

Involvement of splanchnic vascular bed in anaphylactic hypotension in anesthetized BALB/c mice

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Liu W, Takano H, Shibamoto T, Cui S, Zhao Z-S, Zhang W, Kurata Y. Involvement of splanchnic vascular bed in anaphylactic hypotension in anesthetized BALB/c mice. *Am J Physiol Regul Integr Comp Physiol* 293: R1947–R1953, 2007. First published August 22, 2007; doi:10.1152/ajpregu.00904.2006.—Using in vivo and isolated perfused liver preparations of BALB/c mice, we determined the roles of the liver and splanchnic vascular bed in anaphylactic hypotension. Intravenous injection of ovalbumin antigen into intact-sensitized mice decreased systemic arterial pressure (P_{sa}) from 92 ± 2 to 39 ± 3 (SE) mmHg but only slightly increased portal venous pressure (P_{pv}) from 6.4 ± 0.1 cmH₂O to the peak of 9.9 ± 0.5 cmH₂O at 3.5 min after antigen. Elimination of the splanchnic vascular beds by ligation of the celiac and mesenteric arteries, combined with total hepatectomy, attenuated anaphylactic hypotension. Ligation of these arteries alone, but not partial hepatectomy (70%), similarly attenuated anaphylactic hypotension. In contrast, isolated sensitized mouse liver perfused portally at constant flow did not show anaphylactic venoconstriction but, rather, substantial constriction in response to the anaphylaxis-associated platelet-activating factor, indicating that venoconstriction in mice in vivo may be induced by mediators released from extrahepatic tissues. These results suggest that splanchnic vascular beds are involved in BALB/c mouse anaphylactic hypotension. They presumably act as sources of chemical mediators to cause the anaphylaxis-induced portal hypertension, which induced splanchnic congestion, resulting in a decrease in circulating blood volume and, thus, systemic arterial hypotension. Mouse hepatic anaphylactic venoconstriction may be induced by factors outside the liver, but not by anaphylactic reaction within the liver.

anaphylactic shock; hepatic circulation; portal hypertension; splanchnic congestion

ANAPHYLACTIC HYPOTENSION appears to be caused primarily by a decrease in blood flow to the heart, because left ventricular function is relatively well preserved during anaphylactic shock (1, 2). Peripheral circulatory collapse is ascribed to hypovolemia, which results from a decrease in effective circulating blood volume. The latter could be due to vasodilation with the peripheral pooling and increased vascular permeability with a shift of intravascular fluid to the extravascular space (1, 2).

In canine experimental models of anaphylactic shock, an increase in resistance to venous return is important in the pathogenesis of circulatory collapse (27): increased venous resistance decreases venous return, with a resultant decrease in stroke volume and systemic arterial pressure. Indeed, eviscerated dogs did not develop anaphylactic shock (19). In addition, Enjeti et al. (9) reported that the severity of the anaphylactic

shock could be decreased by occlusion of the descending aorta. In dogs, the anaphylaxis-induced increase in venous resistance is partly caused by constriction of the hepatic veins (27, 30), which induces pooling of blood in the liver, as well as in upstream splanchnic organs. In addition, we recently demonstrated that the liver and splanchnic vascular beds are involved in rat anaphylactic hypotension by showing that elimination of splanchnic circulation with total hepatectomy attenuated systemic hypotension induced by the ovalbumin antigen in anesthetized sensitized rats (23). Moreover, substantial hepatic venoconstriction is also observed in anaphylaxis of rabbits (17), guinea pigs (21), cats (4), and calves (16). On the other hand, in the mouse, although immunologic mechanisms for systemic anaphylaxis have been extensively studied (11), the hemodynamic mechanism for anaphylactic shock in mice has not been well clarified because of the technical difficulty involved in measurement of hemodynamic variables in this small animal (3). We hypothesized that, as in dogs and rats, in the mouse, systemic anaphylaxis causes hepatic venoconstriction followed by splanchnic congestion and, possibly, hepatic congestion, resulting in a decrease in venous return and systemic hypotension.

The first purpose of the present study was therefore to determine whether the liver and/or splanchnic vascular bed is involved in anaphylactic hypotension in anesthetized BALB/c mice. To test this hypothesis, we have established anaphylactic hypotension models of mice instrumented for measurement of systemic and hepatic hemodynamics. Changes in systemic arterial pressure and portal pressure were observed in antigen-challenged sensitized mice with the following surgical procedures: 1) hepatectomy alone, i.e., partial (70%) hepatectomy with perfusion of the splanchnic vascular bed preserved; 2) elimination of the splanchnic vascular bed by ligation of the celiac and mesenteric arteries without hepatectomy; 3) elimination of the liver and splanchnic vascular bed, i.e., total hepatectomy combined with elimination of the splanchnic vascular bed by ligation of the celiac and mesenteric arteries; and 4) elimination of the spleen, i.e., splenectomy. In addition, using isolated perfused sensitized livers, we determined whether hepatic anaphylactic venoconstriction could occur within the liver without influence of the systemic circulation. We further determined the hepatic vascular resistance distribution by measuring the sinusoidal pressure with the double-occlusion method (30).

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MATERIALS AND METHODS

Animals. Seventy-six male BALB/c mice (Japan SLC, Shizuoka, Japan) were maintained at 23°C and under pathogen-free conditions on a 12:12-h dark-light cycle and allowed food and water ad libitum. The experiments were approved by the Animal Research Committee of Kanazawa Medical University.

Sensitization. Mice were actively sensitized by a subcutaneous injection of an emulsion consisting of 2 mg of aluminum potassium sulfate adjuvant and 0.01 mg of ovalbumin (grade V, Sigma) dissolved in 0.2 ml of physiological saline. The antigen was injected twice, with 1 wk between injections. Nonsensitized mice were injected with aluminum potassium sulfate adjuvant and ovalbumin-free saline. At 1 wk after the second injection, the mice were used for the following experiments.

Anesthetized mouse experiment. At 1 wk after sensitization, 57 mice (25 ± 2 g body wt) were anesthetized with pentobarbital sodium (100 mg/kg ip) and placed on a thermostatically controlled heating pad (model ATC-101B, Unique Medical), which maintained body temperature at $37 \pm 0.2^\circ\text{C}$ throughout the experiment. The adequacy of anesthesia was monitored by the stability of blood pressure and respiration under control conditions and during a pinch of the hind-paw. Supplemental doses (10% of initial dose) of anesthetic were given intraperitoneally as necessary. The tracheotomy was performed and a tracheal tube (18-gauge stainless needle) was inserted to ensure the airway patency. The right femoral artery was catheterized with a 0.45-mm-OD tapered-tip polyethylene catheter for measurement of mean systemic arterial pressure (P_{sa}) with a pressure transducer (model TP-400T, Nihon-Kohden). The right external jugular vein was catheterized with a 0.67-mm-OD, 0.47-mm-ID polyethylene catheter. The catheter tip was positioned at the confluence of the superior vena cava and the right atrium. This catheter was connected to a Y-type miniature plastic tube; one twig end of the tube was connected via a water-filled polyethylene tube to the pressure transducer for measurement of central venous pressure (P_{cv}), and the other twig end was used to introduce a thin inner polyethylene tube, which was tapered to ~0.3 mm diameter over hot air, for intravenous injection of antigen. Heart rate (HR) was measured by triggering the R wave of the electrocardiogram. The sensitized and nonsensitized animals were randomly divided into mice subjected to gastrointestinal isolation and hepatectomy (GI-HptX), mice subjected to partial hepatectomy (partial HptX), mice subjected to gastrointestinal isolation (GI), mice subjected to splenectomy (SpX), and intact mice.

Thus a mouse was randomly assigned to one of the following groups: 1) GI-HptX-sensitized ($n = 11$), 2) GI-HptX-control ($n = 10$), 3) intact-sensitized ($n = 10$), 4) intact-control ($n = 7$), 5) partial HptX-sensitized ($n = 10$), 6) GI-sensitized ($n = 9$), and 7) SpX-sensitized ($n = 10$). In the GI-HptX mice, through a 3-cm abdominal midline incision (laparotomy), ligation of the celiac artery and the mesenteric artery was followed by total hepatectomy, which consisted of resection of the median and left lateral lobe, the right lateral lobes, and the caudate lobes, as described by Gaub and Iversen (12). In the partial HptX mice, after laparotomy, 70% hepatectomy was performed, with removal of the median and left lateral lobes of the liver (14). In the GI-sensitized and SpX-sensitized mice, after laparotomy, ligation of the celiac artery and mesenteric artery, and splenectomy, respectively, was performed. Then, in the partial HptX, GI-sensitized, SpX-sensitized, and intact mice, a catheter (0.47 mm ID, 0.67 mm OD) was inserted into the main portal vein for continuous measurement of portal venous pressure (P_{pv}). After closure of the abdomen, the baseline measurements were started.

P_{sa} , P_{cv} , P_{pv} , and HR were continuously measured in all mice, except the GI-HptX group, in which P_{pv} was not measured because of total hepatectomy. These hemodynamic variables were continuously displayed on a thermal physiograph (model RMP-6008, Nihon-Kohden). Outputs were also digitally recorded at 20 Hz (PowerLab, ADInstruments). Hemodynamic parameters were observed for ≥ 20

min after surgery until a stable state was obtained. After the baseline measurements, 0.01 mg of the ovalbumin antigen in 100 μl of saline was administered via the inner thin tube with a small dead space, which was set in the jugular vein catheter, as described above.

Isolated perfused mouse liver experiment. Nineteen sensitized ($n = 7$) or nonsensitized ($n = 12$) mice (30 ± 2 g body wt) were anesthetized with pentobarbital sodium (70 mg/kg ip) and mechanically ventilated with room air. A smaller dose of pentobarbital sodium was used in this experiment than in the in vivo experiment, because anesthesia was required only during the short period of liver excision surgery. After laparotomy, heparin (500 mU/g) was injected into the intra-abdominal inferior vena cava (IVC), and at 5 min, blood was withdrawn from the IVC and used as the perfusate. Then the IVC above the renal veins and the hepatic artery were ligated and the portal vein was cannulated with a 20-gauge stainless steel cannula (0.9-mm-OD stainless steel needle) for portal perfusion. After thoracotomy, the supradiaphragmatic IVC was cannulated through a right atrial incision with a stainless cannula of the same size, and portal perfusion was begun with blood diluted with 5% bovine albumin (Sigma) in Krebs solution (118 mM NaCl, 5.9 mM KCl, 1.2 mM MgSO_4 , 2.5 mM CaCl_2 , 1.2 mM NaH_2PO_4 , 25.5 mM NaHCO_3 and 5.6 mM glucose). Heparinized blood obtained from a donor mouse was also added to the perfusate, and finally the liver was perfused with diluted blood at a hematocrit of 5%. Two to 3 min were required to start the perfusion after ligation of the hepatic artery. The liver was rapidly excised, suspended from an isometric transducer (model TB-652T, Nihon-Kohden), and weighed.

The basic method for isolated liver perfusion was described previously (24, 25). The liver was perfused at a constant flow rate in a recirculating manner via the portal vein with the diluted blood. Blood (15 ml) was pumped from the venous reservoir through a heat exchanger (37°C). The height of the reservoir and the blood flow rate could be adjusted independently to maintain the P_{pv} and hepatic venous pressure (P_{hv}) at any desired level. The blood was oxygenated in the reservoir by continuous bubbling with 95% O_2 -5% CO_2 . P_{pv} and P_{hv} were measured with pressure transducers connected to the corresponding sidearm with the reference point at the hepatic hilus. For measurement of double-occlusion pressure (P_{do}), two solenoid valves were placed around the perfusion tubes upstream from the P_{pv} sidearm cannula and downstream from the P_{hv} sidearm cannula (24). Blood flow rate ($\dot{Q}_{pv}$) was measured manually by collection of outflow blood for 1 min just before the baseline measurement and at the end of the experiment to confirm that blood flow was constant during the experimental period. The hepatic vascular pressures and liver weight were monitored continuously and displayed on the physiograph.

Hepatic hemodynamic parameters were observed for ≥ 20 min after the start of perfusion until an isogravimetric state (no weight gain or loss) was obtained by adjustment of $\dot{Q}_{pv}$ and the height of the reservoir at 0–1 cmH_2O P_{hv} . After the baseline measurements, the perfused livers excised from the sensitized mice ($n = 7$) were challenged with 0.01 mg of ovalbumin injected into the reservoir. The livers from nonsensitized mice were also injected with the antigen as the control ($n = 5$). We also examined the vasoreactivity of the perfused mouse livers to platelet-activating factor (PAF; $n = 7$). One milligram of PAF (Sigma) was dissolved in 1 ml of 99.5% ethanol, and this stock solution was stored -20°C . To determine the concentration dependence, we administered PAF as a bolus into the reservoir in a cumulative manner (0.001–10 μM). When P_{pv} peaked after an injection of a given dose of PAF, the double-occlusion maneuver was performed as described below, and then the next-high dose was injected.

The hepatic sinusoidal pressure was measured by the double-occlusion method (29). After 13 s of simultaneous and instantaneous occlusion of the inflow and outflow lines via the solenoid valves, P_{pv} and P_{hv} rapidly equilibrated to a similar or identical pressure, which was P_{do} , with use of Liver software from Biomedical Science. P_{do} was

measured at baseline and at 3, 6, and 10 min after antigen and then at 10-min intervals up to 40 min after antigen. In livers injected with PAF, P_{do} was measured at baseline and at maximal venoconstriction when P_{pv} reached the peak level for each dose of PAF.

The total portal-hepatic venous resistance (R_t), presinusoidal resistance (R_{pre}), and postsinusoidal resistance (R_{post}) were calculated as follows: $R_t = (P_{pv} - P_{hv}) \cdot \dot{Q}_{pv}^{-1}$; $R_{pre} = (P_{pv} - P_{do}) \cdot \dot{Q}_{pv}^{-1}$; and $R_{post} = (P_{do} - P_{hv}) \cdot \dot{Q}_{pv}^{-1}$.

Statistics. Values are means $\pm$ SE. Statistical analysis of the time course-dependent data between the baseline and the postantigen values within groups of the anesthetized mouse experiment and the isolated perfused mouse liver experiment were performed with repeated-measures (2-way) ANOVA. $P < 0.05$ was considered significant. When a significant difference was obtained, post hoc analysis was performed with Bonferroni's posttest correction method (15 comparisons for the anesthetized mouse experiment). Individual points between the intact-sensitized group and the other groups for the anesthetized mouse experiment were compared by the two-tailed multiple t -test with Bonferroni's correction following ANOVA (6 comparisons for P_{sa} and P_{cv} in 7 groups; 4 comparisons for P_{pv} in 4 groups). Individual points between the baseline and the post-PAF groups for the isolated perfused mouse were also compared by the two-tailed multiple t -test with Bonferroni's correction following ANOVA (5 comparisons). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Anesthetized mouse experiment. Figure 1 shows representative examples of the response to an intravenous injection of the ovalbumin antigen in the intact-sensitized and GI-HptX-sensitized groups. Figure 2 shows the summary data of time course changes in P_{pv} , P_{sa} , and P_{cv} of seven groups of anesthetized mice. After an antigen injection in the intact-sensitized group,

P_{sa} and P_{pv} simultaneously began to increase. P_{sa} rapidly increased from the baseline of 92 ± 2 mmHg to the peak of 106 ± 2 mmHg at 2 min after the antigen. Thereafter, P_{sa} decreased progressively to 41 ± 2 mmHg at 10 min and remained at this low level to the end of the experimental period. P_{pv} increased from the baseline of 6.4 ± 0.1 cmH₂O to the peak of 9.9 ± 0.5 cmH₂O at 3.5 min after antigen and gradually decreased to the baseline levels at 40 min. Thus the elevation of P_{pv} persisted for 40 min after antigen. In the present study, this period was designated the portal hypertension period. After antigen, P_{cv} initially increased and then significantly decreased from the baseline of 0.3 ± 0.1 cmH₂O to the nadir of -1.9 ± 0.2 cmH₂O at 8 min.

Ligation of celiac and mesenteric arteries combined with total hepatectomy did not significantly affect the hemodynamic variables in the intact mice (GI-HptX-control). In the GI-HptX-sensitized group, P_{sa} initially increased and then decreased in a manner similar to that in the intact-sensitized group, but to a lesser degree. At 10 min, it decreased from the baseline of 94 ± 1 to 65 ± 2 mmHg, which was significantly higher than the corresponding value of the intact-sensitized group of 41 ± 2 mmHg. Thereafter, it did not decrease so much: the final P_{sa} at 60 min was 53 ± 2 mmHg. Thus P_{sa} from 4 to 60 min after antigen was significantly higher in the GI-HptX-sensitized group than in the intact-sensitized group. In the GI-HptX-sensitized group, P_{cv} also decreased from 0.4 ± 0.1 to -1.7 ± 0.2 cmH₂O; however, the decrease in P_{cv} was significantly smaller than in the intact-sensitized group (Figs. 1B and 2). Then we examined which procedure, i.e., hepatectomy or ligation of the celiac and mesenteric arteries, is

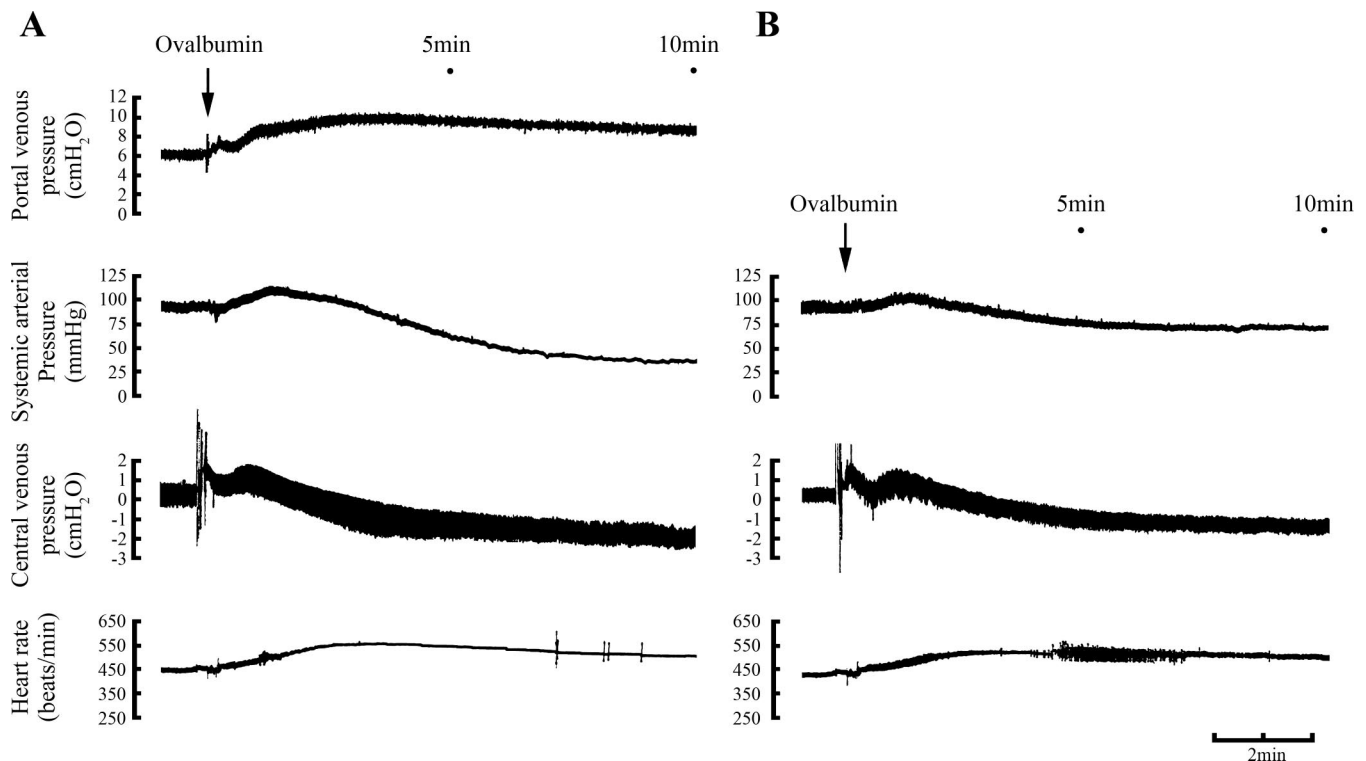


Fig. 1. Representative recording of the response to ovalbumin antigen (0.01 mg) in intact-sensitized (A) and GI-HptX-sensitized (B) groups. Because there was no blood flow in the portal veins due to liver resection, portal venous pressure was not measured in the GI-HptX-sensitized group.

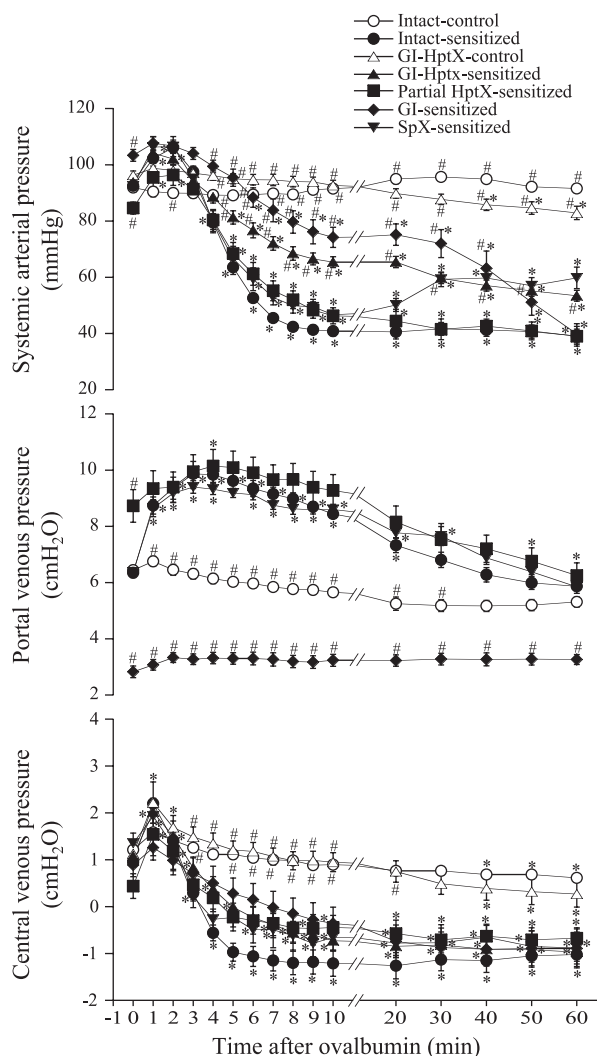


Fig. 2. Summary of changes in systemic arterial pressure, portal venous pressure (P_{pv}), and central venous pressure after antigen injection. P_{pv} was not measured in GI-HptX-control or GI-HptX-sensitized mice because the liver was excised. GI, gastrointestinal ligation; HptX, hepatectomy; SpX, splenectomy. Values are means $\pm$ SE; $n = 7$ intact-control, $n = 9$ GI-sensitized, $n = 10$ intact-sensitized, GI-HptX-control, partial HptX-sensitized, and SpX-sensitized, and $n = 11$ GI-HptX-sensitized. * $P < 0.05$ vs. baseline. # $P < 0.05$ vs. intact-sensitized.

more important for attenuating the decrease of P_{sa} and P_{cv} during anaphylaxis in the GI-HptX-sensitized group.

In the partial HptX-sensitized group, in which 70% of the total liver mass was removed but splanchnic vascular beds were intact, basal P_{pv} was significantly higher than in the intact mice, but other hemodynamic variables were not significantly different (Fig. 2). In this group, an injection of the antigen resulted in a decrease in P_{sa} that was not significantly different from the decrease in the intact-sensitized group (Fig. 2). After antigen, P_{pv} also increased similarly to the intact-sensitized group, although the basal P_{pv} was significantly higher than in the intact-sensitized group because of the partial hepatectomy-induced reduction in vascular surface area. In the SpX-sensitized group, the hemodynamic response to the antigen was not significantly different from that of the intact-sensitized group (Fig. 2). On the other hand, ligation of the celiac and mesen-

teric arteries alone in the GI-sensitized group had beneficial effects on the antigen-induced decrease in P_{sa} that were comparable to those of the GI-HptX-sensitized group (Fig. 2). After antigen, P_{cv} in the GI- and partial HptX-sensitized groups significantly decreased in parallel with a decrease in P_{sa} .

HR significantly increased in all the sensitized groups with administration of the antigen.

Isolated perfused liver experiment. Figure 3 shows the time course of changes in P_{pv} , P_{do} , P_{hv} , liver weight, R_{pre} , and R_{post} in the sensitized, nonsensitized, and PAF-challenged rat livers. Hepatic vascular P_{pv} , P_{do} , and P_{hv} did not change significantly after an injection of 0.01 mg of ovalbumin antigen into the perfusate in a sensitized or nonsensitized mice. Thus hepatic segmental vascular R_{pre} or R_{post} did not increase after antigen. Actually, the other sensitized mouse livers were challenged with a higher dose of 0.1 or 1 mg of ovalbumin, but no significant responses were observed (data not shown). These results indicate that significant anaphylactic venoconstriction did not occur in the perfused liver from sensitized mice. Then we examined whether these perfused liver preparations could respond to the vasoactive substance PAF. As shown in Fig. 3, cumulative administration of PAF substantially increased P_{pv} from the baseline of 6.7 ± 0.1 cmH₂O to the peak of 9.4 ± 0.5 cmH₂O at 1 μ M. P_{do} increased only slightly but significantly after PAF. As a result, PAF almost selectively increased R_{pre} , rather than R_{post} . R_{pre} reached the peak of 1.82 times baseline at 1 μ M, whereas the corresponding R_{post} was only 1.13 times baseline, which is not significantly different from the baseline. The statistically significant R_{post} increase (1.15 times baseline) was observed only at the highest concentration (10 μ M) of PAF. This presinusoidal contraction was accompanied by liver weight loss of -0.16 ± 0.03 g/g liver wt at 10 μ M PAF. These results indicate that the perfused BALB/c mouse livers were substantially responsive to the anaphylactic vasoconstrictor.

DISCUSSION

The two major findings of this study are as follows. 1) Elimination of the blood flow to splanchnic organs, with or without hepatectomy, attenuated the antigen-induced systemic arterial hypotension in anesthetized mice. This suggests that the splanchnic circulation is involved in the genesis of anaphylactic hypotension in mice. 2) P_{pv} increased during anaphylactic hypotension in anesthetized mice. In contrast, the isolated perfused mouse liver did not show substantial venoconstriction in response to antigen. These findings suggest that the anaphylactic portal hypertension in anesthetized mice may be caused not by anaphylactic reaction within the liver but, rather, by extrahepatic factors.

Hepatic venoconstriction is observed during anaphylaxis in dogs (10), rats (15, 23), guinea pigs (21), and rabbits (17). For the first time, we have demonstrated that P_{pv} increased during systemic anaphylaxis in mice. However, the magnitude of portal hypertension was much smaller in our mice than in dogs (10), rats (15, 23), and rabbits (17). P_{pv} after antigen increased only 3.5 cmH₂O from baseline in our anesthetized mice, whereas the antigen-induced increases in P_{pv} were >14 cmH₂O in dogs (10), rats (15, 23), and rabbits (17), although P_{sa} decreased similarly to ~ 40 mmHg among these animals, including the mice in the present study (10, 17, 23). The reason for the smaller increase in P_{pv} in mice is unclear; we assume

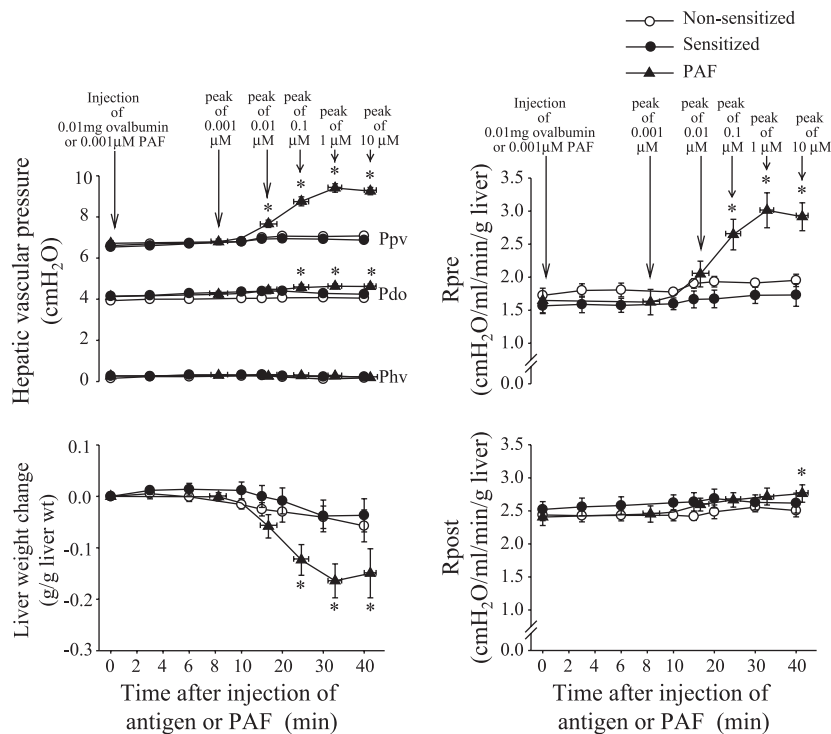


Fig. 3. Vasoreactivity in isolated perfused mouse livers. Time course of changes in P_{pv} , double-occlusion pressure (P_{do}), hepatic venous pressure (P_{hv}), liver weight, presinusoidal resistance (R_{pre}), and postsinusoidal resistance (R_{post}) after an injection of 0.01 mg of ovalbumin antigen into sensitized and nonsensitized control livers and injections of increasing concentrations (0.001–10 μ M) of platelet-activating factor (PAF) into nonsensitized livers. Arrows indicate peak vasoconstriction for each PAF concentration. Time to peak response for each PAF concentration was somewhat varied in individual livers; therefore, average time and its standard errors are presented. Values are means $\pm$ SE; $n = 5$ nonsensitized control and $n = 7$ sensitized and PAF. * $P < 0.05$ vs. baseline.

that it may be the weak responsiveness of mouse hepatic vessels to anaphylactic mediators such as histamine (24), thromboxane A_2 (8), and PAF. Although PAF substantially increased P_{pv} in isolated perfused mouse livers (Fig. 3), the increase was much smaller than that in rats (6), dogs (29), and guinea pigs (22): the peak R_t after PAF was 1.4 times baseline in mice, 3.7 times baseline in rats (6), 3.3 times baseline in dogs (29), and 5.1 times baseline in guinea pigs (22). Further studies on structural and functional mechanisms for weak reactivity of the mouse hepatic vessels are required to determine the amount and distribution of vascular smooth muscle cells and receptors for anaphylactic vasoactive substances and vasoreactivity to those individual substances in the mouse portal and hepatic veins. Another explanation for relatively weak portal hypertension in antigen-challenged anesthetized mice may be related to the absence of antigen-induced venoconstriction in the isolated perfused sensitized mouse livers. In contrast to the perfused livers of mice, those of dogs (10), rats (15, 23), and rabbits (17) showed considerable venoconstriction in response to antigen. These results suggest that, in other anesthetized animals, anaphylactic chemical mediators are released from intrahepatic and extrahepatic tissues, whereas in mice they are released only from extrahepatic tissues. Therefore, the intrahepatic concentrations of chemical mediators might have been smaller in mice than in other animals, resulting in a smaller increase in P_{pv} of mice after antigen. Indeed, mast cells, the key effector cells of anaphylaxis, are histologically undetectable in mouse livers (13); this may account for the absence of anaphylactic venoconstriction of the isolated perfused mouse livers as well as weak portal hypertension of anesthetized mice.

In the GI-HptX- and GI-sensitized groups, anaphylactic hypotension was similarly attenuated. The mechanism for the beneficial effects of GI-HptX or GI on the anaphylactic hypo-

tension is not known. We think that these two groups have two common features: the absence of portal hypertension and the absence of splanchnic vascular beds, which may be involved in the mechanism for the attenuation of anaphylactic hypotension. The first possibility is that anaphylaxis-induced portal hypertension may account for the profound decrease in P_{sa} . Indeed, in the GI-HptX- or GI-sensitized group, portal hypertension did not exist; in the intact-sensitized or the partial HptX-sensitized group, however, P_{pv} increased and P_{sa} substantially decreased (Fig. 2). We speculate the following pathophysiological process: anaphylaxis causes hepatic venoconstriction and portal hypertension, which then causes congestion of the upstream splanchnic organs and, consequently, a decrease in venous return and effective circulating blood volume, and, finally, augmentation of anaphylactic hypotension. Indeed, P_{cv} , an indicator of venous return to the right heart, was significantly greater in the GI-HptX-sensitized group than in the intact-sensitized group after antigen. However, contradictions to this assumption may arise. The first is that the portal hypertension in the present study consistently recovers but systolic blood pressure does not; this outcome was quite different from our previous finding in rats with anaphylactic shock (23). In addition, as discussed above, the increase in P_{pv} was much smaller in our mice than in rats (23). Thus we are uncertain whether the weak portal hypotension in mice causes significant congestion of the splanchnic vascular beds. Finally, we recently showed that a nitric oxide (NO) synthesis inhibitor, nitro-L-arginine methyl ester (L-NAME), significantly attenuated anaphylactic systemic hypotension, but not anaphylactic portal hypertension, in anesthetized BALB/c mice (26). Taken together, these findings suggest that anaphylactic portal hypertension may not play a significant role in anaphylactic hypotension in anesthetized mice.

Another more plausible and possible explanation for the beneficial effects of GI-HptX or GI may be related to the sources of vasoactive chemical mediators released in response to antigens. One of the common factors of both groups was the ligation of the celiac and mesenteric arteries, i.e., elimination of the blood flow to splanchnic organs. It is well known that the gastrointestinal tract contains many mast cells, the effector cell of systemic anaphylaxis (20). A large number of mast cells in the gastrointestinal tract may release substantial amounts of anaphylactic vasoactive substances into the systemic circulation. Removal of these cells could reduce anaphylactic chemical mediator release, resulting in attenuation of the anaphylactic response. In this respect, surgical removal of the spleen, one of the substantial organs of the splanchnic vascular bed, did not significantly improve anaphylactic hypotension, at least in the early phase, in the present study (Fig. 2). This could be explained by the morphological finding that mast cells are very rare in the spleen of the mouse (18); thus the spleen could not release the chemical mediators when the antigen was challenged.

With respect to the effector cells for anaphylaxis, macrophages have also been demonstrated to be involved in systemic anaphylaxis in mice (11). Two distinctive pathways of systemic anaphylaxis in mice have been reported (11): one mediated by mast cells, IgE, and Fc ϵ RI (the classical pathway associated with human allergy) and another mediated by macrophages, IgG, and Fc γ RIII. Another effector cell of macrophages is also abundant in the liver as intravascular resident macrophages, i.e., Kupffer cells. They constitute 80–90% of the fixed tissue macrophages (reticuloendothelial system) and account for ~15% of the liver cells (28).

However, in the present study, partial (70%) hepatectomy did not affect anaphylactic hypotension. This finding was unexpected, because partial hepatectomy must have substantially removed hepatic sources of Kupffer cells, with resultant decrease in release of vasoactive substances, leading to attenuation of hypotension. Thus this finding suggests that the intravascular macrophage is not substantially involved in the present anaphylaxis of ovalbumin-adjuvant sensitized mice. The absence of antigen-induced venoconstriction in the sensitized perfused mouse liver also supports this assumption.

In dogs, in response to hemorrhage, the spleen serves as the blood reservoir and could compensate the hypotension by contracting and expelling the intrasplenic blood to the circulation (5). In the present study, however, splenectomy did not aggravate the antigen-induced hypotension. This suggests that the spleen may not play a compensatory role in mouse anaphylactic hypotension. On the contrary, splenectomy tended to improve the anaphylactic hypotension in the late phase from 30 to 60 min after the injection of antigen (Fig. 2). This finding may reflect a contribution of delayed splenic pooling in sensitized-intact mice simultaneously with the decline in portal hypotension.

One of the purposes of the present study was to determine whether mouse anaphylactic hypotension is accompanied by hepatic congestion due to substantial postsinusoidal constriction, as in canine anaphylactic hypotension (10, 19). To answer this question, we used a mouse perfused liver preparation, which provides information on changes in hepatic vascular resistance distribution and liver blood volume. However, in contrast to the sensitized perfused livers from dogs (29), rats

(15, 23), guinea pigs (21), and rabbits (17), hepatic anaphylactic venoconstriction did not occur in perfused mouse livers. We speculated that hepatic congestion does not occur in anesthetized sensitized mice challenged with antigen on the basis of the following findings. 1) Partial hepatectomy did not attenuate anaphylactic hypotension. If hepatic congestion occurred, partial hepatectomy might have attenuated the pooling of circulating blood, resulting in an increase in venous return and attenuation of systemic hypotension. 2) PAF caused predominant presinusoidal contraction and liver weight loss in isolated perfused mouse livers (Fig. 3). During mouse systemic anaphylaxis, PAF could be released from sensitized mast cells and macrophages (11). In addition, another anaphylactic mediator of histamine also induced liver weight loss in isolated mouse livers perfused at constant flow (24). This liver weight loss, an indicator of reduced hepatic blood volume caused by the anaphylactic mediator, suggests that this chemical mediator also acts to reduce the intrahepatic blood volume in anesthetized mice exposed to systemic anaphylaxis. However, further study on the chemical mediators responsible for mouse anaphylactic hypotension is required.

Cauwels et al. (3) recently reported that anaphylactic shock in conscious mice depends on endothelial NO synthase-derived NO (3). We also reported that the NO synthesis inhibitor L-NAME attenuated anaphylactic systemic hypotension without modulating anaphylactic portal hypertension in anesthetized BALB/c mice (26). This suggests that the beneficial antihypotensive effect of L-NAME is not exerted on the hepatic vessels. We have shown that L-NAME does not affect the basal P_{pv} of hepatic vascular tone in isolated perfused mouse livers, nor did it augment hepatic venoconstriction in response to the anaphylaxis-related mediator of PAF (7) or thromboxane A_2 (8). Further study is required to clarify the relationship between the depressor action of NO and the role of splanchnic vascular beds in mouse anaphylactic hypotension.

Perspectives and Significance

The hemodynamic mechanisms for anaphylactic shock have not been fully clarified. Indeed, there are species differences in the target organs affected by anaphylaxis. Right heart failure takes place in the rabbit and guinea pig, whereas hepatic congestion occurs in the dog (27, 30). On the other hand, we have been proposing that anaphylactic hypotension is accompanied by hepatic venoconstriction, possibly beyond the species differences, and that the liver and splanchnic vascular beds play significant roles in anaphylactic hypotension. Actually, anaphylactic hepatic venoconstriction is observed in dogs (27, 30), rats (23), rabbits (17), guinea pigs (21), cats (4), and calves (16). The anaphylactic portal hypertension may increase the intravascular volume (blood pooling) and the capillary pressure of the upstream splanchnic vascular beds. In addition to the splanchnic pooling, increased capillary pressure and an anaphylaxis-induced increase in vascular permeability synergistically facilitate the transcapillary fluid filtration from the splanchnic vasculature into the interstitium, which may cause a decrease in circulating blood volume, a decrease in venous return, and, finally, hypotension. In this study, we have demonstrated, for the first time, that the mouse also shows hepatic venoconstriction during anaphylactic hypotension, although its magnitude is smaller than that of other species. Furthermore,

the presence of the splanchnic vascular beds, rather than the liver itself, is more important for anaphylactic hypotension in mice. The findings of the present study reinforce the significant roles of the liver and splanchnic vascular beds in the pathogenesis of experimental anaphylactic hypotension. We think that it may be applied to the human case, because anaphylactic hepatic venoconstriction is observed almost beyond species differences. Clinical study is required to examine whether P_{pv} increases during anaphylactic hypotension.

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