Splenic baroreceptors control splenic afferent nerve activity

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Moncrief, Karli, and Susan Kaufman. Splenic baroreceptors control splenic afferent nerve activity. Am J Physiol Regul Integr Comp Physiol 290: R352–R356, 2006. First published October 6, 2005; doi:10.1152/ajpregu.00489.2005.—Stenosis of either the portal or splenic vein increases splenic afferent nerve activity (SANA), which, through the splenorenal reflex, reduces renal blood flow. Because these maneuvers not only raise splenic venous pressure but also reduce splenic venous outflow, the question remained as to whether it is increased intrasplenic postcapillary pressure and/or reduced intrasplenic blood flow, which stimulates SANA. In anesthetized rats, we measured the changes in SANA in response to partial occlusion of either the splenic artery or vein. Splenic venous and arterial pressures and flows were simultaneously monitored. Splenic vein occlusion increased splenic venous pressure (9.5 ± 0.5 to 22.9 ± 0.8 mmHg, n = 6), reduced splenic arterial blood flow (1.7 ± 0.1 to 0.9 ± 0.1 ml/min, n = 6) and splenic venous blood flow (1.3 ± 0.1 to 0.6 ± 0.1 ml/min, n = 6), and increased SANA (1.7 ± 0.4 to 2.2 ± 0.5 spikes/s, n = 6). During splenic artery occlusion, we matched the reduction in either splenic arterial blood flow (1.7 ± 0.1 to 0.7 ± 0.05, n = 6) or splenic venous blood flow (1.2 ± 0.1 to 0.5 ± 0.04, n = 5) with that seen during splenic vein occlusion. In neither case was there any change in either splenic venous pressure (−0.4 ± 0.9 mmHg, n = 6 and +0.1 ± 0.3 mmHg, n = 5) or SANA (−0.11 ± 0.15 spikes/s, n = 6 and −0.05 ± 0.08 spikes/s, n = 5), respectively. Furthermore, there was a linear relationship between SANA and splenic venous pressure (r = 0.619, P = 0.008, n = 17). There was no such relationship with splenic venous (r = 0.371, P = 0.236, n = 12) or arterial (r = 0.275, P = 0.413, n = 11) blood flow. We conclude that it is splenic venous pressure, not flow, which stimulates splenic afferent nerve activity and activates the splenorenal reflex in portal and splenic venous hypertension.

blood flow; splenic vein; portal hypertension; blood pressure

THE SPLEEN CONTRIBUTES to homeostatic regulation of both blood volume and pressure. It is a major site of regulated fluid efflux out of the vasculature and into the extravascular fluid compartment (5, 7). In addition, structural and functional evidence has pointed to the existence of a neural pathway between the spleen and kidneys, through which the spleen may influence renal function (6, 16, 24). In addition, we have shown there to be an inverse relationship between intrasplenic nitric oxide (NO) and mean arterial pressure (MAP) (9), a response that is abolished by either splenic or renal denervation. This led us to propose that intrasplenic hemodynamics might initiate a splenorenal reflex.

Further evidence for such a reflex arose from studies examining the effects of hepatic portal hypertension on splenic and renal hemodynamics. Perturbations of renal hemodynamics are an important pathological consequence of portal hypertension, and it has long been established that the hepatorenal reflex contributes to this dysfunction (17). However, in addition to altering intrahepatic hemodynamics (13, 15), portal hypertension also increases splenic venous outflow pressure (14). We showed that controlled partial occlusion of either the portal or the splenic vein increases both splenic afferent nerve activity and renal efferent nerve activity (14, 19). Thus we proposed that, in addition to the hepatorenal reflex, a splenorenal reflex also contributes to portal hypertension-induced renal dysfunction.

Partial occlusion of either the portal or the splenic vein not only raises splenic venous pressure but also reduces venous outflow from the spleen. The question thus remained as to whether it was increased intrasplenic postcapillary pressure and/or reduced intrasplenic blood flow that triggers the increase in splenic afferent nerve activity. The objective of this study was to distinguish between these possibilities. We compared the relative effects on splenic afferent nerve activity of 1) partial occlusion of the splenic vein, which selectively decreased splenic arterial and venous blood flow and increased splenic venous pressure, and 2) partial occlusion of the splenic artery, which similarly decreased splenic arterial and venous blood flow but caused no change in splenic venous pressure. We were thus able to eliminate flow as a variable when we selectively raised splenic venous pressure.

METHODS

All experimental procedures were approved by the local Animal Welfare Committee according to the guidelines set by the Canada Council on Animal Care. Upon completion of each experiment, all animals were euthanized with an anesthetic overdose of pentobarbital sodium (96 mg iv; MTC Pharmaceuticals, Cambridge, ON).

Animals and Housing

Male Long-Evans rats were purchased from Charles River Canada (St. Foy, QB Canada) and housed in the University of Alberta Animal Facility in a temperature-controlled environment, exposed to light on a 12:12-h light-dark cycle, and given water ad libitum. They were fed a restricted diet of 0.3% sodium rat chow (three pellets daily) for 2 wk before experiments were started, so as to reduce intra-abdominal fat (20). There were three experimental groups: splenic vein-occluded rats (VO; n = 6), and two groups of splenic artery-occluded (AO) rats comprising animals in which arterial blood flow was matched to that observed in the VO group (AOAF, n = 6), and animals in which venous blood flow was matched to that observed in the VO group (AOVF, n = 5).

Surgery

Anesthesia was induced with pentobarbital sodium (62 mg/kg body wt ip) and maintained with Inactin (thiobutabarbital sodium, 80 mg/kg body wt sc; Sigma, St. Louis, MO). Temperature was maintained using Delta-phase heating pads (Braintree Scientific, Braintree, MA).

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The femoral vein and artery were cannulated with Silastic tubing (0.51 mm ID, 0.94 mm OD; Dow Corning, Midland, MI) and polyethylene (PE) tubing (PE-50, 0.58 mm ID, 0.97 mm OD; Becton Dickinson, Sparks, MD) for infusion of isotonic saline (3 ml/h) and measurement of systemic blood pressure, respectively. A midline laparotomy was performed to expose the spleen and its vasculature (Fig. 1). Silastic tubing (0.30 mm ID, 0.64 mm OD) was inserted occlusively into the gastric vein, and its tip was advanced to the junction with the splenic vein. Similarly, PE tubing (PE-10, 0.28 mm ID, 0.61 mm OD) was inserted into the gastric artery and advanced to the junction with the splenic artery. Cannulas were connected to Gould Statham pressure transducers (Gould Electronics, Recording Systems Division, Cleveland, OH) for online measurement of splenic venous and arterial pressure. The splenic vasculature was isolated by ligating all vessels leading to or from other vascular beds (L1/L2; Fig. 1). A cavity was created by securing the edges of the abdominal incision to a wire support ring to contain the warm mineral oil needed to electrically isolate the nerve.

Splenic Vein Occlusion

A balloon occluder was placed around the splenic vein proximal to the junction with the gastric vein; this could be inflated to allow for controlled, partial occlusion of the vessel (splenic vein occlusion, SVO; Fig. 1). Factory-calibrated transit-time flow probes (0.5 V and 1RB series, Transonic Systems, Ithaca, NY) were placed around the splenic artery (0.5 V) and splenic vein (1RB), distal to the junction with the gastric vessels but proximal to the point of branching of the smaller splenic vessels.

Arterial Flow-Matched and Venous Flow-Matched Splenic Artery Occlusions

Surgical procedures for the arterial occlusion groups were similar to those for splenic vein occlusion, except that 1) the balloon occluder was positioned around the splenic artery distal to the junction with the gastric, celiac, and hepatic arteries (SAO; Fig. 1); 2) the gastric artery was occluded, but not cannulated (the placement of the occluder was such that we were unable to obtain downstream arterial pressure measurements in these animals); and 3) single-flow probes were positioned around the vein or artery, for the venous flow-matched group or the arterial flow-matched group, respectively.

Extracellular Nerve Recording

The splenic nerve is located as a bundle between the splenic artery and vein (Fig. 1). Under mineral oil, a section of the splenic nerve was carefully isolated, divided, and sectioned for recording of splenic sensory afferent nerve activity. The distal end of the cut nerve (coming from the spleen) was laid on a bipolar platinum recording electrode. The signals were amplified using an isolation preamplifier (model 11–5407-58) and universal amplifier (model 13–4615-58, Gould Electronics). Nerve activity was recorded online at a sample rate of 10,000 Hz, amplified, and filtered between 100 and 10,000 Hz.

Experimental Protocol

After completion of all surgical procedures, animals were allowed to stabilize for ~30 min. Selection criteria were used to standardize our experimental groups. Inclusion was contingent on achieving baseline values of mean arterial pressure (MAP; 80–105 mmHg), splenic arterial blood flow (1.5–2.5 ml/min), splenic venous blood flow (1.0–2.5 ml/min), splenic venous pressure (SVP; 6–15 mmHg), and splenic afferent nerve activity (SANA; 1–4 spikes/s). Baseline values were recorded for 5 min before occlusion. In VO animals, the balloon occluder was then inflated to increase splenic venous pressure to 20–24 mmHg for 5 min. This value was chosen according to a previously established protocol for partial splenic vein occlusion, mimicking the splenic venous pressure observed in portal hypertension (14). In AO,F animals, the balloon was inflated for 5 min, so as to match the reduction in splenic arterial blood flow observed with SVO. In AO,VF animals, the balloon was inflated for 5 min so as to match the reduction in splenic venous blood flow observed with SVO. The balloon was inflated gradually over a period of ~1 min. For both VO and AO groups, occluded data are the average of a 2-min period, beginning 1 min after the initiation of vessel occlusion.

Data Analysis

Data are presented as means ± SE. All data were recorded and analyzed using WINDAQ/Pro software (DATAQ Instruments, Akron, OH). Nerve activity was quantified as an average discharge rate (spikes/s) based on the visual identification of action potentials in the raw filtered recordings. Compound action potentials could be easily
identified among the background noise, appearing as a distinct broadening of the electrical activity and increased magnitude above background noise. Nerve activity is represented as an average of each 5-min period for baseline, occluded, and recovery data, with recovery values taken 5 min after the end of occlusion. Data were analyzed for multiple comparisons between groups using two-way repeated-measures ANOVA, followed by the Student-Newman-Keuls method for post hoc analysis. Statistical significance was accepted at \( P < 0.05 \).

### Results

Comparisons of baseline values (before vessel occlusion) revealed no significant differences between any of the experimental groups with respect to systemic blood pressure, splenic venous pressure, splenic arterial and venous blood flows, or splenic afferent nerve activity (Table 1).

#### Effects of Venous and Arterial Occlusion on Splenic Venous Pressure, Splenic Arterial Pressure, and MAP

VO and AO had differing effects on splenic venous and arterial pressures. Splenic venous pressure increased during inflation of the balloon occluder around the splenic vein (VO) but returned to baseline after release (Fig. 2), although there were no changes in splenic arterial pressure (baseline \( 87.7 \pm 1.6 \) mmHg, occlusion \( 85.0 \pm 4.4 \) mmHg, release \( 86.3 \pm 3.3 \) mmHg, \( n = 6 \); \( P > 0.05 \)) or MAP (baseline \( 90.2 \pm 2.9 \) mmHg, occlusion \( 86.1 \pm 5.1 \) mmHg, release \( 87.8 \pm 4.2 \) mmHg, \( n = 6 \); \( P > 0.05 \)). Splenic venous pressure did not change after partial occlusion or release of the splenic artery (Fig. 2; \( \text{AO}_{AF} \), \( \text{AO}_{VF} \)). Nor were there any significant changes in MAP (\( \text{AO}_{AF} \): baseline \( 90.7 \pm 2.9 \) mmHg, occlusion \( 91.8 \pm 2.2 \) mmHg, release \( 90.9 \pm 3.5 \) mmHg, \( n = 6 \); \( P > 0.05 \); \( \text{AO}_{VF} \): baseline \( 96.1 \pm 1.2 \) mmHg, occlusion \( 98.3 \pm 2.2 \) mmHg, release \( 100.5 \pm 2.9 \) mmHg, \( n = 5 \); \( P > 0.05 \)).

#### Matched Reduction in Splenic Arterial and Venous Blood Flow

After splenic vein occlusion, splenic arterial blood flow fell from \( 1.7 \pm 0.1 \) to \( 0.9 \pm 0.1 \) ml/min (\( n = 6 \); \( P < 0.05 \)). Using these values as our criteria for the \( \text{AO}_{AF} \) group, we partially occluded the splenic artery so as to match the fall in arterial flow (Fig. 3A). After splenic vein occlusion, splenic venous blood flow fell from \( 1.3 \pm 0.1 \) to \( 0.6 \pm 0.1 \) ml/min (\( n = 6 \)). Similarly, using these values as our criteria for the \( \text{AO}_{VF} \) group, we partially occluded the splenic artery so as to match the fall in venous flow (Fig. 3B). In all three groups, splenic

### Table 1. Baseline values from VO, AO\textsubscript{VF}, and AO\textsubscript{AF} groups

<table>
<thead>
<tr>
<th></th>
<th>VO</th>
<th>AO\textsubscript{VF}</th>
<th>AO\textsubscript{AF}</th>
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<tbody>
<tr>
<td>( n )</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>90.2 ± 2.9</td>
<td>96.1 ± 1.2</td>
<td>90.7 ± 2.9</td>
</tr>
<tr>
<td>SABF, ml/min</td>
<td>1.7 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>SVBF, ml/min</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>SVP, mmHg</td>
<td>9.5 ± 0.5</td>
<td>8.8 ± 1.1</td>
<td>8.3 ± 0.9</td>
</tr>
<tr>
<td>SANA, spikes/s</td>
<td>1.7 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>1.5 ± 0.1</td>
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Values are presented as means ± SE. VO, venous occluded; AO\textsubscript{VF}, arterial occluded/venous flow-matched; AO\textsubscript{AF}, arterial occluded/arterial flow-matched; MAP, mean arterial pressure; SABF, splenic arterial blood flow; SVBF, splenic venous blood flow; SVP, splenic venous pressure; SANA, splenic afferent nerve activity; \( n \) = number.
arterial and venous flows recovered to baseline levels upon deflation of the balloon occluder (Fig. 3, A and B).

**Differential Effects of VO and AO on Splenic Afferent Nerve Activity**

Partial occlusion of the splenic vein caused a marked increase in SANA (Fig. 4, VO). In contrast, partial occlusion of the splenic artery did not cause any significant change in SANA (Fig. 4, AOA_F and AOV_F). In all three groups, SANA returned to baseline after deflation of the balloon occluder. Furthermore, SANA correlated linearly with increases in splenic venous pressure ($r = 0.619, P = 0.008, n = 17$). There was no such relationship between SANA and either splenic arterial blood flow ($r = 0.371, P = 0.236, n = 12$) or splenic venous blood flow ($r = 0.275, P = 0.413, n = 11$).

**DISCUSSION**

Partial occlusion of either the portal or splenic vein causes an immediate increase in splenic venous pressure accompanied by a corresponding fall in splenic blood flow, as well as an increase in splenic afferent nerve activity (14, 19). Our previous findings that intrasplenic NO reflexly alters MAP (9) had raised the possibility that flow-mediated NO release, not pressure, might be the stimulus for splenic afferent nerve activity. To ascertain whether changes in intrasplenic postcapillary pressure or splenic blood flow stimulate afferent nerve activity, we designed the current experiments to eliminate flow as a variable, while we selectively raised splenic venous pressure. We found that partial occlusion of the splenic vein, which was associated with increased splenic venous pressure and decreased splenic blood flow, caused a 27% increase in splenic afferent nerve activity from baseline. By contrast, partial occlusion of the splenic artery, which was accompanied by the same fall in splenic blood flow but which caused no change in splenic venous pressure, had no effect on splenic afferent nerve activity. Regression analysis revealed a positive linear correlation between venous pressure and afferent nerve activity. There was no such relationship between blood flow and nerve activity. Higher splenic arterial flow than venous blood flow has previously been reported and has been ascribed to intrasplenic fluid extravasation (7).

Studies of the spleen have largely focused on its role in immune function. Furthermore, most studies regarding innervation of the spleen and its vasculature have focused on efferent sympathetic innervation and its role in modulating the immune system (2). A substantial proportion (~95%) of nerve fibers in the splenic nerve are sympathetic, most of which originate in the celiac-superior mesenteric ganglion complex (4). Indeed, the very existence of afferent innervation of the spleen had even at one time been questioned, although the authors of one study using fluorescent retrograde tracing did concede that there may be a small number of afferent fibers innervating the hilar region of the spleen and/or the associated vasculature (27). Functionally, a number of studies have clearly demonstrated that afferent fibers do convey sensory information from the spleen (11, 12, 16, 24). Furthermore, evidence for afferent innervation of the spleen has now been demonstrated both histologically (11) and by direct electrophysiological measurement (19).

Our results suggest that intrasplenic postcapillary vascular pressure is monitored and controls splenic afferent nerve activity. This is consistent with findings that there are, in the dog and the cat (6, 16), afferent nerve endings located in the wall of the splenic vein; that is, there are splenic mechanoreceptors, which respond to alterations in venous wall tension. It has long been established that the hepatorenal and renorenal reflexes are sensed and initiated by intrahepatic and intrarenal mechanoreceptors, respectively (1, 10, 21, 22, 25, 28). We propose that a similar mechanism regulates afferent neural discharge rate in the spleen.

Baroreceptors and stretch receptors are present throughout the cardiovascular system, providing regulatory feedback for the maintenance of systemic blood pressure and volume (23). We have previously observed that denervation of the spleen causes a progressive decline in MAP (9). This suggests that the spleen may play a role in the tonic maintenance of blood pressure under normal physiological conditions. In addition, chemical or mechanical stimulation of splenic afferent receptors has been shown to elicit reflex changes in cardiopulmonary sympathetic activity, leading to increased heart rate, contractile force, and systemic blood pressure (6, 16). We have now shown that increased intrasplenic postcapillary pressure initiates an increase in splenic afferent nerve activity. This lends weight to our suggestion that the spleen, like the liver and the kidneys, should be considered a supplementary reflexogenic area involved in normal cardiovascular homeostasis.

**Perspectives**

Our data suggest that, in pathological conditions such as portal hypertension, increased splenic venous pressure would stimulate splenic venous sensory receptors and increase splenic afferent nerve activity. It is tempting to suggest that such a splenic response could serve as a compensatory mechanism to offset the systemic hypotension seen in both experimental and
clinical portal hypertension (3, 26). However, partial occlusion of the splenic or portal vein has previously been shown to cause a significant reduction in mean arterial pressure (14). This portal hypertension-induced fall in systemic pressure stands to worsen the condition by contributing to the already compromised renal and cardiovascular function. It is unlikely to be mediated by our observed increase in splenic afferent nerve activity, since such an increase would be expected to increase renal release (14, 18). Furthermore, we have observed that the fall in MAP observed during splenic vein occlusion persists even after splenic denervation (14). (In these current studies, MAP did tend to fall during SVO but failed to reach significance when baseline, occlusion, and release were analyzed by repeated-measures ANOVA.) We suggest, therefore, that the systemic hypotension associated with splenic and/or portal hypertension is likely not neurally mediated and may instead be caused by the release of an as-yet unidentified hypotensive factor from the spleen (8, 14).

In summary, we have shown that a rise in splenic venous pressure, not a fall in splenic blood flow, is responsible for increasing splenic afferent nerve activity after obstruction of splenic venous outflow. The evidence points to this being mediated through sensory mechanoreceptors located in the walls of the splenic postcapillary vasculature. This study thus establishes that the spleen plays a significant role in cardiovascular regulation by monitoring splanchic (venous) pressure. There is no evidence that splenic blood flow influences splenic afferent nerve activity. We suggest that, in portal hypertension, it is the increased splenic venous outflow pressure, but not the venous stagnation, which initiates an increase in splenic afferent nerve firing, activates the splenorenal reflex, and reduces renal blood flow.

GRANTS

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