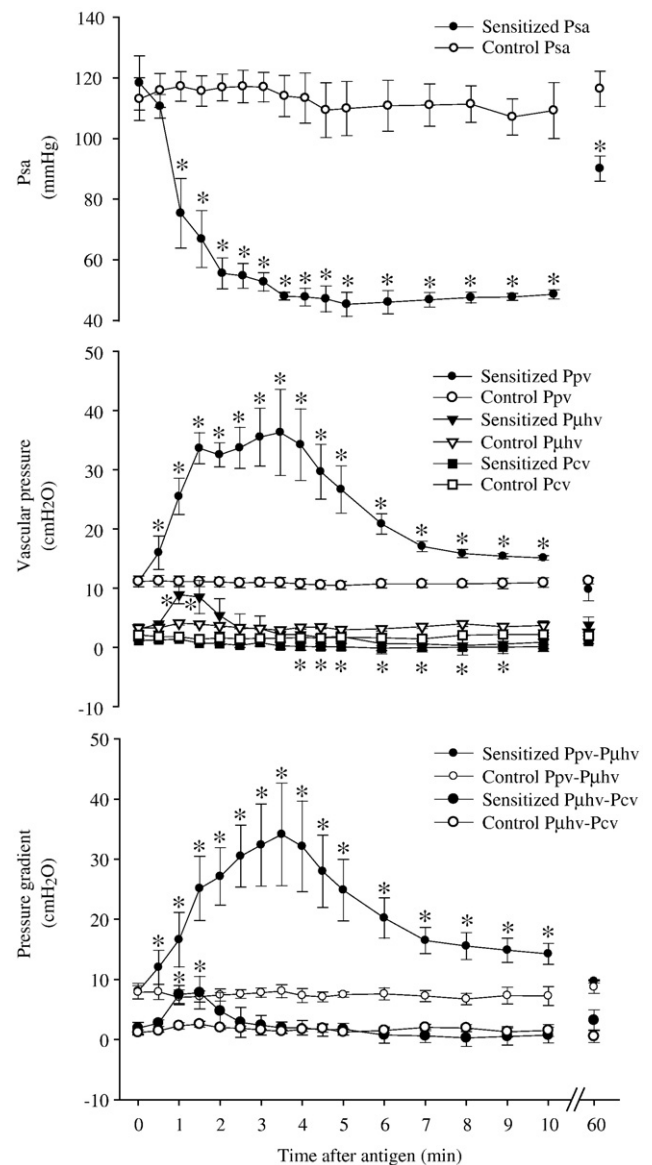


Fig. 3. The summary of time course changes in hemodynamic variables of the sensitized rats ($n=6$, closed symbols) and the control rats ($n=6$, open symbols). Upper panel: Psa. Middle panel: Ppv (circles), Pcv (squares) and Puhv (triangles). Lower panel: the Ppv-to-Puhv gradients (small) and the Puhv-to-Pcv gradients (large circles). Means $\pm$ SE; * $P<0.05$ vs. baseline and the control group.

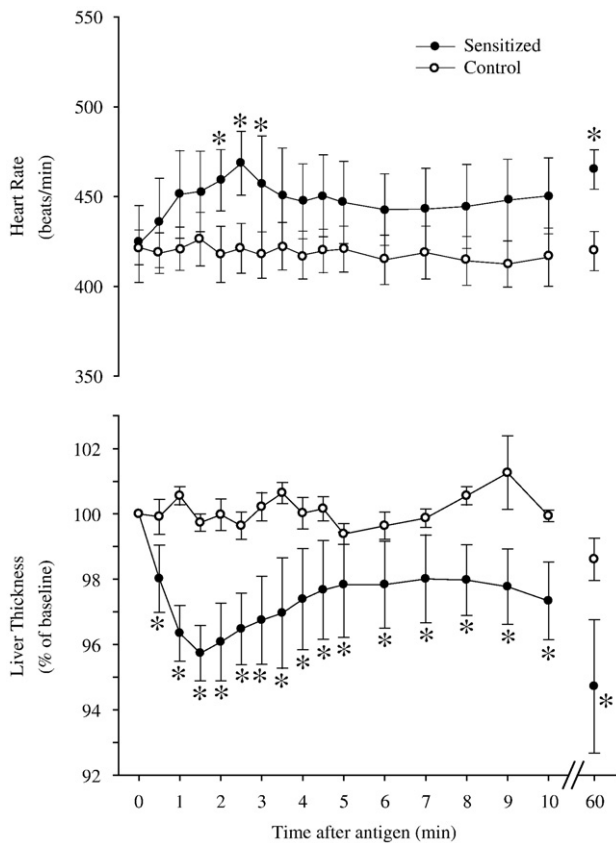


Fig. 4. The summary of time course changes in heart rate and liver thickness of the sensitized rats ($n=6$, closed circles) and the control rats ($n=6$, open circles). Means $\pm$ SE; * $P<0.05$ vs. baseline and the control group.

contrast with the hepatic congestion as observed in canine anaphylactic shock (Essex, 1965).

In the anesthetized rats of the present study, the basal hepatic vascular pressure profile was revealed as follows: Ppv, P_{uhv} and Pcv were 11, 3.1 and 1.2 cmH₂O, respectively. This indicates that the pre-sinusoidal or sinusoidal pressure gradient (Ppv-to-P_{uhv}) comprised 80 $\pm$ 9% of the total portal to vena caval pressure gradient (Ppv-to-Pcv). However, this is not in agreement with the previous findings of Rothe and Maass-Moreno (1998), who reported, using the similar servo-null micropipette system in anesthetized rabbits, that the Ppv-to-P_{uhv} gradient is up to 35% of the Ppv-to-Pcv gradient. This difference from our findings may be attributable to species differences between rat and rabbit. Indeed, we previously demonstrated, by measuring the sinusoidal pressure with the double occlusion pressure (Pdo) in isolated perfused rat liver preparation, that the Ppv-to-Pdo gradient comprised 72 $\pm$ 1% of the total Ppv-to-P_{uhv} (Shibamoto et al., 2005). This finding of the perfused rat livers is consistent with that of the present anesthetized rats.

The most important finding of the present study is that anaphylactic hepatic venoconstriction in *in vivo* anesthetized rats was primarily due to pre-sinusoidal or sinusoidal constriction rather than post-sinusoidal constriction. To our knowledge, this is the first report concerning the site of hepatic venoconstriction during anaphylactic shock in *in vivo* anesthetized rats. This predominant pre-sinusoidal constriction induced by anaphylaxis in anesthetized rats contrasts with the post-sinusoidal constriction in canine anaphylaxis (Essex, 1965; Yamaguchi et al., 1994), which results in hepatic congestion. This finding of anaphylactic pre-sinusoidal constriction in rats is consistent with our previous finding obtained from the isolated ovalbumin-sensitized rat livers perfused at constant flow, in which the ovalbumin antigen caused a substantial increase in Ppv but only a marginal

increase in the sinusoidal pressure: the pre-sinusoidal resistance increased by 250% the baseline, while the post-sinusoidal resistance increased by only 67% the baseline (Shibamoto et al., 2005).

We previously reported using the genetically mast cell-deficient rats that anaphylactic hepatic venoconstriction in anesthetized rats may be caused by vasoactive mediators released from the mast cells (25%) and other cells (75%) (Shibamoto et al., 2007). In addition, mast cells may account for almost hepatic anaphylactic reaction which occurs within the isolated perfused rat liver (Shibamoto et al., 2007). In the subsequent study, we demonstrated that anaphylactic venoconstriction observed in isolated perfused rat livers was caused mainly by cysteinyl leukotrienes and cyclooxygenase products (Cui et al., 2007).

In the present study, a significant increase in P_{uhv} was observed only at the early phase after antigen. This significant constriction of the post-sinusoidal hepatic veins was overwhelmed by a subsequent pre/sinusoidal constriction, which was reflected by a selective increase in the Ppv-to-P_{uhv} gradient (after 2 min in the lower panel of Fig. 3). This initial significant hepatic venous constriction may be caused by the venoconstriction mediators released from hepatic anaphylactic reaction caused by the antigen transported via the hepatic artery. Although uncertain, it is accepted that, in the rat liver, the point of confluence between the hepatic artery and the portal veins is mainly in the sinusoids (Alexander et al., 2001; Watanabe et al., 1994). Thus, the antigen via the hepatic artery reached first the liver at the site of the post-capillary, and there they may react with the hepatic mast cells with resultant release of vasoactive mediator around post-sinusoidal vessels, which could appear as the increase in P_{uhv}. With time, large amounts of mediators released from the splanchnic bed where mast cells are abundantly distributed might enter the portal veins and then constricted vigorously the pre-sinusoidal portal vein system, which turned out the predominant the pre/sinusoidal constriction in the present study.

We estimated the changes in liver blood volume using the ultrasonic crystal dimension measuring system (Greenway and Rothe, 1992). Although this method has a problem in accuracy, it determined whether the liver volume increased or decreased (Greenway and Rothe, 1992). Actually, in the present study, the liver thickness was significantly decreased by approximately 0.5 mm (4%) during anaphylaxis. Hepatic blood volume reduction is consistent with the findings of the isolated perfused livers which were exposed to anaphylactic reaction; the anaphylactic hepatic venoconstriction was accompanied by a decrease in liver weight (5% of the initial liver weight; -0.5 ± 0.4 g $\cdot$ 10 g liver weight⁻¹) (Shibamoto et al., 2005). Hepatic vascular blood loss may be induced either by initial constriction of the post-sinusoidal capacitance veins, as reflected by an increase in the P_{uhv}-to-Pcv gradient, which might expel the intravascular blood to the heart, or by marked anaphylactic pre-sinusoidal constriction, although the mechanism is unknown. It is likely that the blood volume of the sinusoids, which were situated downstream to the constricted pre-sinusoids, might be passively reduced.

The limitations in this study include our inability to puncture the sinusoids and the portal venules in rats. Maass-Moreno and Rothe were able to measure sinusoidal pressure and pre-sinusoidal (portal venule) as well as hepatic post-sinusoidal (hepatic venular) pressures in rabbits (Maass-Moreno and Rothe, 1997; Rothe and Maass-Moreno, 1998). Because the sinusoids compose of most microvessels on the liver surface and contain most of the blood in the liver, measurement of changes in sinusoidal pressure would have been preferred, but these vessels are too small (10 μ m) for stable pressure measurements. We could identify only a few portal venules large enough to penetrate, because the portal venules were embedded in fibrous tissue and extended deeper in the tissue. In addition, it is possible that the pressure profile measured in these surface vessels does not reflect the profile of vessels located further within the liver parenchyma. Finally, we did not measure blood flow to liver in the present study. The pressure gradient changes do not always indicate the changes in

vascular resistances because the pressure gradient could change as a consequence of flow changes at constant vascular resistance. However, we recently found that portal blood flow measured with the ultrasound flow probe (small animal flowmeter, Transonic, Ithaca, NY) reduced to 25% baseline as Ppv began to increase after an injection of antigen into the sensitized rats (unpublished data). Thus, it is suggested that the increase in the pressure gradient observed in the present study is caused by increased vascular resistances or active venoconstriction.

We measured microvascular hepatic venular pressure along with portal venous pressure and liver lobe thickness during anaphylactic shock in anesthetized rats. We found that the antigen injection caused only a transient and minimal increase in P_{uhv} and the substantial and persistent increase in Ppv. This predominant pre/sinusoidal constriction was accompanied by a decrease in the middle lobe thickness, i.e., hepatic volume reduction. We conclude that the portal and sinusoidal vasculatures are the dominant resistance sites of the liver venous vasculature both at basal state and during anaphylaxis-induced venoconstriction in anesthetized rats. This predominant pre-sinusoidal constriction is accompanied by a passive reduction of liver blood volume.

Acknowledgments

This study was supported by a Grant-in-Aid for Scientific Research (18591730, 19592103) from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan. Dr. Wei Zhang was supported by postdoctoral fellowships for foreign researchers of Kanazawa Medical University in 2007.

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