Nitric oxide, but not carbon monoxide, attenuates anaphylaxis-induced postsinusoidal contraction and congestion in guinea pig liver.

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Short title: Nitric oxide and hepatic vascular anaphylaxis in guinea pig liver

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ABSTRACT

The pathophysiology of 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 portally and recirculatingly at constant flow with diluted blood. Using the double occlusion technique to estimate the hepatic sinusoidal pressure (Pdo), 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 NG-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 constriction in isolated guinea pig livers. However, the increases in postsinusoidal resistance and Pdo cause hepatic congestion. Endogenously produced NO, but not CO, modulates these responses.

KEY WORDS

hepatic circulation, antigen, double occlusion pressure, hepatic vascular resistance.
INTRODUCTION

Anaphylaxis is an immediate, type-1 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, angio-edema 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 due to 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 livers 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., 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 into the
perfusing blood (32). 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 hypotension of anaphylaxis (20). NO also
modulates anaphylaxis-induced changes in regional hemodynamics of coronary circulation
(27) and pulmonary circulation (22) 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 gaseous 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 into 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.

In order to clarify the anaphylactic disturbance of hepatic circulation, we herein
established anaphylactic models of isolated portally perfused guinea pig livers, in which the
sinusoidal pressure was measured using the double occlusion method (23, 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
a NOS inhibitor, \( N^3 \)-nitro-L-arginine methyl ester (L-NAME), and a specific inhibitor of CO-generating enzyme heme oxygenase (HO), zinc protoporphyrin IX (ZnPP), whether NO and/or CO modulates the hepatic anaphylaxis.

**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 non-sensitized animals were injected with mixture of complete Freund’s adjuvant (0.5 ml) and physiological saline (0.5 ml) without ovalbumin.

*Isolated Liver Preparation*

Two weeks after sensitization, the animals were anesthetized with pentobarbital sodium (35 mg\( \cdot \)kg\(^{-1} \), 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 intraarterial heparinization (500 U\( \cdot \)kg\(^{-1} \)), 8-9 ml of blood was 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, then portal perfusion was begun with the heparinized autologous blood that was diluted with 5% bovine albumin (Sigma) 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 Hct of 8%. The liver was rapidly excised, suspended from an isometric transducer (TB-652T, Nihon-Kohden, Japan) and weighed continuously throughout the experimental period.

The sensitized and non-sensitized 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 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% O₂ and 5% CO₂. 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. To occlude inflow and outflow perfusion lines simultaneously for measurement of the double occlusion pressure (Pdo), two solenoid valves were placed in a position that each sidearm cannula was between the corresponding solenoid valve and the liver. 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. Bile was collected drop by drop in a small tube suspended from the force transducer (SB-1T, Nihon-Kohden, Japan). One bile drop yielded 0.027 g and the time between drops was measured for determination of the bile flow rate (12). The hepatic vascular pressures, blood flow rate, liver weight and bile weight were monitored continuously and displayed through a thermal physiograph (RMP-6008, Nihon-Kohden, Japan). 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 Pdo.

**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 Phv of 0 - 1 cmH$_2$O, and at a Q of 36 ± 5 ml•min$^{-1}$•10g 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 non-sensitized animals into the Non-sensitized group.

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

2) The ZnPP group (n=6). Instead of L-NAME, ZnPP (10 µM) was administered into the reservoir, and ovalbumin 0.1 mg was injected at 10 min after injection of ZnPP.
3) The Sensitized group (n=7). Ovalbumin 0.1 mg was injected into the reservoir.

4) The Non-sensitized group (n=6). The livers were excised from the non-sensitized animals. Ovalbumin 0.1 mg was injected into the reservoir.

The hepatic sinusoidal pressure was measured by the double occlusion method (23, 32). Both the inflow and outflow lines were simultaneously and instantaneously occluded for 13 sec 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). In each experimental group, Pdo 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 (Rt), portal or presinusoidal (Rpv) and hepatic venous or postsinusoidal (Rhv) resistances were calculated as follows:

\[
R_t = \frac{P_{pv} - P_{hv}}{Q}
\]  
(1)

\[
R_{pv} = \frac{P_{pv} - P_{do}}{Q}
\]  
(2)

\[
R_{hv} = \frac{P_{do} - P_{hv}}{Q}
\]  
(3)

Statistics

All results are expressed as the means ± SD. Analysis of variance followed by Bonferroni’s test was used to test for significant differences. Differences were considered as statistically significant at P values less than 0.05.

RESULTS
The 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 Pdo at baseline state of 25 perfused guinea pig livers was 3.5 ± 0.3 cmH₂O, with Ppv 7.3 ± 0.4 cmH₂O and Phv 0.5 ± 0.3 cmH₂O at Q 36 ± 5 ml•min⁻¹•10g liver wt⁻¹. The calculated Rt was 0.19 ± 0.03 cmH₂O•ml⁻¹•min⁻¹•10g liver wt. The segmental vascular resistances of Rpv and Rhv were 0.11 ± 0.02 and 0.09 ± 0.02 cmH₂O•ml⁻¹•min⁻¹•10g liver wt, respectively, and the Rhv/Rt ratio was 0.44 ± 0.03. This indicates that 56% of the total portal-hepatic venous resistance of the isolated guinea pig livers exists in the portal venous side, which is similar to rabbit livers (15, 23).

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 cmH₂O to the peak of 22.6 ± 2.1 cmH₂O 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•10g liver wt⁻¹ at 10min, as shown in Fig. 2. The double occlusion maneuver performed at 4 min after antigen revealed a higher Pdo than the baseline value. Pdo increased from baseline value of 3.7 ± 0.3 to 7.9 ± 0.5 cmH₂O at 4 min after antigen, followed by a gradual return to the preinjection value, as shown in Fig. 2. At the maximal venoconstriction, the pressure gradient of Pdo-to-Phv was significantly increased from baseline of 3.3 ± 0.3 to 7.4 ± 0.6 cmH₂O, indicating an increase in Rhv. However, the increase in Ppv-to-Pdo gradient from 3.8 ± 0.3 to 14.8 ± 2.1 cmH₂O after antigen was much greater.
than that in the Pdo-to-Phv gradient, indicating a greater increase in Rpv than Rhv. Concomitant with venoconstriction, bile flow decreased to 66 ±11% of the basal flow rate, followed by a gradual recovery to 81 ± 10% at the end of the experimental period.

Figure 3 shows the time course of changes in Rt, Rpv and Rhv after ovalbumin injection. Rt increased in the time course same as Ppv after antigen injection. Rt increased 3-fold from 0.21 ± 0.05 to 0.63 ± 0.10 cmH₂O•ml⁻¹•min⁻¹•10g liver wt at 4 min. After antigen injection, Rpv showed 3.8-fold increase from the baseline of 0.11 ± 0.02 to the peak of 0.43 ± 0.07 cmH₂O•ml⁻¹•min⁻¹•10g liver wt, while Rhv showed only 2.2-fold increase from 0.09 ± 0.02 to the peak of 0.21 ± 0.04 cmH₂O•ml⁻¹•min⁻¹•10g liver wt. The increase of Rpv was significantly greater than that of Rhv.

The 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, Ppv, but not Pdo, increased significantly respectively, resulting in a significant increase in Rpv, but not Rhv. 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 Ppv, 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. Ppv increased markedly from the pre-antigen levels of 8.6 ± 0.6 to the peak of 40.7 ± 4.6 cmH₂O at 4-6 min after antigen. Pdo also significantly increased after antigen (4.1 ± 0.4 vs. 9.5 ± 0.7 cmH₂O; pre-antigen vs. peak). The increase in the Ppv-to-Pdo gradient was much larger than that in the Pdo-to-Phv gradient, suggesting that Rpv increased greater than Rhv. The liver weight increased markedly, reaching the peak of 4.6 ± 1.1 g•10g liver wt⁻¹ at 10min (Fig. 2).

The peak levels of Rt (1.15 ± 0.15 cmH₂O•ml⁻¹•min⁻¹•10g liver wt) 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•10g liver wt), as shown in Fig. 3. This indicates that, the L-NAME pretreatment enhanced anaphylaxis-induced increase in Rt. Although the pretreatment with L-NAME significantly augmented increases in both Rpv and Rhv after antigen, the increase in Rpv (2.2-fold) predominated over that in Rhv (1.2-fold). The increases in Pdo after antigen were highest in the L-NAME groups among all groups studied (Fig. 2), which is consistent with the significant enhancement of Rhv after antigen. Liver weight gain in the L-NAME group was also highest among the groups as 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 deceased to 65 ± 6% of the pre-antigen 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 pre-antigen 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 due to 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 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 splanchnic 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 postsinusoidal constriction (32), while sensitized guinea pig livers 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), which could vigorously contract in response to various mediators of anaphylactic reaction, such as histamine (28), thromboxane A₂ (28), and platelet-activating factor (PAF) (30). We speculate that receptors of these mediators might be localized predominantly in hepatic sublobular veins, which may account for anaphylaxis-induced selective postsinusoidal venoconstriction 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 have not been currently known on the segmental vascular resistances of guinea pig livers. 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 venoconstriction.

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 Rhv. 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), as compared with the blood-perfused livers, perfusate viscosity should be lower and thereby vascular resistance be 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 constriction of regional vasculature such as coronary artery (27) and pulmonary circulation (22). We herein showed that NO inhibition pronounced hepatic anaphylactic venoconstriction. 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 nitric oxide release from the vascular endothelium. Another possibility is related to venoconstriction-induced shear stress, which could generate NO from endothelium (10). In this respect, Macedo and Lautt (16) reported, using in situ cat liver administered of norepinephrine, that L-NAME potentiated venoconstriction 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 venoconstriction 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 venoconstriction, 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 hemoglobin released through hemolysis. It is reported that oxyhemoglobin, oxygenated hemoglobin, inhibits the action of CO (1, 9, 26, 29). The Masterflex roller pump used in the present study should have caused inevitably hemolysis, resulting in release of free hemoglobin into the perfusate. This free hemoglobin might have trapped CO, resulting in prevention of the CO action. However, our preliminary study revealed that the perfusate hemoglobin concentration measured at ten minutes 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 hemoglobin 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 hemoglobin are not well known to exert inhibitory effects on CO action in the
perfused guinea pig livers. We can not exclude the possibility that hemolysis might affect the results of the present study.

No substantial effects of CO on hepatic anaphylactic venoconstriction contrasts 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 observed only 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 Non-sensitized group. This weight gain might be caused by hepatic venoconstriction as evidenced by a significant increase in Rhv. This postsinusoidal contraction could induce the upstream sinusoidal engorgement and increased extravascular fluid filtration caused by an increase in Pdo, the sinusoidal hydrostatic pressure. In the L-NAME group, Pdo after antigen increased to the highest levels among all groups, which indicates that the hepatic microvascular driving pressure for extravascular 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 $R_t$ 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 portal venous resistance 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 affect substantially 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 ($R_{hv}$) 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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REFERENCES


Figure Legends

Figure 1. A representative recording of the response to ovalbumin antigen of a guinea pig liver in the Sensitized group (Sensitized) and the L-NAME group (L-NAME).

Figure 2. The summary of the double occlusion pressure (Pdo) and liver weight changes after antigen injection. Means ± SD; *P<0.05 vs. baseline; #P<0.05 vs. the Sensitized group.

Figure 3. The summary of total hepatic vascular (Rt), portal venous (Rpv) and hepatic venous (Rhv) resistances after antigen injection. Means ± SD; *P<0.05 vs. baseline; #P<0.05 vs. the Sensitized group.
Table 1. Basal hemodynamic variables in isolated perfused guinea pig livers of all groups studied

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonsensitized (n=6)</th>
<th>Sensitized (n=7)</th>
<th>L-NAME (n=6)</th>
<th>ZnPP (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Baseline</td>
<td>Baseline</td>
<td>After</td>
</tr>
<tr>
<td>Ppv (cmH\textsubscript{2}O)</td>
<td>7.2±0.5</td>
<td>7.5±0.5</td>
<td>7.3±0.5</td>
<td>8.6±0.6*</td>
</tr>
<tr>
<td>Phv (cmH\textsubscript{2}O)</td>
<td>0.5±0.2</td>
<td>0.4±0.1</td>
<td>0.6±0.2</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>Pdo (cmH\textsubscript{2}O)</td>
<td>3.3±0.3</td>
<td>3.7±0.3</td>
<td>3.6±0.4</td>
<td>4.1±0.4</td>
</tr>
<tr>
<td>Blood flow (ml/min/10g liver)</td>
<td>36±4</td>
<td>35±5</td>
<td>36±3</td>
<td>36±3</td>
</tr>
<tr>
<td>Rt (cmH\textsubscript{2}O/ml/min/10g liver)</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>
</tr>
<tr>
<td>Rpv (cmH\textsubscript{2}O/ml/min/10g liver)</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>
</tr>
<tr>
<td>Rhv (cmH\textsubscript{2}O/ml/min/10g liver)</td>
<td>0.08±0.02</td>
<td>0.10±0.02</td>
<td>0.09±0.01</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>Bile flow (g/min/10g liver)</td>
<td>0.03±0.01</td>
<td>0.03±0.01</td>
<td>0.03±0.01</td>
<td>0.02±0.01</td>
</tr>
</tbody>
</table>

Values are means±SD. All data were obtained before injection of ovalbumin. After, the data obtained 10 min after injection of L-NAME or ZnPP for the L-NAME or ZnPP group, respectively; *P<0.05 vs. Baseline for the L-NAME and ZnPP groups.
Figure 1

Sensitized

LIVER WEIGHT CHANGE (g)

PORTAL VEIN PRESSURE (cmH2O)

HEPATIC VEIN PRESSURE (cmH2O)

BLOOD FLOW (ml/min)

BILE FLOW (g)

OVALBUMIN

L-NAME

2 min

2 min
Figure 2

- L-NAME
- ZnPP
- Sensitized
- Non-sensitized

Pdo (cmH₂O)

Liver weight change (g/10g liver)

Time after antigen (min)
Figure 3

Rt
(cmH₂O/ml/min/10 g liver)

Rpv
(cmH₂O/ml/min/10 g liver)

Rhv
(cmH₂O/ml/min/10 g liver)

Time after antigen (min)