Brain stem control of arterial pressure in chronic arterial baroreceptor-denervated rats

Schreihofer, Ann M., Satoru Ito, and Alan F. Sved. Brain stem control of arterial pressure in chronic arterial baroreceptor-denervated rats. Am J Physiol Regul Integr Comp Physiol 289: R1746–R1755, 2005. First published August 25, 2005; doi:10.1152/ajpregu.00307.2005.— Interruption of the baroreceptor reflex by transection of afferent nerves (sinoaortic denervation; SAD) or lesions of nucleus tractus solitarius (NTS) elevates sympathetic nerve activity (SNA) and arterial pressure (AP). However, within 1 wk, mean AP returns to normal despite the absence of baroreflexes. In this study, we examine central mechanisms that control AP in chronic baroreceptor-denervated rats. In urethane-anesthetized rats (1.5 g/kg iv) after autonomic ganglionic blockade (5 mg/kg iv chlorisondamine), α₁-adrenergic-mediatedpressor responses (1–100 μg/kg iv phentolamine) were not altered by chronic lesions of NTS, indicating vascular reactivity to sympathetic stimulation is normal. Transection of the spinal cord at T1 profoundly decreased AP and was not further reduced by chlorisondamine in control or denervated rats. Inhibition of the rostral ventrolateral medulla (RVLM) by microinjections of muscimol (100 pmol/side) decreased AP to levels not further reduced by chlorisondamine in control rats, rats with SAD, and rats with NTS lesions. Blockade of GABA_A receptors in the RVLM (50 pmol/side bicuculline) increased AP similarly in control denervated rats. In agreement, inhibition of the caudal ventrolateral medulla (CVLM) by microinjections of muscimol or blockade of glutamatergic inputs (2.7 mmol/side kynurenate) produced comparable increases in AP in control and denervated rats. These data suggest the RVLM continues to drive the SNA that regulates AP in the chronic absence of baroreceptor inputs. In addition, despite the absence of a tonic excitatory input from NTS, in chronic baroreceptor-denervated rats glutamatergic inputs drive the CVLM to tonically inhibit the RVLM. Baroreceptor-independent regulation of the ventralateral medulla may underlie central mechanisms contributing to the long-term control of AP.

caudal ventrolateral medulla; rostral ventrolateral medulla; sinoaortic denervation; kynurenic acid; nucleus tractus solitarius

Several lines of evidence suggest that the return of mean AP to preoperative levels in chronic baroreceptor-denervated rats is associated with a normal sympathetic vasomotor tone (49). First, the decrease in AP produced by autonomic ganglionic blockade, an index of sympathetic vasomotor tone (34), is the same in chronic baroreceptor-denervated and intact rats (3). Second, plasma catecholamine levels are elevated acutely after SAD but are normal in rats with chronic SAD that display a normal mean AP (2, 8, 33). Third, direct recordings of sympathetic nerve activity (SNA) in rats with chronic SAD did not detect differences in the magnitude of SNA between intact rats and rats with chronic SAD (4, 19, 54), in clear contrast to the marked increase in SNA observed acutely after baroreceptor denervation (4, 19).

Surprisingly, little is known about the tonic central neural control of AP in the chronic absence of baroreceptor inputs. Driven by excitatory baroreceptor afferent nerves, the NTS normally provides a powerful tonic inhibitory influence on sympathetic vasomotor tone and AP. However, in rats with chronic SAD, the NTS does not appear to tonically regulate AP. Inhibition of the NTS produces no change in AP in rats with chronic SAD, in contrast to the large rise in AP observed in control animals (42). The apparent lack of tonic activity in the NTS of chronic baroreceptor-denervated rats appears to result from increased tonic inhibition of the NTS, as injection of the GABA_A antagonist, bicuculline, elicits a large depressor response in chronically denervated rats compared with a minimal response in baroreceptor intact rats or acutely denervated rats (21). As observed with rats with chronic SAD, rats with chronic lesions of the NTS also have a normal mean AP (41). These data indicate that, in the chronic absence of baroreceptor inputs or a functional NTS, there are qualitative, fundamental changes in the brain stem control of AP compared with baroreceptor-intact rats. The baroreceptor-independent control of sympathetic vasomotor tone and AP in chronic baroreceptor-denerivated rats may underlie central mechanisms that contribute to the long-term control of a normal AP in baroreceptor-denervated rats, as well as baroreceptor-intact rats.

The brain stem circuitry connecting baroreceptor inputs to sympathetic vasomotor outflow has been well established (see Refs. 7 and 12 for review). Glutamatergic neurons arising from the NTS excite neurons in the caudal ventrolateral medulla (CVLM) (13, 15). In turn, GABAergic neurons from the CVLM inhibit the presympathetic neurons in the rostral ventrolateral medulla (RVLM) (6, 45), which project to sympathetic preganglionic neurons and provide the major drive for arterial baroreceptor reflexes.

ELIMINATION OF ARTERIAL BARORECEPTOR afferent input to the brain by selective sinoaortic denervation (SAD) or destruction of the brain stem site where they terminate in the nucleus tractus solitarius (NTS) markedly increases arterial pressure (AP) (24, 48), primarily by increasing sympathetic vasomotor tone (2, 4, 19, 46). However, the acute hypertensive response to baroreceptor denervation is transient, with mean AP returning to normal levels within 1 wk, despite the persistent absence of baroreflexes (28, 29, 41, 42, 2). Yet, even after AP has returned to normal mean levels, it is quite labile (3, 43), reflecting the loss of the moment-to-moment stabilizing influence of baroreceptor reflexes.

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sympathetic vasomotor tone (7, 14, 40). The aim of the present study was to examine brain stem mechanisms that control AP in chronic baroreceptor-denervated rats, produced either by SAD or by lesions of the NTS, focusing on the role of brain stem structures that are normally involved in the baroreflex control of AP. We used rats with chronic SAD to examine control of AP in the absence of arterial baroreceptor reflexes and tonic regulation by the NTS (42), and we used rats with chronic NTS lesions to examine control of AP in the additional absence of other inputs to NTS (41). First, we tested the hypothesis that sympathetic vasomotor tone in chronic baroreceptor-denervated rats was still governed by supraspinal sites, in particular, the RVLM. Second, because the CVLM is a major regulator of RVLM neuronal activity and is normally driven by baroreceptor inputs through the NTS, we also examined how the CVLM influences AP in rats with chronic baroreceptor denervation.

MATERIALS AND METHODS

Animals. Studies were performed on adult male Sprague-Dawley rats (Zivic-Miller, Allison Park, PA; Harlan, Indianapolis, IN) that were individually housed in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle. Tap water and Purina chow pellets were available ad libitum. Experimental and surgical procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Pittsburgh or the Medical College of Georgia.

Baroreceptor denervations. Rats were denervated either by transection of baroreceptor afferent nerves or bilateral lesions of the NTS where baroreceptor afferent nerves terminate. Rats were anesthetized with halothane (2% in 100% oxygen via a nose cone) and treated with atropine (0.5 mg/kg ip). Rats were also treated with an autonomic ganglionic antagonist (5 mg/kg sc chlorisondamine) and a vasopressin receptor (V1) antagonist (Manning compound, 10 μg/kg sc) to alleviate the acute hypertensive effects of the denervation (41). After denervation surgery, rats were given an antibiotic (0.1 ml im Combi-otic) and saline (10 ml sc) and were allowed to recover for 1–2 wk. To eliminate arterial baroreceptor inputs by transection of baroreceptor afferent nerves, bilateral SAD was performed, as previously described by our group (42). To eliminate cardiac and baroreceptor inputs to the brain, bilateral electrolytic lesions of the baroreceptive portion of the NTS were made, as previously described (36, 39, 41). The dorsal surface of the brain stem was exposed, and a Teflon-coated tungsten electrode (200 μm OD with 375 μm of the tip exposed; A-M Systems, www.a-msystems.com) was inserted into one NTS (0.5 mm lateral from the midline, 0.5 mm rostral to calamus scriptorius, and 0.6 mm below the dorsal surface of the brain stem). A lesion was produced by passing anodal current (1 mA for 15 s) from a constant DC source (Grass Instruments, www.grass-telesfactor.com). The procedure was repeated in the contralateral NTS, and then the wound was closed.

Physiological assessment of denervations. Reflexes were tested in conscious rats 1–2 wk after SAD or lesions of the NTS (41, 42, 43, 50). Rats were anesthetized with halothane, and catheters were implanted into the femoral artery and vein for measurement of AP and heart rate (HR) and administration of drugs, respectively. The free ends of the catheters were tunneled subcutaneously to exit between the scapulae and through a tether attached to a swivel. Rats were allowed to regain consciousness for 1–2 h before reflexes were examined.

To examine arterial baroreceptor reflexes, we measured changes in HR produced by pharmacologically increasing and decreasing AP with phenylephrine (2 μg/kg iv) and sodium nitroprusside (5 μg/kg iv), respectively (41, 43). Rats were considered to be effectively denervated when HR responses to both drugs were totally absent. To examine whether NTS lesions effectively removed vagal cardiac reflexes, we injected phenyl biguanide (50 μg/kg iv) to elicit the Bezold-Jarisch reflex (42). This 5-hydroxytryptamine, receptor agonist produces marked bradycardia and hypotension that are mediated by excitation of cardiopulmonary receptors with vagal afferents that project to the NTS (50). These responses were observed in our control rats and rats with chronic SAD, but they were absent in all rats considered to have effective lesions of the NTS.

Anesthesia preparations. After we tested rat reflexes in the conscious state, rats were anesthetized in one of two ways for experiments. Initially, we chose α-chloralose anesthesia because cardiovascular responses are well maintained, and our prior studies used this preparation. In this case, rats were initially anesthetized with halothane for surgical procedures; then, after completion of surgical procedures, α-chloralose (30 mg/ml solution in warm saline) was infused slowly (60 mg/kg iv, with hourly supplements of 20 mg/kg) as halothane was eliminated. This protocol was used for rats subjected to spinal cord transection.

During initial experiments, we noted that some chronic denervated rats did not tolerate α-chloralose well and had AP levels so far below those observed in their conscious state and those observed in anesthetized control rats that they had to be excluded from further study. In later experiments, urethane (1.5 g/kg iv) was used as an experimental anesthesia because comparable baseline AP levels could be readily achieved in all groups and cardiovascular responses were well maintained. After baroreceptor reflex testing, urethane was infused through the venous catheter (1.5 g·ml⁻¹·kg⁻¹, 50 μl/min). This protocol produced an adequate plane of anesthesia for surgery that was well maintained for the experimental protocols. Adequacy of anesthesia was determined by the absence of a corneal reflex and significant cardiovascular responses (<10 mmHg increase in AP) to firm toe pinch. Supplements of urethane were not needed in the time frame necessary to complete the experimental protocol. Urethane was used for all anesthetized rat experiments except spinal cord transection.

Vascular reactivity in rats with NTS lesions. In order for measurements of acute changes in AP to reflect changes in sympathetic vasomotor tone, vascular reactivity to the α-adrenergic receptor activation at the blood vessels must not be grossly affected by the experimental condition. Previous studies have demonstrated that rats with chronic SAD do not show altered adrenergic vascular reactivity, as suggested by comparable pressor responses to the α-adrenergic agonists methoxamine or phenylephrine (5, 22, 27). Because blood volume is not altered by chronic SAD (29), the same doses calculated by weight could be used for intact and denervated rats. We have previously shown that chronic lesions of the NTS do not alter blood volume (49); however, vascular reactivity to α-adrenergic receptor activation has not been examined in this model. Therefore, we measured pressor responses to a series of doses of phenylephrine in control rats and rats with chronic lesions of the NTS. After testing baroreceptor reflexes in the conscious state, rats were anesthetized with urethane as described in Anesthesia preparations. Once anesthetized, rats were treated with chlorisondamine (5 mg/kg iv) to eliminate endogenous sympathetic vasomotor tone and prevent baroreceptor reflex-mediated responses in intact rats that were absent in denervated rats. Seven doses of phenylephrine were given in a randomized order (1, 2, 4, 8, 16, 24, and 100 μg/kg). Each dose was injected in a 50-μl volume flushed by 200 μl of saline through a dead space of 100 μl in the venous line. The AP was allowed to return to within 10% of baseline levels between each dose.

Spinal transections. To determine whether sympathetic vasomotor tone and AP were still governed by supraspinal drive in chronic baroreceptor-denervated rats, we examined the effect of complete transection of the spinal cord at T9 on mean AP. To demonstrate that the new level of AP was not maintained by remaining sympathetic vasomotor tone, the transection was followed by administration of chlorisondamine (5 mg/kg iv).
After we tested rat baroreceptor reflexes, rats were anesthetized with halothane, artificially ventilated, and placed in a stereotaxic instrument. After a dorsal laminectomy was performed, the dorsal surface of the upper spinal column was exposed. The dorsal portion of vertebra T1 was removed to expose the underlying spinal cord. At the completion of the surgery, α-chloralose was given as described above. In Anesthesia preparations, and the halothane was eliminated. The rat was paralyzed (d-tubocurarin, 0.5 mg/kg iv) and ventilated with 100% oxygen. The spinal cord was transected with a cautery tool (George Tiemann, www.georgetiemann.com) and packed with gel foam. The mean AP and HR were recorded for 2 min before the spinal transection, for 2 min after the spinal transection, and for 2 min after administration of chlorisondamine. At the completion of the experiment, the full transection of the spinal cord was visually confirmed. All rats included in data analyses had complete spinal transections.

Microinjections of drugs into the brain stem. After baroreflex testing was completed, rats were anesthetized with urethane as described above. To expose the brain stem, rats were artificially ventilated and placed in a stereotaxic instrument with the incisor bar set at −11 mm. Shortly before the experiment was started, rats were paralyzed (0.5 mg/kg iv d-tubocurarine).

In rats with chronic SAD, the dorsal surface of the brain stem was exposed as described above (42). The RVLM and CVLM sites were functionally located using a range of previously established coordinates and observing AP responses to microinjection of glutamate (1 nmol; 12–15 injections/side). The coordinates for RVLM sites were 1.7–2.1 mm lateral from the midline, 1.4–1.8 mm rostral to the caudal tip of area postrema, and 2.7–3.1 mm below the dorsal surface of the brain stem with the pipette angled 20° rostrally. Within this range (separating tracts by 0.2 mm), the site producing the largest pressor response to glutamate (>20 mmHg) was selected for further study. The coordinates for CVLM were 1.7–2.1 mm lateral from the midline, 1.1–1.5 mm rostral to caudal tip of the area postrema, and 2.2–2.6 mm below the dorsal surface of the brain stem with no angle applied to the microinjection pipette. Stimulation of CVLM sites with glutamate had to decrease AP >20 mmHg to be further examined.

In rats with chronic lesions of NTS, the dorsal surface of the brain stem was not reliable for locating the RVLM, so this region was approached directly from the ventral surface of the brain stem. In this case, the rat was placed supine in the stereotaxic instrument. To expose the brain stem, portions of the trachea and esophagus were tied off and removed, bone was clipped away, and the dura mater was punctured and excised. Microinjections of glutamate were used to functionally locate the RVLM sites with changes >20 mmHg as the minimum criteria. The coordinates were 1.7–2.1 mm lateral from the midline basilar artery, 0.3–0.7 mm from the ventral surface, and 3.8–4.1 mm rostral to the edge of the occipital foramen.

Control rats were examined using the dorsal and ventral approaches to the brain stem to ensure the magnitude of cardiovascular responses did not vary systematically by the approach used. These data were pooled as no differences were seen.

Drugs were delivered to the brain stem using single-barrel glass pipettes pulled and cut to a tip of 40–50 μm. All drugs were dissolved in artificial cerebral spinal fluid, and pressure was ejected in a volume of 100 nl (42). Once functional sites were located, the pipette was withdrawn, rinsed, and filled with the experimental drug. Muscimol (100 pmol/100 nl), a GABA<sub>A</sub> agonist, was microinjected into RVLM or CVLM to globally inhibit the cell bodies within these regions. Muscimol bicuculline methiodide (50 pmol/100 nl), a GABA<sub>A</sub> receptor agonist, was microinjected to eliminate GABAergic inputs to neurons in the RVLM. Kynurenic acid (2.7 nmol/100 nl), a broad spectrum glutamate receptor antagonist, was microinjected to eliminate glutamatergic inputs to the CVLM. Bilateral injections were made one side at a time with ~1 min between injections. Baseline readings of mean AP and HR were recorded for 3 min before the first injection, and the postinjection values were recorded at the peak or trough occurring within 3 min after the second injection of muscimol or bicuculline.

At the end of the experiment, the pipette was withdrawn and filled with fast green to mark injection sites. After microinjection of the dye (2% in 100 nl), rats were deeply anesthetized with halothane and were quickly decapitated. The brain stem was removed, snap frozen in isopentane on dry ice, cut into 30-μm coronal sections using a cryostat, and mounted onto slides. Sections were examined for the approximate location of injections and included in data analysis if the dye were found within the region of the CVLM or the RVLM as expected. The dorsal medulla was also examined to ensure the lesions were correctly placed within the intermediate NTS (41).

Statistics. To examine potential differences in pressor responses to phenylephrine between control rats and rats with chronic lesions of the NTS, the change in AP was compared between groups using a two-way ANOVA with repeated measures. No post hoc tests were performed, as the F value did not reach significance (P > 0.05). To examine the changes in AP and HR produced by transection of the spinal cord or inhibition of the RVLM followed by chlorisondamine, we performed a two-way ANOVA with repeated measures followed by post hoc tests (t-test with Bonferroni correction for a number of comparisons) to compare mean AP and HR values within and between groups. To examine the changes in AP and HR in response to microinjection of bicuculline into the RVLM or muscimol into the CVLM, we performed a two-way ANOVA followed by post hoc tests (t-test with Bonferroni correction for number of comparisons) to compare mean AP or HR values within and between groups. In all cases, values were considered to be significantly different when P < 0.05.

RESULTS

Effects of baroreceptor denervation on mean AP, HR, sympathetic vasomotor tone, and vascular reactivity. Urethane-anesthetized rats with lesions of the NTS have a normal mean AP and HR (Table 1), as seen in conscious rats with NTS lesions (41). As shown previously in rats with chronic SAD (3, 29), autonomic ganglionic blockade with chlorisondamine produced a normal decrease in AP in rats with chronic lesions of the NTS (Table 1). Together, these data suggest that functional sympathetic vasomotor tone is not chronically altered by central or peripheral arterial baroreceptor denervation.

Autoinhibition ganglion blockade, injections of phenylephrine produced reliable, dose-related increases in AP in control rats and rats with chronic lesions of NTS (Fig. 1). There were no differences between the two groups in the magnitudes of the pressor responses to phenylephrine at any dose examined (Fig. 1). As shown previously for rats with chronic SAD (5, 41), Table 1. MAP and HR before and after autonomic ganglionic blockade in control rats and rats with chronic lesions of the NTS

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<td>Control</td>
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<td>NTS-X</td>
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Values are means ± SE; n = number of rats. Groups of control rats or rats with chronic lesions of the nucleus tractus solitarius (NTS-X) anesthetized with urethane (iv) were given the autonomic ganglionic antagonist chlorisondamine (5 mg/kg iv). Changes in mean arterial pressure (MAP) and heart rate (HR) were recorded 3 min after ganglionic blockade. These rats were subsequently examined for responsiveness to phenylephrine (Fig. 1). Chlorisondamine produced significant decreases in MAP and HR in both groups (*P < 0.05).
22), lesions of the NTS do not appear to alter pressor reactivity to $\alpha$-adrenergic stimulation.

**Supraspinal control of AP in baroreceptor-denervated rats.** Baseline AP in rats with chronic SAD was not different from AP observed in control rats (Figs. 2 and 3A). In control rats, transection of the spinal cord at $T_1$ produced an immediate and profound decrease in AP that was not further reduced by autonomic ganglionic blockade with chlorisondamine (Figs. 2 and 3A). In rats with chronic SAD, transection of the spinal cord produced an equally large decrease in AP, which was not further reduced by chlorisondamine (Figs. 2 and 3A). These data suggest that sympathetic vasomotor tone continues to be driven by supraspinal sites in the chronic absence of arterial baroreceptor inputs.

**Control of AP by the RVLM in baroreceptor-denervated rats.** To locate the RVLM functionally in each rat, glutamate was microinjected systematically across a range of coordinates to locate the site that produced the largest pressor response. In control rats ($n = 20$ injections; 10 rats with one on each side), the average maximal pressor response to glutamate was $36 \pm 3$ mmHg, and the most likely coordinates for locating this maximal response from the dorsal side were 1.9 mm lateral to the midline, 1.6 mm rostral to calamus scriptorius, and 2.7 mm below the dorsal surface of the brain stem or from the ventral side were 1.9 mm lateral to the midline, 4.0 mm rostral to the occipital foramen, and 0.5 mm from the ventral surface. In baroreceptor-denervated rats (including rats with SAD or NTS-X; $n = 11$ rats), the average maximal pressor response to glutamate was significantly larger ($58 \pm 5$ mmHg; $P < 0.05$ vs. control rats), with coordinates comparable to those used in control rats. The pressor responses observed in rats with SAD ($62 \pm 6$ mmHg; $n = 7$ rats) was not different from those observed in rats with NTS lesions ($52 \pm 9$ mmHg; $n = 4$ rats; $P > 0.05$).

As shown previously (32, 40), in baroreceptor-intact rats, bilateral inhibition of the RVLM by microinjections of the GABA$_A$ agonist muscimol produced a marked decrease in AP, which was not further reduced by ganglionic blockade with chlorisondamine (Figs. 3B and 4A). Similarly, in rats with SAD or lesions of the NTS, inhibition of the RVLM produced marked decreases in AP (Figs. 3B and 4B), which were not further reduced by chlorisondamine (Figs. 3B and 4A). These data suggest that the RVLM continues to be the dominant source of drive for sympathetic vasomotor tone in chronic baroreceptor-denervated rats.

In this particular experiment, the rats with SAD had a lower baseline AP than the control rats (Fig. 3B). Inhibition of the RVLM significantly reduced mean AP to the same levels observed in control rats (Fig. 3B), producing a smaller change in AP in rats with SAD ($44 \pm 3$ vs. $61 \pm 4$ mmHg in control rats, $P < 0.05$). In rats with chronic lesions of the NTS, baseline AP and change in AP ($50 \pm 4$ mmHg) after inhibition of the RVLM were comparable to those observed in control rats and rats with chronic SAD (Figs. 3B and 4).

**GABAergic control of the RVLM.** In baroreceptor-intact rats, presympathetic RVLM neurons are tonically inhibited by GABA to decrease sympathetic vasomotor tone and AP, and much of this prominent inhibition is driven by baroreceptor inputs (6, 45). To determine whether chronic baroreceptor denervation alters tonic GABAergic inhibition to these RVLM...
In baroreceptor-intact rats, glutamatergic neurons in the CVLM to produce baroreceptor reflex-mediated responses (1, 26). However, in chronic baroreceptor-denervated rats, the NTS no longer tonically influences AP (42). Therefore, we determined whether the CVLM continues to tonically inhibit the RVLM and whether these CVLM neurons are still tonically activated by glutamate.

The cardiovascular-related region of the CVLM was located by microinjections of glutamate, selecting sites that produced a depressor response >20 mmHg. The average maximal depressor response was -42 ± 2 mmHg in baroreceptor-intact rats and -37 ± 4 mmHg in rats with SAD (P > 0.05).

Bilateral inhibition of the CVLM. Inhibition of neurons in the CVLM by microinjection of the GABA_A agonist muscimol markedly increased AP in baroreceptor-intact rats (67 ± 6 mmHg; Figs. 6B and 7A). This dose of muscimol effectively abolished the depressor response to phenyl biguanide (see Fig. 4).

Fig. 3. Effects of spinal transection or inhibition of the rostral ventrolateral medulla (RVLM) on mean AP in control and chronic baroreceptor-denervated rats. A: in chloralose-anesthetized control rats (n = 5) and rats with chronic SAD (n = 5), acute spinal transection at T1 decreased mean AP, and AP was not further reduced by ganglionic blockade with chlorisondamine. Baseline HR in rats with chronic SAD was not different from control rats (375 ± 27 beats/min in control rats vs. 330 ± 13 beats/min in rats with SAD; P > 0.05). Transection of the spinal cord produced a comparable decrease in HR in both groups (−35 ± 21 beats/min in control rats vs. −34 ± 17 beats/min in rats with chronic SAD; P > 0.05). After spinal transection, chlorisondamine did not significantly alter HR (see Fig. 2). B: in urethane-anesthetized control rats (n = 6), rats with chronic SAD (n = 7), and rats with chronic lesions of the NTS (NTS-X; n = 4), inhibition of the RVLM by bilateral microinjection of muscimol decreased mean AP in all groups, and AP was not further reduced by chlorisondamine. Bars = means ± SE. *Significant decrease from baseline mean AP within group, P < 0.05. †Significantly lower baseline mean AP compared with control rats, P < 0.05. Baseline HR in control rats (407 ± 13 beats/min) was equivalent to that observed in rats with SAD (375 ± 12 beats/min; P < 0.05) and rats with lesions of the NTS (395 ± 35 beats/min; P < 0.05). Inhibition of the RVLM by microinjection of muscimol decreased HR comparably in control rats (−68 ± 7 beats/min), rats with SAD (−64 ± 10 beats/min; P > 0.05), and rats with lesions of the NTS (−61 ± 11 beats/min; P > 0.05; see Fig. 4). Subsequent injection of chlorisondamine produced a small rise in HR in all groups (see Fig. 4; 24 ± 5 beats/min in control rats, 25 ± 9 beats/min in rats with SAD, and 18 ± 11 beats/min in rats with lesions of the NTS).

In a subset of rats to examine the efficacy of the GABAergic receptor blockade, we confirmed that microinjections of bicuculline into the RVLM abolished the depressor responses to phenyl biguanide (in 4 control rats and 4 rats with SAD).

Control of AP by the CVLM in baroreceptor-denervated rats. In baroreceptor-intact rats, glutamatergic neurons in the NTS, which are driven by baroreceptor inputs, tonically activate neurons in the CVLM to produce baroreceptor reflex-mediated responses (1, 26). However, in chronic baroreceptor-denervated rats, the NTS no longer tonically influences AP (42). Therefore, we determined whether the CVLM continues to tonically inhibit the RVLM and whether these CVLM neurons are still tonically activated by glutamate.

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indicating the CVLM was effectively inhibited. In this particular experiment, the rats with chronic SAD had slightly lower baseline AP (Fig. 6B). However, inhibition of the CVLM significantly increased mean AP to the same levels observed in control rats (Figs. 6B and 7B), and the change in AP was not different (85 ± 7 vs. 67 ± 6 mmHg in control rats; \( P > 0.05 \)).

**Blockade of glutamatergic receptors in the CVLM.** Baseline AP was equivalent in control rats and rats with SAD (Fig. 6C). In baroreceptor-intact rats, microinjections of the broad-spectrum glutamate receptor antagonist kynurenate into the CVLM markedly increased AP (55 ± 6 mmHg; Fig. 6C), and this response was not altered by chronic SAD (71 ± 8 mmHg, \( P > 0.05 \); Fig. 6C). Antagonism of glutamatergic receptors in the CVLM produces a rise in AP similar to that seen after inhibition of neurons in the CVLM (Fig. 6B vs. 6C), indicating that glutamate is a major tonic excitatory input for the CVLM neurons that regulate AP.

**DISCUSSION**

The major goal of the present study was to begin to examine the central neural circuits involved in the tonic control of AP in rats with chronic baroreceptor denervation. Although much has been learned about the central neural control of AP during the...
past 30 yr, most of this work has been conducted within the framework of the baroreceptor reflex. The present study sought to gain a greater understanding of factors that may be involved in the long-term, baroreceptor-independent control of AP. The major observations of the present study are that, in the chronic absence of a tonic regulation by baroreceptors or the NTS, a normal mean AP is tonically maintained supraspinally by the RVLM. In addition, baroreceptor-independent glutamatergic inputs to the CVLM drive a GABAergic inhibition of the RVLM to maintain a normal mean AP in chronic baroreceptor-denervated rats.

RVLM drives sympathetic vasomotor control in chronic baroreceptor-denervated rats. Sympathetic vasomotor outflow is normally maintained by supraspinal input, which appears to derive predominantly from the RVLM under resting conditions. Inhibition of the RVLM decreases MAP to \( \sim 65 \text{ mmHg} \), similar to levels observed with spinal cord transection or total autonomic ganglionic blockade (Fig. 3; Refs. 32, 40). The activity of presympathetic neurons in the RVLM is normally restrained by baroreceptor inputs and the NTS. Acutely, after removal of baroreceptor inputs or inhibition of inputs at the NTS, sympathetic vasomotor tone and AP rises by disinhibiting presympathetic neurons in the RVLM (48, 52). Under these conditions, the RVLM continues to tonically drive sympathetic vasomotor tone to maintain AP, as suggested by the profound reduction in renal sympathetic nerve activity after bilateral inhibition of the RVLM in acutely baroreceptor-denervated rabbits (17). However, in chronic baroreceptor-denervated rats, sympathetic vasomotor tone and AP appear to be restored to preoperative levels despite the absence of a great restraint normally provided by baroreceptor inputs and the NTS. To begin to explore how the brain maintains a normal mean AP in the absence of the NTS, we examined whether control of sympathetic vasomotor tone and AP are still driven supraspinally and specifically by the RVLM. The present study shows that inhibition of the RVLM produces decreases in AP that are not further reduced by autonomic ganglionic blockade in chronic baroreceptor-denervated rats produced by either SAD or lesions of the NTS, suggesting that in chronic baroreceptor-denervated rats AP is still maintained by a supraspinal drive of sympathetic vasomotor tone provided by the RVLM.

Although individual sympathetic nerve activities were not measured in the present study, the evoked peaks and troughs in AP measured in the present study are likely to reflect changes in sympathetic vasomotor tone. Manipulations of the CVLM and RVLM briskly alter sympathetic vasomotor nerve activity immediately preceding observed changes in AP in the time frame of the measured AP responses (e.g., Refs. 6, 11, 18, 39). In addition, because adrenergic vascular pressor reactivity is not grossly altered by chronic baroreceptor denervation either by lesions of the NTS or by SAD (5, 22, 27), comparable evoked changes in systemic AP observed among groups in the present study likely reflect similar functional changes in sympathetic vasomotor tone.

GABAergic control of the RVLM by the CVLM in the absence of NTS. The observation that the RVLM remains the predominant site of tonic descending sympathetic vasomotor drive in baroreceptor-denervated rats raises the question of what determines the tonic activity of these neurons. In baroreceptor-intact rats, the tonic activity of RVLM sympathetic vasomotor neurons is the result of a combination of excitatory and inhibitory influences (12, 25, 47). The best-characterized input to presympathetic RVLM neurons is GABAergic, and this inhibitory input is largely driven by baroreceptor afferents relayed to the RVLM through the CVLM (7). Acute inhibition of the CVLM removes baroreceptor-mediated changes in presympathetic RVLM neurons and increases their basal activity, leading to increased sympathetic vasomotor tone and AP (18, 23). However, the CVLM also appears to tonically inhibit the RVLM in the absence of baroreceptor inputs (37). In rats with acute SAD, kainic acid microinjection into the CVLM, which presumably inactivates cell bodies in the region, increases splanchnic nerve activity and AP (10). Similarly, in rabbits with acute lesions of the NTS, blockade of GABAergic receptors in the RVLM by microinjection of bicuculline still produces increases in AP, although the rise is smaller (11). In the present study, inhibition of neuronal cell bodies in the RVLM by local injection of muscimol or blockade of GABAergic input to the RVLM by injection of bicuculline increased AP equally in control and chronically denervated rats (Fig. 6). These observations suggest that baroreceptor inputs and the NTS are not necessary to drive the tonic inhibitory
GABAergic input from the CVLM to the RVLM. Furthermore, in the chronic absence of a drive from baroreceptor inputs, the ability of the CVLM to tonically inhibit the RVLM appears to be comparable to that shown in baroreceptor-intact rats.

In chronic baroreceptor-denervated rats, tonic inhibition of presympathetic RVLM neurons is no longer a result of the tonic influence of baroreceptor inputs to the NTS or the result of any input processed through the NTS. The NTS does not tonically influence AP in rats with chronic SAD (21, 42), and the GABAergic inhibition of presympathetic neurons in the RVLM is intact in rats with chronic lesions of the NTS (Fig. 6A). Such tonic baroreceptor-independent inhibitory actions of the CVLM have been suggested previously even in rats with intact baroreceptor reflexes (9, 15). The origin of this tonic baroreceptor-independent, CVLM-mediated regulation of the RVLM remains unknown but may underlie mechanisms that contribute to the maintenance of a normal resting AP that is defended by baroreceptor reflexes.

Glutamatergic control of CVLM in baroreceptor-denervated rats. The CVLM neurons that inhibit the RVLM are activated tonically by glutamate. Microinjection into the CVLM of kynurenic acid or other drugs that block glutamate receptors increases the activity of presympathetic RVLM neurons, sympathetic nerve activity, and AP (Fig. 6C: Refs. 1, 15). Indeed, blockade of glutamate receptors in CVLM produces as large a pressor response as inhibiting the cell bodies within this region (Fig. 6B vs. Fig. 6C), suggesting glutamate is the primary tonic excitatory input to these CVLM neurons. In rats with intact baroreceptor inputs, much of this glutamatergic input to the CVLM is driven by baroreceptor afferent nerves relayed through a direct projection from NTS. Increasing AP to activate baroreceptor afferents to NTS produces Fos expression in glutamatergic NTS neurons that project to the CVLM (53). In contrast, decreasing AP to unload baroreceptor inputs greatly reduces or silences cardiovascular-related GABAergic CVLM neurons (37, 38). However, blockade of glutamatergic inputs by microinjection of kynurenic acid into the NTS blocks the baroreceptor reflex but produces a smaller rise in AP than blockade of glutamatergic inputs in the CVLM (15), suggesting that the CVLM is also tonically driven by glutamatergic baroreceptor-independent inputs. Clearly, in rats with chronic SAD or lesions of the NTS, inputs independent of the NTS must provide the tonic drive to these cardiovascular-related CVLM neurons. Although glutamatergic inputs to the CVLM are still of primary importance in baroreceptor-denervated rats (Fig. 6C), they are no longer driven by baroreceptor afferent nerves or the NTS.

Other sources of tonic glutamatergic excitation of the cardiovascular-related CVLM neurons are not known. The paraventricular nucleus of the hypothalamus sends a glutamatergic projection to the CVLM (35), and stimulation of the paraventricular nucleus can elicit depressor responses that coincide with activation of individual barosensitive neurons in the CVLM (55), suggesting the paraventricular nucleus neurons may activate the CVLM to decrease AP. Another potential excitatory input to the CVLM may arise from respiratory-related pre-Bötzinger neurons immediately dorsal to the cardiovascular-related CVLM neurons, as lesion of pre-Bötzinger neurons appears to reduce the ability of the CVLM to decrease AP (51). Presympathetic RVLM neurons and sympathetic vasomotor nerves have respiratory-related activity (16) that may be conveyed by respiratory-related excitation of the CVLM. The spinal cord may be another source of excitation to the CVLM because neurons in laminae I-IV in spinal dorsal horn project directly to the region of the CVLM (44). In addition, stimulation of spinal afferents such as those in the greater splanchnic nerve activates baroactivated CVLM neurons, and blockade of glutamatergic receptors in the CVLM by microinjection of kynurenic acid greatly reduces the magnitude of the splanchnic-induced depressor response (30). However, it is not known whether any of these potential inputs directly and tonically activate the GABAergic baroactivated neurons in the CVLM.

Summary and conclusion. Chronic baroreceptor-denervated rats produced either by transection of the baroreceptor afferent nerves or by lesions at their point of termination in the NTS display a normal mean AP. Adrenergic vascular reactivity is not grossly affected by the chronic absence of baroreceptor reflexes, and removal of autonomic tone by ganglionic blockade produces comparable decreases in AP. Together, these data suggest that functional sympathetic vasomotor tone is normal in rats with chronic baroreceptor denervation. In the chronic absence of baroreceptor inputs or the NTS, the RVLM still drives sympathetic vasomotor tone and appears to be tonically restrained by a powerful GABAergic input from the CVLM. Furthermore, in chronic baroreceptor-denervated rats, a tonic drive from the NTS to the CVLM is absent, but the CVLM continues to be tonically activated by glutamate from an unidentified source. These data suggest that baroreceptor-independent glutamatergic inputs to the CVLM play an important role in the long-term maintenance of a normal AP in chronic baroreceptor-denervated rats.

Perspectives

Baroreceptor reflexes are essential for precise acute regulation and stabilization of AP. An essential role for arterial baroreceptors in the long-term control of blood pressure is questionable given that the acute hypertension following baroreceptor denervation is transient, with mean AP returning to normal levels in the chronic absence of baroreceptor reflexes. Previous studies have suggested that mean AP in chronic baroreceptor-denervated rats is supported by a normal sympathetic vasomotor outflow, although these studies have not addressed the central neural circuits involved in this setting of basal tonic sympathetic vasomotor outflow in rats with chronic baroreceptor denervation. We have previously shown that the NTS no longer tonically regulates AP in the chronic absence of baroreceptor inputs, although the present study indicates that the RVLM and CVLM still contribute to baseline sympathetic vasomotor tone much in the same way that they do in baroreceptor-intact rats. However, the glutamatergic baroreceptor-driven input from the NTS to the CVLM is maintained by a different, yet equally powerful, tonic glutamatergic drive in chronic baroreceptor-denervated rats. It remains to be determined whether this adaptation involves the strengthening of existing tonic inputs to the CVLM or the emergence of quiescent inputs in the chronic absence of control by the NTS. These results suggest that the ventrolateral medullary neural systems involved in cardiovascular regulation can function in the chronic absence baroreceptor inputs or the NTS to maintain a relatively normal sympathetic vasomotor outflow. These
baroreceptor-independent circuits may underlie the brain’s ability to provide stable levels of sympathetic vasomotor tone to contribute to the long-term regulation of AP.

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REFERENCES


