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 splenorenal 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 (−0.5 ± 0.1 ml/min; n = 6), splenic denervation (−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 (−0.5 ± 0.1 ml/min; n = 9), which was not influenced by renal denervation (−0.2 ± 0.2 ml/min; n = 6), splenic denervation (−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−1·mmHg−1). 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.

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 hepatorenal 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 spinal splenorenal 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 splenorenal 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-splenic 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 (PVL above) or immediately below (PVL below) the junction with the splenic vein (porto-splenic junction). Occluding below (caudal) to the portal-splenic junction results in selective elevation of mesenteric venous pressure, without influencing the splenic circulation; 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 (PVL above and PVL below) 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 (Vetoquinol, 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).

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Castle Hill, Australia) and analyzed with Chart 5 software with the Spike Histogram Module (ADInstruments).

Animals. Male Long-Evans rats (350–600 g, Charles River, Montreal, QC, Canada) were housed in the University of Alberta Animal Facility for 1 wk before experiments commenced. Animals were exposed to a 12:12-h light-dark cycle in a temperature- and humidity-controlled room. All rats were fed standard 0.3% sodium rat chow and water ad libitum.

Surgery. Anesthesia was induced with pentobarbital sodium (65 mg/kg body wt ip), followed by Inactin [ethyl-(methylpropyl)-malonylthio-urea, 80–100 mg/kg body wt sc; Sigma] to maintain anesthesia after the animal reached a surgical plane. Body temperature was maintained at 37°C with a homeothermic blanket (Harvard Apparatus) or, for the nerve recording experiments, a Deltaphase thermal heating pad (Braintree Scientific, Braintree, MA) to reduce electrical interference.

The femoral vein and artery were cannulated with Silastic [0.51-mm inner diameter (ID), 0.94-mm outer diameter (OD); Dow Corning, Midland, MI] and polyethylene (PE-50, 0.58-mm ID, 0.97-mm OD; VWR International, Mississauga, ON, Canada) tubing for administration of isotonic saline (3 ml/h) and monitoring of systemic blood pressure, respectively. Through a midline laparotomy, the stomach was reflected onto the thorax and the spleen was cleared from its connective tissue attachments to the stomach. Splenic vessels were kept intact. The portal vein was gently exposed down to the level of the superior mesenteric vein. A loose ligature (Prolene 1.5, Ethicon) was placed around the hepatic portal vein, either rostral (PVLA) or caudal (PVLB) to the porto-splenic junction. A cannula (PE-50, 0.58-mm ID, 0.97-mm OD; VWR International) was inserted nonocclusively into the superior mesenteric vein, advanced to the level of the portal vein below the ligature, and secured with tissue adhesive (3M Vetbond; Animal Care Products, St. Paul, MN). This cannula was used to monitor PVP.

Renal/splenic denervation and celiac ganglionectomy. The renal nerves were stripped from the left renal artery and vein, which were subsequently painted with 5% phenol to destroy remaining fibers, as previously described (15). Similarly, the splenic nerves were stripped from the splenic artery and vein distal to the branching of vessels toward the spleen. We have shown (2) that this procedure significantly reduces splenic tissue catecholamine levels. The celiac ganglion supplies nerve fibers to splenic, renal, and mesenteric vascular beds; excision of this ganglion thus interrupts splenic, renal, and mesenteric nerve activity (4, 5). Celiac ganglionectomy was achieved 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 offferent 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 (PVLA) or 9 (PVLB); renal efferent: n = 10 (PVLA) or 9 (PVLB)]. 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 potentials in the raw, filtered recordings (Chart 5 Software, Spike Histogram 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 background 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...
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 was increased by rostral occlusion (PVLA), there was an immediate drop in RBF in the intact animals (−1.2 ± 0.2 ml/min), which was significantly attenuated or abolished in the denervated and splenectomized animals (Fig. 2, top). Elevation of 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. PVLB 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-renal 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 through the spleen (i.e., intestinal-splenorenal 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
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 (11.9 ± 2.7 ml/min) 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 ganglionectomy 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.

**Fig. 5.** Splenic afferent (A) and renal efferent (B) nerve activity (NA) after partial PVL either rostral [PVLA; n = 7 (splenic afferent) or 10 (renal efferent)] or caudal [PVLB; n = 9 (splenic afferent) and 9 (renal efferent)] to the porto-splenic junction. Data are absolute nerve activity (top) and change in nerve activity (bottom). Data are presented as means ± SE. *Significant difference from baseline (RM ANOVA), #significant difference between PVLA and PVLB; P < 0.05.
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.

GRANTS

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