Decompensated hemorrhage activates serotonergic neurons in the subependymal parapyramidal region of the rat medulla

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The rapid loss of blood triggers two successive phases of autonomic responses. Initially arterial pressure (AP) is maintained, principally by an increase in sympathetic outflow to the heart and blood vessels. Beyond 30% blood loss, a second phase (decompensated stage, stage II of hemorrhage) is elicited (21, 49). During that stage, sympathetic tone to most organs except the adrenal medulla is reduced, and the heart rate falls (11, 55). The fall in heart rate combined with hemorrhage-induced sympathoinhibition (HISI) triggers a rapid fall in blood pressure (8). The second stage of hypotensive hemorrhage also heralds a marked reduction in oxygen consumption and a fall in core temperature (25).

The neurophysiological mechanisms underlying HISI are poorly understood. The activation of atrial or ventricular receptors contributes to decompensation (40, 49). Indeed a similar pattern of autonomic responses can be produced without blood loss by artificially reducing venous return (simulated hemorrhage; Ref. 19). However, vagotomization delays rather than abolishes the decompensation phase in conscious rabbits subjected to simulated hemorrhage, suggesting that vagal afferent traffic may not be the only trigger (20).

The cardiac output threshold for phase II induction is elevated by factors that increase baseline sympathetic nerve activity such as reduced blood PO2 and various drugs such as α2-adrenergic antagonists (7, 17).

The rostral ventrolateral medulla (RVLM) probably plays a role in HISI because the resting discharge of many sympathetic neurons is reduced by hypotensive hemorrhage in anesthetized rats (46). Because the activity of RVLM sympathetic neurons is controlled by an extensive central nervous system (CNS) network, the root cause of their inhibition during hypotensive hemorrhage could lie elsewhere within the multiple components of this network. In fact, the contribution of suprapontine regions to HISI, notably the periaqueductal gray matter, has long been suspected (21, 23, 24).

It is probable that many CNS transmitters contribute to HISI although most studies have focused on serotonin and opioids. The notion that serotonin release contributes to HISI originates from the fact that decompensation is delayed in animals treated with a serotonin synthesis inhibitor or with the broad spectrum serotonin receptor antagonist methysergide (18, 36, 48). Further work has suggested that the most critical serotonergic receptors are of the 5-hydroxytryptamine (HT1A) variety (48) and that activation of 5-HT1A receptors specifically within the RVLM makes a notable contribution to HISI (13). However, pharmacological evidence also suggests that the release of opioid peptides in the spinal cord or elsewhere in the CNS contributes to HISI (2, 19, 32).

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The present experiments were designed to test whether HISI is associated with the activation of enkephalinergic, GABAergic, or serotoninergic neurons in the region of the rostral medulla of the rat. Stage II hemorrhage was produced in conscious rats, and neuronal activation was gauged by the presence of Fos-related antigens (3, 12, 14, 45). To assess whether neuronal activation was associated specifically with hypotensive hemorrhage rather than with hypotension alone, the hemorrhaged animals were compared with rats treated with the arterial vasodilator hydralazine. Hydralazine causes normovolemic hypotension and a persistent baroreceptor-mediated activation of the sympathetic vasomotor tone.

MATERIALS AND METHODS

Physiological procedures. Experiments were performed on male Sprague-Dawley rats (Hilltop Laboratories, Scottsdale, PA) weighing 250–350 g. On delivery to the animal care facility, the rats were exposed to a 12:12-h light-dark cycle and given free access to food and water. They were allowed to acclimatize to these conditions for at least 48 h. All experiments were designed in compliance with National Institutes of Health and Institutional Animal Care and Use committee guidelines. The University of Virginia Animal Research Committee approved all protocols and procedures.

Rats were anesthetized with halothane (5% in 100% O2 for induction and 1.8% during surgery) for catheter implantation into the right femoral artery and femoral vein as described previously (52). The catheters were threaded subcutaneously to exit the upper back through a tethering device. An antibiotic (ampicillin, 125 mg/kg im, Bristol-Myers Squibb, Princeton, NJ) and an analgesic (ketorolac, 0.5–0.75 mg/kg ip, Abbott Labs, N. Chicago, IL) were then administered. The next day, rats were subjected to hypotensive hemorrhage (hemorrhage group), received hydralazine intravenously (hydralazine group), or received no further treatment (control group). Each physiological experiment was done using two rats each belonging to a different group. The composition of the pairs was randomized, and the process was repeated until all animals were used. Baseline blood pressure and heart rate measurements were recorded for at least 10 min before experimental treatment.

The hemorrhage group (n = 6) was subjected to a 40% blood withdrawal performed through the arterial line over 3 min. Total blood volume was estimated at 60 ml/kg (56). Further removal of blood, up to 50% of total blood volume, in increments of 0.5 ml was performed to keep blood pressure from rising to baseline levels. The hypotension group (n = 6) received 10 mg/kg of the arterial vasodilator hydralazine (hemorrhage group), or received no further treatment (control group). Each physiological experiment was done using two rats each belonging to a different group. The composition of the pairs was randomized, and the process was repeated until all animals were used. Baseline blood pressure and heart rate measurements were recorded for at least 10 min before experimental treatment.

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For each experiment, at least three additional rats from the same anesthetized group were used to verify that the physiological changes (blood withdrawals) caused by hydralazine were similar to those observed in the hemorrhage group. A total of 10 rats was used per treatment group. The animals were deeply anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip). They were perfused transcardially with 200 ml of 0.9% sodium phosphate-buffered saline (pH 7.4) followed by 500 ml of 4% paraformaldehyde solution in 100 mM sodium phosphate buffer (pH 7.4). Brains were postfixed in paraformaldehyde solution at −4°C for 48 h. The brains were then cut into coronal 30-μm sections on a vibratome microtome and stored in a solution of cryoprotectant (30% RNase-free glycerol, 40% ethylene glycol in 100 mM sodium phosphate buffer, pH 7.4) at −20°C for up to 10 days awaiting histological processing.

Preparation of digoxigenin-labeled RNA probes for histological detection of GAD67 mRNA and preproenkephalin mRNA by in situ hybridization. In situ hybridization was performed using digoxigenin-labeled cRNA probes prepared as described previously (51, 52). The GAD67 riboprobe was transcribed from a 1,132-bp DNA template inserted into the EcoRI site of Bluescript SK+ (Stratagene) (52). The GAD67 riboprobe was transcribed from a 0.75 mg/kg ip, Abbott Labs, N. Chicago, IL) were then administered. The next day, rats were subjected to hypotensive hemorrhage (hemorrhage group), received hydralazine intravenously (hydralazine group), or received no further treatment (control group). Each physiological experiment was done using two rats each belonging to a different group. The composition of the pairs was randomized, and the process was repeated until all animals were used. Baseline blood pressure and heart rate measurements were recorded for at least 10 min before experimental treatment.

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Fig. 1. Anatomic definition of the rostral ventrolateral medulla (RVLM) and subependymal parapyramidal nucleus (SEPPN). Schematic representation of the 3 coronal sections selected for quantitative analysis of Fos-immunoreactive (ir) neurons. The numbers on the right of each section refer to distances (in mm) from bregma according to reference atlas (41). The RVLM region is outlined on left. The SEPPN lies between the base of the brain and the line indicated by the arrowhead. IO, inferior olive; FN, facial nucleus; Amb, nucleus ambiguous; pyr, pyramidal tract; Sol, nucleus of the solitary tract.

Table 1. Phenotype of Fos-ir neurons in the RVLM

<table>
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<th>Hemorrhage</th>
<th>Hydralazine</th>
<th>Controls</th>
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<tr>
<td>Fos-ir (total)</td>
<td>34.5 ± 5.8</td>
<td>47.8 ± 4.0</td>
<td>4.0 ± 0.7†</td>
</tr>
<tr>
<td>Fos-ir/GAD67+</td>
<td>12.5 ± 3.5</td>
<td>15.4 ± 4.2</td>
<td>ND</td>
</tr>
<tr>
<td>Fos-ir/no GAD67</td>
<td>22.0 ± 3.0*</td>
<td>32.4 ± 1.5</td>
<td>ND</td>
</tr>
<tr>
<td>GAD67+/total</td>
<td>285 ± 13</td>
<td>282 ± 19</td>
<td>ND</td>
</tr>
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Values are expressed as mean number of cells per hemisection (± SE); n = 6 for each group. Cell counts were obtained from the rostral ventrolateral medulla (RVLM) region as defined in Fig. 1. Cells present in the subependymal parapyramidal nucleus (SEPPN) were excluded. Bilateral counts were obtained from 3 sections (Fig. 1), generating a single mean number of cells per hemisection per rat. Fos-ir, Fos immunoreactivity; GAD67, GAD67 mRNA. *Significant difference from hydralazine group (t-test). †Significantly different from both experimental groups (1-way ANOVA). ND, not determined.

Table 2. Phenotype of Fos-ir neurons within the SEPPN

<table>
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<th>Hemorrhage</th>
<th>Hydralazine</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fos-ir (total)</td>
<td>18.9 ± 3.1†</td>
<td>3.1 ± 1.2†</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Fos-ir/TryptOH-ir</td>
<td>7.2 ± 0.7*</td>
<td>0.5 ± 0.2</td>
<td>none</td>
</tr>
</tbody>
</table>

Values are means ± SE of cells per hemisection and are derived from bilateral counts obtained from the 3 specific sections defined in Fig. 1. Cell counts were obtained from both sides of the SEPPN region as defined in Fig. 1. Sections from rats subjected to hemorrhage (n = 6) or hydralazine (n = 5) were processed to reveal Fos-ir, tryptophan hydroxylase immunoreactivity (TryptOH-ir), and either GAD67 mRNA or preproenkephalin (PPE) mRNA. In control brains, no determination was made because the results of set 1 indicated virtual absence of Fos staining. The set numbers (1–3) refer to staining protocols performed on separate one-in-six sets of tissue sections from the same animals. †Significant difference from hydralazine group; †Significant difference from control group.

Results

Effect of hemorrhage or hydralazine on blood pressure and heart rate. The first episode of bleeding (removal of an estimated 40% of total blood volume) caused a profound hypotension accompanied by severe bradycardia (Fig. 2A). In most cases including the example shown in Fig. 2A, AP and heart rate began to recover toward baseline levels during the first 5 min. In such cases, rats were kept hypotensive by the additional removal of small amounts of blood (up to three
0.5-ml aliquots). Total blood withdrawal never exceeded 50% of the total estimated blood volume (60 ml/kg; Ref. 56). Each additional bleeding caused a recurrence of the hypotension and bradycardia (Fig. 2A). Injection of hydralazine produced hypotension and tachycardia that were sustained during the entire 2-h period (representative example in Fig. 2B). A parallel statistical analysis of the heart rate values was conducted (1-way ANOVA on ranks) on all time points between −10 min and 2 h (Fig. 3B). All three groups were statistically different. Therefore the bradycardia observed in the hemorrhaged group was statistically significant despite its transient nature. Moreover, throughout the experiment, the heart rate of the hemorrhaged rats was significantly lower than that of the hydralazine-treated rats at all time points despite the fact that blood pressure was generally similar in these two groups.

Expression of Fos-related antigens in the RVLM. Consistent with prior results obtained with the same experimental protocol (46), very few Fos-ir neurons were found in the RVLM of control rats (Table 1). In contrast, 8 to 12 times as many Fos-ir neurons were present in the RVLM of rats subjected to either hemorrhage or hydralazine (Fig. 4, Table 1). Although a very small proportion of all GAD67-labeled neurons was Fos-ir (5%), these cells represented about one-third of all Fos-ir neurons present in the RVLM (Table 1). The number of GABAergic cells expressing Fos after

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**Fig. 2.** Effect of hemorrhage or hydralazine on arterial pressure (AP) and heart rate (HR). Representative examples of a rat subjected to severe hemorrhage (A) and of a rat treated with hydralazine (B). Only the 1st h of recording after the onset of the stimulus (hemorrhage or hydralazine) is shown. Dots above traces indicate times when measurements were made to generate the group data shown in Fig. 3. bpm, Beats/min.

**Fig. 3.** Effect of hemorrhage or hydralazine on AP (A) and HR (B): group data. Each group consisted of 6 rats. Hydralazine infusion or hemorrhage was initiated at time zero. The AP of the control group was significantly different from that of both experimental groups. The AP of the hemorrhage and hydralazine groups were the same except for the first 10 min. The HR of all 3 groups was significantly different. For details on statistical analysis, see RESULTS.
hemorrhage or hydralazine was much greater than the total number of Fos-ir neurons present in the RVLM of control rats, but there was no difference between the two experimental groups. The total number of Fos-ir cells was 30% lower in rats subjected to hemorrhage than in hydralazine-treated rats, but this difference was not statistically significant (Table 1). However, after the Fos-ir neurons with GAD67 mRNA were subtracted, the number of Fos-ir neurons without GAD67 mRNA was significantly higher in hydralazine-treated rats than in hemorrhaged rats (Table 1). To assess the consistency of the counting procedure, we also determined the total number of RVLM neurons that contained GAD67 mRNA. These numbers were the same in both treatment groups (Table 1).

Thus both hypotensive hemorrhage and hypotension cause Fos expression in some GABAergic neurons of the RVLM, but the numbers of activated neurons were the same in both groups. In contrast, hydralazine-induced hypotension produces more Fos expression in non-GABAergic RVLM neurons compared with rats subjected to hemorrhage, suggesting a greater activation of potentially excitatory neurons in the RVLM.

Expression of Fos-related antigens by neurons of the SEPPN. The SEPPN of the hemorrhage group contained large numbers of Fos-ir nuclei whereas Fos immunoreactivity was virtually absent from this region in the hypotension and control groups (Fig. 4, Table 2). As expected, the SEPPN (both the core and lateral wings) contained many serotonergic neurons (examples in Fig. 5, A2, B2, and C2). In the hemorrhage group a large fraction of the Fos-ir nuclei were in serotonergic neurons (40%; Fig. 5, Table 2, set 1).

A second series of sections from the same rats (set 2) was used to detect GAD67 mRNA in addition to Fos and tryptophan hydroxylase immunoreactivities. Control rats were not examined because an absence of Fos immunoreactivity in the SEPPN was observed in set 1 (Table 2). The SEPPN of hemorrhage group contained Fos-ir neurons that expressed GAD67 mRNA, and some of these neurons were serotonergic (example in Fig. 6, A–C, and Table 2). Counts of Fos-ir neurons in the hypotension group were low but also contained a few double-labeled neurons (Fos+/GAD67+ and Fos+/GAD67+/tryptophan hydroxylase+; Table 2, set 2).

In a third series of sections from the same hemorrhage and hypotension groups (set 3), we detected PPE mRNA in addition to Fos and tryptophan hydroxylase...
immunoreactivities (Table 2). The SEPPN of the hemorrhage group contained Fos-ir neurons that expressed PPE mRNA, and some of these neurons were serotonergic (Fig. 6, D–F, and Table 2).

**DISCUSSION**

The present study contains two findings that shed new light on the neural networks that may be selectively engaged by severe hypotensive hemorrhage. First, despite a comparable severity of hypotension, somewhat fewer RVLM neurons with presumed sympathoexcitatory function expressed Fos-related antigens after hypotensive hemorrhage than after isovolemic hypotension. One interpretation of the data is that HISI could be due to the inhibition of a fraction of the RVLM presympathetic neurons. Second, in contrast to normovolemic hypotension, hypotensive hemorrhage activated a group of subependymal parapyramidal cells that release serotonin as well as inhibitory transmitters (enkephalins, GABA). The discussion considers whether the activation of SEPPN neurons could also contribute to HISI or whether this nucleus mediates reflex thermogenic responses to hemorrhage-induced hypothermia.

**Methodological considerations.** The limitations inherent in the Fos methodology have been abundantly discussed by others (12). However, two caveats must be reiterated. First, Fos-ir was detected at a single time point of 2 h after the start of the stimulus. Although this optimal interval was based on prior data (12, 52), the absolute or relative numbers of cells of different types could be affected by selecting a different endpoint. Second, results can also be affected by the type of Fos antibody used. In the present case we used a broad-spectrum antibody that also recognizes Fos B, Fra-1 and Fra-2.

The limitations of the physiological preparation must also be acknowledged. Sympathetic nerve recordings were not made. The only objective criteria that allowed us to suggest that the decompensated phase of hemorrhage and, presumably, HISI had been produced was the initial bradycardia and, at later time points, the fact that heart rate was at or slightly below the prehemorrhage level (Figs. 2 and 3). Another limitation of our protocol is that we cannot precisely determine the duration of the inferred HISI. The limited duration of the bradycardia (Fig. 3) suggests that, in contrast to the hypotension itself, HISI may not be sustained, or at least not with the same intensity, during the full 2 h of the experiments. If so, this would tend to attenuate the differences in Fos expression between the hemorrhaged rats and those subjected to isovolemic hypotension.

**Hypotensive hemorrhage and Fos activation in the RVLM.** Several prior studies have demonstrated that hypotension and hemorrhage cause Fos expression in RVLM neurons, and the present results concur (3, 5, 12, 14, 34, 54). However, other investigators have not examined animals that exhibited sustained bradycardia and long-lasting hypotension, the minimal criteria suggesting that the decompensated stage of hemorrhage may have been reached. In these studies, hemorrhage was more limited in scope (up to 16 ml/kg compared with 24–30 ml/kg in our study), and hypotension, when present, was only of very short duration (3, 5, 12, 14, 34).

Previous investigators have also shown that a large fraction of RVLM neurons that express Fos-related antigens after hypovolemic hypotension or moderate hemorrhage are C1 cells (immunoreactive for tyrosine hydroxylase or phenylethanolamine N-methyltransferase; Refs. 12, 29, 54). Accordingly, we found that, at
the RVLM level investigated, the majority of the Fos-expressing neurons did not contain GAD<sub>67</sub> mRNA, a diagnostic marker of GABAergic somata that is absent from C1 cells (51). Because the number of GABAergic cells that expressed Fos was the same after hydralazine or hemorrhage, these cells are most likely activated by the common denominator to these two stimuli, namely baroreceptor unloading. Very few if any GAD<sub>67</sub> mRNA-containing RVLM neurons project to the thoracic spinal cord (51), suggesting that these RVLM GABAergic neurons are interneurons.

Interestingly, the number of non-GABAergic Fos-ir neurons present within the RVLM was significantly lower in the hemorrhaged rats than in the hydralazine-treated group. A majority of these non-GABAergic neurons must be C1 presympathetic cells (12, 22) given that GAD<sub>67</sub> mRNA is absent from RVLM catecholaminergic neurons (51). At the brain level that we investigated (bregma −11.6 to bregma −12.0 mm) virtually all tyrosine hydroxylase-immunoreactive neurons are C1 cells (42), and ~90% of these cells are bulbospinal and presympathetic (47). Therefore the present study indicates that fewer RVLM presympathetic vasomotor neurons were activated in the rats subjected to decompensated hemorrhage than in the hydralazine-treated ones. This observation must be interpreted cautiously, however.

At face value the results seem congruent with prior evidence that the discharge rate of many RVLM vasomotor presympathetic neurons decreases in anesthetized rats that exhibit HISI (46). In anesthetized rats every RVLM vasomotor presympathetic neuron is not inhibited, consistent with the fact that HISI is not uniform either. For example, adrenal catecholamine release remains very elevated during decompensated hemorrhage (55). Since the RVLM is a major source of presympathetic neurons controlling the adrenal medulla (53), these particular presympathetic neurons should be vigorously activated and thus should express Fos after decompensated hemorrhage. The RVLM presympathetic neurons that expressed Fos after decompensated hemorrhage in the present experiments may control the adrenal medulla and, possibly, other sympathetic efferents not subject to inhibition during decompensated hemorrhage. Alternately, the activation of these neurons may be triggered by the brief initial sympathoactivation that usually precedes HISI.

However, the lower number of Fos-ir neurons present in the RVLM of the rats subjected to hypotensive hemorrhage could also be related to the different hypotensive profiles of the hemorrhage and hydralazine groups (Fig. 1). AUC measurements revealed that the hemorrhaged rats experienced somewhat less hypotension over the course of the 2 h preceding euthanasia. Although the difference in AUC value was not significant, the greater overall hypotension caused by hydralazine could conceivably account for a greater recruitment of RVLM premotor neurons and therefore for the higher number of Fos-ir cells found in the RVLM. This interpretation assumes that the stress and greater initial hypotension caused by hypotensive hemorrhage have less influence on Fos expression in RVLM than a slight sustained difference in baroreceptor unloading during the latter part of the experiment. Activation of serotonergic and other SEPPN neurons.

The SEPPN defined in the present work is a circumscripted portion of the parapyramidal area outlined by Loewy and colleagues (27). It also overlaps but is not identical to the region previously called nucleus interfascicularis hypoglossi (30), also known as the lateral B1 group in reference to the original nomenclature of serotonergic cell groups (for review, see Ref. 6). The region we chose to investigate, the SEPPN, was selected because it can be unambiguously defined by its subependymal location and is quite distinct from the RVLM (Fig. 4). The SEPPN, especially its serotonergic component, projects to the intermediolateral cell column, and thus the SEPPN contains neurons that probably function as presympathetic cells (6, 27, 50) although its sympathetic targets are not precisely defined. There are no published accounts of the pattern of unit activity of these cells, and therefore their function remains speculative at the present time. SEPPN neurons are among the first medullary neurons to be infected after injection of pseudorabies virus (PRV) in the rat’s tail, a strong indication that at least some of these neurons are involved in thermoregulation (50).

However, the parapyramidal area, probably including the SEPPN, is also infected rapidly after injection of PRV into both the adrenal gland and the stellate ganglion (26). This evidence suggests that some SEPPN cells may be involved in coactivating several types of sympathetic outflows, possibly including vascular targets other than skin. Still, the available information leaves considerable uncertainty regarding the wiring of the SEPPN, and the possibility that this structure could be very heterogeneous with regard to its targets should not be dismissed.

Fos experiments performed in rats have indicated clearly that the SEPPN is not activated by stimulation of arterial baroreceptors (15). According to the present data, unloading baroreceptors with hydralazine is also ineffective in triggering Fos expression in the SEPPN. As discussed before, baroreceptor unloading may have been even larger overall in hydralazine-treated rats than in hemorrhaged rats, and yet the latter displayed at least six times as many Fos-ir cells in the SEPPN. Thus, contrary to the presympathetic cells of the RVLM, SEPPN neurons are probably not regulated by arterial baroreceptors. In this respect SEPPN neurons resemble the serotonergic neurons of the raphe magnus (33). The apparent absence of baroreceptor influence suggests that SEPPN cells are not primarily involved in buffering blood pressure, but this characteristic does not exclude a role in regulating specialized vascular beds such as the skin (9, 50).

There is considerable evidence that the SEPPN is regulated by peripheral chemoreceptors and perhaps also by tissue hypoxia, independently of conventional chemoreceptors. Electrical stimulation of the carotid sinus nerve produces massive activation of SEPPN neurons (15) as does hypoxia and increases in inhaled

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CO₂ (5, 15, 35). We did not measure the blood PO₂ of our rats, and therefore we have no evidence for arterial hypoxia, but even in the absence of arterial hypoxia, a severely lowered hematocrit and a reduced blood flow through the carotid bodies could cause significant chemoreceptor stimulation (1). Tissue hypoxia, produced by inhalation of a dose of carbon monoxide presumed to have little or no effect on peripheral chemoreceptors, also causes massive acidification (57).

The SEPPN may also be activated by central chemoreceptors. This nucleus overlaps closely with Schlaefke’s intermediate area, a portion of the ventral medullary surface suspected to contribute to the activation of the respiratory network by hypercapnia (39). Conceivably severe hemorrhage could acidify brain extracellular fluid if an imbalance develops between O₂ delivery and neuronal consumption. However, the SEPPN neurons that express Fos in animals exposed to CO₂-enriched air may not be the serotonergic ones (35). Other evidence indicates that the serotonergic neurons of the parapyramidal region are excited by extracellular acidification (57).

Finally, Fos expression within the SEPPN could also be related to thermoregulation. Hypotensive hemorrhage causes a profound decrease in oxygen consumption that precedes the hypothermia and therefore presumably contributes to it (25). Unfortunately, it is unknown whether the hypothermia is adaptive or results from a failure of thermoregulatory mechanisms. If the latter is true, defensive thermogenesis and cutaneous vasoconstriction may be maximally triggered by hypotensive hemorrhage in a failed attempt to minimize hypothermia. The fact that SEPPN innervates preganglionic neurons that regulate stellate sympathetic efferents and the adrenal medulla is potentially compatible with a role in sympathetically mediated thermogenic responses (26). This role has been attributed recently to bulbospinal neurons located within the raphe pallidus (e.g., 37, 38), a structure that may be functionally related to the SEPPN despite their anatomic separation by the pyramidal tract. Serotonin could conceivably mediate the thermoregulatory effects of SEPPN because this transmitter excites sympathetic preganglionic neurons (28, 31, 43). However, serotonin can also inhibit preganglionic neurons by activating glycinergic interneurons (28), and the hypothesis that SEPPN neurons are uniformly excitatory is not supported by available evidence. For instance, inhibitory transmitters such as enkephalin and GABA are present in many of these cells. Also, only 60% or less of the Fos-labeled nuclei present in the SEPPN after hypotensive hemorrhage could be identified as belonging to serotonergic neurons. These apparent contradictions could be a reflection of the diversity of SEPPN neurons and of their targets.

In summary, hypotensive hemorrhage activates SEPPN neurons. These neurons display a variety of phenotypes compatible with both excitatory (serotonin) and inhibitory (serotonergic, GABAergic, opioidergic) effects on their targets. Although many SEPPN neurons, including serotonergic ones, project to the intermediolateral cell column (27), the rest of their projections is unknown. Their activation by hemorrhage could therefore produce a constellation of effects, including HISI, via the inhibition of selected vasomotor efferents, and cutaneous vasoconstriction, perhaps mediated by the release of serotonin on preganglionic neurons. The activation of opioidergic SEPPN neurons by hypotensive hemorrhage may underlie the recent observation that intrathecal naloxone attenuates HISI (2).

**Perspectives**

Hypotensive hemorrhage and isovolemic hypotension are both associated with decreased AP, but these two pathological situations produce a divergent spectrum of autonomic responses. Hypotension elicited by direct vasodilation stimulates sympathetic tone to the heart and blood vessels to raise cardiac output and peripheral resistance and maintain circulatory function. In contrast, severe blood loss is associated with a seemingly detrimental reversal of this autonomic response pattern. This pattern has been termed decompenatory, a word that implies a failure of normal homeostatic mechanisms, although an adaptive role of the decompenatory phase of hemorrhage is not ruled out. The central mechanisms underlying the decompenatory phase of the responses to blood loss also remain elusive. The present study provides some additional support to the notion that a reduction in the discharge rate of RVLM presympathetic neurons could be involved in the production of HISI (4, 13, 46). Because the GABAergic interneurons present within the RVLM were no more activated by hypotensive hemorrhage than by hydralazine, their contribution to the inhibition of RVLM presympathetic neurons during hemorrhage remains questionable. By contrast, this negative result reinforces prior evidence that 5-HT₁A receptor activation in the RVLM causes or enables HISI (13). Based on this evidence, it has been proposed that serotonin is released by hemorrhage in the RVLM, but the source of the serotonin release remains unclear and its trigger unknown. The present study shows that severe hemorrhage, but not isovolemic hypotension, activates many serotonergic neurons in the SEPPN. The known anatomy of these cells indicates that their activation should increase serotonin release in the spinal cord, but their contribution to the serotonergic innervation of the RVLM is undocumented. Other possibilities include the raphe magnus, a known source of serotonergic innervation of the RVLM (33).

The present experiments emphasize the phenotypic diversity of the SEPPN and indicate that this nucleus is strongly recruited by hypotensive hemorrhage. Because of the known projections of SEPPN to the intermediolateral cell column, the evidence demonstrates that SEPPN must contribute to some of the changes in sympathetic efferent activity associated with hypotensive hemorrhage. The presence of inhibitory transmitters in SEPPN neurons, especially GABA, a transmitter apparently not made by most other medullary
serotonergic neurons (51), suggests that these cells could be contributing to HISI. However, HISI may be restricted to splanchic, cardiac, and muscle vasoconstrictor efferents, and it is far from clear that these particular sympathetic efferents are regulated by SEPPN neurons. Further interpretation will require a much more complete understanding of the input-output connections of SEPPN neurons.

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