

Effects of Hct on L-NAME–induced Potentiation of Anaphylactic Presinusoidal Constriction in Perfused Rat Livers

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Abstract: Effects of hematocrit (Hct) on N^G-nitro-L-arginine methyl ester (L-NAME)–induced modulation of anaphylactic venoconstriction were determined in isolated perfused rat livers. The rats were sensitized with ovalbumin (1 mg), and the livers were excised 2 weeks later and perfused portally and recirculatingly under constant flow at Hct of 0%, 5%, 16%, and 22%. The hepatic sinusoidal pressure was estimated via the double occlusion pressure (Pdo), and the presinusoidal resistance (Rpre) and the postsinusoidal resistance (Rhv) were calculated. The antigen of ovalbumin 0.1 mg was injected into the reservoir at 10 minutes after pretreatment with L-NAME (100 μ M) or D-NAME (100 μ M). Perfusate viscosity, a determinant of vascular resistance and shear stress, was increased in parallel with Hct. In the D-NAME groups, antigen caused predominant presinusoidal constriction. The magnitude of venoconstriction was significantly smaller at Hct 0% than at Hct 5% to 22%, whereas no significant differences were found among Hct 5% to 22%. L-NAME potentiated the antigen-induced increase in Rpre, but not in Rpost at Hct 5% to 22% as compared with D-NAME. But the augmentative effects of L-NAME were similar in magnitude among Hct 5% to 22%. These findings suggest that hepatic anaphylaxis increases production of nitric oxide, which consequently attenuates anaphylactic presinusoidal constriction in rat livers, and that these effects are independent of perfusate Hct or viscosity in blood-perfused rat livers.

Key Words: anaphylactic shock, nitric oxide, viscosity, shear stress, hepatic circulation

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Anaphylactic hypotension is sometimes life-threatening, and is caused by a decrease in effective circulating blood volume.¹ It is reported that the liver is partly involved in animal models of anaphylactic hypotension.^{2,3} Anaphylactic

hepatic venoconstriction, as observed in rats,^{3,4} guinea pigs,⁵ and dogs,^{6,7} causes portal hypertension which then induces congestion of the upstream splanchnic organs, with resultant decrease in venous return and effective circulating blood volume, and finally augmentation of anaphylactic hypotension.

Nitric oxide (NO), a potent vasodilator, regulates the vascular system,^{8,9} and seems to play an important pathophysiologic role in modulating the systemic changes associated with anaphylaxis.¹⁰ We have recently reported that N^G-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, augmented anaphylactic hepatic venoconstriction in guinea pig.⁵ And further, we demonstrated, using the vascular occlusion method for measurement of the hepatic sinusoidal pressure, that the anaphylactic venoconstriction of the presinusoids, but not the postsinusoids, was enhanced by L-NAME.⁵ However, it is not known whether L-NAME also induces augmentation of anaphylactic hepatic venoconstriction in other animals such as rats.

NO is produced by the activation of the NO synthase 3 present in endothelial cells either by hormonal or physical stimuli such as shear stress.^{8,9} The viscosity, an important determinant of shear stress and vascular resistance, highly depends on hematocrit (Hct). Indeed, the vascular resistance increased as Hct elevates at a given blood flow in rat liver, as we previously reported.¹¹ However, it is not well known how the changes in blood viscosity modulate anaphylactic hepatic segmental venoconstriction.

Thus, we herein determined the effect of Hct on the L-NAME–induced modulation of anaphylactic hepatic segmental venoconstriction in rats. To accomplish this purpose, we measured the sinusoidal pressure with the vascular occlusion method, and determined the pre and postsinusoidal resistances during hepatic anaphylaxis in isolated rat livers perfused portally and recirculatingly under constant flow at different Hct of 0%, 5%, 16%, or 22% in the presence of L-NAME or D-NAME (an inactive enantiomer of L-NAME). In addition, we determined the anaphylaxis-induced changes in hepatic oxygen consumption and bile production.

METHODS

Animals and Sensitization

Forty male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) weighing 341 ± 3 g were maintained at 23°C and under

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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. Rats were actively 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).³

Isolated Liver Preparation

Two weeks after sensitization, the animals were anesthetized with pentobarbital sodium (50 mg kg⁻¹, IP) and were mechanically ventilated with room air. The methods for the isolated perfused rat liver preparation were previously described.³ In brief, a polyethylene tube was placed in the right carotid artery. After laparotomy, the hepatic artery was ligated and the bile duct was cannulated with the polyethylene tube (0.5 mm ID, 0.8 mm OD). At 5 minutes after intra-arterial heparinization (500 U kg⁻¹), 8 to 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 cannula (1.3 mm ID, 2.1 mm OD). After thoracotomy, the supra-diaphragmatic inferior vena cava was cannulated through a right atrium incision with a larger stainless cannula (2.1 mm ID, 3.0 mm OD), then portal perfusion was begun with the heparinized blood diluted with 5% bovine albumin (Sigma-Aldrich Co, St Louis, MO) in Krebs solution (118 mM NaCl, 5.9 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.2 mM NaH₂PO₄, 25.5 mM NaHCO₃, and 5.6 mM glucose) at the following Hct levels: 0% (n = 10), 5% (n = 10), 16% (n = 10), and 22% (n = 10). At Hct 5%, only the autologous blood was used, while at Hct 16% and 22%, additional blood was obtained by exsanguination of an anesthetized and heparinized intact donor rat. At Hct 0%, the liver was perfused with only 5% albumin-Krebs solution without blood. The liver was rapidly excised, suspended from an isometric transducer (TB-652T, Nihon-Kohden, Japan), and weighed continuously throughout the experimental period.

The liver was perfused at a constant flow rate in a recirculating manner via the portal vein using perfusate with or without blood that was pumped using a Masterflex roller pump from the venous reservoir through a heat exchanger (37°C). The recirculating perfusate volume was 40 mL. The perfusate was oxygenated in the venous reservoir by continuous bubbling with 95% O₂ and 5% CO₂ (perfusate PO₂ = 300 mm Hg).

The viscosity of the perfusate was measured using cone plate type of viscometer (Biorheolizer, Tokyo Keiki, Japan).

Measurement of Hepatic Vascular Pressures and Vascular Resistances

The portal venous (Ppv) and the 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).⁷ Both the inflow and outflow lines were simultaneously and instantaneously occluded for 17 seconds using the solenoid valves, after which Ppv and Phv rapidly equilibrated to a similar or identical pressure, which was Pdo. Actually, Pdo values were obtained from the digitized data of Ppv and Phv using an original program (LIVER software, Biomedical Science, Kanazawa, Japan). The total portal-hepatic venous (Rt), presinusoidal (Rpre), and postsinusoidal (Rpost) resistances were calculated as follows:

$$R_t = (P_{pv} - P_{hv})/Q \quad (1)$$

$$R_{pre} = (P_{pv} - P_{do})/Q \quad (2)$$

$$R_{post} = (P_{do} - P_{hv})/Q \quad (3)$$

Bile Flow Rate Measurement

Bile was collected drop by drop in a small tube suspended from the force transducer (SB-1T, Nihon-Kohden, Japan). One bile drop yielded 0.018 g and the time between drops was measured for determination of the bile flow rate.³

Hepatic Oxygen Consumption Measurement

To determine the oxygen consumption, the blood oxygen saturation difference between the inflow and outflow blood (ΔSO_2) was monitored continuously with a custom-made oxygen absorption spectrophotometer (ODM-1, Biomedical Science, Kanazawa, Japan), the cuvettes and sensors of which were built in the inflow and outflow perfusion lines.¹² The measured ΔSO_2 was expressed as the oxygen consumption difference meter (ODM) values.¹² ΔSO_2 was calculated using the following regression line equation as determined in our previous study¹²:

$$\Delta SO_2 = 1.5103 \times ODM - 0.0685 \quad (4)$$

Using ΔSO_2 , oxygen consumption (mL min⁻¹ 10 g liver wt⁻¹) was calculated according to the Fick principle by the following equation:

$$\text{Portal blood flow/liver weight (g)} \times 10 \times 1.39 \text{ (ml/g)} \times \text{Hb (g/ml)} \times \Delta SO_2 \quad (5)$$

where Hb is the perfusing blood hemoglobin measured by cyanmethemoglobin method (Hb-B test Wako, Wako, Japan) with a spectrophotometer (UV-1200, Shimadzu, Japan).

Data Recording

The hepatic vascular pressures, blood flow rate, liver weight, bile weight, and ODM values, were monitored continuously and displayed through a thermal physiograph (RMP-6008, Nihon-Kohden, Japan). All outputs were also digitized through the analog-digital converter at a sampling rate of 100 Hz. These digitized values were also displayed and recorded using a personal computer for later determination of Pdo.

Experimental Protocol

Hepatic hemodynamic parameters were observed for at least 20 minutes after the start of perfusion until an isogravimetric state (no weight gain or loss) was obtained by adjusting the portal blood flow rate and the height of the reservoir at a Phv of 0 to 1 cm H₂O, and at a *Q* of 36 ± 0.5 mL min⁻¹ 10 g liver wt⁻¹. Then, the livers perfused at various Hct were further divided into the following 2 groups of the D-NAME and L-NAME groups, in which D-NAME (100 μ M; Sigma) and L-NAME (100 μ M; Sigma), respectively, were administered into the reservoir. Thus, any liver studied was pre-treated with either D-NAME or L-NAME. Ovalbumin 0.1 mg was injected into the reservoir at 10 minutes after injection of D-NAME or L-NAME. In each experimental, measurement was performed up to 30 minutes after antigen.

In each experimental group, the double occlusion was performed at baseline, just before antigen injection, and at the maximal venoconstriction (when Ppv reached the peak), 6, 10, 20, and 30 minutes after injection of antigen.

Statistics

All results are expressed as the means $\pm$ SE. Data were analyzed by 1 and 2-way analysis of variance, using repeated-measures for 2-way comparison within groups. Comparisons of individual points between groups and within groups were made by Bonferroni test. Differences were considered as statistically significant at *P* values less than 0.05.

RESULTS

Perfusate Hct and Viscosity

The perfusate viscosity increased in parallel with Hct from 0% to 22% in both D-NAME and L-NAME groups. The viscosity was 0.879 ± 0.007 mPa s⁻¹ at Hct 0%, 1.232 ± 0.014 mPa s⁻¹ at Hct 5%, 1.706 ± 0.019 mPa s⁻¹ at Hct 16%, and 2.222 ± 0.039 mPa s⁻¹ at Hct 22%.

The Basal Hepatic Vascular Resistances

Figure 1 shows basal Ppv, Rpre, and Rpost. Basal Rpre was higher than basal Rpost in livers of any Hct (0% to 22%), resulting in basal Rpost/Rt ratio of 0.3 to 0.35. The basal Ppv and vascular resistances of Hct 22% groups were significantly greater than those of other Hct groups. D-NAME pretreatment did not change basal Ppv or resistances at any Hct level. However, basal Ppv and Rpre were significantly increased by L-NAME pretreatment at Hct 16% and 22%.

Effects of Hct on the Responses of D-NAME Pretreated Livers to Antigen

Figure 2 shows the summary data of the time-dependent changes in Ppv, Pdo, liver weight, oxygen consumption, and bile flow rate of D-NAME groups. Antigen induced venoconstriction, as reflected by an increase in Ppv. Ppv peaked at 3 minutes after antigen and gradually decreased towards baseline at 30 minutes. The magnitude of venoconstriction at Hct 0% was significantly smaller than that at Hct 5% to 22%. The Ppv peak at Hct 0% was 20.9 ± 1.4 cm H₂O, and that at Hct 5% was 27.8 ± 0.8 cm H₂O. However, no significant differences were found in the peak values of Ppv

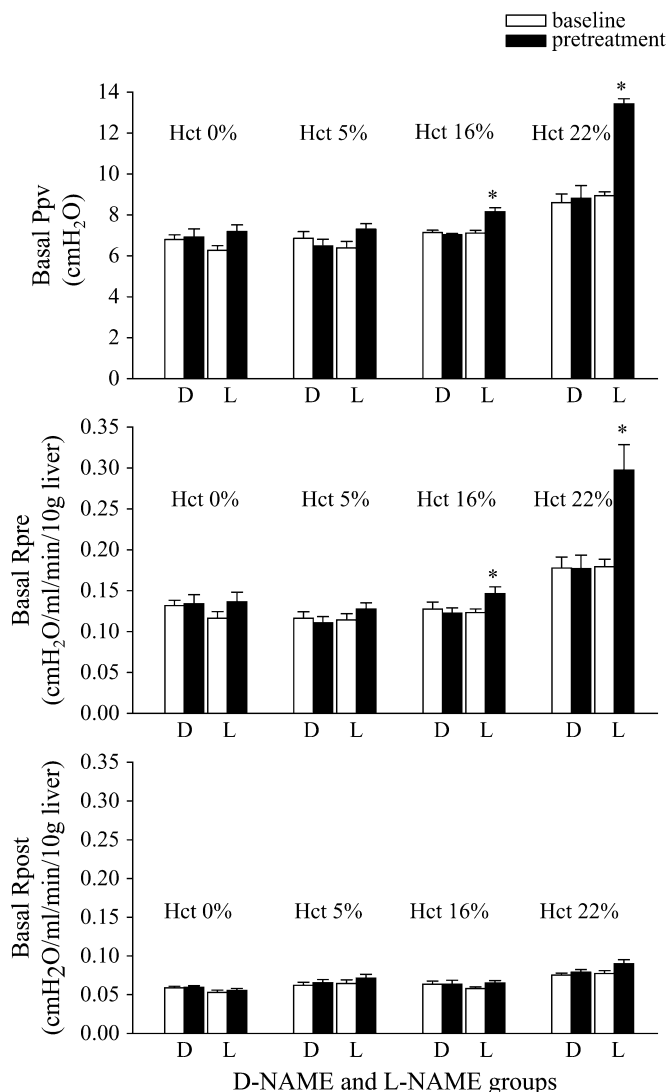


FIGURE 1. The basal portal venous pressure (Ppv), presinusoidal resistance (Rpre), and postsinusoidal resistance (Rpost) of the baseline and after pretreatment with D-NAME (D) and L-NAME (L) at Hct of 0%, 5%, 16%, and 22%. **P* < 0.05 versus the baseline.

after antigen among Hct 5%, 16%, and 22%. Pdo increased slightly but significantly after antigen, and the increase at Hct 0% was smaller than that at Hct 5% to 22%.

Figure 3 shows changes in Rpre and Rpost after antigen. In the D-NAME groups, both Rpre and Rpost significantly increased after antigen. However, the magnitudes of the increases in Rpre were much larger than that in Rpost: At Hct 16%, Rpre increased 0.612 ± 0.067 cmH₂O mL⁻¹ min⁻¹ 10 g⁻¹ liver, whereas Rpost increased only 0.050 ± 0.003 cmH₂O mL⁻¹ min⁻¹ 10 g⁻¹ liver weight⁻¹. The antigen-induced increases in Rpre and Rpost in blood-perfused livers (Hct 5% to 22%) were significantly greater than those in livers perfused without blood (Hct 0%). However, there were no significant differences in increases of Rpre or Rpost among Hct 5%, 16%, and 22%. These results indicate that the antigen induces

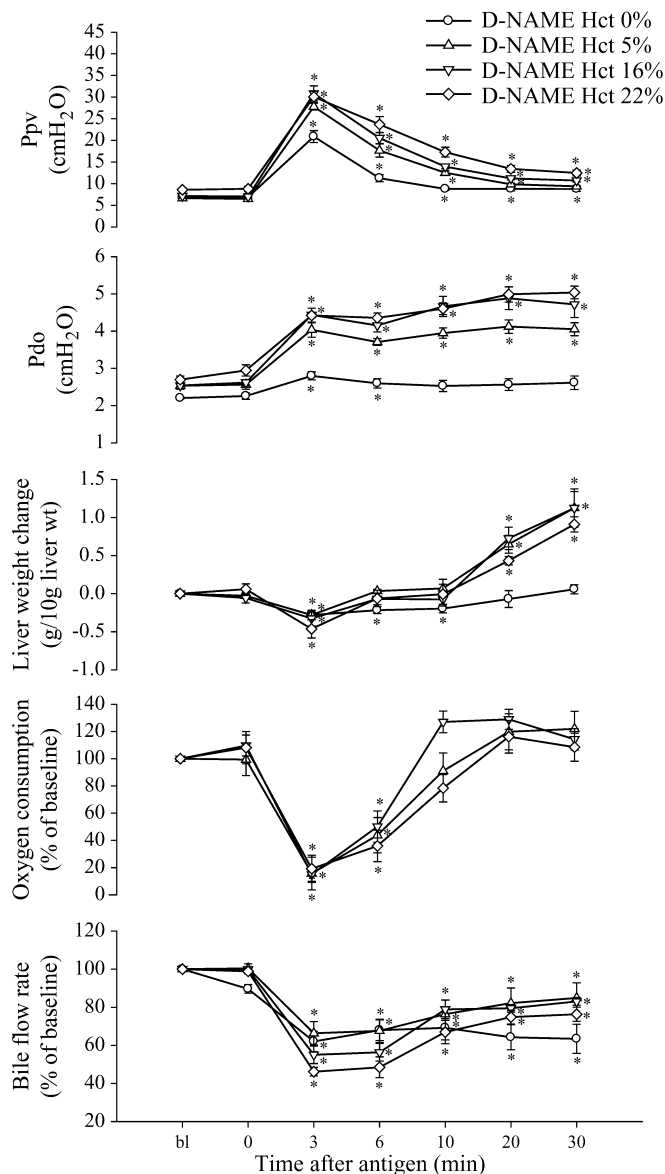


FIGURE 2. The summary data of time course changes in the portal venous pressure (Ppv), double occlusion pressure (Pdo), liver weight change, hepatic oxygen consumption, and bile flow rate after antigen injection at Hct of 0%, 5%, 16%, and 22% in the D-NAME groups. Values are given as mean $\pm$ SE, $n = 5$. * $P < 0.05$ versus the baseline.

predominant presinusoidal constriction independently of blood Hct (5% to 22%) in blood-perfused rat livers.

The liver weight showed a biphasic change of an initial decrease followed by an increase in response to antigen, as shown in Figure 2. The lowest nadir was -0.521 ± 0.054 g 10 g⁻¹ liver at Hct 22%. There were no significant differences in the nadirs among all D-NAME groups.

Bile flow rate decreased Hct dependently, reaching $47 \pm 3\%$ of the baseline of 8.7 ± 0.6 mg min⁻¹ 10 g⁻¹ liver at Hct 22% group, as shown in Figure 2. It did not recover to the baseline at the end of experiment.

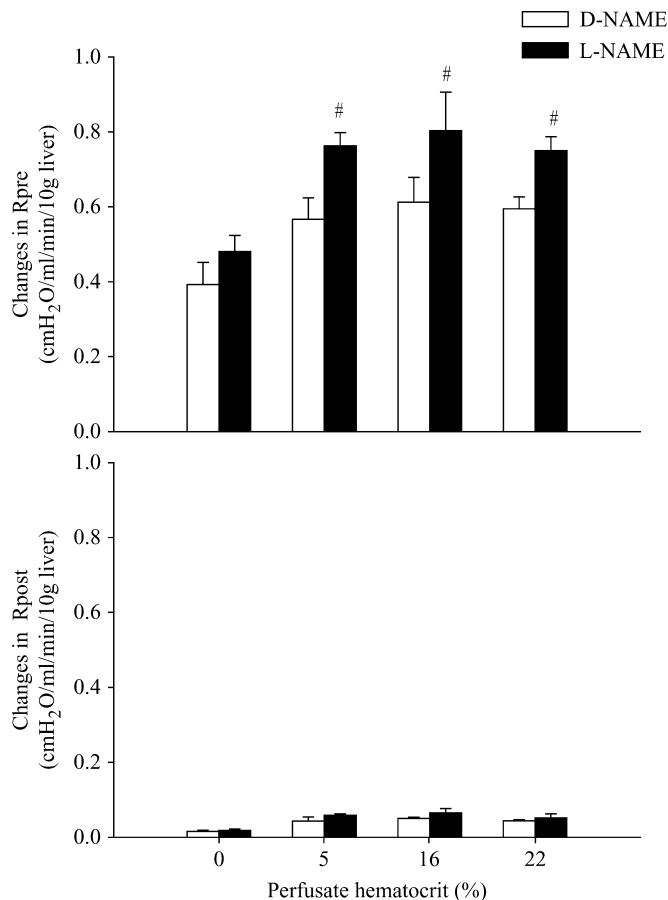


FIGURE 3. The changes in Rpre and Rpost after antigen injection at Hct of 0%, 5%, 16%, and 22% in D-NAME and L-NAME groups. Values are given as mean $\pm$ SE. All values in both D-NAME and L-NAME groups were significantly different from the corresponding baseline, and therefore asterisks were omitted. # $P < 0.05$ versus the D-NAME group.

The basal hepatic oxygen consumption increased as Hct increased: The basal levels were 0.015 ± 0.002 for Hct 5%, 0.070 ± 0.005 for Hct 16%, and 0.096 ± 0.034 mL min⁻¹ 10 g⁻¹ liver for Hct 22%. The oxygen consumption in livers of Hct 0% could not be determined in the present system because of absence of red blood cells in the perfusate. The hepatic oxygen consumption was significantly decreased after antigen injection. It reached the lowest value, $13.5 \pm 4.1\%$ of the baseline of 0.072 ± 0.006 mL min⁻¹ 10 g⁻¹ liver at Hct 16%. Actually, the decreases of hepatic oxygen consumption were similar in magnitudes among Hct 5% to 22%. Oxygen consumption almost recovered to the baseline at 20 minutes after antigen.

Effects of L-NAME on Hepatic Responses to Antigen Under Different Hct

Figure 4 shows the summary data of the time course changes in Ppv, Pdo, liver weight, oxygen consumption, and bile flow rate of the L-NAME groups. As compared with D-NAME groups, the antigen-induced increases in Ppv in the L-NAME groups were qualitatively similar but greater in

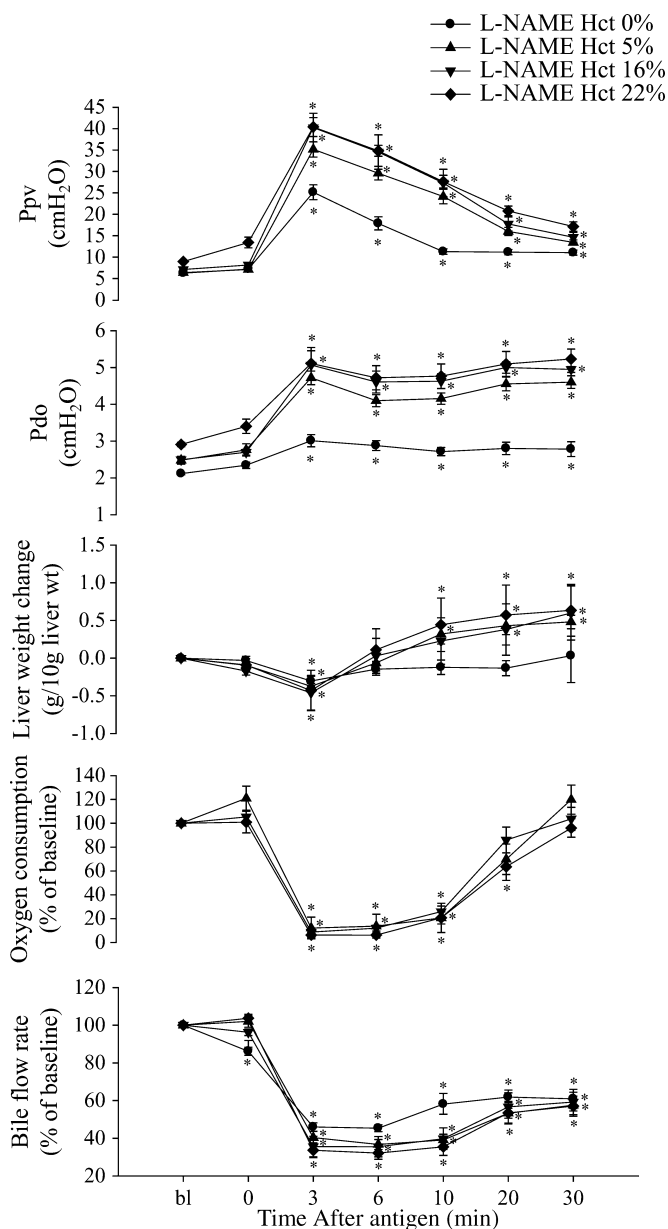


FIGURE 4. The summary data of time course changes in the portal venous pressure (Ppv), double occlusion pressure (Pdo), liver weight change, hepatic oxygen consumption, and bile flow rate after antigen injection at Hct of 0%, 5%, 16%, and 22% in the L-NAME groups. Values are given as mean $\pm$ SE. $n = 5$. * $P < 0.05$ versus the baseline.

magnitude, although the increases in Pdo were comparable. Thus, as shown in Figure 3, the amplitudes of the increase in Rpre, but not in Rpost, were significantly larger in the L-NAME groups than in the D-NAME groups at any Hct level. However, the increases in Rpre were not significantly different among Hct levels of 5% to 22%, although they were significantly higher than those at Hct 0%.

The antigen-induced changes in liver weight, bile flow rate, and oxygen consumption in L-NAME groups were

similar to those in D-NAME groups. However, bile flow rates more profoundly decreased than in D-NAME groups at corresponding Hct level: the nadir, $31.2 \pm 3.6\%$ of the baseline of $8.9 \pm 1.1 \text{ mg min}^{-1} 10 \text{ g}^{-1}$ liver at Hct 22% was much lower than in D-NAME groups. Although there were no significant differences in the nadir of hepatic oxygen consumption in all groups studied, it was much lower at 6, 10, and 20 minutes after antigen in the L-NAME groups than in the D-NAME group, as shown in Figure 4.

DISCUSSION

In the present study, we examined anaphylactic venoconstriction in isolated perfused rat livers pretreated with either L-NAME or D-NAME under various Hct. The first of the main findings is that L-NAME augmented the antigen-induced increase in Rpre, but not Rpost. This suggested that hepatic anaphylaxis increases production of NO, which consequently attenuates presinusoidal constriction in rats. The second is that the magnitudes of the L-NAME-induced augmentation of the anaphylactic presinusoidal constriction were similar at various Hct (5% to 22%), although the perfusate viscosity increased depending on Hct.

In the present study, we first confirmed the previous finding that anaphylaxis induced predominant presinusoidal constriction in rat livers perfused with blood at Hct 12%,³ and further extended it by showing the same result at various Hct ranging 0% to 22%.

The basal levels of Ppv and Rpre of Hct 22% groups were significantly greater than those of other Hct groups (Fig. 1). According to Poiseuille's law, this increase in basal hepatic vascular resistance could be attributed to the perfusate Hct-dependent increase in perfusate viscosity. We have here shown that L-NAME pretreatment increased the basal hepatic resistances, especially Rpre, only at Hct 16% and 22% (Fig. 1). This indicated that NO is not involved in the regulation of basal hepatic vascular tone of isolated rat livers perfused with blood at Hct of 5% or less. This finding is consistent with that on rat livers perfused with a blood-free solution, in which inhibition of NO did not increase basal vascular tone.¹³ It is suggested that the low perfusate viscosity results only in small shear stress and thus low amount of NO production in livers perfused with low Hct. This might account for a negligible role of NO in maintenance of basal vascular tone in livers perfused at Hct of 5% or less.

The present study showed that L-NAME significantly potentiated the antigen-induced increase in Rpre, but not in Rpost, in rat livers, a finding similar to that observed in guinea pig livers.⁵ This suggests that NO might be released selectively from presinusoidal vessels during anaphylaxis, and then attenuated the anaphylactic presinusoidal constriction. The mechanism for this selective vulnerability of presinusoids to NO may be considered as follows: anaphylaxis predominantly constricted presinusoidal vessels as shown in D-NAME groups, where elevated shear stress increased NO release from the same presinusoidal endothelium, leading to relaxation of the adjacent presinusoidal vascular smooth muscle cells in a paracrine manner. Indeed, the wall shear stress in isolated perfused vessels is inversely proportional to the third

power of internal radius theoretically.¹⁴ The decrease in vascular internal radius caused by anaphylactic venoconstriction should result in an increase in shear stress. It would further be increased if turbulence did increase at the constricted site.¹⁴ Thus, the NO release from vascular endothelium of the contracted presinusoids would be increased via the shear stress mechanism during anaphylactic venoconstriction.

In the present study, the magnitudes of the L-NAME-induced potentiation of anaphylactic venoconstriction were similar among livers perfused with blood at Hct 5% to 22% (Fig. 3), although perfusate viscosity increased depending on Hct. However, these results were unexpected, because the Hct-dependent increase in perfusate viscosity could produce more shear-stress,¹⁴ and then increase production of NO, resulting in more dilation of the constricted vessels. Thus, we initially expected that L-NAME-induced potentiation of anaphylactic venoconstriction might increase when the perfusate Hct increased. With respect to the mechanisms for the absence of exaggerated venoconstriction by L-NAME at high Hct, it might be attributed to the concomitant increase in perfusate Hb concentration. It is reported that oxygenated Hb, even in extremely small amounts, inactivates NO, resulting in inhibition of its vasodilatory action.^{15,16} Thus, augmented vasodilatory effect due to the over-produced NO at high perfusate Hct might be attenuated by elevated perfusate Hb, which could inactivate NO. Another possible explanation could be related to the isolated rat liver preparation perfused at a constant flow. It is possible that the hepatic vessels perfused at a low Hct of 5% in the presence of L-NAME contracted mechanically to the maximal levels and could not constrict further even when the perfusate Hct increased. In the present isolated perfused rat liver preparation, Ppv might not be able to increase to the levels higher than 45 cm H₂O. Actually, Ppv did not increase higher than 45 cm H₂O even when supra-physiologically high concentrations (>3 μ M) of PAF, a potent vasoconstrictor, were administered into the similar perfused rat liver preparations.¹⁷

Hepatic oxygen consumption was significantly and markedly decreased to the level less than 20% of baseline, regardless of different perfusate Hct or pretreatment with L-NAME or D-NAME. This finding of anaphylaxis-induced reduction of hepatic oxygen consumption was consistent with previous studies.^{4,12} The mechanisms for this decrease in oxygen consumption may be due to hemodynamic or non-hemodynamic factors. Nonhemodynamic factors may be anaphylaxis-related chemical mediators, such as PAF,^{18,19} TXA₂,^{20,21} and LTD₄,^{19,21} all of which can decrease hepatic oxygen consumption. On the other hand, from the hemodynamic point of view, presinusoidal constriction observed during anaphylactic venoconstriction may account for the decreased oxygen consumption: presinusoidal constriction reduced hepatic vascular surface area, resulting in a decrease in the number of active hepatocytes. Actually, the difference in the recovery of decreased oxygen consumption between the L-NAME (slow recovery) and D-NAME (quick recovery) groups seems to be correlated to the difference in the time course changes in Ppv, hence hepatic venoconstriction, as shown in Figures 2 and 4. Thus, the hemodynamic factors might substantially contribute to the late phase of the anaphylaxis-

induced decrease in oxygen consumption in the L-NAME group.

In summary, ovalbumin-induced anaphylactic hepatic venoconstriction is characterized by predominant presinusoidal constriction in all rat livers with Hct 0% to 22%, and L-NAME pretreatment augmented the antigen-induced venoconstriction, by increasing Rpre, but not Rpost. This suggests that hepatic anaphylaxis increases production of NO, which consequently attenuates anaphylactic presinusoidal constriction in isolated perfused rat livers. However, the magnitude of L-NAME-induced augmentation of anaphylactic venoconstriction was not variable and independent of perfusate Hct and viscosity in blood perfused rat livers. Anaphylaxis reduced hepatic oxygen consumption independently of perfusate Hct 5% to 22%.

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