Role of paraventricular nucleus parvicellular neurons in the compensatory responses to graded hemorrhage

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Role of paraventricular nucleus parvicellular neurons in the compensatory responses to graded hemorrhage. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R278–R285, 1998.—The goal of this study was to determine the role of the parvicellular component of the paraventricular hypothalamic nucleus (PVH) in the compensatory responses to blood loss. Male Sprague-Dawley rats were prepared with bilateral ibotenate lesions of the parvicellular PVH (PVHx; n = 5) or with sham lesions (Sham; n = 8). After >10 days recovery, hemorrhage was performed by gradual withdrawal of 16 ml/kg blood over 34 min via an indwelling femoral arterial catheter while the rats were conscious and unrestrained. Basal serum corticosterone levels, plasma renin concentration (PRC), and heart rate did not differ between PVHx and Sham, whereas basal hematocrit was lower in PVHx than Sham (40 ± 1 vs. 44 ± 1; P < 0.05). After hemorrhage, corticosterone increased fourfold in Sham (P < 0.001) but did not increase significantly in PVHx. However, the blood pressure, heart rate, PRC, and hemodilution responses to hemorrhage were the same in Sham and PVHx during both the normotensive (7–13 ml/kg blood loss) and hypotensive (16 ml/kg blood loss) phases. In conclusion, the parvicellular PVH is essential for the corticosterone response, but not for the cardiovascular or renin responses to blood loss.


MATERIALS AND METHODS

Experiments were performed in male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 280–380 g at the time of stereotaxic surgery. 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), with standard laboratory chow and tap water available ad libitum. All experimental procedures were approved by the University Committee on Animal Research.

Surgical procedures. Surgical procedures were performed under anesthesia (pentobarbital sodium 25 mg/kg with chloral hydrate 128 mg/kg ip) in sterile conditions. Lesioned...
rats received bilateral stereotoxic injections of 200–400 nl ibotenic acid (Research Biochemicals International, Natick, MA; 10 µg/ml in 0.1 M phosphate buffer, pH 7.4) into the PVH (bregma −1.7 mm, lateral ± 1.5 mm at a 7.5° angle, dura −8.0 mm). The ibotenate was injected over a 10-min period using a 500-nl Hamilton syringe, and the needle was then left in place for an additional 10-min period before withdrawal. In sham-lesioned rats, the injection needle was lowered to the same location, but no drug was administered. Femoral arterial catheters were surgically implanted 5–7 days after stereotoxic surgery, as previously described (36).

Experimental procedures. Experiments were performed after at least 4 days had elapsed since catheter implantation. During experiments, each rat was placed in a 10-inch high, 7.5-inch inner diameter Plexiglas 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. 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 between the time at which the catheter extensions were connected and data collection began on the morning of the experiment. Food and water were not available while the rat was in the recording cage.

Blood pressure was recorded via a Century CP01 transducer and Beckman Sensormedics R611 Dynograph recorder. Mean arterial pressure was obtained by electronic integration of the arterial pressure signal.

Hemorrhage protocol. All experiments began in the morning, with the first blood withdrawal performed at 0945–1115. Na⁺ heparin (150 U in 150 µl) was injected into the arterial catheter 15 min before the beginning of the hemorrhage procedure to prevent clotting during blood withdrawal. During the hemorrhage procedure, blood was withdrawn from the arterial catheter at a rate of 1.6 ml/kg body weight per min during an initial 4-min hemorrhage and three subsequent 2-min hemorrhages, each separated by an 8-min observation period during which arterial pressure was recorded. This resulted in a total blood loss of 16 ml/kg body weight over 34 min. An additional 1.2-ml blood sample was collected at 90 min after initiation of the hemorrhage procedure. This sample and blood collected from each of the four hemorrhages were used for hematocrit, plasma Na⁺ and K⁺, renin, and corticosterone determinations. Arterial blood pressure was continuously recorded, except during the periods of blood withdrawal. The rats were returned to housing with food and water available after collecting the ∼90 min blood sample. On the following morning, a final 300-µl blood sample was withdrawn for hematocrit measurement.

Plasma renin, corticosterone, electrolyte, and hematocrit determinations. Blood samples for renin determinations were collected with 0.26 M EDTA solution (30 µl/ml), and the plasma was frozen at −20°C until assay. PRA (rate of formation of angiotensin I from endogenous angiotensinogen) and plasma renin concentration (PRC; rate of formation of angiotensin I in the presence of excess angiotensinogen provided by nephrectomized rat plasma) were determined by radioimmunoassay for angiotensin I and expressed as nangrams angiotensin I formed per milliliter plasma per hour of incubation at 37°C, pH 6.5, as previously described (35). Serum corticosterone concentration was measured by radioimmunoassay using a commercially available kit (Immunochem double-antibody corticosterone radioimmunoassay kit; ICN Biomedicals, Costa Mesa, CA).

For hematocrit and plasma electrolyte determinations, 70- to 90-µl blood samples were collected in triplicate into ammonium-heparin-coated glass microcapillary tubes. After centrifugation for hematocrit determination, the tubes were broken at the interface of plasma and packed cells and the plasma was collected and frozen at −20°C until flame photometry (ILI443; Instrumentation Laboratory, Lexington, MA) for plasma Na⁺ and K⁺ concentration.

Histology. After collection of the final blood sample on the day after the hemorrhage procedure, the rat was anesthetized (pentobarbital sodium 25 mg/kg with chloral hydrate 128 mg/kg, into the arterial catheter) and perfused transcardially with 4% paraformaldehyde in 0.1 M acetate buffer, pH 6.5, followed by 4% paraformaldehyde in 0.1 M borate buffer, pH 9.3. After perfusion, the brain was collected, postfixed for 1–2 h in 4% paraformaldehyde (pH 9.3), soaked overnight in 20% sucrose in phosphate-buffered saline, and then stored frozen at −80°C. The forebrain was sectioned (30 µm sections) through the rostral-to-caudal extent of the hypothalamus with the use of a freezing microtome. Alternate sections were immunocytochemically labeled for corticotropin-releasing factor (CRF), with a light cresyl violet counterstain. CRF immunocytochemistry was performed using Peninsula Laboratories rabbit anti-CRF antiserum (1:34,832 dilution) and immunoreagents from the Vectastain ABC Elite kit (Vector Laboratories) (29). The remaining sections were stained with cresyl violet only.

Bilateral ibotenate lesions of the parvicellular PVH were judged to be complete if all of the following criteria were met: 1) the injection needle tracks entered or contacted the main body of the PVH; 2) cresyl violet-stained tissue showed extensive microgliosis of the entire parvicellular region (except for the periventricular zone) with few healthy PVH neurons other than those that appeared to be magnocellular; and 3) the number of CRF-positive neurons, as estimated by approximate cell counts across the rostral-to-caudal extent of the PVH, was ≥20% of normal (sham lesioned). Of the 16 rats that received ibotenate injections, five were judged to have complete lesions, two had lesions placed distant from the PVH, and three were lost due to catheter failure or poor perfusion. The remaining six rats had partial PVH lesions and served as anatomic controls (see RESULTS).

Statistical analysis. To evaluate the effect of IV ibotenate lesion, statistical comparisons of data from rats with complete lesions versus sham lesions were performed by two-way repeated-measures (RM) ANOVA across all time points, followed by Student-Newman-Keuls individual comparisons. Statistical comparison of data from rats with partial lesions with data from rats with complete or partial lesions (Student-Newman-Keuls analysis based on multifactor RM ANOVA of all 3 groups) was restricted to the control and hemorrhage periods because of missing data in four partially lesioned rats during the recovery period. Corticosterone data were log10 transformed to adjust for unequal variance across time.

All data are expressed as the mean ± SE.

RESULTS

Histology of PVH lesions. Histology of brain sections from the eight rats in the sham-lesioned group showed evidence of needle tracks within or in close proximity to the PVH. However, the PVH appeared otherwise intact (Fig. 1A).

Five rats were judged to have complete bilateral lesions of the parvicellular PVH. In each case, the injection needle track was visualized within or in contact with the main body of the PVH. Light microscopy evaluation of cresyl violet-stained sections showed extensive microgliosis and loss of neurons in all parvi-
cellular regions of the PVH with the exception of the neurons immediately adjacent to the third ventricle in the periventricular zone. In contrast, the magnocellular neurons appeared to be largely intact in most (4 of 5) rats. However, there was extensive cytoarchitectural remodeling, such that the magnocellular neurons appeared less densely packed and had migrated to a more medial position (Fig. 1B). Selective destruction of parvicellular neurons, with cytoarchitectural remodeling and sparing of the magnocellular neurons, has been previously reported by Herman and Weigand (14) for PVH ibotenate lesions (14). CRF immunocytochemistry was performed as an additional marker for parvicellular neurons (Fig. 1). The number of CRF-positive neurons visualized throughout the rostral-caudal extent of the PVH was reduced by ~80–90% compared with sham-lesioned controls. All rats with complete lesions of the parvicellular PVH showed neuronal damage and gliosis in the region dorsal and lateral to the PVH, with relatively little damage ventral, rostral, or caudal to the PVH. There was no evidence of damage to the dorsomedial or ventromedial hypothalamic nuclei, and only two of five rats with complete lesions showed evidence of damage to the anterior hypothalamic nucleus.

In brain sections from six of the ibotenate-injected rats, needle tracks were visualized near the PVH, but ibotenate injection resulted in only partial (~50%) destruction of the parvicellular PVH. However, the pattern of microgliosis dorsal and lateral to the PVH in rats with partial lesions was similar to that of complete PVH lesions. Data from this group of rats, therefore, served as an anatomic control for the nonspecific effects of ibotenate damage to regions adjacent to the PVH.

Basal values in PVH-lesioned and sham-lesioned rats. PVH-lesioned rats did not differ from sham-lesioned rats or rats with partial lesions for body weight or plasma Na⁺ and K⁺ concentration (Table 1). However, basal (prehemorrhage) hematocrit was slightly lower in PVH-lesioned rats than sham-lesioned rats...
Basal values for mean arterial pressure, heart rate, plasma renin activity, and plasma corticosterone concentration also did not differ between lesioned and sham-lesioned rats (see Figs. 3–5).

Response to hemorrhage. During the hemorrhage period, blood was withdrawn intermittently to a cumulative blood loss of 16 ml/kg over 34 min, as shown in Fig. 2. PVH ibotenate lesion did not alter the effect of hemorrhage on either mean arterial blood pressure or heart rate (ANOVA, \( P > 0.40 \); Fig. 3). In all three groups of rats, MAP remained within the normotensive range for blood losses of up to 13 ml/kg and decreased significantly after 16 ml/kg blood loss. All three groups of rats also showed a significant bradycardia after 16 ml/kg blood loss. Furthermore, there was no significant difference between sham-lesioned rats and rats with complete bilateral lesions for the magnitude of decrease in blood pressure or heart rate after 16 ml/kg blood loss or for blood pressure and heart rate recovery.

PRA and PRC each increased significantly in all three groups of rats after 6–16 ml/kg blood loss (Fig. 4). Both PRA and PRC determinations were performed in view of evidence that plasma angiotensinogen (renin substrate) concentration is decreased by lesions of the PVH region (19). The increases in PRA and PRC were nearly identical in PVH-lesioned rats compared with rats with partial (<50%) PVH lesions. There was no statistically significant overall difference in PRA values for PVH-lesioned versus sham-lesioned rats (RM ANOVA across all time points, \( P > 0.10 \) for lesion and lesion-by-hemorrhage interaction effects), although the PRA increase was greater in sham-lesioned rats than in rats with either complete or partial PVH lesions during the initial hypotensive phase of the hemorrhage response (at +32 min; Newman-Keuls, \( P < 0.05 \)). This attenuation of the PRA increase was not specifically due to loss of parcellular PVH neurons, because it occurred in rats with both complete and incomplete lesions, and was probably caused by deficits in plasma renin substrate concentration. The effect of hemorrhage on PRC (rate of angiotensin formation in the presence of excess exogenous renin substrate) did not differ between sham-lesioned rats and rats with either complete or partial lesions, indicating that destruction of parcellular PVH neurons does not alter the increase in plasma renin levels in response to graded blood loss.

In contrast to the lack of effect of PVH lesion on the blood pressure, heart rate, and renin responses to blood loss, PVH lesion significantly attenuated the corticoste-
rone response to hemorrhage (Fig. 5). Plasma corticosterone concentration was increased by hemorrhage in sham-lesioned rats and rats with incomplete PVH lesions \((P < 0.01\), but not in PVH-lesioned rats \((P = 0.16\)). The corticosterone response to hemorrhage was significantly reduced in PVH-lesioned rats compared with sham-lesioned rats \((\text{RM ANOVA}, P < 0.05)\).

Both lesioned and sham-lesioned rats showed a progressive decrease in hematocrit that began during the hemorrhage period and continued over the following 24 h \((P < 0.001; \text{Fig. 6})\). PVH-lesioned rats had a lower hematocrit than sham-lesioned rats both before and after hemorrhage \((P < 0.02)\). However, the extent of posthemorrhage hemodilution did not differ between PVH-lesioned and sham-lesioned rats. By 24 h posthemorrhage, percent hematocrit had decreased by 12 ± 1 in sham-lesioned rats and by 10 ± 1 in PVH-lesioned rats, indicating that vascular refilling (blood volume restitution) was not substantially altered by PVH lesion. Water intake during the 24 h after hemorrhage also did not differ between PVH-lesioned rats \((60 ± 7 \text{ ml})\) and sham-lesioned rats \((55 ± 5 \text{ ml})\).

**DISCUSSION**

In this study, ibotenate lesion of the PVH resulted in extensive loss of parvicellular neurons. Ibotenate lesion significantly attenuated the corticosterone response to blood loss, providing functional evidence for the efficacy of the lesion procedure. The attenuated corticosterone response was most likely due to loss of the CRF-containing neurons and confirms that the parvicellular...
PVH plays an essential initiating role in the glucocorticoid response to blood loss. However, bilateral parvicellular PVH lesions did not alter the effects of hemorrhage on blood pressure, heart rate, or plasma renin levels.

There are two phases in the acute response to a progressive hemorrhage, which are dependent on the volume of blood lost. During the initial phase (nonhypotensive hemorrhage), mean arterial pressure is maintained at normotensive levels despite the decrease in blood volume. This phase is typified by increased systemic vascular resistance and increased renal nerve activity (33). PRA also increases significantly as a consequence of sympathetic activation (4). The second phase (hypotensive hemorrhage) ensues when blood loss reaches a critical volume. During this phase, arterial pressure falls precipitously as a consequence of systemic vasodilation. The hypotensive phase is accompanied by bradycardia and a profound decrease in renal sympathetic nerve activity, yet adrenomedullary catecholamine release increases significantly. PRA is further increased as a consequence of reduced renal perfusion pressure and increased adrenomedullary catecholamine release (33). Thus heart rate, renal nerve activity, and adrenomedullary responses differ markedly between nonhypotensive and hypotensive hemorrhage, indicating that different central nervous system pathways are activated during these two phases.

The present study evaluated both phases of the acute compensatory response to graded hemorrhage. If parvicellular PVH projections to sympathetic preganglionic neurons were to play a critical role during blood loss, then PVH lesion would be expected to compromise the ability to maintain arterial pressure and increase renin release during the nonhypotensive phase of the response to hemorrhage, when sympathetic activation is most prominent. However, bilateral ibotenate PVH lesions did not alter the ability to maintain normotensive arterial pressure during blood loss, the volume of blood that could be withdrawn before a significant decrease in pressure occurred, or the increase in PRA and PRC during nonhypotensive hemorrhage. Thus the parvicellular PVH plays no essential role in the reflex compensations that defend arterial pressure and increase renin release during the initial phase of the response to blood loss. Furthermore, the minimum arterial pressure achieved after a 16 ml/kg blood loss, the bradycardia and further increase in PRC that accompany hypotensive hemorrhage, and the rate of recovery of arterial pressure and heart rate after blood loss were unaffected by ibotenate lesion of the parvicellular PVH. The lack of effect of PVH ibotenate lesion on the responses to hypotensive hemorrhage is consistent with the observation of Darlington et al. (11) that knife cut deafferentation of the PVH prevents the increase in ACTH but does not affect the heart rate, plasma catecholamine, or PRA response to a rapid severely hypotensive hemorrhage.

The acute compensatory responses to blood loss are followed by gradual replacement of the lost blood volume by transcapillary fluid shifts from the interstitial spaces into the plasma compartment. The process of plasma volume expansion occurs over a time course of 12–24 h after hemorrhage and results in hemodilution and a consequent reduction in hematocrit. The rate and extent of plasma volume replacement depend on a complex profile of endocrine, metabolic, and vascular responses to blood loss (10, 36). Studies performed in anesthetized fasted dogs indicate that a significant increase in plasma glucocorticoid concentration is required to permit complete plasma volume replacement (30). In the present study, PVH ibotenate lesion significantly attenuated the hemorrhage-induced increase in plasma corticosterone concentration. However, hematocrit decreased similarly in sham-lesioned and ibotenate-lesioned rats at 1 h and at 24 h posthemorrhage, indicating that plasma volume replacement was not altered by parvicellular PVH lesion. A similar observation was made by Bealer (3), who showed that electrolytic lesions of the preoptic recess abolished the corticosterone response to a rapid 13 ml/kg hemorrhage but did not attenuate plasma volume replenishment during the 24-h posthemorrhage period. Thus in the conscious rat, with food and water freely available before and after the hemorrhage procedure, a significant increase in plasma glucocorticoid concentration may not be necessary for posthemorrhage plasma volume expansion.

There is evidence to indicate that parvicellular PVH neurons mediate inhibition of renal sympathetic nerve activity when blood volume is acutely expanded (13, 22). In the present study, parvicellular PVH lesions did not alter the increase in PRC during the normotensive phase of blood loss, during which time renal nerve activity is known to increase in intact conscious animals (33). Because renin release is stimulated by increased renal nerve activity, any deficit in the increase in renal nerve activity would be expected to result in an attenuated renin response to nonhypotensive hemorrhage. Thus our data provide no evidence to suggest that the PVH plays an essential role in mediating the increased renal sympathetic nerve activity that occurs when blood volume is acutely reduced. However, basal hematocrit was slightly lower in PVH-lesioned rats than in sham-lesioned controls. This suggests that plasma volume may have been modestly expanded in rats with PVH ibotenate lesions and implies that the parvicellular PVH may modulate blood volume under resting conditions.

In summary, although Fos immunolabeling studies demonstrate that parvicellular PVH neurons are activated by decreased blood volume both in the presence and absence of decreased arterial pressure (5), the functional role of this neuronal population in cardiovascular regulation remains uncertain. As with any study involving chronic neuronal lesions, it is possible that, in the ibotenate-lesioned rats, another neuronal population compensated for the lesion and assumed the functions normally performed by the parvicellular PVH. Nonetheless, our data indicate that while the parvicellular PVH is essential for the corticosterone response to blood loss, it is not essential for the cardiovascular or...
renin responses during either the compensated (nonhypotensive) or hypotensive phases of hemorrhage.

Perspectives

PVH lesions attenuate hypertension in several experimental models, including most models of sodium- and volume-dependent hypertension (7, 12, 15, 27, 39). In most cases the ameliorative consequences of PVH lesion have been attributed to loss of the parvicellular component, rather than to loss of magnocellular vasopressin neurons. This implies that chronic parvicellular PVH dysfunction can disrupt fluid/electrolyte status and arterial pressure regulation. There is only sparse evidence, however, to support the premise that the parvicellular PVH normally plays an essential role in the autonomic responses to cardiovascular challenge. Perhaps the most compelling evidence that parvicellular PVH neurons modulate the response to altered blood volume is that of Lovick et al. (22) and Haselton et al. (13), in which excitotoxin lesions of the parvicellular PVH were shown to attenuate the renal vasodilation and inhibition of renal nerve activity normally elicited by acute plasma volume expansion. However, these results were obtained in anesthetized animals and must therefore be extrapolated to the conscious state with caution, as anesthesia is known to alter the control of renal nerve activity by the PVH (18). In contrast, parvicellular PVH neurons are known to serve as a necessary relay for the cardiovascular and renin responses to certain behavioral stressors. Ibotenate lesion of the PVH reduces the tachycardiac response to foot shock (6) and prevents the increase in PRC that normally accompanies a conditioned fear response (32). This suggests that the primary function of cardiovascular information relayed to parvicellular PVH autonomic projection regions may be to modulate behavioral effects on autonomic function.

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