Disordered central cardiovascular regulation in portal hypertensive and cirrhotic rats

DAISHENG SONG,1 KEITH A. SHARKEY,2 DEANNE R. BREITMAN,1 YIKUN ZHANG,1 AND SAMUEL S. LEE1
1Liver Unit, Gastrointestinal Research Group, Department of Medicine and 2Neuroscience Research Group, Department of Physiology and Biophysics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Received 10 December 1999; accepted in final form 2 October 2000

Disordered central cardiovascular regulation in portal hypertensive and cirrhotic rats. Am J Physiol Gastrointest Liver Physiol 280: G420–G430, 2001.—Portal hypertension due to either prehepatic portal hypertension or cirrhosis is associated with cardiovascular derangement. We aimed to delineate regulatory mechanisms in the brain stem cardiovascular nuclei in rat models of prehepatic portal hypertension and cirrhosis.

Neuronal activation in the nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM) were assessed by immunohistochemical staining for the immediate-early gene product Fos. In the same sections, catecholaminergic neurons were counted by tyrosine hydroxylase (TH) staining. Ninety minutes after hypotensive hemorrhage (or no volume challenge), the animals were killed for Fos and TH medullary staining. These protocols were repeated after capsaicin administration. The NTS of unchallenged sham-operated rats had scant Fos-positive cells (3.6 ± 0.4 cells/section), whereas hemorrhage significantly increased Fos staining (91.8 ± 14). In contrast, the unchallenged portal hypertensive and cirrhotic groups showed increased Fos staining (14.3 ± 5.8 and 32.8 ± 2.8, respectively), which hemorrhage did not alter significantly. The numbers of TH-positive cells were similar in the three unchallenged groups; double labeling revealed that ~50% of TH-positive cells were activated by hemorrhage in the sham and cirrhotic rats but not the portal hypertensive rats. Similar patterns of Fos and TH staining were observed in the VLM. Capsaicin treatment not only significantly reduced the Fos-positive neuron numbers in portal hypertensive and cirrhotic rats but also attenuated hemorrhage-induced Fos and double-positive cells in both NTS and VLM. These results suggest that disordered trafficking in capsaicin-sensitive nerves and central dysregulation contribute to blunted cardiovascular responsiveness in cirrhosis and prehepatic portal hypertension.

Hyperdynamic circulation; Fos; tyrosine hydroxylase; cirrhosis; nucleus of the solitary tract; ventrolateral medulla; capsaicin; sensory afferent nerves

CIRRHOSIS AND PORTAL VENOUS hypertension in humans and experimental animal models of liver disease are associated with cardiovascular disturbances. The circulation becomes hyperdynamic, characterized by increased cardiac output and decreased peripheral vascular resistance. Moreover, abnormal cardiovascular homeostasis is also manifested by blunted responsiveness of blood vessels to various vasoconstrictor and dilator influences and attenuated reflexes such as baroreflexes (7, 39). For example, in both cirrhosis and chronic portal venous hypertension, there is a decreased pressor sensitivity and impaired chronotropic responses to catecholamines (30, 42). In rats, superior mesenteric arterial blood flow is elevated, and the mesenteric constrictor response to an a-adrenoceptor agonist is blunted (20). Neural factors have been postulated to be involved in the genesis of this disturbed cardiovascular function, especially the sympathetic system, which shows indirect evidence of overactivity, as judged by elevated serum catecholamine levels (21).

However, the precise mechanisms underlying these cardiovascular anomalies remain unclear.

c-fos is an immediate-early gene that has important roles in cellular signal transduction and transcriptional regulation (15). Immunohistochemical detection of the c-fos protein product, Fos, has been used successfully as a metabolic marker to identify neuronal groups and trace neuronal pathways that have been activated by physiological and pathophysiological stimuli (16, 44).

The nucleus of the solitary tract (NTS) in the medulla of the brain stem is the primary site of termination of cardiovascular inputs from the periphery and has important roles in regulating cardiovascular function by controlling reflex activity such as baroreflexes and chemoreflexes (45, 47). Another important cardiovascular control area is found in the ventrolateral medulla (VLM), which regulates sympathetic activity through spinal projections onto preganglionic neurons (24, 47). Many studies have demonstrated that the NTS and VLM are highly responsive to hemorrhage and sustained administration of vasodilator or vasoconstrictor agents (3, 14, 23). Fos protein, used as an indicator of neuronal activation in the NTS and VLM,
significantly increases after activation of baroreceptors (17, 36, 38).

Our previous study showed that Fos expression in NTS and VLM was significantly increased, and the central response to volume challenge was blunted in cirrhotic rats (11). However, it is not clear if this phenomenon is due to portal hypertension per se or some effect of cirrhosis and hepatic insufficiency. Because both prehepatic portal hypertension and cirrhosis with portal hypertension are associated with a similar pattern of cardiovascular anomalies, including hyperdynamic circulation and blunted reflexes, we hypothesize that disturbed function of medullary cardiovascular nuclei is a critical pathogenic mechanism. Moreover, by examining tyrosine hydroxylase (TH) immunoreactivity, which stains central catecholaminergic neurons, we sought to clarify the role of medullary catecholaminergic mechanisms in the pathogenesis of circulatory abnormalities. In particular, dual staining for Fos and TH in the same neurons would allow us to determine the extent of catecholaminergic neuronal activation in response to a significant cardiovascular challenge, namely hemorrhage, in normal rats and those with portal hypertension.

Finally, because another of our previous studies had shown that lifelong denervation of the sensory afferent nerves by neonatal capsaicin treatment ablated the hyperdynamic circulation of portal hypertensive and cirrhotic rats (31), we wondered if the peripheral afferent signal to the brain stem nuclei was carried through these sensory afferent nerves. We therefore performed studies with capsaicin denervation in adult rats to test these ideas. Capsaicin administration in large doses to adult rats has been known to cause a selective dysfunction of afferent neurons with thin, mostly unmyelinated fibers (12).

We chose the following two rat models of portal hypertension: the chronic bile duct-ligated (BDL) rat, which exhibits portal hypertension but also biliary cirrhosis and hepatocellular insufficiency at 3–4 wk postligation, and the portal vein-stenosed (PVS) rat, which has a pure prehepatic portal hypertension without hepatic damage or dysfunction. The advantage of using these models is that the cardiovascular abnormalities have been well delineated in a large number of previous studies (9, 28, 29).

MATERIALS AND METHODS

Animals and treatment protocols. The protocol was approved by the University of Calgary Faculty of Medicine Animal Care Committee, and all animals received humane care in accordance with guidelines established by the Canadian Council on Animal Care. Male Sprague-Dawley rats were anesthetized with inhalational halothane, and through a midline abdominal incision the common bile duct or portal vein was ligated to induce cirrhosis or portal hypertension as previously described (29, 40). In brief, for BDL, the infrahepatic common bile duct was doubly ligated with 3-0 silk. The needle was then immediately withdrawn, leaving a graded stenosis of the portal vein. A control group of rats was subjected to sham operation, which included only the halothane anesthesia, abdominal incision, and gentle manipulation but not ligation of the bile duct or portal vein. In each rat, before the incision was sutured, 0.1 mg gentamicin was applied to the abdomen. After the operation, the rats were injected with 30,000 units of benzathine penicillin G intramuscularly. BDL rats also received 0.25 mg vitamin K<sub>1</sub> subcutaneously immediately after the operation and weekly thereafter until the time of study. Hemodynamic and brain stem staining studies were done 18–21 days after PVS, 24–28 days after BDL, and 18–25 days after sham operation.

Capsaicin treatment. Separate groups of normal adult rats weighing 150–200 g were treated with a total dose of 100 mg/kg capsaicin (Sigma), which was given in two injections (50 + 50 mg/kg sc) over two consecutive days. Control rats received equal volumes of vehicle (10% ethanol, 10% Tween 80, and 80% physiological saline, vol/vol/vol). After a recovery period of 7 days, the efficacy of capsaicin denervation was tested by administration of a drop of 0.33 mM capsaicin into one eye and then observation of any wiping movements. All rats with any wiping movements were excluded from further experiments. The animals without wiping movements were then subjected to one of the three surgeries described above (sham operation, BDL, or PVS). At the end of the postsurgical periods described above, just before the hemodynamic studies, continuing capsaicin denervation was tested again by the capsaicin eyedrop. All rats at this point demonstrated lack of wiping movements. Preliminary studies showed that vehicle treatment did not induce any discernible hemodynamic or brain stem immunohistochemical staining changes in sham, PVS, or BDL rats, so further studies were not done in these animals.

Hemodynamic measurements and tissue preparation. For the hemodynamic and brain stem staining studies, the animals were anesthetized with 50 mg/kg ip pentobarbital sodium (MTC Pharmaceuticals, Mississauga, ON). To avoid Fos expression due to stress, rats that struggled or squealed during the injection were removed from further study. The right femoral artery and vein were cannulated with PE-50 tubing (Becton-Dickinson, Parsippany, NJ). A few minutes before any incision, topical lidocaine 2% ointment (Astra Pharma, Mississauga, ON) was applied locally to avoid Fos expression due to pain. Blood pressure and heart rate were recorded from the femoral artery using a polygraph recorder (model 2400; Gould, Oxnard, CA), calibrated at each use with a pressure manometer. After obtaining baseline blood pressure, hemorrhage was induced by manually withdrawing 12 ml blood/kg body wt (estimated 20% of blood volume) at a rate of 2 ml/min in one-half of the rats from each group (n = 5 in each group). The other one-half of the rats (n = 5 in each group) were treated as unchallenged controls: their right femoral arteries and veins were cannulated, but no blood was withdrawn.

After 90 min, the rats were infused intravenously with an excess of pentobarbital sodium (30 mg/kg). This injection caused a cessation of respiration, but the heart continued beating. The rats were then perfused with 1 l/kg body wt of cold PBS at pH 7.4 followed by 1 l/kg of cold 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. The brain stems were carefully removed from the rats, postfixed overnight in 4% paraformaldehyde at 4°C, and then cryoprotected in 30% sucrose in 0.1 M phosphate buffer from 1 to 3 days at 4°C. Serial 30-μm sections of brain stem were made using a cryostat, and sections were analyzed for Fos and TH immunoreactivity using single- and double-labeling protocols. Ad-
jacent sections were used for cresyl violet staining to reveal the nuclear boundaries.

**Fos immunohistochemistry.** Sections were processed for the immunohistochemical detection of Fos using rabbit anti-Fos polyclonal antisera, directed against residues 7–14 of human Fos (Oncogene Science, Manhasset, NY). Sections were incubated in a blocking serum, consisting of 1.5% normal goat serum (Vector Laboratories, Burlingame, CA) diluted in PBS containing 0.4% Triton X-100 for 1 h at room temperature. The blocking serum was removed, and the sections were incubated with the primary antibody, rabbit anti-Fos polyclonal antisera diluted 1:20,000 with blocking serum, for 48–72 h at 4°C. Specificity was tested using liquid phase preabsorption. In this case, the diluted primary antibody was preabsorbed overnight (4°C) with the Fos peptide (100 µg/ml antibody), residues 7–14 (Oncogene Science), before being incubated as above. Sections were washed with PBS for 10 min at room temperature and then incubated with secondary antibody, biotinylated goat anti-rabbit IgG (1:200; Vector), for 30 min at room temperature. After the sections were washed in PBS for 10 min, they were incubated in Vectastain Elite ABC Reagent (Vector) for 30 min at room temperature. The sections were then washed in PBS for 10 min and incubated in diaminobenzidine-nickel peroxidase substrate (Vector) until the reaction was complete. The sections were then washed three times with PBS and mounted on chrome-alum (Sigma Chemicals, St. Louis, MO)-coated slides. The slides were left to air-dry overnight and were then dehydrated and covered with a coverslip.

**Immunofluorescence staining.** Fos immunoreactivity was detected using the rabbit anti-Fos polyclonal antisera. TH detection was performed with rabbit anti-TH antisera raised against purified bovine adrenal TH (Eugene Tech International, Ridgefield Park, NJ). Although both primary antibodies are raised in rabbits, double labeling was achieved by consecutive application of each antibody. Because Fos is restricted to the nucleus and TH to the cytoplasm, there is no possibility of overlap.

After being blocked in 1.5% normal goat serum (Vector) for 1 h at room temperature, the sections were subjected to sequential immunofluorescence labeling of Fos and TH. After first primary antibody incubation with anti-Fos for 48 h at 4°C, sections were washed three times in PBS and then incubated in cytomegalovirus 3-conjugated AffiniPure donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA), diluted 1:200 in PBS, for 60 min at room temperature. After PBS washing, the sections were incubated in the second primary antibody, rabbit anti-TH (1:500), for 48 h at 4°C. FITC-conjugated goat anti-rabbit IgG (Incstar, Stillwater, MN) was then used as above as the secondary antibody to visualize the TH immunoreactivity. After 60 min of washing at room temperature, sections were finally washed for 10 min three times in PBS containing 0.1% Triton X-100 and mounted on slides with phosphate-buffered glycerol.

**Histology.** For cresyl violet staining, sections were mounted on chrome-alum-coated slides and allowed to air-dry overnight. After being dehydrated in alcohol and stained with cresyl violet, the sections were finally covered with coverslips and Entellan. The result was a purple staining of the Nissl substance in the cytoplasm.

**Quantification.** The diaminobenzidine- and nickel-stained or immunofluorescent-labeled Fos cells were identified by their size, shape, and color, which were discernible from the background stain. The relevant brain stem nuclei were identified using the cresyl violet-stained sections in conjunction with the atlas of Paxinos and Watson (41). Meanwhile, double labeling with neuron-specific enolase and Fos was done in preliminary studies to rule out the possibility of Fos expression from neuroglial cells instead of neurons (36). Results showed that the majority of Fos-positive cells in this study were neurons. All slides were analyzed together to decrease the variability in counting error. Counting was done visually at ×200 magnification. Sections for analysis were taken between the appearance of the obex at interaural 5.30 mm and bregma 14.30 mm and before the appearance of the fourth ventricle at interaural 4.40 mm and bregma 13.40 mm; thus three sections were analyzed with 0.30 mm between each section. The counts used were the means of the three sections.

**Statistical analysis.** Results are expressed as means ± SE. Differences between groups were analyzed by nonparametric Kruskal-Wallis ANOVA, followed by Dunn’s post hoc test. P < 0.05 was considered significant.

**RESULTS**

**Hemodynamic measurements.** Both BDL and PVS groups showed lower baseline blood pressure compared with sham controls, but the differences were not statistically significant (data not shown). None of the unchallenged groups of rats displayed any significant change in mean arterial pressure or heart rate throughout the 90-min experiment (data not shown). The mean blood pressure dropped significantly in all three groups 3 min after hemorrhage and generally showed a similar pattern of recovery with near restoration of baseline pressures starting by 30 min after hemorrhage (Fig. 1A). The PVS group showed a slightly attenuated drop in blood pressure after hemorrhage compared with either the control or BDL groups. Capsaicin treatment attenuated the magnitude of hypotension in all three groups (no significant difference compared with baseline; Fig. 1B). There was no statistically significant change in heart rate among any hemorrhaged groups. However, BDL rats exhibited a slightly faster heart rate at baseline and maintained this relative bradycardia compared with the other groups (Fig. 1C). Capsaicin-treated BDL rats did not differ from the other groups in baseline heart rate or response to hemorrhage (Fig. 1D).

**Immunohistochemistry.** To aid interpretation of the photomicrographs (Figs. 2 and 3), a schematic representation of the brain stem nuclei and subnuclei is presented in Fig. 4. In preliminary studies, we observed no quantitative or qualitative differences between preparations single labeled for either Fos or TH or double labeled for Fos and TH. In some cases, Fos immunoreactive nuclei were observed “bleeding through” the TH labeling, but since TH labeling is cytoplasmic this did not pose a problem for interpretation. The pattern of Fos and TH labeling in the sham-operated control and hemorrhaged groups was found to be similar to that previously published in normal animals (3, 4, 14). Fos labeling was very sparse in unchallenged rats but increased after hemorrhage in the following three regions of the medulla: the area postrema, the NTS, and the VLM. In these studies, our interest was focused on the central autonomic regions of the NTS and VLM, which are also major cat-
echolaminergic nuclei (43, 45, 47). The pattern of TH labeling we observed in controls is very similar to that previously described in these regions (22).

NTS. Fos immunoreactive nuclei were sparsely distributed in sham-operated control rats (3.6 ± 0.4). After hemorrhage, there was a marked increase in Fos expression in the NTS (91.8 ± 14, P < 0.001; Figs. 2 and 5). This was predominantly located in the commissural, dorsal, and dorsolateral subnuclei and the lateral portions of the medial subnucleus of the NTS (Fig. 2) and was mostly absent from the other subnuclear regions such as the subnucleus gelatinosus, which receives predominantly gastric input, and the ventral regions of the NTS, which receive respiratory input.

Both portal-stenosed and cirrhotic rats showed increased basal Fos expression in NTS (14.2 ± 1.4 and 32.8 ± 2.8, both P < 0.05 vs. sham control), but hemorrhage did not further increase Fos staining (Figs. 2 and 5). The subnuclear distribution of Fos in the portal hypertensive animals was similar to that in sham-operated animals. Capsaicin treatment not only significantly reduced the responsiveness to hemorrhage in sham controls but also significantly attenuated baseline Fos expression in PVS and BDL rats (8.2 ± 2.2 and 8.0 ± 2.9, as shown in Figs. 3 and 5). Indeed, as immediately obvious from Fig. 5, capsaicin treatment eliminated all changes in Fos expression such that all six groups of animals (unchallenged and hemorrhaged sham rats, PVS and BDL groups) showed similarly low levels of Fos-staining cells.

There was an increased basal TH expression in BDL rats, but in no groups did hemorrhage alter the pattern of staining. After capsaicin treatment, the basal expression of TH was modestly reduced compared with untreated rats, but the differences did not reach significance (Fig. 6).

However, the dual-staining studies revealed interesting patterns (Fig. 7). The numbers of dual Fos- and TH-positive cells increased significantly, as expected, after hemorrhage in the sham-operated rats and also in the BDL rats. However, in the portal-stenosed rats, no significant change in the number of double-labeled cells was noted after hemorrhage. These patterns were the same both in terms of absolute numbers and also as a percentage of the TH cells that were also Fos labeled (percentage data not shown). Again, capsaicin treatment blunted hemorrhage-induced responses in both the sham and BDL groups and eliminated any differences between the six groups.

VLM. A virtually identical pattern of Fos distribution was found in the VLM (Figs. 5 and 8). Only a few Fos-positive cells were found in unchallenged sham controls (3.8 ± 0.6). Hemorrhage significantly increased Fos expression (47.4 ± 6.6, P < 0.001). Compared with unchallenged sham controls, significantly increased numbers of Fos-immunoreactive cells were found in unchallenged PVS and BDL groups (19.8 ± 4.5 and 12.5 ± 2.2, respectively, P < 0.05 vs. sham control). Again, Fos staining showed no significant change after hemorrhage in the PVS and BDL rats. In virtually identical fashion to the NTS, capsaicin significantly blunted hemorrhage-induced Fos expression in sham controls and attenuated Fos induction in both PVS and BDL groups (9.9 ± 2.3 and 7.5 ± 2.3, Fig. 5).

Total numbers of TH-positive cells in the three unchallenged groups and after hemorrhage, with and without capsaicin treatment, are shown in Fig. 6. In unchallenged rats, total TH immunoreactivity was sig-
significantly higher in the PVS group (21.4 ± 2.5) compared with the BDL group (7.7 ± 2.3, \(P < 0.05\)).

Similar to Fos expression, there were no significant changes in total TH counts in any group after hemorrhage before and after capsaicin treatment (data not shown). Double-labeling studies showed a significant increase in double-labeled cells only in the sham and BDL rats after hemorrhage but not in the PVS group (Fig. 7). A similar pattern to NTS was observed after capsaicin treatment: the hemorrhage response was blunted in both sham control and BDL groups.

**DISCUSSION**

Many previous studies have examined the issue of cardiovascular anomalies in portal hypertension and cirrhosis by focusing on the blood vessels. Vascular hyporesponsiveness to dilator and constrictor influences had been previously thought to be mediated by either a structural or local humoral abnormality in the blood vessel, such as overproduction of the vasodilator nitric oxide (8, 39). However, accumulating evidence has suggested that central nervous regulatory mecha-
nisms are abnormal in liver disease (2, 11, 32). For example, central sympathetic tone was suggested to be involved in the pathogenesis of portal hypertension, because the centrally acting sympatholytic drug clonidine reduced portal pressures in patients with cirrhosis (37). In BDL cirrhotic rats, evidence of deregarded sympathetic cardiovascular tone had been documented (26). More recently, a study in PVS rats showed that enzymatic activity of TH is significantly increased in rat brain homogenates (32). Our previous study also showed that Fos expression in the NTS and VLM was significantly increased, and neuronal responses to both hemorrhage and volume expansion were blunted in cirrhotic rats (11). The present study attempted to further elucidate the mechanisms underlying this blunted central response. Specifically, we tried to address three questions. 1) Are blunted central mechanisms associated with portal hypertension per se, or some aspect of hepatic failure? 2) What is the role of central sympathetic neuronal activation in each condition? 3) Are capsaicin-sensitive afferent neurons involved in triggering the central nervous system (CNS) activation?

By studying two models of portal hypertension before and after capsaicin treatment, with and without liver cirrhosis, we were able to show that Fos immunoreactivity as an indicator of neuronal activation is attenuated by portal hypertension per se. However, even though both experimental models share common circulatory disturbances, including portal hypertension and hyperdynamic circulation, it is clear that many aspects of the cardiovascular system, including
central neural regulation, are markedly different between these two models. In the present study, the total TH and double-labeling studies showed a significantly different pattern of central regulation between the two animal models. The number of baseline total TH-immunopositive neurons in the BDL rats was higher in the NTS and lower in the VLM, whereas there were no significant differences in the PVS rats. The exact significance of the divergent baseline TH immunoreactivity in the cirrhotic NTS and VLM remains unclear. However, this discrepancy probably reflects the different roles of the NTS and VLM in cardiovascular regulation. The NTS, especially the dorsal and medial areas, is the central projection site of primary baroreceptors and plays a key role in mediating and regulating hemodynamic function. In addition to the peripheral afferent inputs, NTS neurons also receive inputs from other brain nuclei, suggesting that the NTS critically shapes afferent baroreceptor input to CNS and the performance characteristics of the overall reflex (47). The VLM is the major vasomotor area that projects directly to the spinal cord. Compared with the NTS, it has an important role in the direct regulation of sympathetic activity and cardiovascular responses (19, 34, 45–48). In summary, the NTS can be considered primarily an afferent integrative center for cardiovascular input, whereas the VLM is an efferent output center. After capsaicin treatment, baseline expression of TH was reduced to a modest extent, but these changes did not reach statistical significance.

In no group did total TH activity change within the 90-min time frame of these studies, even with a marked sympathetic stimulus such as severe hypotension. The explanation is that TH mRNA requires several hours in neurons to be expressed as protein, and our protocol was designed to examine the peak of Fos expression that occurs in <2 h. Thus, by means of double labeling, we would be able to determine the extent of activation of the catecholaminergic neurons containing TH, in response to a sympathetic stimulus. To our knowledge, this is the first study to directly
examine central sympathetic neural activation in either cirrhosis or portal hypertension.

The double-labeling studies showed that, as expected, sympathetic neurons in the cardiovascular control centers become activated in sham-operated control rats with a volume challenge. In the cirrhotic rats, although total Fos-immunoreactive responses were indeed blunted, the extent of activation of TH neurons in the NTS and VLM appeared intact. This of course suggests that attenuated responsiveness of other types of neurons in the NTS and VLM contributes to the overall blunted central cardiovascular regulation. In contrast, the PVS rats showed no activation of TH-immunoreactive neurons after hemorrhage in either the VLM or NTS. These disparate results in the two experimental models accord well with a previous study by Abergel and colleagues (1), who found that, after removal of central sympathetic tone by spinal pithing, the entire hyperdynamic circulation in PVS rats is eliminated, whereas BDL rats continue to show some degree of hyperdynamic circulation. In other words, the circulatory derangement in BDL rats is only partially dependent on central sympathetic tone, and other neural or local factors play a role, whereas, in the portal-stenosed rat, hyperdynamic circulation is wholly dependent on sympathetic neural influence. Similar to its effects on Fos expression, capsaicin treatment attenuated dual-labeled neurons in sham controls in both NTS and VLM regions. Furthermore, this treatment also blunted the hemorrhage response in BDL groups, indicating that intact capsaicin-sensitive innervation is essential for neuronal activation in the CNS after hemorrhage.

What is the mechanism by which portal hypertension could induce neuronal activation in the medullary nuclei? Several possibilities may be entertained. Hepatic venous congestion was shown two decades ago by Kostreva et al. (27) to affect cardiovascular reflexes. They demonstrated that vena caval constriction activates hepatic afferent nerves with resultant reflex increases in renal and cardiopulmonary sympathetic efferent activity (27). This theory, while attractive, would not explain the current results, as our rats with prehepatic portal hypertension do not have hepatic venous congestion.

Another possibility is that certain humoral factors may increase in animals with portosystemic shunting, such as these portal hypertensive and cirrhotic rats. Substances such as nitric oxide or ammonia are known to have CNS effects. The latter can induce changes in extracellular brain glutamate levels and thus activate Fos (19). From the current results, we cannot definitively comment on a possible role of any such humoral factors in central activation.

In view of the present results and our previous studies with capsaicin, we believe that a more likely mechanism is via mesenteric venous congestion or distention, which is known to stimulate visceral afferent nervous activity (25). The increased afferent signals would then be processed and integrated in the NTS and VLM, and spinal efferent signals would induce vasoactive changes. In this regard, a study that we performed a few years ago may have some bearing. We found that neonatal denervation of the primary afferent nerves with capsaicin blocked the later development of the hyperdynamic circulation in adult life when the portal vein stenosis or bile duct ligation was done in these rats (31). Because of these observations, we examined the effects of capsaicin denervation in adult rats in the present study. The present results clearly demonstrated the dramatic effect of capsaicin in completely eliminating not only the central activation to hemorrhage in normal rats but also the baseline central Fos activation in PVS and BDL rats. In other words, capsaicin treatment rendered the central neuronal patterns identical in all three groups (controls, PVS, and BDL). This observation, along with our previous study, indicates that intact primary afferent innervation is prerequisite for the development or maintenance of hyperdynamic circulation and central neuronal activation, thereby strongly suggesting that the peripheral signal to the medullary cardiovascular regulation in liver disease might be mediated by sympathetic nervous system.
control nuclei is carried by primary afferent nerves. The present results also identified the likely mechanism whereby neonatal capsaicin treatment in our previous study eliminated the hyperdynamic circulation in portal hypertensive and cirrhotic rats. As we had speculated in a previous report (31), interruption of the peripheral-to-central afferent signaling, presumably from the gut/liver to the medullary nuclei, clearly results in elimination of the increased central neuronal activation and consequent normalization of the deranged hemodynamics.

We believe that our present capsaicin data are valid as they are remarkably internally consistent, showing reduction across all nuclei and groups. Moreover, the results in the sham-operated controls are entirely consistent with previous studies demonstrating that baroreflexes and other cardiovascular reflexes are blunted with capsaicin treatment (10, 33). Indeed, the Fos results in the capsaicin-treated sham controls showing complete abrogation of neuronal activation in the NTS and VLM in response to hemorrhage offer mechanistic insight into the genesis of this phenome-
non. It seems that capsaicin-induced interruption of the afferent arm of the reflex arc results in elimination of any signal to be processed in the medullary cardiovascular nuclei and thus no significant baroreflex. This finding, while novel, is hardly surprising given what is already known about capsaicin effects on primary afferent nerves.

It is known that Fos expression can be induced by many stimuli, including stress (13, 17). To reduce this possibility, we eliminated any rats that had struggled or squealed during the anesthetic injection from further study. The lack of Fos expression in both NTS and VLM regions of the sham controls is strong evidence that we were successful in this regard. Another limitation of this work may come from the use of pentobarbital sodium anesthesia, which can suppress heart rate and blood pressure (5, 6, 28, 29). To avoid Fos expression due to pain, the level of anesthesia was necessarily relatively deep, and this likely explains the low blood pressures at baseline in all three groups, as well as the lack of any detectable change in heart rates after the significant hypotensive challenge. However, we are confident that the level of anesthesia did not affect the patterns of neuronal activation in our study because the control animals responded, as expected, with significant increases in Fos and dual TH and Fos activation in response to the hypotensive stimulus.

In conclusion, we detected increased Fos immunoreactive cells and a blunted Fos response to hemorrhage in both prehepatic portal hypertension and cirrhosis, suggesting central involvement in the cardiovascular disturbance in these models. However, the pattern of central sympathetic activation differed between portal hypertensive and cirrhotic rats, suggesting that sympathetic regulation is generally intact in the latter and attenuated in the former. Finally, the capsaicin results suggest that the peripheral signal to initiate central activation is carried through primary afferent nerves.

This work was funded by research operating grants from the Medical Research Council of Canada. D. R. Breitman was supported by a Summer Studentship award from the Alberta Heritage Foundation for Medical Research (AHFMR). K. A. Sharkey and S. S. Lee are AHFMR Senior Scholars.

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