

Interactions Between Area Postrema and Sympathetic or Respiratory-Related Neurons in the Rostral Ventrolateral Medulla

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I. Introduction

The rostral ventrolateral medulla contains sympathoexcitatory neurons that are considered to contribute to baseline sympathetic vasomotor tone and respiratory-related neurons, which contribute to the generation of respiratory motor pattern. The area postrema, located in the dorsomedial medulla, is the most caudal of the circumventricular organs and is best recognized as the central “chemoreceptor trigger zone” for vomiting. More recently, neurons in this structure have been shown to play an important role in regulating cardiovascular function. The studies described in this paper determine the role of area postrema neurons in modifying the discharge patterns of sympathetic or respiratory-related neurons in the rostral ventrolateral medulla in rabbits.

II. Area Postrema Interactions with Sympathetic-Related Neurons in the Rostral Ventrolateral Medulla

Sympathoexcitatory neurons in the rostral ventrolateral medulla provide a tonic excitatory drive to vasomotor preganglionic sympathetic neurons and

thereby contribute to the maintenance of baseline sympathetic vasomotor tone (1–3). The neurons discharge with patterns consistently and temporally related to postganglionic sympathetic nerve activity (1–6). Many of the neurons make monosynaptic excitatory projections to preganglionic sympathetic neurons in the intermediolateral cell column (1–5,7). These sympathetic-related neurons also form the final common pathway for relaying reflex and descending changes in sympathetic nerve activity. Activation of the arterial baroreceptors (2–6,8,9), peripheral carotid chemoreceptors (10), low-pressure receptors in the heart and lung (11), nociceptors (12), and somatosensory receptors (13,14) has been shown to modulate the activity of neurons in the rostral ventrolateral medulla, as has activation of higher brain centers such as the lateral hypothalamus (15, 16). Therefore, it seems likely that most changes in sympathetic nerve activity are relayed, ultimately, by synaptic input onto these sympathetic neurons in the rostral ventrolateral medulla.

The area postrema is situated on the dorsal surface of the medulla over the fourth ventricle. In rats and rabbits, the structure spans the midline, extending from the calamus scriptorius to the obex, while in mammals other than rodents, the area postrema is bilateral (17). The cell types in the area postrema include small neurons (7 to 15 μm), flattened ependymal cells, astrocytes, and a few oligodendroglia. Because neurons in the area postrema do not have a blood-brain barrier, they are susceptible to the influences of hormones and peptides in the peripheral circulation. Previous studies have suggested that circulating substances with cardiovascular effects—including vasopressin (18, 19), endothelin (20), and angiotensin II (21)—excite neurons in the area postrema. This susceptibility to blood-borne substances led to the hypothesis that area postrema neurons, responding to humoral influences, may modulate autonomic reflex function.

A role for area postrema in regulating cardiovascular function has been demonstrated by the effects of either stimulation or destruction of area postrema neurons on sympathetic nerve activity (22,23), arterial blood pressure (24,25), and baroreflex function (18,22,26). Electrical stimulation at 10 to 40 Hz or chemical stimulation with glutamate of area postrema neurons decreases sympathetic nerve activity in rabbits without changing arterial blood pressure (22,23), decreases arterial blood pressure in rats (25,27), and increases arterial blood pressure in dogs (24). Stimulation of area postrema neurons either by circulating vasopressin or by direct background electrical excitation augments baroreflex-mediated and volume expansion-evoked inhibition of sympathetic nerve activity (18,19). Area postrema neurons are required for the progression of chronic angiotensin-induced hypertension in normotensive rats (28) and for the full expression of hypertension in spontaneously hypertensive rats (29). The neural circuitries for relaying these cardiovascular changes have not been investigated, but area postrema neurons project to a number of other brain regions with known cardiovascular functions.

Anatomical and electrophysiological studies have shown that area postrema neurons project to the medial aspect of the nucleus tractus solitarius (NTS) (29), have reciprocal connections with neurons in the lateral parabrachial nucleus (32), and also project to the rostral ventrolateral medulla (31) and to the hypothalamic paraventricular nucleus (29). Since changes in sympathetic nerve activity are relayed by sympathetic neurons in the rostral ventrolateral medulla and since area postrema neurons appear to fine-tune reflex control of sympathetic nerve activity, it seems likely that sympathetic neurons in the rostral ventrolateral medulla may be an important potential target for area postrema modulation. Accordingly, we determined, in rabbit, the effect of single electrical pulses delivered to the area postrema on the activity of sympathetic neurons in the rostral ventrolateral medulla. Sympathetic neurons exhibited discharge patterns that were synchronized to sympathetic nerve activity as determined by spike-triggered averaging; they exhibited a cardiac rhythm as determined by post R-wave analysis and were inhibited by activation of the baroreceptors produced by intravenous bolus injections of phenylephrine.

Single sequential stimuli delivered at 1 Hz to the area postrema produced an initial excitation followed by an inhibition of sympathetic neurons in the rostral ventrolateral medulla. An example is shown in Figure 1A. The area postrema-evoked increase in unit activity had an onset latency of 22 ms and peak latency of 26 ms. The excitation was followed by an inhibition that had an onset of 45 ms and lasted 123 ms. Figure 1B shows the post-R wave averages of arterial blood pressure and sympathetic nerve activity and the post-R wave histogram of unit activity. The neuron exhibited a cardiac rhythm and was most likely to discharge in late diastole ~ 35 ms after the R wave trigger (Fig. 1B, lowest panel). The cardiac rhythm of sympathetic nerve activity (Fig. 1B, middle panel) was delayed with respect to the cardiac rhythm of the unit (Fig. 1B, lowest panel). Figure 1C illustrates the temporal relationship between the unit activity and renal sympathetic nerve activity. Sympathetic nerve activity was locked to the unit discharge and peaked ~ 110 ms after the unitary spike. In contrast, sympathetic nerve activity exhibited no consistent temporal relationship to random triggers delivered with the same average frequency as that of the medullary neurons. Activation of the baroreceptors with an IV bolus injection of phenylephrine inhibited the unit and sympathetic nerve activity in parallel (Fig. 1D).

Area postrema stimuli delivered at 1 Hz also produced an excitation followed by a pronounced inhibition of averaged sympathetic nerve activity (Fig. 2). The peak increase in averaged sympathetic nerve activity occurred 190 ms after the stimuli and exceeded the mean peak for the excitatory response of the medullary sympathetic neurons to area postrema stimulation (peak latency = 29 ± 10 ms) by about 160 ms. The area postrema-evoked excitation of sympathetic nerve activity was followed by an inhibition. The postexcitatory inhibition occurred at 238 ms after the stimulus and exceeded the inhibition onset

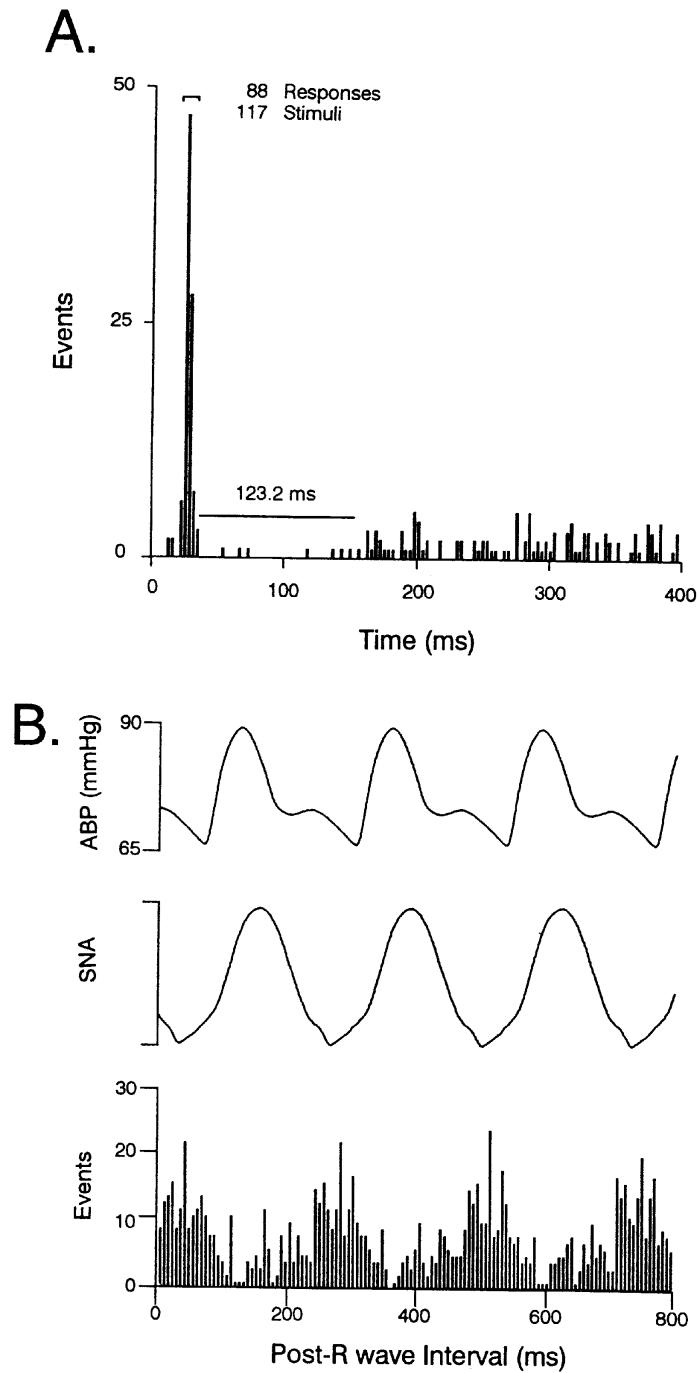
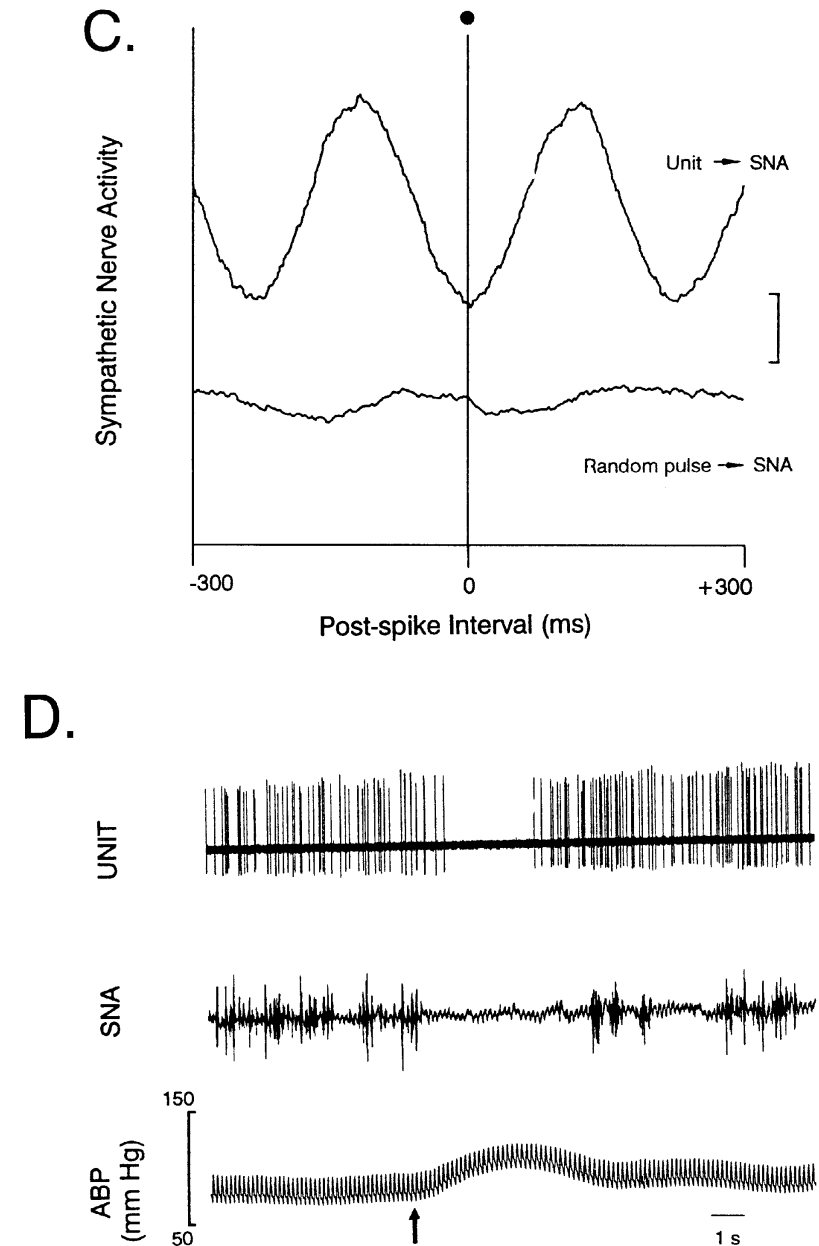


Figure 1 (A) Poststimulus time histogram of response of a sympathetic neuron in the rostral ventrolateral medulla to sequential stimuli delivered to area postrema ($28 \mu\text{A}$, 0.3 ms , 1 Hz). Bin width is 3.2 ms . (B) Post-R wave averages (500 triggers) of arterial blood pressure (ABP) and sympathetic nerve activity (SNA) and post-R wave histogram (234 triggers) of unit activity. Vertical calibration is $200 \mu\text{V}$ for SNA. Bin width is 6.4



ms for the histogram. (C) Average of sympathetic nerve activity triggered by 700 spontaneous spikes of unit and 700 random pulses of the same average frequency. Peaks in upper trace exceed those in the lower by more than ninefold. Vertical calibration is $50 \mu\text{V}$ for SNA. (D) Simultaneous inhibition of medullary unit activity and sympathetic nerve discharge (SNA filtered at 1 to 1000 Hz) by IV bolus injection of phenylephrine (arrow). (From Ref. 6.)

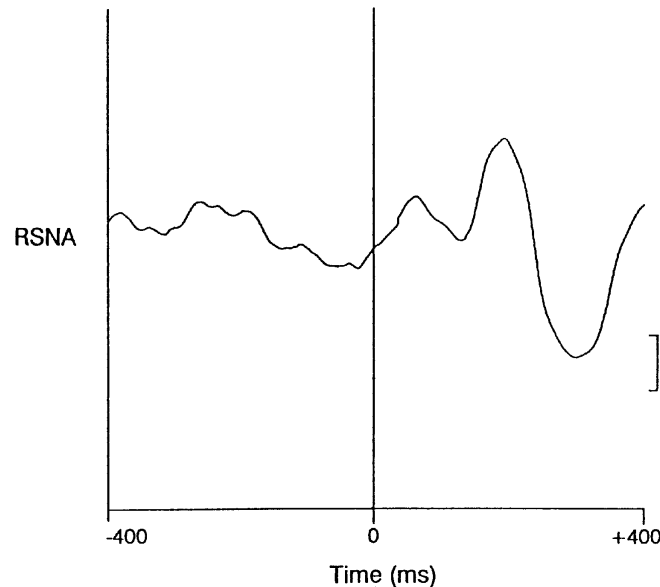


Figure 2 Average of renal sympathetic nerve activity (RSNA) triggered by 50 stimuli applied to the area postrema. Area postrema stimulation ($25 \mu\text{A}$, 0.3 ms , 1 Hz) evoked an excitatory peak in averaged RSNA followed by an inhibition.

for the sympathetic medullary neurons (onset = $53 \pm 21 \text{ ms}$) by about 174 ms . These findings suggest that the biphasic responses of sympathetic neurons in the rostral ventrolateral medulla are transmitted to postganglionic sympathetic nerves. Sympathetic neurons were recorded from 1.5 to 2.5 mm rostral to the obex, ventral to the nucleus ambiguus at the rostral pole of the inferior olive. This distribution coincides with the electrophysiological localization of bulbo-spinal sympathetic neurons in the rostral ventrolateral medulla in the rabbit by Yu-Wen et al. (34) and Terui et al. (35).

Since higher-frequency stimulation (10 to 40 Hz) has been shown to decrease multifiber postganglionic sympathetic nerve activity in the rabbit (22, 23), we tested the responses of 5 medullary neurons to area postrema pulses delivered at the higher frequencies of 10 to 20 Hz for 3 s . Each pulse evoked a spike and a period of inhibition; however, after the stimulator was turned off, the cell remained inhibited for much longer (~ 1 to 6 s) compared with the duration of inhibition following sequential stimuli delivered at 1 Hz ($\sim 127 \text{ ms}$). These findings suggest a frequency-dependent response of the sympathetic nervous system to area postrema stimulation; low-frequency stimulation (1 Hz) evoked an excitatory/inhibitory response, while higher-frequency stimulation (10 to 40 Hz) resulted in a prolonged period of inhibition.

The biphasic response of the sympathetic medullary neurons to area postrema stimulation raises the possibility that the excitation and inhibition were

relayed over different pathways to the rostral ventrolateral medulla. Assuming a conduction distance of 6 mm from the center of the area postrema to the recording sites in the rostral ventrolateral medulla in the rabbit and an onset latency of $\sim 22 \text{ ms}$ for the excitatory response, the estimated axonal conduction velocity would be $\sim 0.3 \text{ m/s}$. This slow conduction velocity suggests that the impulses that elicited the excitatory responses were transmitted either directly from the area postrema to the rostral ventrolateral medulla via small, unmyelinated fibers or via a polysynaptic pathway. With an onset latency of $\sim 53 \text{ ms}$ for the inhibition, the calculated axonal conduction velocity is $\sim 0.1 \text{ m/s}$, suggesting that the impulses that elicited the inhibition were transmitted directly via slower-conducting unmyelinated fibers or via a longer pathway than was the excitatory response.

The ability of area postrema neurons to fine-tune sympathetic responses to afferent inputs from other sources will most likely depend on the relative timing and/or the magnitude of the afferent inputs from the area postrema neurons and from the other afferent sources onto sympathetic medullary neurons. The capacity to evoke both an excitatory and inhibitory response of sympathetic medullary neurons may provide a means whereby area postrema neurons, in a time-dependent manner, can shape changes in sympathetic nerve activity by modifying the responses of sympathetic medullary neurons to a variety of other converging afferent inputs. A case in point is the ability of area postrema neurons to fine-tune baroreflex regulation of sympathetic nerve activity. Excitation of neurons in the area postrema, either by direct application of L-glutamate or by background electrical stimulation (22), augments baroreflex-mediated sympathoinhibition by increasing the gain of the curve relating decreases in sympathetic nerve activity to increases in arterial blood pressure. The baroreflex-sympathetic pathway is known to involve synapses in the NTS (33), caudal ventrolateral medulla (34,35), and the rostral ventrolateral medulla (2-4,7-9, 35). Therefore, area postrema neurons may modulate baroreflex-mediated sympathoinhibition by altering baroreceptor afferent input at any of those synapses. We have recently provided electrophysiological evidence in rabbits of time-dependent facilitatory interactions between convergent excitatory inputs from area postrema and baroreceptor afferents at the level of the NTS (36). Area postrema neurons may also augment baroreflex-mediated sympathoinhibition at the level of the rostral ventrolateral medulla; this augmentation may also depend on the timing of the inputs from the baroreceptors and area postrema neurons. For example, arrival of inhibitory volleys from the baroreceptors during the excitatory response of medullary neurons to activation of area postrema neurons might result in an occlusive interaction between the two inputs, thereby diminishing baroreceptor-mediated sympathoinhibition. On the other hand, arrival of baroreceptor input during the period of area postrema-evoked inhibition of the sympathetic medullary neurons might result in a facilitative interaction between the two inputs, thereby augmenting either the magnitude and/or the duration of baroreceptor-mediated inhibition. A

model illustrating known interactions between area postrema and baroreceptor afferents in the NTS and the potential interactions between area postrema and sympathetic neurons in the rostral ventrolateral medulla is shown in Figure 3.

The second issue regarding the modulatory role for area postrema neurons of sympathetic nerve activity involves the magnitude of the input from area postrema neurons relative to that converging from other sources. Once again, the best-described interaction is between area postrema and baroreceptor afferents. Studies of baroreflex function in rabbits have suggested that area postrema-evoked decreases in renal sympathetic nerve activity are prominent only when baroreceptor afferent activity is intact (23). If tonic baroreceptor activity augments area postrema-evoked sympathoinhibition, then a steady-state increase in baroreceptor traffic to sympathetic neurons in the rostral ventrolateral medulla might augment the inhibitory responses of medullary sympathetic neurons to area postrema stimulation and diminish the excitatory responses. Accordingly,

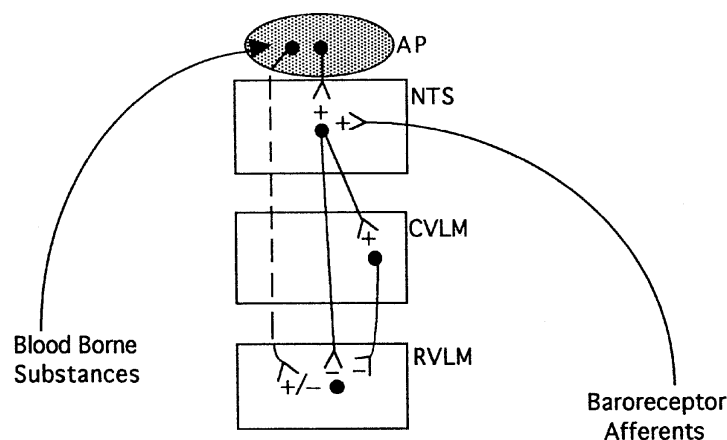


Figure 3 A model illustrating potential interactions between area postrema and medullary sympathetic neurons. Blood-borne substances excite neurons in the area postrema (AP). These AP neurons project to the nucleus tractus solitarius (NTS), where they interact in a facilitative manner with baroreceptor afferents onto neurons in the baroreflex pathway. Area postrema neurons also interact with neurons located more distally in the baroreflex-sympathetic pathway in the rostral ventrolateral medulla (RVLM). The dashed line from AP to the RVLM indicates that the number of intervening synapses is unknown. There is currently no evidence that AP neurons interact with the baroreflex-sympathetic pathway at synapses in the caudal ventrolateral medulla (CVLM). The biphasic AP input to these sympathetic neurons in the RVLM allows for area postrema neurons to modify sympathetic neuronal responses to baroreceptor input in a time-dependent manner. In a broader sense, the biphasic nature provides a mechanism whereby area postrema, responding to multiple blood-borne agents, can modulate either inhibitory or excitatory responses of medullary sympathetic neurons to afferent traffic evoked reflexly or from higher brain sites.

we performed experiments in which we compared the magnitude of the excitatory response and the duration of the inhibitory response of sympathetic medullary neurons under control conditions and during steady-state activation of the baroreceptors produced by steady-state phenylephrine infusions. An example from those findings is shown in Figure 4. During the phenylephrine infusions, the increase in the peak evoked activity was diminished and the dura-

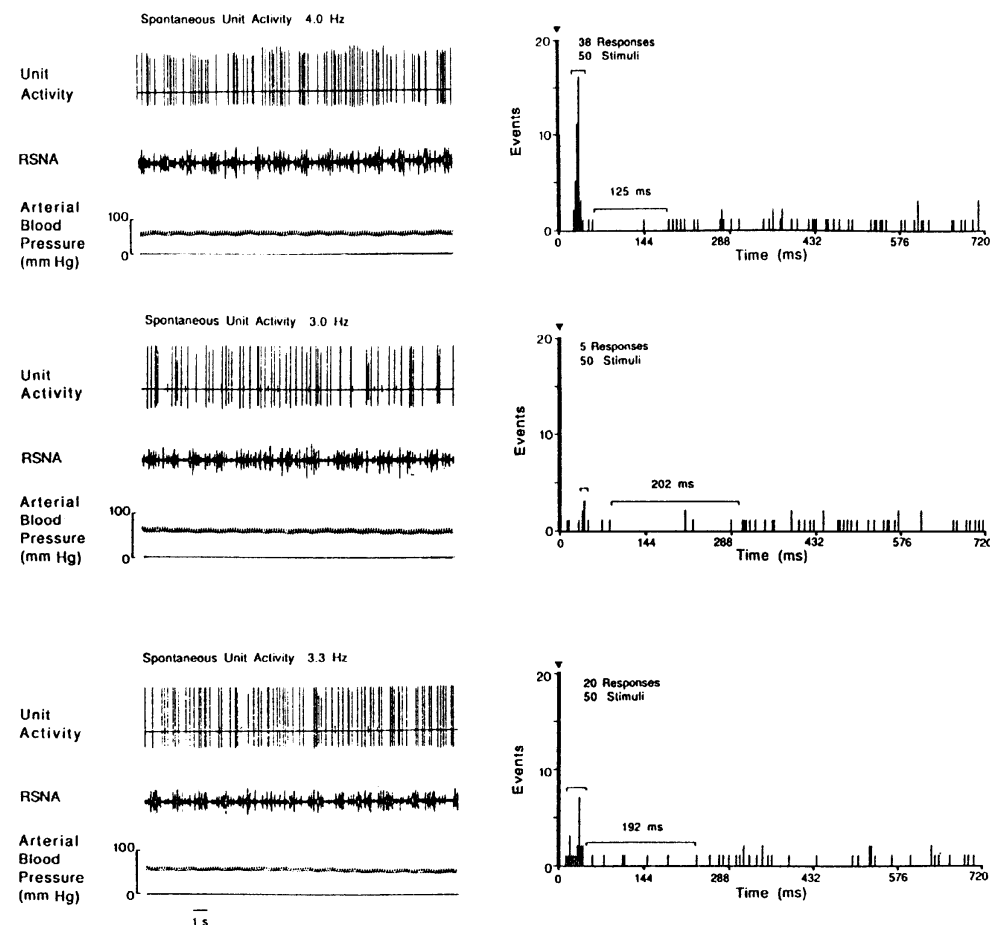


Figure 4 Example of a sympathetic neuron that was inhibited by phenylephrine infusion. Before infusion of phenylephrine (top panel), 50 area postrema stimuli ($10 \mu\text{A}$, 0.7 ms , 1 Hz) evoked 38 responses during the peak excitation and produced an inhibition that lasted 125 ms. Spontaneous activity of the neuron was 4.0 Hz. During phenylephrine infusion (middle panel), spontaneous activity was decreased to 3.0 Hz, and 50 stimuli evoked only 5 responses during the peak excitation, but they evoked a longer inhibition of 202 ms. Phenylephrine infusion was stopped and the neuron exhibited a partial recovery (bottom panel). Spontaneous activity increased to 3.3 Hz, and 50 area postrema stimuli evoked 20 responses during the peak excitation and an inhibition that lasted 192 ms. RSNA, renal sympathetic nerve activity.

tion of the spike-free period was longer. Both changes were proportional to the decrease in background neuronal activity but nonetheless suggest that increasing the magnitude of baroreceptor afferent input might favor the inhibitory response of sympathetic neurons in the rostral ventrolateral medulla to area postrema input.

III. Area Postrema Interactions with Respiratory-Related Neurons in the Rostral Ventrolateral Medulla

The rostral ventrolateral medulla also contains neurons involved in the control of respiration. The longitudinal column of respiratory cells is divided into the ventral respiratory group (VRG), which extends rostrally into the pre-Bötzinger and the Bötzinger complex. The VRG can be further subdivided into the rostral VRG (rVRG), which extends rostral to the obex in the vicinity of the nucleus ambiguus. The rVRG contains primarily inspiratory neurons with augmenting patterns (IAug), although inspiratory neurons with decrementing (IDec) and plateau patterns (IPlat) have also been recorded (37). Many neurons in the rVRG are bulbospinal, although some project to the contralateral VRG and to the dorsal respiratory group (DRG). In the rabbit, the rVRG extends from ~0.5 to 2.0 mm rostral to the obex (38). We have recorded the activity of inspiratory neurons in this region and have determined that at least some IAug, IDec, and IPlat project to the phrenic motor nucleus (39). Neurons in the VRG are thought to be involved in generating or shaping respiratory pattern. During vomiting, the discharge patterns of medullary respiratory neurons are modulated to synchronize respiratory muscles for vomiting (40); inspiratory neurons, with medullary arborizations, are excited, while bulbospinal inspiratory neurons are inhibited. Since the area postrema is regarded as the "chemoreceptor trigger zone" for vomiting, being required for the production of emesis in a variety of models (41), we determined whether area postrema stimulation might alter the discharge patterns of respiratory neurons in the rVRG. Single sequential stimuli delivered at 1 Hz to the area postrema failed to alter the discharge patterns of respiratory-related neurons in the VRG. An example is shown in Figure 5. The inspiratory neuron was inhibited by lung inflation, showing prolonged bursts and shortened interburst intervals when lung inflation was withheld and being silenced when the lungs were hyperinflated (Fig. 5A). The neuron showed no responses to area postrema stimulation (Fig. 5B). Twenty-three respiratory-related neurons were tested for responses to area postrema stimuli, and 20 of those 23 were further characterized by their responses to maintained lung inflation. None received area postrema input, but all received lung volume-related input: 9 were excited by lung inflation and discharged continuously when inflation was maintained for three cycles; 11 were either silenced or exhibited a shortened burst duration when inflation was maintained for three cycles. Neurons with respiratory-related discharge were routinely encountered just dorsal

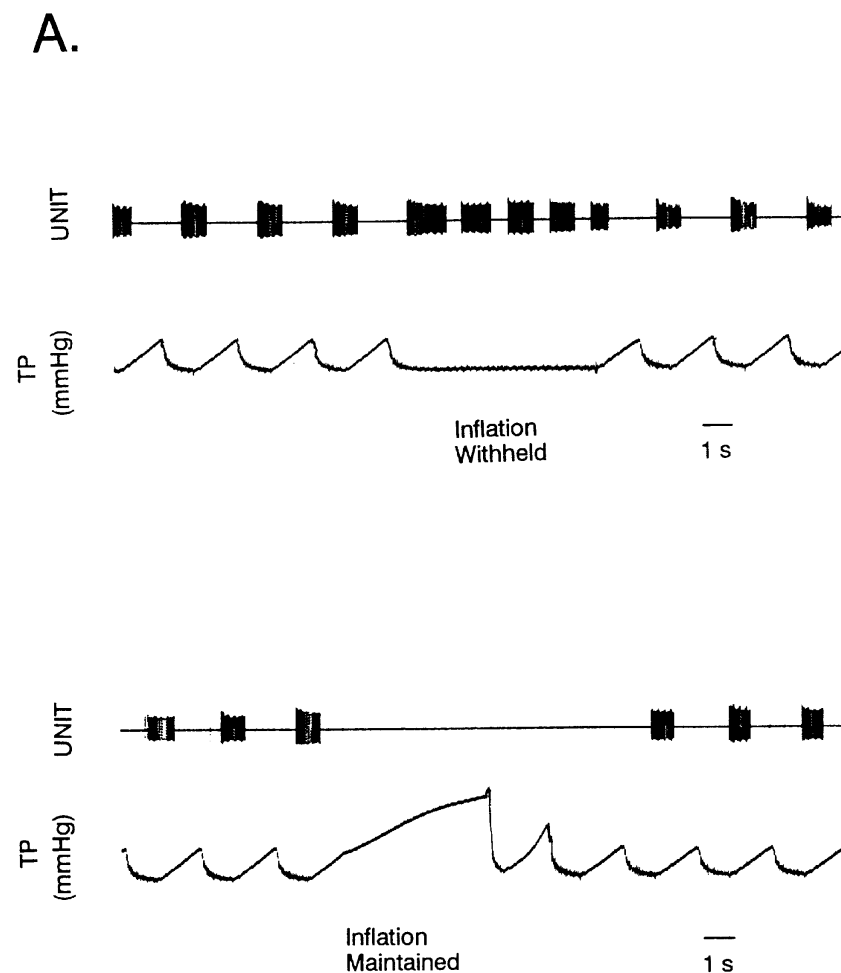


Figure 5 Example of a neuron in the rVRG that discharged with respiratory periodicity and was unaffected by area postrema stimulation. (A) Discharge pattern of the neuron in response to manipulation of lung volume. TP, tracheal pressure. (B) Poststimulus time histogram of unit response to single sequential area postrema stimuli (155 triggered sweeps; 25 μ A, 0.3 ms, 1 Hz). Bin width is 3.2 ms for histogram.

and ~100 to 200 μ m medial to sympathetic neurons. This region, located ~1 to 2 mm rostral to the obex, is consistent with the functionally (38) and anatomically identified (42) rVRG in rabbits. These findings suggest that low-frequency stimuli delivered to the area postrema may not alter respiratory pattern through neurons in the VRG.

IV. Summary

Area postrema neurons can provide both excitatory and inhibitory inputs to sympathetic neurons in the rostral ventrolateral medulla. The biphasic nature

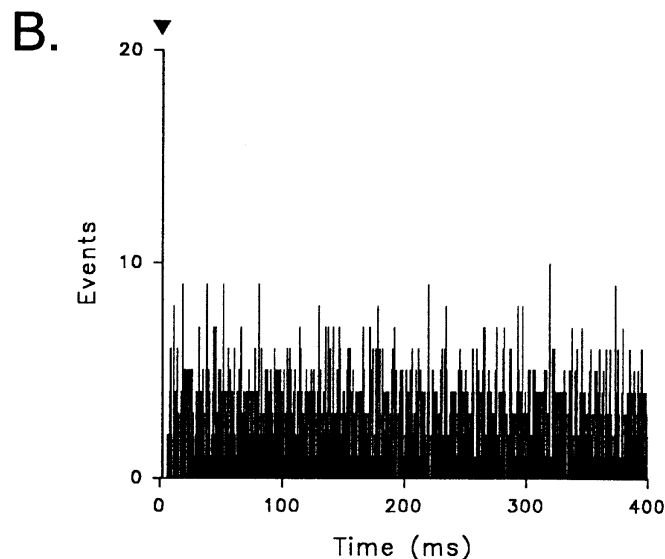


Figure 5 Continued

of the responses of sympathetic medullary neurons to area postrema stimulation may provide a mechanism whereby area postrema neurons, responding to a variety of humoral or neuronal influences, can fine-tune sympathetic nerve activity. In contrast, although the area postrema has been shown to be critical for emesis in a variety of animal models, excitation of area postrema neurons may not play a role in reorganizing the patterns of respiratory neurons in the VRG to accommodate the vomiting reflex.

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The Caudal Pressor Area

A New Region of the Ventrolateral Medulla Involved in Cardiovascular Regulation

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I. Introduction

A central question in cardiovascular regulation is: What are the mechanisms responsible for the generation and maintenance of vasomotor tone and arterial blood pressure (ABP) regulation? Evidence gathered over the last 25 years has unequivocally established that normal levels of arterial blood pressure in anesthetized animals depend upon the activity of small groups of neurons located in discrete regions of the ventrolateral medulla (VLM) (reviewed in Refs. 1 and 2).

The first conclusive evidence for a discrete localization of neurons involved in these functions was obtained by Feldberg and Guertzenstein (3). They demonstrated that several drugs known to be selectively active on cell bodies were able to evoke changes in ABP when applied topically to a restricted area of the ventral medullary surface situated just caudal to the trapezoid bodies and rostral to the roots of the twelfth cranial nerve (4). Most importantly, bilateral applications of the inhibitory amino acid glycine in this area produced a decrease in ABP equivalent to that observed immediately after spinal transection. Accordingly, stimulation of neurons in this area evoked an increase in

†Deceased.

ABP, whereas bilateral electrolytic lesions reduced ABP to spinal levels (5). This region corresponds to the area described in the pioneer work of Schläfke and Loeschke (6). Studying regions of the VLM that are associated with respiratory control, they demonstrated that bilateral cooling of these areas produced not only respiratory arrest but also a step decrease in ABP (6).

Since then, numerous studies using the cat (7,8), rat (9–12), and rabbit (13,14) have been conducted and have confirmed and extended the original observation. In the rat, this area has been identified as the rostral ventrolateral medulla (RVLM) (12,15–17). RVLM neurons project directly to the intermediolateral cell column (16,18–20) and are responsible for the tonic excitation of sympathetic preganglionic neurons (7,21–23).

A few years after the original observations of the RVLM were made, another area with cardiovascular activity was identified in the cat's VLM by the same group (24,25). Localized caudally to the RVLM, in close proximity to the rootlets of the twelfth nerve, this area was identified as a vasodepressor area. Stimulation of this area resulted in hypotension, while its inhibition produced marked increases in ABP (9,25–27). The caudal ventrolateral medulla (CVLM) contains tonically active sympathoinhibitory neurons. Lesions or functional inactivation of this area results in increases in sympathetic nerve activity and hypertension (9,28–30). The CVLM acts through connections with the RVLM (11,28–31).

The first evidence suggesting the existence in the ventrolateral tegmentum of a third area with vasomotor activity was obtained by Feldberg and Guertzenstein (32). Working in cats under chloralose anesthesia (60 mg/kg) these authors demonstrated that topical application of leptazol on caudal sites of the VLM produced depressor responses. However, when chloralose anesthesia was increased to 90 to 120 mg/kg, a distinction between two areas in this region was detectable: a rostral area at the twelfth cranial nerve rootlets where leptazol produced its usual depressor effect, and a more caudal area, between the twelfth nerve rootlets and the first cervical nerve, where pressor effects were obtained. Furthermore, in this condition (i.e., deep anesthesia), the most caudal area was shown to be tonically active, since its inhibition by bilateral application of sodium pentobarbital produced a decrease in blood pressure. An increase in blood pressure, in response to stimulation of these caudal sites of the VLM, was later reported in anesthetized or decerebrate cats (33,34) and rats (35). However, researchers were unable to demonstrate any tonic activity from this area.

Recently, we have carried out a series of studies (36–39) to determine the functional characteristics of the caudalmost ventrolateral medulla. In addition, we have also investigated its role in the maintenance of arterial blood pressure as well as its interactions with the other two cardiovascular areas of the ventral medullary surface.

II. Methods

Experiments were performed on male adult rats (Wistar 250 to 350 g) that had been anesthetized with urethane (1.2 g/kg, IP) or on unanesthetized animals that had previously been subjected to midcollicular transection under ether anesthesia. The trachea and femoral vein were cannulated for mechanical ventilation and drug infusions, respectively. Pulsatile ABP was recorded from a cannula inserted into a femoral artery. Mean arterial pressure (MAP) was obtained by filtering the ABP signal, and heart rate (HR) was measured by a cardiometer that was also triggered by the ABP signal. The animals were paralyzed with gallamine (Flaxedil, 4 mg/kg, IV) and mechanically ventilated. Temperature was maintained at 37°C by a heating table. Microinjections were performed through micropipettes (OD 0.2 mm) and were placed inside guide cannulas. The length of the micropipettes and the guide cannulae was adjusted to allow only the micropipettes to be inserted into the brain tissue. The guide cannulas were directed to the desired stereotaxic position using the calamus scriptorius (CS) as a reference. Vertical positioning was obtained by slowly lowering both the micropipette and the guide cannula until a slight displacement between them was observed. Postmortem histology demonstrated that this procedure consistently permitted the placement of the micropipette tip next to the VLM surface. Microinjections, consisting of 200 nL of 0.1 M sodium phosphate buffer, L-glutamate (L-Glu, 50 or 100 nmol), glycine (Gly, 50 or 100 nmol), GABA (50 nmol), plus kynurenic acid (KYN, 2 nmol) or bicuculline methiodide (BIC, 0.2 nmol) were dissolved. In experiments investigating possible interactions between different areas of the VLM, a pair of micropipettes was implanted in the RVLM (2.6 mm rostral and 2.1 mm lateral to the CS) or CVLM (1.0 mm rostral and 1.8 mm lateral to the CS). In both areas, vertical positioning was obtained by lowering the micropipettes until they reached the VLM surface, as described above.

III. Localization of the Caudal Pressor Area (CPA)

Initially, to identify pressor sites within the CVLM, the VLM was mapped in terms of ABP responses to unilateral microinjections of the excitatory amino acid L-Glu (50 nmol/200 nL). The area surveyed extended from 2.0 mm caudal to 1.0 mm rostral to the CS and 1.0 to 3.0 mm lateral to the midline. Injection sites were marked and plotted in schematic maps of the VLM surface. Each animal received no more than two applications, one on each side of the brainstem. Results obtained in these experiments demonstrated that, in urethane-anesthetized rats, stimulation of a restricted area of the caudalmost portion of the VLM consistently evoked increases in ABP. These pressor responses ranged from 15 to 60 mm Hg, had a latency of 2 to 5 s, and peaked within 20 s; the

ABP remained above resting levels for up to 4 min. Heart rate responses were variable and inconstant; however, tachycardia was noted most frequently (10 to 20 bpm).

Figure 1 summarizes results obtained from 10 animals with L-Glu microinjected in several sites of the VLM. The areas from which pressor responses were obtained are shown by the circles drawn with continuous lines on the left side, while the circles drawn with interrupted lines on the right correspond to areas in which blood pressure was not affected or from which a depressor response was obtained. These depressor responses most likely resulted from stimulation of sympathoinhibitory neurons of the CVLM. The area defined by the pressor responses to L-Glu lies between the rootlets of the twelfth cranial and

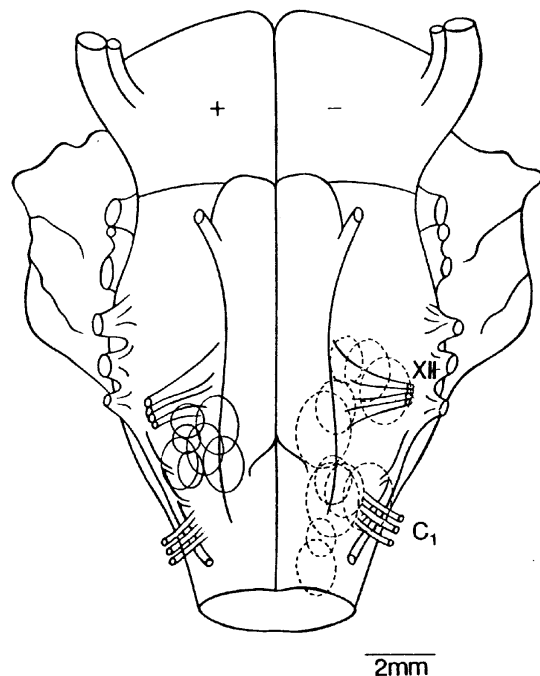


Figure 1 Diagram representing the rat's ventral medullary surface, showing localization of pressor sites in the caudal ventrolateral medulla. In urethane-anesthetized, paralyzed, and artificially ventilated rats ($n=10$), the ventrolateral medulla was mapped out to localize sites where microinjections of L-glutamate (L-Glu, 50 nmol/200 nL) produce pressor responses. Each animal received no more than two applications, one on each side of the brainstem. Each ovoid is the result of a single application. Regardless the actual site of application, ovoids drawn on the left side (+) with continuous lines indicate sites where L-Glu produced increases in arterial blood pressure >15 mm Hg. Ovoids drawn on the right side (-) with interrupted lines indicate sites where blood pressure was unaffected or depressor responses were obtained. XII and C₁: rootlets of twelfth cranial and first cervical nerves, respectively. (From Ref. 38.)

the first cervical nerve, laterally to the pyramides, and just medial to the spinal rootlets of the eleventh cranial nerve. The actual area is probably smaller than indicated by the ovals drawn with continuous lines. This is the case, since the rostral portion of the cranialmost and the lower portion of the caudalmost circles have to be excluded from being responsible for the pressor responses obtained when microinjections were performed in this region. Furthermore, ovoids drawn at the right show that the region between the two nerves is free from interrupted ovals (i.e., no negative results were obtained from this area). This area corresponds approximately to the following stereotaxic coordinates: 0.5 to 1 mm caudal and 1.5 to 2.5 mm lateral to the CS.

Within this pressor area, increasing the L-Glu dose to 100 nmol did not cause further increases in ABP or HR responses. Bilateral applications did not result in any increase in the magnitude of pressor responses but did regularly result in their prolongation.

Since the first observation of pressor sites in the caudalmost medulla by Feldberg and Guertzenstein (32), other studies have confirmed and extended that observation. An incidental observation of pressor responses to L-Glu in caudal sites of the VLM was reported by Dembowski et al. in cats (33). In this study, pressor responses to L-Glu stimulation were observed in about one-third of all animals. Curiously, in these animals researchers were often unable to obtain hypotension in response to stimulation of the CVLM. However, in our experience, pressor and depressor effects could be observed in the same animal and always in separate, distinct areas. A similar result (i.e., pressor and depressor sites in the same animal) was recently described in cats (34). The area defined by the pressor responses to L-Glu in our study corresponds closely to the area described previously in rats by Gordon and McCann (35). This area is designated by them as the caudal pressor area (CPA), a term that we employ in this paper. Contrary to the original observation (32), the level of anesthesia was not a relevant factor in any of these studies.

IV. Cardiovascular Responses to CPA Inhibition

In order to verify that CPA neurons have a tonic pressor activity, we studied the effects of inhibition of CPA neurons on the basal levels of ABP and HR. Unilateral microinjection of the inhibitory amino acid glycine (Gly, 50 nmol/site) into the CPA produced marked decreases in ABP, often accompanied by bradycardia. Depressor responses ranged from -15 to -45 mm Hg, and occasionally—although they are rarely reached—levels equivalent to those observed after spinal transection. As observed with L-Glu, bilateral microinjections increased duration but not magnitude of depressor responses. ABP and MAP records of Figure 2A illustrate a representative example. In a urethane-anesthetized rat, bilateral application of glycine (50 nmol/200 nL) produced a decrease in blood pressure reaching 50 mm Hg within 30 s after the injections. Blood pressure

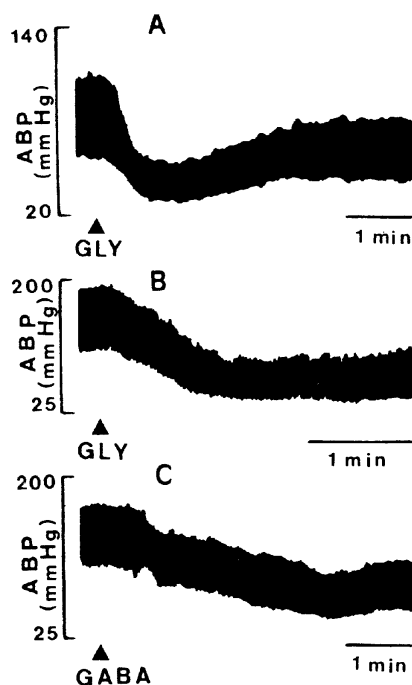


Figure 2 Effects of CPA inhibition by bilateral microinjections of glycine (Gly, 50 nmol/site) on the pulsatile arterial blood pressure (ABP, mm Hg) in a urethane-anesthetized (A) or in an unanesthetized (B) decerebrate rat. In a urethane-anesthetized rat (C), CPA was inhibited by bilateral application of GABA (50 nmol/site).

remained below basal levels for 6 min. Contrary to what was previously observed in cats by Feldberg and Guertzenstein, depressor responses to CPA inhibition were not dependent on the level of anesthesia. This was demonstrated by the fact that in unanesthetized decerebrate animals, CPA inhibition by bilateral application of glycine produced depressor responses similar to those obtained in anesthetized animals (Fig. 2B).

Results obtained in this series of experiments demonstrated that CPA neuronal activity is required for maintenance of basal levels of ABP. Although previous studies have demonstrated the presence of pressor sites in the caudalmost VLM, they have failed to demonstrate any tonic activity for this area (34,35). Since these prior studies have utilized GABA as an inhibitory agent, we tested to see if CPA activity was selectively sensitive to Gly or whether it could also be suppressed by GABA. Results obtained demonstrated that bilateral application of GABA (50 nmol/200 nL) into CPA resulted in depressor responses similar to those obtained with Gly (Fig. 2C). An explanation for this discrepancy between the present study and a previous study (35), conducted in rats, could be that previous applications were made at the level of the caudalmost

rootlets of the twelfth cranial nerve. However, in the present study, applications were localized slightly more caudal to this level between the twelfth cranial and first cervical nerve rootlets. Another possible explanation is that in this study we utilized larger volumes and doses than in previous studies. Also in our study, drugs were applied immediately dorsal and spread over the ventral surface, closely resembling topical applications, and covered a larger portion of the CPA. It is possible that the combination of these factors allowed us to simultaneously inhibit a larger number of CPA neurons and to demonstrate CPA tonic activity.

Results obtained with CPA excitation or inhibition indicate that these areas exhibit the similar functional characteristics of RVLM (i.e., both are tonic sympathoexcitatory areas). Seeking to distinguish between RVLM and CPA, we compared the effects of inhibition of each of these two areas on the pressor response evoked by bilateral carotid occlusion.

V. Effects of CPA Inhibition on the Pressor Response to Carotid Occlusion

In urethane-anesthetized rats, the common carotid arteries were exposed and carefully dissected free from surrounding tissues bilaterally. Bilateral carotid occlusion was obtained by placing miniature clamps on the arteries for 1 min. The carotid occlusion pressor response (COPR) was defined as the mean increase in MAP during the occlusion, compared before and 1 min after bilateral inhibition of the CPA or RVLM.

Data in Table 1 summarize the results obtained in these experiments. Before CPA inhibition, ABP was 87 ± 6 mm Hg and COPR averaged 23 ± 3 mm Hg/s.

Table 1 Effects of CPA or RVLM Inhibition by Glycine (100 nmol/site) on the Carotid Occlusion Pressor Response (COPR)^a

Treatment	N	Before inhibition		After inhibition	
		Control (MAP)	COPR (mm Hg/s)	Control (MAP)	COPR (mm Hg/s)
CPA inhibition	6	87 ± 5.9	23 ± 2.7	53 ± 5.9^b	21 ± 4.4
RVLM inhibition	6	93 ± 9.5	28 ± 3.7	45 ± 5.3^b	$8 \pm 3.0^{b,c}$

^aValues are means \pm SE. Comparisons of basal mean arterial blood pressure (MAP, mm Hg) and the mean increase in MAP during the carotid occlusion pressor response (COPR, mm Hg/s) before and during inhibition by bilateral microinjection of glycine into the caudal pressor area (CPA) and the rostral ventrolateral medulla (RVLM).

^b $P < 0.05$ compared with control.

^c $P < 0.05$ CPA compared with RVLM.

One minute after bilateral application of Gly (100 nmol/site) into the CPA, ABP decreased to 53 ± 6 mm Hg, a level significantly lower than the control, but the COPR was not modified (21 ± 4 mm Hg/s). Conversely, before RVLM inhibition, ABP was 93 ± 9 mm Hg and COPR averaged 28 ± 4 mm Hg/s, respectively. One minute after RVLM inhibition by bilateral application of Gly (100 nmol/site), ABP decreased to 45 ± 5 mm Hg (a level similar to that obtained by CPA inhibition); however, COPR was markedly reduced (8 ± 3 mm Hg/s).

Although the mechanisms involved in the carotid occlusion pressor response are not completely clear, it is accepted that this reflex is due not only to suppression of baroreceptor activity but also to stimulation of peripheral and central chemoreceptors and to cerebral ischemia. Inhibition of RVLM neurons markedly reduced the COPR, indicating that this area is involved in all neural pathways underlying these responses, as was previously shown by others (40–44). On the other hand, the maintenance of COPR during CPA inhibition indicates that, in this condition, at least one of these pathways bypasses the CPA and is thus responsible for the observed sympathoexcitation.

VI. Putative Pathways Involved in the CPA Cardiovascular Responses

To investigate the pathways underlying cardiovascular responses to CPA excitation or inhibition, we examined possible interactions between CPA and the other two cardiovascular areas of the VLM.

Initially we examined whether or not CPA pressor responses were dependent on the activity of RVLM neurons. For this purpose, two pairs of micropipettes were implanted in the RVLM and CPA of anesthetized rats, utilizing the methods described above. Pressor responses to CPA stimulation by L-Glu (50 nmol/site) were evaluated before and 1 min after RVLM inhibition by Gly (100 nmol/site). Results obtained in a typical experiment are illustrated in Fig. 3A. Bilateral stimulation of the CPA with L-Glu produced a 35-mm Hg pressor response (Fig. 3A, left). Twenty minutes later, when ABP had returned to control values, the RVLM was inhibited by bilateral application of Gly. Once the evoked hypotension was fully established (about 1 min later), stimulation of the CPA with the same dose of L-Glu produced only a 5-mm Hg rise in ABP (Fig. 3A, right). Similar results obtained in a group of six animals are summarized in Table 2. During RVLM inhibition, pressor responses to CPA stimulation were reduced from 29 ± 4 to 10 ± 4 mm Hg ($P < 0.05$).

Conversely, the effects of RVLM stimulation are not inhibited by CPA blockade, as shown by a typical experiment in Fig. 3B. Initially, the RVLM was stimulated with L-Glu, producing a 60-mm Hg increase in ABP (Fig. 3B, left). After a recovery period of 20 min, CPA was inhibited by Gly (100 nmol/site) applied bilaterally; once the hypotension was established, the RVLM was

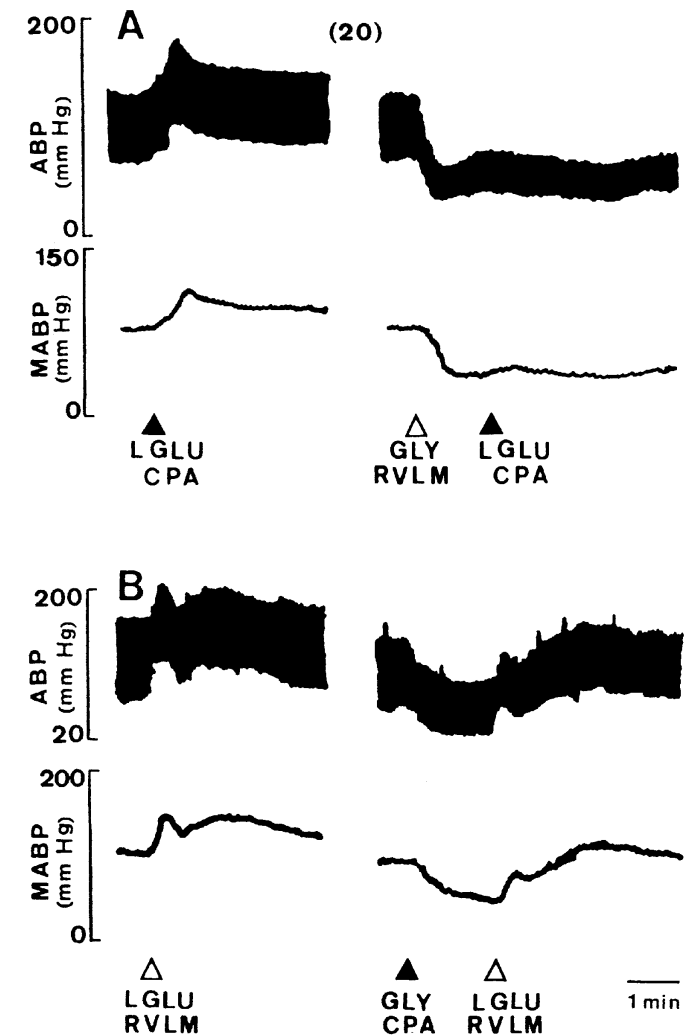


Figure 3 Interactions between the CPA and RVLM responses. (A) The CPA was stimulated by bilateral application of L-glutamate (L-Glu, filled triangles, 50 nmol/site) before (left) and during (right) RVLM inhibition by glycine applied bilaterally (Gly, open triangle, 100 nmol/site). (B) RVLM was stimulated by bilateral application of L-glutamate (open triangles, 50 nmol/site) before (left) and during (right) CPA inhibition with glycine bilaterally applied (filled triangles, 100 nmol/site). Numbers on the top of the tracings indicate time in minutes between successive records. (From Ref. 38.)

again stimulated, producing a 50-mm Hg increase in ABP (Fig. 3B, right). In a group of similar experiments ($n = 6$), pressor responses to RVLM stimulation averaged 34 ± 8 mm Hg before and 60 ± 9 mm Hg during CPA inhibition (Table 2).

These results showed that cardiovascular responses to CPA stimulation depend on the activity of sympathoexcitatory neurons in the RVLM. Pressor

Table 2 Interactions Between CPA and RVLM^a

	N	Before inhibition		After inhibition	
		Basal (MAP)	Stim. (MAP)	Basal (MAP)	Stim. (MAP)
CPA stimulation (L-Glu, 50 nmol/site) combined with RVLM inhibition (Gly, 100 nmol/site)	6	100 ± 4.8	+29 ± 4.1	53 ± 9.1 ^b	+10 ± 5.1 ^b
RVLM stimulation (L-Glu, 50 nmol/site) combined with CPA inhibition (Gly, 100 nmol/site)	6	100 ± 8.5	+34 ± 8.3	63 ± 6.2 ^b	+60 ± 8.7 ^b

^aValues are means ± SE. Comparisons of effects of RVLM inhibition on the pressor responses to CPA stimulation (top) and CPA inhibition on the pressor responses to RVLM stimulation (bottom).

^b*P* < 0.05 compared with control.

responses to CPA stimulation were markedly reduced during RVLM inhibition; whereas pressor responses to RVLM stimulation were not dependent on CPA activity. They suggest that CPA effects are mediated by RVLM. Another possibility would be that CPA neurons project directly into the intermediolateral cell column, contributing to the maintenance of tonic excitation of sympathetic preganglionic neurons (SPN). In that case, the reduction of CPA pressor response during RVLM inhibition would only reflect the sudden withdrawal of an excitatory drive and a marked reduction in SPN excitability. The RVLM, however, provides a greater proportion of this drive and would have the ability to overcome the effects of CPA inhibition. This possibility, although appealing, seems unlikely, since various studies have failed to detect direct projections from this region (CPA) to the intermediolateral cell column (15,16,18,45).

We next addressed the question of whether pressor responses to CPA stimulation were due to excitation of RVLM neurons by glutamatergic synapses. In preliminary experiments, pressor responses to CPA stimulation were compared before and after microinjections of the broad-spectrum glutamate antagonist kynurenic acid (KYN, 2 nmol/site), into the RVLM bilaterally. Results obtained in these experiments (not shown) indicated that CPA pressor responses were not affected by this procedure. Curiously, in some experiments where KYN was injected into sites within the CVLM, it was shown to be effective in blocking increases in ABP during CPA stimulation. These results suggested that CPA responses were dependent on glutamatergic synapse(s) within the CVLM. These experiments, however, were somewhat disputable, since it was not possible to completely exclude the chance that these effects were only due to diffusion of the KYN from the injection sites to the CPA itself. We overcame this inconvenience by utilizing a different experimental approach. We

tested not the pressor but the depressor effects of CPA inhibition during bilateral application of KYN into the CVLM.

VII. Glutamatergic Synapses Within the CVLM Are Required for the Depressor Responses Produced by CPA Inhibition

In a urethane anesthetized rat, bilateral inhibition of the CPA with Gly (100 nmol/site) produced the already described depressor response (Fig. 4A, left). After ABP had returned to control levels, kynurenic acid (KYN, 2 nmol/200 nL)

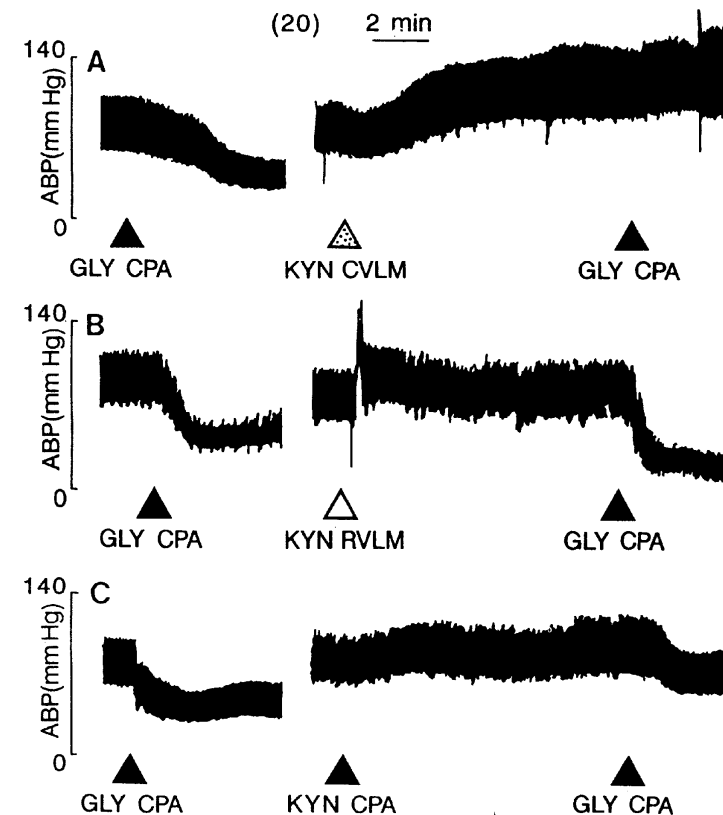


Figure 4 Effects of glutamatergic blockade on the depressor responses to CPA inhibition. In anesthetized rats, CPA was initially inhibited by bilateral microinjections of glycine (Gly, 100 nmol/site, black triangles, left). The glutamate antagonist Kynurenic acid (KYN, 4 nmol/site) was then applied to (A) the caudal ventrolateral medulla (CVLM, dotted triangle); (B) the rostral ventrolateral medulla (RVLM, open triangle) or (C) the CPA itself (black triangle), and Gly application was repeated (black triangles on the right side). Numbers on the top of the tracings indicate time in minutes between successive records.

