Vagus nerve is involved in lack of blood reflow into sinusoids after rat hepatic ischemia

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Vagus nerve is involved in lack of blood reflow into sinusoids after rat hepatic ischemia. Am J Physiol Heart Circ Physiol 278: H1565–H1570, 2000.—Although recovery of microcirculation is an important determinant for ischemia-reperfusion injury, little information is available about hepatic blood flow after ischemia. To examine regulatory mechanisms of postischemic hepatic microcirculation, we studied the sinusoidal blood flow in sinusoids after portal triad clamping of rat livers for 5, 15, or 30 min. Hepatic tissue blood flow and erythrocyte blood flow in sinusoids were measured using a laser-Doppler flowmeter and an intravital microscope, respectively. There was a time of no blood flow (lag time) in sinusoids after declamping, dependent on the ischemic time. Cholinergic blockade agents eliminated the lag time, whereas nerve stimulation at the hiatus esophagus or on the hepatoduodenal ligament during reperfusion prolonged it. Chemical denervation with 10% phenol or surgical denervation on the hepatoduodenal ligament eliminated the lag time. The prolongation of lag time by nerve stimulation was completely abrogated by truncal vagotomy. These results suggest that the cholinergic vagus nerve is involved in causing the lag time of sinusoidal blood flow in hepatic ischemia-reperfusion.

ischemia-reperfusion; blood flow; portal triad clamping; hepatic microcirculation

MATERIALS AND METHODS

Reagents. E5880, a platelet-activating factor antagonist, was a gift from Eizai (Tokyo, Japan). Vinblastine and allopurinol were purchased from Sigma Chemical (St. Louis, MO). All other chemicals were of analytic grade.

Animals and procedures. Male Lewis rats weighing 200–250 g were deprived of food and had free access to water for 12 h prior to the experiments. All animal experiments were conducted in accordance with our institutional guidelines for the care and use of laboratory animals. Rats were anesthetized with pentobarbital sodium (40 mg/kg body wt ip) and allowed to breathe spontaneously. Catheters were inserted into the femoral artery and a tributary of the superior mesenteric artery for monitoring the systemic arterial and portal venous pressures, respectively. Physiological saline (1.5 ml·h⁻¹·kg body wt⁻¹) was infused with an infusion pump (model 11, Harvard Apparatus, South Natick, MA) from a catheter inserted in the femoral vein throughout the experiments.

PTC was performed for 5, 15, or 30 min by gently placing a microvascular clip on the hepatoduodenal ligament, in which the hepatic artery, portal vein, and bile duct are present (11). After transient ischemia for the indicated times, the clip was released and hepatic tissue blood flow was measured using a laser-Doppler flowmeter or an intravital microscope. In separate experiments, we measured the postischemic hepatic blood flow after PTC using portal-systemic shunt rats.
as described by Meredith and Wade (18) to rule out the effects of gastrointestinal congestion. Briefly, the spleen was subcutaneously transpositioned with the splenic vasculature, which is reported (18) to enable anastomosis to develop between the splenic capsule and the abdominal wall. After 4 days, PTC was performed to measure the postischemic hepatic blood flow and portal venous pressure. Clamping of the hepatoduodenal ligament other than the bile duct was performed as follows: the common bile duct was gently isolated, and a microvascular clip was placed on the hepatoduodenal ligament without clamping the bile duct. Nerve stimulation using an electronic stimulator (SEN-3101, Nihon Kohden, Osaka, Japan) was performed by placing a bipolar platinum electrode around either the hepatoduodenal ligament or the hiatus esophagus with rectangular monophasic pulses of 10-Hz frequency, 2-ms duration, and 40-V amplitude, from 10 min before declamping until 20 min after declamping (10, 12).

In separate experiments, a catheter was inserted into the vena cava through the jugular vein and blood samplings were made before PTC (for 15 min) and 5 min after PTC. The plasma endothelin-1 level was measured by RIA as previously reported (22).

Measurement of hepatic microcirculation. A laser-Doppler flowmeter (ALF 21, Advance, Tokyo, Japan) was used for monitoring hepatic tissue blood flow. After a baseline recording of 10 min, we performed the ischemic procedures for the indicated times. The postischemic hepatic blood flow was monitored for 30 min after release of the microsurgical clip. Hepatic blood flow monitoring was conducted in the same area of the left lateral lobe.

Measurements of blood flow of the hepatic artery and the portal vein in the hepatoduodenal ligament were performed with an ultrasonic transit-time volume flowmeter (USTF; T206, Transonic Systems, Ithaca, NY) using a miniature flow probe, a 2SB-reflector probe. USTF was placed on the hepatic side of the hepatoduodenal ligament to measure the blood flow in the hepatoduodenal ligament. PTC was performed for 15 min by gently placing a microvascular clip on the intestinal side of the hepatoduodenal ligament.

The apparatus and the analytic method for using the intravital microspectroscope were essentially the same as those previously described (14, 22, 26). Three units were combined with a microscope: 1) a computer-controlled scanning spectrophotometer for measuring the visible spectra of tissues and erythrocytes to obtain the O2 saturation (SO2) of Hb flowing in single sinusoids, 2) two photomultipliers for measuring the erythrocyte velocity (v) according to the dual-spot cross-correlation method, and 3) a CCD camera and an image analyzer for measuring the sinusoid diameter (D) and length of the sinusoid. To examine the changes in Hb content and oxygenation in the left lateral lobe, we guided transmitted light from a 340-µm spot diameter to a microspectroscope through a 5-mm-diameter optical fiber, instead of a 400-µm-diameter fiber, which enabled measurement of the spectral changes in the liver at a depth of 3–5 mm. The difference in absorption between 577 and 586 nm was used for monitoring changes in blood oxygenation, where 577 nm is the a-band peak of oxygenated Hb and 588 nm is the isosbestic point of oxygenated and deoxygenated Hb. The difference in absorption between 586 and 603 nm was used for monitoring changes in the Hb concentration, where 603 nm was used as the reference wavelength because of the small difference in molar extinction coefficient between oxygenated and deoxygenated Hb.

Drugs and treatment. Heparin (20 IU·kg body wt.−1·h−1) and trinitroglycerin (30 µg·kg body wt.−1·h−1) were infused intravenously from 30 min before the experiments until the end of experiments. E5880 (13 mg/kg body wt), a platelet-activating factor (PAF) inhibitor, was injected intramuscularly 12 h and 1 h before the experiments. Vinblastine (1.6 mg/kg body wt) dissolved in distilled water was intraperitoneally administered to the rats 5 and 2 days before the experiments. The administration of vinblastine decreased the number of white blood cells from 6,800 ± 1,270 (mean ± SD, n = 5) to 1,780 ± 430 per cubic millimeter. Allopurinol (50 mg/kg body wt), which reduces oxygen radical formation by inhibiting xanthine oxidase, was administered intraperitoneally 1 h before the experiments. Atropine (5 mg/kg), scopolamine (10 mg/kg), or phenoxybenzamine (5 mg/kg) and propranolol (20 mg/kg) were administered intraperitoneally 20 min before the experiments (10, 19). In the last group, rats with hypotenion shock were excluded from the study. Guanethidine (100 mg/kg) was administered intraperitoneally 18 h before the experiments (19). Chemical denervation was performed by applying 10% phenol on the hepatoduodenal ligament 20 min before the experiments as described by Cucchiara et al. (3). For surgical denervation, the hepatoduodenal ligament was completely dissected and skeletonized except for the hepatic artery, portal vein, and bile duct (3). Truncal vagotomy was performed at the hiatus esophagus.

RESULTS

Lack of blood flow in sinusoids after declamping. Total clamping of the hepatoduodenal ligament stopped hepatic tissue blood flow and the mean arterial blood pressure decreased from 100 ± 8 (mean ± SD; n = 5) to 60 ± 10 (n = 5) mmHg, whereas the portal venous pressure was increased from 8 (mean of five different experiments) to 45 mmHg. After we released the microsurgical clip, the systemic blood pressure was restored to the preclamping level (Fig. 1). The recovery of the systemic blood pressure appeared to be faster than that of hepatic tissue blood flow. There was a transient time of no hepatic tissue blood flow (lag time) after the microvascular clip was declamped (Fig. 1). The length of the lag time appeared to depend on the time of ischemia (Table 1). The portal venous pressure and blood flow in the hepatoduodenal ligament were measured (Fig. 2). After declamping, the portal venous pressure returned to the preclamping level within a few seconds. Blood flow in the hepatoduodenal ligament, which mainly consisted of portal blood flow (11), showed an initial transient blood flow and then rapidly decreased to the basal line, which was followed by a gradual increase in the blood flow. PTC decreased the liver thickness, and declamping engorged the liver and increased the liver thickness. After hepatic tissue blood flow was initiated, the liver size and thickness were gradually normalized. The blood levels of endothelin-1 before and after PTC were 1.6 ± 0.8 (n = 6) and 2.2 ± 1.4 (n = 6) pg/ml, respectively, and there was no significant difference between their values (P = 0.1664).

Because laser-Doppler flowmetry is sensitive to blood flow changes in the hepatic artery and to blood flow...
changes after venous stasis in areas near the liver surface (1, 2), we directly measured sinusoidal blood flow (erythrocyte velocity) using a microspectroscope (Fig. 3). After PTC, the microspectroscope demonstrated collapse of sinusoids within several minutes and a lack of erythrocytes flowing in sinusoids. There were erythrocytes in the central veins, terminal portal veins, and other large vessels, where the blood showed a to-and-fro movement. Erythrocytes flowed back into collapsed sinusoids 2–7 min after declamping as observed by the laser-Doppler flowmeter (Fig. 1 and Table 1). To confirm that these changes in sinusoidal blood flow occurred not only in superficial sinusoids but also in more deeply located sinusoids, we evaluated changes in hepatic Hb content and oxygenation of the liver at 3- to 5-mm thicknesses using transmitted light and an intravital microspectroscope. Data were not shown, but increases in hepatic Hb content and oxygenation were initiated 1 min after declamping. The results were consistent with the presence of lag time as observed by the laser-Doppler flowmetry in principle (Fig. 1), but deeply located sinusoids appeared to have faster recovery of blood flow than superficially located sinusoids (Table 1). In the present study, the lag time was defined as the time during which there was no erythrocyte flow in sinusoids between declamping and the reflow of erythrocytes. The lag time appeared to depend on the time of ischemia (Table 1).

Effects of drugs and nerves. To elucidate the mechanism of lag time formation, we examined the effects of several humoral and nervous factors on sinusoidal blood flow. In the present study, the lag time was defined as the time during which there was no erythrocyte flow in sinusoids between declamping and the reflow of erythrocytes. The lag time appeared to depend on the time of ischemia (Table 1).

![Fig. 1. Recovery of hepatic tissue blood flow after temporary portal triad clamping (PTC).](http://ajpheart.physiology.org/)

![Fig. 2. Changes in afferent vessel blood flow and blood pressure after temporary PTC.](http://ajpheart.physiology.org/)

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**Table 1. Lag time after transient portal triad clamping measured by laser-Doppler flowmeter or by in vivo microspectroscope**

<table>
<thead>
<tr>
<th>Ischemic Time</th>
<th>Laser-Doppler Flowmeter</th>
<th>In Vivo Microspectroscope</th>
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</thead>
<tbody>
<tr>
<td>5 min (n = 5)</td>
<td>Lag time, min 0 ± 0°</td>
<td>Final flow, % of initial values 86.3 ± 17.9</td>
</tr>
<tr>
<td>15 min (n = 5)</td>
<td>1.9 ± 1.2°</td>
<td>58.5 ± 7.9</td>
</tr>
<tr>
<td>30 min (n = 5)</td>
<td>9.0 ± 6.6°</td>
<td>44.8 ± 3.6</td>
</tr>
<tr>
<td>15 min + 10% phenol</td>
<td>0 ± 0°</td>
<td>ND</td>
</tr>
<tr>
<td>15 min + nerve stimulation at the hiatus esophagus</td>
<td>6.2 ± 1.1°</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of experiments. °P < 0.05 vs. 5-min and 15-min ischemia; †P = 0.0022 vs. 5-min ischemia; ‡P = 0.0001 vs. 5-min ischemia; §P = 0.0591 vs. 15-min ischemia; †lag time was not detectable in these conditions. °Same data as presented in Table 2. ND, not determined. No significant difference was found between other pairs.
blood flow. Although the portal-systemic shunt operation inhibited the increase in the portal pressure during PTC (mean portal pressure = 11 mmHg; n = 5), the lag time was not significantly affected (Table 2). Furthermore, partial clamping of the left lateral lobe also avoided portal congestion and caused a similar lag time (mean = 2.1 min, n = 2). Extrahepatic bile duct obstruction was reported (19) to be associated with decreased portal blood flow. However, the lag time was not eliminated by clamping the hepatoduodenal ligament other than the choledocus (Table 2). An anticoagu-

Table 2. Effects of various agents and procedures on lag time after 15-min portal triad clamping (measured by laser Doppler flowmeter)

<table>
<thead>
<tr>
<th>Procedures and Drugs</th>
<th>Lag Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (15-min ischemia)</td>
<td>1.9 ± 1.2</td>
</tr>
<tr>
<td>Portal-systemic shunt</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>No bile duct obstruction during PTC</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>Heparin (20 U/kg)</td>
<td>1.1 ± 1.1</td>
</tr>
<tr>
<td>E5880 (two doses of 13 mg/kg)</td>
<td>1.1 ± 1.3</td>
</tr>
<tr>
<td>Trinitroglycerol (30 mg·kg⁻¹·h⁻¹)</td>
<td>1.8 ± 2.1</td>
</tr>
<tr>
<td>Allopurinol (50 mg/kg)</td>
<td>1.1 ± 1.0</td>
</tr>
<tr>
<td>Vinblastine (two doses of 1.6 mg/kg)</td>
<td>3.0 ± 2.8</td>
</tr>
<tr>
<td>Atropine (5 mg/kg)</td>
<td>0 ± 0*†</td>
</tr>
<tr>
<td>Scopolamine (10 mg/kg)</td>
<td>0 ± 0*†</td>
</tr>
<tr>
<td>Guanethidine (100 mg/kg)</td>
<td>1.7 ± 1.3</td>
</tr>
<tr>
<td>Phenoxbenzamine (5 mg/kg) + propranolol (20 mg/kg)</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>10% Phenol treatment on hepatoduodenal ligament</td>
<td>0 ± 0*†</td>
</tr>
<tr>
<td>Surgical denervation in hepatoduodenal ligament</td>
<td>0 ± 0*†</td>
</tr>
<tr>
<td>Truncal vagotomy</td>
<td>0 ± 0*†</td>
</tr>
<tr>
<td>Nerve stimulation at hepatoduodenal ligament</td>
<td>6.2 ± 1.1*</td>
</tr>
<tr>
<td>Vagal nerve stimulation at hiatus esophagus</td>
<td>5.5 ± 1.2‡</td>
</tr>
<tr>
<td>Nerve stimulation at celiac truncus</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>Truncal vagotomy + nerve stimulation at hepatoduodenal ligament</td>
<td>0 ± 0*†</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 5 experiments). PTC, portal triad clamping. *P < 0.0001 vs. control; †lag time was not detectable in this condition; ‡P = 0.0008 vs. control.

DISCUSSION

The liver receives 67–80% of its blood flow from the portal vein and 20–33% from the hepatic artery. Extrahepatic vascular pressures are 80–120 mmHg in the hepatic artery and 7–10 mmHg in the portal vein.
Intrinsic regulation of hepatic arterial flow appears to be in the terminal arteriole and is reported (15) to be regulated by the hepatic arterial buffer response. In contrast, portal blood flow is not considered to be regulated by local mechanisms but rather by the amount of blood received from the splanchnic circulation (15, 17). We showed that the major initial blood flow after PTC comes from the portal vein (11). In the present study, recovery of hepatic blood flow after transient ischemia was evaluated. By PTC, the systemic blood pressure showed a sudden and then a gradual decrease, and its recovery after declamping appeared to be faster than that of hepatic blood flow (Fig. 1). A sudden decrease in the systemic blood pressure was observed in portal-systemic shunt rats (data not shown). These data suggest that portal congestion may not be the main cause of a decrease in systemic blood pressure during PTC. In contrast, the portal venous pressure was increased by PTC and then gradually decreased during PTC. After declamping, the portal venous pressure rapidly decreased with transient blood flow in the hepatoduodenal ligament, but there was no detectable hepatic tissue blood flow in liver surface (Fig. 2). The portal blood pressure rapidly decreased with initial blood flow in the hepatoduodenal ligament measured by USTF and with transient hepatic congestion. Although we have no direct evidence, initial transient blood flow in the hepatoduodenal ligament, hepatic congestion, and a rapid decrease in portal blood pressure after declamping may suggest that deeply located sinusoids have blood flow after declamping whereas superficially located sinusoids have no flow (lag time), and thus there may be heterogeneity of sinusoidal blood flow after PTC.

Sinusoidal erythrocyte flow is irregular or intermittent and sometimes shows reversed flow even under physiological conditions (16). Clamping the hepatic artery and portal vein on the hepatoduodenal ligament caused interruption of sinusoidal erythrocyte flow and the collapse of sinusoids within several minutes. By declamping, sinusoids were refilled with flowing erythrocytes after a substantial lag time. Although vasoconstriction may occur in the presence of oxygen radicals (21), data from the study indicated that leukocytes themselves and radicals derived from leukocytes or xanthine oxidase may not be causative (Table 2). An increase in endothelin levels might be causative (22, 28), but we could not detect any increase in the blood levels of endothelin-1 in contrast to an endotoxin model (22). It is reported (23) that acute biliary obstruction causes hemodynamic changes in the liver. The results indicated that acute biliary obstruction does not affect the presence of the lag time (Table 2). Furthermore, portal congestion during PTC is not considered to be involved in lag time formation because portal-systemic shunt operation and partial clamping of the left lateral lobe could not eliminate it.

Hepatic blood flow and vascular resistance respond to both neural and humoral factors. Stimulation of hepatic sympathetic fibers derived from the splanchnic nerves is reported to constrict the hepatic vasculature via an \( \alpha_2 \)-adrenergic mechanism, which results in reduction of hepatic blood flow and hepatic blood volume, whereas stimulation of the parasympathetic fibers, the vagus nerve, is reported to open previously closed sinusoids (7). Stimulation of the hepatic branch of the vagus nerve or the topical application of cholinergic agonists, however, constricts portal venules and central veins, which results in a drastic decrease in sinusoidal blood flow (13, 25). Noradrenergic nerve terminals were reported (6) to be present in the periphery of cholinergic neurons and to cause vasoconstriction. Thus nervous regulation of the hepatic vascular response is complex and still controversial. Our data showed that an irreversible \( \alpha_2 \)- and \( \alpha_1 \)-adrenergic antagonist (phenoxybenzamine) failed to eliminate the lag time and that cholinergic blocking agents and procedures of cholinergic nerve block eliminated the lag time (Table 2). Thus a cholinergic mechanism, rather than an adrenergic mechanism, may be responsible for the lag time. However, it is still difficult to interpret the mechanism by which proximal truncal vagotomy eliminated the prolonged lag time induced by distal nerve stimulation (Table 2), because in these experimental conditions the nerve stimulation is considered to have similar effects on hepatic blood flow even after the proximal nerve was cut (10). Afferent nerves may be partly involved in this process; however, this could not solely account for the phenomenon because efferent nerves to the liver were proximally cut and distally stimulated. Afferent nerves plus other mechanisms may be involved.

The presence of a lag time after transient ischemia has not been reported in other organs, probably because of the lack of detailed investigations of ischemic tissue microcirculation. It is unknown whether there is a lag time after a short time of ischemia, i.e., 5-min ischemia, because the two methods used in this study needed 10–15 s for stable measurements. In this connection, it is interesting that complete collapse of sinusoids required several minutes. The reasons why the lag time was dependent on ischemic time are not elucidated in the present investigation. Species differences may be another important issue in this kind of study. For example, blood flow patterns and their nervous regulation are different among rats, dogs, cats, and humans. One other issue is that the use of anesthetics, e.g., the pentobarbital sodium in the present study, may affect the vascular response and blood flow after ischemia. However, in clinical settings, ischemia-reperfusion of the liver usually occurs under anesthesia. The clinical significance of the lag time after transient ischemia has not been sufficiently addressed; however, the present findings indicate that even after the clamps are removed, there still exists a part of the liver with no blood flow, where hepatic tissue hypoxia continues.

In summary, there was an ischemic time-dependent period of no blood flow in hepatic sinusoids after PTC. The cholinergic vagus nerve seemed to be involved in causing the lag time.
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