Active and passive liver microvascular responses from angiotensin, endothelin, norepinephrine, and vasopressin

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Rothe, Carl F., and Roberto Maass-Moreno. Active and passive liver microvascular responses from angiotensin, endothelin, norepinephrine, and vasopressin. Am J Physiol Heart Circ Physiol 279: H1147–H1156, 2000.—Vasoconstrictor agents may induce a decrease in hepatic vascular volume passively, by decreasing distending pressure, or actively, by stimulating contractile elements of capacitance vessels. Hepatic venular resistance was estimated in anesthetized rabbits from hepatic venular pressure (P_{hvv}) by servo-null micropipette, inferior vena cava pressure, and total hepatic blood flow (F_{hv}) by ultrasound flow probe. Changes in liver volume were estimated from measures of liver lobe thickness. Angiotensin (ANG) II, endothelin (ET)-1, norepinephrine (NE), and vasopressin (VP) were infused into the portal vein at a constant rate for 5 min. We conclude that ANG II and NE induced active constriction of hepatic capacitance vessels, because the liver lobe thickness decreased significantly even though P_{hvv} and portal venous distending pressure (P_{pv}) increased. All four agents increased splanchic and hepatic venous resistances in similar proportions. With VP, P_{hvv} and P_{pv} decreased, but with ET-1, P_{hvv} and P_{pv} increased. However, lobe thickness was not significantly changed by either drug during the infusion compared with the 2-min control period. Thus VP and ET-1 have only minor effects on hepatic capacitance vessels. ET-1, at 0.04 μg·min⁻¹·kg body wt⁻¹, caused an increase in systemic arterial blood pressure, but erythrocyte movement through the sinusoids in some animals stopped.

vascular capacitance; hepatic venular pressure; servo-null micropressure pipette for pressure; presinusoidal resistance; venoconstriction

THE SPLANCHNIC VASCULATURE, and especially the liver, potentially contributes a large fraction of the blood mobilized under conditions of stress (p. 1539 of Ref. 13). Vasoactive hormones may induce this blood redistribution, but the mechanisms are not well defined. By measurement of total hepatic blood flow and the pressure in the hepatic venules, which collect the blood from the hepatic sinusoids, it is possible to localize changes in microvascular resistance. Also, by measurement of changes in liver lobe thickness, to estimate changes in liver volume, it is possible to partition, under some conditions, active changes in vascular capacitance properties from passive changes in stressed blood volume. A passive change in vascular volume is a change in stressed volume related to a change in distending pressure of the vessel. The stressed volume of a vascular segment is defined as the product of the compliance of the segment and the transmural distending pressure (31–33, 36, 37). An active change of vascular volume connotes a change in activity of the smooth muscle in the blood vessel walls (16, 32, 33, 37) or in the activity of contractile elements in the stellate cells of the hepatic sinusoids (3, 18, 20, 46). Thus vasoactive agents may induce active changes in the unstressed volume or compliance of the sinusoids or other blood vessels. The unstressed volume of a segment is defined as the volume at a transmural pressure of zero. The unstressed volume is calculated by extrapolating the relatively linear segment of the pressure-volume relationship over the normal operating range to zero transmural pressure. Experimentally, the unstressed volume is the difference between the total volume and the stressed volume (31–33).

Shoukas and Sagawa (36, 37) developed a technique to measure systemic vascular compliance and explicitly noted that the carotid sinus baroreceptor reflex could vary the vascular compliance or the unstressed vascular volume or both. In their early studies, they reported that the reflex caused no significant change in systemic vascular compliance (37), but their later studies and those of others have reported small changes in compliance (4, 7, 14). Greenway and co-workers (12, 16) have concluded that changes in splanchic bed unstressed volume, and not compliance, provide the major blood volume reserve. In the studies reported here, we did not attempt to measure changes in compliance, which would have required measuring the change in distending pressure in response to a change in volume without any change in contractile element activity. Active venoconstriction may be assumed if the contained volume decreased in conjunction with an increase in distending pressures.

In a preceding study, we examined the mechanisms of capacitance and resistance responses to norepinephrine (NE) and the vasodilatory drugs isoproterenol,
lates VP secretion to increase its blood concentration is also a vasoconstrictor. Severe hemorrhage stimulates VP secretion by increasing the outflow resistance. Adenosine also passively induced engorgement by reducing upstream resistance and so increasing flow to increase distending pressure, thereby passively increasing blood volume.

These hepatic microvascular capacitance and resistance responses to various vasoactive agents are not well known because of the difficulty of simultaneously measuring the blood flow, volume (or diameter), and pressure within the hepatic venules, sinusoids, and portal venules. We used the complex servo-null micropipette technique to measure hepatic venular pressures because we have reported (25) that a catheter placed in a hepatic vein to measure intrahepatic venular pressure gives erroneously high values. Lautt et al. (24), using retrograde hepatic venous catheters retracted ~5 mm from the wedge position, reported that hepatic intralobar pressures were insignificantly different from portal venous pressure, thereby placing almost all of the resistance between the portal vein and vena cava in the hepatic outflow veins. Our direct measurements suggest that the portal venule and hepatic venous resistances are similar (26, 34).

The methods used in this study were very similar to those we used to determine the normal pressures within the microscopic portal venules, sinusoids, and hepatic venules of individual lobules (26). A brief review of the anatomic and physiological characteristics of the hepatic circulation and the mechanisms related to redistribution of blood to or from the liver was recently published (34) as was a brief characterization of vascular capacitance (33).

The four vasoactive drugs studied have different systemic actions and were expected to selectively stimulate different mechanisms influencing liver capacitance. NE is released in response to stress, excitement, or vigorous physical activity. It actively reduces the blood volume of the liver but does not change the compliance (13, 22). Angiotensin (ANG) is an important participant in both the regulation of salt and water excretion by the kidneys and the regulation of arterial blood pressure via changes in total peripheral resistance. It is involved in some forms of hypertension. Angiotensin (ANG) is one of the most potent vasoconstrictors known (45). However, the physiological role of ET continues to be unclear. Vane (40) hypothesized that it might be contributing to disease states such as hypertension. Its primary function may be related only to serious pathology and cell death in which local necrotic areas are vascularity isolated from the remainder of the body to reduce the spread of toxins. Vasopressin (VP), the antidiuretic hormone, influences water excretion but at high concentrations is also a vasoconstrictor. Severe hemorrhage stimulates VP secretion to increase its blood concentration >50-fold (8). This helps sustain the systemic arterial blood pressure (8) and has been considered to participate in arterial pressure stabilization (6).

Our hypotheses were as follows: 1) the four vasoactive agents, ANG, ET, NE, and VP, cause active constriction of the hepatic capacitance vessels; and 2) the hepatic venous resistance responds in the same direction and proportion to each of the four vasoactive agents as the splanchnic resistance.

**METHODS**

This study, approved by the Indiana University Animal Care and Use Committee (study MD285b), closely followed the procedures used in our recent study (34). New Zealand White rabbits [3.6 ± 0.3 kg (means ± SD), n = 11 rabbits, 2 of which were males] were sedated with 4 mg/kg chlorpromazine. Under local anesthesia, a central ear artery and vein were cannulated to measure systemic arterial pressure (Psa) and to infuse the anesthetic. A solution of 10 mg/ml α-chloralose and 100 mg/ml urethane was infused intravenously for 30 min to a dose of 50 mg/kg body wt chloralose and 500 mg/kg body wt urethane. We infused additional anesthetic if the rabbit exhibited any unexpected movement or a response to a stimulus such as touching the eye or pinching a toe. Rabbits were chosen to provide a preparation large enough for insertion of catheters for the various measurements without interference of normal flow, yet small enough to be able to position the animal under the microscope.

Lactated Ringer (70%) and 6% dextran-70 with 5% dextrose (30%) were infused at a rate of 11 ± 1 ml·h⁻¹·kg body wt⁻¹ to replace fluid losses. The animals were allowed to breathe spontaneously unless the arterial pressure or respiratory rate decreased (6 of 11 animals), at which time positive-pressure artificial respiration was used (34).

To measure the portal venous pressure (Ppv), a 1.0-mm outer diameter (OD) polyethylene catheter was advanced from a cecal vein into the portal vein to within 3 cm of the liver hilum. To measure the abdominal vena caval pressure (Pvcav), a 1.3-mm-OD catheter was advanced through a branch of the right jugular vein and positioned ~2 cm upstream from the diaphragm at the level of the hepatic venous outflow. The liver lobe was exposed and supported as previously described (26, 34). Microvascular pressure was measured with the use of a servo-null micropipette pressure-measuring system (model 5A; Instruments for Physiology and Medicine, San Diego, CA) in conjunction with a microscope, video camera, and recorder (34). The field was epi-illuminated. The diameters of the hepatic venules were measured from the calibrated video image (1 mm = 1.55 μm). Hepatic venules were identified as microvessels fed from sinusoids in a convergent flow pattern.

The pressure transducers were calibrated and zero-referenced to the middle of the right atrium and continuously flushed with saline (34). The servo-null micropressure system was calibrated by placement of the pipette in a chamber partially filled with 0.9% NaCl and pressurized at 0, 10.0, and 20.0 mmHg with the use of a water manometer. The pressure data resolution was 0.08 mmHg; the relative uncertainty was estimated to be <0.2 mmHg (34).

The total hepatic blood flow (Fhv) was measured with a 6-mm-wide ultrasound transit-time flow probe (Transonics, Ithaca, NY) placed around both the portal vein and the hepatic artery within 2 cm of the hepatic hilum. The surgical trauma required to adequately set up the preparation for microvascular pressure measurements limited our options for accurately measuring hepatic blood flow. Reduction of movement at the puncture site to less than ~10 μm during...
each breath required positioning of a lobe on a rigidly supported stationary surface. The liver was further isolated from the movement of the diaphragm with a barrier, with some risk of restricting hepatic blood flow. Because the rabbit hepatic arterial anatomy is complex and up to 1.5 h can be required to isolate the artery (2), we chose not to risk possible catastrophic failure and additional trauma but rather placed both vessels in the flow probe. Even so, once the lobe was adequately positioned for the microvessel pressure measurements, the probe was inaccessible and so could not be repositioned. This reduced the reliability and accuracy of our total hepatic flow data but did provide evidence of partial vascular occlusion as we manipulated the isolation barrier. We often saw small flow pulsations in synchrony with the heart rate in the $F_{hv}$ recording, thus substantiating the inclusion of the hepatic artery.

Liver lobe thickness changes were obtained by measurement of the distance between the microscope focus point at the pipette tip in the vessel and the stationary support table with the use of a variable differential transformer (resolution 8 $\mu$m) attached to the microscope head to sense the position of the micropipette tip. To keep the ~5-$\mu$m pipette centered in the 50-$\mu$m-diameter vessels required continuous refocusing during the responses to the vasoactive agents. Otherwise, movements of the vessel, related to respiratory activity of the animal, could place the pipette tip next to the vessel wall, thereby distorting the conductivity field and leading to intolerable noise and loss of signal. During NE infusion, the thickness decreased ~350 $\mu$m, and lateral movement often occurred. The focusing for thickness estimates was not subjective, because during the responses to drugs, the operator was required to control the pipette position by use of a three-dimensional micromanipulator, move (with the other hand) the table holding the animal to keep the field containing the puncture site visible, and repetitively refocus the microscope (by use of a foot control) to keep the tip in view. The data were recorded for later objective analysis. A percent change in thickness underestimates the corresponding change in volume, because the thickness measurement was only in one dimension, whereas a volume change is likely to be in three dimensions (15).

**Data acquisition.** The raw signals from all transducers were averaged with a low-pass four-pole Bessel filter ($f_c = 0.18$ Hz) and digitized every 0.1 min with the use of a 12-bit converter (Keithley series 500). The last 0.1-min interval in each minute of data was used as the value for that minute. The hepatic venous resistance ($R_{hv}$) was computed as follows:

$$R_{hv} = (P_{vhv} - P_{hvy})/F_{hv},$$

where $P_{vhv}$ is hepatic venular pressure (by servo-null micropipette). Splanchnic bed resistance ($R_{pspl}$) was computed as follows:

$$R_{pspl} = (P_{pspl} - P_{hv})/F_{pspl}.$$

**Protocol.** The micropipette was immersed in the fluid pool above the vessel to establish the transmural pressure baseline and was then inserted into the vessel. We used a 2 min control period, a 5 min period of drug infusion, and a 3 min recovery period. The drugs were infused via the $P_{pv}$ catheter from a microsyringe at up to 193 $\mu$l/min with an uncertainty of ~1 $\mu$l/min (34). The pressure flushing lines were clamped periodically for 0.2 min to measure and verify the constancy of the flow-related pressure offset (0.5–2.5 mmHg).

On the basis of pilot runs for three of the four agents, we chose a single infusion rate that gave unambiguous responses and three rates for ET. We then could compare the hepatic vascular responses to all four vasoactive agents in the same animal. The microvascular pressure technique was too complex and time consuming for use of the five to eight different doses of each of the four drugs to develop dose-response curves in the same animal.

NE (1 mg/ml base diluted to 260 $\mu$g/ml with 5% dextrose; Abbott) was infused first at 4.7 $\mu$g·min$^{-1}$·kg body wt$^{-1}$ as a test of the system and the responsiveness of the rabbit. ET-1 (E-7764 diluted in saline to 1 $\mu$g/ml; Sigma) was infused last because of its long-lasting effects. ANG II (A9525 diluted in saline to 10 $\mu$g/ml; Sigma) and VP (54.5 $\mu$g/ml Pitressin diluted to 2.18 $\mu$g/ml with saline; Parke-Davis) were used in random order. About 10 min was provided between runs for recovery.

**Statistics.** The average of the effects of each drug during the last 3 min of infusion was compared with the preceding 2 min control periods and expressed as differences or as percent change from control. A Student’s $t$-test of the change was computed. Differences were considered significant if $P < 0.05$. Data variability is described by the standard deviation (SD).

**RESULTS**

The livers at the end of the experiments, which were drained of blood and without the gall bladder, were 2.53 ± 0.36% of the body weight. The $F_{hv}$ averaged 954 ± 167 ml·min$^{-1}$·kg liver wt$^{-1}$. The thickness of the liver lobe at the site of measurement averaged 5.6 ± 2.0 mm. The hepatic venule diameters averaged 54 ± 17 $\mu$m.

Control data from the 2 min period before the drug infusions were obtained for 63 runs from 11 rabbits (Table 1). Mean $P_{sn}$ and $P_{pv}$ were similar to those reported earlier (34). The combined hepatic inflow (assumed to equal outflow, $F_{hv}$) averaged 24.4 ± 8.7 ml·min$^{-1}$·kg body wt$^{-1}$ and was lower than in the earlier study. The $P_{vhv}$ averaged 1.9 mmHg less than the $P_{pv}$ and 2.0 mmHg higher than the $P_{hvy}$. The venous pressure gradient across the liver ($P_{pv} - P_{hvy}$) averaged only 3.9 mmHg, a value similar to that reported before (26, 34). The high coefficient of variability for flow (Table 1) accounts for some, but not all, of the uncertainty of the computed resistances. The portal venous concentration of the drugs was estimated as the ratio of the rate of drug infusion to the rate of blood flow.

NE was infused via the portal vein at 4.7 ± 0.6 $\mu$g·min$^{-1}$·kg body wt$^{-1}$ to administer a dose of 138 nmol/kg body wt over the 5 min infusion period. The $P_{sa}$, $P_{pv}$, and $P_{hvy}$ were increased significantly, but the liver lobe thickness (THK) decreased 7% (Fig. 1 and Table 1). The $P_{hvy}$ heart rate (HR), and ratio of the $P_{pspl}$ to $P_{hvy}$ gradient to the total $P_{pspl}$ to $P_{hvy}$ gradient (Grad) were not significantly changed by this infusion rate. The $F_{hv}$ significantly increased after ~3 min of infusion. After the first minute of infusion, there were marked increases in both $R_{pspl}$ and $R_{hvy}$ (Table 1). The 54-$\mu$m hepatic venules and the abdominal vena cava; Fig. 1). Within 3 min after the infusion was stopped, all variables, except $P_{pv}$, $F_{hv}$, and THK, had returned to control. The increase in $R_{pspl}$ and $R_{hvy}$ strongly suggests that the liver extracted only part of the infused vasopressor agent. The responses were less than those reported earlier (Table 1 of Ref. 34) because the infusion rates were less.

ANG II was infused at 0.38 ± 0.11 $\mu$g·min$^{-1}$·kg body wt$^{-1}$ to administer a total dose of 1.8 nmol/kg body wt. This caused $P_{sa}$, $P_{pv}$, and $P_{vhv}$ to increase
Table 1. Hepatic vascular responses to intraportal vein infusion of various vasoactive agents

<table>
<thead>
<tr>
<th>Title</th>
<th>Control</th>
<th>CV, %</th>
<th>NE</th>
<th>ANG II</th>
<th>VP</th>
<th>ET-1</th>
<th>ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>63</td>
<td>10–12</td>
<td>19–21</td>
<td>17–19</td>
<td>20–22</td>
<td>10–11</td>
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</table>

**Values (average of 3.0-, 4.0-, and 5.0-min responses) are means ± SD; n = no. of experimental runs using 11 rabbits (each run is average of the 3 experimental observations).** For control data: $P_{sv}$, systemic arterial pressure (mmHg); $P_{pv}$, portal venous pressure (mmHg); $P_{mv}$, hepatic venous pressure (mmHg); $P_{avv}$, abdominal venous pressure at liver (mmHg); CV, coefficient of variation; NE, norepinephrine; ANG II, angiotensin II; VP, vasopressin; ET-1, endothelin-1; $F_{hv}$, hepatic venous flow from sum of hepatic arterial and portal venous flow (ml/min kg body wt); $P_{mvv}$, hepatic venous pressure [(P$_{mv}$ - P$_{av}$)/P$_{mv}$]; $R_{mv}$, hepatic venous conductance (ml-mmHg·min⁻¹·kg body wt⁻¹); $R_{sp}$, splanchnic vascular resistance [(P$_{sa}$ - P$_{pv}$)/P$_{pv}$] in units of 1.000 (mmHg·min·kg BW·ml⁻¹); P$_{sv}$, hepatic venous pressure [(P$_{avv}$ - P$_{av}$)/P$_{avv}$]; P$_{avv}$, hepatic venous pressure [(P$_{mv}$ - P$_{mvv}$)/P$_{mv}$]. For experimental data: pressure change from experimental-run control or % change from control for run, except for ET-1 data, which are referred to initial control for lowest dose. Dose (nmol/kg BW) is calculated as cumulative dose for ET-1. Portal vein concentration (conc) is estimated as ratio of infusion rate to $F_{hv}$; *P < 0.01 and †P < 0.001 compared with control values; § not significant.

(Fig. 2 and Table 1). The large increases in $R_{hv}$ and $R_{pl}$ lead to a small decrease in $F_{hv}$ even though the perfusion pressure increased. The decrease in liver thickness, in conjunction with an increase in distending pressures (P$_{plv}$ and P$_{pv}$), suggests that most of the reduction in vascular capacitance was active. The significant decrease in HR was associated with the increased P$_{sa}$. Only P$_{avv}$ and Grad were not changed significantly. By 3 min after the end of the infusions, the variable values returned almost to control.

VP was infused at 0.047 ± 0.026 μg·min⁻¹·kg body wt⁻¹ (equal to 0.043 nmol·min⁻¹·kg body wt⁻¹) to administer a total dose of 0.22 nmol/kg body wt. This caused $R_{hv}$ and $R_{sp}$ to increase so much that $F_{hv}$ decreased to 60% of control even though P$_{sa}$ increased 28% (Fig. 3 and Table 1). As a consequence of the decrease in $F_{hv}$, $P_{pv}$ and $P_{plv}$ also significantly decreased. Lobe THK was not changed even though the distending pressure was decreased just downstream from the sinusoids and in the portal vein. Most variables had not returned to control by 3 min after the VP infusion was stopped.

ET-1, when infused at a low rate (0.0012 ± 0.0006 μg·min⁻¹·kg body wt⁻¹ rate), caused $R_{sp}$ to significantly decrease to 91.6 ± 9.6% of control and flow to increase 6.8 ± 10.0%. Even at these low infusion rates, $P_{pv}$ increased 0.98 ± 0.26 mmHg, suggesting a portal venule increase in resistance. No other variables were significantly changed. Because of the cumulative effects of ET-1 and because ET-1 was infused at the end of the experiment, the data in Table 1 and Figs. 4 and 5 are expressed as the change from the initial 2-min control period before any ET-1 administration.

When ET-1 was infused at a moderate rate of 0.0109 ± 0.0067 μg·min⁻¹·kg body wt⁻¹ (equal to 0.0044 nmol·min⁻¹·kg body wt⁻¹) to administer a cumulative dose of 0.046 ± 0.004 nmol/kg body wt, $R_{sp}$ increased to 36 ± 47% above the control value (recorded before the low infusion rate started), and $F_{hv}$ decreased 23 ± 22% below control (Table 1). Furthermore, this dose caused a marked increase in R$_{sv}$, and thus P$_{plv}$ increased 0.84 ± 1.32 mmHg (Fig. 4). $P_{pv}$ also increased 2.0 ± 1.1 mmHg, suggesting portal venule constriction. Liver lobe THK was not significantly changed compared with the preinfusion control period, even though P$_{pv}$ and $R_{hv}$ were increased, but the lobe THK was significantly less than the initial control average. At these moderate infusion rates, ET-1 is a potent vaso- and venoconstrictor, increasing $R_{hv}$ and $R_{sp}$. However, the P$_{sa}$ decreased and the HR increased slightly (Table 1). The changes in all variables, except P$_{avv}$ and Grad, were statistically significant ($P < 0.001$) compared with the initial control.

During the ~25 min between the initial control observation, the low and moderate infusions of ET-1, and the control period before the high infusion rate (see below), a total of 0.112 ± 0.010 μg/kg body wt of ET-1 was infused. The splanchnic vascular resistances had increased: P$_{sa}$ to P$_{avv}$ ($R_{sp}$), 78%; and P$_{plv}$ to P$_{avv}$ ($F_{hv}$), 321%. This caused hepatic distending pressures to increase: $P_{pv}$ to 2.7 mmHg and P$_{plv}$ to 1.7 mmHg, but the P$_{sa}$ did not change. The $F_{hv}$ decreased 36%. All changes, except P$_{sa}$, were significantly different from the initial control. By the end of the moderate infusion, the lobe THK had decreased to a significant 4.9% of initial control average but only an insignificant 2.2%
compared with the control period for the moderate
infusion.

When ET-1 was infused at $0.0435 \pm 0.0042 \, mg \cdot min^{-1} \cdot kg \cdot body \cdot wt^{-1}$ (equal to $4.4 \, pmol \cdot min^{-1} \cdot kg \cdot body \cdot wt^{-1}$) to administer a relatively high cumulative
dose averaging $0.136 \pm 0.012 \, nmol/kg \cdot body \cdot wt$, $R_{spl}$, $R_{mPV}$, and $R_{hv}$ increased markedly (Fig. 5). (Please
note the changes in scale for the resistances, lobe THK, $P_{avc}$, and $P_{pv}$ and also the residual changes in most
variables at the start of the high ET-1 run, which had
carried over from the previous infusions of ET-1.) During the 5 min of ET infusion, $P_{avc}$ progressively in-
creased, whereas $F_{hv}$ decreased even more from the
value at preinfusion control. The hepatic lobe THK did
not significantly change compared with the preinfusion control. The hepatic lobe THK started a progressive increase in parallel with the
increase in $P_{pv}$ and $P_{mPV}$ (Fig. 5).

By 3 min after the end of the high infusion rate (total
dose $0.329 \pm 0.030 \, \mu g/kg \cdot body \cdot wt$), the resistances
were much higher compared with the initial control:
$R_{spl}$, 158%; $R_{mPV}$, 514%; and $R_{hv}$, 678%. This caused the
hepatic pressures to increase: $P_{pv}$ to 6.9 mmHg, $P_{mPV}$ to
3.2 mmHg, and $P_{avc}$ to 8.2 mmHg. The $F_{hv}$ was only 45%
of the initial control. The lobe THK was then only 0.5% less than the initial control (Fig. 5). The small decrease in $P_{avc}$, although not significant at 3–5 min, progres-
sively and significantly decreased after the infusion
was stopped, suggesting blood sequestration. From
these data, we conclude that the net influence on the
vascular capacitance vessels is minor, but with the
large increases in distending pressure, the contractile
elements must have been activated by the infusion of
ET-1 to resist a volume change. The resistances both
upstream ($R_{mPV}$) and downstream ($R_{hv}$) from the
hepatic venules markedly increased, thus confirming
that ET-1 acts in a similar manner on venous as well as
arterial and presinusoidal vessels. The onset of re-
sponse to the infusion of ET-1 was slower than that of
the other vasoactive agents, and the full response was
often not evident until several minutes after the infu-
sion was stopped. The recovery was slower. The vari-
ability between experiments was large (Table 1).

A striking observation in some experiments using
ET-1 was the stopping of erythrocyte movement in the
sinusoids even at low drug-infusion rates. We made
a crude visual estimation of erythrocyte velocity com-
pared with the pre-ET velocities by assigning one of six
categories: 100% = no change; 90% = just-detectable
decrease; 75% = clear decrease; 50% = about one-half;
20% = very sluggish; and 5% = no movement, or back
and forth. These data, at the corresponding dose, were
fitted with an exponential curve starting at 100% of
control and decreasing to an asymptote of zero (Fig. 6).
The 50% velocity reduction (estimated by interpo-
ation) occurred at a cumulative dose of 0.16 $\mu g/kg \cdot body \cdot wt$.Fig. 1. Cardiovascular responses to norepinephrine (NE) infused via
the portal vein at $4.6 \pm 0.6 \, \mu g \cdot min^{-1} \cdot kg \cdot body \cdot wt^{-1}$. $P_{avc}$, systemic
arterial pressure (as percentage of control); $F_{hv}$, hepatic venous
outflow (as percentage of control); $P_{pv}$, portal venous pressure; $P_{mPV}$, hepatic venular pressure; $P_{avc}$, abdominal vena cava pressure
(change from control in mmHg); $R_{spl}$, splanchic vascular resistance
($P_{avc} - P_{pavc}$/$F_{hv}$); $R_{mPV}$, hepatic venular resistance
($P_{pv} - P_{hv}$/$F_{hv}$); HR, heart rate; Thick, liver lobe thickness
(percentage of control) at site of $P_{mPV}$ measurement. During the 3- to
5-min periods of infusion, all variables significantly changed from
control except $P_{avc}$ and HR (see Table 1).

Fig. 2. Cardiovascular responses to angiotensin (ANG) II infusion
via the portal vein at $0.38 \pm 0.11 \, \mu g \cdot min^{-1} \cdot kg \cdot body \cdot wt^{-1}$. For
explanation of abbreviations, see legend to Fig. 1. During the 3- to
5-min periods of infusion, all variables significantly changed from
control except $P_{avc}$ (see Table 1).
wt. (The moderate cumulative dose averaged 0.112 ± 0.010 μg/kg body wt, and the high dose averaged 0.328 ± 0.012 μg/kg body wt.) The same function was fitted to the Fhv data. A 50% reduction in Fhv occurred at 0.37 μg/kg body wt. In the last three experiments, even higher doses were administered, but these three rabbits were relatively resistant to further reductions in hepatic blood flow.

DISCUSSION

All four vasoactive agents used cause a significant increase in R_hv as well as R_spl (Table 1). NE and ANG II caused an active reduction in vascular capacitance by reducing liver volume (lobe THK) in conjunction with increased distending pressures (P_{uhl} and P_{pv}). VP caused the R_spl to more than double, thereby decreasing blood flow and, consequently, P_{uhl} and P_{pv}, but lobe THK was not significantly changed (Table 1). ET-1 also increased all resistances and decreased flow, but P_{uhl} and P_{pv} increased. The lack of change in lobe THK could either be from a lack of response by the contractile elements of the venous vessels or from a balance between a reduction in volume by wall contraction and an increase in distending pressure by a downstream increase in resistance to flow. [A 10% change in volume of a segment of the vasculature with a relatively large cross-sectional area (e.g., 20-μm venules with a cross section of 37,000 cm² and composing 25% of the total volume; see Table 2 of Ref. 30) will have a much smaller net effect on resistance than a 10% change in volume of larger vessels.]

There were no significant changes in the fraction of the P_{uhl}-to-P_{ave} gradient with respect to the total P_{pv}-to-P_{ave} gradient (Grad; Table 1), suggesting that the pre- and postsinusoidal vessels were similarly sensitive to each of the agents. As a corollary, the R_spl increased, as did the R_hv. The resistance across the portal venules and sinusoïds [(P_{pv} - P_{uhl})/F_{hv}] must also have increased, because P_{pv} increased more than P_{uhl} with the assumption that the hepatic arterial flow was negligible or changed in proportion to the change in F_{hv}.

Baroreceptor control of HR was functioning in our rabbits. When the mean P_{sa} increased, suggesting incomplete extraction from the blood by the liver of the vasoactive agents infused, the HR consequently decreased. However, the highly significant increase in P_{sa} by NE did not lead to a significant change in HR. During the moderate infusion rate of ET, P_{sa} decreased and HR increased.

NE. As expected (10, 11, 34, 35), NE caused an active reduction in liver size and thus can activate the liver to be a rapid and effective blood volume reservoir. The hepatic vascular capacitance response to NE was clearly active, because the volume was decreased even though the distending pressures (P_{uhl} and P_{pv}) and F_{hv}...
increased. The infusion rate via the portal vein of NE (4.7 g·min⁻¹·kg body wt⁻¹) was high, but our goal was to define clearly the characteristics of the response. In both our earlier study (34) and this one, the Fhv, during the first minute of infusion of NE decreased but then increased above control as the Psa increased. Using an isolated, autologous blood-perfused dog liver preparation, Shibamoto et al. (35) reported that a bolus injection of NE into the circulating perfusate decreased the liver weight by >20%, even though the Ppv and sinusoidal pressure increased. (The concentration of NE in the perfusate was calculated to be 4.3 μM, an even higher concentration than we used; see Table 1.) Sinusoidal pressures were estimated via a triple-occlusion method and at control averaged 5.2 mmHg, with the postsinusoidal resistance making up 54% of the total Psv-to-Pasc gradient. That value is similar to the 53% that we found for the faction that Pshv, composed of the total gradient at control (Table 1). We note that Greenway et al. (10) concluded on p. H992 that the “sympathetic control of the hepatic venous bed is mediated through the hepatic innervation, and circulating catecholamines play at most a minor role.”

ANG II. The decrease in lobe THK in conjunction with significant increases in Pshv and Ppv (Fig. 2) suggests that ANG II can induce an active constriction of the vasculature of the liver. Greenway and Lautt (11) reported an ~42% reduction in liver volume during infusion of ANG II at 0.5 μg·min⁻¹·kg body wt⁻¹ into femoral veins of cats. The intestinal vascular conductance was reduced to ~25% of control (11). The Ppv was increased only ~2 mmHg. Bulkley et al. (5) have concluded that ANG II acts proportionally more on the Rspl than on other arteries and therefore is the agent leading to serious gastric ischemia in cardiogenic shock. Pang and Tabrizchi (29), using conscious rats, reported that ANG II increases the mean circulatory filling pressure. Our data suggest that the liver participates in the process.

VP. VP caused such a large increase in splanchnic and other arterial resistances that even though Psa increased, the Fhv, Ppv, and Pshv decreased. We assume that the hepatic arteries were stimulated to a similar degree as the intestinal and stomach arteries. Greenway and Lautt (11) reported only an ~10% reduction in liver volume during infusion of VP at 10 μU·mg⁻¹·kg⁻¹. (With the assumption of a potency of 367 U/mg, their infusion rate was about one-half of what we used.) In our study, there was no significant change in lobe THK even though Ppv decreased 0.6 mmHg. The intestinal vascular conductance reduction to ~25% of control is similar to our 350% of control Rspl.

Our data confirm the study of Pang and Tabrizchi (29), which suggests that VP is a much more potent arteriolar vasoconstrictor than a venoconstrictor, because they found a relatively small increase in mean circulatory filling pressure compared with that of the Psa at comparable infusion rates. Trippodo (39) also concluded on p. 923 that VP had a “relatively small overall venoconstricting action.” Wallace et al. (42) reported that at plasma VP levels of 500 pg/ml (~0.5 nmol/l) did not change either systemic or pulmonary vascular conductance reduction to ~25% of control Rspl.

Fig. 5. Cardiovascular responses to high infusion rate of ET-1 infused at 0.044 ± 0.004 μg·min⁻¹·kg body wt⁻¹. (Cumulative dose of 0.326 ± 0.030 μg/kg body wt = 0.136 ± 0.012 nmol/kg body wt.) Abbreviations as in Fig. 1. (Note change in scale for Rshv. Thick, Pshv, and Ppv.) During the 3- to 5-min periods of infusion, all variables significantly changed from control except Pasc (see Table 1).

Fig. 6. Red blood cell (RBC) velocity (A) and Fhv (B) in the microvessels at progressively increasing doses of ET-1 expressed as μg/kg body wt of cumulative dose (the high dose averaged 0.33 ± 0.03 μg/kg body wt). Semilogarithmic curves were least-squares fitted with an initial value of 100 and asymptote of zero. For RBC velocity: 31 observations, 50% response at 0.16 μg/kg body wt, rate constant = 4.32, and R² = 0.721. For Fhv: 36 observations, 50% response at 0.37 μg/kg body wt, rate constant = 2.01, and R² = 0.867. Linear regression (lines not shown) for RBC velocity: zero intercept = 55.8%, and slope = −33.4·μg⁻¹·kg⁻¹ (P = 0.02, r² = 0.159). Linear regression for Fhv: zero intercept = 74.6%, and slope = −45.6·μg⁻¹·kg⁻¹ (P = 0.1, r² = 0.097).
compliance, but the large increase in total peripheral resistance caused a marked increase in $P_{na}$. Welt and Rutlen (44) found, during VP infusion in pigs, a decrease in splanchnic vascular capacity related to the reduced splanchnic blood flow. Because of the lack of a significant change in lobe THK during the infusion of VP at high rates, we conclude that VP has a relatively much smaller influence on the capacitance vessels than the resistance vessels.

ET. ET influenced the presinusoidal portal venules of the liver, in that both the moderate and high infusion rates increased $P_{pv}$ and the portal venular and splanchnic capacitances and decreased $F_{hv}$. In addition, however, the $P_{shv}$ and the $R_{hv}$ were also increased, and so some of the increase in $P_{pv}$ was from the increase in $P_{shv}$. Although the trend during the high ET infusion rates was a decrease in flow and erythrocyte velocity (Fig. 6), the between-animal variability was high for both variables, in part because of the uncertainty of our measurement technique. ET can apparently influence all contractile elements of the liver and not just the presinusoidal resistance.

Over ten thousand papers related to ET have been published since the discovery of this vasoconstrictor peptide by Yanagisawa et al. (45) in 1988. They reported that the ED$_{50}$ of ET is at least an order of magnitude lower than that reported for ANG II, VP, or neuropeptide Y (45). It causes an increase in $P_{sa}$ that lasts for about an hour. It acts directly on smooth muscle cells but does require calcium.

Kaneda et al. (19) perfused rat livers with a solution containing 1 nmol/l ET-1 and observed a heterogeneous contraction of the 40- to 80-μm-diameter distal segments of preterminal portal venules so intense as to obliterate the lumen, as well as strong contractions of larger proximal segments. This redistributes flow and might provide a protective mechanism. They detected no constriction of the sinusoids, central venules, or sublobular veins or evidence of liver engorgement from the intraportal infusion of ET-1. Furthermore, they found that retrograde perfusion containing ET-1 caused neither vascular constriction nor elevation of the perfusion pressure. However, Ito et al. (18) reported that topically applied ET-1 to mouse liver microvasculature caused constriction of sinusoids as well as portal and central venules, and Kawada et al. (20) found that cultured stellate cells from hepatic sinusoids were stimulated by ET-1 even at concentrations of 0.1 nmol/l. Aharinejad et al. (1), using vascular casting, found that tufts of smooth muscle in 100- to 250-μm sublobular veins in dog livers constricted in response to 1 μg/kg body wt of ET-1. At that dose, the sublobular vein diameters were decreased 39%, $P_{sa}$ increased 43%, and $P_{pv}$ increased 57%. ET-1 causes a small increase in mean circulatory filling pressure (41), suggesting an active reduction in vascular capacitance. Wang et al. (43) have studied isolated rabbit livers perfused with a 5% albumin-Krebs solution at a constant rate. They estimated the sinusoidal (capillary) pressure ($P_{do}$) as the equilibrium pressure of portal venous ($P_{pv}$) and large hepatic vein ($P_{hv}$) after a sudden occlusion of both the portal venous (inflow) and hepatic venous (liver outflow) cannulas. (The hepatic artery was ligated throughout the experiment.) ET-1 caused an increase in $P_{pv}$ and portal venous resistance [$R_{pv} = (P_{pv} - P_{do})/F_{hv}$]. However, they reported that the $R_{hv}$ [$R_{hv} = (P_{do} - P_{hv})/F_{hv}$] was not changed even after injecting 5 μg of ET-1 into the perfusate, giving a calculated perfusate concentration of 10 nmol/l. At this high concentration, $P_{pv}$ increased by 13 mmHg, and the liver weight decreased ~10% during the constant perfusion; yet the increase of $P_{do}$ of 0.4 mmHg from the baseline of 4.0 mmHg was not statistically significant. With retrograde perfusion with the 10 nmol/l of ET-1, there was a large 4.5-mmHg increase in $P_{do}$ and the liver weight increased 15%, suggesting a passive distension from the constant perfusion and a hepatic venous constriction. In our study, a portal vein blood concentration of ~0.7 nmol/l caused a 4-mmHg increase in $P_{pv}$ and also a highly significant increase in $P_{shv}$ of 1.9 mmHg resulting from a 662% increase in $R_{hv}$.

Zhang et al. (46) infused 1 mM ET-1 into isolated rat livers and observed a significant sinusoidal constriction at the sites of Ito cells, but they computed, not measured, the distending pressure in the sinusoids. Ohuchi et al. (28), using the same concentration in their nonrecirculating rat liver perfusion system, reported hepatocyte damage and also found that the volume of the sinusoids and space of Disse decreased from ~0.27 and 0.14 ml/g liver to 0.07 and 0.07 ml/g liver, respectively. (Indicator dilution techniques with $^{51}$Cr-labeled rat blood cells and $^{125}$I-labeled albumin were used.) In another part of the study, they reported that 88% of the ET-1 was trapped during a single pass through untreated liver. Bauer et al. (3) also reported a profound and long-lasting reduction in erythrocyte velocity in sinusoids during infusion of ET-1 at 0.010 nmol/l. The decrease in sinusoidal diameter may have been a passive result of a decreased sinusoidal pressure related to a reduced blood flow. Measurement of pressure within the microvessels is essential for distinguishing between active and passive capacitive responses to drugs.

ET has been recommended as a more effective vasoconstrictor than VP or ANG II to direct blood from normal hepatic tissue to tumors during treatment with cytotoxins, because the duration of action is longer: 20.7, 8.6, and 4.2 min, respectively (17). Under some conditions, the blocking of ET actions may be useful. For example, hepatic ischemia evokes ET-mediated vasoconstriction of the hepatic bed, including the sinusoids, but pretreatment with the ET blocker bosentan prevents the posts ischemic sinusoidal constriction in rats (38).

Limitations of methods used. Limitations of our experimental approach to measure hepatic microvascular pressure have been, in part, described in previous reports (26, 34). Because the sinusoids contain most of the blood in the liver, we would have preferred to measure changes in sinusoidal pressure, but these ~10-μm-diameter vessels are too small for long-term, reliable pressure measurements. Use of an isolated in vitro liver preparation would have simplified the prob-
blem of controlling movement and interpreting the results, which occurred with recirculation of drug. However, isolated, artificially perfused preparations are much less representative of the live animal because of inadequate oxygenation, missing nutrients, absence of innervation, release of toxic materials or accumulation of waste materials, and possible abnormal osmotic and oncotic environment (2, 13).

Because we needed to access and preserve the integrity of the liver surface, we could not measure the liver volume with a plethysmograph or pairs of ultrasonic crystals. Localized measurements, including our lobe THK technique, are subject to increased variability with respect to a total liver volume measurement but not to significant systematic error (15). Volume measurements using indicator dilution techniques are clearly the most direct method. However, virtually all chemical indicators are extracted to some extent by the liver and so cannot be used. Only labeled erythrocytes or 2- to 5-μm-diameter spheres, which pass through the liver without loss and which do not cross into the space of Disse, would give accurate blood volume data (9). Obtaining such data without interfering with the microvascular pressure measurements would have been difficult.

The hepatic arterial flow (F_{ha}) in the rabbit is relatively less than that of other animals. Alexander et al. (2) found that a hepatic arterial perfusion rate of 20% of the total provided a reasonable hepatic arterial pressure. The data of Mastai et al. (27) suggest that F_{ha} provides only ~10% of the F_{hv}.

Our computation of R_{spl} was based on the pressure gradient from the systemic arteries to the abdominal vena cava (P_{sa} - P_{avr}). A measure of F_{ha} would have helped characterize the hepatic vasculature. An hepatic arterial buffer response tends to modulate hepatic arterial blood flow to maintain a constant total hepatic arterial buffer response tends to modulate hepatic arterial pressures. ET-1, at 0.04 μg·min⁻¹·kg body wt⁻¹, caused a 13% increase in P_{sa}, but the increase in hepatic portal venule resistance was so great that the erythrocyte movement through the sinusoids in some animals stopped. The hepatic microvascular responses to ET are highly heterogeneous.

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