Location of the Area Postrema Pressor Pathway in the Dog Brain Stem

KAREN L. BARNES AND CARLOS M. FERRARIO

SUMMARY Electrical stimulation of the dog’s area postrema (AP) induces a response that mimics the pressor response produced by intravertebral infusion of low-dose angiotensin II, which causes an increase in mean arterial pressure associated with transient tachycardia and increased peripheral resistance. The present study investigated in morphine-chloralose anesthetized dogs whether: 1) the characteristics of the AP pressor response are influenced by the presence of carotid sinus afferents; 2) structures rostral to the medulla influence the AP pressor response; and 3) the pressor pathway is initiated by neurons within the AP. Since bilateral cervical sinovagal denervation, which potentiated the phenylephrine pressor response, did not affect the pressor response to AP stimulation, the data provide evidence for an inhibitory influence exerted upon the central baroreflex mechanism by the AP pressor mechanism. The unaltered AP pressor response after midcollicular transection suggests that the efferent pathway is contained within the brain stem caudal to the pons. Finally, the elimination of the pressor response following kainic acid microinjection into the AP provides evidence that the AP pressor mechanism is initiated by neurons within the AP, rather than by fibers of passage from other pressor centers. These results suggest that the AP produces its facilitation of central sympathetic vasomotor outflow via a pathway contained within the medulla. (Hypertension 6: 482-488, 1984)

KEY WORDS • pressor response • autonomic nervous system • vagal afferents • carotid sinus afferents • midcollicular transection • kainic acid

Previous studies by Ferrario et al. and Joy and Lowe have revealed that in the mongrel dog the area postrema (AP) mediates the pressor effects of angiotensin II (ANG II) infused via the vertebral artery. The possibility that the effects of ANG II may involve activation of a pressor pathway in the vicinity of the AP has received support recently from the work of Barnes et al., who showed that electrical stimulation of the AP produced a pressor response with hemodynamic features bearing a striking resemblance to the cardiovascular effects obtained by infusing small amounts of ANG II into the vertebral arteries. Investigation of the efferent peripheral pathways that mediate the pressor effects of electrical stimulation of the AP confirmed that the major contributor to the rise in arterial pressure was increased peripheral resistance. These and other studies of the long-term hemodynamic alterations produced by removal of the AP have provided important information regarding the possible role of the AP pressor pathway in the regulation of bulbospinal mechanisms that influence efferent sympathetic nerve activity. The present study investigated whether: 1) the characteristics of the AP pressor response are influenced by the presence of carotid sinus afferents; 2) the structures rostral to the medulla oblongata influence the AP pressor response; and 3) the pressor pathway is initiated by neurons within the AP.

Methods

Nineteen mongrel dogs (weight, 14 ± 1 kg) were anesthetized with chloralose (60 mg/kg i.v.) after premedication with morphine (2 mg/kg i.m.). Catheters were placed in a femoral artery to monitor arterial pressure with a strain gauge transducer (Statham P-23db, Gould-Statham, Oxnard, California) and in a femoral vein for the infusion of drugs. The dog’s head was then fixed in a stereotaxic headholder with the nose tipped downward at 45° to expose the AP after the foramen magnum had been opened through a dorsal midline incision.

The effect of complete baroreceptor denervation upon the AP pressor responses was evaluated in nine bilaterally vagotomized dogs by comparing the responses before and after section of the carotid sinus nerves. In another four animals the participation of structures rostral to the medulla in the efferent pressor pathway was determined by transection of the brain stem at the midcollicular level. The remaining six dogs underwent microinjection of the neurotoxin kainic acid into the AP to destroy neurons within the AP.
Central Nervous System Stimulation

We used monopolar-stimulating electrodes made of Teflon-coated stainless steel wire (Medwire Corporation, Mt. Vernon, New York; coated diameter, 0.127 mm). Only the flat circular cross section of stainless steel (0.076 mm o.d.) was exposed for stimulation since the wire was insulated to the tip. A large alligator clip on the neck muscles served as reference electrode. The stimulating electrode was positioned within the AP under visual guidance with a Zeiss surgical microscope. Stimuli were cathodal monophasic rectangular pulses of constant current (0.2 msec pulse duration, 50 Hz, 20 sec train duration), delivered from a Pulsar 6b stimulator through a CCIU-8 RF stimulus isolation unit (Fredrick Haer Company, Brunswick, Maine). The electrode was lowered in 0.1 mm increments beneath the brain-stem surface until a pressor response characteristic of AP stimulation was obtained with stimulus strength set at 50 μA. After an optimal pressor point was identified, control responses were obtained at approximately 2, 5, and 8 times threshold (20, 50, and 80 μA) by using a predetermined random order. After the experimental treatment, the three stimuli were given in reverse order to counterbalance any long-term trends in the animal’s condition during the experiment.

Cervical Sinovagal Denervations

In nine animals, the right and left vagus nerves were cut in the neck. The carotid sinus nerves were then isolated just below the jugular foramen as described previously. With a bipolar electrode (Rhodes NE-200, David Kopf, Tujunga, California), an electro-neurogram was obtained for each isolated nerve to confirm that it carried action potentials that discharged in synchrony with the arterial pressure pulse. Four of the nine dogs were positioned in the stereotaxic frame to allow placement of a stimulating electrode in the lateral hypothalamus (AP = 18–19, L = 4, D = 31–35 mm below cortical surface) through a small burr hole. When an optimal hypothalamic pressor response had been obtained, the electrode was fixed to the skull with dental cement. The animal’s head was then tipped to 45° to allow placement of the AP-stimulating electrode. The hypothalamic pressor responses and AP pressor responses at approximately 2, 5, and 8 times threshold current were then compared before and after bilateral transection of the carotid sinus nerves. In the remaining five dogs, pressor responses to intravenous phenylephrine (1 and 4 μg/kg i.v.) and electrical stimulation of the AP were assessed before and after carotid sinus denervation.

Midcollicular Transection

The possible importance of structures rostral to the colliculi in the AP pressor mechanism was investigated in an additional four dogs. Stimulation of the AP was repeated before and after complete transection of the brain stem at the midcollicular level, later verified by inspection of the brain stem. After control AP pressor responses had been obtained at 20, 50, and 80 μA, a portion of the left occipital cortex was removed through a left occipitoparietal craniotomy to allow visualization of the brain stem at the level of the tentorium. Transection of the brain stem was achieved by blunt dissection with a suction tip covered with a cottonoid; the cottonoid was left in place to prevent bleeding. The stimulus-response curve was reassessed 1 hour after transection. The pressor response to bilateral carotid occlusion was determined before and after midcollicular transection to allow comparison of the effects of transection on the baroreceptor reflex mechanism with those on the AP pressor mechanism.

Microinjection of Kainic Acid

In four of the remaining dogs, AP stimulus response curves at 20, 50, and 80 μA were determined first by stimulating the right AP and then the left AP in the intact animal. Then kainic acid (10 nmol in 1.0 μl phosphate-buffered saline) was slowly microinjected during a period of 20 minutes into the right half of the AP, and 1 μl of the phosphate-buffered saline alone was injected into the left AP as a control for the mechanical effects of microinjection. The solutions were administered through a glass micropipette (20 μm tip diameter) with a Picospritzer II (General Valve Corporation, Fairfield, New Jersey). Within each half of the AP, the pipette was first positioned at the site where the electrode that generated the control AP stimulus response curve had been located. Then four microinjections of 0.1 μl of solution were administered over 10 minutes, for a total of 0.4 μl. Next the electrode was moved 0.5 mm rostrally, lowered into the AP, and an additional 0.3 μl of solution was injected over 5 minutes. The final 0.3 μl of solution was injected into the AP at a third site 0.5 mm caudal to the first site. This procedure distributed the volume of the microinjected solution optimally over the rostrocaudal extent of the AP and minimized the possibility of diffusion into adjacent regions such as the nucleus tractus solitarius (NTS). The dose of kainic acid was selected on the basis of studies by Olney, who showed that this amount was sufficient to produce histological evidence of neuronal death within 3 hours after microinjection, with sparing of axons and terminals 1 to 3 weeks after injection. Four hours later, the AP stimulus response curves were repeated in the left AP and then the right AP. In the remaining two animals, both the AP stimulus-response curves and unilateral and bilateral occlusions of the common carotid arteries were repeated before and after microinjection of kainic acid into the right half of the AP.

Cardiovascular Responses

Arterial pressure was recorded in the abdominal aorta from a cannula introduced via a femoral artery; heart rate was monitored with a biotachometer triggered by the R-R interval of the simultaneously recorded electrocardiogram. For each AP stimulus, the control level of mean arterial pressure or heart rate was computed as the mean value for the 20 seconds immediately before
the stimulus onset. Because the maximum change in heart rate generally did not coincide with the peak pressor response, both the maximum heart rate response and the heart rate change associated with the peak pressor response were analyzed. The latencies of the peak pressure rise and the maximum heart rate change were also compared across the stimulus conditions.

Histological Verification

At the conclusion of the experiment, a small marking lesion (200 μA DC, 15 sec) was made through the stimulating electrode to verify the site of stimulation. The brain stem was removed and fixed in 10% buffered formalin. Frozen sections were cut at 50 μm intervals, stained with neutral red and Luxol fast blue, and examined microscopically to confirm the postioning of the electrode tip in the AP. We have shown previously that a characteristic AP pressor response can be obtained from stimulation sites throughout the anatomical extent of the AP. In four dogs receiving hypothalamic stimulation, the rostral diencephalon was cut and stained similarly to verify the location of the stimulating electrode in the lateral hypothalamus. In the dogs receiving microinjections of kainic acid and phosphate buffer, the AP was examined carefully to make sure that there was no evidence of physical damage that could account for the disappearance of the pressor response in the right half of the AP.

Statistical Evaluation

All results are expressed as means ± SEM. In experiments consisting of more than two conditions, the data were subjected to analysis of variance for repeated measures on the same subjects. Scheffe's method was used to calculate the 95% confidence intervals for all contrasts between the conditions in each group of dogs. Statistical comparisons between pairs of means were made with the two-tailed Student's t test for paired variates. Linear regression analyses and correlation coefficients were calculated by the method of least squares. Differences and correlation coefficients were considered to be significant at \( p < 0.05 \).

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<th>Table 1. Magnitude and Latency of the Pressor Response to Electrical Stimulation of the Area Postrema in Nine Vagotomized Dogs Before and After Carotid Sinus Denervation</th>
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<td>Change in mean arterial pressure (mm Hg)</td>
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Values are means ± SEM, \( F \) values, and probabilities from analysis of variance with repeated measures. \( F \) values were highly significant for an effect of stimulus current level on change in mean arterial pressure (48.62, \( p < 0.0001 \)) and change in heart rate (13.54, \( p < 0.0005 \)), but were not significant for either response latency measure.

Results

Effects of Carotid Sinus Nerve Section on the Area Postrema Pressor Response in Vagotomized Dogs

In nine bilaterally vagotomized dogs, baseline mean arterial pressure (140 ± 9 mm Hg) and heart rate (134 ± 10 bpm) were not significantly different from those that we have reported previously in vagotomized dogs undergoing autonomic blockade. Subsequent bilateral sectioning of the carotid sinus nerves increased mean arterial pressure to 160 ± 9 mm Hg (0.10 > \( p > 0.05 \)), but there was no further augmentation of the tachycardia (143 ± 13 bpm).

In dogs with bilateral vagotomy, electrical stimulation of the AP produced a rapid rise in mean arterial pressure accompanied by tachycardia. When the pressor responses during AP stimulation at 20, 50, and 80 µA were compared before and after removal of the carotid sinus nerves, the peak change in arterial pressure was unaltered (Table 1, Figure 1). Although there was a tendency for the tachycardia to decrease, these changes did not attain statistical significance by the analysis of variance (Table 1). Table 1 also shows that baroreceptor denervation did not alter the latencies from stimulus onset of the peak changes in mean arterial pressure and heart rate. Figure 1 displays the relationship of the increases in mean arterial pressure and heart rate to the stimulus current before and after carotid sinus denervation.

The effects of carotid sinus denervation upon the hypothalamic pressor response and upon the increase in mean arterial pressure produced by intravenous phenylephrine in previously vagotomized dogs were examined to determine whether elimination of the baroreceptor reflexes was responsible for any potentiation of responses to pressor stimuli. The peak increases in mean arterial pressure during stimulation of the lateral hypothalamus at 2, 5, and 8 times threshold current in four vagotomized dogs were 26 ± 4, 39 ± 5, and 46 ± 4 mm Hg. Following bilateral removal of the carotid sinus nerves the pressor responses to the same hypothalamic stimuli were 32 ± 10, 37 ± 8, and 45 ± 10 mm Hg. Although there was a tendency for the
pressor response at the lowest stimulus current to increase following carotid sinus denervation, the differences did not attain significance. At the higher levels of stimulation, the pressor responses were essentially the same before and after elimination of the carotid sinus nerves. In contrast, the pressor response to intravenous phenylephrine was significantly increased in five dogs following carotid sinus denervation for both the 1 μg/kg and 4 μg/kg doses (before: 37 ± 5 and 71 ± 9 mm Hg; after: 56 ± 8 and 100 ± 4 mm Hg, p < 0.05).

Midcollicular Transection

In four dogs, the AP was stimulated before and after transection of the brain stem at the midcollicular level, as verified histologically. Midbrain transection did not significantly alter the resting levels of mean arterial pressure or heart rate (before: 118 ± 9 mm Hg, 55 ± 6 bpm; after: 110 ± 7 mm Hg, 60 ± 9 bpm). Stimulation of the AP at three current levels also yielded pressor responses that were unchanged by midcollicular transection, as shown in the individual example of Figure 2 and the averages in Figure 3. The peak increases in mean arterial pressure during AP stimulation at 20, 50, and 80 μA were 19 ± 1, 30 ± 3, and 40 ± 4 mm Hg in the intact animal and 20 ± 0.3, 32 ± 3, and 46 ± 6 mm Hg after transection. Peak heart rate changes were 15 ± 4, 31 ± 7, and 50 ± 10 bpm before and 18 ± 6, 26 ± 5, and 42 ± 5 bpm after midbrain disconnection. Linear regression analysis of the relationship of peak increase in mean arterial pressure to stimulus current indicated that the same equation fit the data before and after midcollicular transection; the same was true for the relationship of peak heart rate increase to stimulus current before and after transection. Furthermore, the latencies of the peak changes in mean arterial pressure and heart rate were unaltered by midcollicular transection. The increase in mean arterial pressure produced by bilateral common carotid occlusion was slightly but not significantly reduced following midbrain transection (before: 38 ± 5 mm Hg, after: 29 ± 5 mm Hg; p > 0.2).

Microinjection of Kainic Acid

Resting mean arterial pressure in these animals rose from 123 ± 5 to 227 ± 7 mm Hg within 10 minutes following microinjection of kainic acid into the right half of the AP. This significant hypertension was most likely due to the excitatory effects of kainic acid upon the AP neurons that initiate the pressor pathway, effects that have been postulated by Olney to constitute the actual mechanism of kainic acid neurotoxicity. Four hours later, the blood pressure had fallen to 148 ± 11 mm Hg, a value not significantly different from that recorded before kainic acid injection. In contrast, heart rate rose slowly from 45 ± 5 to 115 ± 14 bpm (p < 0.05) by 1 hour after kainic acid injection and remained at that level for the duration of the experiment. The stimulus-response curve for mean arterial pressure evoked from the left half of the AP was not altered following microinjection of phosphate-buffered saline into that side (28 ± 8, 37 ± 9, and 46 ± 7 mm Hg before; 29 ± 7, 40 ± 7, and 46 ± 6 mm Hg after). On the other hand, the heart rate responses were converted from tachycardia to bradycardia (11 ± 5, 17 ± 9, and 23 ± 7 bpm before; -2 ± 10, -8 ± 13, and -11 ± 11 bpm after). Before microinjection of kainic acid, the pressor responses during stimulation of the right AP at 20, 50, and 80 μA were 21 ± 4, 35 ± 4, and 56 ± 5 mm Hg. Four hours after microinjection of kainic acid into the right half of the AP, the pressor responses during stimulation of that side were abolished (0 ± 0, 2 ± 1, and 3 ± 1 mm Hg). The tachycardia accompanying the preinjection pressor responses was also eliminated after kainic acid administration (before: 14 ± 5, 33 ± 13, and 36 ± 10 bpm; after: -1 ± 1, -1 ± 5, and -2 ± 7 bpm). These data are displayed in Figure 4.

In two animals, the pressor responses during both unilateral and bilateral occlusion of the common carotid arteries were determined before and after microinjection of kainic acid into the right AP. In both animals, neither occlusion of the right carotid (before: 26 mm Hg; after: 26 mm Hg) nor occlusion of both carotid arteries (before: 40 mm Hg; after: 40 mm Hg) revealed any change in the pressor responses 4 hours after delivery of kainic acid into the AP. Careful histological
examination of both sides of the AP failed to reveal any significant physical trauma to the right half of the AP that could account for the selective disappearance of the AP pressor response from the right AP following microinjection of kainic acid (Figure 5).

Discussion

Previous experiments in the dog\(^3\) have demonstrated that electrical stimulation of the AP produces a pressor response that resembles the hemodynamic effects of small doses of ANG II given into the vertebral arter-
In both cases, the predominant hemodynamic cause of the rise in arterial pressure was an increase in total peripheral resistance. The present study has evaluated several alternative hypotheses to uncover the efferent pathway by which the AP pressor mechanism acts upon bulbospinal neurons to elicit changes in sympathetic vasomotor discharge.

One question concerns the possible participation of the baroreceptor reflex. Our previous investigation of the effects of vagotomy upon the AP pressor response demonstrated that the magnitudes of both the rise in arterial pressure and the increase in heart rate during AP stimulation were not significantly changed following vagotomy. The present study reveals that complete removal of buffer reflexes produced by bilateral transection of the carotid sinus nerves in dogs previously vagotomized did not alter either the magnitudes or the latencies of the increases in blood pressure and heart rate produced by electrical stimulation of the AP. These data might suggest that the baroreceptor reflexes do not act to oppose the increases in sympathetic activity triggered via the AP pressor pathway. However, the
significant potentiation of the pressor effects of intravenous phenylephrine, which contrasts with the unaltered AP pressor response, provides evidence for an inhibitory influence exerted upon the central baroreflex mechanism by the AP pressor mechanism. Other investigators have suggested a similar baroreflex inhibition mediated by ANG II acting on the AP. Fukiyama\textsuperscript{10} proposed that ANG II acting centrally at the AP may modulate activity in the baroreceptor pathway in the region of the NTS. Moreover, Sweet and Brody\textsuperscript{11} found that intravertebral, but not intravenous, ANG II attenuated the hindlimb vasodilator response to intravenous norepinephrine. Their results suggested a central inhibition of the baroreceptor pathway by angiotensin acting on the AP.

Another question concerns the possible involvement of regions rostral to the colliculi, such as the hypothalamus, in the efferent pathway for the AP pressor mechanism. It is conceivable that stimulation of the AP activates neurons that project rostrally to the pressor regions of the hypothalamus and relay there with hypothalamic efferents that are known to project via the lateral brain stem to the medullary and/or spinal cord vasomotor neurons.\textsuperscript{12} However, the failure of midcollicular brain-stem transection to alter the AP pressor response appears to exclude this possibility. Indeed, the AP pressor response at three levels of stimulus current was actually slightly increased after the transection, in contrast to the modest decrease in the pressor response to bilateral carotid sinus hypotension after transection. This finding provides another parallel with the pressor effects of intravertebral ANG II, shown by Gildenberg et al.\textsuperscript{13} to be unaffected by midbrain transection.

A further possibility that the AP pressor mechanism results from stimulation of efferent fibers passing through the AP from some distant pressor region such as the hypothalamus had to be investigated separately, since after midcollicular transection the axons remained electrically excitable as long as 2 weeks after disconnection from their soma. To evaluate the possibility of such a pathway, kainic acid was microinjected into the AP to destroy its intrinsic neurons while sparing fibers of passage. McGee and McGeer\textsuperscript{14} have recently reviewed the ability of kainic acid to destroy neurons within the AP and to spare axons when microinjected slowly into the tissue. Since the pressor responses during electrical stimulation of the left AP were unaltered by microinjection of phosphate buffer, while the responses from the right AP were abolished after microinjection of kainic acid, these data suggest that neurons in the AP mediate the pressor mechanism.

To be sure that the disappearance of the AP pressor response following microinjection of kainic acid was not due to leakage of the neurotoxin into the adjacent NTS, which might have destroyed the baroreflex mechanism on that side, we tested unilateral and bilateral carotid occlusion responses in two dogs before and after microinjection of kainic acid into the right AP. Since the carotid occlusion pressor responses were unaffected by kainic acid and since histological examination showed no damage to any brain-stem structure, we conclude that damage to the baroreceptor mechanism cannot account for the elimination of the AP pressor response following kainic acid.

These results provide evidence that the AP pressor pathway is initiated by neurons within the AP rather than by fibers of passage from other pressor centers. In addition, the pathway is contained within the brain stem caudal to the colliculi, since it is unaltered by midbrain transection. Furthermore, the lack of potentiation of the pressor response after elimination of the cardiovascular afferents may suggest that the AP pressor mechanism is not inhibited by the baroreflexes, but itself acts to inhibit the baroreceptor reflex mechanism. Our present results and those of our earlier anatomical studies\textsuperscript{15} of the efferent projections of AP neurons suggest that the most likely explanation for the efferent pathway of the AP pressor mechanism is a pathway that projects to the brain stem vasomotor centers, with a probable synapse in the medial nucleus tractus solitarii.

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