Activation of lateral parabrachial nucleus neurons restores blood pressure and sympathetic vasomotor drive after hypotensive hemorrhage

Martha L. Blair and Deanne Mickelsen
Department of Pharmacology and Physiology, University of Rochester
School of Medicine and Dentistry, Rochester, New York

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Blair, Martha L., and Deanne Mickelsen. Activation of lateral parabrachial nucleus neurons restores blood pressure and sympathetic vasomotor drive after hypotensive hemorrhage. Am J Physiol Regul Integr Comp Physiol 291: R742–R750, 2006. First published March 30, 2006; doi:10.1152/ajpregu.00049.2006.—Lesions of the lateral parabrachial nucleus (LPBN) impair blood pressure recovery after hypotensive blood loss (Am J Physiol Regul Integr Comp Physiol 280: R1141, 2001). This study tested the hypothesis that posthemorrhage blood pressure recovery is mediated by activation of neurons, located in the ventrolateral aspect of the LPBN (VL-LPBN), that initiates blood pressure recovery by restoring sympathetic vasomotor drive. Hemorrhage experiments (16 ml/kg over 22 min) were performed in unanesthetized male Sprague-Dawley rats prepared with bilateral ibotenate lesions or guide cannulas directed toward the external lateral subnucleus of the VL-LPBN. Hemorrhage initially decreased mean arterial pressure (MAP) from ~100 mmHg control to 40–50 mmHg, and also decreased heart rate. In animals with sham lesions, MAP returned to 84 ± 4 mmHg by 40 min posthemorrhage, and subsequent autonomic blockade with hexamethonium reduced MAP to 53 ± 2 mmHg. In contrast, animals with VL-LPBN lesions remained hypotensive at 40 min posthemorrhage (58 ± 4 mmHg) and hexamethonium had no effect on MAP, implying a deficit in sympathetic tone. VL-LPBN lesions did not alter the renin response or the effect of vasopressin V1 receptor blockade after hemorrhage. Posthemorrhage blood pressure recovery was also significantly delayed by VL-LPBN infusion of the ionotropic glutamate receptor antagonist kynurenic acid. Both VL-LPBN lesions and VL-LPBN kynurenate infusion caused posthemorrhage bradycardia to be significantly prolonged. Bradycardia was reversed by hexamethonium or atropine, but did not contribute to posthemorrhage hypotension. Taken together, these data support the hypothesis that stimulation of VL-LPBN glutamate receptors mediates spontaneous blood pressure recovery by initiating restoration of sympathetic vasomotor drive.

The hypotensive phase also initiates compensatory increases in renin, vasopressin, and epinephrine secretion rates (39). Spontaneous blood pressure recovery from hemorrhagic hypotension is accomplished by the combined actions of elevated plasma angiotensin and vasopressin concentrations, and restoration of sympathetic (α1-adrenergic) vasoconstrictor drive to the arterial and venous circulations (13, 34, 41, 43). Finally, blood volume is restored to basal levels by transcapillary fluid shifts that begin immediately after blood loss, resulting in significant plasma volume expansion even within the first hour posthemorrhage (1, 8, 46).

The central nervous system (CNS) neurotransmitter systems and neuroanatomical pathways that underlie the initial hypotension, sympathoinhibition, and bradycardia associated with blood loss have been actively investigated (9, 42, 43, 44). In contrast, the CNS pathways that reverse sympathoinhibition and drive spontaneous blood pressure recovery have previously received little attention. However, a recent study from our laboratory demonstrates that the lateral parabrachial nucleus (LPBN), an autonomic integrative center located in the dorsolateral pons, plays an essential role in mediating spontaneous blood pressure recovery following hypotensive blood loss (2). In that study, conscious rats with bilateral ibotenic acid lesions of the LPBN were subjected to a slow hemorrhage of 16 ml/kg blood withdrawal. LPBN ibotenate lesions did not affect the ability to maintain normotensive arterial blood pressure during the initial phase of blood loss or the volume of blood that could be withdrawn before a significant decrease in pressure occurred. However, in rats with complete lesions of the ventrolateral aspect of the LPBN (VL-LPBN; the external lateral subnucleus of the LPBN, and immediately adjacent region), blood pressure failed to recover following hypotensive blood loss over the course of the 40-min posthemorrhage recovery period. The deficit in blood pressure recovery could not be attributed to deficits in the renin or vasopressin response to blood loss or the extent of posthemorrhage plasma volume expansion.

We therefore propose that following a hypotensive blood loss, spontaneous blood pressure recovery is mediated by activation of a population of VL-LPBN neurons that reverses sympathoinhibition and restores sympathetic drive to the vasculature. The present experiments were designed to test this hypothesis. To verify that spontaneous blood pressure recovery requires activation of LPBN neurons and to test the hypothesis that spontaneous blood pressure recovery is mediated by stimulation of LPBN ionotropic glutamate receptors, the ionotropic glutamate receptors; atropine; vasopressin receptor antagonist; heart rate; sympathetic nervous system

DURING PROGRESSIVE BLOOD loss, arterial blood pressure is initially maintained at normotensive levels by peripheral vasoconstriction that is primarily mediated by increased sympathetic nervous system activity. Hypotension ensues when blood loss reaches a critical volume, triggering a precipitous decline in total peripheral resistance that is caused by withdrawal of sympathetic vasoconstrictor drive, and is accompanied by bradycardia (39). This hypotensive response serves the adaptive purpose of providing a window of time during which low perfusion pressure reduces bleeding and facilitates hemostasis.

Address for reprint requests and other correspondence: M. L. Blair, Dept. of Pharmacology and Physiology, Univ. of Rochester Medical Center, Box 711, 601 Elmwood Ave., Rochester, NY 14642 (e-mail: martha_blair@urmc.rochester.edu).

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glutamate receptor antagonist kynurenic acid was infused directly into the VL-LPBN immediately following hypotensive blood loss and the time course of blood pressure and heart rate recovery was compared with animals that received vehicle infusion only. The hypothesis that the VL-LPBN is essential for posthemorrhage restoration of sympathetic drive was tested by pharmacologic blockade of autonomic activity in animals with and without bilateral ibotenate lesions of the VL-LPBN. To avoid the confounding effects of anesthesia on CNS, autonomic and neuroendocrine function, these experiments were performed while the animals were unanesthetized and unrestrained.

MATERIALS AND METHODS

Experiments were performed in male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 225–250 g at the time of arrival at the University of Rochester vivarium. The rats were housed in individual cages in the University of Rochester vivarium with a 12:12-h light-dark cycle (lights on 0600–1800 h), with standard laboratory chow and tap water available ad libitum. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee. Each animal was used in only one hemorrhage experiment.

Surgical preparation. All surgical procedures were performed while the animals were anesthetized with Equithesin anesthesia (pentobarbital 25 mg/kg with chloral hydrate 128 mg/kg ip), using aseptic conditions. Animals prepared with LPBN lesions received bilateral stereotoxic injections of 300–400 nl ibotenic acid (10 μg/μl in 0.1 M phosphate buffer, pH 7.4; Sigma or Tocris) directed toward the external lateral subnucleus of the LPBN (0.4 mm caudal to interaural line, ±2.0–2.2 mm lateral, –6.2 mm dura, incisor bar –3.3 mm). The ibotenate was injected over a 10-min period, by using a 500-μl Hamilton syringe with a 25-gauge needle. The needle was then left in place for an additional 10-min period before being withdrawn. In sham-lesioned rats, the injection needle was lowered to the same location, but no drug was administered. Catheters were surgically implanted in the left femoral artery and femoral vein as previously described (46). 5–8 days after stereotoxic surgery.

For VL-LPBN kynurenic acid infusion experiments, rats were first prepared with stereotoxically-placed bilateral 22-gauge guide cannulas (Plastics One, Roanoke, VA; 6.3-mm length) directed toward the external lateral subnucleus of the LPBN (0.4 mm caudal to interaural line, lateral ± 2.8 mm with a 4-degree angle toward midline; incisor bar –3.3 mm). Two stainless steel jeweler’s screws were placed on each side of the skull adjacent to the guide cannula to serve as an anchor, and the area was secured with cranioplastic cement. A femoral arterial catheter was surgically implanted on the day of stereotoxic surgery as previously described (46).

Additional animals to be utilized for atropine infusion experiments and for verification of the efficacy of the vasopressin V1 antagonist were prepared with left femoral artery and femoral vein catheters (46) but had no prior stereotoxic surgery.

General experimental procedures. Hemorrhage experiments were performed after at least 5 days had elapsed since catheter implantation (12–15 days after ibotenate lesion stereotoxic surgery). During hemorrhage experiments, each rat was placed in a recording cage that permitted the animal to move about freely. The rat was adapted to the experimental conditions by being placed in the recording cage for 1–2 h on at least 1 day before the experiment and was permitted an additional 30-min adaptation period after connecting the arterial catheter to the blood pressure recording apparatus on the morning of the experiment before beginning the control measurements. Food and water were not available while the rat was in the recording cage. The catheters were connected to tubing extensions that permitted blood to be withdrawn and arterial blood pressure to be recorded without restraining or otherwise disturbing the rat. Blood pressure was recorded with an ADInstruments disposable transducer and PowerLab data acquisition system, using a data sampling rate of 400 Hz. Heart rate was calculated from the arterial pressure signal using the rate meter software included in the PowerLab data acquisition package.

Hemorrhage procedure. Hemorrhage experiments were performed in the morning, beginning at 0930–1200 h. Sodium heparin (150 units in 150 μl) was injected into the arterial catheter 8 min before beginning the hemorrhage procedure to prevent clotting during blood withdrawal. The hemorrhage procedure consisted of three sequential blood withdrawals. Blood was withdrawn from the arterial catheter at a rate of 1.6 ml·kg⁻¹·min⁻¹ for two 4-min withdrawals and an additional 2-min withdrawal, each separated by a 6-min observation period. This resulted in a total blood loss of 16 ml/kg over 22 min. Blood pressure and heart rate were continuously recorded beginning 30 min before blood withdrawal and for at least 60 min after completing the hemorrhage.

Experimental protocol for animals with LPBN ibotenate lesions. To test the hypothesis that the VL-LPBN is essential for posthemorrhage restoration of sympathetic drive, animals prepared with bilateral ibotenate or sham VL-LPBN lesions were first exposed to a 16-ml/kg blood withdrawal and then received an intravenous infusion of the autonomic ganglion blocker hexamethonium chloride (Sigma; 10 mg in 100 μl saline) at 40 min posthemorrhage. To determine whether VL-LPBN lesions altered the contribution of vasopressin to blood pressure maintenance after hemorrhage and hexamethonium treatment, a vasopressin V1 receptor antagonist ([d(CH₂)₅]¹,Tyr(Me)²,Arg⁸)-vasopressin; BACHEM; 5 μg in 100 μl saline) was infused intravenously at 45 min posthemorrhage, 5 min after hexamethonium administration. In addition, plasma renin activity was measured (Gamma-Coat plasma renin activity radioimmunoassay kit; DiaSorin, Stillwater, MN) in blood samples collected during each of the three hemorrhage blood withdrawals and from an additional 1-ml blood sample collected 5 min after administration of the vasopressin antagonist. Hematocrit was measured from triplicate 70-μl samples collected in microcapillary tubes at the start of the first hemorrhage blood withdrawal and again at the end of the experiment, 5 min after V1 antagonist infusion.

Efficacy of the V1 antagonist was confirmed in a separate group of four intact animals, by demonstrating blockade of the pressor response to intravenous vasopressin infusion ([Arg⁸]-vasopressin acetate salt, Sigma; 30 ng in 100 μl saline) administered 5 min after intravenous infusion of the V1 antagonist (5 μg in 100 μl saline). Two of these animals were subsequently utilized in the atropine infusion studies, 48–72 h after the V1 antagonist test.

Experimental protocol for intact hemorrhaged animals with atropine infusion. To determine whether reversal of vagally-mediated bradycardia can contribute to blood pressure recovery from hemorrhagic hypotension, intact animals were first exposed to 16 ml/kg hemorrhage and then received an intravenous infusion of either atropine methylbromide (300 μg in 100 μl saline; Sigma) or isotonic saline (100 μl) at 10 min posthemorrhage. Blood pressure and heart rate were then observed until termination of the experiment at 60 min posthemorrhage.

Experimental protocol for animals with VL-LPBN kynurenic acid infusion. To test the hypothesis that spontaneous blood pressure recovery is mediated by stimulation of VL-LPBN ionotropic glutamate receptors, animals were first prepared with bilateral guide cannulas directed toward the external lateral subnucleus of the LPBN. On the day of the experiment, a 28-gauge injector needle that extended 1.5 mm beyond the tip of the guide cannula was secured in the guide cannula 25 min before beginning the 16-ml/kg hemorrhage procedure. The drug was infused from a 1-μl Hamilton syringe by using a modified Razell infusion pump, through a 30-cm length of calibrated microdialysis tubing (1.2 μl/100 mm; CMA, Stockholm, Sweden) that was filled with 10 mM kynurenic acid and then attached to the injector needle. The volume of drug infused was verified by movement of Evans blue dye, separated from the drug by an oil
droplet, down the calibrated tubing. Kynurenic acid (Sigma) was first prepared in a 100-mM stock solution by dissolving the drug in 5 M NaOH, and then bringing the solution to volume with isotonic saline and adjusting the pH with 2 N HCl, and stored at −20°C. On the day of the experiment, a drug aliquot was thawed and diluted to 10 mM in artificial cerebrospinal fluid (in mM: 120 NaCl, 25 NaHCO3, 3.3 KCl, 1.2 Na2HPO4, 1.8 CaCl2, 2.4 MgSO4, pH 7.4) before infusion.

The ionotropic glutamate receptor antagonist kynurenic acid (10 mM) was infused beginning 3 min after completion of the hemorrhage, at a rate of 3 nM in 300 nl over 15 min, followed by continuous infusion of 2 nM in 200 nl/h for the remainder of the 90-min posthemorrhage observation period. Hematocrit was measured from triplicate 70 μl samples collected in microcapillary tubes at the start of the first hemorrhage blood withdrawal and again at 90 min posthemorrhage.

Experiments were performed in an additional group of animals prepared with bilateral guide cannulas to determine whether LPBN kynurenic infusion altered blood pressure or heart rate in the basal state (drug time-control experiments). The protocol for drug time-control experiments was identical to that described above for hemorrhaged animals with kynurenic infusion, except that no blood was withdrawn. Kynurenic acid was infused into the parabrachial region at the same rate as in hemorrhaged animals (3 nM in 300 nl over 15 min, followed by continuous infusion of 2 nM in 200 nl/h for the remainder of the experiment). These animals were not utilized in any other experimental protocol.

Histology. At completion of each experiment, the animals were deeply anesthetized with Equithesin (pentobarbital 25 mg/kg with chloral hydrate 128 mg/kg infused into the arterial catheter) and transcardially perfused (4% paraformaldehyde) as previously described (23). The brain was then postfixed for 1–2 h in 4% paraformaldehyde followed by 20% sucrose in phosphate-buffered saline for 24–48 h and subsequently stored frozen at −80°C. The brain was cut in 30-μM coronal sections by using a freezing stage sliding microtome. Sections were stored in cryoprotectant at −20°C until histological processing. Sections spaced at 90-μM intervals were stained with cresyl violet for identification of infusion and lesion sites.

Ibotenate lesions were judged to be correctly placed, if the following criteria were met: (i) The injection site localized to the ventrolateral half of the LPBN, at the level bordered dorsally by the ventral spino cerebellar tract and corresponding to bregma −8.88 to −9.36 mm in the fifth edition of the atlas of Paxinos and Watson (33); (ii) cresyl violet-stained tissue showed extensive microgliosis and neuronal loss within and around the external lateral subnucleus throughout the rostral-caudal extent of the LPBN region bordered dorsally by the ventral spino cerebellar tract; and (iii) the volume of the LPBN was reduced in the ventrolateral half of the region between the ventral spino cerebellar tract and superior cerebellar peduncle. Animals in which the lesions were misplaced on one or both sides of the brain, but were within or adjacent to the parabrachial complex, were utilized as anatomical controls in the data analysis.

Kynurenic acid and vehicle infusion sites were judged to be correctly placed if the infusion site was in the ventrolateral half of the LPBN, at the level bordered dorsally by the ventral spino cerebellar tract and corresponding to bregma −8.88 to −9.36 mm in the atlas of Paxinos and Watson (33). The infusion needle track was clearly visible in cresyl violet-stained sections, and the region surrounding the infusion site showed an increased distance between neurons. Animals in which the kynurenic acid infusion sites were misplaced on one or both sides of the brain, but were within or adjacent to the parabrachial complex, were utilized as anatomical controls in the data analysis.

Statistical analysis. Statistical analyses were performed by SPSS SigmaStat 3.0 software. The effect of VL-LPBN ibotenate lesion, kynurenic infusion, or atropine on the blood pressure, heart rate, hematocrit, and renin response to hemorrhage was statistically evaluated by multifactorial repeated-measures (RM) analysis of variance (ANOVA). When the multifactorial RM ANOVA showed significant effects of hemorrhage, individual Holm-Sidak comparisons of the prehemorrhage control value with each subsequent sampling time were performed for each group of animals, using the pooled variance estimate computed by single-factor RM ANOVA for that group. Individual Holm-Sidak comparisons across groups at each time point, using the pooled variance from one-way ANOVA at each time, were performed when the multifactorial RM ANOVA showed significant treatment effects or a significant treatment-by-hemorrhage interaction effect. For all hemorrhage protocols, these analyses were performed on blood pressure and heart rate data obtained from 10 min before hemorrhage (prehemorrhage control period) until 1 h posthemorrhage. Additional single-factor ANOVA and Holm-Sidak comparisons were performed as described in results and the figure legends. The null hypothesis for individual comparisons was rejected only when the unadjusted P value for that comparison was less than the critical level determined by the Holm-Sidak method (14), using an overall significance level of P < 0.05. All data are expressed as the means ± SE.

RESULTS

Histology of LPBN lesions. Complete VL-LPBN ibotenate lesions (n = 5) caused extensive bilateral damage to the external lateral subnucleus of the LPBN (Fig. 1A), as indicated by neuronal loss, microgliosis, and narrowing of the zone bordered by the ventral spino cerebellar tract and superior cerebellar peduncle. All correctly placed injection sites were localized either within or adjacent to the external lateral subnucleus, at the level shown as 9.00–9.24 mm caudal to bregma in Fig. 1B. In animals with partial lesions (anatomical controls, n = 6), a substantial portion of the external lateral subnucleus was spared on one (n = 3) or both (n = 3) sides of the brain. Misplaced injection sites were localized within 8.7 to 9.3 mm caudal to bregma, and were either within the dorsomedial half of the LPBN, the rostral end of the VL-LPBN, or immediately adjacent to the LPBN (Fig. 1B).

Effect of VL-LPBN lesions on responses to hemorrhage. VL-LPBN ibotenate lesions did not alter the initial response to 16 ml/kg blood loss but significantly impaired posthemorrhage blood pressure recovery and altered the response to subsequent autonomic blockade (Fig. 2; RM ANOVA, P < 0.01 for hemorrhage-drug, lesion, and interaction effects). In all three groups of animals, blood pressure remained at normotensive levels during the posthemorrhage recovery period, animals with sham or partial lesions (anatomical controls, n = 6), a substantial portion of the external lateral subnucleus was spared on one (n = 3) or both (n = 3) sides of the brain. Misplaced injection sites were localized within 8.7 to 9.3 mm caudal to bregma, and were either within the dorsomedial half of the LPBN, the rostral end of the VL-LPBN, or immediately adjacent to the LPBN (Fig. 1B).

Autonomic blockade with hexamethonium at 40 min posthemorrhage had markedly different effects on animals with complete VL-LPBN lesions compared with sham or partial lesions (Fig. 2). In animals with sham or partial lesions, hexamethonium caused an abrupt blood pressure decrease of 31 ± 4 and 35 ± 4 mmHg, respectively. In contrast, blood pressure did not change (−2 ± 6 mmHg) after hexamethonium treatment in animals with complete VL-LPBN lesions (ANOVA, P < 0.001 for comparison between groups of magnitude of blood pressure decrease). Furthermore, the blood pressure of sham and partial lesion animals after autonomic blockade was nearly identical to that of animals with complete
bilateral VL-LPBN lesions either before or after autonomic blockade, indicating that hemorrhaged animals with complete VL-LPBN lesions had a marked deficit in sympathetic tone.

Hemorrhage also resulted in a bradycardia in all three groups of animals (Fig. 2). As was the case for blood pressure, VL-LPBN lesions significantly altered the time course of heart rate recovery, as well as the response to autonomic blockade (RM ANOVA hemorrhage-drug effect, \( P < 0.001 \); interaction effect, \( P < 0.05 \)). In animals with sham or partial LPBN lesions, heart rate recovered after hemorrhage in parallel with blood pressure recovery and showed no change in response to hexamethonium administration at 40 min posthemorrhage. In contrast, heart rate remained significantly depressed throughout the posthemorrhage recovery period in animals with complete VL-LPBN lesions (Fig. 2), and hexamethonium caused an abrupt 74 \pm 14 beat/min increase in heart rate (ANOVA, \( P < 0.001 \) for comparison between groups of magnitude of change of heart rate). Hexamethonium blocks neurotransmission not only at sympathetic ganglia, but at parasympathetic ganglia as well. Thus the increase in heart rate seen after hexamethonium in hemorrhaged animals with VL-LPBN lesions demonstrates that they had a persistent vagally-mediated bradycardia throughout the posthemorrhage recovery period.

Administration of a vasopressin V1 antagonist 5 min after hexamethonium administration caused a further decrease in blood pressure of \( \sim 10 \) mmHg in all groups of animals, indicating that animals with complete VL-LPBN lesions did not have a deficit in vasopressin-mediated vasoconstrictor tone. In addition, plasma renin activity increased to the same extent during hemorrhage and after hexamethonium and V1 receptor blockade in all three groups of animals (Table 1; RM ANOVA hemorrhage-drug effect, \( P < 0.001 \); lesion and interaction effects, \( P > 0.30 \)). Hematocrit also decreased to the same extent in all groups of animals, indicating that LPBN lesions did not alter the extent of blood volume restoration by transcapillary fluid flux (Table 2; RM ANOVA hemorrhage effect, \( P < 0.001 \); lesion effect, \( P = 0.09 \); interaction effect, \( P > 0.10 \)). Taken together, these data imply that VL-LPBN lesions impair spontaneous blood pressure recovery by disrupting the restoration of appropriate autonomic tone to the heart and vasculature.

**Effect of reversal of vagal bradycardia on blood pressure recovery in intact animals.** To determine the extent to which the persistent vagal bradycardia may have contributed to sustained posthemorrhage hypotension in animals with VL-LPBN lesions, experiments were performed to determine whether reversal of bradycardia can improve blood pressure during hemorrhagic hypotension. The muscarinic cholinergic antagonist atropine was infused intravenously 10 min after hemorrhage in intact animals (\( n = 5 \); Fig. 3). Atropine caused an immediate heart rate increase of >100 beats/min (\( P < 0.01 \), Holm-Sidak), and heart rate remained significantly higher than in hemorrhaged saline-control animals (\( n = 5 \)) throughout the remaining 40 min of the posthemorrhage recovery period (RM ANOVA, hemorrhage, and drug-by-hemorrhage interaction effects; \( P < 0.001 \)). However, blood pressure was unaffected by atropine treatment. Blood pressure did not differ between atropine-treated and saline control animals at any time after drug administration (RM ANOVA, hemorrhage effect, \( P < 0.001 \); drug and interaction effects, \( P > 0.10 \)), indicating that bradycardia does not play a significant role in hemorrhagic hypotension. Therefore, the sustained posthemorrhage hypotension of animals with VL-LPBN lesions cannot be attributed to their persistent bradycardia and must instead be due to a sustained deficit in sympathetic drive to the vasculature.
Localization of kynurenic acid infusion sites. To test the hypothesis that posthemorrhage blood pressure recovery requires activation of ionotropic glutamate receptors located in the VL-LPBN, experiments were performed to determine the effect of VL-LPBN kynurenic acid infusion on spontaneous blood pressure recovery after hemorrhage. In animals with correctly placed kynurenic acid infusion sites (n = 5), the infusion sites were centered within the ventrolateral half of the LPBN, between 8.9 and 9.3 mm caudal to bregma, on both sides of the brain (Fig. 4). In animals with misplaced infusion sites (anatomical controls, n = 6), the infusion sites were centered outside the target area on one (n = 2) or both (n = 4) sides of the brain (Fig. 4). The distribution of correctly placed vehicle infusion sites (n = 6) was similar to that of correctly placed kynurenic acid infusion sites.

Effect of VL-LPBN kynurenic acid infusion on blood pressure recovery after hemorrhage. In these experiments, kynurenic acid infusion began 3 min after completion of the 16 ml/kg hemorrhage. All groups of animals showed an initial hypotensive response to hemorrhage (blood pressure decrease to 40–50 mmHg) accompanied by bradycardia (Fig. 5). Animals that received VL-LPBN vehicle infusion or had kynurenic acid infused into regions outside the LPBN showed a gradual return to normotension and their blood pressure stabilized at 80–90% of prehemorrhage control levels within 30 min posthemorrhage. In contrast, kynurenic acid infusion directly into the VL-LPBN caused a significant delay in blood pressure recovery (RM ANOVA; hemorrhage effect, P < 0.001; lesion effect P = 0.05, lesion-by-hemorrhage interaction effect, P < 0.01). Blood pressure was significantly lower at 15–35 min posthemorrhage in animals that received VL-LPBN kynurenic acid infusion than in those that received VL-LPBN vehicle infusion or kynurenic acid infusion into regions outside the LPBN (Fig. 5). Whereas mean arterial pressure had returned to at least 80% of basal levels within 26 ± 7 min posthemorrhage in animals that received vehicle infusion into the VL-LPBN and within 17 ± 2 min posthemorrhage in those receiving kynurenic acid infusion into regions outside the LPBN, blood pressure did not reach 80% of basal levels until 55 ± 10 min posthemorrhage in animals in which kynurenate was

Table 2. Hematocrit values before and after hemorrhage for animals with VL-LPBN ibotenate lesions or kynurenic acid infusion

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Before Hemorrhage</th>
<th>End of Hemorrhage</th>
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<tbody>
<tr>
<td>Sham</td>
<td>41.0 ± 0.6</td>
<td>32.1 ± 0.6*</td>
</tr>
<tr>
<td>Partial VL-LPBN lesion</td>
<td>42.2 ± 0.8</td>
<td>33.4 ± 0.5*</td>
</tr>
<tr>
<td>Complete bilateral VL-LPBN lesion</td>
<td>43.5 ± 0.2</td>
<td>33.8 ± 0.4*</td>
</tr>
<tr>
<td>VL-LPBN vehicle infusion</td>
<td>43.3 ± 0.8</td>
<td>37.1 ± 0.6*</td>
</tr>
<tr>
<td>Kynurenic acid infusion outside VL-LPBN</td>
<td>41.8 ± 0.9</td>
<td>35.3 ± 1.0*</td>
</tr>
<tr>
<td>VL-LPBN kynurenic acid infusion</td>
<td>42.9 ± 1.2</td>
<td>36.6 ± 0.7*</td>
</tr>
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Values are means ± SE. Hematocrit is expressed as percent. *P < 0.01 vs. before hemorrhage.
infused directly into the VL-LPBN (P < 0.01, Holm-Sidak test). Animals that received VL-LPBN kynurenate infusions also showed sustained bradycardia, with a heart rate that averaged 70–80 beats/min lower than in the other two groups of animals at 15–35 min posthemorrhage (Fig. 5). These data provide further evidence that VL-LPBN neurons play an essential role in initiation of the CNS-mediated responses that support posthemorrhage blood pressure recovery and support the hypothesis that this response is mediated by VL-LPBN ionotropic glutamate receptors.

Effect of LPBN kynurenic acid infusion on blood pressure recovery in nonhemorrhaged control animals. Additional experiments were performed to determine whether LPBN kynurenic acid infusion alters blood pressure or heart rate in the basal state. In these experiments, kynurenic acid was infused into the parabrachial region at the same rate as in hemorrhaged animals, but no blood was withdrawn. Kynurenic acid infusion had no effect on basal blood pressure (Fig. 6) or heart rate (data not shown) when infused for 40 min either directly into the VL-LPBN (n = 6) or into regions adjacent to the LPBN (n = 5).

DISCUSSION

The CNS orchestrates a complex sequence of neural and hormonal compensations in response to blood loss. The sympathoinhibition and bradycardia associated with hypotensive blood loss appear to be mediated by CNS serotonergic and opioid receptors (11, 12, 16, 31, 40, 43–45) that may be located in the rostral ventrolateral medulla (10, 43) and periaqueductal grey (3, 9, 42), respectively. However, there is little known about the neuroanatomical pathways and CNS neurotransmitter systems that reverse sympathoinhibition and restore blood pressure back to normotensive levels after hemorrhagic hypotension has developed.

Our data indicate that spontaneous blood pressure recovery is initiated by CNS pathways that differ from those that are involved in the initial compensatory responses to blood loss. In both the present and previous (2) studies, bilateral ibotenate lesions of the ventrolateral aspect of the LPBN did not affect either the ability to maintain normotensive arterial blood pressure during the initial phase of blood loss, or the volume of blood that could be withdrawn before a significant decrease in pressure occurred. Thus the VL-LPBN does not contribute to the immediate compensatory responses to small-volume blood loss, nor does it contribute to the hypotensive response to larger blood losses. However, spontaneous blood pressure recovery...
recovery from hemorrhagic hypotension is significantly impaired in animals with bilateral VL-LPBN lesions. Whereas hemorrhaged sham lesion and anatomical control animals readily restored their blood pressure back to normotensive levels, animals with VL-LPBN lesions remained markedly hypotensive, with a mean arterial pressure that was \(40\) mmHg below basal levels, even at 40 min posthemorrhage. This implies that spontaneous blood pressure recovery from hemorrhagic hypotension is mediated by a neuronal population within the VL-LPBN.

The pontine parabrachial complex has extensive connectivity with brain stem sites involved in autonomic regulation and serves as the primary relay center for transfer of visceral sensory information to the forebrain (19, 27, 38). The parabrachial complex is composed of at least eleven discrete subnuclei (5). There is ample evidence to indicate that the external lateral subnucleus, located in the ventrolateral aspect of the LPBN, is the region of the parabrachial complex most likely to play a critical role in spontaneous blood pressure recovery. The external lateral subnucleus is a major target of viscerosensory projections from the nucleus of the tractus solitarius (NTS) (20), which is the primary relay site for sensory input from arterial baroreceptors, cardiopulmonary receptors, and peripheral chemoreceptors. The external lateral subnucleus of the LPBN also receives direct projections from other brain regions involved in autonomic regulation including the area postrema (20) and periaqueductal grey (26). These projections provide the neuroanatomical substrate for hypotension- and hypovolemia-related sensory input to the VL-LPBN. LPBN neurons are activated by blood loss, and by multiple other stimuli associated with hemorrhagic hypotension. The increase in parabrachial nucleus Fos expression elicited by hemorrhage (7, 24), isovolemic hypotension (22, 35), high plasma levels of angiotensin (48), chemoreceptor activation (47), and tissue hypoxia (21) is localized primarily to the external lateral subnucleus, confirming that this region is the primary parabrachial nucleus recipient of excitatory input conveying sensory information associated with hypertensive blood loss.

In this study, spontaneous blood pressure recovery was impaired by bilateral VL-LPBN lesions only when the lesions caused extensive bilateral damage to the external lateral subnucleus throughout the rostral-caudal extent of the LPBN region bordered dorsally by the ventral spinocerebellar tract. In contrast, blood pressure recovery in animals with partial VL-LPBN lesions did not differ from that of animals with sham lesions. Animals with partial lesions were prepared identically to those with complete lesions, but histological analysis showed that their lesion sites were slightly displaced from the VL-LPBN target region. The only consistent difference between partial lesions and complete VL-LPBN lesions is that partial lesions spared portions of the external lateral subnucleus on one or both sides of the brain, thus supporting the premise that the neuronal population that mediates spontaneous blood pressure recovery resides in the external lateral subnucleus.

Cardiovascular afferent information is relayed from the NTS to the VL-LPBN in part by glutamatergic neurons (25, 36, 37).

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**Fig. 5.** Effect of VL-LPBN kynurenic acid infusion on MAP and HR after hemorrhage. Hatched bars indicate time of blood withdrawal (16 ml/kg). Kynurenic acid infusion (Kyn) began at 3 min posthemorrhage (arrow). VL-LPBN Kyn infusion \((n = 5; \text{Kyn LPBN; black circle})\) delayed MAP and HR recovery after hemorrhage compared with Kyn infusion into areas outside of the LPBN \((n = 6; \text{Kyn Miss; black triangle})\) or LPBN vehicle infusion \((n = 6; \text{shaded square})\). \(^*P < 0.05\) vs. Kyn Miss or vehicle LPBN at same time. \(^{a}P < 0.05\) vs. prehemorrhage control at this and all subsequent measurement times through 15 min posthemorrhage for Kyn Miss or vehicle LPBN; \(^{b}P < 0.01\) vs. prehemorrhage control MAP at this and all subsequent measurement times through 45 min posthemorrhage for Kyn LPBN; \(^{c}P < 0.01\) vs. prehemorrhage control HR at this and all subsequent measurement times through 35 min posthemorrhage for Kyn LPBN.

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**Fig. 6.** Effect of VL-LPBN infusion on MAP in nonhemorrhaged control animals. Kynurenic acid was infused either directly into the VL-LPBN (black circle; Kyn LPBN, \(n = 6\)) or into areas adjacent to the LPBN (triangle; Kyn Miss, \(n = 5\)) at the same rate as in hemorrhaged animals.
These NTS projections activate parabrachial neurons via ionotropic glutamate receptors (25, 37). In addition, ionotropic glutamate receptor subunits are densely localized to the VL-LPBN within the external lateral subnucleus (6). Therefore, to test the hypothesis that spontaneous blood pressure recovery is mediated by activation of a neuronal population within the VL-LPBN, we performed experiments in which the ionotropic glutamate receptor antagonist kynurenic acid was infused directly into the VL-LPBN immediately after completion of a 16-ml/kg hemorrhage. VL-LPBN kynurenate infusion caused a significant delay in blood pressure recovery; the time elapsed between the end of hemorrhage and restoration of mean arterial pressure to 80% of basal levels was twice as long in animals with VL-LPBN kynurenate infusion as in vehicle-infused animals, and threefold that of animals in which kynurenate was infused into adjacent regions outside the LPBN. This further confirms the essential role of the VL-LPBN in posthemorrhage blood pressure recovery and supports the hypothesis that spontaneous blood pressure recovery is mediated by activation of VL-LPBN ionotropic glutamate receptors.

It is of interest to note that spontaneous blood pressure recovery was more effectively prevented (or delayed) by ibotenate lesions than by VL-LPBN kynurenic acid infusion; the average blood pressure at 20–35 min posthemorrhage was 20 mmHg lower in animals with bilateral VL-LPBN ibotenate lesions than in animals that received VL-LPBN kynurenate infusion (55 ± 3 vs. 75 ± 5 mmHg; P < 0.02; Student’s t-test). This is not surprising, given that kynurenate infusion did not begin until after the hemorrhage was completed. It is possible that the glutamate receptor antagonist did not reach all of the critical population of neurons before the process of blood pressure recovery was initiated. In addition, there are numerous neurotransmitters and receptor types within the VL-LPBN (e.g., see Refs. 4, 15, and 18), suggesting that additional VL-LPBN neurotransmitters may be involved in initiating spontaneous blood pressure recovery.

The LPBN has the capacity to initiate sympathoexcitation. Electrical or glutamate stimulation of the LPBN elicits increases in arterial pressure (5, 28) that can be blocked by guanethidine or hexamethonium and are accompanied by increased renal sympathetic nerve activity and decreased hindlimb blood flow (28, 29, 32). LPBN neuronal activation also can attenuate baroreflex suppression of heart rate and renal sympathetic nerve activity (17, 30). Both the pressor and baroreflex inhibition response to LPBN stimulation are mediated by the rostral ventrolateral medulla (28, 30), which is the primary source of excitatory drive to sympathetic preganglionic neurons. In addition, stimulation of discrete sites within the ventrolateral aspect of the LPBN, either within or adjacent to the external lateral subnucleus, elicits prominent hypertensive and tachycardic responses (5). The short latency and large magnitude of this response suggest that it is mediated by intense sympathetic activation.

Our data indicate that the VL-LPBN mediates spontaneous blood pressure recovery by restoring sympathetic vasmotor drive. In animals with sham or partial VL-LPBN lesions, blood pressure decreased to ~40 mmHg immediately following 16 ml/kg blood loss but recovered nearly to normotensive levels within 40 min posthemorrhage. Subsequent autonomic blockade with hexamethonium caused blood pressure to fall to hypotensive levels. In contrast, animals with complete VL-LPBN lesions remained hypotensive at 40 min posthemorrhage, and their blood pressure did not change after following autonomic blockade, consistent with a significant deficit in sympathetic tone. Subsequent vasopressin V1 receptor blockade caused a modest further decrease in blood pressure that was of similar magnitude in all groups of animals (~10 mmHg), and plasma renin activity increased to the same extent in all groups both during hemorrhage and after hexamethonium and V1 antagonist administration. The extent of plasma volume expansion by transcapillary refilling, as indicated by decreased hematocrit, also did not differ between groups. This implies that the delay in blood pressure recovery in animals with complete VL-LPBN lesions is caused by impaired restoration of sympathetic drive.

Hexamethonium blocks neurotransmission not only at sympathetic ganglia, but at parasympathetic ganglia as well. Thus hexamethonium administration abolishes both sympathetic control of the heart and vasculature and vagal control of heart rate. The hypertensive response to blood loss is accompanied by a vagally-mediated (atropine-sensitive) bradycardia. This decrease in heart rate serves to prolong ventricular filling time and defend stroke volume; consequently, the bradycardia is thought to contribute little to the acute decrease in cardiac output that accompanies the onset of hemorrhagic hypotension (39). In our study, all groups of animals showed a significant bradycardia after 16 ml/kg blood loss. In animals with sham or partial lesions, heart rate recovered in parallel with blood pressure recovery, whereas animals with complete bilateral VL-LPBN lesions remained bradycardic throughout the posthemorrhage recovery period. To determine whether reversal of vagal bradycardia plays a significant role in spontaneous blood pressure recovery, additional experiments were performed in intact animals subjected to 16 ml/kg blood loss. Administration of the muscarinic cholinergic receptor antagonist atropine during hemorrhagic hypotension caused an immediate increase in heart rate, but had no effect on blood pressure. Therefore, the sustained posthemorrhage hypotension of animals with VL-LPBN lesions cannot be attributed to their persistent bradycardia and must instead be due to a sustained deficit in sympathetic drive to the vasculature.

In conclusion, the results of this study support the hypothesis that the VL-LPBN initiates spontaneous blood pressure recovery by restoring sympathetic drive to the vasculature. The impaired posthemorrhage blood pressure recovery seen in animals with VL-LPBN lesions demonstrates the limited efficacy of elevated levels of vasoconstrictor hormones (angiotensin, vasopressin) and restoration of blood volume secondary to transcapillary fluid shifts, in the face of a profound deficit in sympathetic vasmotor drive. The neuronal population that initiates spontaneous blood pressure recovery appears to be localized to the external lateral subnucleus of the LPBN and to be activated during hemorrhagic hypotension by stimulation of ionotropic glutamate receptors.

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