Endothelin-1 selectively contracts portal vein through both $\text{ET}_A$ and $\text{ET}_B$ receptors in isolated rabbit liver

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Wang, Hong-Gang, Toshishige Shibamoto, and Takashige Miyahara. Endothelin-1 selectively contracts portal vein through both $\text{ET}_A$ and $\text{ET}_B$ receptors in isolated rabbit liver. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1036–G1043, 1997.—We determined the constrictive effect of endothelin (ET)-1 on the hepatic vascular resistance distribution and the receptor subtype responsible for the effect in isolated rabbit livers perfused via the portal vein with 5% albumin-Krebs solution. The sinusoidal pressure was estimated using the double vascular occlusion pressure. The basal portal venous resistance comprised 59% of the total portal- hepatic venous resistance. In response to a bolus injection of ET-1 (0.05–5 µg), which led to a final concentration of 0.1–10 nM in the recirculating perfusate, the portal venous resistance increased in a dose-dependent manner, whereas the hepatic venous resistance did not change significantly at any concentration. This hepatic vasoconstriction was associated with liver weight loss. The selective portal venous constriction induced by ET-1 was confirmed in livers perfused retrogradely from the hepatic vein to the portal vein. The ET-1-induced hepatic vasoconstriction was significantly attenuated by the selective $\text{ET}_A$ receptor antagonist BQ-123 (1 µM). The $\text{ET}_B$ receptor antagonist BQ-788 (1 µM) also attenuated the constriction at ET-1 concentrations less than 10 nM. The combination of BQ-123 and BQ-788 tended to inhibit the hepatic vasoconstriction more effectively than BQ-123 alone. These results suggest that ET-1 selectively contracts the portal vein via both $\text{ET}_A$ and $\text{ET}_B$ receptors, with predominance of $\text{ET}_A$ receptor in isolated albumin-Krebs-perfused rabbit livers.

double vascular occlusion pressure; sinusoidal pressure; hepatic vascular resistance; hepatic circulation

There are species differences in the primary site of hepatic vascular resistance, that is, pre- or postsinusoidal vessels. Lautz and co-workers (14, 15) reported, using catheter methods in cats and dogs, that the major site of vascular resistance is at the level of the small hepatic veins. However, the catheter method has an inherent disadvantage in which the catheter itself may add resistance to the local venous flow (16). In contrast, the micropuncture techniques revealed that the primary resistance site in the rat liver exists in the portal-sinusoidal vasculature with only a minimal hepatic venous resistance (21, 25). More recently, Bohlen et al. (3) reported that when the micropipette venule pressure was measured in livers of rats and puppies, the hepatic venous circuit contributed 44% and 31% of the total vascular resistance, respectively. We have recently reported that the vascular occlusion pressure represents the sinusoidal pressure, thus permitting the computation of the vascular resistance distribution (24, 29, 32). The double-occlusion technique revealed that the portal vascular resistance comprised 43–47% of the total hepatic vascular resistance in isolated canine livers (29, 32). On the other hand, with respect to rabbit livers, Rothe and co-workers (3, 17) showed that 43% of the total hepatic venous vascular resistance exists in the hepatic vein. However, any further study on the hepatic vascular resistance distribution has not been reported.

Endothelin (ET)-1, a 21-amino acid peptide, belongs to a family of isopeptides produced by endothelial cells (33) and other cell types such as macrophages (4). ET can evoke potent and long-acting vasoconstriction in multiple vascular beds (23). Recently, ET-1 has been shown to produce a sustained pressor response of hepatic circulation in vivo (1) and in isolated livers (7, 27). However, to our knowledge, there are no reports determining in which segments of the hepatic circulation ET-1 does constrict.

The specific ET receptors $\text{ET}_A$ and $\text{ET}_B$ have been identified in the rat liver (12). In general, the $\text{ET}_A$ receptor mediates vasoconstriction, whereas the $\text{ET}_B$ receptor mediates vasodilation as well as vasoconstriction (18, 26). In rat livers, Ito cells express both $\text{ET}_A$ and $\text{ET}_B$ receptors, whereas Kupffer cells and sinusoidal endothelium express only $\text{ET}_B$ receptor (12). Zhang et al. (34) reported that sinusoidal constriction was mediated by $\text{ET}_A$ receptors and presinusoidal constriction by $\text{ET}_B$ receptors in rat liver (34). On the other hand, ET-1-induced hepatic arterial constriction was markedly reduced by the $\text{ET}_A$ receptor antagonist, whereas the $\text{ET}_B$ receptor antagonist had no effect in isolated canine livers (5). However, there are no data on the specific ET-1 receptor responsible for the ET-1-induced vascular constrictive response in rabbit liver. The availability of the selective specific $\text{ET}_A$ receptor antagonist BQ-123 and the $\text{ET}_B$ receptor antagonist BQ-788 enables us to clarify the $\text{ET}_A$- or $\text{ET}_B$-mediated action on rabbit hepatic vessels.

Using the double vascular occlusion method, we determined the vascular resistance distribution at resting state in isolated rabbit livers perfused with 5% albumin-Krebs buffer under a constant perfusion flow by way of the portal vein with ligation of the hepatic artery. The second purpose of the present study was to determine the constrictive effect of ET-1 on hepatic vascular resistance distribution and liver weight change. We used the reverse perfusion technique to clarify more accurately the hepatic vascular response to ET-1. Finally, we determined the ET receptor subtype responsible for the vascular constrictive response to ET-1 of the rabbit liver.
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MATERIALS AND METHODS

Isolated liver preparation. Twenty-nine rabbits weighing 2.2 ± 0.1 kg were anesthetized with pentobarbital sodium (30 mg/kg iv) and mechanically ventilated with room air. Catheters were placed in the left jugular vein and in the right carotid artery. After thoracotomy and laparotomy, loose ligatures were placed around the hepatic artery, the portal vein, the inferior vena cava, and the common bile duct. At 5 min after heparinization (500 U/kg iv), the rabbit was rapidly bled through the carotid arterial catheter. After ligation of the aforementioned vessels and bile duct, the liver was rapidly excised and weighed. Then, the portal vein and the hepatic vein were cannulated with plastic cannulas (3 mm ID), while the hepatic artery was ligated to simplify analysis of the intrahepatic vascular circuit. The common bile duct was also cannulated with polyethylene tubing. Perfusion was begun within 5 min after excision of the liver.

The cannulated liver was suspended from an electric balance (LF-6, Murakami Koki) and perfused via the portal vein at a constant perfusion flow rate of 157 ± 4 ml/min (n = 29) with 5% bovine serum albumin (Sigma) in Krebs-Henseleit buffer in a recirculating fashion. The perfusate was maintained at 37°C using a water bath and was oxygenated in the outflow reservoir by continuous bubbling with 95% O₂-5% CO₂ (inflow perfusate PO₂, 300 mmHg). A bubble trap was placed in the inflow line. The portal (Pₚv) and hepatic venous (Pₜv) pressures were measured using pressure transducers (Gould) referenced to the level of the portal vein at the hepatic hilus. The flow rate and the height of the venous reservoir could be adjusted independently to maintain Pₚv and Pₜv at any desired level. The perfusion flow rate (Q) was measured with an electromagnetic flowmeter (MFV 1200, Nihon Kohden), and the flow probe was positioned in the venous outflow line. To occlude the portal and hepatic venous lines simultaneously for measurement of the double-occlusion pressure (Pdo), solenoid valves were placed around the perfusion catheters upstream from the Pₚv sidearm cannula and downstream from the Pₜv sidearm cannula. Bile flow was continuously collected in a small tube suspended from the force transducer (45196A, NEC-Sanei). Initially, the isogravimetric state (no weight gain or loss) was obtained by adjusting Pₚv and Pₜv to a level within the normal perfusion range, as described below. The experiments were performed in adherence to the guidelines of the Physiological Society of Japan for the use of experimental animals and to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services Publication No. NIH 85–23, Revised 1985).

Experimental protocol. Hepatic hemodynamic parameters were observed for at least 20 min after the start of perfusion until an isogravimetric state was reached at a Pₚv of 6–9 mmHg, a Pₜv of 0–2 mmHg, and a Q of 0.21 ± 0.05 (SEM) l·min⁻¹·100 g liver⁻¹ (n = 29). The total volume of the recirculating perfusate was 200 ml. After the baseline measurements, the perfused livers were divided into the following groups. In the ET-1 (anterograde perfusion) group (n = 6), 0.05, 0.5, 1.5, or 5 µg of ET-1 was injected as a bolus into the portal line and attained the final perfusate concentration of 0.1, 1, 3, or 10 nM, respectively. In the retrograde perfusion group (n = 6), the liver was perfused retrogradely from the hepatic vein, and ET-1 was injected into the inflow (hepatic venous) line in the same manner as the ET-1 anterograde perfusion group. In the ET receptor antagonist groups, the effect of BQ-123, a specific ET₆ receptor antagonist, and BQ-788, a specific ET₃ receptor antagonist, either alone or in combination, on the ET-1-induced hepatic vascular constrictor response was studied. Ten minutes after addition of 130 µg of either BQ-123 (n = 6), BQ-788 (n = 6), or combined BQ-123 and BQ-788 (n = 5), which yielded a final concentration of 1 µM, ET-1 (0.05–5 µg) was injected into the portal line. The concentration of BQ-123 (1 µM) or BQ-788 (1 µM) is optimal to block the ET₆ or ET₃ receptor subtype as previously reported (6, 34). The groups were studied in random order.

The hepatic sinusoidal pressure was measured by the double-occlusion method (29, 32). Both the inflow and outflow cannulas were simultaneously occluded, after which Pₚv and Pₜv rapidly equilibrated to a similar or identical pressure, which was the Pdo. In each experimental group, Pdo was measured at baseline and at the time when Pₚv reached the maximal level after an injection of ET-1.

The total portal-hepatic venous resistance (Rₚv), portal venous resistance (Rₚv), and hepatic venous resistance (Rₜv) were calculated as follows

\[ R_1 = \frac{(P_{pv} - P_{tv})}{Q} \]  \hspace{1cm} (1)
\[ R_{pv} = \frac{(P_{pv} - P_{do})}{Q} \]  \hspace{1cm} (2)
\[ R_{tv} = \frac{(P_{do} - P_{tv})}{Q} \]  \hspace{1cm} (3)

Drugs. BQ-123 and BQ-788 were provided by Banyu Pharmaceutical (Tsukuba, Japan). ET-1 (human, porcine) was purchased from Sigma Chemical (St. Louis, MO). BQ-123 was dissolved in saline (1 µmol/ml), and BQ-788 was dissolved in dimethyl sulfoxide (1 µmol/ml). ET-1 was dissolved in distilled water and stored as a stock (10 nmol/ml) at the temperature of −20°C.

Statistics. All results are expressed as means ± SE, unless particularly stated. Student’s t-test was used to determine the significance of differences between the baseline and peak values. Comparisons of a given variable between the groups were performed using analysis of variance followed by Duncan’s multiple-range test. A P value < 0.05 was considered significant.

RESULTS

Basal hepatic vascular resistance distribution in isolated rabbit liver. The initial wet liver weight measured immediately after excision was 79 ± 3 g (n = 29). The Pdo at baseline state of 23 anterogradely perfused livers was 3.9 ± 0.1 mmHg, with Ppv 8.1 ± 0.2 mmHg and Ptv 1.1 ± 0.4 mmHg at Q of 0.23 ± 0.01 l·min⁻¹·100 g liver⁻¹. The calculated Rtv was 32.6 ± 1.6 mmHg·l⁻¹·min⁻¹·100 g liver⁻¹. The segmental vascular resistances of Rpv and Rtv were 19.7 ± 1.3 and 13.0 ± 0.6 mmHg·l⁻¹·min⁻¹·100 g liver⁻¹, respectively (n = 23), and the corresponding Rtv/Rtv was 0.41 ± 0.02. This indicates that 59% of the total portal-hepatic venous resistance of the isolated rabbit livers exists in the portal venous side.

In six retrogradely perfused livers, the Pdo at baseline state was 4.1 ± 0.2 mmHg. The corresponding segmental vascular resistances of Rpv and Rtv were 23.9 ± 4.6 and 12.5 ± 1.2 mmHg·l⁻¹·min⁻¹·100 g liver⁻¹, respectively. The Rtv/Rtv of 0.37 ± 0.05 in the retrogradely perfused...
livers was not significantly different from that in the anterogradely perfused livers.

Effects of ET-1 on hepatic hemodynamics, liver weight, and bile flow. Figure 1 shows a representative example of the response to an injection of 5 µg ET-1 (10 nM) of an anterogradely perfused rabbit liver. Soon after an injection of ET-1, vasoconstriction occurred, as evidenced by an increase in $P_{pv}$, with a concomitant decrease in $Wt$. $P_{hv}$ and $Q$ did not change because of the constant flow rate perfusion. The double-occlusion maneuver at 5 min after ET-1 injection revealed a $P_{do}$ of 4.4 mmHg, not markedly different from the baseline value of 4.0 mmHg. At baseline, the pressure gradient between $P_{pv}$ and $P_{do}$, 4.5 mmHg, was slightly greater than that between $P_{do}$ and $P_{hv}$, 2.8 mmHg. However, after ET-1 injection, the former gradient ($P_{pv} - P_{do}$) was much increased to 18.8 mmHg as compared with the latter gradient ($P_{do} - P_{hv}$) of 3.2 mmHg. This finding suggests that ET-1 caused predominantly an increase in $R_{pv}$. This dominant portal venous constriction was also observed in the retrogradely perfused liver injected with ET-1 as shown in Fig. 2. An increase of the inflow pressure of $P_{hv}$, an indication of hepatic vasoconstriction, was accompanied by increases in $P_{do}$ and $Wt$. The latter findings indicated an increase of downstream vascular resistance, i.e., constriction of the portal vein.

Hepatic vasoconstriction peaked within 5 min after an injection of ET-1, as shown in Figs. 1 and 2. Thereafter, hemodynamic variables gradually returned to the preinjection levels within 30 min after injection except in the livers exposed to the highest concentration of 10 nM, in which hepatic vasoconstriction persisted after 30 min. Figure 3 shows the peak changes in the inflow pressure, $P_{do}$ and $Wt$ in anterogradely and retrogradely perfused livers. The $Wt$ decreased dose-dependently along with an increase in the inflow pressure ($P_{pv}$), but $P_{do}$ did not significantly change at ET-1 concentrations ranging from 0.1 to 10 nM in the anterogradely perfused liver. Actually, 10 nM ET-1 caused a decrease in $Wt$ by $9.1 \pm 1.9 \text{ g/100 g liver}$ with an increase in the inflow pressure by $12.6 \pm 1.4 \text{ mmHg}$. On the other hand, in the retrograde perfusion livers, at concentrations <10 nM, $Wt$ tended to decrease, and $P_{do}$ tended to increase, but not significantly. At 10 nM, $Wt$ and $P_{do}$ significantly increased by $15.1 \pm 7.7 \text{ g/100 g liver}$ and $4.5 \pm 1.3 \text{ mmHg}$, respectively.

Figure 4 shows the peak changes in $R_{pv}$ and $R_{hv}$ at various concentrations of ET-1 in the anterograde and retrograde perfusion groups. In the anterograde perfusion group, $R_{pv}$ increased significantly at concentrations higher than 0.1 nM, reaching the maximum level of $329 \pm 26 \%$ of baseline at 10 nM. In contrast, $R_{hv}$ did not change significantly even at the highest concentration of 10 nM. In the retrogradely perfused livers, no significant changes were observed in $R_{pv}$ or $R_{hv}$ except for the highest concentration of 10 nM. There were significant differences in $R_{pv}$ between the anterograde and retrograde perfusion groups at ET-1 concentrations of 1 and 3 nM. In contrast, 10 nM ET-1 produced a similar response of $R_{pv}$ and $R_{hv}$ to that in the anterograde group, although the $R_{hv}$ tended to increase. The $R_{hv}/R_{p}$ decreased to $15 \pm 1$ and $24 \pm 3 \%$ in the anterograde and retrograde perfusion groups, respectively.

The basal bile flow rate was $0.07 \pm 0.01 \text{ g·min}^{-1}·100 \text{ g liver}^{-1}$ in the 23 anterogradely perfused livers. Bile flow significantly decreased to $51 \pm 11$ and $3 \pm 3 \%$ of the baseline at 3 and 10 nM ET-1, respectively, along with a decrease in $Wt$ and an increase in $P_{pv}$ 5–30 min after ET-1 injection. After that, bile flow at 3 nM ET-1 returned toward the basal level ($81.5 \pm 22 \%$) as $Wt$ and $P_{pv}$ returned toward the preinjection level, whereas it kept at the low level at 10 nM even 30 min after

![Fig. 1. Representative recording of a response to an injection of 5 µg endothelin-1 (ET-1; 10 nM) administered from portal vein of an anterogradely perfused liver.](image-url)
injection. In the retrogradely perfused livers, at concentrations <10 nM, no significant changes were found in bile flow, but at 10 nM, bile flow increased transiently and then decreased to 49 ± 13%.

Effects of ET receptor antagonists on the ET-1-induced hepatic vascular constriction response. Figure 5 summarizes peak changes in Ppv after an injection of ET-1 with and without the ET receptor antagonists BQ-123 and/or BQ-788. ET-1-induced increase in Ppv was significantly attenuated by BQ-123 with the concentration of 1 µM (by 64% at 10 nM ET-1). BQ-788 (1 µM) also attenuated the Ppv elevation at concentrations less than 10 nM ET-1. The combination of BQ-123 and BQ-788 tended to inhibit the hepatic vasoconstriction elicited by the highest concentration (10 nM) of ET-1 more effectively by 75% than BQ-123 alone.

DISCUSSION

We determined the vascular resistance distribution at resting state, and the constrictive effects of ET-1 on the segmental resistance distribution in the isolated rabbit livers perfused via the portal vein with 5% bovine serum albumin-Krebs Henseleit buffer. The first major finding is that 59% of the total portal-hepatic venous resistance exists in the portal vein and 41% in the hepatic vein at resting state. The second is that ET-1 selectively contracts the portal vein. Finally, the ET-1-induced hepatic vasoconstriction is mediated by both ETA and ETB receptors in the rabbit livers.

We have recently shown that the Pdo represents the hepatic sinusoidal pressure in isolated blood-perfused canine livers and that the portal vascular resistance comprised 43–47% of the total hepatic vascular resistance at resting state (29, 32). In the subsequent study, using the triple vascular occlusion method in isolated canine livers perfused bivascularly via the portal vein and the hepatic artery, we demonstrated that postsinusoidal vascular resistance comprises approximately one-half of the total liver vascular resistance (24). These previous studies coincidentally suggested that sinusoid is located in the midpoint of the longitudinal vascular resistance of the canine livers. In the present study, we have shown, using the same vascular occlusion method, that 59% of the total portal-hepatic venous resistance exists in the portal vein, and 41% in the hepatic vein at resting state of isolated rabbit liver. This result is consistent with that of Rothe and co-workers (3, 17), who reported using a micropipette servo-null pressure measurement technique that 43% of the total resting resistance of in vivo rabbit liver occurs in the hepatic vein as evaluated by pressure dissipation. However, it should be pointed out that the liver of the present study was perfused with blood-free albumin-Krebs solution, whereas the Rothe liver was perfused with whole blood in the in vivo state. This difference in the character of perfusate between the two studies produces the differences in absolute hepatic vascular resistance values, and the criticism may be provoked that the similarity of the Rpv/Rt value may be accidental. However, in the lung, the pre-to-postcapillary resistance ratio is independent of the perfusate character, that is, blood or cell-free perfusate, despite the presence of the differences in absolute vascular resistance values (22). Thus we believe that the present study confirmed Rothe's proposal: the portal and sinusoidal vasculature is the dominant resistant site, and postsinusoidal vessels also contain considerable resistance in rabbit livers.

In the present study we have not only shown that ET-1 causes an increase in portal venous pressure in
isolated perfused rabbit livers as previously reported in the perfused rat (7, 27) and dog (31) livers, but also first demonstrated that the ET-1-induced hepatic vasoconstriction occurs almost exclusively in the portal vein. The latter conclusion was derived from the following finding: in the anterogradely perfused livers, \( P_{\text{do}} \) did not change significantly as the inflow pressure of \( P_{\text{pv}} \) increases dose-dependently. The selective portal vein constriction was subsequently confirmed by the results obtained from the retrograde perfusion livers, in which 10 nM ET-1 caused an increase in \( P_{\text{do}} \) in association with an increase in the inflow pressure of \( P_{\text{pv}} \). Taken together, regardless of the direction of perfusion, ET-1 contracts almost selectively the portal vein in the rabbit livers, as shown in Fig. 4.

Fig. 3. Peak changes in inflow pressure, double-occlusion pressure \( (P_{\text{do}}) \), and liver weight \( (Wt) \) at concentrations of 0.1–10 nM ET-1 in anterogradely and retrogradely perfused livers. Solid bars, anterogradely perfused livers \((n = 6)\); open bars, retrogradely perfused livers \((n = 6)\). *\( P < 0.05 \) from baseline. +\( P < 0.05 \) from retrogradely perfused group.

Fig. 4. Peak changes in portal \( (R_{\text{pv}}) \) and hepatic \( (R_{\text{hv}}) \) venous resistances at concentrations of 0.1–10 nM ET-1 as expressed by percentage of baseline in anterograde and retrograde perfused livers. Solid bars, anterogradely perfused livers \((n = 6)\); open bars, retrogradely perfused livers \((n = 6)\). *\( P < 0.05 \) from baseline. +\( P < 0.05 \) from retrogradely perfused group.

Fig. 5. Effects of BQ-123 and BQ-788 used either alone or in combination at concentration of 1 µM on peak changes in portal venous pressure \( (P_{\text{pv}}) \) in response to ET-1 \((0.1–10 \text{nM})\). •, ET-1 group \((n = 6)\); ○, BQ-788 group \((n = 6)\); △, BQ-123 group \((n = 6)\); □, combined BQ-123 and BQ-788 group \((n = 5)\). #\( P < 0.05 \) vs. ET-1 group. *\( P < 0.05 \) vs. combined BQ-123 and BQ-788 group.
In the present study, using albumin-Krebs perfusate with no blood cells, we found an increase in portal venous pressure by 3.2 mmHg at a concentration of 1 nM ET-1. In contrast, the same concentration of ET-1 causes a greater increase in portal venous pressure by 4.3 mmHg in the isolated liver perfused with 5% autologous red blood cells in Krebs-Henseleit buffer (34). In addition, in the in vivo study, there was also a greater increase by 4.9 mmHg in the portal venous pressure at 20 min after a continuous intraportal infusion of ET-1 at 10 pmol/min, the total receiving dose of 0.2 nmol (1), which was the same as the bolus dose of the present study. These results may suggest that high viscosity of blood and/or erythrocyte plugging of the narrowed sinusoids may account for the greater increase in vascular resistance in the previous studies, as compared with the present study.

The mechanism by which ET-1 constricts almost selectively presinusoidal vessels or portal vein is not known. The first may be ascribed to constriction of Ito cells, which are highly contractile and located around the endothelial cells throughout the sinusoid, and their multiple cellular appendages reach out to wrap around sinusoid (30). Indeed, the Ito cell has been reported to contract in response to ET-1 (34). However, Zhang et al. (34), using intravitral microscopy and measuring portal venous pressure, have clearly shown that ET-1 produced an increase in the portal venous pressure and a decrease in sinusoidal diameter that is mediated by the contraction of Ito cell. However, when BQ-123 was pretreated, the ET-1-induced decrease in sinusoidal diameter was completely abolished, but the increased portal venous pressure was not affected. This finding indicates that the substantial sinusoidal constriction does not contribute to the ET-1-induced increase in portal venous pressure, that is, total portal-hepatic venous vascular resistance. Thus the role of sinusoid constriction is restricted in local distribution of flow rather than in the control of total portal resistance, as suggested by Zhang et al. (34). However, the double-occlusion technique of the present study cannot distinguish whether the increase in inflow pressure was caused by the constriction of portal vein or of both portal vein and sinusoids. If rabbit Ito cells are preferentially located in the portal side of the sinusoids, or the perportal regions of the sinusoids, and are able to contract in response to ET-1, the constriction of the Ito cells in sinusoids might be involved in the elevation of portal pressure and possibly presinusoidal constriction. The second possibility may be related to the upstream binding of ET-1, which could reduce the concentration of ET-1 in downstream vessels (2). However, this is not likely because the upstream resistance of $R_{pv}$ did not increase in the retrogradely perfused livers when ET-1 was injected into the inflow line of the hepatic vein. Finally, the different distribution of ET receptors may account for the selective presinusoidal constriction of ET-1. ET-1 receptors responsible for hepatic vasoconstriction may be rich on the smooth muscle cells in the portal vein as compared with that in the hepatic vein of rabbit liver. Indeed, Gondo et al. (8) found that strong binding sites of $^{125}$I-labeled ET-1 were present in the periportal region, as based on light-microscopic autoradiographic observations in rats. This predominant existence of the receptors in the portal vein may contribute to the selective portal venous constriction of ET-1.

In the present study, Wt decreased in response to ET-1 in the anterogradely perfused livers, whereas in the retrogradely perfused livers, it increased along with an increase in $P_{pv}$. The exact mechanism for the Wt loss in anterogradely perfused livers is not known in the present study. However, it might be caused by a decrease in blood of the portal vein, which might be expelled by portal vein constriction, and/or a decrease in the sinusoidal blood due to derecruitment of the sinusoid, which might be caused by heterogeneous portal venule contraction. The latter possibility seems to be consistent with the finding of the overall patchy coloring of liver surface, reflecting inhomogeneity of perfusion, after injection of dye and ET-1 into the rat liver (2). On the other hand, with respect to the weight gain in the retrograde perfusion group, the increase in $P_{vd}$ could induce initially an increase in vascular capacitance followed by an increase in fluid movement from the intravascular space to the extravascular space. In cat livers, an intravascular volume continues to increase for as long as 20 min, and fluid filtration also persisted at a steady state throughout the period of increased sinusoidal pressure (11).

ET-1 plays an important role in the pathogenesis of liver damage (10, 20). ET-1 was released from the liver after warm ischemia-reperfusion injury and apparently participated in systemic and local hemodynamic changes that affected survival (20). ET-1 antiserum injection diminished significantly the microcirculatory disturbance in liver after ischemia-reperfusion and prevented liver injury (10). It was demonstrated that plasma ET concentration in patients with cirrhosis with ascites was significantly elevated. However, in patients without ascites, no significant difference was observed compared with normal control (28). In this study, we determined that ET-1 contracts selectively the portal vein in rabbit liver. It is possible that the constriction of the portal vein, which could lead to decreased oxygen delivery to the tissue and eventually to hypoxia, in part, contributes to mechanisms of liver damage and promotes the development of liver disease.

Generally, the bile flow reflects metabolic functions of hepatocytes and viability of perfused liver (9, 20). In the present study, the basal bile flow rate was 0.07 ± 0.01 g·min$^{-1}$·100 g liver$^{-1}$ in the 23 anterograde perfusion livers. This is similar to 0.09–0.11 ml·min$^{-1}$·100 g liver$^{-1}$ reported previously in perfused rabbit liver (19). We found that the bile flow decreased dose-dependently after injection of ET-1 at the concentrations higher than 1 nM along with elevation of $P_{pv}$. This finding is consistent with the previous reports on rat (13, 27). It has been reported that administration of vasodilatory agents such as a nitric oxide donor, a prostaglandin I$ _2$ analog, and papaverine restored the biliary flow rate to normal values along with the recovery of $P_{pv}$ (13, 27). Thus the reduced bile flow in response to ET-1 may be a phenomenon secondary to vasoconstriction (13, 27). Although vasoconstriction may be one of the mecha-
nisms for the ET-1-induced reduction in bile flow, further investigation is necessary to rule out the direct effect on hepatocytes or bile duct.

In our study, the hepatic vasoconstrictive responses to the low concentrations of ET-1 in the retrograde perfusion group were significantly weaker than that in the anterograde perfusion group. There are two possible explanations for this difference. One possibility may be related to the clearance of ET-1 during sinusoidal perfusion in the retrograde group. Under the retrograde perfusion, ET-1 might be bound to the ET receptors in sinusoids (12), resulting in clearance of the peptide (2) before it arrived at the constriction site of the portal vein. Thus the portal vein in retrogradely perfused livers might be exposed to a lower concentration of ET-1 than in anterogradely perfused livers, with resultant weaker constriction in the retrograde perfusion group. Another possibility, a Starling resistor-type mechanism, might be exerted in the outflow vessels of the portal vein in the retrograde perfusion. Maass-Moreno and Rothe (17) have recently shown that an increase in hepatic outflow pressure, which is induced by partially occluding the caudal thoracic vena cava, did not cause an increase in hepatic venular pressure until the abdominal vena cava pressure exceeded a critical level, that is, break pressure, and that a similar phenomenon was also observed in sinusoids and portal venules. This phenomenon was considered as a Starling resistor-type mechanism. If this mechanism might occur in retrogradely perfused livers, the inflow pressure would not increase until the sinusoidal pressure increased over the break pressure, which was caused by constriction of outflow vessel of the portal vein.

The specific ET receptors, ETA receptor and ETB receptor, have been identified in the rat liver (12), in which Ito cells express both ETA and ETB receptors, and Kupffer cells and sinusoidal endothelium only ETB receptor (12). Zhang et al. (34) reported that constriction of the rat hepatic vessels is mediated by both the ETA and ETB receptors (34). We have shown that the concentration-dependent vasoconstriction caused by ET-1 was significantly attenuated by the selective ETA receptor antagonist BQ-123. BQ-788, the ETB receptor antagonist, also attenuated the hepatic vasoconstriction at ET-1 concentrations less than 10 nM. The combination of BQ-123 and BQ-788 tended to inhibit the hepatic vasoconstriction more effectively than BQ-123 alone. These results suggested that the ET-1-induced constriction of the hepatic vessels in the rabbit livers is mediated through both ETA and ETB receptors, although ETA receptor predominated over ETB receptor. This finding appears to be basically consistent with Zhang's results on rat hepatic vessels.

In conclusion, we have shown that 59% of the total portal-hepatic venous resistance exists in the portal vein and 41% in the hepatic vein at the resting state in isolated rabbit livers perfused by 5% albumin-Krebs solution. ET-1 selectively contracts the portal vein mediated through both ETA and ETB receptors, with predominance of ETA receptor over ETB receptor. However, it remains unknown whether Ito cell-induced sinusoidal constriction, as observed in rat liver, contributes to these rabbit hepatic vasoconstrictive responses to ET-1.

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