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.

Portal hypertension is associated with profound perturbations of renal and cardiovascular homeostasis, the underlying mechanisms of which are still poorly understood. Clinically, the increase in hepatic portal pressure may be caused by prehepatic block (portal vein thrombosis), by intrahepatic block (cirrhosis), or by posthepatic block (hepatic vein outflow obstruction; see Ref. 12). Although the hemodynamic changes within the liver differ greatly according to the site of obstruction, the hemodynamic effects are remarkably consistent, namely a hyperdynamic circulation. There is also evidence that, despite there being an increase in total blood volume (5, 26, 32), central blood volume may be reduced (13). Clearly, the hemodynamic responses cannot derive solely from changes in stimulation of intrahepatic pressoreceptors, since in some cases intrahepatic pressure is increased (intra- and posthepatic block) and in the others it is decreased (prehepatic block). We have recently described how the spleen can influence blood pressure, plasma volume, and renal function (1, 7–9, 15, 17, 18). Our evidence suggests that changes in blood pressure/flow within the spleen alter fluid efflux from the intravascular space in the lymphatic system. We hypothesized that some of the changes in cardiovascular homeostasis observed in portal hypertension arise, not only from the liver, but also from the spleen, the venous effluent of which flows in the portal vein.

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...
were killed with an anesthetic overdose of pentobarbital issued by the Canada Council on Animal Care. All animals with the splenic vein; PVL B, portal vein ligation caudal to junction C (Homeothermic blanket, Harvard Apparatus, Canada). Inactin [Byck, ethyl-(1-methylpropyl)malonyl-thio-urea, 80 tobarbital sodium (60 mg/kg body wt ip) and maintained with diet and water ad libitum. Controlled environment and maintained on a 0.3% sodium light on a 12:12-h cycle in a humidity- and temperature-controlled weight. They were exposed to (450 flow). In some rats, flow probes (1RB series; Transonic Systems, Ithaca, NY) were positioned around the splenic artery and vein (to measure occlusively in the portal vein and secured with tissue adhesive (polyethylene, 0.58 mm ID, 0.97 mm OD) was inserted non-pervasively into the portal vein, either rostral (PVL A; Fig. 1) or caudal (PVL B; Fig. 1) to the junction with the splenic vein. The splenic nerve was isolated from the 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 Vetbond; 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.

Spleen denervation. The spleen was denervated according to the method of Lindblom et al. (20). Briefly, the spleen nerves were visualized using Toluolidine 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).

Spleen 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 LPF-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
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 ml/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 system, it would be attenuated by splenectomy. This was not the case: we found that splenectomy did not alter the increase in intrasplenic fluid extravasation that we had observed post portal vein ligation (30).

**Fig. 3.** Effect of hepatic portal vein ligation caudal to the junction with the splenic vein ($n = 8$). A: MAP. B: PVP. C: blood flow in the splenic artery (filled bars) and vein (open bars). D: A-V. Vertical bars delineate means ± SE. *P < 0.05 compared with baseline value.

**Fig. 4.** Effect of hepatic portal vein ligation rostral to the junction with the splenic vein ($n = 6$) in splenic denervated animals. A: MAP. B: PVP. C: blood flow in the splenic artery (filled bars) and vein (open bars). D: A-V. Vertical bars delineate means ± SE. *P < 0.05 compared with baseline value.
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 venous outflow, and there should be no change in intrasplenic 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 neurally 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 observed 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 microvascular 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 unlikely that the stimulus for fluid efflux arises from presoreceptors 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 hepatorenal reflex pathway (24). It is thus conceivable that there is also a hepatosplenic reflex pathway, whereby changes in intrahepatic blood flow could influence splenic 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 presoreceptors has been shown to increase mesenteric afferent nerve activity and reduce renal blood flow (4, 23) through intense renal vasoconstriction (14). This has led to the suggestion that there may be an intestinal/renal reflex pathway (14, 23). Our data thus suggest that there may also be an intestinal/splenic reflex pathway that, in the face of intestinal congestion, mediates an increase in fluid extravasation from the splenic vasculature. Under normal physiological conditions, such a reflex would aid in clearing fluid from a plethoric splanchic 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 increase 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 treatment to selectively eliminate primary afferent (sensory) 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 splanchic circulation, may influence systemic blood pressure. However, clarification of the role that these reflexes play in the perturbations of long-term pathophysiological conditions, such as cirrhosis of the liver, must be approached cautiously given that the clinical condition is associated with many structural and metabolic changes apart from the simple rise in portal venous pressure. In addition, experiments would have to be done in conscious, instrumented rats, since surgery 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 afferent 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), contributions 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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REFERENCES