Lesions of Epinephrine Neurons in the Rostral Ventrolateral Medulla Abolish the Vasodepressor Components of Baroreflex and Cardiopulmonary Reflex

ANTONIO R. GRANATA, M.D., DAVID A. RUGGIERO, PH.D., DONG H. PARK, PH.D., TONG H. JOH, PH.D., AND DONALD J. REIS, M.D.

SUMMARY Epinephrine-containing neurons of the rostral ventrolateral medulla (RVL) (the Cl group of Hokfelt) in the rat are primarily unilaterally innervated by neurons in the nucleus tractus solitarius (NTS) and in turn project to autonomic spinal neurons. In this study, we investigated whether the Cl area of the RVL mediates the vasodepressor responses (VDR) induced by either electrical stimulation of the vagus nerve or carotid sinus stretch. In all experiments, Cl neurons were localized immunocytochemically with antibodies to phenylethanolamine N-methyltransferase (PNMT). Bilateral lesions of the Cl area decreased arterial pressure (AP) and heart rate (HR) to spinal cord transection levels and blocked the VDR induced by vagal stimulation and carotid sinus stretch. Combined lesions of the contralateral NTS and Cl area ipsilateral to the stimulated vagus nerve maintained AP and HR at normal levels, and totally blocked the VDR to vagal stimulation and carotid sinus stretch. Since projections from the vagus nerve to NTS are bilateral and those from NTS to Cl unilateral, the combined contralateral NTS/ipsilateral Cl lesions isolated and interrupted the ipsilateral NTS-Cl pathway and, therefore, blocked the baroreceptor reflex. The results are consistent with the hypothesis that neurons in the NTS synapsing in or projecting through the Cl area mediate the baro- and cardiopulmonary mechanoreceptor reflex.

(Hypertension 5 (supp V): V-80-V-84, 1983)

KEY WORDS • baroreceptor reflex • epinephrine neurons • rostral ventrolateral medulla • vagal stimulation

It has been proposed that neurons in the lower brain stem are essential for maintaining normal levels of arterial pressure (AP). Small bilateral lesions in a restricted region of the rostral ventrolateral medulla (RVL) decrease AP to similar levels reached by spinal cord transection. Neurons in this area project to the spinal cord while electrical or chemical stimulation of this area increase AP. Neurons concentrated in this area of the RVL contain phenylethanolamine N-methyltransferase (PNMT), the enzyme that catalyzes the conversion of norepinephrine to epinephrine. These neurons compose the Cl group described by Hökfelt et al. It has been recently proposed in this laboratory that these Cl neurons in the RVL project principally to the intermediolateral column of the spinal cord, where preganglionic neurons are situated, and in turn receive a direct innervation from cardiovascular subdivisions of NTS. Moreover, since electrical or chemical stimulation of these Cl neurons elevates AP, while neuronal blockade drops AP to levels comparable to those produced by spinal cord transection, it has been proposed that Cl neurons appear necessary for maintaining normal levels of AP and may constitute a vasomotor center. The purpose of this study was to test the hypothesis that the Cl neurons in the RVL medulla mediate the vasodepressor response (VDR) elicited by baroreflex and other cardiopulmonary mechanoreceptor stimulation. Since bilateral neuronal blockade of the Cl area drastically reduces AP, it would have been impossible to determine in this preparation any reflex vasodepressor response. Therefore, in this paper, we sought to develop a model in which we could isolate the baroreceptor reflex arc while maintaining normal levels of systemic AP.
Materials and Methods

Studies were performed on male Sprague-Dawley rats anesthetized with urethane (1.5–1.8 g/kg, i.p.). The AP was recorded via a femoral catheter inserted in the aorta, and the heart rate (HR) was computed from the arterial pressure wave. After the trachea was cannulated, the animals were paralyzed with tubocurarine chloride (0.12 mg/kg. i.m.) and artificially ventilated while the body temperature was maintained at 37°C.

One vagus nerve was exposed through a longitudinal midline incision in the ventral surface of the neck, dissected free of surrounding connective tissue, and transected distal to the superior laryngeal nerve. The wound was closed and the animal placed supine, with the head inclined downward by 45° in a stereotaxic frame. An occipital craniotomy was performed, and the caudal portion of the fourth ventricle was exposed by incising the dura and arachnoid, and gently retracting the posterior vermis. The central end of the vagus nerve was approached dorsally by separating the posterior neck muscles. The nerve was then placed on a platinum wire electrode and maintained in a pool of warmed paraffin oil enclosed by a skin flap. The vagus nerve was stimulated with rectangular pulses (2 msec duration; 10 sec train at 5 Hz) with stimulus currents, usually adjusted at 10 times the threshold for eliciting a change in AP, which was usually 60 to 80 μA.

The carotid sinus baroreceptors were stimulated naturally (stretched) by pulling a ligature looped around the common carotid artery downward, toward the heart for 15 seconds. Statistical evaluation was performed by using the paired Student’s t test.

Lesions were placed in different sites of the brain by passage of a DC anodal current of 400 μA for 15 to 30 seconds through an insulated stainless steel electrode (100 μm o.d.) with an exposed tip of about 150 μm. Lesions were placed unilaterally or bilaterally in the RVL at a site 1.5 to 2 mm rostral to the calamus scriptorius and 2 mm lateral from the midline. Unilateral lesions were combined with lesions in the nucleus tractus solitarius (NTS) at the level of the calamus scriptorius in the same animal. Lesions of the NTS were placed contralateral to the stimulating electrode of the vagus nerve or carotid sinus stretched and contralateral to the C1 area lesioned. Control lesions were placed in other areas adjacent to the C1 area.

In paralyzed and artificially ventilated animals, reflex changes in AP and HR evoked by vagal stimulation and carotid sinus stretch were recorded before and at least 15 minutes after placement of lesions.

At the end of the experiment, an extra dose of urethane was administered and the animals were perfused through the heart with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed and postfixed for 30 minutes in the same fixative. A sliding microtome was used to cut 40 μm transverse sections through the medulla, and then the peroxidase-antiperoxidase technique was used to process sections for immunocytochemical demonstration of dopamine-β-hydroxylase (DBH) or PNMT.

Results

Bilateral electrolytic lesions in an area of the RVL where C1 neurons were located resulted in a marked fall in arterial pressure (AP) and heart rate (HR) (table 1). The magnitude of the fall in AP did not differ from that produced by spinal cord transection. In four rats, AP and HR were both reduced after spinal cord transection, from 97.2 ± 10.6 mm Hg to 50.1 ± 6.5 mm Hg (p < 0.01) and from 360.1 ± 16.1 bpm to 225.6 ± 20.1 bpm (p < 0.01) respectively. Results indicated that the lesion interrupted all excitatory outflow to sympathetic preganglionic neurons proceeding from the brain stem.

Bilateral lesions of the C1 area also abolished the vasodepressor and bradycardiac response elicited by supramaximal electrical stimulation of the proximal limb of the cut vagus nerve (table 1). Such lesions also abolished the reflex changes in AP and HR produced by stretching the carotid sinus (table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control Before lesion</th>
<th>Vagal stim (mean change)</th>
<th>After lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAP</td>
<td>HR</td>
<td>MAP</td>
</tr>
<tr>
<td>Bilateral C1 area lesions</td>
<td>4</td>
<td>98.5 ± 8.2</td>
<td>380.8 ± 30.2</td>
</tr>
<tr>
<td>Contralateral NTS and ipsilateral C1 area lesions</td>
<td>4</td>
<td>96.9 ± 6.6</td>
<td>368.0 ± 15.2</td>
</tr>
</tbody>
</table>

*Values are means ± SEM; No. = number of experiments; MAP = mean arterial pressure (mm Hg); HR = heart rate (bpm). Unilateral vagal stimulation was performed with a pulse of 2 msec duration, 5 Hz and ×10 threshold current intensity; 10-second duration of stimulus train. *p = < 0.025. †p = < 0.01 compared with before lesion controls.

In all cases, ipsilateral and contralateral refer to the side of the vagal stimulation.

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TABLE 1. Effects on Cardiovascular Responses to Electrical Stimulation of the Unilateral Cervical Vagus Nerve on Arterial Pressure and Heart Rate of Bilateral C1 Area Lesions or Ipsilateral C1 Area Plus Contralateral Nucleus Tractus Solitarius (NTS) Lesions

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TABLE 2. Effects on Cardiovascular Responses to Baroreceptor Stimulation by Carotid Sinus Stretch of Bilateral C1 Area Lesions or Ipsilateral C1 Area Plus Contralateral NTS Lesions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Before</th>
<th>Carotid sinus stretch (mean change)</th>
<th>After</th>
<th>Carotid sinus stretch (mean change)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>MAP</td>
<td>HR</td>
<td>Control</td>
</tr>
<tr>
<td>Bilateral C1 area lesions</td>
<td>4</td>
<td>98.9±8.5</td>
<td>346.6±20.8</td>
<td>-27.8±6.1</td>
<td>233.3±1.3</td>
</tr>
<tr>
<td>Contralateral NTS and ipsilateral C1 area lesions</td>
<td>5</td>
<td>95.8±10.5</td>
<td>355.0±7.5</td>
<td>-30.5±7.5</td>
<td>340.5±1.1†</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

The carotid sinus baroreceptors were stimulated naturally (stretched) by pulling the ligature looped around the common carotid artery downward toward the heart for 12 seconds. *p < 0.025, †p < 0.01 compared with before lesion controls. In all cases, ipsilateral and contralateral refer to the side of the carotid sinus stretch. See table 1 for abbreviations.

Locations of the lesions were carefully mapped in five rats in which C1 lesions were placed. Tissues throughout the medulla were stained with PNMT in order to relate the lesions to the locations of the C1 neurons. In all cases in which bilateral lesions of the C1 area in the RVL resulted in a fall of AP and HR, and the reflex vasodepressor response and bradycardia to electrical stimulation of the vagus nerve or carotid sinus stretch were abolished, the lesions destroyed the majority of C1 neurons on both sides. The results confirmed that neurons in the C1 region are tonically active and totally responsible for maintaining the AP. However, the blockade of the baroreceptor response after bilateral C1 lesions could have been due to the low level of AP, which might have precluded any further decrease in AP.

Figure 1. Schematic representation of a lesion of the nucleus tractus solitarius (NTS) on the right side (upper diagram), and a lesion of the contralateral C1 area (lower diagram) in one experiment. Note that the lesion in the NTS (upper) is located in its caudal aspect and destroys approximately the medial one-half of its structure. Included within the area destroyed are dopamine B-hydroxylase (DBH) immunoreactive cells of the A2 group and portions of the dorsal vagal nucleus and nucleus gracilis. The lesion in the C1 area (lower) is placed at the level of the rostral ventrolateral medulla underlying the retrofacial nucleus. Abbreviations: A1 = (noradrenergic) cell group of the caudal ventrolateral medulla; A2 = (noradrenergic) cell group of the caudal nucleus tractus solitarius; C1 = PNMT cell group of epinephrine synthesizing cell bodies; DMX = dorsal motor nucleus of the vagus; IVN = inferior vestibular nucleus; LRN = lateral reticular nucleus; MAO = medial accessory olive; NC = nucleus cuneatus; NPS = nucleus parasilisarius; NPvc = nucleus reticularis parvocellularis; NTSr = nucleus tractus solitarius pars rostralisis; RA = nucleus retroambiguus; RPa = nucleus raphe palidus; RVL = rostral ventrolateral medulla; STN; and STNr = spinal trigeminal nucleus pars caudalis and pars oralis; STT = spinal trigeminal tract.
To assess the importance of the C1 area in the baroreceptor response, and still maintain AP at normal levels, a lesion in the right NTS was first performed. Therefore, now only the left NTS/C1 projection would be able to convey the vasodepressor response induced by the left vagus nerve stimulation. In the same animal, the lesion in the right NTS placed at the level of the calamus scriptorius was followed by a rostral lesion in the left C1 area (fig. 1). After partial deafferentation of the baroreceptor fibers produced by the right NTS lesion, the AP was moderately increased; the subsequent left C1 lesion then resulted in a secondary fall of AP, so that, after both lesions, the AP and HR did not differ from that of unlesioned controls (tables 1 and 2). In these experiments, the combined NTS/C1 lesions entirely abolished the reflex vasodepressor and bradycardic responses elicited by electrical stimulation of the left vagus nerve (table 1). The reflex fall of AP and HR produced by stretch of the ipsilateral carotid sinus was also abolished (table 2).

To determine if locations of the lesions in the medulla which abolished the baroreflex response were selective to the C1 area, we made lesions in areas adjacent to C1. The reflex changes in AP produced by vagal stimulation or carotid sinus stretch were not abolished in rats with lesions in the NTS contralateral to the stimulated vagus nerve combined with ipsilateral lesions of either the medullary raphe (two rats), nucleus reticularis gigantocellularis pars ventralis or pars dorsalis (three rats), the nucleus reticularis parvocellularis overlying the C1 area (two rats), ventrolateral tegmentum lateral and rostral to the C1 area (three rats), or spinal trigeminal nucleus (at and caudal to the level of the C1 group) (three rats).

In addition, neurons were also localized in the caudal ventrolateral medulla (CVL) at the level of the calamus scriptorius, which demonstrated dopamine-β-hydroxylase (DBH) but no PNMT immunoreactivity. These DBH neurons belong to the A1 group of Dahlstrom and Fuxe. In three rats, an ipsilateral lesion of the A1 area combined with a lesion of the contralateral NTS did not abolish the vasodepressor response induced by vagal stimulation or carotid sinus stretch.

**Discussion**

In this study, we sought to test the hypothesis that the area of rostral ventrolateral medulla (RVL) containing PNMT neurons mediates the vasodepressor response to stimulation of arterial baroreceptors and other afferent fibers of the vagus nerve. This hypothesis has been based on evidence obtained in this laboratory that: 1) the area of the RVL containing PNMT neurons, the C1 area of Hökfelt et al., is directly innervated by strong primarily unilateral projections from cardiovascular subdivisions of the NTS; and 2) C1 neurons in the RVL project selectively to the intermediodorsal column in the spinal cord, where preganglionic neurons are located.

Bilateral electrolytic lesions of the C1 area of the RVL resulted in a fall of AP and HR to levels comparable with the decrease of AP and HR produced by spinal cord transection. The lesions destroyed, in all cases, the bulk of the PNMT neurons bilaterally. These results then support the hypothesis that C1 neurons are tonically active and represent a tonic vasomotor center. Bilateral lesions of the C1 area also blocked the reflex vasodepressor response to vagal stimulation as well as the reflex decrease in AP and HR induced by carotid sinus stretch.

However, the remarkable hypotension induced by bilateral C1 area lesions (even lower than that produced by reflex stimulation) made it impossible in these experiments to assess if indeed the baroreflex was suppressed or only masked by the fall of AP.

To isolate the baroreceptor reflex arc while maintaining normal levels of systemic AP, lesions were placed in the NTS contralateral to the stimulated vagus nerve. The reason for this rested on the hypothesis that projections from baroreceptors and other cardiopulmonary mecanoreceptors in the vagus nerve to the NTS are bilateral, whereas those from the NTS to C1 area are unilateral. Therefore, an NTS lesion is placed contralateral to the side of vagal stimulation, the ipsilateral C1 area is isolated and the baroreflex will be mediated only via this C1 area.

The unilateral lesion of NTS resulted in a moderate increase of AP, related to the partial unilateral interruption of central baroreceptor terminals. When the contralateral (to the side of vagal stimulation) NTS lesion was paired with a lesion of the C1 area on the ipsilateral side of the brain, the elevated AP returned to levels comparable to control, but the vasodepressor responses to stimulation of the vagus nerve, or to carotid sinus stretch were abolished. However, since in this condition, the resting AP was maintained at control levels, the abolition of the baroreflex could not be attributed to the intense hypotension associated with bilateral C1 lesions. The present results are therefore consistent with a view that the relay from NTS mediating the baroreflex is bilateral, because of the decussation of baroreceptor fibers in the NTS and, second, that the pathway from the NTS to the C1 area is unilateral.

Lesions placed in areas adjacent to C1, including the noradrenergic neurons of the A1 group located in the caudal ventrolateral medulla, failed to modify either the vasodepressor response to vagal stimulation or the reflex responses to carotid sinus stretch. These results support the view that lesions of C1 area selectively block the baroreceptor reflex.

Vagal afferent stimulation with frequencies of 50 and 100 Hz elicit an increase of AP. Bilateral lesions of the C1 area as well as the combined lesions of the right NTS/left C1 area completely blocked the pressor responses elicited by left vagus nerve stimulation (Granata, A.R., Ruggiero, D.A., and Reis, D.J., unpublished results). Therefore, the RVL where PNMT neurons are located also mediates the pressor responses elicited by electrical stimulation of the vagus nerve with higher frequencies.

In conclusion, the present studies are consistent with the hypothesis that neurons originating in the cardio-
vascular NTS and synapsing in or projecting through the rostral ventrolateral medulla where Cl adrenergic neurons are situated (the Cl area) mediate the vasodepressor response from baro- and cardiopulmonary mechanoreceptors.

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Lesions of epinephrine neurons in the rostral ventrolateral medulla abolish the vasodepressor components of baroreflex and cardiopulmonary reflex.
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Hypertension. 1983;5:V80
doi: 10.1161/01.HYP.5.6_Pt_3.V80
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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