Lesions in Rostral Ventromedial or Rostral Ventrolateral Medulla Block Neurogenic Hypertension

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Abstract  Neurogenic hypertension results from the removal of inhibitory baroreceptor afferent input to vasomotor systems in the central nervous system. We sought to determine whether the bilateral destruction of neurons in the rostral ventrolateral or rostral ventromedial medulla, made using microinjections of N-methyl-D-aspartic acid (30 nmol in 200 nL), would block the acute increase in arterial pressure after sinoaortic deafferentation in pentobarbital-anesthetized rats. Bilateral lesions of the rostral ventrolateral or rostral ventromedial medulla decreased mean arterial pressure (107±4 to 78±5 and 115±3 to 94±3 mm Hg, respectively). In rostral ventrolateral or rostral ventromedial medulla lesioned rats, sinoaortic deafferentation failed to increase arterial pressure. Sham lesions or lesions placed rostral to the rostral ventrolateral or rostral ventromedial medulla did not significantly lower arterial pressure. Subsequent sinoaortic deafferentation significantly increased mean arterial pressure (109±3 to 145±4 and 109±5 to 141±3 mm Hg, respectively). In eight rats we used an infusion of angiotensin II to return arterial pressure to control levels after lesion of the rostral ventrolateral (n=4) or rostral ventromedial (n=4) medulla. In these animals, sinoaortic deafferentation failed to increase arterial pressure. We conclude that neurons in the rostral ventrolateral and rostral ventromedial medulla are involved in the normal maintenance of arterial pressure and the development of hypertension after sinoaortic deafferentation in pentobarbital-anesthetized rats. (Hypertension. 1994;24:91-96.)

Key Words  • hypertension, experimental • central nervous system • aspartic acid • rats • medulla oblongata

The loss of inhibitory baroreceptor input to vasomotor centers in the central nervous system produces an increase in arterial blood pressure (AP), which is referred to as neurogenic hypertension. Experimentally, neurogenic hypertension can be produced by surgically interrupting peripheral baroreceptor afferent nerves (principally those within the carotid sinus and aortic depressor nerves), a procedure referred to as sinoaortic deafferentation (SAD).1-3 Neurogenic hypertension can also be produced by lesioning the nucleus tractus solitarius (NTS), the primary site of baroreceptor afferent termination in the central nervous system.4-7 In either model the initial increase in AP is mediated by an increase in central sympathetic outflow.8 However, in the NTS model a centrally mediated increase in vasopressin release is also involved in the hypertensive response.7,9 Whether AP remains chronically elevated in these models of neurogenic hypertension is controversial,1-2,5,10-13 although lability of AP is a common chronic feature.1,2,5,10-13

The location or locations of those neurons responsible for the increase in sympathetic nerve activity after the removal of inhibitory baroreceptor influences have not been completely identified. Several studies indicate that sympathetic networks rostral to the brain stem are involved in the genesis of neurogenic hypertension.5,14-17

Given the importance of medullary sympathetic networ0ks in the normal maintenance of neurogenic vasomotor tone,18,19 it is reasonable to expect that these networks also play an important role in the hypertensive response to SAD or NTS lesion. The purpose of this study was to determine whether sympathetic networks in the medulla are involved in the acute increase in AP after SAD. In particular, these studies examined the involvement of two groups of sympathetic vasomotor neurons located in the rostral ventrolateral medulla (RVLM) and rostral ventromedial medulla (RVMM). The RVLM contains sympathetic bulbospinal neurons that play an important role in the maintenance of neurogenic vasomotor tone (for reviews see References 18 and 19). The activity of these sympathetic neurons is under baroreceptor reflex control.18-21 Moreover, Benarroch et al22 showed that bilateral lesion of the RVLM reverses the hypertensive response elicited by NTS lesion. However, RVLM lesion does not prevent the increased release of vasopressin after NTS lesion.22 Studies by Brody and colleagues (Varner et al23 and Cox and Brody24) suggest that the RVMM (located rostral and medial to the RVLM) also contains neurons involved in the maintenance of neurogenic vasomotor tone. However, it is not known whether their activity is under baroreceptor reflex control.

In the present studies we used N-methyl-D-aspartic acid (NMDA)–induced lesions of the RVLM or RVMM to determine whether neurons in either region are involved in the acute hypertension and tachycardia produced by SAD in anesthetized rats.

Methods

Subjects

Forty-three male Sprague-Dawley rats (Harlan Sprague Dawley Inc) weighing 250 to 350 g were used in these studies. Before the experiments the animals were housed in groups

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with free access to food and water. All experimental procedures were in accordance with the University of Iowa and National Institutes of Health guidelines for the care and use of laboratory animals.

Surgery

Rats were anesthetized with pentobarbital (45 to 50 mg/kg IP). Anesthetic was supplemented (15 to 20 mg/kg IP or IV) as dictated by the presence of corneal reflex and/or cardiovascular responses to surgical procedures. The femoral artery and vein were catheterized for the recording of AP and administration of drugs, respectively. The arterial line was connected to a pressure transducer and an oscillographic recorder (model R 611, Beckman Instruments), which recorded pulsatile and mean arterial pressure (MAP). Heart rate (HR) was derived from the AP pulse by a cardiotachometer (model 9857B, Beckman). The trachea was cannulated and the rat mechanically ventilated (model 683, Harvard Apparatus) with a stroke volume of 2.5 mL and rate of 60 to 70 breaths per minute. Body temperature was maintained at 37.5 ± 1°C with a controlled heating pad (K-Module, American Pharmaseal).

Rats were placed in a stereotaxic head frame (David Kopf Instruments) with the bite bar 17 mm below interaural zero. The dorsal surface of the medulla was exposed by reflecting the scalp and removing the occipital bone and underlying dura. The calamus scriptorius was used as the rostral-caudal and medial-lateral stereotaxic reference point (A0). To locate the ventral surface of the medulla, the pipette was slowly lowered under microscopic guidance until a slight bend was observed in the pipette. The pipette was then retracted until the bend straightened, and this point served as the reference point for all dorsal-ventral movements.

SAD was performed with the use of a variation of the method of Krieger. Briefly, the carotid sinus, aortic depressor, and superior laryngeal nerves were bilaterally sectioned. The superior cervical ganglia were removed bilaterally and the adventitia stripped from the carotid bifurcation.

Microinjection

The RVLM and RVMM were stimulated and lesioned by microinjection of NMDA through stereotaxically placed three-barrel glass micropipettes (0.58-mm internal diameter, 1.0-mm outer diameter, 50- to 100-μm tip diameter) using a pneumatic pressure injection system (model 800, World Precision Instruments). The speed and volume of injection were controlled by observing the fluid meniscus inside the pipette with the use of a stereo microscope fitted with a calibrated reticule in the eyepiece. Microinjections into the RVLM were made 2.5 to 2.6 mm rostral and 0.9 to 1.0 mm lateral to A0 and 1.5 mm above the ventral surface of the medulla. Microinjections into the RVMM were made 1.8 to 2.0 mm rostral and 2.0 mm lateral to A0 and 0.5 to 0.8 mm above the ventral surface of the medulla. The term chemical inactivation is to be considered synonymous in this report. Lesion sites were marked by microinjection of 20 to 30 nL of a 5% solution (in saline) of fluorescent rhodamine microspheres (Luma Fluor Inc) through an adjacent barrel of the pipette. In some cases the fluorescent marker was contained in the NMDA lesion (5% concentration).

The effectiveness of the lesion was verified by showing that the microinjection of NMDA (40 ng/50 nL) into the lesion site failed to increase MAP 2.5 to 3 hours later. In addition, NMDA (40 ng/50 nL) was microinjected into the other vasomotor region ipsilateral to the lesioned area to determine whether the lesion had inactivated both vasomotor areas. After stimulation, the rats were removed from the stereotaxic instrument, and SAD was performed. AP was continuously monitored during the SAD procedure and for 15 to 30 minutes after deafferentation was completed. Pressure measurements were taken at the peak of the hypertensive response, which was usually reached between 2 and 7 minutes after SAD. The ganglionic blocking agent trimethaphan (5 mg/kg IV) was used to determine the level of neurogenic cardiovascular tone after SAD. In a second group of eight rats, after bilateral lesion of either the RVLM or RVMM, MAP was returned to prelesion levels with an intravenous infusion of angiotensin II (Ang II, 0.1 to 0.5 μg/kg per minute) before SAD.

Two control groups were used in this study. In one group, sham lesions were made by bilateral microinjection of 200 nL normal saline into either the RVLM (n = 4) or RVMM (n = 4). In the second group (n = 6), sites rostral to the RVMM or RVLM were lesioned bilaterally with the use of NMDA (30 nmol/200 nL). Injections rostral to the RVLM were made 1.9 to 3.1 mm rostral and 2.0 mm lateral to A0 and 0.7 to 1.5 mm above the ventral surface. Lesions rostral to the RVMM were made 3.5 to 3.6 mm rostral and 1.0 mm lateral to A0 and 1.5 mm above the ventral surface.

Histology

Fifteen to 30 minutes after SAD the rats were deeply anesthetized and killed by intracardiac perfusion with saline followed by 10% buffered formalin. The brains were removed and stored in buffered formalin for a minimum of 2 days. Serial frozen sections were cut with the use of a freezing microtome. Fluorescently labeled lesion sites were identified in unstained sections with the use of a Leitz microscope and photographed. The sections were then stained with cresyl violet and the lesion sites identified.

Drugs

Drugs used were pentobarbital (Butler), NMDA (Sigma Chemical Co), trimethaphan (Roche Laboratories), and Ang II (Sigma).

Data Analysis

AP and HR response values were calculated directly from the polygraphic recordings. MAP and HR levels before and after lesion were computed as an average of five measures taken at 1-minute intervals. Changes in MAP and HR elicited by SAD and drug injections were measured at maximum peak responses.

Statistical Analysis

Data are represented as mean ± SEM. Changes in MAP and HR during control, after lesion, and after SAD within the lesion and control groups were analyzed by repeated-measures ANOVA followed by the Student's modified t test using the Bonferroni correction for multiple comparisons. The same analysis was used for comparisons of MAP and HR values between lesion and control groups. The criterion for statistical significance was set at a value of P < .05.

Results

Fig 1 summarizes MAP and HR levels in the sham lesion (n = 8), control NMDA lesion (n = 6), RVLM lesion (n = 11), and RVMM lesion (n = 10) groups before lesion, after lesion, and after subsequent SAD. Resting
MAP was not significantly different in any of the experimental groups before lesion. Sham lesions or control NMDA lesions made rostral to the RVLM or RVMM failed to alter MAP significantly. Bilateral chemical inactivation of the RVLM significantly reduced MAP from 107±4 to 78±5 mm Hg. Chemical inactivation of the RVMM also significantly reduced MAP from 115±3 to 94±3 mm Hg. SAD significantly increased MAP in sham and control lesion rats to 145±4 and 141±3 mm Hg, respectively. However, the hypertensive response to SAD was blocked by prior bilateral lesion of either the RVLM or RVMM. Ganglionic blockade significantly reduced MAP in the sham (140±4 to 43±7 mm Hg) and control (138±2 to 45±5 mm Hg) lesion groups after SAD. MAP was also significantly reduced in the RVLM (75±3 to 37±4 mm Hg) and RVMM (86±4 to 31±2 mm Hg) lesioned rats. MAP level after ganglionic blockade was not significantly different among the four experimental groups.

Resting HR was not significantly different among the four experimental groups (Fig 1). Sham lesions did not change resting HR. Control NMDA lesions or lesions of the RVMM significantly increased HR from 334±22 to 361±20 and 354±14 to 388±19 beats per minute, respectively. Bilateral lesions of the RVLM failed to alter HR. HR increased after SAD only in the control NMDA lesion animals.

In a second group of experiments the hypotension produced by NMDA lesion of the RVLM (n=4) or RVMM (n=4) was reversed before SAD with the use of an intravenous infusion of Ang II. Fig 2 summarizes these results. Resting MAP in the RVLM and RVMM lesion groups were not significantly different before lesion, nor were they different than the levels in the sham and control NMDA lesion groups shown in Fig 1. RVLM lesion significantly reduced MAP 92±7 mm Hg. RVMM lesion also reduced MAP (107±3 to 97±6 mm Hg) although in this group of animals this decrease was not significant. An Ang II infusion was used to return MAP to prelesion levels. Subsequent SAD failed to increase MAP in the RVLM or RVMM lesioned rats.

To assess the effectiveness of the lesions, we injected NMDA (40 ng/50 nL) into lesion sites before and 2.5 to 3 hours after chemical inactivation. Representative AP and HR responses elicited by the microinjection of NMDA into the RVLM before lesion and 3 hours after lesion of the same site are shown in the left and middle traces of Fig 3, respectively. In 10 experiments the microinjection of NMDA into the RVLM before lesion increased MAP by 42±5 mm Hg. Chemical inactivation of the RVLM virtually eliminated (3±2 mm Hg) these responses. Similarly, in nine rats the pressor responses elicited by stimulation of the RVMM (46±10 mm Hg, n=10; in one rat the RVMM was stimulated bilaterally) were abolished (3±1 mm Hg) after lesioning the same site. The magnitude of the pressor responses elicited by stimulation of the RVLM or RVMM (36±5 mm Hg, n=7) was not significantly affected (32 ±2 mm Hg) by sham lesions. In most cases (69%), bradycardia accompanied the pressor responses elicited by stimulation of the RVLM or RVMM before lesion. Although not directly tested, these responses were probably reflex in origin.

RVLM lesion failed to block the NMDA-induced pressor response elicited by stimulation of the ipsilateral RVMM (Fig 3, right). In nine rats, after NMDA lesion of the RVLM, stimulation of the ipsilateral RVMM by microinjection of NMDA increased MAP 37±3 mm Hg (n=13, RVMM was stimulated bilaterally in four rats). Similarly, in eight rats, stimulation of the
RVLM after lesion of the ipsilateral RVMM increased MAP 36±6 mm Hg (n=8).

The photomicrographs in Fig 4 show a site where NMDA was microinjected to inactivate the RVLM in one rat. The histologically identified sites of the bilateral NMDA lesions in this study are summarized in Fig 5. The location of those lesions that prevented neurogenic hypertension after SAD are shown by filled circles. Effective lesions in the RVLM were centered ventral to the nucleus ambiguus and caudal to the posterior pole of the facial nucleus. Effective lesions in the RVMM were located more medial and rostral in the nucleus gigantocellularis reticularis. Lesion sites rostral to the RVLM and RVMM that failed to prevent neurogenic hypertension are shown by the open circles.

Discussion

This study has shown that the NMDA-induced inactivation of either RVLM or RVMM neurons prevents the acute development of neurogenic hypertension after SAD. The lack of a hypertensive response likely reflects the loss of baroreceptor-sensitive sympathoexcitatory neurons in these regions. RVLM-spinal sympathoexcitatory neurons play a critical role in the maintenance of sympathetic tone (for reviews see References 18 through 20). Moreover, their discharges are inversely related to the level of baroreceptor reflex activity.18-20 Benarroch et al22 have shown that during the acute hypertensive response produced by NTS lesion, electrolytic lesions or the microinjection of kainic acid into the RVLM returns AP to prehypertensive levels. These lesions did not block the increase in vasopressin release produced by NTS lesion.22

The presence of sympathoexcitatory neurons in the RVMM is less well documented. Studies from this laboratory have demonstrated that activation of RVMM neurons by the use of microinjections of NMDA increase AP.23 The axonal projections of these neurons have not been determined. Whether the discharges of vasomotor neurons in the RVMM are under baroreceptor reflex control is not known, although functionally unidentified neurons in the region of the RVMM respond to changes in the level of AP.27 Two lines of evidence suggest that RVMM neurons are involved in the maintenance of neurogenic vasomotor tone. First, the microinjection of glycine into the RVMM decreases AP in pentobarbital-anesthetized rats.23 Second, as demonstrated in the present study, the bilateral inactivation of RVMM neurons significantly lowered MAP in most rats (Fig 1).

The major question arising from this study is why bilateral lesions of either the RVLM or RVMM prevent the development of neurogenic hypertension after SAD. The simplest explanation is that the RVLM and RVMM are contained in the same efferent sympathetic pathway. Interruption of this pathway at either site...
would be expected to decrease resting AP and block the hypertensive response after SAD. However, this model does not account for the observation that after bilateral lesion of one vasomotor region, NMDA-induced activation of the other intact vasomotor region increased AP. This observation suggests that the vasomotor outputs from the RVLM and RVMM descend to the spinal cord over separate pathways.

Although bilateral lesion of either area significantly decreased AP, ganglionic blockade revealed that a significant amount of neurogenic vasomotor tone remained in these animals. Presumably, this remaining tone was generated by the other intact vasomotor region. Cox and Brody have reported that bilateral inactivation of both the RVLM and RVMM using microinjections of lidocaine lowers AP to spinal levels. Although the intact vasomotor region continues to generate vasomotor tone, it lacks sufficient reserve capacity to increase AP after SAD. The pressor responses elicited by stimulation of the intact region most likely resulted from the activation of neurons not involved in the maintenance of neurogenic vasomotor tone under the conditions of these experiments. Even after AP was returned to prelesion levels by Ang II infusion (in an effort to increase baroreceptor tone on neurons in the intact region), SAD still failed to produce hypertension. Angiotensin infusion was chosen because circulating Ang II reportedly has little effect on the sympathetic components of the baroreceptor reflex. Why inactivation of the RVMM failed to significantly decrease AP in this rat group before Ang II infusion is not clear. In these rats the RVMM may not have been as involved in the maintenance of AP, or the lesions may not have been as complete as in the first rat group. Nonetheless, these RVMM lesions were sufficient to block the hypertensive response to SAD.

Whether there are reciprocal connections between the RVLM and RVMM that are involved in the control of neurogenic vasomotor tone is not known. However, our study indicates that both regions are capable of independently influencing neurogenic vasomotor tone and the integrity of both sites is required to produce hypertension after SAD.

It is difficult to evaluate the size of the nonfunctional zone associated with an acute neurochemical lesion because the normally used markers of these lesions, such as the appearance of cell loss and/or gliosis, require several days to appear. In the present study the extent of the lesions was not functionally mapped by using microinjections of NMDA because of the fear that repeated pipette penetrations would destroy large portions of the medulla, possibly preventing an increase in MAP after SAD. However, three observations suggest that the NMDA microinjections into one site did not inactivate both the RVLM and RVMM. First, after inactivating the RVLM, NMDA microinjection into the RVMM ipsilateral to the lesion elicited a pressor response, indicating that the microinjection had not destroyed neurons in the RVMM. Similarly, pressor responses were elicited by the NMDA microinjection into the RVLM after inactivating the ipsilateral RVMM. Second, histological reconstruction of long-term lesions (7 days) of the RVLM made by using the same NMDA volumes and concentrations as used in the present study did not involve both the RVLM and RVMM. Finally, the distance between control NMDA injections that failed to block neurogenic hypertension and the RVLM or RVMM was often less than the distance between the RVLM and RVMM.

In conclusion this study has shown that both the RVLM and RVMM, the forebrain has also been shown to play a significant role in the development of neurogenic hypertension by using SAD or NTS lesion. Whether the forebrain contribution to neurogenic hypertension after SAD involves the RVLM and/or RVMM has not been directly tested. This possibility is supported by anatomic evidence. The RVLM receives afferent inputs from a number of brain regions involved in cardiovascular function, including the parabrachial nucleus, NTS, Kollik-Fuse, periaqueductal gray, lateral hypothalamus, and paraventricular nucleus. The afferent projections to cardiovascular responsive regions in RVMM have not been documented.

In conclusion this study has shown that both the RVLM and RVMM are involved in the development of
neurogenic hypertension elicited by SAD in pentobarbital-anesthetized rats. These studies have also provided further evidence that the RVMM is involved in the maintenance of neurogenic vasomotor tone in this preparation.

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