Chronic Lesion of Rostral Ventrolateral Medulla in Spontaneously Hypertensive Rats

Elisardo C. Vasquez, Stephen J. Lewis, Kurt J. Varner, and Michael J. Brody†

We studied the effects of chronic selective neuronal lesion of rostral ventrolateral medulla on mean arterial pressure, heart rate, and neurogenic tone in conscious, unrestrained spontaneously hypertensive rats. The lesions were placed via bilateral microinjections of 30 nmol/200 nl N-methyl-D-aspartic acid. The restimulation of this area with N-methyl-D-aspartic acid 15 days postlesion failed to produce a pressor response. One day postlesion, the resting mean arterial pressure was significantly decreased in lesioned rats when compared with sham rats (100±7 versus 173±4 mm Hg, p<0.05). Fifteen days later, the lesioned group still showed values significantly lower than the sham group (150±6 versus 167±5 mm Hg, p<0.05). No significant heart rate differences were observed between the sham and lesioned groups. The ganglionic blocker trimethaphan (5 mg/kg i.v.) caused similar reductions in mean arterial pressure in both lesioned and sham groups. The trimethaphan-induced hypotension was accompanied by a significant bradycardia in lesioned rats (—32±13 beats per minute) but a tachycardia in sham rats (+33±12 beats per minute) 1 day postlesion. Therefore, rostral ventrolateral medulla neurons appear to play a significant role in maintaining hypertension in conscious spontaneously hypertensive rats. Spinal or suprabulbar structures could be responsible for the gradual recovery of the hypertension in the lesioned rats. (Hypertension 1992;19[suppl II]:II-154–II-158)

It has long been recognized that the rostral ventrolateral medulla (RVLM) contains neurons necessary for maintaining normal resting arterial pressure and heart rate (HR). In the past it was shown that interruption of nerve impulse activity in RVLM neurons using tetrodotoxin, inhibitory amino acids, or electrolytic lesions causes a collapse of arterial pressure and HR1–3 to levels produced by spinal cord transections.4 Activation of this area using electrical stimulation or excitatory amino acids causes elevations of arterial pressure and HR.1,2,5,6 Cochrane and Nathan7 examined the effects of bilateral electrolytic lesion of the RVLM on arterial pressure and HR of conscious rats, showing that this lesion did not cause hypertension in rats that were fully recovered from anesthesia. Because these lesions destroy both cell bodies and fibers of passage, the results are somewhat difficult to interpret. Considering the limitations of this technique, we recently introduced a method for selectively lesioning neurons within the RVLM while preserving fibers of passage using the neurotoxin N-methyl-D-aspartic acid (NMDA).8,9 By using this approach, we have shown that bilateral NMDA-induced lesions within the RVLM cause a significant reduction of arterial pressure in normotensive rats 1 day postlesion but that arterial pressure recovers within 7 days. Although the role of the RVLM in the maintenance of arterial pressure in normotensive rats is well established, few studies have investigated the possible involvement of this brain region in the etiology of hypertension.10,11 Studies have suggested that a centrally mediated increase in sympathetic outflow could contribute to the maintenance of hypertension in spontaneously hypertensive rats (SHRs)12; however, the responsiveness of RVLM neurons seems to be normal in anesthetized SHRs.11 Therefore, the aim of the present work was to investigate the effects of chronic selective neuronal lesion in RVLM on resting arterial pressure and HR in conscious SHRs.

Methods

Experiments were performed in male 16–20-week-old SHRs (n=16) weighing 300–350 g, purchased from Harlan Sprague Farms. All experimental pro-
Pecisures were in accordance with the University of Iowa and National Institutes of Health guidelines for the care and use of animals. The animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and placed on a heating pad to ensure proper body temperature (American Medical System, Cincinnati, Ohio). The femoral artery and vein were catheterized for arterial pressure measurements and drug injection, respectively, and the free end of each cannula was exteriorized through an incision at the back of the neck. The trachea was intubated to permit artificial ventilation with a rodent respirator (Harvard Apparatus, South Natick, Mass.).

The animal was mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, Calif.) with the head inclined downward and the bite bar set at -17 mm. The occipital bone was removed and the atlanto-occipital membrane opened. The calamus scriptorius served as stereotoxic zero for rostral-caudal and lateral coordinates. The RVLM region was localized 2 mm lateral to the midline, 0.7 mm dorsal to the ventral surface of the brain, and 1.8-2.0 mm rostral to the calamus scriptorius. Bilateral microinjections into the RVLM were done using three-barreled glass micropipettes (0.58 mm i.d.) pulled to an outside tip diameter of approximately 50 µm. NMDA (30 nmol/200 nl) was delivered by a pneumatic picopump (PV 800, World Precision Instruments, Sarasota, Fla.). In sham-operated rats, 200 nl of saline was microinjected. The RVLM area was functionally identified by the pressor response elicited by the microinjection of NMDA (40 ng/50 nl) through a barrel of a three-barreled micropipette. The volume of NMDA or saline injected was determined by measuring the movement of the fluid meniscus in the pipette through the graticule of a dissecting microscope (Olympus Corp., Lake Success, N.Y.). The animals were allowed to recover 24 hours postlesion in conscious, unrestrained rats. Resting mean arterial pressure (MAP), pulsatile pressure, and HR were measured 1, 6, 10, and 15 days postlesion in conscious, unrestrained rats. Resting MAP and HR were considered only after an initial period of 1-2 hours of continuous recording of these parameters to allow the animals to adapt to the measurement conditions. MAP was measured with disposable pressure transducers (Cobe Laboratories, Lakewood, Colo.). HR was measured by a cardiotachometer triggered from the arterial pulse pressure signal. Neurogenic tone was quantified by injecting the ganglionic blocker trimethaphan (5 mg/kg i.v.) and measuring the changes in MAP and HR.

At the end of the chronic experiments, the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and perfused through the ascending aorta with saline followed by 10% buffered formalin. The brains were removed, cut in sections of 40 µm, and stained with cresyl violet. The extent of selective neuronal damage was quantified microscopically by measuring the area of gliosis and cell loss in the coronal sections. Statistical analysis was done using a two-way repeated-measures analysis of variance followed by the Tukey procedure for post hoc comparisons. All data are reported as mean±SEM. Differences were considered significant at values of p<0.05.

Results

Before the lesions, NMDA (40 ng/50 nl)–induced pressor responses were similar in sham and lesioned groups (+27% versus +26%, respectively, n=4) but were completely attenuated in lesioned rats 15 days postlesion (sham versus NMDA lesion, +23% versus +1%, n=4). Figure 1 summarizes the site and extent of bilateral lesion in the eight RVLM lesioned rats. For each animal, the extent of the lesion was determined by the presence of gliosis and cell loss resulting from the neurotoxic action of NMDA. The lesions were bilaterally centered at 11.8 mm posterior to bregma, 2 mm lateral to midline, and 0.7 mm dorsal to the ventral surface of the brain stem. The extension of the lesion was approximately 1 mm in diameter. Figure 2 shows the effects of this lesion on resting MAP, pulsatile pressure, and HR in the same rat, compared with a sham rat, 1-15 days postlesion. The group averages of the lesion effects are shown in Figure 3. One day postlesion, resting MAP was substantially decreased (p<0.01) in the SHR lesioned group (100±7 mm Hg) when compared with the SHR sham group (173±4 mm Hg). The lesioned animals gradually recovered from the hypotension, but after 15 days, MAP was still lower than in the sham group (150±6 versus 167±5 mm Hg, p<0.05). No significant differences were observed in resting HR between sham and lesioned SHR rats.
The contribution of neurogenic tone to the maintenance of resting MAP and HR was analyzed using the ganglionic blocker trimethaphan. Figure 4 summarizes the effects of this agent on resting MAP and HR. Trimethaphan caused similar reductions in MAP in both lesioned and sham SHR groups. However, the changes in resting HR after the ganglionic blockade were in opposite directions 1 day postlesion; the SHR sham group showed an increase of 33 ± 12 beats per minute and the lesioned SHR group a decrease of 32 ± 13 beats per minute.

**Discussion**

It has been proposed that a centrally mediated sympathetic hyperactivity contributes to the maintenance of hypertension in SHRs. However, the origin of this increase in sympathetic outflow is not yet known. This study is the first showing the effects of chronic lesion of RVLM on arterial pressure in SHRs. It was demonstrated that these lesions markedly reduce MAP in the hypertensive strain. The RVLM in the rat is centered 2 mm lateral to the midline and 0.7 mm dorsal to the ventral surface of the brain stem, overlapping the C1 adrenergic region. Efferent projections from RVLM neurons reach the thoracolumbar intermediolateral cell column, providing a direct input to the preganglionic sympathetic neurons. As previously reviewed by others, the

**Figure 2.** Representative tracings illustrate temporal evolution of resting mean arterial pressure (MAP), pulsatile pressure (PP), and heart rate (HR) in a sham lesioned (SHAM) and a rostral ventrolateral medulla (RVLM) lesioned rat.

**Figure 3.** Histograms show chronic effects of selective neuronal lesion of rostral ventrolateral medulla (RVLM) on resting mean arterial pressure (MAP) and heart rate (HR) in conscious, unrestrained spontaneously hypertensive rats. Values represent mean ± SEM. **Significant differences between RVLM lesioned and sham lesioned rats; p < 0.01.

**Figure 4.** Histograms show effects of ganglionic blockade on mean arterial pressure (MAP) and heart rate (HR) in conscious sham lesioned (sham) and rostral ventrolateral medulla (RVLM) lesioned rats. Values (mean ± SEM) represent MAP and HR changes after trimethaphan (5 mg/kg i.v.). **Significant differences between RVLM lesioned and sham lesioned rats; p < 0.01.
integrity of the RVLM has been considered crucial for the neurogenic maintenance of resting arterial pressure and HR.

In a previous study, we introduced the use of NMDA as a novel method of obtaining selective neuronal lesion in RVLM, contrasting with the electrolytic lesion method used by others, which destroys both neurons and fibers of passage. Figures 2 and 3 clearly illustrate the critical role played by RVLM in the maintenance and generation of high levels of AP in this model of spontaneous hypertension. One day postlesion, the resting MAP in RVLM lesioned rats was drastically reduced to levels considered to be normotensive in other rat strains. The MAP of these animals did gradually recover; however, 15 days postlesion, there was still a significant reduction in MAP (10%) when compared with sham rats. (MAP was not monitored for longer than 15 days because of the technical limitations of the direct AP measurement.) This finding sharply contrasts with our previous study of chronic selective neuronal lesions of RVLM in Sprague-Dawley rats and that of chronic electrolytic lesions of RVLM in Long-Evans rats, which showed a significant reduction in MAP and a complete recovery of normal values by 2–7 days postlesion. The present studies did not determine the mechanisms responsible for the recovery of the arterial pressure in the RVLM lesioned rats. A greater role of NMDA-resistant RVLM neurons in the tonic expression of arterial hypertension and a more important role of other brain or spinal areas could contribute to the expression of this phenomenon. Recently, we also reported that in barbiturate-anesthetized Sprague-Dawley rats, acute NMDA-induced lesion of neurons in RVLM blocks the development of neurogenic hypertension induced by sinoaortic baroreceptor denervation.

Although no significant differences were observed in mean resting HR between the RVLM lesioned SHR and sham SHR groups, three rats displayed a definite tachycardia, especially 1 day postlesion. This could be caused by the observed partial lesion of nucleus ambiguus, which is one of the origins of cardiac vagal preganglionic neurons.

Administration of the autonomic ganglionic blocking agent trimethaphan produced a similar decrease in MAP, which was significant in both RVLM lesioned and sham groups at 1, 6, 10, and 15 days postlesion. This finding is similar to those reported in normotensive Sprague-Dawley and Long-Evans rats. Considering that the resting MAP, previous to the administration of the ganglionic blockade, is statistically different in both groups, one could argue that the relative decrease in MAP after trimethaphan was higher in the RVLM lesioned groups (41%) than in the sham group (30%). However, although the trimethaphan-induced hypotension was accompanied by a tachycardic response in the sham group, the RVLM lesioned rat showed a significant bradycardic response, 1 day postlesion. This bradycardia in the RVLM lesioned rats could contribute to the relatively greater decrease in MAP. The tachycardia observed in the sham rats could be caused, at least in part, by behavioral reaction to the trimethaphan. The animals intensively scratch the dorsal neck and head region immediately after trimethaphan. In RVLM rats, this behavioral activity was very attenuated in the first days postlesion. The ganglionic blocking agent chlorisondamine has a similar hypotensive effect as trimethaphan, but it was avoided because the hypotensive and bradycardic effect of this drug lasts more than 1 hour, and lesioned rats do not survive the administration of this agent on the first postlesion day.

Finally, this study showed that the chronic selective neuronal lesion of the RVLM substantially reduces the resting MAP in conscious SHRs, although the cardiovascular neurogenic tone seems not to be altered. Three further points need to be mentioned. First, the effectiveness of the RVLM lesion was confirmed in this study by the finding that 15 days postlesion the bilateral chemical restimulation of the lesion area with NMDA failed to evoke a pressor response. Second, the neuronal loss in RVLM was histologically confirmed in the coronal sections. Third, in preliminary studies using fluorescent tracers, we demonstrated that NMDA lesions virtually abolished the RVLM cells projecting to the spinal cord of the rat (unpublished observation from our laboratory). Therefore, it is possible that other non-RVLM medullary, spinal, or supramedullary centers take over the cardiovascular role previously played by the RVLM.

Acknowledgments

We thank Richard Shaffer and Sandra Boutelle for their technical support and Joanie Rogers for secretarial assistance in the preparation of this manuscript.

References

5. Dampney RAL, Kamada M, Reis DJ: Profound hypotension and abolition of the vasomotor component of the cerebral ischemic response produced by restricted lesions of medulla oblongata: Relationship to the so-called tonic vasomotor center. Circ Res 1979;45:63–70

10. Kubo T, Nagura J, Kihara M, Miru Y: Cardiovascular effects of L-glutamate and gamma-aminobutyric acid injected into the rostral ventrolateral medulla in normotensive and spontaneously hypertensive rats. *Arch Int Pharmacodyn Ther* 1986;279:150–161

11. Smith JK, Barron KW: Cardiovascular effects of L-glutamate and tetrodotoxin microinjected into the rostral and caudal ventrolateral medulla in normotensive and spontaneously hypertensive rats. *Brain Res* 1990;506:1–8


**KEY WORDS** • medulla oblongata • blood pressure • ganglionic blockade • neurotoxins • trimethaphan • spontaneously hypertensive rats
Chronic lesion of rostral ventrolateral medulla in spontaneously hypertensive rats.
E C Vasquez, S J Lewis, K J Varner and M J Brody

Hypertension. 1992;19:II154
doi: 10.1161/01.HYP.19.2_Suppl.II154

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/19/2_Suppl/II154

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/