Hemorrhagic shock primes the hepatic portal circulation for the vasoconstrictive effects of endothelin-1

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1Department of Anesthesiology and Critical Care Medicine, University of Freiburg, D-79106 Freiburg; and 2Department of Anesthesiology and Critical Care Medicine, University of the Saarland, D-66421 Homburg, Germany

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Pannen, Benedikt H. J., Stephan Schroll, Torsten Loop, Michael Bauer, Alexander Hoetz, and Klaus K. Geiger. Hemorrhagic shock primes the hepatic portal circulation for the vasoconstrictive effects of endothelin-1. Am J Physiol Heart Circ Physiol 281: H1075–H1084, 2001.—To test whether hemorrhagic shock and resuscitation (HSR) alters the vascular responsiveness of the portohepatic circulation to endothelins (ETs), we studied the macro- and microcirculatory effects of the preferential ETA receptor agonist ET-1 and of the selective ETB receptor agonist sarafotoxin 6c (S6c) after 1 h of hemorrhagic hypotension and 5 h of volume resuscitation in the isolated perfused rat liver ex vivo using portal pressure-flow relationships and epifluorescence microscopy. Although HSR did not cause major disturbances of hepatic perfusion per se, the response to ET-1 (0.5 × 10−9 M) was enhanced, leading to greater increases in portal driving pressure, total portal resistance, and zero-flow pressures and more pronounced decreases in portal flow, sinusoidal diameters, and hepatic oxygen delivery compared with time-matched sham shock controls. In sharp contrast, the constrictive response to S6c (0.25 × 10−9 M) remained unchanged. Thus HSR primes the portohepatic circulation for the vasoconstrictive effects of ET-1 but does not alter the effects of the ETB receptor agonist S6c. The enhanced sinusoidal response may contribute to the subsequent development of hepatic microcirculatory failure after secondary insults that are associated with increased generation of ET-1.

endothelin receptors; microcirculation; portal vein; pressure-flow relationship; sarafotoxin 6c

HEMORRHAGIC SHOCK IS FREQUENTLY associated with a poor prognosis, even if adequate volume resuscitation results in a restoration of systemic hemodynamics. For example, a recently published prospective study (17) in patients with hemorrhagic shock revealed an overall mortality of >50%. This high mortality rate seems primarily to be due to the subsequent development of multiple organ failure (39). Among those patients that develop multiple organ failure, the incidence of liver failure has been reported to exceed 60% (39). Moreover, based on the central role the liver plays in the organism’s metabolic and immunological response to injury (37), the occurrence of liver failure is frequently associated with a further deterioration of the prognosis (19).

This raises the question of which pathophysiological mechanisms may be responsible for the development of liver injury after hemorrhagic shock and resuscitation (HSR). Although various factors have been implicated, accumulating evidence suggests that the occurrence of hepatic microcirculatory failure may be one major determinant. For example, Wang et al. (47) described a progressive impairment of hepatic microvascular blood flow after hemorrhagic shock despite adequate fluid resuscitation. In addition, physical prevention of microvascular shutdown using a flow-controlled reperfusion mode largely prevents parenchymal cell necrosis after ischemia-reperfusion of the liver, i.e., the degree of microcirculatory failure determines the extent of lethal hepatocyte injury (10). Moreover, the occurrence of sinusoidal perfusion failure after HSR is associated with deteriorations of the hepatic mitochondrial redox state and bile flow, enzyme release from the liver, and hepatocyte necrosis (2, 36).

It is of particular interest to note that recent evidence from our and other laboratories suggests that endothelins (ETs), a family of isopeptides that can exert potent and long-acting vasoconstriction (49), may play a crucial role in the dysregulation of hepatic microvascular perfusion (12). Exogenous ETs can cause a sustained increase in total portal resistance and a decrease in portal flow in the normal liver (14, 51). A video microscopic study (8) of the hepatic microcirculation has revealed that these vasoconstrictive effects of ETs involve both extrasinusoidal and sinusoidal sites. The pattern of microcirculatory disturbances caused by exogenously administered ETs closely resemble the pattern of changes observed in the liver under pathological conditions such as endotoxemia (30, 35) or ischemia followed by reperfusion (21). Moreover, under these conditions, ET levels are increased, and administration of ET antiserum or pharmacological blockade of ET receptors reduces microvascular injury in the liver (16, 32, 33, 48).

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However, it remains unclear how an initial insult such as compensated and reversible HSR may lead to the subsequent development of progressive hepatic microcirculatory and organ failure. Evidence suggests that subtle changes occur in cells or even organs after exposure to a primary minor stimulus that produces hardly detectable injury in itself, e.g., short periods of ischemia, but that may aggravate the response to a secondary insult (13, 22). Consequently, in addition to increased generation, an enhanced response to ETs could contribute to this phenomenon, as previously shown in other models of hepatic stress such as chronic ethanol consumption or endotoxemia (6, 35). Therefore, we hypothesized that HSR, even if it is not primarily associated with profound impairments of sinusoidal perfusion, may alter the vascular responsiveness of the portohepatic circulation and prime the liver for the deleterious vasoconstrictive effects of ETs.

To test this hypothesis, we used a rat model of in vivo HSR and studied the effect of hemorrhagic shock on the intrinsic portal contractile response to ETs in the isolated perfused liver ex vivo. This methodological approach allows the combined analysis of 1) total portal resistance, 2) flow-dependent and -independent components of portal resistance using multiple-point pressure-flow (P-Q) relationships, and 3) hepatic microcirculation via direct in situ video microscopy without any confounding systemic hemodynamic effects of ETs.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River; Sulzfeld, Germany), weighing between 300 and 350 g, were used for all experiments. The experimental protocol was approved by the local animal care and use committee, and all animals received humane care according to the criteria outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 86-23, Revised 1985). Rats were fasted for 6 h before the induction of anesthesia but allowed free access to water. After anesthesia was induced with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), animals were placed supine on a heating pad (38–40°C) to maintain body temperature. After a tail vein was cannulated, an infusion of Ringer solution (10 ml·kg	extsuperscript{-1}·h	extsuperscript{-1}) was started to compensate for evaporative losses during the surgical preparation. Anesthesia was maintained with supplemental intravenous bolus injections of pentobarbital sodium (5 mg/kg body wt) when indicated by any evidence of spontaneous muscle activity or lessening of the anesthetic plane. A tracheotomy was performed, and animals were allowed to breathe spontaneously. A fluid-filled polyethylene-50 catheter was inserted into the left carotid artery and connected to a pressure transducer (MX 860, Medex Medical; Lancashire, UK), and arterial blood pressure was continuously measured.

Hemorrhagic shock protocol. A nonlethal nonheparinized pressure-controlled model of compensated HSR was used. After surgical instrumentation and a stabilization period of 10 min, animals in shock groups were bled to a mean arterial pressure of 40 mmHg in <5 min. The rate of blood withdrawal was 2 ml/min. Shed blood was collected in syringes containing citrate-phosphate-dextrose solution (0.14 ml/ml of blood) (Sigma; St. Louis, MO). Mean arterial pressure was maintained at 40 ± 4 mmHg by intermittent withdrawal of 0.3–0.4-ml aliquots of blood at infusion of respective aliquots of Ringer solution. After 60 min of hemorrhagic hypotension, resuscitation of the animals was performed with 60% of the shed blood withdrawn (reinfused during the first 20 min of resuscitation), and twice the maximal bleedout volume was given as Ringer solution during the first hour of resuscitation, i.e., 200% of the shed blood volume. During the second hour of resuscitation, the infusion rate of the Ringer solution was lowered to a volume equaling the maximal bleedout volume, i.e., 100% of the shed blood volume. Thus the total volume of Ringer solution administered during the first 2 h of resuscitation equals 300% of the shed blood withdrawn and 500% of the amount of shed blood reinfused. For the remaining experimental period, the infusion rate of Ringer solution was kept constant at the basal rate of 10 ml·kg	extsuperscript{-1}·h	extsuperscript{-1}. This resuscitation regimen ensures complete recovery and maintenance of mean arterial pressure until the end of the experiment. Time-matched sham shock animals were anesthetized, completely instrumented as described above, and received a constant infusion of Ringer solution of 10 ml·kg	extsuperscript{-1}·h	extsuperscript{-1} during the whole observation period but did not undergo hemorrhage (sham shock groups).

Isolated perfused liver system. A pressure-limited recirculating isolated liver perfusion system was employed as previously described (10, 34). Briefly, the perfusate [5% rat red blood cells (RBC) in Krebs-Henseleit bicarbonate buffer (KHB); pH 7.4] was pumped from an outflow reservoir through a Silastic tubing oxygenator (gassed with a mixture of 95% O	extsubscript{2}-5% CO	extsubscript{2}) into an overflow chamber that served as the inflow reservoir. A heat exchanger was used to warm the perfusate to 37°C. An ultrasonic in-line flow probe (Transonic; Ithaca, NY) was placed ahead of the inlet cannula and connected to a flowmeter (T-206, Transonic) to measure the total flow rate (Q	extsubscript{t}). Portal inlet pressure (P	extsubscript{inlet}) was measured via a pressure transducer (Transpac Transducer, Abbott; Wiesbaden, Germany) connected to a T-fitting in the inlet cannula. The outflow cannula drained into the outflow reservoir. Another pressure transducer was connected to a second T-fitting in the vena caval outlet cannula to measure outlet pressure (P	extsubscript{outlet}). Both pressure transducers were calibrated simultaneously and zeroed against a column of fluid open to the atmosphere at the level of the cannula tips. The two cannulas were positioned at the level of the abdominal posterior vena cava. Signals were recorded using the WinDaq 200 personal computer-based data-acquisition system (Dataq Instruments; Akron, OH). Total portal resistance (R	extsubscript{p}) was calculated from (P	extsubscript{inlet} – P	extsubscript{outlet})/Q	extsubscript{t}.

Multiple-point P-Q relationships were generated essentially as we have previously described (35). The flow rate was adjusted to 0, 2.5, 5, 10, 15, 20, and 25 ml/min, and P	extsubscript{inlet} was measured under steady-state conditions including direct measurement of the inlet pressure at zero flow (P	extsubscript{inlet}-o). During the generation of each P-Q relationship, P	extsubscript{outlet} was continuously monitored and kept at a value of −1 cmH	extsubscript{2}O by adjusting the height of the outflow reservoir. The slope of the P-Q relationship (slopeP-QR) reflects the flow-dependent incremental resistance of the portohepatic system.

Allogeneic rat blood was obtained for each experiment from a donor animal after cannulation of the left carotid artery under pentobarbital anesthesia. RBC were separated by centrifugation at 3,000 g for 3 min. The supernatant (plasma and buffy coat) was discarded. The RBC pellet was washed in 0.9% saline solution, resuspended in an equal volume of KHB, filtered through a Pall PL50 leucocyte removal filter (Pall Newquay; Cornwall, UK), and stored at 4°C for no longer than 30 min before use.
**Experimental protocol.** On the basis of the time course of shock-induced hepatic expression of vasoactive mediators (3, 5), baseline measurements and all subsequent pharmacological interventions were performed at 6 h after shock induction (i.e., 1-h shock, 5-h resuscitation; shock groups) or in time-matched sham shock experiments (1-h sham shock, 5-h sham resuscitation; sham shock groups). At 30 min before baseline, a laparotomy was performed, and livers were isolated and perfused in situ via the portal vein essentially as previously described (10, 34). The initial flow rate was set to 30 ml/min, and the perfusate hematocrit was adjusted to ~5% by addition of rat RBC prepared as described above. Fluorescein isothiocyanate-labeled bovine serum albumin was added to the perfusate for assessment of sinusoidal boundaries and visualization of RBC in the sinusoids via negative contrast (26). Subsequently, the flow rate was reduced to 20 ml/min, and, after a 15-min stabilization period, all baseline measurements were obtained.

Three different series of experiments were conducted. In the first series, immediately after baseline, a single dose of the preferential ETA receptor agonist ET-1 (Sigma) was added to the perfusate of livers isolated from either sham shock or shock animals (n = 9 per group) to achieve a final ET-1 concentration of 0.5 × 10⁻⁹ M. In a second experimental series, the selective ETB receptor agonist sarafotoxin 6c (S6c; Sigma) was added (sham shock, n = 6 experiments/group; shock, n = 5 experiments/group). A S6c perfusate concentration of 0.25 × 10⁻⁹ M was chosen, because pilot experiments with sham control livers had shown that the peak increase in Rı induced by this S6c dose was similar in magnitude to the peak response to an ET-1 perfusate concentration of 0.5 × 10⁻⁹ M. In a third series, 1 ml of vehicle (H₂O) was added to the perfusate of livers from either sham shock or shock animals as a control (n = 5 per group). Posttreatment measurements were performed after 10 min, because pilot experiments have shown that the macro- and microhemodynamic effects of ET-1 and S6c reached a stable plateau after 5 min and that this lasted for 5–10 min.

**Epifluorescence liver microscopy.** Videomicroscopy of the liver was performed essentially as reported in detail previously (10, 34). Briefly, the liver preparation was positioned on the stage of a Zeiss Axiootech fluorescence microscope (Axio- tech Variol 100 HD; Carl Zeiss; Jena, Germany) and viewed with a ×40 water-immersion objective (Zeiss Achroplan, Carl Zeiss). The surface of the left liver lobe was epi-illuminated with a fiber-optic illuminator (KL 1500, Schott Glawerke; Wiesbaden, Germany) similar to the oblique transillumination procedure described by MacPhee et al. (24), and a randomly chosen acinus was brought into focus so that sinusoids of zone 3 could be observed. This region of the acinus was chosen because the parallel arrangement of sinusoids permits unambiguous quantitative assessment of the sinusoidal microcirculation (11). With the same area in focus, the liver was epi-illuminated with a 100-W mercury lamp with 450–490 nm of excitation and 520-nm emission band-pass filters, which allows visualization of fluorescein isothiocyanate-conjugated RBC within hepatic sinusoids. All microscopic images were projected onto a charge-coupled device video camera (FK 6990 IQ-S, Pieper; Schwerte, Germany). On-line digital contrast enhancement was performed on all images for improved clarity using an Argus-20 image processor (Hamamatsu Photonics; Hamamatsu, Japan). Processed images were recorded for off-line analysis using a S-VHS video recorder (Panasonic AG 7550E, Matsushita Electrical Industrial; Osaka, Japan).

Assessment of sinusoid diameter (Dₛ) and RBC velocity (Vₛ) was done off-line during video playback as we have previously described (34) using digitized frame-by-frame analysis with the Lobulus image analysis system (Medics, Homburg, Germany) at a specimen-to-monitor ratio of 1:2,400 (32). Pre- and posttreatment measurements were performed within the same segments of the same sinusoids.

**Hepatic oxygen delivery and consumption.** To obtain estimates of total hepatic oxygen delivery (DO₂) and consumption (VO₂), 200 µl of perfusate were simultaneously withdrawn from the inflow and outflow tubing of the isolated perfused liver system into gas-tight syringes. Subsequently, hemoglobin concentration, oxygen saturation, and oxygen partial pressure were determined within each sample using an automated blood gas analysis system (ABL 625, Radiometer Medical A/S; Copenhagen, Denmark), which had been calibrated for rat blood using an OSM 3 hemoximeter (Radiometer Medical A/S). Estimates of hepatic DO₂ and VO₂ were derived using standard and previously described equations (31).

**Data analysis.** Data are presented as means ± SE. Raw data at baseline are provided in Table 1. Statistical differences from baseline were determined using a paired t-test. Differences between shock and sham shock groups were tested using an unpaired t-test within each experimental series. When criteria for parametric tests were not met (Kolomogorov-Smirnov test for normality and/or Levene-Mediane test for equal variance failed), the respective nonparametric tests (i.e., Wilcoxon signed-rank test or Mann-Whitney rank sum test) were used. All individual P-Q relationships were analyzed by linear regression to obtain the regression slope. A P value <0.05 was considered to indicate a significant difference. All statistical tests were performed using the SigmaStat software package (Jandel Scientific; San Rafael, CA).

## RESULTS

**Hepatic macrohemodynamics, microhemodynamics, and oxygenation after HSR.** The model of compensated hemorrhagic shock used in the present study did not cause major disturbances of portal venous hemodynamics in the isolated perfused liver after 5 h of resuscitation.

### Table 1. Hepatic macrohemodynamics, microhemodynamics, and oxygenation in sham shock and shock groups at baseline

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<thead>
<tr>
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<th>Sham Shock</th>
<th>Shock</th>
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<tr>
<td>P_inlet − P_outlet, cmH₂O</td>
<td>8.8 ± 0.34</td>
<td>9.2 ± 0.50</td>
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<tr>
<td>Qₘ, ml/min</td>
<td>20.1 ± 0.04</td>
<td>20.1 ± 0.03</td>
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<tr>
<td>Rₛ, cmH₂O·min·ml⁻¹</td>
<td>0.44 ± 0.017</td>
<td>0.46 ± 0.025</td>
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<tr>
<td>Pₐ − cmH₂O</td>
<td>1.9 ± 0.15</td>
<td>1.6 ± 0.21</td>
</tr>
<tr>
<td>SlopePQR, cmH₂O·min·ml⁻¹</td>
<td>0.27 ± 0.019</td>
<td>0.27 ± 0.017</td>
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<tr>
<td>Dₛ, μm/μm</td>
<td>12.3 ± 0.35</td>
<td>12.5 ± 0.34</td>
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<tr>
<td>Vₛ, μm/s</td>
<td>406 ± 30</td>
<td>407 ± 41</td>
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<tr>
<td>DO₂, μl/min</td>
<td>710 ± 26.7</td>
<td>783 ± 24.4</td>
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<tr>
<td>VO₂, μl/min</td>
<td>361 ± 12.7</td>
<td>374 ± 19.9</td>
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Values are means ± SE. Livers were obtained from rats subjected to hemorrhagic shock (1 h; mean arterial pressure: 40 ± 4 mmHg) and volume resuscitation (for 5 h; shock group, n = 19 experiments) or from time-matched sham shock animals (sham shock group, n = 20 experiments), and isolated and perfused via the portal vein. After a stabilization period of 15 min, all baseline measurements were obtained. P_inlet − P_outlet, inlet pressure minus outlet pressure; Qₘ, total portal flow rate; Rₛ, total portal resistance; Pₐ, zero-flow inlet pressure; slopePQR, regression slope of multiple-point pressure-flow relationships; Dₛ, sinusoid diameter; Vₛ, red blood cell velocity; DO₂, hepatic oxygen delivery; VO₂, hepatic oxygen consumption.
citation. This is illustrated by the experimental data summarized in Table 1. At this time point, all macro- and microhemodynamic parameters measured, as well as hepatic \( \text{DO}_{2} \) and \( \text{VO}_{2} \), did not significantly differ between the shock and sham shock groups.

**Effect of HSR on the change in total portal vein resistance in response to ET-1 and S6c.** Whereas vehicle did not cause any major changes in the macrohemodynamic parameters measured, administration of ET-1 increased \( p_{\text{inlet}} - p_{\text{outlet}} \) (Fig. 1), caused a decrease in \( q_{t} \) (Fig. 2), and caused a respective increase in \( r_{t} \) (Fig. 3) in all experimental groups. However, the resistive response to ET-1 was more pronounced in livers from animals that underwent HSR compared with livers from sham shock animals (Figs. 1–3). Addition of the preferential \( \text{ET}_{A} \) receptor agonist S6c to the perfusate also resulted in increases in \( p_{\text{inlet}} - p_{\text{outlet}} \) and \( r_{t} \) (Figs. 1 and 3) and decreases in \( q_{t} \) compared with baseline (Fig. 2). Despite this similarity, livers from shock animals showed different behaviors in response to the two vasoconstrictive agents. In contrast with the enhancing effect of HSR on the portal contractile response to ET-1, the macrohemodynamic changes induced by S6c were either unaffected (\( q_{t} \) and \( r_{t} \); Figs. 2 and 3) or even attenuated (\( p_{\text{inlet}} - p_{\text{outlet}} \); Fig. 1) after HSR compared with the respective sham control group.

**Effect of HSR on ET-1- and S6c-induced changes in portal vein \( P-Q \) relationships.** The administration of vehicle to the perfusate did not have a significant effect on \( p_{Q=0} \) (Fig. 4) or the slope \( \text{slope}_{Q=0} \) in livers from sham shock controls or shock animals (Fig. 5). In contrast, both vasoactive agents caused increases in the flow-independent (Fig. 4) and flow-dependent components (Fig. 5) of \( r_{t} \) in sham control and shock groups. HSR did not alter the effect of ET-1 or S6c on the slope \( \text{slope}_{Q=0} \) (Fig. 5). However, the changes in \( p_{Q=0} \) were differentially affected after shock (Fig. 4). Whereas HSR did not influence the magnitude of the increase in \( p_{Q=0} \...
after S6c, the increase in $P_{Q=0}$ in response to ET-1 was much more pronounced after shock compared with sham shock.

**Effect of HSR on the hepatic microcirculatory response to ET-1 and S6c.** Direct microscopic observation of the hepatic microcirculation within the intact organ revealed a significant narrowing of sinusoids in response to ET-1 that could not be observed in the vehicle-treated groups (Table 2 and Fig. 6). The magnitude of sinusoidal narrowing after ET-1 was much more pronounced in livers from animals that underwent HSR compared with those that had been obtained from sham shock animals (Fig. 6). Representative high-power video micrographs illustrating the enhanced ET-1-induced decrease in sinusoidal dimensions that could be observed after HSR are depicted in Fig. 7. Whereas S6c also caused a reduction in $D_s$, its microcirculatory effects were not altered by HSR, i.e., the decrease in $D_s$ was similar in the sham and the shock group (Table 2 and Fig. 6). Whereas $V_{RBC}$ remained unchanged in the two vehicle groups, administration of ET-1 caused a decrease in $V_{RBC}$ in the sham control group that could not be observed in the shock group ($-106 \pm 37$ vs. $-6 \pm 40 \mu m/s$, $P < 0.05$). In contrast, addition of S6c to the perfusate resulted in an increase in $V_{RBC}$ in livers from sham shock animals ($169 \pm 48 \mu m/s$, $P < 0.05$) but did not cause any significant changes in $V_{RBC}$ in livers from shock animals.

**Influence of HSR on the changes in hepatic oxygenation after ET-1 and S6c.** Estimates of total hepatic $D_{O2}$ and $V_{O2}$ remained unchanged in the two vehicle groups (Fig. 8, A and B). In contrast, both ET-1 and S6c caused substantial reductions in $D_{O2}$ (Fig. 8A). Whereas the negative effect of S6c on $D_{O2}$ tended to be attenuated after HSR, the impairment of the hepatic oxygen supply in response to ET-1 was more pronounced under these conditions compared with respective sham shock controls (Fig. 8A). Although the changes in $V_{O2}$ did not reach statistical significance, there was a tendency toward a lower $V_{O2}$ in particular after ET-1 administration in the shock group, whereas $V_{O2}$ tended to increase on S6c administration after shock (Fig. 8B).

**DISCUSSION**

Accumulating evidence suggests that even a subtle initial insult that does not cause overt disturbances in organ perfusion, function, or integrity may nevertheless significantly alter the responsiveness to a secondary noxious stimulus, thus leading to the subsequent development of organ dysfunction or failure (13, 23). However, the pathophysiological mechanisms responsible for this “two-hit” phenomenon remain to be identified. Maintenance of the integrity of the microcirculation is crucial to prevent liver injury (10). Therefore, in addition to changes in hepatic metabolic activity, energy status, or immune function, alterations in the control of nutritive microvascular blood flow could provide a mechanistical basis for the negative priming effect exerted by hemorrhagic shock (28).
Table 2. Mean $D_s$ in individual isolated perfused rat livers from sham shock or shock animals before and after vehicle, ET-1, or S6c.

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<th>Sham Shock</th>
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<td></td>
<td>Baseline</td>
<td>10 min</td>
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<tr>
<td><strong>Vehicle</strong></td>
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<tr>
<td>6.6 ± 0.38</td>
<td>7.4 ± 0.44</td>
<td>12.1 ± 2.76</td>
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<tr>
<td>13.4 ± 0.29</td>
<td>12.7 ± 0.34*</td>
<td>10.1 ± 0.65</td>
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<tr>
<td>13.5 ± 0.60</td>
<td>13.9 ± 0.72</td>
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<tr>
<td>11.5 ± 0.83</td>
<td>11.3 ± 0.66</td>
<td>15.2 ± 1.25</td>
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<tr>
<td>15.8 ± 2.12</td>
<td>14.6 ± 1.87</td>
<td>11.8 ± 1.06</td>
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<tr>
<td><strong>ET-1</strong></td>
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<tr>
<td>11.0 ± 0.45</td>
<td>9.6 ± 0.60</td>
<td>12.3 ± 1.73</td>
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<tr>
<td>10.5 ± 1.68</td>
<td>8.8 ± 1.63*</td>
<td>12.1 ± 1.01</td>
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<td>14.5 ± 0.39</td>
<td>10.6 ± 0.60*</td>
<td>14.4 ± 0.19</td>
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<tr>
<td>9.0 ± 0.85</td>
<td>6.3 ± 0.64*</td>
<td>15.8 ± 0.92</td>
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<td>11.0 ± 0.89</td>
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<td>8.5 ± 0.62</td>
<td>6.4 ± 0.76*</td>
<td>14.7 ± 1.03</td>
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<td>12.1 ± 1.18</td>
<td>12.9 ± 0.82</td>
<td>10.0 ± 0.75</td>
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<td>13.8 ± 1.34</td>
<td>11.4 ± 0.65</td>
<td>15.3 ± 1.57</td>
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<td>11.7 ± 1.21</td>
<td>8.8 ± 1.08*</td>
<td>12.9 ± 1.12</td>
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<tr>
<td><strong>S6c</strong></td>
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<td>16.2 ± 1.51</td>
<td>11.0 ± 0.83*</td>
<td>12.9 ± 1.11</td>
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<td>15.3 ± 0.54</td>
<td>11.5 ± 0.40*</td>
<td>13.2 ± 0.18</td>
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<tr>
<td>14.7 ± 0.82</td>
<td>10.7 ± 1.10*</td>
<td>12.7 ± 0.36</td>
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<tr>
<td>12.6 ± 1.51</td>
<td>7.1 ± 0.94*</td>
<td>8.5 ± 0.80</td>
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<tr>
<td>12.6 ± 0.83</td>
<td>9.1 ± 0.85*</td>
<td>9.7 ± 0.45</td>
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<tr>
<td>11.9 ± 1.52</td>
<td>8.0 ± 1.04*</td>
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Values are means ± SE of all sinusoids analyzed in each individual liver. Livers were obtained from rats subjected to hemorrhagic shock (1 h; mean arterial pressure: 40 ± 4 mmHg) and volume resuscitation (for 5 h; Shock group, n = 19 experiments) or from time-matched sham shock animals (sham shock group, n = 20 experiments), and isolated and perfused via the portal vein. After a stabilization period of 15 min, baseline measurements were performed. Posttreatment measurements were obtained at 10 min after the administration of either vehicle (1 ml H$_2$O), endothelin-1 (ET-1; 0.5 × 10$^{-9}$ M), or sarafotoxin 6c (S6c; 0.25 × 10$^{-9}$ M). *$P < 0.05$ vs. respective baseline value (within each experiment).

of hepatic perfusion under these experimental conditions, the responsiveness to ET-1 was substantially enhanced, i.e., the increase in $P_{inlet} - P_{outlet}$ and $R_t$ as well as the decrease in $Q_t$ after ET-1 was much more pronounced in livers from animals that underwent HSR compared with livers from sham shock animals. A similar increase in the portohepatic contractile response to ETs has also been described after chronic ethanol consumption (6) or endotoxemia (35). It is important to note that these are very different pathological entities. For example, whereas endotoxemia causes a generalized inflammatory response, inflammation is a delayed and much less pronounced event after HSR. Therefore, the similar increase in the contractile response to ET-1 observed under these different conditions may be considered to reflect a rather uniform general remodeling of the hepatic hemodynamic responsiveness that occurs after diverse pathological stimuli.

Identification of the sites of altered resistance regulation could contribute to the characterization of the mechanisms responsible for the HSR-mediated increase in the portohepatic contractile response to ET-1. Thus we combined the analysis of portal venous P-Q relationships with the direct observation of the sinusoidal microcirculation using epifluorescence video microscopy. Previous studies (1, 27) have provided substantial indirect evidence that changes in the flow-independent component of portal venous resistance, i.e., $P_Q=0$, primarily reflect changes in resistance that occur in a downstream sinusoidal or post sinusoidal compartment. On the other hand, changes in the flow-
dependent component of portal resistance, i.e., of the slopePQR, reflect changes in resistance that occur upstream from the site of the PQ0 in a vessel where a positive PQ0 higher than the actual P outlet exists (9, 27). Therefore, the increase in PQ0 and slopePQR, associated with a respective decrease in Ds and VRBC that could be observed in livers from sham shock animals in response to ET-1, strongly supports the results of previous studies (8, 50) in the normal liver: that ET-1 acts at both sinusoidal and extrasinusoidal sites. It is of great interest to note that the changes in the components of Rt after ET-1 were differentially affected by HSR. Whereas the ET-1-induced increase in PQ0 was much greater after HSR compared with sham, the increase in slopePQR was comparable in both groups. On the basis of the assumptions summarized above, these data would suggest that the enhancement of the contractile response to ET-1 after HSR occurred primarily within the sinusoidal compartment of the liver. This is further supported by the fact that in situ microscopic measurements of Ds did indeed reveal that the sinusoidal narrowing caused by ET-1 was much more pronounced after HSR compared with sham while, at the same time, the reduction in VRBC was attenuated. This raises the question of which factors could be responsible for the fact that manifestation of the enhanced responsiveness to ET-1 after HSR occurred primarily within the sinusoidal compartment.

On one hand, this could be the result of a HSR-induced increase in the contractility of hepatic stellate cells (HSC) (46). These nonparenchymal liver cells are localized within the space of Disse and have been suggested to act as liver-specific pericytes with vasoregulatory properties at the level of the sinusoids (18, 38). For example, HSC can actively contract in response to ET-1 in vitro as well as in the intact liver in situ (20, 50). Moreover, activation of HSC has been shown to be associated with a specific increase in the sensitivity to ET-1 (40, 43). Therefore, the results of the present study would be internally consistent with the notion that the enhancement of the ET-1-mediated sinusoidal narrowing after HSR may result from a hypercontractile state of HSC. On the other hand, McCuskey (25) recently suggested that diameters of sinusoids might also decrease due to passive recoil when inflow is reduced via an increase in presinusoidal resistance and intrasinusoidal distending pressure falls. Thus the enhanced sinusoidal narrowing in response to ET-1 after HSR could also reflect a hypercontractility of smooth muscle cells located within the terminal portal venules. However, portal venular constriction, e.g., in response to the α1-adrenoreceptor agonist phenylephrine, has been shown to be associated with an isolated decrease in slopePQR in the absence of any significant changes in PQ0 (35). Because this was not the case under the experimental conditions of the present study, the sum of these data favor a predominant role of the sinusoidal compartment in the HSR-induced enhancement of the vasoconstrictive response to ET-1.

The biological effects of ETs are mediated through different receptors that are heterogeneously expressed among liver cells. This raises the question of which receptors could be involved in the sensitizing process after HSR. Whereas HSC and hepatocytes express ETA and ETB receptors, only the ETB receptor can be found
PORTAL CONTRACTILE RESPONSE AFTER HEMORRHAGIC SHOCK

Fig. 8. Effect of hemorrhagic shock on changes in estimates of total 
hepatic oxygen delivery (A) and consumption (B) in isolated perfused 
rat livers in response to either vehicle (1 ml H2O), ET-1 (0.5 × 10^{-9} 
M), or S6c (0.25 × 10^{-9} M). Hemoglobin concentration, oxygen 
saturation, and oxygen partial pressure were determined within 
perfusate samples withdrawn from the inflow and outflow tubing to 
calculate estimates of oxygen delivery and consumption. Posttreat-
ment measurements were performed 10 min after pharmacological 
intervention. Livers were obtained from rats subjected to hemor-
rhagic shock and volume resuscitation (shock groups) or from time-
matched sham shock animals. Data represent means ± SE; n = 5–9 
experiments/group. *P < 0.05 vs. respective baseline value (within 
each group); #P < 0.05 vs. respective sham shock group (within each 
series).

on Kupffer and endothelial cells (18). Both ET_{A} and 
ET_{B} receptor agonists may cause contraction of the 
respective hepatic target cells (42, 50). However, the 
relative contribution of the two receptors may largely 
vary depending on the actual experimental or patho-
logical conditions. Thus we also studied the effect of 
HSR on the contractile response to the selective ET_{B} 
receptor agonist S6c. In sharp contrast with the en-
hancing effect of HSR on the response to ET-1, the 
portohepatic hemodynamic effects of S6c were un-
changed or even slightly attenuated after shock. In this 
regard, it is of particular interest to note that Reinher 
et al. (40) recently proposed that activation of HSC 
may induce the appearance of a high-affinity ET_{A} re-
ceptor subtype, whereas in quiescent HSC a low-affin-
ity ET_{A} receptor subtype prevails (40). However, these 
putative receptor subtypes have not been dissected at 
the molecular level, and neither ET_{A} nor ET_{B} receptor 
antagonists were used in the present study. Therefore, 
the sensitizing effect of HSR on the contractile re-
sponse to ETs cannot be ascribed to a specific ET 
receptor. In addition, it is important to note that only a 
single dose of ET-1 and S6c was tested. On the basis of 
the results of previous dose-response determinations 
of ET-1 in the portal circulation (7), an ET-1 dose was 
chosen that caused a half-maximal increase in portal 
resistance to allow the detection of both increases as 
well as decreases in the contractile response. Conse-
quently, a dose of S6c was chosen for comparison, 
which caused an increase in R_{t} in the normal liver of a 
similar magnitude as that of ET-1 did. However, based 
on this experimental design, we cannot exclude the 
possibility that the differential effects of HSR on the 
portohepatic contractile response may vary depending 
on the actual concentration of ET-1 or S6c.

The results of the present study were obtained using 
an isolated perfused liver system. This experimental 
approach was chosen to allow the analysis of the in-
trinsic hepatic hemodynamic response to ETs in the 
absence of confounding factors such as varying levels of 
anesthesia, changes in sympathetic outflow, differ-
ences in hematocrit, or systemic hemodynamics. De-
note these advantages, the use of such a system may 
be associated with significant alterations of hepatic 
perfusion. For example, in agreement with previous 
results, mean D_{s} were ~0.5–2 μm wider, and D_{s} 
showed a larger variability in the isolated perfused 
liver in vitro compared with the normally perfused 
liver in vivo (4, 8, 51). Therefore, factors that could 
contribute to these phenomena, such as an inhomoge-
neous washout of vasoactive mediators, denervation, 
or altered intra- and extravascular pressure gradients, 
may in turn have affected the response to the agonists. 
Consequently, further in vitro and in vivo studies will 
be needed to more precisely define the mechanisms of 
the sensitization of the liver to ET-1 after HSR.

Various pathological conditions that frequently fol-
low upon hemorrhagic shock, such as endotoxemia, 
hypoxemia, or recurrent periods of hemorrhagic hypotension, have been shown to result in increases in 
systemic or hepatic levels of ET-1 (29, 41, 44, 48, 52). 
On the basis of the results of the present study, expo-
sure of the liver to increased amounts of ET-1 after 
HSR-mediated priming of the portohepatic circulation 
may cause critical perturbations of nutritive hepatic 
blood flow. The potential functional significance of 
these findings is further supported by the fact that the 
increased responsiveness to ET-1 was associated with 
respective impairments of hepatic oxygenation.

In conclusion, our results demonstrate that HSR 
primed the portohepatic circulation for the vasocon-
strictive effects of ET-1 via a mechanism that predom-
nantly involves the sinusoidal compartment. These 
findings could, at least in part, explain why compen-
sated and reversible HSR frequently leads to the sub-
sequent development of progressive hepatic microcir-
culatory and organ failure in response to a secondary 
insult.

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