Role of the locus ceruleus in baroreceptor regulation of supraoptic vasopressin neurons in the rat

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Received 27 May 1999; accepted in final form 9 February 2000.

Grindstaff, Ryan J., Regina R. Grindstaff, Margaret J. Sullivan, and J. Thomas Cunningham. Role of the locus ceruleus in baroreceptor regulation of supraoptic vasopressin neurons in the rat. Am J Physiol Regulatory Integrative Comp Physiol 279: R306–R319, 2000.—The goal of this study was to identify the source of baroreceptor-related noradrenergic innervation of the diagonal band of Broca (DBB). Male Sprague-Dawley rats underwent sinoaortic denervation (SAD, n = 13) or sham SAD surgery (n = 13). We examined Fos expression produced by baroreceptor activation and dopamine-β-hydroxylase immunofluorescence in hindbrain regions that contain noradrenergic neurons. Baroreceptors were stimulated by increasing blood pressure >40 mmHg with phenylephrine (10 μg · kg⁻¹ · min⁻¹ iv) in sham SAD and SAD rats. Controls were infused with 0.9% saline. Only the locus ceruleus (LC) demonstrated a baroreceptor-dependent increase in Fos immunoreactivity in dopamine-β-hydroxylase-positive neurons. In a second experiment, normal rats received rhodamine-labeled microsphere injections in the DBB (n = 12) before phenylephrine or vehicle infusion. In these experiments, only the LC consistently contained Fos-positive cells after phenylephrine infusion that were retrogradely labeled from the DBB. Finally, we lesioned the LC with ibotenic acid and obtained extracellular recordings from identified vasopressin neurons in the supraoptic nucleus. LC lesions significantly reduced the number of vasopressin neurons that were inhibited by acute baroreceptor stimulation. Together, these results suggest that noradrenergic neurons in the LC participate in the baroreflex activation of the DBB and may thus be important in the baroreflex inhibition of vasopressin-releasing neurons in the supraoptic nucleus.

Fos; supraoptic nucleus; sinoaortic deafferentation

BODY FLUID HOMEOSTASIS is regulated through the coordinated interactions of neural, behavioral, and hormonal systems. Vasopressin plays a key role in maintaining this fluid balance. Vasopressin is synthesized and released from magnocellular neurons located in the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) in the hypothalamus, which project to the posterior pituitary. The SON and PVN also contain a separate population of magnocellular neurons that release the hormone oxytocin into the peripheral circulation (53). A number of physiological stimuli have been identified that selectively regulate the activity of vasopressin or oxytocin neurons in the SON (52, 53). One such stimulus is the activation of peripheral baroreceptors by an acute increase in blood pressure (BP), which selectively inhibits the spontaneous activity of vasopressin neurons. In fact, this response is so reliable that it has traditionally been used to discriminate vasopressin neurons from oxytocin neurons during in vivo electrophysiology experiments (52, 53). Although the BP sensitivity of vasopressinergic neurosecretory cells in the SON has been recognized for some time, the nature of the neural pathway that brings baroreceptor information to the SON has not been defined.

The results of several studies indicate that the diagonal band of Broca (DBB), which is located in the telencephalon, is a critical component of this pathway. The DBB contains neurons that are activated by stimulating peripheral baroreceptors (31). Electrical stimulation of the DBB selectively inhibits SON vasopressin neurons (30), and the DBB- and baroreceptor-evoked inhibition of SON vasopressin neurons are mediated by GABA A receptors (30, 56). Ibotenic acid lesions of the DBB significantly decrease the number of SON vasopressin neurons that are inhibited by baroreceptor stimulation (11). On the basis of this evidence, it appears that the DBB is an integral part of the neural pathway bringing baroreceptor information to the SON. It was subsequently demonstrated that the DBB does not project directly to the SON but, rather, to a region adjacent to the SON, the perinuclear zone (PNZ) of the SON (29, 58). The existence of inhibitory interneurons in the PNZ is supported by electrophysiology experiments showing that excitotoxic lesions of the PNZ, which spare the excitotoxin-resistant SON neurons, block the DBB- and baroreceptor-evoked inhibition of SON vasopressin neurons (45). Thus, although two forebrain components of this pathway have been successfully identified, it has not been determined how baroreceptor information reaches the DBB.

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Several studies indicate that the baroreceptor projection to the DBB may involve noradrenergic neurons. For example, the depletion of DBB norepinephrine significantly decreases the number of vasopressin-releasing SON neurons that are inhibited by baroreceptor stimulation (12). Likewise, injections of norepinephrine into the DBB selectively inhibit the activity of vasopressin neurons in the SON (13). In addition, microdialysis studies demonstrate that the norepinephrine content of the DBB is significantly increased during drug-induced baroreceptor activation (5). Although these results indicate that a noradrenergic mechanism in the DBB is involved in the baroreflex inhibition of SON vasopressin neurons, the source of the norepinephrine remains unresolved.

Three regions in the medulla and the pons contain noradrenergic neurons and innervate the DBB: the A2 region of the nucleus of the solitary tract (NTS), the A1 region of the caudal ventrolateral medulla (CVL), and the locus ceruleus (LC) (32, 38, 55, 59). In vivo electrophysiology experiments and studies using the c-fos technique to assess neural activation indicate that each of these areas contains neurons that are activated by changes in BP (3, 8, 9, 26, 41, 42, 44, 47). The majority of the neurons in each of these three noradrenergic areas is activated by decreases in BP (22, 25, 26, 54). These data appear to argue against any of these noradrenergic cell groups providing the DBB with excitatory input related to baroreceptor activation. However, some studies do suggest that these regions may contain a population of neurons that are activated by baroreceptor stimulation (8, 9, 41, 42, 44, 46) as well. Therefore, it is possible that one or more of these regions contain a small population of noradrenergic neurons that project to the DBB and are activated by peripheral baroreceptor stimulation.

One possible source of baroreceptor-related noradrenergic projections to the DBB is the LC. Banks and Harris (4) demonstrated that thermal and microlesions (6-OHDA) lesions of the LC, a noradrenergic nucleus located in the dorsal pons, block the baroreceptor-induced inhibition of SON vasopressin neurons. A more recent study (19) disputes the findings of Banks and Harris, attributing their results to the destruction of fibers of passage. The A2 cell group located in the NTS may provide the DBB with baroreceptor-related innervation (55), although this hypothesis has not been critically tested. Studies on the A1 region of the ventrolateral medulla (18) do not support a strong role for this region in the baroreceptor-mediated inhibition of vasopressin neurons in the SON. Thus the source of noradrenergic input to the DBB remains unresolved.

The purpose of the present study was to determine which area in the pons or medulla provides the DBB with the baroreceptor information that is necessary for the baroreflex inhibition of vasopressin neurons in the SON. In experiment 1 we investigated whether any of the noradrenergic regions that project to the DBB contain neurons activated by baroreceptor stimulation. Next, in experiment 2, we tested whether the baroreceptor-sensitive neurons in these areas project to the DBB. On the basis of the results of experiments 1 and 2, we conducted experiment 3, which consisted of electrophysiology experiments, in rats with excitotoxic lesions of the LC to determine the functional importance of the LC in the pathway that mediates the baroreceptor-mediated inhibition of SON vasopressin neurons.

MATERIALS AND METHODS

Animals

Experiments were conducted on male Sprague-Dawley rats weighing 250–350 g (Harlan, Indianapolis, IN). Animals were maintained in a temperature-controlled environment on a 12:12-h light-dark cycle. All surgical procedures and experimental protocols were carried out as approved by the Institutional Animal Care and Use Committee at the University of Missouri-Columbia in accordance with the guidelines of the Public Health Service, American Physiological Society, and Society for Neuroscience.

Experiment 1: Effect of 2 h of Phenylephrine-Induced Hypertension on Fos Expression in Sham and Sinusoidal-Deafferented Rats

Sinoaortic deafferentation. Rats underwent bilateral sinoaortic deafferentation (SAD) as described by Kreiger (35). Briefly, rats were anesthetized with ketamine (1 ml/kg ip), and a midline incision was made in the ventral surface of the neck. The muscles were retracted, and the carotid artery was identified. The aortic depressor nerve was severed, and the superior laryngeal nerve was transected at its convergence with the vagus. The superior cervical sympathetic nerve was transected caudal to the superior cervical ganglion. The carotid bifurcation was stripped of adventitia and nerve fibers and painted with 10% phenol in ethanol. This procedure was then repeated on the contralateral side. The sham surgical operation involved only exposing the relevant nerves and arteries. On awakening, SAD animals were given chlorisondamine (0.9 mg sc) and flunixin (Banamine, 2 mg/kg sc), a ganglionic blocker and a nonsteroidal anti-inflammatory, respectively, to aid in postsurgical recovery. Animals were monitored daily for weight gain and general welfare.

Rats were allowed ≥12 days for recovery and prepared with femoral artery and venous catheters for the measurement of BP and drug infusions, respectively. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and catheters constructed from fused PE-10 and PE-50 tubing were inserted into the femoral artery and femoral vein. The distal ends of the catheters were tunneled subcutaneously and externalized at the nape of the neck. While the animals were anesthetized, the efficacy of the SAD surgery was tested by examining the changes in heart rate (HR) in response to infusions of the peripherally acting α-adrenoceptor agonist phenylephrine (10 μg · kg⁻¹ · min⁻¹ iv) and sometimes sodium nitroprusside (5 μg/kg iv). Successfully denervated animals did not display a reflex change in HR in response to infusions of the peripherally acting α-adrenoceptor agonist phenylephrine (10 μg · kg⁻¹ · min⁻¹ iv) and sometimes sodium nitroprusside (5 μg/kg iv). Successfully denervated animals did not display a reflex change in HR in response to increases or decreases in BP of ≥40 mmHg. The arterial catheters were then filled with a saturated sucrose solution containing heparin (1,000 U/ml). The venous catheters were filled with heparinized 0.9% sodium chloride. The rats were returned to their individual cages and allowed an additional 2 days to recover. Sham and SAD rats were randomly divided into two experimental groups: sham-phenylephrine (n = 6), sham-saline (n = 7), SAD-phenylephrine (n = 8), and SAD-saline (n = 5).
Infusion protocol. On the day of the infusion experiment, the arterial catheter was connected to a pressure transducer for recording of mean arterial pressure (MAP) and HR on a computer running Atlantis data acquisition software. After BP and HR had stabilized (~15–60 min), baseline recordings were acquired for ≥ 20 min before the start of the infusion protocol. Infusion pumps (model 975, Harvard) were used to deliver isotonic saline vehicle or phenylephrine (10 μg·kg⁻¹·min⁻¹) through the femoral venous catheter for 2 h at a flow rate of ~0.0096 ml/min. This rate of infusion was periodically adjusted to maintain an MAP increase of 40–50 mmHg in phenylephrine-infused rats. Infusion rate was likewise adjusted in the vehicle-infused animals, inasmuch as they were being infused with the same infusion pump. Animals were continuously monitored during the infusion periods to ensure that there were no signs of behavioral arousal or distress. At the end of the 2-h infusion period, rats were anesthetized with pentobarbital sodium (50 mg/kg iv) and immediately perfused transcardially with 0.3–0.5 liter of 0.1 M PBS followed by 4% paraformaldehyde in 0.1 M PBS (4°C, 0.5 liter). The brains were removed and placed in a vial containing 30% sucrose in PBS for 2 days for cryoprotection. A cryostat was used to section brains into 40-μm coronal sections that were collected into a vial containing 0.1 M PBS. The sections were processed for Fos and dopamine-β-hydroxylase (DBH) immunocytochemistry.

Fos immunocytochemistry. Free-floating sections from each brain were processed for Fos immunocytochemistry by use of a rabbit polyclonal anti-Fos antibody (Oncogene Science, Cambridge, MA). First, the sections were incubated in 0.3% hydrogen peroxide in distilled water for 30 min at room temperature, then they were rinsed for 30 min in 0.1 M PBS. Sections then were incubated for 2 h at room temperature in PBS diluent [3% normal horse serum (Sigma Chemical, St. Louis, MO) in 0.1 M PBS containing 0.25% Triton X-100]. After this step, the sections were incubated with the rabbit polyclonal anti-Fos antibody, diluted to 1:30,000 in PBS diluent, for 48 h at 4°C. After two 30-min rinses in 0.1 M PBS, the sections were incubated with a biotinylated horse anti-rabbit IgG (Vector, Burlingame, CA), diluted to 1:200 in PBS diluent, for 2 h at room temperature. The tissue then was reacted with an avidin-peroxidase conjugate (ABC Vectastain kit, Vector) and PBS containing 0.04% 3,3′-diaminobenzidine hydrochloride and 0.04% nickel ammonium sulfate (both from Sigma Chemical). This reaction was terminated by rinsing the tissue with 0.1 M PBS.

DBH immunofluorescence. After the sections were processed for Fos immunoreactivity, they were again incubated in PBS diluent for 1 h at room temperature. Then sections were incubated with a mouse monoclonal DBH antibody (Chemicon International, Temecula, CA), diluted 1:1,000 in PBS diluent, for 48 h at 4°C. After two 30-min rinses with 0.1 M PBS, the sections were incubated with a biotinylated horse anti-rabbit IgG (Vector, Burlingame, CA), diluted to 1:200 in PBS diluent, for 2 h at room temperature. The tissue then was reacted with an avidin-peroxidase conjugate (ABC Vectastain kit, Vector) and PBS containing 0.04% 3,3′-diaminobenzidine hydrochloride and 0.04% nickel ammonium sulfate (both from Sigma Chemical). This reaction was terminated by rinsing the tissue with 0.1 M PBS.

Experiment 2: Colocalization of Phenylephrine-Induced Fos Expression in Brain Stem Neurons Retrogradely Labeled From the DBB

DBB injections. Normal rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and injected with 300 nl of rhodamine-labeled fluorescent latex microspheres (LumaFluor, Naples, FL) into the vertical limb of the DBB (level skull coordinates: 0.6 mm anterior to bregma and 7.3 mm ventral to dura at the midline). Each injection was made through 30-gauge hypodermic tubing connected to a 5-μl microsyringe (Hamilton, Reno, NV) over a 3-min period. The injector was left in place for 5 min and then was withdrawn over an additional 5-min period. At least 1 wk after microinjections into the DBB, animals were anesthetized with pentobarbital sodium (50 mg/kg ip) for implantation of indwelling catheters as described above.

The DBB-injected rats were divided randomly into two experimental groups: phenylephrine-infused and saline vehicle-infused animals. These groups were then infused, and BP and HR were recorded as described for experiment 1. After the infusions, the rats were anesthetized and perfused, and their brains were sectioned and processed for Fos and DBH immunoreactivity, as previously described. Sections were examined using light microscopy to identify Fos-positive cells, verify injection sites within the DBB, and analyze patterns of retrograde labeling.
D9H immunoﬂuorescence was used to anatomically deﬁne the rostral-caudal levels of the LC. Neurons containing rhodamine-labeled microspheres were identiﬁed as projecting to the DBB. D9H-positive neurons that contained rhodamine beads and expressed Fos were identiﬁed. Regions of the brain were deﬁned, and images were collected as previously described, with the exception of the IVL, which was not included in the analysis because it did not contain cells retrogradely labeled from the DBB. The number of sections used to analyze each region was the same as for experiment 1. Each area was then analyzed for the presence of Fos-positive retrogradely labeled neurons. The total number of retrogradely labeled, Fos-positive neurons in each rat was determined for each region. Each retrogradely labeled, Fos-positive neuron was also examined for D9H immunoﬂuorescence.

Experiment 3: Effect of Excitotoxic Lesions of the LC on Baroreceptor-Mediated Inhibition of SON Neurons

LC injections. Male Sprague-Dawley rats (300–360 g body wt) were anesthetized with pentobarbital sodium (50 mg/kg ip) before surgery. After placement in a stereotaxic frame, three unilateral stereotaxic injections of ibotenic acid (200 nl, 5 μg/μl dissolved in 0.1 mM PBS) were each delivered into the LC (coordinates: 10.6, 10.3, and 10.0 mm caudal to bregma, 1.2 mm lateral to bregma, and 7.5 mm ventral to bregma). This volume was injected over a 5-min period by use of a 30-gauge steel injector connected to a 5-μl Hamilton syringe. After each injection, the injector was left in the tissue for 4–5 min, then it was withdrawn over a second 5-min period. Although such unilateral lesions were made in the left or right LC, lesions were made primarily in the left LC to facilitate recording from the more accessible ipsilateral SON (see Electrophysiology protocol). Control injections (sham lesions) were made by injecting the same volume of PBS vehicle into the LC by use of the same coordinates described for the lesion. Lesions were also made in areas immediately adjacent to the LC, including the subceruleus region ventral to the LC and the medial PBN lateral and dorsal to the LC. Ibotenic acid injections penetrating the fourth cerebral ventricle resulted in immediate respiratory failure. Animals were monitored daily after the surgery to ensure their general health and were allowed a 4–5-day recovery period before electrophysiology experiments (11, 45).

Electrophysiology protocol. Rats that were lesioned or sham lesioned were anesthetized with pentobarbital sodium (50 mg/kg ip), and catheters were placed in the left femoral artery and vein for BP and phenylephrine infusion, respectively. Another catheter was placed in the left jugular vein for supplementary infusion of pentobarbital sodium to maintain anesthesia at a level sufﬁcient to suppress a withdrawal reﬂex (2–5 mg/kg every 20–30 min as needed). Next, a transpharyngeal surgical approach was used to expose the SON and pituitary (11–13, 45). A pressure foot was placed on the ventral surface of the hypothalamus to stabilize the area surrounding the SON. A bipolar stimulating electrode was placed in the posterior pituitary to antidromically activate neurosecretory neurons in the SON. The criteria used for antidromic activation included a constant-latency, all-or-none response at threshold and evidence of collision cancellation between spontaneously occurring action potentials and action potentials evoked antidromically from the posterior pituitary (11–13, 45).

Extracellular action potentials from SON neurons were recorded using glass micropipettes ﬁlled with 3 M sodium chloride. The signals were ampliﬁed (model 2400 extracellular preampliﬁer, Dagan, Minneapolis, MN), ﬁltered, and relayed through a window discriminator to an analog-to-digital converter (CED 1401, Cambridge Instruments) and then to a Pentium computer running Spike2 data acquisition software (Cambridge Instruments). Antidromic activation was produced using a posterior pituitary bipolar electrode discharging single 1-Hz supraphreshold current pulses (0.1-ms duration, intensity ≤10 mA). BP and HR were recorded using Spike2 data acquisition software. Phasic, antidromically identiﬁed SON neurons ipsilateral to the stereotaxic injections were tested for baroreceptor sensitivity by elevating arterial BP with injections of phenylephrine (10 μg/10 μl iv). Extracellular recordings were made from the SON ipsilateral to the injected LC, because previous studies indicate that experimental manipulations of the LC affect only ipsilateral SON neurons (4, 19).

Only antidromically activated neurons exhibiting phasic patterns of activity characteristic of vasopressin neurons (11, 13, 45) were tested. Peripheral baroreceptors were stimulated by raising BP ≥40 mmHg with a bolus injection of phenylephrine (10 μg/10 μl). Previous studies demonstrated that this stimulus will inhibit 95–100% of phasic neurons in the SON (11–13, 45). Each phasic neuron tested was allowed to discharge at least two spontaneous phases before an injection of phenylephrine. Phenylephrine was injected in the 5–15 s immediately after the initiation of a spontaneous phase or during a sustained phasic burst. A cell was determined to be sensitive to the increase in BP if its activity ceased during the 20 s immediately after the onset of a BP increase (11, 13, 45). Cells were tested for baroreceptor responsiveness no more than ﬁve times. As many as six phasically active neurons were recorded from any one animal. The ﬁring properties of each cell and the characteristics of each BP change were statistically analyzed.

Histology. After the conclusion of the electrophysiological experiments, each rat was given an overdose of pentobarbital sodium (50 mg/kg iv) and perfused transcardially with PBS and then with 4% paraformaldehyde. Their brains were subsequently removed and placed in a vial containing 30% sucrose-PBS solution for cryoprotection. After the brains were sectioned in a cryostat at 40 μm, sections were mounted on gelatin-coated glass slides and stained with Giemsa (Merck, Rahway, NJ). Lesioned areas were differentiated by neuronal loss and glial inﬁltration. The size of the lesion was determined by measuring the area of the remaining LC on the lesioned side and comparing it with the untreated contralateral side. These comparisons were made along the rostral-caudal extent of the LC. The difference between the lesioned and the untreated LC was expressed as percent decrease in the area of the LC on the lesioned side. The brains from the vehicle-injected control rats were also analyzed to determine whether the injections were made in the region of the LC.

Statistics

Values are means ± SE. Statistical analyses were performed using Abstat (Anderson-Bell) and SigmaStat (Jandel, San Rafael, CA) statistical analysis software. MAP and HR data were analyzed initially using three-way mixed-effects ANOVA. Follow-up tests involved two-way ANOVA and Student-Newman-Keuls tests to determine differences among the groups. Data from the cell counts were analyzed similarly for differences among groups for each brain region. When the data failed the test for normality of variance, they were analyzed by the nonparametric Kruskal-Wallis ANOVA and nonparametric Dunn’s follow-up test on ranks to determine differences among experimental groups. For the electrophys-
Table 1. Mean arterial pressure and heart rate of sham SAD and SAD rats before and during 2 h of isotonic saline or phenylephrine infusion

<table>
<thead>
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<th>Mean Arterial Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
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<td>Baseline</td>
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<td>Sham SAD</td>
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<tr>
<td>Isotonic saline</td>
<td>7</td>
<td>121.0±3.9</td>
</tr>
<tr>
<td>Phenylephrine</td>
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<td>112.6±4.2</td>
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<td>SAD</td>
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<td>Isotonic saline</td>
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</tr>
<tr>
<td>Phenylephrine</td>
<td>8</td>
<td>120.8±3.6</td>
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</tbody>
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Values are means ± SE. SAD, sinoaortic deafferented. *P < 0.05 vs. saline-infused controls. †P < 0.05 vs. all other infusion treatments.

RESULTS

Experiment 1: Effect of 2 h of Phenylephrine-Induced Hypertension on Fos Expression in Sham and SAD Rats

Effects of infusions on cardiovascular parameters. The MAP measurements that were obtained from unanesthetized SAD rats demonstrated more variability than those obtained from intact rats. Despite this increase in lability, there were no differences in baseline MAP or HR among the groups (Table 1). The intravenous infusions of phenylephrine produced a significant increase in BP that was comparable among SAD and sham SAD rats (Table 1). This increase in BP was associated with a significant bradycardia only in the sham SAD rats (Table 1). We also observed a smaller but statistically significant increase in BP in the SAD rats that were infused with saline compared with the sham SAD rats infused with saline (Table 1).

Table 2. Average number of Fos-positive neurons and Fos- and ΔβH-positive neurons per section by nuclei

<table>
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<tr>
<th></th>
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<tr>
<td></td>
<td>Fos</td>
<td>Fos + ΔβH</td>
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<tr>
<td></td>
<td>Saline</td>
<td>Pheny</td>
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<tr>
<td>NTS</td>
<td>29.0±7.3</td>
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<tr>
<td>CVL</td>
<td>6.7±0.7</td>
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<td>IVL</td>
<td>6.5±1.2</td>
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</tr>
<tr>
<td>PBN</td>
<td>22.4±6.3</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>cLC</td>
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<td>4.9±1.3</td>
</tr>
<tr>
<td>mLC</td>
<td>5.4±1.5</td>
<td>5.4±1.5</td>
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<tr>
<td>rLC</td>
<td>2.5±0.4</td>
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<td></td>
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Values are means ± SE. CVL, caudal ventrolateral medulla; IVL, intermediate ventrolateral medulla; cLC, caudal locus ceruleus; mLC, medial locus ceruleus; rLC, rostral locus ceruleus; NTS, nucleus of the solitary tract; PBN, parabrachial nucleus; ΔβH, dopamine-β-hydroxylase. *P < 0.05 vs. all other groups for the same area and cell type.
neurons in the LC were also positive for DbH immunoreactivity, which is consistent with studies indicating that all LC neurons are noradrenergic (14).

Fos-positive neurons in the PBN were predominantly located in the external lateral portion of the nucleus. Whereas DbH-positive neurons were observed occasionally (1 in every 2–3 40-μm sections), there were no Fos-positive neurons also labeled with DbH in this region.

**Experiment 2: Colocalization of Phenylephrine-Induced Fos Expression in Brain Stem Neurons Retrogradely Labeled From the DBB**

**Effects of infusions on cardiovascular parameters.** The effects of control and phenylephrine infusions on MAP and HR were comparable to the results of experiment 1. Infusion of isotonic saline did not alter MAP or HR throughout the 2-h infusion period (MAP: 97.2 ± 5.0 and 95.8 ± 5.0 mmHg for baseline and infusion, respectively; HR: 361.3 ± 11 and 361 ± 7.2 beats/min for baseline and infusion, respectively, all P > 0.05). The phenylephrine infusion significantly increased BP compared with baseline (MAP: 100.1 ± 0.1 and 150.6 ± 4.7 mmHg for baseline and infusion, respectively, P < 0.05). This increase in BP was accompanied by a significant reflex bradycardia compared with the controls that was maintained for the duration of the phenylephrine infusion (356.3 ± 8.8 and 264 ± 7.2 beats/min for baseline and infusion, respectively, P < 0.05).

**Retrograde labeling and Fos immunoreactivity.** Histological analysis of the injection sites indicated that there were 12 animals with injections restricted to the vertical limb of the DBB (7 phenylephrine-infused and 5 saline-infused animals). Effective spread of rhodamine microspheres from the center of the injection sites reached ~300 μm in diameter, creating a teardrop-shaped injection field (Fig. 2). Such injections typically demonstrated minimal reflux of the beads up
the cannula tract. The remaining rats \( (n = 11) \) were injected in areas surrounding the vertical limb of the DBB, including the medial septum, the lateral septum, and the adjacent preoptic region.

DBB injections produced retrograde labeling in the NTS, CVL, LC, and PBN. In the NTS, retrogradely labeled neurons were found mostly in the caudal portions, corresponding to the following coordinates: from calamus (bregma \(-14.60 \) mm) to obex (bregma \(-13.00 \) ). A moderate number of retrogradely labeled neurons were observed in the CVL in the more caudal aspects of this cell column. All regions of the LC contained neurons that were retrogradely labeled from the DBB. More labeled neurons were observed in the caudal and medial portions of the LC. In the PBN, retrograde labeling was seen in the central lateral part of the nucleus. This contrasted with the distribution of Fos-positive cells, which were located more in the external lateral part of the PBN.

In rats that were not injected in the DBB, patterns and distributions of retrograde labeling in the brain stem regions were different from the labeling observed in rats that were injected in the DBB. Injections of rhodamine-labeled microspheres that were ventral and rostral to the DBB produced almost no retrogradely labeled neurons in the NTS, CVL, PBN, or LC. Injections into the medial septum were associated with limited retrograde labeling in these regions. Injections that were caudal to the DBB in the preoptic region produced patterns of retrograde labeling that included the PBN and LC, but with only minimal labeling in the NTS and CVL. Such injections displayed no patterns of double-labeled, Fos-positive neurons in these regions. Injections that were placed in the septum lateral to the DBB displayed intense retrograde labeling in the NTS and CVL region but little retrograde labeling in the PBN and LC.

Intravenous infusion of phenylephrine resulted in a significant increase in the number of Fos-positive nuclei in the NTS, CVL, PBN, and LC, as described in experiment 1 (Table 3). However, the numbers of Fos-positive neurons that also were retrogradely labeled from the DBB differed among these regions (Table 3).

In all but one phenylephrine-infused animal, there were no neurons in the NTS expressing Fos that also were retrogradely labeled. Similarly, in four of the five animals infused with isotonic saline, no retrogradely labeled NTS neurons were observed to express Fos (Table 3). In six of the seven rats, the CVL did not have Fos-positive neurons that also were retrogradely labeled from the DBB. In one phenylephrine-infused rat, however, retrograde labeling was observed in two Fos-positive neurons in the CVL (Table 3). Basal Fos expression associated with isotonic saline infusion was not colocalized in retrogradely labeled CVL neurons. Although the observed retrogradely labeled neurons were also in the external lateral PBN and central lateral PBN, none of the Fos-positive nuclei were colocalized in neurons that were retrogradely labeled from the DBB (Table 3). Likewise, there were no retrogradely labeled, Fos-positive neurons in the control rats in the PBN.

The LC was the only region to consistently display retrogradely labeled Fos-positive neurons in phenylephrine-infused rats (Table 3). Particularly, the cLC and mLc consistently contained 4–10 double-labeled neurons per rat (Fig. 3), whereas the rLC consistently contained 1–3 double-labeled neurons per rat. Several retrogradely labeled cells were also observed that were not Fos positive. Saline-infused control rats contained no retrogradely labeled Fos-positive neurons in the LC (Table 3).

Retrogradely labeled, Fos-positive neurons were further characterized by using DβH immunofluorescence to identify noradrenergic neurons. All the neurons in the LC that were Fos positive and retrogradely labeled from the DBB were also positive for DβH immunoreactivity. Although the NTS and CVL contained DβH-immunofluorescent neurons, none of these cells were retrogradely labeled from the DBB and Fos positive.

### Table 3. Number of Fos-positive neurons in different brain regions of phenylephrine- and saline-infused intact rats

<table>
<thead>
<tr>
<th></th>
<th>Fos-Positive Neurons</th>
<th>Retrogradely Labeled Fos-Positive Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Phenylephrine</td>
</tr>
<tr>
<td>NTS</td>
<td>12.9±4.8</td>
<td>97.5±20.1*</td>
</tr>
<tr>
<td>CVL</td>
<td>3.8±0.8</td>
<td>21.6±0.8*</td>
</tr>
<tr>
<td>PBN</td>
<td>29.2±8.9</td>
<td>65.7±6.1*</td>
</tr>
<tr>
<td>cLC</td>
<td>8.4±2.3</td>
<td>30.6±5.6*</td>
</tr>
<tr>
<td>mLc</td>
<td>11.5±2.4</td>
<td>36.7±5.9*</td>
</tr>
<tr>
<td>rLC</td>
<td>4.1±1.6</td>
<td>11.8±1.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. saline-infused control group.
cleus, and medial and lateral PBN. A third group of animals injected with PBS vehicle into and in the vicinity of the LC (n = 11) exhibited no signs of neuronal damage beyond minor mechanical damage from the injector. Inclusion in this group was dependent on verification of cannula tract penetration into the same regions as in animals with successful LC lesions.

**Electrophysiology.** Data were obtained from a total of 73 antidromically activated SON neurons displaying the phasic firing pattern characteristic of putative vasopressin neurons (see MATERIALS AND METHODS). Extracellular recordings were obtained from 30 phasically active neurons in animals that received PBS vehicle injections in the LC. Brief phenylephrine-induced increases in BP transiently inhibited the activity of all 30 phasic SON neurons tested (Fig. 6A). Recordings were made from 15 phasic SON neurons in rats with control lesions. All 15 of these neurons were inhibited by acute increases in BP (Fig. 6B).

In the two rats with lesions that destroyed less than one-half of the LC at each level, only seven of nine phasic neurons were inhibited by increases in BP that were sufficient to inhibit all the phasic neurons from vehicle-injected and lesion-control rats (Table 4). This...
decrease in the number of phasically active SON neurons that were inhibited by acute baroreceptor stimulation was statistically significant compared with the vehicle-injected controls (Table 4). Recordings were obtained from 19 phasic SON neurons in the rats with ibotenic acid lesions of the LC. Of the 19 neurons tested for BP sensitivity, 7 were not inhibited by phenylephrine-induced elevations in MAP (Table 4). An example of this phenomenon is shown in Fig. 6C. This reduction in the number of phasically active neurons displaying baroreceptor-induced inhibition of activity was significantly different from vehicle-control and lesion-control groups (Table 4; \( P < 0.01 \)). The data from the firing properties and the BP measurements are summarized in Table 5. There were no differences in any of the firing characteristics of the phasic neurons, baseline BP, or magnitude of the BP increases produced by phenylephrine among the groups (Table 5).

**DISCUSSION**

The DBB has been shown to be an integral component of the pathway regulating the baroreceptor-induced inhibition of vasopressin release (11–13, 31, 52).

Fig. 6. Continuous rate meter recordings (spikes/s) and corresponding blood pressure (BP) traces depicting phasically active neurons from the supraoptic nucleus (SON) from a vehicle-injected control rat (A), a lesion-control rat (B), and a rat with a lesion of the LC (C). In vehicle-control and lesion-control rats, transient phenylephrine-induced increases (open boxes) in BP produce characteristic cessations in spontaneous neuronal activity. In an LC-lesioned rat, comparable phenylephrine-induced increases in BP do not influence neuronal activity.
How the peripheral baroreceptor information is processed and relayed from the NTS to the DBB remains unclear, although a noradrenergic mechanism is implicated (4, 5, 12, 13). The goal of the present study was to determine the source of any noradrenergic baroreceptor afferents to the DBB.

Experiment 1 explored the role of high-pressure arterial baroreceptors in the activation of neurons in hindbrain regions that contain catecholamines. Fos immunoreactivity, an index for neuronal activation, was combined with DbH immunofluorescence to analyze these brain regions in SAD and sham SAD rats in response to phenylephrine- or saline-vehicle infusion. Phenylephrine infusion significantly increased the expression of Fos in the NTS, CVL, IVL, PBN, and LC of baroreceptor-intact sham SAD animals compared with vehicle-infused sham SAD and vehicle- and phenylephrine-infused SAD rats, suggesting that the increase in Fos expression was due to the activation of arterial baroreceptors. The results demonstrate that the increase in BP increased Fos expression in noradrenergic neurons only in the LC. Experiment 2 examined these regions for noradrenergic, baroreceptor-sensitive neurons that project to the DBB. In experiment 2, using the same phenylephrine infusion protocol, we combined retrograde tract tracing from the DBB and DbH immunofluorescence with Fos immunocytochemistry after baroreceptor activation. Our major finding is that noradrenergic, baroreceptor-sensitive neurons that project to the DBB were found exclusively in the LC.

On the basis of these results, we conducted experiment 3, in which we tested the functional significance of the baroreceptor-sensitive projection from the LC to the DBB. Excitotoxic lesions of the LC were made, and extracellular recordings from cells in the SON were obtained to test whether the LC contributes to the baroreceptor-mediated inhibition of vasopressin neurons in the SON. The results of this study indicate that ibotenic acid lesions of the LC significantly attenuate the number of SON vasopressin neurons that are inhibited by acute baroreceptor stimulation. This result is consistent with the hypothesis that the LC is the source of noradrenergic, baroreceptor-related innervation to the DBB. Nonetheless, the decrease in the number of vasopressin SON neurons inhibited by baroreceptor stimulation in the rats with LC lesions was not as great as the decreases that have been observed after ibotenic acid lesions of the DBB (11) or the PNZ (45) or catecholamine depletion of the DBB (12).

In experiments 1 and 2, baroreceptor activation was associated with an increase in Fos expression in the NTS, CVL, IVL, and PBN. In each of these regions, the increase in Fos expression was observed primarily in neurons that were not DbH positive. These results are consistent with a number of studies that have shown that increased arterial pressure produced by intravenous phenylephrine is sufficient to produce increased Fos immunoreactivity in these areas (3, 8, 9, 26, 42, 44, 50). That these increases occurred in noradrenergic neurons also is consistent with earlier studies (8, 9). We demonstrated for the first time in the rat that the elevation in Fos expression observed in each of these nuclei after phenylephrine infusion is dependent on intact baroreceptors. These findings are in agreement with the LC-lesioned rats. Statistical analysis with 1-way ANOVA with follow-up tests using Student-Newman-Keuls test for multiple comparisons revealed no statistically significant differences between groups (P > 0.05). For values without normal distributions, Kruskal-Wallis 1-way ANOVA revealed no statistical differences (P > 0.05).

### Table 4. Analysis of blood pressure sensitivity in antidromically activated phasic SON neurons in vehicle-control, lesion-control, and LC-lesioned groups

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Neurons Inhibited</th>
<th>Neurons Not Inhibited</th>
<th>% Neurons Inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-control</td>
<td>11</td>
<td>30</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Lesion-control</td>
<td>7</td>
<td>15</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Partial LC lesion</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>78†</td>
</tr>
<tr>
<td>LC lesion</td>
<td>8</td>
<td>12</td>
<td>7</td>
<td>63*</td>
</tr>
</tbody>
</table>

Values represent number of neurons and their response to a 10-μl bolus of phenylephrine sufficient to raise blood pressure ≥40 mmHg; number of neurons showing normal blood pressure sensitivity are expressed as a percentage of the total neurons tested; n, number of animals. SON, supraoptic nucleus; LC, locus ceruleus. *Significantly different from vehicle-control and lesion-control number of animals. SON, supraoptic nucleus; LC, locus ceruleus.

### Table 5. Discharge and hemodynamic properties of phasic neurons evaluated from vehicle-control, lesion-control, and LC-lesioned rats

<table>
<thead>
<tr>
<th></th>
<th>Vehicle-Control (n = 11)</th>
<th>Lesion-Control (n = 7)</th>
<th>LC Lesions (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cells</td>
<td>30</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Interburst interval, s</td>
<td>24.7±3.1</td>
<td>30.7±4.3</td>
<td>25.0±4.3</td>
</tr>
<tr>
<td>Firing rate, spikes/s</td>
<td>6.4±0.5</td>
<td>5.7±0.5</td>
<td>6.0±0.6</td>
</tr>
<tr>
<td>Burst length, s</td>
<td>52.2±4.5</td>
<td>56.5±7.7</td>
<td>42.6±3.8</td>
</tr>
<tr>
<td>ΔMAP, mmHg</td>
<td>76.7±2.4</td>
<td>83.2±5.5</td>
<td>73.0±2.5</td>
</tr>
<tr>
<td>Baseline MAP, mmHg</td>
<td>80.6±2.1</td>
<td>86.8±4.0</td>
<td>83.2±1.9</td>
</tr>
<tr>
<td>Peak MAP, mmHg</td>
<td>157.4±3.2</td>
<td>170.0±3.7</td>
<td>156.2±3.0</td>
</tr>
<tr>
<td>Latency of inhibition</td>
<td>3.7±0.5</td>
<td>3.7±0.0</td>
<td>4.8±0.8</td>
</tr>
<tr>
<td>Cessation period, s</td>
<td>64.3±7.3</td>
<td>52.9±8.5</td>
<td>66.9±11.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. MAP, mean arterial pressure; BP, blood pressure. Rats with partial LC lesions are pooled with the LC-lesioned rats. Statistical analysis with 1-way ANOVA with follow-up tests using Student-Newman-Keuls test for multiple comparisons revealed no statistically significant differences between groups (P > 0.05). For values without normal distributions, Kruskal-Wallis 1-way ANOVA revealed no statistical differences (P > 0.05).
with those of Potts et al. (50), who conducted a similar experiment using rabbits.

There was no evidence in our studies that the NTS, CVL, and PBN contain baroreceptor-sensitive neurons that project to the DBB. This suggests that these regions do not contribute direct baroreceptor-related afferents to the DBB that participate in the baroreceptor-mediated inhibition of vasopressin neurons in the SON. However, the limitations of Fos immunocytochemistry do not allow us to completely rule out a role for these nuclei in the baroreceptor-mediated regulation of DBB activity. Failure to observe Fos immuno-reactivity in a neuron does not necessarily mean that the stimulus has not changed the activity of that neuron. Inhibition, adaptation, or insufficient stimulation are each mechanisms that do not increase Fos expression in neurons (21). We did observe a significant increase in Fos in these areas, as previously reported (3, 8, 9, 26, 42, 44, 50), indicating that our stimulus was sufficient to produce Fos expression in these regions. Therefore, our failure to observe Fos-positive, retrogradely labeled neurons in the NTS, CVL, and PBN was probably not due to insufficient stimulation. Nonetheless, the Fos immunocytochemistry technique is not without its limitations (21), and the results of these experiments must be interpreted within the limitations of this technique.

Electrophysiology studies have provided little support for the hypothesis that the A1 region of the CVL or PBN is involved in the baroreceptor-mediated inhibition of SON vasopressin neurons. For example, inhibiting the A1 region does not significantly decrease the number of vasopressin neurons that are inhibited by baroreceptor stimulation (18), and, furthermore, most experimental evidence indicates that the A1 region mediates activation of SON neurons (15, 16, 17, 25, 27, 37, 39, 51). Similarly, electrical stimulation of the PBN has been reported to increase the activity of SON neurons (28). Although chemical stimulation of the PBN does inhibit SON neurons, this inhibition of neurons in the SON is associated with a pressor response to the glutamate injection in the PBN (20). Therefore, the lack of baroreceptor-sensitive neurons projecting to the DBB from the CVL or PBN is consistent with other reports in the literature. We also did not observe evidence of a baroreceptor-sensitive projection from the NTS to the DBB. Because the NTS is the primary target for peripheral baroreceptor afferents, it is a necessary part of any pathway that would bring baroreceptor information to the SON. Our observations suggest that its role in this pathway does not involve a direct baroreceptor-sensitive projection to the DBB. This is consistent with an earlier study by McAllen and Harris (40) that indicated the involvement of a long-latency, polysynaptic pathway in the baroreceptor projection to the SON.

Of the regions we investigated, only the LC contains a population of noradrenergic, baroreceptor-sensitive neurons that project to the DBB. Furthermore, in the present study the increase in Fos-positive neurons in the LC was not observed in SAD rats infused with phenylephrine. This indicated that the increase in Fos observed in the LC was dependent on intact baroreceptors and not the result of some nonspecific effect of the phenylephrine infusion. However, there is some controversy surrounding the arterial baroreceptors’ ability to influence the activity of LC neurons. In this study and others, baroreceptor activation resulted in increased Fos expression in the LC (8, 41, 44); yet others see no change in Fos expression in the LC (26, 50). These discrepancies may be explained, in part, by differences in experimental protocols. First, our study increased BP ~50 mmHg for 2 h, whereas similar studies did not raise BP to comparable levels for as long a time period. Differences in the thickness of the brain sections and the specificity of Fos antibodies may likewise contribute to these differences. Also, we used DβH immunofluorescence to define the LC and examined the entire rostral-caudal extent of the nucleus. These approaches were not used in most of the previous studies (26, 41, 44, 50).

Although the observed number of Fos-positive LC neurons retrogradely labeled from the DBB was small, this does not necessarily reflect the physiological significance of this projection. First, our injections of rhodamine microspheres into the DBB were confined to the vertical limb of the DBB, which is a fairly large structure. As a result, not all the neurons projecting to the DBB may have been exposed to labeled beads or transported enough beads to intensely backlabel all afferent neurons. Moreover, the limited spread and minimization of uptake by fibers of passage, the primary advantage of latex microspheres (54), may have contributed to the relatively low number of retrogradely labeled cells. Nevertheless, our results indicate that the LC has noradrenergic projections to the DBB that are activated by stimulating baroreceptors.

We attempted to address the aforementioned limitations with experiment 3, in which we lesioned the LC and recorded directly from putative vasopressin neurons in the SON during acute baroreceptor stimulation. This maneuver significantly decreased the number of vasopressinergic SON neurons that were inhibited by baroreceptor stimulation. Injections of the same volume of vehicle into the LC (vehicle control) or lesions of regions adjacent to the LC (lesion control) did not significantly alter the number of baroreceptor-sensitive phasic SON neurons. Therefore, it is unlikely that the observed decrease in the number of baroreceptor-sensitive SON neurons in LC-lesioned rats is the result of nonspecific mechanical damage or the loss of neurons in neighboring parts of the dorsal pons. Furthermore, it is unlikely that the observed effect on baroreflex inhibition of vasopressin neurons is attributable to damaged fibers of passage coursing through the dorsal pons. Ibogenic acid, at the concentration used here, has been shown to destroy neuronal perikarya while leaving fibers of passage intact (10, 24, 45).

Although the ibogenic acid lesions of the LC significantly reduced the number of vasopressin neurons in the SON that were inhibited by baroreceptor stimula-
tion, the majority (67%) of the vasopressinergic neurons in LC-lesioned rats was still inhibited by the increases in BP. One possible explanation for this result may be the difficulty in producing a complete lesion of the LC with ibotenic acid. Although at least one-half to two-thirds of the more caudal and medial aspects of the LC were effectively lesioned, even with three different injections along the rostral-caudal plane of the LC, we had only two rats with complete lesions of the LC. The proximity of the LC to the fourth cerebral ventricle made complete destruction of the LC very difficult. As mentioned in Materials and Methods, this procedure was associated with a high mortality rate, presumably because of the ibotenic acid leaking into the ventricle. Complete destruction of the LC may be required to entirely eliminate the baroreceptor sensitivity of vasopressinergic SON neurons. Furthermore, although anatomic evidence suggests that LC projections to the DBB are unilateral (2, 32, 55, 59), it is possible that there is some integration of LC input at the level of the DBB such that the effect of a unilateral LC lesion may not be of the same magnitude as that observed in previous studies (11, 12, 45). It is also possible that the role of norepinephrine in the DBB is modulatory in nature.

The possibility that complementary pathways relay baroreceptor information to the SON is another possible explanation for this partial effect. Our study did not reveal significant baroreceptor-induced Fos expression colocalized in NTS, A1, or PBN neurons retrogradely labeled from the DBB. However, the possibility that these areas may relay information cannot be discounted because of the limitations of Fos immunocytochemistry. Moreover, it is possible that the DBB may receive afferent baroreceptor information from forebrain regions, such as the bed nucleus of the stria terminalis (BST) (60). Wilkinson and Pittman (60) identified a population of neurons in the BST that were antidromically activated from the DBB/ventral septal area, and their firing was altered by variations in BP. How baroreceptor information is relayed to the BST and how this connection is integrated with other signals at the level of the DBB remain to be determined.

The results from our experiments support the hypothesis that the LC participates in the baroreceptor-mediated inhibition of SON vasopressin by providing the DBB with noradrenergic, baroreceptor-related afferents. Although the LC has traditionally been described as involved in arousal and the detection of changes in the internal environment (47), the role of the LC in cardiovascular regulation has been somewhat controversial. The results of several studies report inhibition and excitation of LC neurons after stimulation of arterial baroreceptors (2, 8, 22, 41, 44, 46, 50). Anatomic evidence suggests that the LC receives projections from the NTS (7), although existence of this connection is not universally accepted (1). Furthermore, the role of the LC in the regulation of vasopressin release also is controversial. For example, electrical stimulation of the LC is reported to increase circulating vasopressin levels (56), and a population of LC neurons is excited by decreases in BP (22). Together these studies indicate that the LC contains neurons that may facilitate vasopressin release.

However, other studies provide evidence that is consistent with the hypothesis that the LC is involved with the baroreceptor-mediated inhibition of vasopressin neurons. It has been shown that a small population of LC neurons is excited by increases in BP (8, 44, 46) and aortic depressor nerve stimulation (41, 43). Banks and Harris (4) provided the first evidence supporting a role for the LC in the transmission of baroreceptor information to the SON: they showed that thermal and 6-OHDA lesions of the LC completely blocked baroreflex inhibition of SON neurons ipsilateral to the lesion. Subsequently, it has been suggested that these results were due to the destruction of fibers of passage (19). In the study by Banks and Harris, some of the controls necessary to reduce nonspecific effects of 6-OHDA were not used, leaving their study open to this criticism (33). Consistent with this hypothesis, injections of glutamate into the LC do not produce vasopressin release (57). In a more recent study, GABA was microinjected into the LC while extracellular recordings were obtained from the SON (19). In this study, injections of GABA into the LC, which would inhibit neurons without influencing fibers of passage, did not influence the baroreceptor-mediated inhibition of vasopressin neurons in the ipsilateral SON. This approach also has its limitations. The volume of the microinjections used in their study may not be sufficient to inhibit a very large portion of the LC, given its considerable rostral-caudal extent (23), although Day and Sibbald (19) contend that their GABA injections were large enough and of high enough concentration to more than adequately cover the LC and any adjacent regions. Nevertheless, the inability to precisely determine the spread of the injectate and the functional concentration of GABA delivered to the region of interest is a limitation of this technique. Although Day and Sibbald did verify the placement of the injections in the region of the LC, the method that they used did not provide an indication of how large an area was covered by their injections. In addition, the fact that there were neither effects of the GABA injections on any SON neurons nor effects of glutamate injections on cardiovascular parameters at concentrations seen by others (reviewed in Ref. 19) indicates that the microinjections may not have covered a sufficient portion of the LC.

To reconcile the apparently disparate results from these two studies, we used an excitotoxin, ibotenic acid, to lesion the LC. This method has been shown to leave fibers of passage intact (10, 24, 45) and is verifiable using a conventional histology (11, 45). Although quantification of the extent of the LC destroyed by ibotenic acid injections by use of DβH immunofluorescence would have been desirable, we could not obtain consistent DβH immunostaining because of the invasive nature of the surgery required for recording from SON neurons and minor swelling of the brain after several hours of recording. As a result, Giemsa was used to stain for nissl substance in the brain. The large, dark
somases of the catecholaminergic neurons defining the LC are easily distinguishable from nearby regions by this staining method (Fig. 4). Our results indicate that lesions of the LC significantly reduce the baroreceptor-induced inhibition of vasopressin neurons, which is consistent with the earlier study by Banks and Harris (4).

Perspectives

Our experiments indicate that the LC is most likely involved in the baroreceptor-mediated inhibition of vasopressinergic SON neurons by acting at the DBB. Although this baroreceptor input to the LC must originate in the NTS, it is possible that the NTS does not project directly to the LC (1). If this is indeed the case, at least one more synapse exists between the LC and the NTS. Thus the pathway from the peripheral baroreceptors to the SON appears to be complex and polysynaptic, as predicted by McAllen and Harris (40). This contrasts with the relatively simpler brain stem circuits involved in baroreceptor regulation of the autonomic nervous system (15). The differences in these pathways may be related to the physiological role of the baroreceptors in regulating autonomic outflow vs. the regulation of circulating levels of the hormone vasopressin. The arterial baroreceptors are traditionally characterized as regulating the moment-to-moment output of the autonomic nervous system to buffer changes in BP. In fact, neurons in the rostral ventrolateral medulla and the caudal ventrolateral medulla, two important components of the autonomic baroreflex circuit, have been shown to have activity that is synchronized to the cardiac cycle (reviewed in Ref. 15). This type of acute buffering would require a relatively simple neural circuit that involves rapid neurotransmission that transmits the information from the baroreceptors with a high degree of fidelity. Vasopressin, on the other hand, is a hormone with a half-life reported to be as short as 1 min or as long as 8 min, depending on the species and experimental conditions (34, 36), and its circulating levels are dependent on the output of neurosecretory neurons in the hypothalamus (6, 52, 53). Although changes in plasma osmolality have been described as the most salient stimulus involved in determining the circulating levels of vasopressin, a number of other physiological stimuli have been identified that can alter the activity of magnocellular neurosecretory cells (53). Work from Bourque’s laboratory (6) suggests that the osmotic regulation of SON neurons involves direct projections from the organum vasculosum of the lamina terminalis, which use glutamate as the primary neurotransmitter. This pathway is more similar to the circuit mediating baroreflex control of the parasympathetic limb of the autonomic nervous system than the pathway bringing baroreceptor information to the SON. This could be because osmolality is more directly involved in the moment-to-moment regulation of magnocellular neurosecretory cells than are the arterial baroreceptors and that the relationship between arterial pressure, the activity of magnocellular neurosecretory neurons, and circulating vasopressin is more complex.

The authors thank Karen Higgs, Jim Baker, and Arej Sawani for excellent technical assistance and Dr. Kathleen Curtis for assistance with SAD surgeries.

This work was done during the tenure of a Predoctoral Fellowship Award from the American Heart Association, Missouri Affiliate (R. J. Grindstaff), and was supported by National Heart, Lung, and Blood Institute Grants R29-HL-55692 (J. T. Cunningham), K02-HL-06820 (J. T. Cunningham), and T32-HL-0721 (R. R. Grindstaff) and a grant-in-aid from the American Heart Association, Missouri Affiliate (J. T. Cunningham).

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