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LIVER VOLUME, AS ASSESSED BY FOUR ULTRASONIC CRYSTALS ARRANGED TO FORM A TETRAHEDRON, DECREASES DURING ANAPHYLACTIC SHOCK IN ANESTHETIZED RATS

Hiromichi Takano, Toshishige Shibamoto, Wei Zhang, and Yasutaka Kurata

Department of Physiology II, Kanazawa Medical University, Uchinada, Japan

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ABSTRACT—We determined the hepatic volume change in anaphylactic hypotension by using four ultrasonic crystals in anesthetized rats. The hepatic volume was measured with four ultrasonic crystals arranged to form a tetrahedron on the liver surface. Before *in vivo* experiments, using isolated perfused rat liver preparations, we compared the measured liver volume changes with the whole-liver weight changes during hepatic blood flow rate changes and venoconstriction induced by norepinephrine. The measured relative change of the tetrahedron volume (V[utc]; percentage changes of the initial volume) was closely correlated with the liver weight change (W; percentage changes of the initial liver weight): V(utc) = 0.85W − 4.11 (r^2 = 0.67). Then, we measured the liver weight and the tetrahedron volume during hepatic anaphylaxis in isolated perfused liver excised from the rats sensitized with ovalbumin. An injection of the antigen into the perfusate caused anaphylactic venoconstriction, liver weight loss (1.1 ± 0.3 g; 9% ± 1%), and the tetrahedron volume reduction (12% ± 4%). Finally, we measured the liver volume change during anaphylactic hypotension in anesthetized ovalbumin-sensitized rats. When the antigen was i.v. injected into anesthetized rats, along with systemic hypotension and hepatic venoconstriction, the liver tetrahedron volume decreased by 6% ± 2% from baseline. In conclusion, we established a method to measure the hepatic volume by using four ultrasonic crystals forming a tetrahedron. Using this ultrasonic crystal method, we demonstrated that liver volume decreases during anaphylactic hypotension in anesthetized rats.

KEYWORDS—Anaphylaxis, hepatic circulation, hepatic venoconstriction, ultrasonic crystal, blood mobilization, perfused rat liver

INTRODUCTION

The liver serves as a blood reservoir in that it contains up to 15% of the total blood volume, has a large compliance (1–3), and is located close to the heart. Stopping liver blood inflow causes approximately 5 mL of blood per kilogram of body weight to be passively transferred to the central circulation (1). Hepatic blood could be expelled into the central circulation so as to compensate circulatory failure during hemorrhagic shock (4). Actually, increases in hepatic sympathetic nerve activity or plasma norepinephrine, both of which are observed in hemorrhagic shock, can reduce hepatic blood volume. Contrary to the hemorrhagic shock, in canine anaphylactic shock, pooling of blood in liver is induced by hepatic venoconstriction, especially selective constriction of postsinusoidal hepatic veins (5–7). However, it is uncertain whether hepatic congestion occurs in anaphylactic shock models of other species such as rats. It is not known whether the hepatic blood volume increases or decreases during experimental anaphylactic shock *in vivo* in animals other than dogs.

The changes in hepatic volume in experimental animals were previously measured by several methods including the volume plethysmography (8) and ultrasonic crystals (9). The plethysmographic technique was applied to relative large animals such as cats and dogs (1, 2, 8, 10, 11) but not small animals of rats or mice because of the inherent technical difficulty. In contrast, ultrasonic crystals were used to measure the liver thickness as an indicator of the liver volume by measuring the distance of each of the pair of crystals attached on the liver surface (9). This crystal measurement does not need invasive surgery and could be applied to the livers of small animals. However, there was possible unreliability to estimate the hepatic blood volume changes by sonometric crystals (9) because liver thickness measurement was performed at a limited area only, and furthermore liver dimension inhomogeneity existed (9). We propose the more reliable application of ultrasonic crystals by determining the volume of the tetrahedron that was formed by four crystals attached on the ventrodorsal sides of liver surfaces.

Thus, as the first purpose of the present study, the changes in the liver volume determined by four ultrasonic crystals were compared with those by direct weighing of the liver during changes in blood flow and norepinephrine injections in isolated perfused rat livers. This allowed examination of the relationships between three-dimensional change and the overall weight change of liver during hepatic capacitance changes. The second purpose was to determine the liver volume changes by this ultrasonic crystal method during hepatic anaphylaxis in isolated perfused rat livers. Finally, we determined the liver volume changes during anaphylactic hypotension in anesthetized rats.

MATERIALS AND METHODS

Animals

Thirty-four male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) weighing 352 ± 3 g were maintained at 23°C and pathogen-free conditions on 12/12-h dark/light cycles and received food and water *ad libitum*. The

Address reprint requests to Toshishige Shibamoto, MD, PhD, Department of Physiology II, Kanazawa Medical University, Uchinada 920-0293, Japan. E-mail: shibamo@kanazawa-med.ac.jp.

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experiments conducted in this study were approved by the animal research committee of Kanazawa Medical University.

Liver volume-weight relation in perfused rat liver

Isolated liver preparation—The animals were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) and were mechanically ventilated with room air. The method for the isolated perfused rat liver preparation was previously described (12). In brief, a polyethylene tube was placed in the right carotid artery. After laparotomy, the hepatic artery was ligated, and the bile duct was cannulated with the polyethylene tube (0.5 mm inner diameter [ID], 0.8 mm outer diameter [OD]). At 5 min after intra-arterial heparinization (500 U/kg), 8 to 9 mL of blood was withdrawn through the carotid arterial catheter. The intra-abdominal inferior vena cava above the renal veins was ligated, and the portal vein was cannulated with a stainless cannula (1.3 mm ID, 2.1 mm OD). After thoracotomy, the supradiaphragmatic inferior vena cava was cannulated through a right atrium incision with a larger stainless cannula (2.1 mm ID, 3.0 mm OD), then portal perfusion was begun with the heparinized blood diluted with 5% bovine albumin (Sigma-Aldrich Co, St Louis, Mo) in Krebs solution (118 mM NaCl, 5.9 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 25.5 mM $NaHCO_3$, and 5.6 mM glucose) at hematocrit 10% ± 1%. The liver was rapidly excised, suspended from the isometric transducer (TB-652T; Nihon-Kohden, Tokyo, Japan), and the initial liver weight was recorded. There followed a continuous measurement of liver weight throughout the experimental period.

The liver was perfused with blood at a constant flow rate in a recirculating manner via the portal vein. The blood 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 blood was oxygenated in the venous reservoir by continuous bubbling with 95% O_2 and 5% CO_2 (perfusate Po_2, 300 mmHg).

The portal venous (Ppv) and the hepatic venous pressures were measured using pressure transducers (TP-400T; Nihon Kohden) attached by a sidearm to the appropriate cannulas, with the reference points at the hepatic hilus. The portal hepatic flow rate was measured with an electromagnetic flowmeter (MFV 1200; Nihon Kohden), and the flow probe was positioned in the inflow line.

The hepatic vascular pressures, blood flow rate, and liver weight were digitized through the analog-digital converter at a sampling rate of 100 Hz. These digitized values were displayed and recorded using a personal computer.

Liver volume measurement

Four 1-mm ultrasonic microtransducer crystals (Sonometrics, Ontario, Canada) were applied to the ventrodorsal surface of the middle lobe of the liver; one crystal (crystal no. 1 [Cr1]) was attached on the ventral surface of the lobe and the other three (Cr2, Cr3, and Cr4) were attached on the dorsal surface, as shown in Figure 1A. Each crystal was attached by a single stitch of 7.0 silk ligature close to the junction of the crystal and its wires. The crystals were connected to a digital ultrasonic crystal measurement system (Sonometrics). Each crystal acts as a transmitter and a receiver. Thus, each length of six pairs of crystals (a, b, c, d, e, f) was simultaneously measured to calculate the volume of the tetrahedron: a, b, c, d, e, and f indicate the length of Cr1-to-Cr2, Cr1-to-Cr3, Cr1-to-Cr4, Cr3-to-Cr4, Cr2-to-Cr4, and Cr2-to-Cr3, respectively, as shown in Figure 1B.

The volume of the tetrahedron (V) was calculated by the following equation:

$$\begin{aligned} 144V^2 = {} & a^2d^2(b^2 + c^2 + e^2 + f^2 - a^2 - d^2) \\ & + b^2e^2(c^2 + a^2 + f^2 + d^2 - b^2 - e^2) \\ & + c^2f^2(a^2 + b^2 + d^2 + e^2 - c^2 - f^2) \\ & - b^2c^2d^2 - c^2a^2e^2 - a^2b^2f^2 - d^2e^2f^2. \end{aligned} \quad (1)$$

Protocol of the perfused liver experiment

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 portal blood flow rate and the height of the reservoir at a hepatic venous pressure of 0 to 1 cm H_2O. Thereafter, the following two experimental protocols were used to compare the change in the volume of the ultrasonic crystal tetrahedron with that in the liver weight: 1) blood flow change: in six livers, portal blood flow rates were initially decreased in a stepwise manner from 35 to 15 mL/min. After return to the baseline blood flow rate, it was increased to 40 mL/min; 2) norepinephrine-induced hepatic venoconstriction: norepinephrine (0.001 – 1 μM; n = 6) was administered into the reservoir.

Hepatic anaphylaxis in perfused rat liver

Hepatic anaphylaxis was evoked in perfused livers from the sensitized rats, as previously described (12). In brief, rats were actively sensitized by the s.c. injection of 1 mg ovalbumin (grade V; Sigma) dissolved in physiologic saline (0.5 mL) and complete Freud adjuvant (0.5 mL) (n = 5; the sensitized group). The nonsensitized rats were s.c. injected with adjuvant only but not the antigen (n = 5; the control group). Two weeks later, the livers were excised and perfused, and measurements of hepatic hemodynamics and liver volume were performed as previously described. Hepatic anaphylaxis was induced by an injection of the antigen (0.1 mg) into the perfusate.

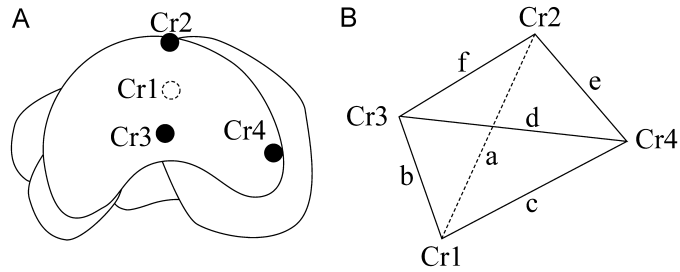

FIG. 1. **Schemas of four crystals attached to the liver surfaces of the left lobe (A) and of the tetrahedron composed of four crystals (B)**. A, Three crystals (Cr2, Cr3, and Cr4) were attached on the upper surface and the other crystal (Cr1) on the lower surface.

Anaphylactic shock in anesthetized rat

Surgical preparation—Rats were actively sensitized in the same manner as for the isolated perfused liver experiment. The animals were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally) and placed supinely on a thermostatically controlled heating pad (BWT-100; Bio Research Center, Nagoya, Japan) that maintained body temperature at 37°C ± 0.5°C throughout the experiment. The adequacy of anesthesia was monitored by the stability of blood pressure and respiration under control conditions and during tail pinch. Supplemental doses of anesthetic (10% of initial dose) were given intraperitoneally if necessary.

Arterial pressure (Psa) was recorded from the right carotid artery. The abdomen was opened by a midline incision, and Ppv was recorded from a cannula inserted through the small vein draining the appendix. To inject an antigen to cause anaphylactic shock, the right external jugular vein was catheterized. The reference level was set at the level of the right atrium. Pressures were measured using transducers (TP-400T; Nihon Kohden) and digitally recorded and displayed continuously at 20 samples per second (Power Lab, AD Instruments, Australia).

Liver volume measurement—The respiratory movement of the diaphragm affected the ultrasonic crystal measurements. Therefore, a diaphragm-shaped lead plate was inserted to separate the liver from the diaphragm. Four ultrasonic crystals were applied to the surface of the middle lobe of the liver in the same manner as in the isolated perfused liver experiment (Fig. 1).

Protocol of the anesthetized rat experiment

In the sensitized rats (n = 6; the anaphylactic shock group) and the nonsensitized rats (n = 6; the control group), hemodynamic parameters were observed for at least 20 min after surgery until a stable state was obtained. After the baseline measurements, ovalbumin antigen (0.6 mg) was i.v. injected in animals of both groups. The Psa and Ppv were continuously measured with pressure transducers. The length of six pairs of crystals was also simultaneously measured. After the experiments, the volume of the tetrahedron of the crystals was calculated, as previously described.

Statistics

All results are expressed as the mean ± SE. Statistical analysis was performed by repeated-measures ANOVA. Comparison of individual points within groups was made by the ANOVA followed by the Bonferroni post-test correction method. Comparison of individual points between two groups was made by Student *t* test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Relationship between the volume measured with four ultrasonic crystal probes and the weight in isolated livers

The initial liver weight measured at the beginning of the perfusion experiment was 11.7 ± 1.1 g (n = 12). The basal volume of the tetrahedron formed by four crystals was 102 ± 22 μL, ranging from 35 to 280 μL, whereas the length of a pair of crystals was 12 ± 0.4 mm, ranging from 5.7 to 20 mm.

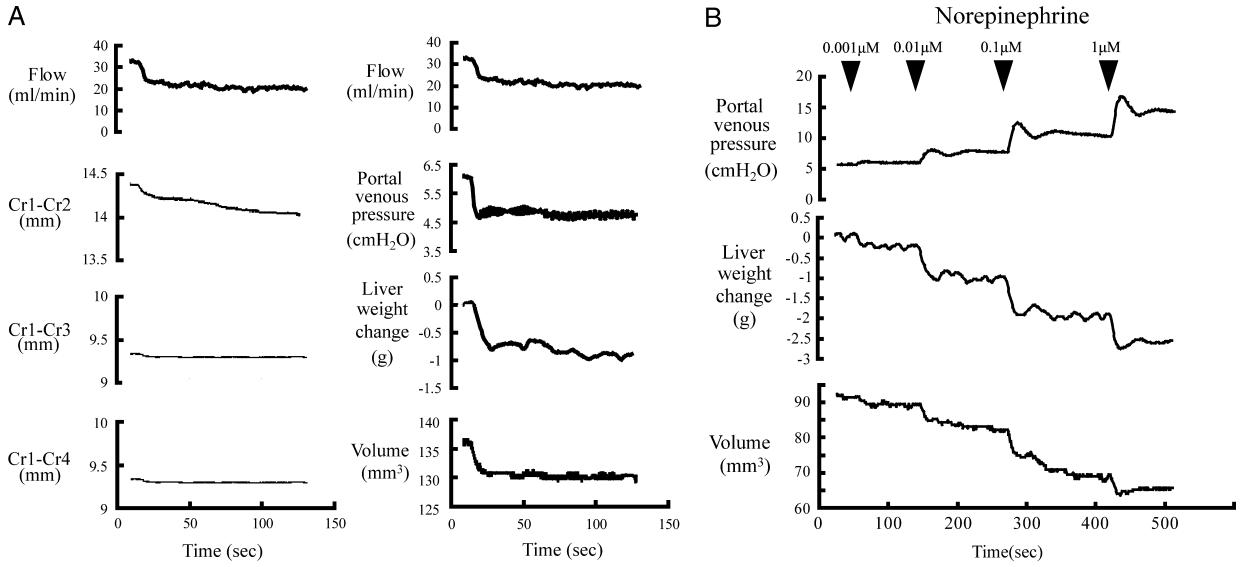

FIG. 2. **A, Representative recordings of the changes in the lengths of Cr1-to-Cr2 (Cr1–Cr2), Cr1-to-Cr3 (Cr1–Cr3), and Cr1-to-Cr4 (Cr1–Cr4), Ppv, liver weight change, and the calculated tetrahedron volume when the blood flow was decreased from 35 to 15 mL/min in an isolated perfused rat liver. B, Representative recordings of the changes in Ppv, liver weight changes, and the tetrahedron liver volume in response to norepinephrine administered cumulatively from 0.001 to 1 μM in an isolated perfused rat liver.**

Figure 2A shows representative recordings of the changes in the crystal lengths, Ppv, liver weight change, and the calculated tetrahedron volume when the blood flow was decreased from 35 to 15 mL/min in an isolated perfused rat liver. When the blood flow was decreased, the liver weight and each length of the pair of crystals decreased. However, each change in the liver thickness was not uniform in magnitude among regions; the length of Cr1-to-Cr2 was decreased more than that of Cr1-to-Cr3 or Cr1-to-Cr4, as shown in Figure 2A (left). In this perfused liver, the volume of the tetrahedron formed by the four crystals decreased in a manner similar to the decrease in the liver weight and Ppv (Fig. 2A, right).

When norepinephrine (0.001 – 1 μM) was injected into the perfusate, along with increases in Ppv, the liver weight and the tetrahedron volume similarly decreased in a dose-dependent manner, as shown in Figure 2B.

Figure 3 shows the relationship between the tetrahedron volume changes measured with the four ultrasonic crystal probes (V[utc]; percentage changes of the initial volume) and the measured liver weight changes (W; percentage changes of the initial liver weight). These pooled data were obtained from intact isolated perfused livers in which the blood flow was changed (n = 6) or norepinephrine (n = 6) was injected, as previously described. The regression line equation is:

$$V(utc) = 0.85\ W - 4.11(\ r^2 = 0.67) \qquad (2)$$

where V(utc) is the percentage changes in the tetrahedron volume to the initial tetrahedron volume, and W is the percentage changes in liver weight. The V(utc) was closely correlated with W. This indicates that the whole-liver weight change can be estimated by the changes in the tetrahedron volume measured by the crystals in isolated perfused rat livers.

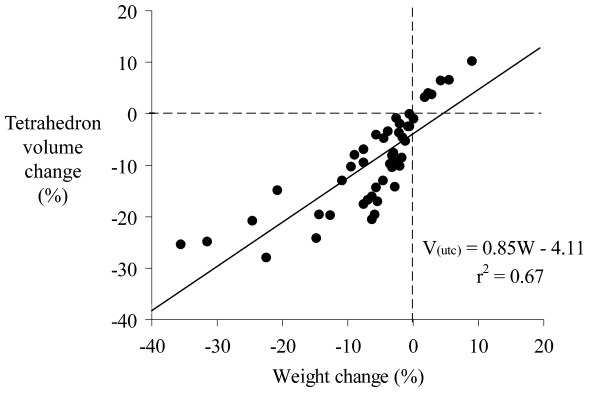

FIG. 3. **The relationship between the percentage changes in the tetrahedron volume (percentage volume change) measured with four ultrasonic crystal probes (V[utc]) and the measured liver weight (percentage weight change [W])**. The regression line equation was V(utc) = 0.85 W + 4.11 (r^2 = 0.67).

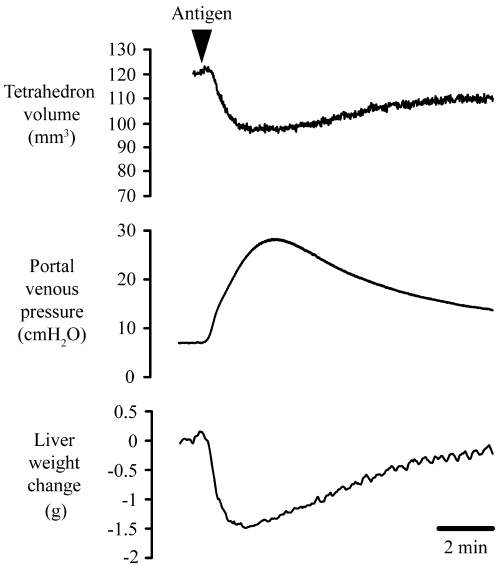

FIG. 4. **Representative recordings of the anaphylactic response of a perfused rat liver**. The ovalbumin antigen (0.1 mg) was injected into the perfusate of an isolated blood-perfused liver from a sensitized rat.

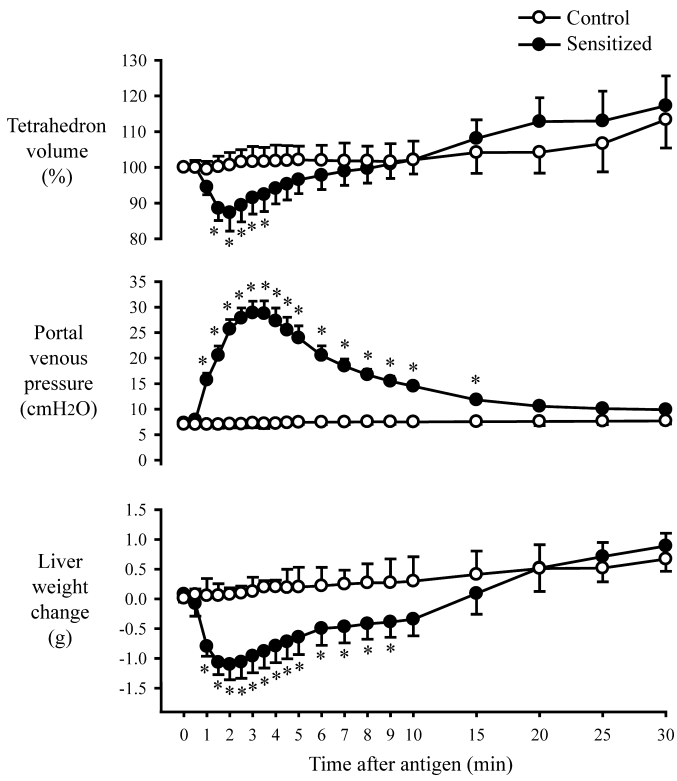

FIG. 5. **The summary of the time course changes in the calculated tetrahedron liver volume, Ppv, and liver weight changes in the anaphylaxis (sensitized; closed circles) and control (control; open circles) groups of the perfused rat livers**. *$P < 0.05$ vs. baseline.

The response of the blood-perfused livers to antigen

An antigen injection into the perfused rat livers caused hepatic venoconstriction and liver weight loss, as shown in Figures 4 and 5. Within 1 min after antigen, hepatic venoconstriction occurred as evidenced by an increased Ppv. The Ppv gradually increased from the baseline of 7.3 ± 0.2 cm H_2O to the peak value of 28.8 ± 2.3 cm H_2O at 3 min after antigen (Fig. 5). The liver weight decreased to the nadir of −1.1 ± 0.3 g (−9% ± 2%) below baseline at 2 min after antigen and then increased to 1.2 ± 0.4 g (9% ± 3%) at 60 min. In parallel with this liver weight changes, the tetrahedron liver volume

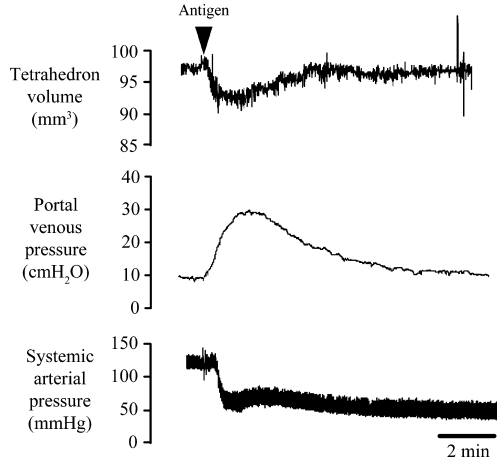

FIG. 6. **Representative recordings of the anaphylactic response of an anesthetized rat**. The ovalbumin antigen (0.6 mg) was i.v. injected into an anesthetized rat sensitized with ovalbumin.

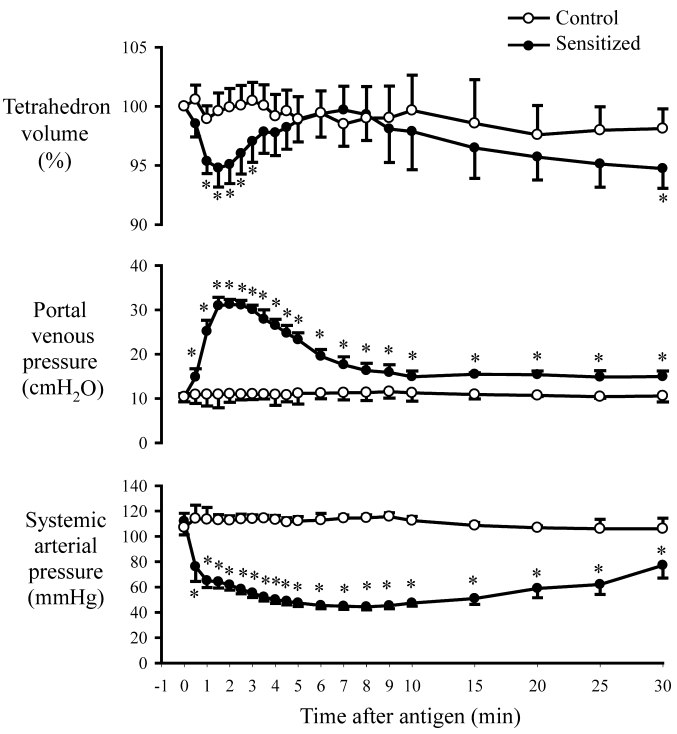

FIG. 7. **The summary of the time course changes in the calculated tetrahedron liver volume, Ppv, and systemic arterial pressure in the anaphylaxis (sensitized; closed circles) and control (control; open circles) groups of the anesthetized rats**. *$P < 0.05$ vs. baseline.

also decreased to the nadir values of 87% ± 5% of baseline at 3 min after antigen and then returned to baseline at 60 min.

The response of the anesthetized rats to antigen

Figure 6 shows a representative example of the response to an i.v. injection of the ovalbumin antigen in an anesthetized rat sensitized with ovalbumin. Figure 7 shows the summary data of time course of percentage change in tetrahedron volume, Ppv, and Psa of anesthetized rats. After an antigen injection in the sensitized group, Psa and Ppv simultaneously began to decrease and increase, respectively. The Psa rapidly decreased from the baseline of 112 ± 12 mmHg to 65 ± 5 mmHg at 1 min after antigen and then continued to progressively decrease to the nadir of 44 ± 3 mmHg at 8 min, followed by a gradual recovery to 77 ± 10 mmHg at 60 min. The Ppv increased from the baseline of 10.5 ± 0.6 cm H_2O to the peak of 31.2 ± 1.2 cm H_2O at 2 min after antigen and then decreased to 15 ± 1.3 cm H_2O at 10 min. After that, the Ppv remained at this level. The tetrahedron liver volume decreased to the nadir value of 94% ± 2% of baseline at 1.5 min after antigen and then increased to 99% ± 2% of baseline at 7 min, followed by a gradual decrease. The tetrahedron liver volume of 94% ± 2% of baseline at 60 min after antigen in the sensitized group was not significantly different from the corresponding value of the nonsensitized rats of the control group, in which the liver volume also tended to decrease at the end of the experimental period.

DISCUSSION

In the present study, we established the method to measure the hepatic volume by using four ultrasonic crystals forming a tetrahedron. The change of the liver tetrahedron volume

measured with this method was closely correlated with the whole-liver weight change in isolated perfused liver during the interventions of the blood flow change and norepinephrine administration. Using this ultrasonic crystal method, the liver volume reduction was shown along with liver weight loss during hepatic anaphylaxis of perfused liver. Finally, we for the first time demonstrated that liver volume decreases during anaphylactic hypotension in anesthetized rats.

Measurement of the hepatic volume is important to understand the pathophysiology of circulatory shock. Redistribution of blood from the liver to the heart changes the filling pressure of the right side of the heart and, therefore, cardiac output. The liver blood volume changes could be assessed from liver lobe diameters measured by pairs of the ultrasonic crystals. Although this sonomicrometry has the advantage of not disturbing liver position or blood flow, a relative thickness change measured at different sites may be larger or smaller than the relative change in total volume (9). Greenway and Rothe (9) reported that different responses could be obtained from two sets of crystals at the same time on the same liver. They attributed this possible unreliability of the ultrasonic crystal method to liver dimension inhomogeneity (9): the change in shape of the liver during active constriction of the capacitance vessels is complex and not uniform. If a large portal or hepatic vein exists between the crystals, variability apparently became exaggerated because thickness changes in larger veins may differ from microvascular thickness changes. Similar variability between measurements of liver thickness at two different points on the same liver was reported by Risoe et al. (13–15). To solve this problem derived from the liver regional inhomogeneity, they calibrated the thickness changes postmortem at the end of each experiment by infusing known volumes and constructed the thickness-volume relation in each preparation of anesthetized dogs (13–15). Furthermore, in their subsequent study, this volumetric calibration method was improved by applying to live *in vivo* animals (16), resulting in avoiding possible postmortem distortion of hepatic configuration (14, 15). However, these volumetric calibrations seem to be difficult for small animals such as rats. In this study, we measured the hepatic volume by constructing the tetrahedron with four ultrasonic crystals. This method is not invasive and also easily performed in rats.

We here tried to estimate the whole-liver weight changes by measuring the small tetrahedron volume change, but not the total liver volume. Although we sampled only a small part of the total liver volume, the regression coefficient between the whole-liver weight changes and the tetrahedron liver volume changes measured with four crystals was 0.85h, which suggests adequate but not so high. One of the reasons for the incomplete coincidence may be that the measured volume changes of the middle lobe may not represent those of the whole volume because the middle lobe was located relatively upper part in the isolated perfused liver preparation; its volume may be affected by gravitational effect. Thus, the tetrahedron of the middle lobe might have underestimated the whole-liver volume. However, the experiment on hepatic anaphylaxis in the isolated perfused liver demonstrated that the V-W relation works well. According to the V-W relation, the measured tetrahedron volume reduction of 13% (Fig. 5) corresponds to 10% of the whole-liver weight loss. The latter value of 10% was very close to the measured whole-liver weight loss of 9% (−1.1 g). Therefore, we think that the whole-liver change could be estimated by the tetrahedron volume change. However, the application of this V-W relation in perfused livers to the *in vivo* animals needs further investigation.

In the present isolated rat livers perfused at constant flow, Ppv increased and both liver volume and liver weight decreased in response to norepinephrine and anaphylaxis. It should be pointed out that reduction of the intrahepatic blood was evoked in rat livers, in which blood flow was not decreased but constant. This indicates that constriction of the hepatic vessels actively reduced the liver blood volume. These findings are consistent with that of our previous studies (12, 17). We demonstrated using the hepatic vascular occlusion methods (12, 17, 18) that the hepatic venoconstriction induced by norepinephrine and anaphylaxis was caused by predominant presinusoidal constriction. With the constant perfusion of the liver, the mechanism of the liver blood volume reduction is unknown. It is likely that the blood volume of the sinusoids, which were situated downstream to the selectively constricted presinusoids, might be reduced as well as postsinusoidal veins.

The main finding of the present study is that liver volume decreases during anaphylactic hypotension in anesthetized rats. This liver volume reduction may be presumably caused by active anaphylactic constriction of hepatic vessels, as previously discussed. This is derived from the finding of isolated livers perfused at constant flow, in which a decrease in liver weight was also observed independently of influences of the autonomic nervous system or hepatic blood flow. As another mechanism, increased sympathetic nerve activity in response to a fall of Psa induced by systemic anaphylaxis may account for a decrease in liver volume (3). Electrical stimulation of the hepatic sympathetic nerves causes a decrease in liver volume in dogs (10, 11) and cats (8). Hepatic nerve stimulation expels up to 50% of hepatic blood volume (3, 8, 11). Indeed, Potas et al. (19) reported that in anaphylactic hypotension in pentobarbital-anesthetized Sprague-Dawley rats sensitized by bovine serum albumin, renal sympathetic nerve activity increased during anaphylactic hypotension. In contrast to the renal sympathoexcitatory response to anaphylactic hypotension in the rat, sympathoinhibition was observed in anesthetized dogs during anaphylactic hypotension (20, 21). These differences could reflect species differences. Finally, a decrease in portal blood flow, resulting from decreased arterial flow to splanchnic vascular bed, could passively decrease a distending pressure of the hepatic vasculature, resulting in a decrease in liver blood volume. Indeed, this mechanism might at least in part contribute to the reduction of the liver volume of the anesthetized rat. The fall of Psa after antigen should have caused decreases in celiac and mesenteric arterial flow, which might lead to decreased portal blood flow (22).

The hepatic vascular blood volume reduction could serve as autotransfusion, which might counteract the decrease in circulating blood volume and systemic hypotension in rats. Although the anaphylactic liver itself might have expelled

the intrahepatic reserve blood to the systemic circulation and then contributed to supplementation of circulating blood volume, anaphylactic hypotension had occurred. This suggests that the blood mobilized from the liver is not enough to fully compensate for the blood pressure fall induced by systemic anaphylaxis.

Anaphylactic hepatic venoconstriction in anesthetized rats may be caused by vasoactive mediators released from the mast cells (25%) and other cells (75%) (23). This evidence was derived from the *in vivo* study for mast cell–deficient rats and the corresponding wild-type rats. In addition, mast cells may almost account for hepatic anaphylactic reaction, which occurs within the liver (23). In the subsequent study, we demonstrated that anaphylactic venoconstriction observed in isolated perfused rat livers was caused mainly by cysteinyl leukotrienes and cyclooxygenase products (24), which may be released from sensitized mast cells (23).

In summary, we have described a method to measure the hepatic volume changes by constructing a tetrahedron with four ultrasonic crystals in the middle lobe of the rat liver. We first compared, using the isolated perfused rat liver preparations, the volume changes measured by ultrasonic crystals with the whole-liver weight changes during hepatic blood flow rate changes and venoconstriction induced by norepinephrine. The percentage change of the tetrahedron volume (V[utc]) was closely correlated with the percentage changes in liver weight (W): $V(utc) = 0.85W - 4.11$ ($r^2 = 0.67$). In the second series of the experiment, liver volume reduction was observed along with anaphylactic venoconstriction and liver weight loss in hepatic anaphylaxis of isolated perfused livers. Finally, using this ultrasonic crystal method, we demonstrated that liver volume decreased by 6% from baseline during anaphylactic hypotension in anesthetized rats. This method can be applied to the studies to determine the liver volume changes in circulatory shock caused by hemorrhage (4) and endotoxin (25), sympathetic nerve activation (10, 11, 15), and liver ischemia-reperfusion injury (26).

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