Effect of portal hypertension on splenic blood flow, intrasplenic extravasation and systemic blood pressure

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Kaufman, Susan, and Jody Levasseur. Effect of portal hypertension on splenic blood flow, intrasplenic extravasation and systemic blood pressure. Am J Physiol Regul Integr Comp Physiol 284: R1580–R1585, 2003. First published February 6, 2003; 10.1152/ajpregu.00516.2002.—We have previously shown that intrasplenic fluid extravasation is important in controlling blood volume. We proposed that, because the splenic vein flows in the portal vein, portal hypertension would increase splenic venous pressure and thus increase intrasplenic microvascular pressure and fluid extravasation. Given that the rat spleen has no capacity to store/release blood, intrasplenic fluid extravasation can be estimated by measuring the difference between splenic arterial inflow and venous outflow. In anesthetized rats, partial ligation of the portal vein rostral to the junction with the splenic vein caused portal venous pressure to rise from 4.5 ± 0.5 to 12.0 ± 0.9 mmHg (n = 6); there was no change in portal venous pressure downstream of the ligation, although blood flow in the liver fell. Splenic arterial flow did not change, but the arteriovenous flow differential increased from 0.8 ± 0.3 to 1.2 ± 0.1 ml/min (n = 6), and splenic venous hematocrit rose.

Mean arterial pressure fell (101 ± 5.5 to 95 ± 4 mmHg). Splenic afferent nerve activity increased (5.6 ± 0.9 to 16.2 ± 0.7 spikes/s, n = 5). Contrary to our hypothesis, partial ligation of the portal vein caudal to the junction with the splenic vein (same increase in portal venous pressure but no increase in splenic venous pressure) also caused the splenic arteriovenous flow differential to increase (0.6 ± 0.1 to 1.0 ± 0.2 ml/min; n = 8). The increase in intrasplenic fluid efflux and the fall in mean arterial pressure after rostral portal vein ligation were abolished by splenic denervation. We propose there to be an intestinal/hepatic/splenic reflex pathway, through which is mediated the changes in intrasplenic extravasation and systemic blood pressure observed during portal hypertension.

Normal portal pressure is 4–8 mmHg, sufficient to maintain flow to the hepatic sinusoids. When pressure exceeds 10 mmHg, portal hypertension is deemed to be present (12). We have shown that efflux of isoncotic fluid from the spleen is influenced, not by changes in capillary permeability but by intrasplenic microvascular hydrostatic pressure (3, 16, 30). We have also reported that partial ligation of the portal vein (PVL A; Fig. 1) raises splenic microvascular pressure (30). The current experiments were originally designed to confirm our initial observation that this was associated with increased intrasplenic fluid efflux. As a control, we ligated the portal vein caudal to the junction with the splenic vein (PVL B; Fig. 1) to cause the same changes in portal venous pressure and intrahepatic pressure, but no change in splenic venous outflow pressure. To our surprise, we still found an increase in intrasplenic fluid extravasation. We also observed a small, but consistent, fall in mean arterial pressure (MAP). We concluded that these changes probably involve a splanchnic reflex. Accordingly, we measured splenic afferent nerve activity and repeated the portal vein ligation experiments after denervating the spleen.

MATERIALS AND METHODS

The experimental procedures were approved by the local Animal Welfare Committee in accordance with the guidelines presented.
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sodium at the completion of each experiment.

Animals and housing. A total of 37 male Long-Evans rats (450–550 g) was obtained from Charles River Canada. They were housed in the University Animal Facility for several weeks until they had gained sufficient weight. They were exposed to light on a 12:12-h cycle in a humidity- and temperature-controlled environment and maintained on a 0.3% sodium diet and water ad libitum.

Anesthesia and surgery. Anesthesia was induced with pentobarbital sodium (60 mg/kg body wt ip) and maintained with Inactin [Byck, ethyl-(1-methylpropyl)malonyl-thio-urea, 80 mg/kg body wt sc]. Body temperature was maintained at 37°C (Homeothermic blanket, Harvard Apparatus, Canada). All procedures were done under a Zeiss dissecting microscope. Cannulas were inserted in the femoral artery (polyethylene, 0.58 mm ID, 0.97 mm OD) and vein (0.51 mm ID, 0.94 mm OD; Silastic, Dow Corning). The arterial cannula was used for measuring systemic blood pressure. The venous cannula was used to administer isotonic saline (3 ml/h) to replace evaporative and surgical losses.

The spleen was cleared from its attachments to the stomach. The stomach was then delivered through the abdominal incision, laid on the thorax of the rat, and covered in plastic wrap, care being taken to avoid undue traction on the abdominal organs. To ensure that the splenic artery and vein supplied and drained only the spleen, all branches running from the splenic vessels to the pancreas, stomach, and other surrounding tissue were ligated and divided. A loose ligature was placed around the hepatic portal vein, either rostral (PVL A; Fig. 1) or caudal (PVL B; Fig. 1) to the junction with the splenic vein.

Blood pressure, blood flow, and hematocrit. A cannula (polyethylene, 0.58 mm ID, 0.97 mm OD) was inserted non-occlusively in the portal vein and secured with tissue adhesive (3M Vebond; Animal Care Products, St. Paul, MN); this cannula was used to monitor portal venous pressure. Transit-time flow probes (Transonic 1RB series) have been validated for measurement of absolute blood flow in the rat and found to be accurate and highly reproducible (31).

After surgery was completed, the rat was allowed to stabilize for 30–45 min. Baseline readings of systemic blood pressure, portal venous pressure, and splenic arterial and venous blood flow were recorded for 20 min. The portal venous snare was then tightened until portal venous pressure rose to 12–15 mmHg. Hemodynamic recordings were made for a further 20 min. The data, which are presented, were averaged over the last 10 min of each recording session. In a small group of rats (n = 4), cannulas were inserted in the left gastric artery and vein to sample splenic arterial and venous blood for measurement of hematocrit.

Splenic denervation. The spleen was denervated according to the method of Lindblom et al. (20). Briefly, the splenic nerves were visualized using Toluidine blue, and a 2- to 3-mm length of nerve was resected. Great care was taken not to damage the pancreatic tissue. The sham control animals were subjected to the same procedure, except that the nerve was not interrupted. We have previously confirmed that this procedure reduces splenic tissue catecholamine levels to nearly undetectable levels (10).

Splenic afferent nerve recording. A separate group of rats was used for these experiments (n = 5). The abdominal cavity was filled with mineral oil, and the spleen nerve was isolated and divided. The distal end of the nerve was placed on bipolar platinum recording electrodes and stabilized with Kwik-Cast (WPI, Sarasota, FL). The nerve signal was amplified and filtered between 100 and 1,000 Hz (Leaf Electronics QT-B; WPI LFP-30). Output from the amplifier was fed to a loudspeaker and displayed on a personal computer (10-kHz sampling rate; WinDaq, Dataq Instruments, Akron, OH). After stabilization (20 min), afferent nerve activity was recorded on-line. Later (20 min), the portal venous ligature was tightened until portal venous pressure measured between 12 and 15 mmHg. Nerve recording continued for a further 20 min.

Data acquisition and analysis. Blood pressure and flow were recorded on-line (DI-400; Dataq Instruments) and analyzed using Dataq’s own software (WinDaq).

Analysis of data and statistics. The difference between splenic arterial inflow and venous outflow (A-V differential) was used to estimate intrasplenic fluid efflux. Throughout this study, means ± SE are presented. Student’s t-test for paired data was used to examine the statistical significance of changes in MAP, portal venous pressure, and splenic blood flow. ANOVA was used to test whether there were significant differences in baseline values of MAP, splenic blood flow, and A-V differential between the three experimental groups. Statistical significance was accepted at P < 0.05.

RESULTS

There were no significant differences between any of the three groups of rats [PVL A (n = 6), PVL B (n = 8), and PVL A plus splenic denervation (n = 6)] with respect to baseline blood pressure (101 ± 5, 101 ± 3, 108 ± 8 mmHg), splenic arterial blood flow (2.1 ± 0.2, 1.6 ± 0.2, and 2.3 ± 0.4 ml/min), or A-V differential (0.8 ± 0.2, 0.7 ± 0.1, 0.5 ± 0.3 ml/min), respectively.

Partial ligation of the portal vein caused portal venous pressure to increase from 6.5 ± 0.4 to 13.3 ± 0.3 mmHg (n = 33, including rats used for measurement of splenic nerve activity and portal venous blood flow).

When the portal vein was partially ligated rostral to the junction with the splenic vein (PVL A; Fig. 1), there

![Fig. 1. Anatomy of splenic circulation and placement of ligatures on hepatic portal vein. PVL A, portal vein ligation rostral to junction with the splenic vein; PVL B, portal vein ligation caudal to junction with the splenic vein.](image-url)
was a small but significant and consistent fall in systemic blood pressure (Fig. 2A). Portal pressure rose (Fig. 2B). There was no change in pressure on the downstream (liver) side of the ligature (baseline: 6.2 ± 1.4 mmHg; occlusion: 6.9 ± 2.2 mmHg, n = 2), although portal venous flow in the liver fell from 15.1 ± 2.5 to 5.9 ± 1.7 ml/min (n = 4). There was no change in splenic arterial blood flow, but splenic venous outflow tended to fall (Fig. 2C). This resulted in a significant increase in the A-V flow differential (Fig. 2D). The splenic venous hematocrit rose from 46.5 ± 0.6 to 50.5 ± 0.5% (n = 4).

When the portal vein was partially ligated caudal to the junction with the splenic vein (PVL B; Fig. 1), systemic blood pressure fell again. Although the change in MAP did not reach significance in the PVL B group reported in Fig. 3, inclusion of the data from the rats used to measure changes in portal blood flow revealed that there was indeed a significant fall, i.e., that the failure to reach significance in Fig. 3 was a type I error, corrected by increasing the sample size (baseline MAP: 101 ± 1.7 mmHg; MAP during PVL B: 94 ± 3 mmHg; n = 16). Again, there was no change in portal venous pressure downstream of the stenosis (baseline: 4.9 ± 0.8 mmHg; occlusion: 4.9 ± 0.7 mmHg, n = 4), although portal venous blood flow fell from 11.7 ± 0.6 nl/min to 8.6 ± 0.4 nl/min (n = 5). There was no change in splenic arterial blood flow, but splenic venous outflow fell (Fig. 3C). This resulted in a significant increase in the A-V flow differential (Fig. 3D).

When the portal vein was partially ligated (PVL A; Fig. 1) in the splenic denervated rats, there was no change in systemic blood pressure (Fig. 4A), nor were there any changes in splenic arterial or venous blood flow (Fig. 4C). There was no change in the A-V flow differential (Fig. 4D).

There was an increase in splenic afferent nerve activity from 5.6 ± 0.9 to 16.2 ± 0.7 spikes/s (n = 5) in response to partial portal vein ligation (portal venous pressure upstream of the ligature rose from 8.5 ± 0.9 to 14.8 ± 0.5 mmHg).

DISCUSSION

Partial ligation of the portal vein, rostral to the junction with the splenic vein (PVL A; Fig. 1), did not change splenic arterial blood flow. However, splenic venous blood flow tended to fall. This resulted in a significant increase in the difference between inflow and outflow. Because we ensure that blood can only enter and leave the spleen through the splenic artery and vein, respectively, and because the rat spleen has no capacity to store blood (27, 30), we have reasoned that the A-V differential is a measure of intrasplenic fluid extravasation and splenic lymphatic outflow (1–3, 7, 9, 16). The increase in splenic venous hematocrit observed in this, and previous studies (17), also supports our contention that the splenic A-V flow differential reflects significant fluid extravasation within the splenic circulation. Furthermore, we have observed that portal vein occlusion causes a gross distension of the splenic lymph duct (7) and an increase in lymph flow in the extrasplenic microvasculature circulation (intravital microscopy, unpublished observation).

Intrasplenic fluid extravasation is not dependent on changes in microvascular permeability, since the splenic circulation is freely permeable at all times to plasma proteins (7). Rather, fluid efflux is controlled by
intrasplenic hemodynamics by altering the relative tone of the splenic pre- and postcapillary arterioles and venules, i.e., in much the same manner as renal glomerular filtration rate is determined by glomerular filtration pressure and glomerular afferent/efferent arteriolar vascular tone. We had previously shown that portal vein ligation and elevation of portal venous pressure cause an increase in intrasplenic microvascular pressure (30). This was associated with an increase in intrasplenic fluid extravasation. We had reasoned that, if the observed increase in intrasplenic efflux was directly caused by pressure “backup” along the splenic...
venous outflow tract, then partial portal vein ligation
caudal to the junction with the splenic vein (PVL B; 
Fig. 1) should permit free drainage of the splenic ve-
nous outflow, and there should be no change in intra-
splenic fluid extravasation. This is not what we found. 
Caudal ligation also caused a significant increase in 
fluid extravasation. In light of our previous finding 
that the splenic nerves influence plasma volume (2), 
we investigated whether the portal hypertension-induced 
increase in intrasplenic fluid extravasation was neu-
really mediated.

Portal vein ligation, rostral to the junction with the 
splenic vein (PVL A; Fig. 1), was repeated in splenic 
denervated animals. Although portal venous pressure 
increased to the same extent, there was no fall in 
splenic venous blood flow and no measurable increase 
in fluid extravasation. We propose therefore that the 
increase in splenic fluid extravasation normally ob-
erved in portal hypertension is induced not by a direct 
mechanical increase in back pressure to the spleen but 
by a neurally mediated increase in intrasplenic micro-
vascular pressure, i.e., by altering the balance of pre- 
to postcapillary resistance.

Given that portal vein ligation did not alter hepatic 
portal pressure downstream of the ligature, it is un-
likely that the stimulus for fluid efflux arises from 
pressoreceptors in the liver. On the other hand, both 
rostral and caudal portal vein stenosis caused a fall in 
portal venous flow in the liver (9.6 ± 1.2 ml/min after 
PVL A and 3.2 ± 0.5 ml/min after PVL B). Such a 
change in intrahepatic portal flow has been shown to 
elicit a reduction in renal blood flow through the hep-
torenal reflex pathway (24). It is thus conceivable that 
there is also a hepatosplenic reflex pathway, whereby 
changes in intrahepatic blood flow could influence 
spinal efferent nerve activity and intrasplenic fluid 
extravasation.

Regardless of the position of the ligature, portal vein 
stenosis would also cause an increase in intestinal 
venous outflow pressure. Activation of intestinal pres-
sooreceptors has been shown to increase mesenteric 
afferent nerve activity and reduce renal blood flow (4, 
23) through intense renal vasocostriction (14). This 
has led to the suggestion that there may be an intesti-
nal/renal reflex pathway (14, 23). Our data thus sug-
gest that there may also be an intestinal/splenic reflex 
pathway that, in the face of intestinal congestion, me-
diates an increase in fluid extravasation from the 
splenic vasculature. Under normal physiological condi-
tions, such a reflex would aid in clearing fluid from a 
plethoric splanchnic circulation.

In addition to increasing splenic fluid efflux, portal 
hypertension caused a fall in systemic blood pressure 
(Fig. 2). Hypotension has been reported both clinically 
and in experimental models of portal hypertension (5, 
6, 25). It has been suggested that this is caused by a fall 
in total peripheral resistance (28) because of an in-
crease in such vasodilatory factors like nitric oxide 
(21). Our finding that splenic denervation abolished 
the portal hypertension-induced fall in systemic blood 
pressure suggests that this explanation may be over-
simplistic. Indeed, we have ourselves already reported 
that the spleen may reflexly control systemic blood 
pressure (10). Furthermore, neonatal capsaicin treat-
ment to selectively eliminate primary afferent (sen-
sory) innervation has been reported to prevent the 
development of a hyperkinetic circulation in portal 
hypertensive rats (19). We confirmed that, indeed, 
splenic afferent nerve activity did increase in response 
to portal vein ligation. Thus changes in splenic afferent 
nerve activity, elicited by hemodynamic changes in the 
splanchnic circulation, may influence systemic blood 
pressure. However, clarification of the role that these 
reflexes play in the perturbations of long-term patho-
physiological conditions, such as cirrhosis of the liver, 
must be approached cautiously given that the clinical 
condition is associated with many structural and met-
abolic changes apart from the simple rise in portal 
venous pressure. In addition, experiments would have 
to be done in conscious, instrumented rats, since sur-
gery and pentobarbital sodium anesthesia are known to 
blunt reflex control of the cardiovascular system, 
probably at the level of the vasomotor center of the 
brain (22).

In conclusion, we have demonstrated that partial 
ligation of the portal vein induces an increase in fluid 
extravasation from the splenic circulation. We propose 
that the hepatic afferent nerves, the mesenteric affer-
ent nerves, and splenic efferent nerves make up a 
complex of intrasplanchic reflex pathways, through 
which are mediated changes in intrasplenic vascular 
tone and microvascular pressure. Although there is 
evidence that some of these reflex pathways may pass 
directly through the dorsal root ganglia (11), contribu-
tions from the cardiovascular regulatory centers in the 
brain cannot be ignored (29). Portal vein ligation also 
elicited a fall in MAP, which was associated with an 
increase in splenic afferent nerve activity and which 
was abolished by splenic denervation. We suggest that 
this reflex pathway could contribute to the fall in blood 
pressure associated with portal hypertension.

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