Effects of Norepinephrine and Histamine on Vascular Resistance in Isolated Perfused Mouse Liver

Toshishige SHIBAMOTO, Sen CUI, Zonghai RUAN, and Yasutaka KURATA
Department of Physiology, Kanazawa Medical University, Uchinada Ishikawa, 920-0293 Japan

Abstract: Mice have frequently been used for a variety of physiological studies because of the development of genetic engineering. However, the characteristics of hepatic vessels such as the vascular resistance distribution and the reactivity to various vasoconstrictors are not known in mice. We therefore determined the basal levels of segmental vascular resistances and the effects of histamine and norepinephrine on the vascular resistance distribution of mice. The liver of male non-inbred ddY mice was excised and perfused via the portal vein with 5% bovine albumin-Krebs solution at a constant flow rate. The sinusoidal pressure was measured by the double occlusion pressure and used to determine the presinusoidal ($R_{pre}$) and postsinusoidal ($R_{post}$) resistances. The basal $R_{post}$ comprised 53 ± 1% of the total hepatic vascular resistance. The norepinephrine and histamine increased $R_{pre}$ in a greater magnitude than $R_{post}$ with liver weight loss. However, the response to histamine was weaker than that to norepinephrine. Moreover, histamine-induced vasoconstriction showed tachyphylaxis. In conclusion, the presinusoidal and postsinusoidal resistances of mouse livers were similar in magnitude. The presinusoidal vessels predominantly contract in response to norepinephrine and histamine in mouse livers. [The Japanese Journal of Physiology 55: 143–148, 2005]

Key words: double occlusion pressure, isolated perfused mouse liver, sinusoidal pressure.

The passive blood mobilization to and from the liver, which influences the venous return to the heart, is critically dependent on the location and magnitude of intrahepatic vascular resistances in relation to the compliances [1]. There are species differences in the distribution of the hepatic vascular resistance. In canine livers, the presinusoidal resistance comprises approximately 50% of the total liver vascular resistance [2], but it comprises 56% and 59% in guinea pig [3] and rabbit livers [4–6], respectively, and more than 60% in rat livers [7, 8]. However, the basal hepatic vascular resistance distribution of mouse livers is not known, although mouse has been frequently used in physiological studies because of the development of genetic engineering.

Species differences are also found in the primary site of hepatic vasoconstriction. By using the vascular occlusion methods for measurement of the hepatic sinusoidal pressure [2, 9], we have recently shown that the hepatic longitudinal vascular responsiveness to vasoactive substances differs among different species, such as dogs, rabbits, rats, and guinea pigs [4–6, 9–11]. Histamine predominantly contracts the postsinusoidal veins with resultant hepatic congestion in dogs [9, 10] and guinea pigs [11], but this substance constricts presinusoidal vessels in rabbits [4]. In rat livers, histamine did not contract or dilate hepatic vessels [11]. On the other hand, norepinephrine predominantly contracts the presinusoidal veins over the postsinusoidal veins in dogs, rabbits, rats, and guinea pigs [4, 7, 9, 11]. However, the effects of these vasoconstrictors have not been determined on hepatic vascular resistance distribution in mice. Furthermore, norepinephrine is released during the critical circumstances of sympathoexcitation, such as hemorrhagic shock, but histamine could be released during liver transplantation and systemic anaphylaxis and thereby cause a disturbance of hepatic circulation [12].

Therefore we have herein established the isolated perfused mouse liver preparation, which permits the measurement of hepatic vascular pressures, including sinusoidal pressure and liver weight. We determined the basal hepatic vascular resistance distribution and the effects of histamine and norepinephrine on segmental vascular resistances in mouse livers.
Methods

The study protocol was approved by the Animal Research Committee of Kanazawa Medical University in Uchinada, Japan. Thirty-two male specific-pathogen-free outbred ddY mice (43 ± 0.5 [SE] g; SLC Co., Hamamatsu, Japan), one of the most popular mouse strains in Japan, were anesthetized with pentobarbital sodium (50 mg/kg, ip) and mechanically ventilated with room air. After laparotomy, the bile duct was cut and the hepatic artery was ligated. At 5 min after an injection of heparin (500 mU/g) into the intraabdominal inferior vena cava (IVC), the IVC above the renal veins was ligated, and the portal vein was cannulated with a stainless cannula (OD 1.2 mm, ID 1.0 mm) for portal perfusion. After thoracotomy, the supradiaphragmatic IVC was cannulated through a right atrial incision with the same size stainless cannula, and portal perfusion was then begun with 5% albumin-Krebs buffer. The liver was rapidly excised, then suspended from an electric balance and weighed.

The basic method for liver perfusion was described previously [4], but each apparatus was the minimum size. The liver was perfused at a constant flow rate in a recirculating manner via the portal vein with the albumin-Krebs buffer that was pumped by a Masterflex pump from the venous reservoir through a heat exchanger (37°C). The recirculating blood volume was 30 ml. The height of the reservoir and the perfusate flow rate could be adjusted independently to maintain the portal and hepatic venous pressures at any desired level. The perfusate was oxygenated in the reservoir by continuous bubbling with 95% O₂ and 5% CO₂. We measured the portal venous (Ppv) and the hepatic venous (Phv) pressures with pressure transducers connected to the corresponding side arm with the reference points at the hepatic hilus. To measure the double occlusion pressure (Pdo), we placed two solenoid valves around the perfusion tubes upstream from the Ppv sidearm cannula and downstream from the Phv sidearm cannula [4]. The perfusate flow rate (Q) was measured manually by collecting outflow perfusate for 1 min just before the baseline measurement. The same measurement was done at the end of the experiment to confirm the constancy of perfusate flow during the experimental period. The hepatic vascular pressures and liver weight (W) were monitored continuously and displayed through a thermal physiograph.

Hepatic hemodynamic parameters were observed for at least 20 min after the start of perfusion, during which an isogravimetric (no liver weight gain or loss) state was reached. After the baseline measurements, the perfused livers were challenged with either histamine (Sigma) or norepinephrine (Bitartrate salt, Sigma). They were injected as a bolus into the reservoir to attain the final perfusate concentration of 0.001–30 µM and 1–1,000 µM, respectively. The volume of each injected agent was adjusted to less than 0.5 ml.

The hepatic sinusoidal pressure was measured by the double occlusion method [2]. The inflow and outflow lines were simultaneously and instantaneously occluded with the solenoid valves, after which Ppv and Pdo rapidly equilibrated to a similar or identical pressure, which was Pmv. In each experimental group, Pdo was measured at baseline and maximal vasoconstriction.

The total portal-hepatic venous (Rt), presinusoidal (Rpre), and postsinusoidal (Rpost) resistances were calculated as follows:

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

All results are expressed as the mean ± SEM. The comparisons were made with Student’s t-tests. A p value of less than 0.05 was considered significant.

Results

The final wet liver weight measured immediately after experiments was 1.91 ± 0.02 g. The Pdo at the baseline states of 32 perfused mouse livers was 2.3 ± 0.1 mmHg, with Ppv 4.0 ± 0.1 mmHg and Phv 0.5 ± 0.05 mmHg at Q 2.3 ± 0.05 ml/min/g liver wt. The calculated Rt was 1.55 ± 0.04 mmHg/ml/min/g liver wt. The segmental vascular resistances of Rpre and Rpost were 0.74 ± 0.03 and 0.81 ± 0.02 mmHg/ml/min/g liver wt, respectively, and the Rpost/Rpre ratio was 0.53 ± 0.01. This indicates that 53% of the total portal-hepatic venous resistance of the isolated mouse livers exists in the postsinusoids.

Norepinephrine and histamine produced qualitatively the same responses: Ppv increased substantially, but
Vascular Resistance in Perfused Mouse Liver

$P_{pv}$ either did not change or increased only minimally, with liver weight loss, as shown in Figs. 1 and 2. In livers treated with either norepinephrine or histamine, the $P_{pv}$-$P_{pv}$ gradient increased in a greater magnitude than the $P_{do}$-$P_{do}$ gradient, a finding indicating that $R_{pre}$ was predominantly increased over $R_{post}$. However, the response to histamine was weaker than that to norepinephrine. Moreover, histamine-induced vasoconstriction showed tachyphylaxis (Fig. 2). Figure 3 shows the peak levels in $R_{pre}$, $R_{post}$, $R_{av}$, and $Wt$ changes after injections of norepinephrine and histamine. $R_{av}$ increased in a dose-dependent manner at 0.001–30 µM norepinephrine, reaching the maximum level of 151 ± 3% of baseline at 30 µM. This increase in $R_{av}$ at 30 µM was mainly due to an increase in $R_{pre}$ because the maximum levels of $R_{pre}$ was 193 ± 3% of the baseline, and the corresponding levels of $R_{post}$ was only 117 ± 3% of the baseline, as shown in Fig. 3. Histamine did not cause vasoconstriction until the concentration increased to 100 µM, as shown in Fig. 3. Even at 1,000 µM histamine, $R_{pre}$ increased to only 145 ± 9% of the baseline, whereas $R_{post}$ did not change significantly. Immediately after norepinephrine or histamine, the liver weight decreased and then gradually returned to the baseline. The maximal liver weight losses after injections of norepinephrine at 30 µM and histamine at 1,000 µM were approximately 0.15 and 0.03 g/g liver wt, respectively.

**Discussion**

There is a species difference in the distribution of segmental vascular resistances in the livers of animals, including dogs, rabbits, guinea pigs, and rats. We have recently shown by measuring the sinusoidal pressure, using the triple vascular occlusion method [9] and the double occlusion method [2], in isolated canine livers that $R_{post}$ comprises approximately half of $R_{av}$. In contrast, $R_{pre}$ in the other animals is greater in magnitude than $R_{post}$. Actually, we subsequently demonstrated that 59% of $R_{av}$ exists in presinusoidal vessels in isolated rabbit livers [4, 5]. This agrees with the study of Maass-Moreno and Rothe [13], who reported that in intact rabbit livers the pressure gradient from the hepatic sinusoids averaged 59% of the total $P_{pv}$ to the abdominal vena caval pressure gradient. Similar segmental vascular resistance distribution was found in guinea pig livers, in which $R_{pre}$ comprises 61% of $R_{av}$ [3, 11]. The rat livers show more marked predominance of $R_{pre}$ over $R_{post}$; $R_{pre}$ is 69% of $R_{av}$ [11]. In the present study, for the first time we reported that the basal vascular resistance distribution of mouse livers was similar to that of canine livers because $R_{pre}$ comprises 47% of $R_{av}$.

Species differences are also found in the hepatic vascular responsiveness to vasoactive substances. With respect to responses to histamine, this substance predominantly contracts the postsinusoidal vessels in dogs [9, 10, 14, 15] and guinea pigs [11]. On the other hand, in rabbit livers, histamine selectively increases $R_{pre}$ in isolated-perfused liver [4], and this vasoactive amine also significantly increases $R_{post}$ in in vivo preparations [19]. In contrast, histamine did not contract the hepatic vessels in rat livers [11, 16–18]. In the present study we showed that histamine selectively contracts the presinusoidal vessels of mouse livers, a finding similar to the results of studies on rabbit livers. The difference in the vasoconstrictive site for histamine might be ascribed, at least in part, to the different distribution of functionally active receptors between the presinusoidal vessels and the hepatic veins. The absence of vasoconstrictive responses to histamine in rat livers may be due to a lack of functional histamine receptors in rat hepatic vascular smooth muscles.

In the present study, the concentration of histamine required to produce significant hepatic vasoconstriction was 100 µM. The responsiveness of mouse hepatic vessels to histamine seems to be much weaker than to norepinephrine because the lower concentration of 0.1 µM norepinephrine can induce significant hepatic vasoconstriction, as shown in Fig. 3. Furthermore, 1,000 µM histamine increased $R_{av}$ only to 1.2-fold baseline, whereas the 100 times lower concentration of 10 µM norepinephrine increased $R_{av}$ to 1.4-fold baseline. A similar result was observed in isolated rabbit liver [4].

In contrast to histamine, norepinephrine predominantly contracts presinusoidal vessels over postsinusoidal vessels in dogs [7, 9], rabbits [4, 19], guinea pigs [11], and rats [11]. Actually, Rothe and colleagues, using the microopipette servonull pressure measurement technique, have recently demonstrated that the increase in the presinusoidal resistance is greater than in the postsinusoidal resistance in dogs [7], rats [7], and rabbits [19] during norepinephrine infusion. We added
new evidence that a similar response to norepinephrine was observed in isolated mouse livers. It is well known that norepinephrine causes a reduction in liver blood volume in cats [20], dogs [9, 21], rabbits [4, 19], guinea pigs [11], and rats [11]. In this respect, the present study showed that mouse livers also respond to norepinephrine with a reduction of liver weight, suggesting a decrease in liver blood volume. These findings suggest that norepinephrine, a mediator of the sympathetic nervous system, causes predominant presinusoidal constriction with a resultant decrease in liver blood volume beyond the species differences. The physiological significance of this finding is that the primitive response to life-threatening insults, which cause sympathoexcitation, may be similar among animals. In this respect, histamine, a mediator released from mast cells...
in response to allergy or anaphylaxis, does not seem to be essential to life; therefore the hepatic responses may differ among animals.

In the present study, a decrease in $W_t$ accompanied predominant presinusoidal vessel constriction, when norepinephrine or histamine was injected into mouse livers. The mechanism for this decrease cannot be currently clarified. However, it may be related to possible heterogeneous portal venule constriction. If heterogeneity existed in portal venule constriction among the hepatic lobules, that is, some vessels were closed and others open, the blood volume of sinusoids that was distal to the closed portal venules could be passively reduced because of a decrease in the distending pressure of the sinusoids. In contrast to this passive change in liver volume, another possibility exists that contractile elements exist in the walls of the hepatic sinusoids that may be stimulated by norepinephrine as well as endothelin [22–24]. Rothe and Maass-Moreno [24] infused norepinephrine into in vivo rabbit and found a decrease in liver volume, even though $P_{pv}$ and hepatic venule pressure increased. This is clear proof of an active response and not a passive response to distending pressure.

As shown in Fig. 2, in response to the second bolus injection of histamine at 1 mM, hepatic venoconstriction was not observed, but the liver weight transiently decreased. The mechanism for this venoconstriction-independent decrease is unknown. We assumed that high osmolarity resulting from a bolus injection of 1 mM histamine might account for the decrease in liver weight: The hyperosmotic solution with 1 mM histamine might have caused osmosis and liver cell volume shrinkage, resulting in transient liver weight loss. Since the presence of structural pores of the sinusoidal endothelium enables free and rapid movement of drug and water molecules between the intravascular spaces and Disse’s spaces, it is expected that an intravascular hyperosmolarity could easily cause osmosis at hepatocytes and at sinusoidal endothelial cells. Water derived from these cells might rapidly diffuse into the intravascular space through the endothelial pores and might be carried away extrahepatically via the blood stream. Indeed, we observed that a bolus injection of the solution with nonvasoactive sucrose at 1 mM, the volume and osmolarity of which are the same as those of the histamine solution, caused venoconstriction-independent liver weight loss in isolated perfused liver (data not shown).

There are limitations of the methods used in the present study. First, the livers were perfused via only the portal vein because of the technical difficulty to perfuse the hepatic artery. With the hepatic artery occluded, no clues were provided for the sensitivity of the hepatic arterioles to histamine and norepinephrine. Second, the livers were perfused with 5% bovine albumin-Krebs solution, but not with blood. There is a possibility that the oxygen sensitivity of the most metabolically active tissue may be limited. However, we confirmed previously that the bubbling with 95% oxygen of the albumin-Krebs perfusate produced the inflow perfusate $P_{O_2}$, 300 mmHg [5]. We believe that the delivery of oxygen to the liver was adequate.

In conclusion, by measuring the hepatic sinusoidal pressure with the double occlusion method, we determined the basilar vascular resistance distribution and the effects of histamine and norepinephrine on the segmental vascular resistances in isolated mouse livers perfused with blood-free albumin Krebs buffer. The presinusoidal and postsinusoidal resistances of mouse livers were similar in magnitude. In response to norepinephrine and histamine, presinusoidal vessels predominantly contract with a resultant decrease in hepatic vascular volume in mouse livers.

This work was supported by a Grant for Collaborative Research from Kanazawa Medical University (C2003-1, C2004-1, C2005-1) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan (No. 15591665).

REFERENCES

8. Ling YQ, Shibamoto T, Honda T, Kamikado C, Hironaka E, Hongo M, and Koyama S: Increased sinusoidal
pressure is associated with early liver weight gain in ischemia-reperfusion injury in isolated perfused rat liver. J Surg Res 88: 70–77, 2000