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

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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 H]:H-193-II-197)
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 i.p.); 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, Fullerton, Calif.) as pulsatile and mean arterial pressure (MAP). Heart rate was derived from the arterial pulse using a Beckman 9857B cardiotaohonieter. 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 used as the stereotaxic zero point.

Glycine (500 mM) 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 microinjection 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 gradicule 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)
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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 by -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, 0 to 25 mm Hg; 1, 26 to 45 mm Hg; 2, more than 45 mm Hg; No change in MAP (0 to ±5 mm Hg) is shown by 0. AcS7, 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.

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

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References


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