NO, but not CO, attenuates anaphylaxis-induced postsinusoidal contraction and congestion in guinea pig liver

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Ruan, Zonghai, Toshishige Shibamoto, Tomohiro Shimo, Hideaki Tsuchida, Tomonobu Koizumi, and Matomo Nishio. NO, but not CO, attenuates anaphylaxis-induced postsinusoidal contraction and congestion in guinea pig liver. Am J Physiol Regul Integr Comp Physiol 286: R94–R100, 2004. First published October 2, 2003; 10.1152/ajpregu.00648.2002.—The pathophysiology of the hepatic vascular response to anaphylaxis in guinea pig is not known. We studied effects of anaphylaxis on hepatic vascular resistances and liver weight in isolated perfused livers derived from guinea pigs sensitized with ovalbumin. We also determined whether nitric oxide (NO) or carbon monoxide (CO) modulates the hepatic anaphylaxis. The livers were perfused portalily and recirculatingly at constant flow with diluted blood. With the use of the double-occlusion technique to estimate the hepatic sinusoidal pressure (Pss), portal venous resistance (Rpv) and hepatic venous resistance (Rhv) were calculated. An antigen injection caused venoconstriction characterized by an increase in Rpv greater than Rhv and was accompanied by a large liver weight gain. Pretreatment with the NO synthase inhibitor Nω-nitro-L-arginine methyl ester, but not the heme oxygenase inhibitor zinc protoporphyrin IX, potentiated the antigen-induced venoconstriction by increasing both Rpv and Rhv (2.2- and 1.2-fold increase, respectively). In conclusion, anaphylaxis causes both pre- and postsinusoidal constrictions in isolated guinea pig livers. However, the increases in postsinusoidal resistance and Pss cause hepatic congestion. Endogenously produced NO, but not CO, modulates these responses.

hepatic circulation; antigen; double occlusion pressure; hepatic vascular resistance

Anaphylaxis is an immediate, type-I hypersensitivity reaction that occurs after exposure of sensitized organisms and tissues to sensitizing antigen. The most common life-threatening feature of acute anaphylaxis is cardiovascular collapse and shock, although there are other life-threatening effects, including bronchospasm, angioedema, and pulmonary edema (24). Cardiovascular manifestation includes a rapid and precipitous decrease in systemic arterial pressure with a concomitant decrease in cardiac output (5). Anaphylactic hypotension is primarily caused by alterations in the systemic circulation that influence blood flow to the heart because left ventricular function is relatively well preserved during anaphylactic shock (5). Peripheral circulatory collapse is ascribed to hypovolemia, which results from a plasma volume loss. The latter could be the result of vasodilation with the peripheral pooling in large-capacity splanchnic venous beds and increased vascular permeability with a shift of intravascular fluid to the extravascular space.

In canine experimental models of anaphylactic shock, congestion of the liver and the upstream splanchnic organs is important in the pathogenesis of circulatory collapse. Actually, eviscerated dogs did not develop anaphylactic shock (17). Enjeti et al. (5) reported that the severity of the anaphylactic shock could be decreased by occluding the descending aorta. Canine anaphylactic hepatic congestion is caused by constriction of postsinusoidal hepatic veins. Yamaguchi et al. (32), using the vascular occlusion method in isolated perfused livers derived from naturally sensitized dogs, demonstrated that selective postsinusoidal constriction occurs during hepatic anaphylaxis induced by injection of the Ascaris suum antigen in the perfusing blood. On the other hand, it has not been known so far whether in other species than dogs anaphylactic reaction causes constriction of postsinusoidal hepatic veins, resulting in hepatic congestion and pooling of circulating blood.

Nitric oxide (NO) is important in regulating blood flow by exerting vasodilatory actions in multiple vascular beds (19), including the hepatic circulation (18). With respect to the effects of NO on anaphylaxis, NO contributes to acute hypertension of anaphylaxis (20). NO also modulates anaphylaxis-induced changes in regional hemodynamics of coronary circulation (27) and pulmonary circulation (23) by serving as an endogenously released vasodilator. However, it has not been known whether NO modulates the change in hepatic hemodynamics during anaphylaxis.

In addition to NO, another endogenously generated gas, carbon monoxide (CO), may also exert local vasodilatory effects in the liver (6). It is reported that CO is continuously generated in livers and thereby contributes to maintenance of normal hepatic vascular tone (26). Moreover, CO is released in the hepatic circulation in response to various stressful stimuli (2, 3). However, there are no studies that determined the role of CO in the hepatic anaphylaxis.

To clarify the anaphylactic disturbance of hepatic circulation, we herein established anaphylactic models of isolated portalantly perfused guinea pig livers, in which the sinusoidal pressure was measured using the double-occlusion method (22, 32). The first purpose of the present study was to determine effects of anaphylaxis on hepatic vascular resistance distribution and liver weight changes. The second purpose was to determine, using an NO synthase inhibitor [Nω-nitro-L-arginine methyl ester (L-NAME)] and a specific inhibitor of CO-generating enzyme heme oxygenase (HO) [zinc protoporphyrin IX (ZnPPIX)], whether NO and/or CO modulates the hepatic anaphylaxis.

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MATERIALS AND METHODS

Sensitization

Twenty-five male Hartley guinea pigs weighing 351 ± 31 (SD) g were used in this study. The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University. Guinea pigs were actively sensitized by the intraperitoneal 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 physiological saline (0.5 ml). The nonsensitized animals were injected with a mixture of complete Freund’s adjuvant (0.5 ml) and physiological saline (0.5 ml) without ovalbumin.

Isolated Liver Preparation

After sensitization (2 wk), the animals were anesthetized with pentobarbital sodium (35 mg/kg ip) and mechanically ventilated with room air. A polyethylene tube was placed in the right carotid artery. After laparotomy, the cystic duct and the hepatic artery were ligated, and the bile duct was cannulated with the polyethylene tube (1.0 mm ID, 1.3 mm OD). At 5 min after intra-arterial heparinization (500 U/kg), 8–9 ml blood were withdrawn manually with a plastic syringe through the carotid arterial catheter. The intra-abdominal inferior vena cava (IVC) above the renal veins was ligated, and the portal vein was cannulated with a stainless cannula (2.1 mm ID, 3.0 mm OD) for portal perfusion. After thoracotomy, the supradiaphragmatic IVC was cannulated through a right atrium incision with the same size stainless cannula, and then portal perfusion was begun with the heparinized autologous blood that was diluted with 5% bovine albumin (Sigma) in Krebs solution (in mM: 118 NaCl, 5.9 KCl, 1.2 MgSO 4 , 2.5 CaCl 2 , 1.2 NaH 2 PO 4 , 25.5 NaHCO 3 , and 5.6 glucose) at Hct of 8%. The liver was rapidly excised, suspended from an isometric transducer (TB-2100; Nihon-Kohden). One bile drop yielded 0.027 g, and the time between drops was measured for determination of the bile flow rate (12). The hepatic sinusoidal pressure was measured by the double-occlusion method (22, 32). Both the inflow and outflow lines were simultaneously and instantaneously occluded for 13 s using the solenoid valves, after which Ppv, and P hv rapidly equilibrated to a similar or identical pressure, which was P do. Actually, P do values were obtained from the digitized data of Ppv and P hv, using an original program (LIVER software; Biomedical Science, Kanazawa, Japan). In each experimental group, P do was measured at baseline and 4-, 6-, 10-, and then at 15-min intervals for 120 min after an injection of ovalbumin.

The sensitized and nonsensitized 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 height of the reservoir and the portal blood flow rate (Q) could be adjusted independently to maintain the portal and hepatic venous pressures at any desired level. The perfused blood was oxygenated in the venous reservoir by continuous bubbling with 95% O 2 and 5% CO 2 . The portal venous (Ppv) and hepatic venous (Phv) pressures were measured using pressure transducers (TP-4000T; Nihon-Kohden) attached by sidearm to the appropriate cannulas with the reference points at the hepatic hilus. To occlude inflow and outflow perfusion lines simultaneously for measurement of the double-occlusion pressure (P do), two solenoid valves were placed in a position so that each sidearm cannula was between the corresponding solenoid valve and the liver. Portal Q was measured with an electromagnetic flowmeter (MFV 1200; Nihon-Kohden), and the flow probe was positioned in the inflow line. Bile was collected drop by drop in a small tube suspended from the force transducer (SB-1T; Nihon-Kohden). One bile drop yielded 0.027 g, and the time between drops was measured for determination of the bile flow rate (12). The hepatic vascular pressures, Q, liver weight, and bile weight were monitored continuously and displayed through a thermal physiograph (RMP-6008; Nihon-Kohden). Outputs were also digitized by the analog-digital converter at a sampling rate of 100 Hz. These digitized values were displayed and recorded using a personal computer for later determination of P do.

Experimental Protocol

Hepatic hemodynamic parameters were observed for at least 20 min after the start of perfusion until an isogravimetric state (no weight gain or loss) was obtained by adjusting the flow rate and the height of the reservoir at a P do of 0–1 cmH 2 O and at a Q of 36 ± 5 ml/min-1·10 g liver wt-1. After the baseline measurements, the perfused livers excised from the sensitized animals were randomly assigned to one of the following three groups and that from the nonsensitized animals into the nonsensitized group.

The L-NAME group (n = 6). Before the injection of ovalbumin, L-NAME (100 μM) was administered in the reservoir. Ovalbumin (0.1 mg) was injected in the reservoir at 10 min after injection of L-NAME.

The ZnPP group (n = 6). Instead of L-NAME, ZnPP (10 μM) was administered in the reservoir, and ovalbumin (0.1 mg) was injected at 10 min after injection of ZnPP.

The sensitized group (n = 7). Ovalbumin (0.1 mg) was injected in the reservoir.

The nonsensitized group (n = 6). The livers were excised from the nonsensitized animals. Ovalbumin (0.1 mg) was injected in the reservoir.

The hepatic sinusoidal pressure was measured by the double-occlusion method (22, 32). Both the inflow and outflow lines were simultaneously and instantaneously occluded for 13 s using the solenoid valves, after which Ppv, and P hv rapidly equilibrated to a similar or identical pressure, which was P do. Actually, P do values were obtained from the digitized data of Ppv and P hv, using an original program (LIVER software; Biomedical Science, Kanazawa, Japan). In each experimental group, P do was measured at baseline and 4-, 6-, 10-, and then at 10-min intervals for 120 min after an injection of ovalbumin.

The total portal-hepatic venous (Rpv), portal or presinusoidal (Rpv), and hepatic venous or post sinusoidal (Rsv) resistances were calculated as follows

\[ R_v = \frac{(P_{pv} - P_{hv})}{Q} \]  
\[ R_{pv} = \frac{(P_{pv} - P_{do})}{Q} \]  
\[ R_{hv} = \frac{(P_{do} - P_{hv})}{Q} \]

Statistics

All results are expressed as means ± SD. ANOVA followed by Bonferroni’s test was used to test for significant differences. Differences were considered as statistically significant at P values <0.05.

RESULTS

Effect of antigen injection on hepatic hemodynamic variables, bile flow, and liver weight

The final wet liver weight measured immediately after experiment was 12 ± 2 g. The P do at the baseline state of 25 perfused guinea pig livers was 3.5 ± 0.3 cmH 2 O, with Ppv 7.3 ± 0.4 cmH 2 O and P hv 0.5 ± 0.3 cmH 2 O at Q 36 ± 5 ml/min-1·10 g liver wt-1. The calculated Rv was 0.19 ± 0.03 cmH 2 O·ml-1·min-1·10 g liver wt-1. The segmental vascular resistances of Rpv and Rsv were 0.11 ± 0.02 and 0.09 ± 0.02 cmH 2 O·ml-1·min-1·10 g liver wt-1, respectively, and the Rsv-to-Rpv ratio was 0.44 ± 0.03. This indicates that 56% of the resistance of the portal venous side, which is similar to rabbit livers (15, 22).

Figure 1 shows a representative example of the response to ovalbumin. Within 1 min after antigen injection, venoconstriction occurred, as reflected by an increase in Ppv. Ppv increased from the baseline of 7.5 ± 0.5 cmH 2 O to the peak of 22.6 ± 2.1 cmH 2 O within 4–6 min after antigen. At the same time, the liver weight showed a gradual increase, reaching the peak of 2.4 ± 0.8 g/10 g liver wt at 10 min, as shown in Fig. 2. The
3.8-fold increase from the baseline of 0.11 of 0.43. Venoconstriction, bile flux to P do gradient from 3.8 from 0.21 indicating a greater increase in R pv than R hv. Concomitant with ZnPP, after injection of L-NAME, P pv, but not P do, increased the portal veins rather than hepatic veins in basal states of primarily presinusoidal vessels and that endogenous NO dilates liver wt.

Figure 1 shows a representative example of the response to ovalbumin antigen after pretreatment with L-NAME. P pv increased markedly from the preantigen levels of 8.6 ± 0.6 to the peak of 40.7 ± 4.6 cmH2O at 4–6 min after antigen. P do also significantly increased after antigen (4.1 ± 0.4 vs. 9.5 ± 0.7 cmH2O preantigen vs. peak). The increase in the P pv-P do gradient was much larger than that in the P do-P hv gradient, suggesting that R pv increased greater than R hv. The liver weight increased markedly, reaching the peak of 4.6 ± 1.1 g/10 g liver wt at 10 min (Fig. 2).

The peak levels of R (1.15 ± 0.15 cmH2O·ml⁻¹·min⁻¹·10 g liver wt⁻¹) in the L-NAME group were 1.8-fold greater than that in the sensitized group (0.64 ± 0.09 cmH2O·ml⁻¹·min⁻¹·10 g liver wt⁻¹), as shown in Fig. 3. This indicates that the L-NAME pretreatment enhanced the anaphylaxis-induced increase in R. Although the pretreatment with L-NAME significantly augmented increases in both R pv and R hv after antigen, the increase in R pv (2.2-fold) predominated over that in R hv (1.2-fold). The increases in P do after antigen were highest in the L-NAME groups among all groups studied (Fig. 2), which is consistent with the significant enhancement of R hv after antigen. Liver weight gain in the L-NAME group was also highest among the groups shown in Fig. 2. In contrast, the ZnPP pretreatment did not affect the anaphylactic changes in any variables studied.

The basal bile flow rate was 0.04 ± 0.02 g·10 g liver wt⁻¹·min⁻¹ (n = 25). The bile flow in the ZnPP group decreased to 65 ± 6% of the preantigen levels transiently as concurrent with venoconstriction, a finding similar to the sensitized group. In the L-NAME group, the bile flow rate

**Fig. 1.** Representative recording of the response to ovalbumin antigen of a guinea pig liver in the sensitized group (Sensitized; A) and the N^6^-nitro-L-arginine methyl ester (L-NAME; B) group.

Effect of L-NAME and ZnPP on Basal Hepatic Circulation and Hepatic Anaphylaxis

Table 1 shows the summary data of hemodynamic variables at baseline and 10 min after administration of L-NAME or ZnPP. After injection of L-NAME, P pv, but not P do, increased significantly, resulting in a significant increase in R pv, but not R hv. Liver weight decreased only slightly but significantly after L-NAME. These findings indicate that L-NAME constricts primarily presinusoidal vessels and that endogenous NO dilates the portal veins rather than hepatic veins in basal states of isolated blood-perfused guinea pig liver. In contrast, administration of ZnPP, an inhibitor of HO, did not significantly change P pv, suggesting that CO does not play a significant role in the maintenance of the basal vascular tone of isolated blood-perfused guinea pig livers.

Figure 1 shows a representative example of the response to ovalbumin antigen after pretreatment with L-NAME. P pv increased markedly from the preantigen levels of 8.6 ± 0.6 to the peak of 40.7 ± 4.6 cmH2O at 4–6 min after antigen. P do also significantly increased after antigen (4.1 ± 0.4 vs. 9.5 ± 0.7 cmH2O preantigen vs. peak). The increase in the P pv-P do gradient was much larger than that in the P do-P hv gradient, suggesting that R pv increased greater than R hv. The liver weight increased markedly, reaching the peak of 4.6 ± 1.1 g/10 g liver wt at 10 min (Fig. 2).

The peak levels of R (1.15 ± 0.15 cmH2O·ml⁻¹·min⁻¹·10 g liver wt⁻¹) in the L-NAME group were 1.8-fold greater than that in the sensitized group (0.64 ± 0.09 cmH2O·ml⁻¹·min⁻¹·10 g liver wt⁻¹), as shown in Fig. 3. This indicates that the L-NAME pretreatment enhanced the anaphylaxis-induced increase in R. Although the pretreatment with L-NAME significantly augmented increases in both R pv and R hv after antigen, the increase in R pv (2.2-fold) predominated over that in R hv (1.2-fold). The increases in P do after antigen were highest in the L-NAME groups among all groups studied (Fig. 2), which is consistent with the significant enhancement of R hv after antigen. Liver weight gain in the L-NAME group was also highest among the groups shown in Fig. 2. In contrast, the ZnPP pretreatment did not affect the anaphylactic changes in any variables studied.

The basal bile flow rate was 0.04 ± 0.02 g·10 g liver wt⁻¹·min⁻¹ (n = 25). The bile flow in the ZnPP group decreased to 65 ± 6% of the preantigen levels transiently as concurrent with venoconstriction, a finding similar to the sensitized group. In the L-NAME group, the bile flow rate
decreased markedly to 59 ± 15% of the preantigen levels within 6 min after antigen.

**DISCUSSION**

The main findings of the present study are that the anaphylactic reaction in isolated perfused guinea pig livers was characterized by increases in both of pre- and postsinusoidal resistances, accompanied by liver weight gain, and that L-NAME, but not ZnPP, augmented this anaphylaxis-induced venoconstriction and hepatic congestion.

It is well known that the hepatic vascular responses to anaphylaxis of rats (7) and dogs (31, 32) are characterized by constriction of the hepatic vessels. In dogs, anaphylactic constriction of the hepatic vessels is accompanied by severe hepatic congestion resulting from vigorous postsinusoidal contraction (32). We herein showed that the hepatic anaphylaxis of guinea pigs also causes venoconstriction and congestion. Furthermore, the double vascular occlusion technique revealed that both pre- and postsinusoidal vessels contracted in response to antigen, although the presinusoidal constriction was greater in magnitude than the postsinusoidal constriction. The significant postsinusoidal constriction, as reflected by an increase in

![Fig. 2. Summary of the double-occlusion pressure (P_{do}; A) and liver weight changes (B) after antigen injection. Data are means ± SD. P < 0.05 vs. baseline (*) and vs. the sensitized group (#).](image)

![Fig. 3. Summary of total hepatic vascular (R_{t}; A), portal venous (R_{pv}; B), and hepatic venous (R_{hv}; C) resistances after antigen injection. Data are means ± SD. P < 0.05 vs. baseline (*) and vs. the sensitized group (#).](image)
Pdo, may account for hepatic congestion, as evidenced by the profound liver weight gain. These findings may indicate that anaphylaxis-induced congestion of liver and upstream splanchic organs could contribute to a decrease in circulating blood volume and thereby to anaphylactic hypotension in guinea pigs.

It has been shown that there is a species difference between dog and guinea pig in hepatic vessels that constrict preferentially during anaphylaxis; sensitized canine livers show selective presinusoidal constriction (32), whereas sensitized guinea pig livers show predominant presinusoidal constriction. The mechanism by which such a species difference occurred is not known. However, canine postsinusoidal hepatic veins contain anatomically smooth muscle sphincters in hepatic sublobular veins (4) that could vigorously contract in response to various mediators of anaphylactic reaction, such as histamine (28), thromboxane A2 (28), and platelet-activating factor (PAF; see Ref. 30). We speculate that receptors of these mediators might be localized predominantly in hepatic sublobular veins, which may account for anaphylaxis-induced selective postsinusoidal vasoconstriction in canine livers. In guinea pig livers, the predominant presinusoidal constriction induced by anaphylaxis may be caused by these substances. However, effects of these vasoconstrictors on the segmental vascular resistances of guinea pig livers have not been currently known. In addition, the localization of smooth muscle sphincter is not known in guinea pig livers. Further study is required to identify the chemical mediators responsible for this hepatic anaphylactic vasoconstriction.

We studied the modulating effects of NO on basal hepatic vascular tone in blood-perfused guinea pig livers. We found that l-NAME increased basal levels of Rpv but not Rpv. This finding indicates that endogenous basal production of NO may cause dilatation of primarily the portal side of the guinea pig hepatic vascular bed. These results contrast with the findings on the isolated rat livers perfused with a blood-free solution, in which inhibition of NO did not increase basal vascular tone (25). This discrepancy may be attributed to a difference in the mode of liver perfusion, especially the perfusate. We previously observed that l-NAME increased basal Ppv in the blood-perfused rat liver but not in blood-free perfused rat liver, although both rat livers were otherwise perfused in the same manner at a constant flow rate (unpublished observation). In blood-free perfused livers (25), compared with the blood-perfused livers, perfusate viscosity should be lower and thereby vascular resistance lower, which might generate only small shear stress and thus low levels of NO produced. This might account for a negligible role of NO in maintenance of basal vascular tone in blood-free perfused livers (25).

Synthesis of NO is stimulated during anaphylaxis (13). Indeed, inhibition of NO synthesis aggravates anaphylactic vasoconstriction. The exact mechanism for an activation of NO synthesis during hepatic anaphylaxis remains unclear. However, we assume that both effects of chemical mediators and shear stress could explain the increased NO production during hepatic anaphylaxis in this study. Indeed, most mediators of anaphylaxis, such as histamine (11, 14), leukotrienes (21), thromboxane A2 (14), and PAF (8), all stimulate NO release from the vascular endothelium. Another possibility is related to vasoconstriction-induced shear stress, which could generate NO from endothelium (10). In this respect, Macedo and Laust (16) reported, using in situ cat liver administered norepinephrine, that l-NAME potentiated vasoconstriction only under constant flow perfusion, where shear stress could increase, but not under constant pressure perfusion, where shear stress could not increase. In the present study, the hepatic perfusion flow was held constant during vasoconstriction, and thus shear stress could have increased, resulting in increased NO release.

CO could serve as a vasodilator and is endogenously generated by hepatocytes via the constitutively expressed HO-2 (26). In the present study, inhibition of CO synthesis by ZnPP did not significantly alter either the basal hepatic vascular pressures or the anaphylactic hepatic vasoconstriction, indicating that CO does not modulate hepatic circulation at basal levels or during anaphylaxis in isolated blood-perfused guinea pig livers. The former finding is not consistent with that on the isolated rat livers perfused with a blood-free solution, in which inhibition of CO increased basal vascular tone (25). No apparent action of CO on basal hepatic vascular tone in the present study might be attributed possibly to perfusate free Hb released through hemolysis. It is reported that oxyhemoglobin, oxygenated Hb, inhibits the action of CO (1, 9, 26, 29). The Masterflex roller pump used in the present study should have caused inevitable hemolysis, resulting in release of free Hb in the perfusate. This free Hb might have trapped CO, resulting in

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### Table 1. Basal hemodynamic variables in isolated perfused guinea pig livers of all groups studied

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<tr>
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<th>Nonsensitized Baseline (n = 6)</th>
<th>Sensitized Baseline (n = 7)</th>
<th>l-NAME (n = 6) Baseline</th>
<th>l-NAME (n = 6) After</th>
<th>ZnPP (n = 6) Baseline</th>
<th>ZnPP (n = 6) After</th>
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<tr>
<td>Rpv, cmH2O</td>
<td>7.2±0.5</td>
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<td>0.4±0.1</td>
<td>0.6±0.2</td>
<td>0.8±0.3</td>
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<td>0.6±0.3</td>
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<tr>
<td>Blood flow, ml/min⁻¹·10 l g liver⁻¹</td>
<td>36±4</td>
<td>35±5</td>
<td>36±3</td>
<td>36±3</td>
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<td>35±3</td>
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<tr>
<td>Rpv, cmH2O·ml⁻¹·min⁻¹·10 g liver⁻¹</td>
<td>0.18±0.04</td>
<td>0.21±0.05</td>
<td>0.19±0.02</td>
<td>0.23±0.03*</td>
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<tr>
<td>Rpv, cmH2O·ml⁻¹·min⁻¹·10 g liver⁻¹</td>
<td>0.11±0.03</td>
<td>0.11±0.02</td>
<td>0.10±0.01</td>
<td>0.13±0.02*</td>
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<td>0.12±0.02</td>
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<tr>
<td>Rpv, cmH2O·ml⁻¹·min⁻¹·10 g liver⁻¹</td>
<td>0.08±0.02</td>
<td>0.10±0.02</td>
<td>0.09±0.01</td>
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<tr>
<td>Blood flow, g/min⁻¹·10 g liver⁻¹</td>
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Values are means ± SD; n, no. of animals. l-NAME, N⁶O₂-nitro-l-arginine methyl ester; ZnPP, zinc protoporphyrin IX; Ppv, Ppv, and Ppv, portal venous, hepatic venous, and double-occlusion pressures, respectively; Rpv, Rpv, and Rpv, total, portal venous, and hepatic venous resistance, respectively. All data were obtained before injection of ovalbumin. After data obtained 10 min after injection of l-NAME or ZnPP for the l-NAME or ZnPP groups, respectively. *P < 0.05 vs. baseline for the l-NAME and ZnPP groups.
NITRIC OXIDE AND HEPATIC VASCULAR ANAPHYLAXIS IN GUINEA PIG LIVER

prevention of the CO action. However, our preliminary study revealed that the perfusate Hb concentration measured at 10 min after baseline measurement, when effects of ZnPP on basal hepatic circulation were evaluated, was only 17.6 ± 4.4 mg/dl (2.6 ± 0.6 µM, n = 7). Moreover, at 120 min after antigen, the end of the experimental period, the perfusate Hb concentration (61.9 ± 12.6 mg/dl; 9.1 ± 1.9 µM) does not seem to reach the levels enough to inhibit the CO action, because the biological action of CO can be blocked by oxyhemoglobin at concentrations of 25–100 µM (1, 9, 26, 29). However, exact concentrations of Hb are not well known to exert inhibitory effects on CO action in the perfused guinea pig livers. We cannot exclude the possibility that hemolysis might affect the results of the present study.

No substantial effects of CO on hepatic anaphylactic venoconstriction contrast with recent reports that circulatory shock, such as endotoxemia (3) and hemorrhagic shock (2), induced HO-1 in the liver, which results in attenuation of the increased hepatic vascular resistance. It seems likely that CO might not modulate hepatic circulation if it could not be generated substantially by inducible HO-1 under stressful perturbations. In the present study, we only observed an acute short phase of anaphylaxis, in which HO-1 could not have been induced because it takes more than several hours to induce HO-1 after oxidative stress (2, 3).

In this study, liver weight gain was observed after antigen in all groups except the nonsensitized group. This weight gain might be caused by hepatic venoconstriction, as evidenced by a significant increase in $R_{pv}$. This postsinusoidal contraction could induce the upstream sinusoidal engorgement and increased extracellular fluid filtration caused by an increase in $P_{do}$, the sinusoidal hydrostatic pressure. In the L-NAME group, $P_{do}$ after antigen increased to the highest levels among all groups, which indicates that the hepatic microvascular driving pressure for extracellular filtration was also highest. This assumption may account for the marked liver weight gain in the L-NAME group.

We found that the bile flow transiently decreased during hepatic anaphylaxis. The mechanism for this cholestasis is not known from the present study. This cholestasis could be caused by venoconstriction, which often causes the heterogeneous perfusion, resulting in partial anoxia and finally cholestasis. Actually reduced bile flow in the present study was dependent on venoconstriction in that in the L-NAME group where the antigen-induced increase in $R_{pv}$ was greatest among groups studied, the decrease in bile flow was also greatest. The direct effect of chemical mediators released during anaphylaxis on the bile-producing system might contribute to cholestasis in the present study.

The limitation of the present study is related to the use of a constant-flow portal perfusion with diluted, recirculating blood. Hepatic arterial perfusion with normally oxygenated blood would improve the metabolic milieu of the liver. With perfusion at prevailing arterial pressures, the large increase in $R_{pv}$ would markedly reduce total hepatic blood flow and change the magnitude of the responses to NO. If the blood flow had not been constant, the 46% increase in liver weight with anaphylaxis and L-NAME would have been less. Another shortcoming is related to the perfusate, which included a foreign protein of bovine albumin for oncotic pressure control. However, the bovine albumin is routinely used as perfusate for a variety of isolated perfused organ studies. We believe that it might not substantially affect the results of the present study.

In summary, this study demonstrated that the hepatic vascular anaphylaxis in guinea pig isolated perfused liver is characterized by hepatic congestion caused by increases in postsinusoidal resistance ($R_{ps}$) and the sinusoidal pressure ($P_{do}$). The large increase in presinusoidal resistance ($R_{pv}$) is the primary cause of splanchnic bed congestion that leads to the serious reduction of circulating volume in anaphylactic shock. Finally, blocking NO, but not CO, increases basal tone and potentiates the anaphylaxis-induced hepatic congestion by acting mainly on the postsinusoidal vessels of guinea pig livers.

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