Splenic reflex modulation of central cardiovascular regulatory pathways

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Moncrief K, Hamza S, Kaufman S. Splenic reflex modulation of central cardiovascular regulatory pathways. Am J Physiol Regul Integr Comp Physiol 293: R234–R242, 2007. First published March 29, 2007; doi:10.1152/ajpregu.00562.2006.—The splenorenal reflex induces changes in mean arterial pressure (MAP) and renal function. We hypothesized that, in addition to spinal pathways previously identified, these effects are also mediated through central pathways. We investigated the effect of elevated splenic venous pressure on central neural activation in intact, renal-denervated, and renal + splenic-denervated rats. Fos-labeled neurons were quantified in the nucleus of the tractus solitarius (NTS), paraventricular nucleus (PVN), supraoptic nucleus (SON), and subfornical organ (SFO) after 1-h partial splenic vein occlusion (SVO) in conscious rats bearing balloon occluders around the splenic vein, telemetric pressure transducers in the gastric vein (splenic venous pressure), and abdominal aorta catheters (MAP). SVO stimulated Fos expression in the PVN and SON, but not NTS or SFO of intact rats. Renal denervation abolished this response in the parvocellular PVN, while renal + splenic denervation abolished activation in the magnocellular PVN and the SON. In renal-denervated animals, SVO depressed Fos expression in the NTS and increased expression in the SFO, responses that were abolished by renal + splenic denervation. In intact rats, SVO also induced a fall in right atrial pressure, an increase in renal afferent nerve activity, and an increase in MAP. We conclude that elevated splenic venous pressure does induce hypothalamic activation and that this is mediated through both splenic and renal afferent nerves. However, in the absence of renal afferent input, SVO depressed NTS activation, probably as a result of the accompanying fall in cardiac preload and reduced afferent signaling from the cardiopulmonary receptors.

baroreflex; portal hypertension; kidney

There is increasing evidence that the spleen is intimately involved in maintaining cardiovascular homeostasis. Our recent finding that splenic baroreceptors control splenic afferent nerve activity (31) suggests a mechanism by which the spleen may act as a monitor of the splanchic circulation; these vascular beds play an important role in modulating both cardiac preload (by controlling splanchnic venous tone), and cardiac afterload (by controlling splanchnic arterial tone) (18, 26). Partial occlusion of the splenic vein is known to activate splenic afferent and renal sympathetic nerves through a spinal pathway, the splenorenal reflex (19, 20, 28, 31). We proposed that increased splenic venous pressure could also influence central pathways via neural afferent signals from the spleen and/or the kidney. Such pathways could contribute to the central cardiovascular dysregulation observed in portal hypertension, a condition characterized by splanchic venous congestion and, specifically, an increase in splenic venous outflow pressure (19, 45).

The present study was designed to investigate these possibilities by examining whether stimulation of splenic baroreceptors activates known cardiovascular regulatory centers in the brain and whether central neural activation under these circumstances is affected by prior renal and/or splenic denervation. We have focused on regions of the brain known to subserve autonomic and neuroendocrine regulation of the cardiovascular system: the nucleus of the tractus solitarius (NTS) in the brain stem, the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus, and the subfornical organ (SFO). The NTS is the primary relay station through which cardiovascular afferent inputs are received in the brain stem and subsequently transmitted to higher brain centers in the hypothalamus such as the PVN (11, 46). The NTS is thus an integral player in hypothalamic autonomic regulation and is known to respond to cardiovascular manipulations such as hemorrhage or infusions of vasoactive agents (11, 23, 46).

The PVN integrates a variety of inputs from autonomic centers in the spinal cord, brain stem, and forebrain (48) (Fig. 1). The PVN also receives input from the circumventricular organs, including the SFO and organum vasculosum of the lamina terminalis (OVLT) (Fig. 1); these structures lack a blood-brain barrier, and are thus directly affected by circulating messengers in the bloodstream, such as angiotensin II (29). The PVN is subdivided into parvocellular and magnocellular components, distinguishable on the basis of anatomical location and cellular content (47). Neurons located within the parvocellular PVN project to the median eminence, from where they may release a number of hormonal factors important in cardiovascular regulation (38, 47, 48, 50), and to the preganglionic autonomic neurons in the spinal cord (42). Neurons from the magnocellular division of the PVN project to the posterior pituitary and produce either oxytocin or vasopressin (12, 47). The SON receives input from the ascending pathways activated by cardiac and renal afferent nerves (13, 43), and it too controls secretion from the posterior pituitary.

Immunohistochemical detection of Fos, the protein product of the immediate-early gene c-fos, is an established method of identifying neurons that have been activated by physiological and pathophysiological stimuli (17, 32, 37, 41). The objective of this study was to determine the relative contribution of the spleen and kidney to central signaling during activation of the splenorenal reflex by partial SVO. We studied the pattern of Fos expression in pairs of control vs. splenic-vein occluded rats in three groups: intact; bilaterally renal-denervated (to remove renal afferent input, thereby isolating the effect of splenic afferent activity); and renal + splenic-denervated (to determine whether there was any nonneural contribution). Splenic venous...
METHODS

All experimental procedures were approved by the local Animal Welfare Committee according to the guidelines set by the Canada Council on Animal Care. Central neural activation (Fos protein expression) was measured in conscious rats previously prepared with splenic venous balloon occluders. The study comprised three experimental groups which were further divided into pairs of control and experimental subsets: 1) intact: nonoccluded controls (n = 6) and splenic vein-occluded rats (n = 5); 2) renal-denervated: nonoccluded controls (n = 5) and splenic vein-occluded rats (n = 6); and 3) renal + splenic-denervated: nonoccluded controls (n = 6) and splenic vein-occluded rats (n = 5). Right atrial pressure and renal afferent nerve activity were also measured in separate groups of anesthetized animals.

Animals and Housing

Male Long Evans rats were purchased from Charles River Canada (St. Foy, QC, Canada) and housed in the University Animal Facility in a temperature-controlled environment, exposed to light on a 12:12-h light-dark cycle, and maintained on a 0.3% sodium diet and water ad libitum.

Drugs

All surgical procedures were carried out aseptically under pentobarbital sodium anesthesia (62 mg/kg body wt ip; MTC Pharmaceuticals, Cambridge, ON, Canada). Temperature was maintained using Delta-phase heating pads (BrainTree Scientific, Braintree, MA). Following surgery, all animals were given an analgesic, buprenorphine hydrochloride (Buprenex injectable; 0.02 mg/kg body wt im; Reckitt and Colman Pharmaceutical, Richmond, VA), for postoperative pain management.

Surgery and Chronic Instrumentation: c-fos Experiments

A PA-C10 telemetric pressure transducer (0.4 mm OD; Data Sciences International, Arden Hills, MN) was inserted occlusively into the gastric vein, and its tip was advanced to the junction with the splenic vein for continuous measurement of splenic venous pressure in the conscious rat. During the in vivo experimental protocol, splenic venous pressure was transmitted to a signal receiver that was connected to a personal computer for online data acquisition using WINDAQ/Pro software (DATAQ Instruments, Akron, OH).

A vascular balloon occluder (VO-1.5N, model 18080–01; Fine Science Tools, North Vancouver, BC, Canada) was implanted and secured around the splenic vein between the gastric and hepatic portal vein. The actuating tube was passed through the abdominal muscle and exteriorized at the nape of the neck, providing a means for inflation and controlled partial occlusion of the splenic vein in the conscious animal.

Bilateral renal denervation and splenic denervation were carried out as previously described (1, 22). Briefly, the renal and/or splenic nerves were isolated and sectioned. After cutting away all visible nerve fibers in either case, a 10% phenol solution was painted on the area to ensure complete destruction of the nerves.

The abdominal aorta was cannulated nonocclusively with MicroRenathane tubing (model MRE-033; 0.36 mm ID, 0.84 mm OD; BrainTree Scientific, Braintree, MA), secured with a 6-0 Prolene suture in the adventitia of the aorta plus two 4-0 silk sutures in the adjacent psoas muscle, and exteriorized at the nape of the neck. During the in vivo experiment, the exteriorized aortic catheter was attached to a Statham pressure transducer (Gould Electronics, Recording Systems Division, Cleveland, OH) for online monitoring of MAP using WINDAQ/Pro software (DATAQ Instruments, Akron, OH).

Protocol: Chronic c-fos Experiments

Animals recovered in their own cages following surgery and on the 4th day were relocated to a metabolic cage for acclimatization to experimental conditions. The in vivo experiment was carried out on the 5th day. Conscious animals were allowed to stabilize for ~30 min, followed by a 30-min baseline recording of MAP and splenic venous pressure. In splenic vein-occluded animals, the balloon occluder was then inflated and clamped to increase and maintain splenic venous pressure at 20–24 mmHg. This value was chosen according to a previously established protocol for partial SVO, mimicking the effects of portal hypertension (19). Control (nonoccluded) animals received the same treatment with the exception of balloon inflation. After 1 h, rats received an anesthetic overdose of pentobarbital sodium (200 mg/kg body wt ip; MTC Pharmaceuticals, Cambridge, ON, Canada) and were prepared for fixation. It is customary when studying c-fos activation in the central nervous system to maintain the stimulus for 1 h to allow ample time for the Fos protein to be expressed (23).

Tissue Processing: c-fos Experiments

Animals were perfused transcardially with ~200 ml ice-cold 0.9% physiological saline followed by 500 ml ice-cold 4% paraformaldehyde in 0.1 M PBS (pH 7.2). Brain tissue was removed and postfixed in 1:1 solution of 30% sucrose and 4% paraformaldehyde.

Fig. 1. Schematic of a sagittal view of the brain illustrating the main nuclei and neural pathways involved in autonomic and neuroendocrine control of the cardiovascular system. Sensory input from cardiovascular and visceral receptors is received and relayed between the brain stem and hypothalamic cardiovascular regulatory centers, ultimately controlling sympathetic outflow to the periphery. Peripheral afferent nerves are shown as dashed lines. Sympathetic outflow tracts are shown as dotted lines. IML, intermediolateral cell column; NTS, nucleus of the tractus solitarius; PVN, paraventricular nucleus; SFO, subformical organ; OVLT, organum vasculosum of the lamina terminals; SON, suprachiasmatic nucleus.
for 1 h at 4°C. Brains were then transferred to 20% sucrose for cryoprotection overnight at 4°C. The following day, serial transverse sections (40 μm) of hypothalamus and brain stem were cut in a cryostat and placed free-floating in PBS (pH 7.2) for subsequent immunohistochemical processing.

**Immunohistochemistry: c-fos Experiments**

Fos staining was carried out using the Vectastain Elite ABC Kit (Rabbit IgG, model PK-6101; Vector Laboratories, Burlingame, CA). Floating sections were incubated in PBS-diluted Vectastain blocking serum (1.5% normal goat serum) for 30 min at room temperature. The tissue was rinsed and transferred to a 1:200 solution of primary antibody, anti-Fos protein [Ab-2], polyclonal rabbit IgG (cat. no. PC05, Calbiochem/EMD Biosciences, San Diego, CA), in 0.3% Triton X-100/PBS. Due to enhanced sensitivity of the Vectastain Elite ABC Kit permitting shortened primary antibody incubation times, sections were incubated in primary antibody for 1 h at room temperature with agitation. Punctuated with 10-min PBS rinses, tissues were incubated sequentially for 1 h at room temperature in PBS-diluted 1:200 Vectastain biotinylated secondary antibody solution (Biotinylated Goat Anti-Rabbit IgG) combined with Vectastain normal blocking serum, followed by PBS-diluted 1:100 Vectastain Elite ABC Reagent (avidin-biotin peroxidase complex). Sections were then washed in PBS and incubated in diaminobenzidine peroxidase for 10 min at room temperature to visualize Fos labeling as a brown reaction product. Sections were rinsed in PBS and mounted on glass microscope slides, air dried overnight, and protected with a coverslip.

**Quantification and Analysis: c-fos Experiments**

Neurons labeled for Fos protein were examined with light microscopy by counting labeled cell nuclei in the NTS, PVN, SON, and SFO manually (Fig. 2). All slides were analyzed together to decrease variability leading to counting error. Relevant hypothalamic and brain stem nuclei were identified in accordance with the atlas of Paxinos and Watson (36). Every other section was analyzed and counts from both hemispheres were combined for each section per animal. Data were then averaged for control and experimental groups to obtain the number of neurons per section positive for Fos. To aid in analysis, brain stem regions were objectively divided into three levels relative to the reference point of bregma: rostral (−11.60 mm), intermediate (−13.30 mm), and caudal (−14.60 mm). Since the intermediate-caudal regions of the NTS have previously been identified as critical for cardiovascular regulation (39), only sections between −14.30 and −12.80 mm were analyzed, for an average of 21 ± 2 sections per animal. The PVN was analyzed at approximately −1.80 mm, corresponding to an intermediate level (i.e., mid-PVN) which is divisible

![Fig. 2. Representative photomicrographs showing immunohistochemical labeling of Fos in neurally intact nonoccluded controls (A, C, and E) and splenic vein-occluded (B, D, and F) animals. The numbers of labeled neurons in the PVN and SON are shown to significantly increase, whereas those in the NTS decrease in response to splenic vein occlusion. Scale bar = 50 μm.](http://ajpregu.physiology.org/)
into two subregions consisting of magnocellular and parvocellular neurons. On average, 14 ± 1 sections were analyzed for Fos labeling in the PVN of each animal. For the SON, an average of 13 ± 1 sections per animal were analyzed at approximately −1.80 mm. For the SFO, an average of 6 ± 0.4 sections per animal were analyzed between −1.60 mm and −1.08 mm.

**Right Atrial Pressure**

Separate groups of animals were used for these experiments. Rats were anesthetized with pentobarbital sodium (62 mg/kg body wt ip), plus Inactin (80 mg/kg body wt, sc). Cannulae were placed in the femoral artery (polyethylene, 0.58 mm ID × 0.97 mm OD) and vein (Silastic, 0.51 mm ID × 0.94 mm OD; Dow Corning) for measurement of systemic blood pressure and infusion of isotonic saline (3 ml/h) respectively. The right atrium was cannulated (polyethylene, 0.58 mm ID × 0.97 mm OD) via the right jugular vein for measurement of right atrial pressure. The gastric vein was cannulated (Silastic, 0.30 mm Id × 0.64 mm OD; Dow Corning) for measurement of splenic venous pressure, and a snare (6.0 prolene; Ethicon) was placed loosely around the splenic vein at its junction with the hepatic portal vein; this allowed for controlled partial occlusion of the splenic vein (19). After 30-min stabilization, there was a 20-min (baseline) period after which the splenic venous snare was tightened until splenic venous pressure reached 22–24 mmHg. Splenic venous pressure and right atrial pressure were monitored continuously. Mean values were measured over two 10-min periods, starting 1 min after occlusion.

**Renal Afferent Nerve Activity**

Separate groups of rats were used for these experiments. The animals were anesthetized and prepared with femoral arterial and venous cannulae, a gastric vein cannula, and a splenic venous snare, as described above. The intestines were placed gently back into the abdominal cavity and retracted to the animal’s right side with moist gauze so as to expose the left renal blood vessels and create an abdominal “well,” which could be filled with heavy mineral oil (Laboratoire Atlas, Montreal, QC, Canada). A branch of the renal nerve was then isolated and cut proximally (i.e., away from the kidney) to allow for recording of afferent nerve activity. The ends of cut nerves were 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 personal computer (sampling rates 10 kHz; PowerLab, ADInstruments).

After a 30- to 35-min stabilization period, renal afferent nerve activity was recorded online for 20 min, after which the splenic venous ligature was tightened to elevate splenic venous pressure to ~20–24 mmHg. Nerve activity was recorded for a further 20 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) (19).

**Statistical Analysis**

Results are presented as means ± SE. In vivo blood pressure data were analyzed using Student’s t-test for paired data. Differences in numbers of labeled neurons between groups and variations in rostral-caudal distribution of labeled neurons were analyzed by two-way ANOVA. The Bonferroni t-test was used for post hoc analysis of multiple comparisons vs. controls. For all tests, statistical significance was accepted at P < 0.05.

**RESULTS**

**Hemodynamic Measurements**

Baseline blood pressure data represent the average of a 5-min period immediately preceding the occlusion stimulus. Occluded values represent the average of a 5-min period midway through the 1-h period of SVO.

Comparisons of baseline values (prior to vessel occlusion) revealed no significant differences between any of the experimental groups with respect to MAP or splenic venous pressure (Table 1). In addition, statistical analysis of control (nonoccluded) groups revealed no significant changes in MAP or splenic venous pressure from baseline values over time. For these reasons, results for blood pressure responses are presented as a change from baseline for all experimental groups.

**Splenic Venous Pressure: c-fos Experiments**

Inflation of the balloon occluder around the splenic vein significantly increased splenic venous pressure in all three experimental groups compared with baseline values (Fig. 3A). There were no significant differences among SVO groups with respect to baseline or occlusion values of splenic venous pressure (i.e., equivalent stimulus for splenic baroreceptor activation). No significant changes were observed for the three (nonoccluded) control groups with respect to splenic venous pressure.

**Mean Arterial Pressure and Heart Rate: c-fos Experiments**

Partial occlusion of the splenic vein in the conscious rat significantly increased MAP from baseline in the intact animals (115.7 ± 1.7 to 124.4 ± 2.6 mmHg; n = 5; P < 0.05; Fig. 3B). This was accompanied by an increase in heart rate (baseline experimental: 374 ± 14 beats/min; SVO: 421 ± 14 beats/min; n = 5, P < 0.05). There were no significant changes in MAP or heart rate for the intact time-controlled animals. Nor did MAP change in the renal-denervated or renal + splenic-denervated animals i.e., the increase in MAP observed with SVO in the intact animals was abolished by renal denervation alone, and by renal + splenic denervation.

**Right Atrial Pressure**

SVO invariably induced a fall in right atrial pressure (baseline: 3.2 ± 0.4 mmHg; 1st 10-min period of occlusion: 3.0 ± 0.4 mmHg; 2nd 10-min period of occlusion: 3.0 ± 0.4 mmHg; n = 9, P < 0.05).

**Renal Afferent Nerve Activity**

SVO induced a progressive increase in renal afferent nerve activity (baseline: 21.8 ± 3.3 spikes/s; 1st 10-min period of Table 1. Baseline values of splenic venous pressure and MAP from control and experimental intact, RD, and RSD animals

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>No.</th>
<th>SVP, mmHg</th>
<th>MAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>14.2 ± 2.0</td>
<td>105.1 ± 3.4</td>
</tr>
<tr>
<td>SVO</td>
<td>6</td>
<td>9.3 ± 1.4</td>
<td>115.7 ± 1.7</td>
</tr>
<tr>
<td>RD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>16.4 ± 1.4</td>
<td>100.6 ± 2.3</td>
</tr>
<tr>
<td>SVO</td>
<td>6</td>
<td>13.3 ± 2.1</td>
<td>105.9 ± 7.1</td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>10.4 ± 1.5</td>
<td>110.4 ± 4.4</td>
</tr>
<tr>
<td>SVO</td>
<td>4</td>
<td>9.8 ± 0.3</td>
<td>108.7 ± 7.1</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE. RD, renal-denervated; RSD, renal + splenic-denervated; Control, nonoccluded animals; SVO, splenic vein-occluded animals; MAP, mean arterial pressure; SVP, splenic venous pressure.
occlusion: 26.3 ± 4.5 spikes/s; 2nd 10-min period of occlusion: 27.5 ± 5.4 spikes/s; n = 8, P < 0.05).

Effect of Denervations on Numbers of Fos-Positive Neurons in Control Animals

NTS. Analysis of rostral-caudal distribution of labeled neurons in the NTS revealed no variation between levels (data not shown). Data are therefore presented as the number of labeled nuclei per section for the intermediate-caudal regions. There was no difference in renal-denervated controls compared with the intact group (Fig. 4). There was, however, a significantly reduced number of neurons labeled for Fos in renal + splenic-denervated controls compared with both the intact and renal-denervated groups (P < 0.05).

PVN. There were no differences between the control groups (intact, renal-denervated; renal + splenic-denervated) with regard to numbers of Fos-labeled neurons in the SON (Fig. 6A).

SON. There were no differences between the control groups (intact, renal-denervated; renal + splenic-denervated) with regard to numbers of Fos-labeled neurons in the SON (Fig. 6A).
SON. SVO induced a significant increase in the number of Fos-positive neurons in the SON of intact and in renal-denervated animals ($P < 0.05$; Fig. 6A). Denervation of the spleen plus kidney abolished this response.

SFO. SVO induced a significant reduction in the number of Fos-positive neurons in the SFO of intact animals, but an increase in the renal-denervated group (Fig. 6B). Denervation of the spleen plus kidney abolished this response.

**DISCUSSION**

We have previously demonstrated that partial occlusion of the portal or the splenic vein causes an immediate increase in splenic venous pressure, which is accompanied by an increase in splenic afferent nerve activity (19, 31). Enhanced splenic afferent nerve activity causes a reflex increase in renal sympathetic nerve activity, leading to a reduction in renal blood flow (5, 19, 20). Given that portal hypertension (caused by portal vein stenosis) is known to alter central Fos protein expression (45), we wished to investigate the role of the spleen and kidney in this central dysregulation. We found that partial occlusion of the splenic vein in the conscious rat does affect central neuronal activation and that this depends on the integrity of both renal and splenic innervation. Specifically, we attribute the central effects of SVO to increases in renal and splenic afferent signaling; the former would increase as a result of the increased renal sympathetic nerve activity (34) and the latter as a result of the increased splenic venous pressure (31).

With regard specifically to the current study of cardiovascular control, the PVN is known to receive input from at least three neural pathways: 1) direct connection from the SFO/OVLT mediated by angiotensinergic neurons (25, 40, 49); 2) baroreceptor-mediated pathway via activation of low-pressure or volume receptors, relayed through the brain stem (2–4); and 3) direct projection of renal afferents (7–9, 14, 15). In our experiments, the response of parvocellular PVN neurons to partial SVO was entirely dependent on intact renal nerves. On the other hand, enhanced activity of both the SON and the magnocellular PVN neurons persisted after renal denervation, but was abolished when splenic neural input was removed. We suggest that, whereas the SVO-induced activation of parvocellular PVN neurons originated solely from the kidney via afferent nerves to the NTS (44), the effects on the SON and magnocellular PVN neurons were dependent upon direct input from both renal (10) and splenic afferent nerves. This difference may reflect the functional differences of parvocellular PVN vs. SON and magnocellular PVN neurons; parvocellular PVN neurons exert their actions through ascending pathways to the anterior pituitary and descending pathways to the brain stem and spinal cord (48), whereas both SON and magnocellular PVN neurons project to the posterior pituitary, inducing release of vasopressin (48). We suggest that the spleen, as an accessory to the kidneys in blood volume regulation, contributes to homeostasis through a direct neural connection to the magnocellular region of the PVN and to the SON. Together, information from the spleen and kidneys would be integrated in the hypothalamus and subsequently translated into neurohormonal control of blood volume.

The increase in splenic afferent nerve activity caused by SVO failed to induce an increase in NTS Fos. These data suggest a lack of direct input from the splenic afferent nerves.
to the NTS, which is consistent with evidence that visceral afferents reach the brain stem primarily via the vagus (Xth cranial) nerve and that few, if any, of such vagal afferents innervate the spleen (33). On the other hand, the NTS is the site of termination of both renal and baroreceptor/volume afferent nerves (16, 35, 39). It therefore appears that NTS activity is predominantly influenced by renal and cardiac afferents, with negligible input from the splenic afferent fibers. In the renal denervated animals, when input from the NTS was presumably derived exclusively from the cardiac receptors, SVO decreased NTS activity. This is consistent with the fall in right atrial pressure which we observed during SVO. Although this fall was small, it must be recognized that, at these low pressures in such a compliant tissue as the atrium, a small fall in intraluminal pressure would reflect a pronounced change in atrial dimensions, and thus in atrial volume receptor afferent signal-

We propose that, in the intact animals where SVO did not alter NTS activation, the inhibitory influence of decreased atrial volume receptor afferent nerve activity was offset both by the increased input from the renal afferent nerves, and by the increase in MAP. In the renal denervated animals, there was no input from the renal afferent nerves. Nor was there any increase in MAP. The inhibitory effect of decreased atrial distension therefore caused a fall in NTS activation (Fig. 7).

The SFO, like the other circumventricular organs, is activated by circulating hormones, such as angiotensin II. We propose that the fall in activation that we observed in the intact animals during SVO was secondary to the rise in MAP. This would have reduced plasma renin activity and circulating angiotensin II, thus reducing SFO activation. In the renal denervated animals, we did not observe any increase in MAP. There would therefore have been no fall in circulating angiotensin II levels. We might thus have anticipated that there would be no change in SFO activation. However, there was increased Fos expression in the SFO of the renal denervated animals. Splenic (plus renal) denervation abolished the response. This suggests that the splenic afferent nerves may directly activate SFO neurons.

**Perspectives**

We observed a fall in right atrial pressure during SVO, suggesting that there was a reduction in venous return to the heart. This could arise from increased splanchic venous pooling (splanchnic venous dilation) and/or reduced splanchnic vascular conductance (splanchnic arterial constriction). Indeed, nonadrenergic noncholinergic pathways, such as nitricergic and CGRP-releasing nerves, are stimulated in the mesenteric vasculature in portal hypertension, and have been implicated in the profound splanchnic venulectasis typical of the condition (21, 30). We propose that these changes in mesenteric vascular tone are mediated through a splenointestinal reflex, by which an increase in splenic venous pressure increases splenic afferent nerve activity, and initiates a mesenteric (venous) vasodilation, a mesenteric arterial constriction, and a reduction in cardiac preload. The increase in splenic venous pressure secondary to increased portal venous pressure, may thus contribute to the central and peripheral cardiovascular dysfunction associated with portal hypertension by exacerbating splanchnic pooling and central underfilling (Hamza S and Kaufman S, unpublished observation).

We observed an SVO-induced increase in MAP in the conscious rats, which was dependent upon intact renal and splenic nerves. This could potentially have been attributable to increased plasma renin activity caused by the reflex increase in renal sympathetic nerve activity. However, such an increase in circulating angiotensin II would be reflected by an increase in SVO activation (27). Such was not the case. In the intact animals, Fos expression in the SFO was decreased. We propose that the increased MAP was sympathetically mediated. The significant increase in heart rate observed during SVO is consistent with such a hypothesis. Moreover, it is well established that increased renal afferent nerve activity can, through activation of centers in the posterior hypothalamus, induce an increase in sympathetic outflow and an increase in systemic blood pressure (6). Indeed, this mechanism is now believed to be responsible for the genesis of hypertension in chronic renal failure. As a follow up to these studies, it would be most valuable to measure the changes in global sympathetic outflow, total peripheral resistance, and plasma renin activity during SVO in conscious animals.

We conclude that increased splenic venous pressure does induce central neural activation, both directly via the splenic afferent nerves, and also indirectly via the renal afferent nerves. The latter are activated by an increase in renal sympathetic nerve activity mediated through the splanchnorenal reflex pathway (34). Our data suggest that, whereas there is a lack of direct input from the splenic afferent nerves to the NTS, these nerves do influence both magnocellular PVN and SON neural activation. Our findings also point to an indirect effect of SVO to depress neural activation of the NTS, secondary to an accompanying fall in right atrial pressure. In conclusion, we have shown that increased splenic venous outflow pressure, such as occurs during portal hypertension, initiates marked alterations in activation of the central neural pathways control-
ling cardiovascular regulation. We suggest that these perturbations contribute to the central cardiovascular dysregulation observed in portal hypertension.

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