Effect of mesenteric vascular congestion on reflex control of renal blood flow

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Hamza SM, Kaufman S. Effect of mesenteric vascular congestion on reflex control of renal blood flow. Am J Physiol Regul Integr Comp Physiol 293: R1917–R1922, 2007. First published August 22, 2007; doi:10.1152/ajpregu.00180.2007.—Portal hypertension initiates a splanorenal reflex, whereby increases in splenic afferent nerve activity and renal sympathetic nerve activity cause a decrease in renal blood flow (RBF). We postulated that mesenteric vascular congestion similarly compromises renal function through an intestinal-renal reflex. The portal vein was partially occluded in anesthetized rats, either rostral or caudal to the junction with the splenic vein. Portal venous pressure increased (6.5 ± 0.1 to 13.2 ± 0.1 mmHg; n = 78) and mesenteric venous outflow was equally obstructed in both cases. However, only rostral occlusion increased splenic venous pressure. Rostral occlusion caused a fall in RBF (−0.2 ± 0.2 ml/min; n = 9) that was attenuated by renal denervation (−0.05 ± 0.1 ml/min; n = 6), splenectomy (−0.2 ± 0.1 ml/min; n = 11), celiac ganglionectomy (−0.3 ± 0.1 ml/min; n = 9), and splenectomy (−0.5 ± 0.1 ml/min; n = 6). Caudal occlusion induced a significantly smaller fall in RBF (−0.02 ± 0.1 ml/min; n = 9), which was not influenced by renal denervation (−0.2 ± 0.2 ml/min; n = 6), splenectomy (−0.1 ± 0.1/ml/min; n = 7), celiac ganglionectomy (−0.1 ± 0.3 ml/min; n = 8), or splenectomy (−0.3 ± 0.1 ml/min; n = 7). Renal arterial conductance fell only in intact animals subjected to rostral occlusion (−0.007 ± 0.002 ml·min<sup>−1</sup>·mmHg<sup>−1</sup>). This was accompanied by increases in splenic afferent nerve activity (15.0 ± 3.5 to 32.6 ± 6.2 spikes/s; n = 7) and renal efferent nerve activity (32.7 ± 5.2 to 39.3 ± 6.0 spikes/s; n = 10). In animals subjected to caudal occlusion, there were no such changes in renal arterial conductance or splenic afferent/renal sympathetic nerve activity. We conclude that the portal hypertension-induced fall in RBF is initiated by increased splenic, but not mesenteric, venous pressure, i.e., we did not find evidence for intestinal-renal reflex control of the kidneys.

port hypertension; spleen; renal sympathetic nerve activity

PORTAL HYPERTENSION (PH), which is often present in chronic liver disease, is characterized by a pathological elevation in portal venous pressure (PVP) (>10 mmHg). Although end-stage renal failure is common in chronic liver disease, there is no intrinsic renal disease (16). There is evidence, however, that increased sympathetic nervous activity contributes to PH-induced renal dysfunction (1, 11). Indeed, it has been established that renal function may be controlled by a heporenal reflex, whereby elevated PVP/reduced portal venous flow triggers increased hepatic afferent/renal efferent sympathetic nerve activity (8, 17–19, 22).

Given that the splenic vein drains into the portal vein, any increase in PVP is associated with a parallel increase in splenic venous pressure (26). Increased intrasplenic pressure induces an increase in splenic afferent nerve activity, which induces an increase in systemic blood pressure through two distinct pathways. It activates a splanorenal reflex to increase renal sympathetic nerve activity and stimulate renin release (6), and it alters central neural control of sympathetic outflow (23). In the latter study, we showed that elevated splenic venous pressure induces activation of paraventricular and supraoptic nuclei of the hypothalamus, both of which are known to be important in cardiovascular homeostasis (25). In addition, the PH-induced obstruction of splenic venous outflow induces a fall in renal blood flow (RBF) that is mediated through the splanorenal reflex increase in renal sympathetic nerve activity (12).

Increased portal pressure also impedes blood draining from the gut. Thus it has been proposed that mesenteric congestion may contribute to renal (dys)function through activation of an intestinal-renal reflex (3, 10, 13, 21, 28). Previous studies led us to believe that selective mesenteric congestion could alter splenic function, i.e., an intestinal-splenic reflex (14). In the present study we investigated whether increased mesenteric venous pressure could influence RBF either directly to the kidney (intestinal-renal reflex) or indirectly through the spleen (intestinal-splenorenal reflex).

We used acute partial portal vein ligation (PVL) in rats to observe the effect of elevated PVP on RBF. As previously described (14), the portal vein was partially occluded above (PVLA) or immediately below (PVLB) the junction with the splenic vein (porto-splenic junction). Oclusion below (caudal) to the porto-splenic junction results in selective elevation of mesenteric venous pressure, without influencing the splenic pressure; thus any effect arising from this occlusion implicates the intestine. We measured the consequences of these maneuvers on PVP, mean arterial blood pressure (MAP) and RBF. The effects of renal denervation, splenic denervation, celiac ganglionectomy (i.e., combined functional splenic, mesenteric, and renal denervation), and splenectomy were studied. In a separate group of animals, we recorded the effects of occlusion (PVLA and PVLB) on splenic afferent and renal efferent nerve activity. Contrary to our hypothesis, selective mesenteric congestion did not modulate renal sympathetic nerve activity or RBF, either directly (intestinal-renal reflex) or indirectly (intestinal-splenorenal reflex). We conclude that the mesenteric vascular bed does not play a critical role in regulating RBF in PH.

MATERIALS AND METHODS

All experimental procedures were approved by the local Animal Welfare Committee in accordance with the guidelines issued by the Canada Council on Animal Care. All animals were euthanized with an anesthetic overdose of pentobarbital sodium (Vetobarbitol, 96 mg ip; Lavaltrie, QC, Canada) at the end of each experiment. Data were recorded online (DATAQ Instruments, Akron, OH) and analyzed with WINDAQ software (DATAQ Instruments), except for nerve activity data, which were recorded with PowerLab equipment (ADInstruments, Australia).
Portal venous blood flow is smaller after PVLB (but also decreases portal venous blood flow, which may induce a rounding tissues. The unpaired celiac ganglion was then excised from sur-
of the superior mesenteric artery were stripped from their respective
teric and celiac arteries. The nerves along the celiac artery and trunk
nerve activity (4, 5). Celiac ganglionectomy was achieved by blunt
supplies nerve fibers to splenic, renal, and mesenteric vascular beds;
reduces splenic tissue catecholamine levels. The celiac ganglion
toward the spleen. We have shown (2) that this procedure significantly
subsequently painted with 5% phenol to destroy remaining fibers, as
was used to monitor PVP.
The portal vein below the ligature, and secured with tissue adhesive
clusively into the superior mesenteric vein, advanced to the level of
caudal (PVLB) to the porto-splenic junction. A cannula (PE-50,
the superior mesenteric vein. A loose ligature (Prolene 1.5, Ethicon)
kept intact. The portal vein was gently exposed down to the level of
its connective tissue attachments to the stomach. Splenic vessels were
were tied off individually with 4-0 silk suture and completely ligated
PVLA or PVLB. Branches of vessels leading directly into the spleen
no difference in the fall in portal venous blood flow into the liver after
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PVL not only increases intraportal venous pressure
intraportal venous pressure and portal venous blood flow, which may induce a
hepatoportal reflex-mediated change in renal function (22). The fall in
portal venous blood flow is smaller after PVLB (~3.2 ± 0.6 ml/min) than after
PVL ( ~9.6 ± 2.4 ml/min) (14), since the former does not
impede splenic venous outflow into the portal vein. To control for this,
a group of animals were splenectomized, thus ensuring that there was
no difference in the fall in portal venous blood flow into the liver after
PVL or PVLB. Branches of vessels leading directly into the spleen
were tied off individually with 4-0 silk suture and completely ligated
with fine tissue scissors. The spleen could thus be completely removed
while maintaining the splenic vascular arcade intact.
Renal blood flow and portal venous blood flow. A factory-calibrated
flow probe (IRB series, Transonic Systems, Ithaca, NY) was positioned around the left renal artery and covered in conducting gel. Zero-flow reading was confirmed before use by placing the probe in a nonturbulent water bath. A 30- to 35-min stabilization period was allowed, during which time, body temperature, MAP, and RBF were monitored. Portal venous blood flow was similarly measured with a 3RB Transonic flow probe.
Experimental protocol (renal blood flow). Five experimental groups were subjected to either PVLA (portal vein occlusion rostral to the junction with the splenic vein) or PVLB (portal vein occlusion caudal to the junction with the splenic vein): intact control rats [n = 9 (PVLB) and 9 (PVL)], renal denervated rats [n = 6 (PVLB) and
6 (PVL)], splenic denervated rats [n = 11 (PVL) or 7 (PVLB)], celiac ganglionectomized rats [n = 9 (PVLB) or 8 (PVL)], and splenectomized rats [n = 6 (PVL) or 7 (PVLB)]. After the stabilization period, baseline RBF, MAP, and PVP were recorded for 20
min, after which the portal ligature was tightened to effect partial portal vein occlusion and elevation of PVP to 12–15 mmHg (i.e., experimental PH). PVP, MAP, and RBF were then recorded for a further 10 min.
Whole fiber nerve recording. For determination of splenic afferent nerve activity, the intestines were placed gently back into the abdominal
cavity after portal vein cannulation and covered with moist gauze. The splenic arcade was then isolated over this gauze, and the edges of
the abdominal wall were sutured to a premade steel support ring
to create an abdominal “well” (3.0 cotton, Davis-Geck, American
Cyanamid). This was then filled with heavy mineral oil (Laboratoire
Atlas, Montreal, QC, Canada). The splenic vessels were gently exposed by blunt dissection under the mineral oil. Great care was taken to
ensure that the nerves were at no time exposed to air. An ~1-cm
length of splenic nerve was exposed and carefully dissected from the
vessels and surrounding tissues with fine forceps (no. 5, Dumont,
0.05 × 0.01 mm; Fine Science Tools, Vancouver, BC, Canada). The
proximal end (close to midline) of the nerve was cut with fine tissue
scissors for determination of afferent nerve activity. The protocol for
renal nerve isolation was similar, except that the left renal vessels
were exposed by gently retracting the intestines to the animal’s right
with moist gauze. This essentially formed a space adequate to fill with
mineral oil. A branch of the renal nerve was then isolated as for the
splenic nerve; however, the nerve was cut distally (i.e., closer to
the kidney) to allow for recording of efferent nerve activity. The ends of
cut nerves were then placed onto bipolar silver-platinum electrodes,
and the nerve signal was amplified (preamplifier, Gould) and filtered
between 100 and 10,000 Hz. Output from the amplifier was fed to a
loudspeaker and displayed on a PC (sampling rates: renal efferent 4
kHz, splenic afferent 10 kHz; PowerLab, ADInstruments).
Experimental protocol (nerve activity). Separate groups of rats
were used for these experiments [splenic afferent: n = 7 (PVLB) or
9 (PVL); renal efferent: n = 10 (PVLB) or 9 (PVL)]. After a 30-
to 35-min stabilization period, either splenic afferent or renal efferent
nerve activity was recorded online for 20 min, after which the portal
venous ligature was tightened to elevate PVP to 12–15 mmHg. Nerve
activity was recorded for a further 10 min. Analysis of nerve activity
was based on average firing rate (spikes/s) of identified action poten-
tials in the raw, filtered recordings (Chart 5 Software, Spike Histo-
gram Module, ADInstruments) (12). Initially, background noise was
determined by recording postmortem signals at the end of each
experiment. Because this was not different from determining back-
ground noise directly from the recorded nerve trace (as recommended
by ADInstruments), this method was used instead for subsequent
experiments.
Data analysis. Results are based on the first 10 min of each 20-min
recording period. Data were analyzed with one-way ANOVA (Figs.
1–3) or Student’s t-test (Fig. 5). Two-way ANOVA was used for
comparing data between PVLA and PVLB treatments. Significance
was accepted at P < 0.05.
RESULTS
Portal venous pressure and flow. Mean baseline PVP for all
animals in the RBF study was 6.5 ± 0.1 mmHg (n = 78). This
was elevated to 12–15 mmHg (mean 13.2 ± 0.1 mmHg) by

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partial occlusion of the portal vein. There were no significant differences between the experimental groups with respect to the baseline or experimental PVP values. In the splenectomized animals, there was no difference between the fall in portal venous blood flow subsequent to PVLA (−9.9 ± 1.9 ml/min; n = 4) and PVLB (−11.9 ± 2.7 ml/min; n = 3; P = 0.553).

Mean arterial blood pressure. Baseline MAP was similar in the intact, renal, and splenic denervated animals but lower in the celiac ganglionectomized animals (Fig. 1). MAP fell (intact, renal denervated, celiac ganglionectomized, splenectomized) or tended to fall (splenic denervated) from baseline after PVLA and PVLB (Fig. 1).

Renal blood flow. Baseline RBF was similar in intact, renal/splenic denervated, and splenectomized animals but lower in celiac ganglionectomized animals (Fig. 2). When PVP by caudal occlusion (PVLB) in the intact animals resulted in a significantly smaller drop in RBF (−0.5 ± 0.1 ml/min) compared with that observed after PVLA (Fig. 2, bottom). There were no changes in RBF in the denervated and splenectomized animals after PVLB (Fig. 2, bottom).

Renal conductance. Renal conductance (K) was calculated as the ratio of flow (Q) to renal perfusion pressure (P): K = Q/P. During PVLA, renal arterial conductance dropped significantly from baseline in the intact animals (−0.007 ± 0.002 ml·min⁻¹·mmHg⁻¹; Fig. 3, left). This change was completely abolished after renal denervation, splenic denervation, celiac ganglionectomy, and splenectomy (Fig. 3, left). Renal conductance did not change in any of the groups subjected to PVLB (Fig. 3, right).

Nerve activity. Mean baseline PVP for all animals in this section was 6.6 ± 0.2 mmHg (n = 35). There was no statistical difference between the baseline values of either splenic afferent or renal efferent nerve activity (P = 0.064). As in the RBF study above, PVP was elevated to 12–15 mmHg (mean 13.7 ± 0.3 mmHg; n = 35). Mean baseline MAP for all animals was 96.2 ± 1.9 mmHg, which fell to 90.4 ± 1.2 mmHg during PVLA (P < 0.05) or to 88.6 ± 5.5 mmHg during PVLB. PVLA caused a significant increase in activity in both splenic afferent (Figs. 4A and 5A) and renal efferent (Figs. 4C and 5B) nerves. This was not observed after PVLB (Fig. 4, B and D, and Fig. 5).

DISCUSSION

The fall in renal arterial conductance observed in intact animals after PVLA was completely abolished by renal denervation, by splenic denervation, by celiac ganglionectomy, and by splenectomy, and renal conductance did not change in any of the groups (intact, denervated, or splenectomized) during PVLB. Moreover, although both splenic afferent and renal efferent nerve activity increased during PVLA, this increase was not observed during PVLB. Had mesenteric congestion triggered a direct neural reflex (i.e., intestinal-splenic reflex), we would have observed an increase in renal efferent nerve activity and a fall in renal conductance during PVLB. Similarly, had mesenteric congestion triggered an indirect neural reflex via the spleen (i.e., intestinal-splenoportal reflex), we would have observed increases in both splenic afferent and renal efferent nerve activity during PVLB. We did not observe any changes in nerve activity or renal arterial conductance with PVLB. It appears, therefore, that after PVL, the intestine does not initiate either direct or indirect neural reflexes to control RBF. The residual fall in RBF observed in the denervated

![PVLA](image1.png)

![PVLB](image2.png)
animals subjected to PVLA or PVLB may be attributed to the fall in MAP, because there was no change in renal arterial conductance. On the basis of these observations, we conclude that selective mesenteric congestion alone does not play a role in regulating RBF. By contrast, the fall in renal conductance in the intact animals after PVLA confirms our previous findings that there is neural modulation of RBF mediated through the spleen.

It is known that the hepatorenal reflex may be elicited by changes in intrahepatic blood flow (22). Thus PVLA could potentially, by reducing portal venous blood flow, have initiated the change in renal vascular conductance through the hepatorenal reflex. PVLB does not cause such a marked fall in intrahepatic blood flow as PVLA because blood continues to flow unimpeded from the spleen into the portal vein and liver. The failure of PVLB to increase renal vascular conductance could then have been attributed to the smaller fall in intrahepatic blood flow. We eliminated the contribution of the spleen to changes in intrahepatic blood flow by splenectomizing the animals. Despite the fact that the fall in blood flow was then just as great as that observed after PVLA in the intact animals (9.6 ± 2.4 ml/min) (14), PVLB still failed to elicit a change in renal vascular conductance. We conclude therefore that the fall in renal vascular conductance elicited by PVLA was mediated primarily through the splenorenal reflex, rather than through the hepatorenal reflex.

The role of the mesenteric vascular bed as a reflexogenic region has been investigated by others (3, 10, 13, 28). The most
extensive study to date of intestinal-renal reflex regulation of RBF in PH was done by Miller et al. (21). They found that occlusion of the superior mesenteric vein in dogs (equivalent to PVL in our experiments) caused a profound reduction in cardiac filling pressure and output and a fall in RBF. Normalization of cardiac hemodynamics by intravenous fluid resuscitation did not restore RBF. Although splanchic ganglionectiony did not prevent the fall in RBF, normalization of cardiac indexes in these animals did partially restore RBF toward normal. The authors concluded that the renal perturbations observed in PH are due to an intestinal-renal reflex initiated by intestinal venous congestion (21). There are a few points to be considered as to why our results are at variance with this conclusion. First, in the absence of measures of systemic blood pressure or renal vascular conductance in the studies of Miller et al., it is impossible to conclude whether there was any change in renal vascular tone. Second, it was not noted by these investigators whether or not their dogs had been splenectomized, so it is probable that the spleen was intact. This is critical, because the dog spleen differs in structure and function from that of human and rat (20, 27). Sympathetic nerve stimulation in the dog has been shown to result in active expulsion of a large volume of blood (splenic contraction) (9), which would greatly complicate interpretation of the results of Miller et al.’s study. Third, their experimental protocol was very different from our own. The superior mesenteric vein of the dogs was completely occluded for 2 min (21). By contrast, we only partially occluded the portal/superior mesenteric vein for 10 min, while measuring PVP throughout to ensure that there was the same degree of PH in all animals. Complete occlusion of the superior mesenteric vein would have deprived the liver of its main blood supply, thus potentially causing ischemia of the hepatic tissues and subsequent metabolic derangement, which could ultimately have affected RBF. Fourth, they did not measure nerve activity, which makes it difficult to conclusively establish the presence of a functioning neural reflex.

Perspectives and Significance

Pathophysiologically, we have evidence that the splenorenal reflex can contribute to PH-induced renal dysfunction. However, this reflex may also have an important role in normal physiology. We have shown that increased splenic venous pressure (in the absence of changes in blood flow) elevates splenic afferent nerve activity (24). However, a previous study from this laboratory (6) prompted us to consider that changes in splenic venous blood flow and intrasplenic nitric oxide (NO) biosynthesis may also be important, and may be responsible for activating the splenorenal reflex. While we acknowledge that this requires further investigation, we propose that whereas increased splenic pressure initiates changes in central control of systemic blood pressure (23), the splenorenal reflex may be initiated by a reduction in splenic venous blood flow. The concept that there might be different types of nerve signaling within a single nerve is not without precedence: DiBona (7) has shown that within the renal sympathetic nerves specific subgroups of nerve fibers convey differential information encoded in the frequency domain of the firing.

Normally the changes in splenic blood flow and pressure are congruent. Thus in hypervolemia there is increased intrasplenic and mesenteric pressure and flow. The rise in intrasplenic pressure would increase signaling from the intrasplenic pressor receptors and initiate a reflex reduction in systemic blood pressure. In the absence of any fall in splenic blood flow, there would be no increase in renal efferent nerve activity. There would also be no reason physiologically for the increased mesenteric blood volume to initiate a reflex to conserve renal salt and water. By contrast, in hypovolemia there would be reduced intrasplenic and mesenteric pressure and flow. The reduction in splenic blood flow would, through reduced intrasplenic NO biosynthesis, induce a splenorenal reflex increase in renal sympathetic nerve activity. This would restore systemic blood pressure/volume both by increasing renin release and by reducing RBF and increasing renal salt and water retention.
We propose that in PH there is a unique combination of increased splenic and mesenteric venous pressure and reduced splenic venous flow. It is this latter phenomenon that initiates the increase in renal sympathetic nerve activity and renal dysfunction. Under these circumstances, there would be no physiological basis for suggesting that either the increased intrasplenic or mesenteric venous pressure should initiate reflexes to reduce renal salt and water excretion, as indeed our data show. We did not find any evidence that the mesenteric vascular congestion associated with PH contributes to increasing renal vascular resistance.

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REFERENCES


