Identification of Cardiovascular Neurons in the Rostral Ventromedial Medulla in Anesthetized Rats

Kurt J. Varner, Darin S. Rutherford, Elisardo C. Vasquez, and Michael J. Brody†

Recent studies have identified a region in the rostral ventromedial medulla (RVMM) of rats that appears to be involved in cardiovascular function. Since these studies used either microinjection of lidocaine or electrical stimulation, the exact contribution of intrinsic neurons as opposed to fibers of passage could not be determined. The present study was performed to map the location of neurons in RVMM from which changes in mean arterial pressure could be elicited by the microinjection of the excitatory amino acid analogue N-methyl-D-aspartic acid (NMDA) (20 ng/50 nl), which selectively activates cell bodies in barbiturate-anesthetized rats. Microinjection of NMDA into RVMM most often (53%) elicited pressor responses (31 ± 7 mm Hg). On the basis of these responses, RVMM was determined to encompass a large portion of the nucleus gigantocellularis 0.5–1.5 mm lateral to the midline, 0.5–3.5 mm above the ventral surface, and extending from the rostral to the caudal pole of the facial nucleus. Depressor responses (−21 ± 3 mm Hg) were found at all levels of RVMM but were most concentrated and of the largest magnitude in the rostral and caudal poles of RVMM. Microinjection of the inhibitory neurotransmitter glycine (500 mM) was used to determine whether neurons in RVMM were contributing to the maintenance of arterial pressure. Microinjection of glycine decreased arterial pressure (−15 ± 2 mm Hg) throughout most of RVMM. Unexpectedly, increases in mean arterial pressure (24 ± 3 mm Hg) were elicited by microinjection of glycine into the same region in RVMM in which NMDA most frequently elicited pressor responses. These studies show that 1) RVMM contains neurons capable of influencing vasomotor tone, 2) at least some of these neurons contribute to the maintenance of neurogenic tone in anesthetized rats, and 3) microinjection of glycine into RVMM can increase mean arterial pressure by an as yet undetermined mechanism. (Hypertension 1992;19[suppl II]:II-193–II-197)

It has long been recognized that neural networks in the rostral medulla are essential for the maintenance of neurogenic vasomotor tone in a number of species. Within the rostral medulla a number of subregions have recently been identified, each of which contains neurons capable of controlling cardiovascular function. These subregions include the rostral ventrolateral medulla (RVLM) (see Reference 1), dorsal medial medulla,2–4 and midline raphe nuclei (pallidus, obscurus, and magnus).5,6 Recently our laboratory has identified an additional subregion in the rostral ventromedial medulla (RVMM) in rats that also appears to be involved in cardiovascular regulation. Preliminary studies indicate that RVMM lies medial to and in the same rostral-caudal plane as RVLM.7–9 Bilateral inactivation of either RVLM or RVMM using microinjections of the local anesthetic lidocaine elicits equal reductions in arterial pressure in urethane-anesthetized rats.7–9 Combined inactivation of both regions virtually eliminates neurogenic vasomotor tone.9 Although both regions are equally involved in the neurogenic maintenance of arterial pressure, studies using microinjections of lidocaine or electrical stimulation have shown that these two regions differentially control cardiovascular function. RVLM and RVMM have been shown to differ with respect to their 1) sensitivity to changes in respiratory tidal volume,8 2) control of regional blood flow,7 3) control of the level of activity on individual sympathetic nerves,9 and 4) control of heart rate.10 Since electrical stimulation and lidocaine affect both axons of passage and cell bodies, it is not clear
from previous studies whether RVMM contains the cell bodies of neurons capable of influencing cardiovascular function. Therefore, in the present study we used microinjection of the excitatory amino acid N-methyl-D-aspartic acid (NMDA), which selectively activates cell bodies, to determine whether RVMM contains neurons capable of influencing arterial pressure. In the second study, we microinjected the inhibitory neurotransmitter glycine into RVMM to determine whether RVMM contains the cell bodies of neurons involved in the maintenance of neurogenic vasomotor tone in anesthetized rats.

**Methods**

Experiments were performed in 18 male Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) weighing 300–400 g. All experimental procedures were performed in accordance with the University of Iowa and National Institutes of Health guidelines for the care and use of laboratory animals.

Rats were anesthetized with pentobarbital (40–50 mg/kg ip) and the femoral artery and vein were cannulated for the measurement of arterial pressure and the administration of drugs, respectively. Arterial pressure was recorded using a CDX pressure transducer (Cobe, Arvada, Colo.) and was displayed on a Beckman recorder (model R611, Beckman Instruments, Inc., Fullerton, Calif.) as pulsatile and mean arterial pressure (MAP). Heart rate was derived from the arterial pulse using a Beckman 9857B cardiotaohonometer. The trachea was cannulated, and the animal was allowed to respire naturally. Anesthesia was supplemented as dictated by the presence of corneal reflex and/or cardiovascular responses to surgery.

The rats were placed in a stereotaxic head frame (David Kopf Instruments, Tujunga, Calif.) with the bite bar 17 mm below interaural zero. The dorsal surface of the medulla was exposed by removing the occipital bone and dura. The calamus scriptorius was identified as the stereotaxic zero point.

Glycine (500 nM) and NMDA (20 ng) were microinjected stereotaxically into RVMM using one- or three-barrel glass micropipettes (0.58 mm i.d., 50–80 μm composite tip diameter) connected to a pneumatic injection system (model PV 800, World Precision Instruments, Sarasota, Fla.). Normal saline was injected as a vehicle and volume control. In all cases, the injection volume of 50 nl was delivered over 5–10 seconds. The speed and volume of injection were controlled by watching the fluid meniscus in the pipettes with a stereo microscope (Olympus, Deer Park, N.Y.) with a graticule in the eyepiece. Pipette penetrations were made from 1.5–4.0 mm rostral to the calamus scriptorius, 0.5–2.0 mm lateral to the midline, and 0.5–4.0 mm dorsal to the ventral surface of the medulla. Injections were made along pipette tracks with a minimum of 500 μm between injections in a given track. In several instances depressor and pressor responses could be elicited by the microinjection of NMDA into sites separated by only 500 μm. Pipette tracks were separated by a minimum of 500 μm and no more than six tracks were made in each animal. Selected injection sites were marked by the injection of rhodamine microspheres (5–10% solution, Luma Fluor Inc., New City, N.Y.) contained in the NMDA or control saline solutions. Selected sites of glycine injection were marked by injecting 20–30 nl India ink contained in the third barrel of the pipette.

At the end of each experiment the rats were killed by intracardiac perfusion with saline followed by 10% buffered formalin. Brains were removed and stored in buffered formalin for 2 days after which serial 40-μm frozen coronal sections were cut. Injection sites containing rhodamine were identified directly using a fluorescent microscope (Ernest Leitz, GMBH, Wetzlar, FRG) and photographed. The sections were then stained with cresyl violet to identify nuclear groups. Since not all injection sites were marked in each experiment, careful records were kept of the coordinates of all injections. Sites of injection were reconstructed from histologically identified pipette tracks using the ventral end of the tracks (ventral surface of the medulla) as a reference point. Injection sites marked with rhodamine were used to verify the depth of injection sites along specific tracks and to identify the individual tracks.

Maximum changes in MAP and heart rate elicited by microinjection of drugs were measured directly from polygraphic records. Only those responses that occurred within 30 seconds of the injection were analyzed. Data are reported as mean±SEM.

**Results**

**N-Methyl-D-Aspartic Acid Injections**

A total of 176 sites were stimulated by microinjecting NMDA (20 ng/50 nl) in 14 animals. Stimulation of 93 (53%) sites elicited pressor responses (31±7 mm Hg), whereas stimulation of 37 (21%) sites elicited depressor responses (−21±3 mm Hg). Stimulation of 46 (26%) sites elicited changes (increases or decreases) in arterial pressure of 0–5 mm Hg. Control injections of saline failed to elicit changes in MAP of more than ±5 mm Hg. Heart rate responses accompanying the changes in arterial pressure most often appeared to be of reflex origin, and therefore were not systematically studied.

Figure 1 is a map of histologically identified sites of NMDA microinjection into RVMM illustrating the magnitude and direction of the MAP response elicited at each site. Equivalent changes in pressure were elicited from both sides of the brain. For clarity, depressor responses have been grouped on the left and pressor responses on the right side of each section.

At each level of RVMM shown in Figure 1, the relative distribution (percent) of pressor, depressor, and nonresponsive sites was calculated. The proportion of pressor-responsive sites in RVMM increased from the rostral (P=10.52) to the caudal (P=11.96)
FIGURE 1. Schematic drawings show coronal sections of the rat medulla summarizing sites of N-methyl-D-aspartic acid (NMDA) injection (20 ng in 50 nl) in 14 rats. Sections are taken from the atlas of Paxinos and Watson. P values indicate distance (mm) caudal to bregma. Symbols on the left of each section show the location and magnitude of depressor responses elicited by NMDA: ○, 0 to −5 mm Hg; ●, −6 to −25 mm Hg; ■, −26 to −45 mm Hg; ▲, more than −45 mm Hg. Location and magnitude of increases in mean arterial pressure are shown by the symbols on the right side of each section: ○, 0 to 5 mm Hg; ●, 6 to 25 mm Hg; ■, 26 to 45 mm Hg; ▲, more than 45 mm Hg. Acc7, accessory facial nucleus; Amb, nucleus ambiguus; VII, facial nucleus; Gi, gigantocellular reticular nucleus; g7, genu of the facial nerve; Pyr, pyramids; RVL, nucleus reticularis ventrolateralis; Sp5, spinal tract of the trigeminal nerve.

Pole (29%, 57%, 45%, 75%, and 41%, respectively), where P value indicates distance (mm) caudal to bregma. Conversely, the proportion of depressor sites was greatest at the rostral and caudal poles and lowest toward the center of RVMM (rostral-caudal: 38%, 20%, 19%, 8%, and 35%, respectively). The rostral-caudal distribution of nonresponsive sites (33%, 23%, 36%, 18%, and 24%, respectively) showed no particular pattern.

Glycine Injections
To determine whether neurons in RVMM were involved in the tonic maintenance of arterial pressure, the inhibitory neurotransmitter glycine was microinjected into those regions in RVMM from which increases in arterial pressure were elicited by the microinjection of NMDA. Glycine was injected into a total of 81 sites in RVMM in four rats. Sites in RVMM into which glycine was microinjected are mapped in Figure 2. Microinjection of glycine decreased MAP −15 ± 2 mm Hg (n = 19). Depressor sites were scattered from 10.52 to 11.6 mm caudal to bregma. Unexpectedly, in 32 sites the microinjection of glycine increased MAP 24 ± 3 mm Hg. These pressor responses were elicited from the same region in which microinjection of NMDA elicited the largest proportion of pressor responses (compare section 11.6 in Figures 1 and 2).

Discussion
This study has provided the first systematic mapping of the location of neurons in RVMM in the anesthetized rat capable of influencing neurogenic vasomotor tone. Mapping revealed that the vasoactive portion of RVMM is centered in nucleus gigantocellularis, 0.5–1.5 mm lateral to the midline, 0.5–3.5 mm above the ventral surface, and extending roughly the rostral-caudal length of the facial nucleus. The largest increases in arterial pressure were elicited from the ventral half of nucleus gigantocellularis 11.3–11.6 mm caudal to bregma. Mapping indicated that cardiovascular reactive neurons in RVMM are located substantially more dorsal and rostral than was previously indicated by studies using microinjections of the local anesthetic lidocaine. Previous studies indicate that RVMM is in the same medial-lateral and dorsal-ventral plane as RVLM. The appreciable separation between RVLM and RVMM reduces the possibility that the arterial pressure responses attributed to the activation of neurons in RVMM also involved activation of neurons in RVLM. In addition, the fact that depressor and pressor responses were elicited from sites separated by 500 μm using 50 nl injections of NMDA further suggests that responses elicited by stimulation of RVMM did not result from the activation of neurons in RVLM.

The borders of RVMM overlap portions of a number of previously identified cardiovascular responsive areas in the rostral medial medulla. Minson and colleagues, using microinjections of l-glutamate,
FIGURE 2. Schematic drawings show coronal sections of the rat medulla summarizing sites of glycine injection (50 nl of 500 mM) into rostral ventromedial medulla (RVMM) in four rats. Sections are redrawn from the atlas of Paxinos and Watson.13 P values indicate distance (mm) caudal to bregma. Sites from which increases and decreases in mean arterial pressure (MAP) were elicited are shown by filled and open symbols, respectively. The magnitude of MAP responses (increases or decreases) is shown by symbol shape: □, 0 to 25 mm Hg; ■, 26 to 45 mm Hg; ▲, more than 45 mm Hg; No change in MAP (0 to ±5 mm Hg) is shown by ○. Ac7, accessory facial nucleus; VII, facial nucleus; Gi, gigantocellular reticular nucleus; g7, genu of the facial nerve; Pyr, pyramids; RVL, nucleus reticularis ventralis; Sp5, spinal tract of the trigeminal nerve.

identified pressor sites in the lateral portion of nucleus raphe magnus (B3) in the ventromedial medulla of rats. Pressor responses elicited by L-gluta-

cmate injection into this region are blocked by prior treatment with 5,7-dihydroxytryptamine but not by electrolytic lesions of RVL. Pressor responses are also elicited by glutamate stimulation in nucleus gigantocellularis dorsal to the B3 neurons. These pressor responses are only partially attenuated by pretreatment with the serotonin neurotoxin 5,7-dihydroxytryptamine. The pressor areas identified by Minson and colleagues6 overlap the caudalmost and ventral portions of RVMM. Willette and colleagues11 also elicited pressor responses using microinjections of L-glutamate in the ventral and lateralmost portions of nucleus gigantocellularis in rats.

Several investigators using electrical and chemical stimulation have identified a pressor region in the dorsal medial medulla in rats, cats, and rabbits.2-4 The majority of these pressor sites are within the nucleus reticularis parvocellularis and overlap the dorsalmost portion of RVMM. Yardley and coworkers4 identified additional pressor areas extending rostral and lateral to RVMM in the rat. Microinjection of glycine or muscimol into dorsal medial medulla fails to consistently decrease arterial pressure,4 leading to the conclusion that neurons in the dorsal medial medulla are not required for the generation of vasomotor tone.

Microinjection of NMDA elicited depressor responses scattered throughout RVMM. The largest proportion of depressor sites and largest depressor responses were elicited from the caudal and rostral poles of RVMM. A similar mixture of pressor and depressor sites is reported in the dorsal medial medulla and in regions overlapping rostral and lateral portions of RVMM.4 Other investigators have reported more discrete depressor regions, particularly in the caudal nucleus gigantocellularis and nucleus reticularis ventralis,11 lying caudal to RVMM.

Bilateral inactivation of RVMM using microinjection of the local anesthetic lidocaine decreased MAP by up to 50 mm Hg, suggesting that RVMM is responsible for a significant portion of neurogenic vasomotor tone in anesthetized rats.7-9 However, since lidocaine inactivates both axons and cell bodies it is not clear whether neurons in RVMM are involved in the maintenance of arterial pressure. The present observation that microinjection of glycine decreased arterial pressure suggests that RVMM contains the cell bodies of neurons involved in the maintenance of neurogenic tone in anesthetized rats. Glycine inhibits neuronal transmission via actions on synaptic networks and not axons of passage. Based on the magnitude of the hypotensive responses, glycine was apparently not as effective in reducing MAP as was lidocaine. This may reflect the fact that large volumes of lidocaine (200 nl) were injected bilaterally into RVMM, whereas glycine was injected unilaterally in smaller volumes (50 nl). Thus, lidocaine undoubtedly inactivated more neurons in RVMM, producing a greater decrease in MAP. Alternatively, the axons of tonic vasomotor neurons in other brain
regions may pass through RVMM. These axons would be inactivated by lidocaine but not glycine.

A surprising result of this study was the observation that microinjection of glycine into the major pressor region in RVMM (defined using NMDA) elicited pressor responses. These responses were also found to be blocked by the glycine antagonist strychnine (unpublished observation from our laboratory). This is not the first report that microinjection of glycine mimics the arterial pressure response elicited by an excitatory amino acid. Talman and colleagues recently reported that microinjection of either glycine or L-glutamate into the same site in the nucleus tractus solitarius elicits similar decreases in arterial pressure.

At the present time the mechanism responsible for the glycine-mediated pressor response has not been determined. From these studies, we conclude that the RVMM contains the cell bodies of neurons that when activated are capable of influencing vasomotor tone. Since microinjection of glycine elicited decreases in MAP, it appears that at least some of these neurons contribute to the maintenance of neurogenic tone in anesthetized rats. Whether these neurons are more or less involved in cardiovascular regulation in conscious rats remains to be determined. Finally, microinjection of glycine into a specific brain region, the RVMM, increases MAP by an undetermined mechanism.

Acknowledgments

We thank Dr. Stephen J. Lewis for his editorial comments and Joanie Rogers for typing the manuscript.

References


**KEY WORDS** • blood pressure • N-methyl-D-aspartic acid • glycine • medulla oblongata
Identification of cardiovascular neurons in the rostral ventromedial medulla in anesthetized rats.
K J Varner, D S Rutherford, E C Vasquez and M J Brody

Hypertension. 1992;19:II193
doi: 10.1161/01.HYP.19.2_Suppl.II193

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/II193