Physiological and Pharmacological Characterization of the Area Postrema Pressor Pathways in the Normal Dog

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SUMMARY Previous studies have implicated the area postrema (AP) as a site responsible for the centrally mediated neurogenic effects of angiotensin II. To clarify its role further we characterized in mongrel dogs: 1) the onset and offset transients of the pressor response due to electrical stimulation of the AP; 2) the peripheral efferent autonomic pathways; and 3) the immediate and long-term hemodynamic effects of AP ablation. Large pressor responses (+30 ± 4 mm Hg) due to increased peripheral resistance were obtained at low stimulus strength (20–80 mA) via electrodes stereotaxically lowered into the area postrema. Rises in arterial pressure were due to augmented sympathetic activity because they were prevented by sympathetic ganglionic blockade and were unaffected by either vagotomy or beta-adrenergic blockade. In conscious, trained, instrumented dogs thermocoagulation of the AP caused transient hypertension (40 ± 1%) and tachycardia. Subsequently, mild hypotension due to decreases in cardiac output and peripheral resistance was a feature persisting for 4 additional weeks of measurements. The mild changes in resting hemodynamics were associated with a large fall in the normal lability of heart rate and cardiac output sustained for the entire period of observation. The hypotensive effects of AP ablation were accompanied by significantly reduced vasoconstrictor effects of I.V. injected angiotensin II at all doses tested (0.5–5 ng). Vascular responses to I.V. norepinephrine (5–40 ng) were augmented. Since electrical stimulation causes hypertension and ablation results in sustained hypotension, the data indicate that the AP participates in the regulation of blood pressure by the central nervous system. (Hypertension 1: 235–245, 1979)

KEY WORDS • nucleus tractus solitarius • area postrema • angiotensin II • vascular reactivity • arterial pressor response • cardiac output • hemodynamic variability

At the caudal end of the ala cinera between the funiculus separans and the clava there is a small strip of tissue bathed by cerebrospinal fluid called the area postrema (AP), which on microscopic examination is found to be rich in blood vessels, neurons and glia. It's previously known function was participation in the integration of the neurally elicited emetic response.

There is now reason to believe that the AP may also play a role in the neural control of cardiovascular function, acting to enhance central vasomotor sympathetic activity in response to increased blood levels of angiotensin II. The present study was undertaken to further our knowledge of the function of the AP in the regulation of arterial blood pressure by: 1) characterizing in the dog the hemodynamic consequences of electrical stimulation of the AP; 2) assessing the efferent autonomic pathways involved in the expression of this cardiovascular response; and 3) investigating the long-term effects of permanent inactivation of this structure on the arterial blood pressure and cardiac output of awake trained dogs.

Methods

Experiments were performed in 25 mongrel dogs weighing between 18 and 24 kg. Fifteen of the 25 animals were used in acute experiments, while the remaining 10 were used to assess the long-term hemodynamic changes following ablation of the AP. Six of the 15 dogs used in acute experiments and all 10 chronic animals were instrumented several weeks beforehand with an electromagnetic flowmeter around the ascending aorta using a technique described elsewhere. Arterial pressure was obtained from a catheter inserted into either an iliac (chronic experiments) or femoral artery (acute experiments).

In both acute and chronic experiments (aseptic technique), the AP was exposed after placing the dog's head in a stereotaxic head-holder with the nose tipped downward 45°. In acute experiments, animals were anesthetized with chloralose (60 mg/kg, I.V.) after
premedication with morphine (2 mg/kg, I.M.). In chronic experiments, the animals were anesthetized with halothane. After exposure of the foramen magnum via a midline incision from the crest of the occiput to the C-2 region, the atlanto-occipital membrane was transected. A small portion of the occipital bone overlying the cerebellum was removed with a rongeur to facilitate exposure of the fourth ventricle. With the aid of a dissecting microscope, the pia was opened and the caudal fourth ventricle was exposed by gentle retraction of the cerebellum. After identification of the AP, either electrodes were inserted under visual guidance (acute experiments), or the AP was coagulated bilaterally (chronic experiments) from the obex to its most rostral part, by means of a technique described previously.* Briefly, the AP was coagulated under visual guidance with the fine tip of a wire filament heated to 200°C. The lesion was circumscribed within the anatomical boundaries of the structure as assessed by later histological verification. After placement of lesions both the dura and atlanto-occipital membranes were sutured with 4-0 silk; muscles were approximated with interrupted 2-0 sutures, and the skin was closed with a continuous run of 1-0 silk.

**Acute Experiments**

**Electrical Stimulation**

Hemodynamic characterization of the effects of electrical stimulation of the AP was undertaken in six dogs. In all experiments monopolar stainless steel, Teflon-insulated electrodes (outside diameter 76 μm) were placed under direct vision into the AP. Details of the technique are described elsewhere.† Constant current stimulation (Frederick Haer Co., Brunswick, Maine) was given in trains of 0.2 msec pulses (20-70 Hz) for 10 seconds. A large alligator clip on the neck muscles served as reference electrode.

With stimulus current set between 50 and 80 μA, electrodes were lowered in 0.1-mm increments from the brain-stem surface until a maximum response characteristic of the region being studied was obtained. At the completion of each study a small marking lesion (200 μA, 500 Hz or DC, 15 sec) was placed to verify the site stimulated. Then, with current again set between 50 and 80 μA, the electrode was lowered in 0.1-mm steps until the characteristic response pattern disappeared. At this point another marking lesion was made at the bottom of the electrode tract. Two to four tracts were made for each study.

**Pharmacological Evaluation**

These studies were carried out in nine dogs. Before exposure of the AP, both carotid arteries and vagus nerves were isolated in the neck with loose ligatures placed around each structure. In five of the nine dogs, pressor responses to AP stimulation were compared before and after unilateral and bilateral vagotomy and after I.V. administration of propranolol at a dose of 0.6 mg/kg. Adequacy of beta-blockade was confirmed by the absence of any change in heart rate following injection of isoproterenol (1 μg I.V.). In four other bilaterally vagotomized dogs, pressor responses were determined before and after ganglionic sympathetic blockade. An initial dose of pentolinium (3 mg/kg) was given intramuscularly, followed 15 minutes later by a second dose of 3 mg/kg, I.V. Adequacy of ganglionic blockade was judged by the abolition of the pressor response to carotid occlusion.

**Chronic Experiments**

Ten dogs (22 ± 1 kg) were brought to the laboratory for daily training and conditioning after they had been certified by a veterinarian to be in good health and fully recovered from all surgical procedures. During recording sessions animals were housed in a pen situated in a darkened laboratory, shielded from ambient visual or auditory stimuli. Each recording session lasted from a minimum of 60 minutes to 3 hours, during which time the dogs were confined to the pen but were free to turn about or rest quietly.

At the completion of a 15- to 42-day control period the animals were anesthetized again with halothane and mechanically respirated. The lower portion of the medulla was exposed surgically as described above. The AP was heat coagulated bilaterally in seven dogs and just touched with the cold tip of the cautery in three others. The latter animals served as sham-operated controls.

In four of the seven AP-lesioned animals and the three sham-operated controls measurements of arterial pressure and cardiac output were obtained immediately after termination of the anesthesia. In one of these seven dogs hemodynamic monitoring was continued for the first 7 consecutive hours after ablation of the AP. In the three AP-lesioned animals not monitored immediately, measurements were resumed 24 hours after operation and continued almost daily for an average of 25 ± 6 days (range: 7 to 39 days).

**Hemodynamic Monitoring**

Beat-by-beat recordings of phasic arterial pressure and aortic blood flow were stored on an FM analog tape recorder for later processing by a digital computer (Nova-2, Data General Corp., Waltham, MA). During playback the aortic pressure and blood flow wave forms were fed into an A-D converter with 12-bit resolution. Signals were digitized at the rate of 208 samples/sec. The high resolution of the A-D converter permitted discrimination of aortic blood flow and pressure samples to 0.204 ml/sec/bit and 0.122 mm Hg/bit, respectively. The computer was programmed to isolate a cardiac cycle based on the aortic blood flow wave form. The program determined the systolic and diastolic pressures as the highest and lowest values during each heart cycle. Mean arterial pressure was computed by summing all digitized pressures during a beat divided by the number of samples. Heart rate was computed as the reciprocal of the product of the number of samples within a cardiac cycle times the sampling interval (4.8 msec). Beat stroke volume was
Statistical Evaluation

The mean and the standard deviation of the mean values of each variable were computed for each recording session and from the grouping of recording sessions at designated time intervals (i.e., control, 1 week after AP ablation, etc.). The mean of all session standard deviations during a designated time period was computed for each dog to estimate hemodynamic stability during those recording sessions as suggested by Ito and Scher. \(^8\) We will refer to this value as an index of intra-session variability.

Dose-Response Curves

On three different occasions before and at least once weekly after either ablation of the AP or sham operation, pressor responses to both norepinephrine (10, 20 and 40 \(\mu\)g) and angiotensin II (0.5, 1, 2 and 5 \(\mu\)g) were investigated. Dose-response curves were determined at the end of a recording session following insertion of a butterfly 21-gauge needle into a limb vein. Drugs were diluted in 0.9% sodium chloride and injected as a 1-ml bolus via the side port of an I.V. infusion line. On no occasion were the animals aware of the procedure. Norepinephrine doses were calculated to give about the same increases in arterial blood pressure as those produced by angiotensin II.

Histological Examination

At the completion of all experiments the chest was opened, and a large cannula was inserted into the left ventricle via the apex. The aorta was then ligated just below the subclavian artery and a large slit was made in the right atrium. First saline and then 10% buffered formalin (1000 ml/min) was perfused into the isolated cephalad circulation of the dog until all blood had been cleared. The brain stem was then removed and placed in a mixture of 30% sucrose in 10% formalin for a minimum of 2 weeks. Serial sections of the medulla were cut at 25-\(\mu\) to 50-\(\mu\) intervals. These were stained with cresyl violet and Luxol fast blue according to the method of Klflver and Barrera. \(^9\) Alternate sections were stained with neutral red and Luxol fast blue.

Statistical Techniques

Statistical comparison between experimental and control mean values was made with Student’s \(t\) test for either paired or unpaired data, as appropriate. \(^10\) In the acute studies involving pharmacological assessment of efferent pathways of the AP response, a one-way analysis of variance for repeated measures on the same subjects was employed. Scheffe’s method \(^11\) was used to calculate the 95% confidence intervals for all contrasts between the three conditions. Differences were considered significant for \(p \leq 0.05\).

Results

Hemodynamic Characteristics of the Pressor Response due to Electrical Stimulation of the AP

Figure 1 illustrates the time course of the changes in mean arterial pressure, heart rate, cardiac output and total peripheral resistance to a 10-second application of a low-current (76 ± 6 \(\mu\)A, 52 ± 3 Hz) electrical stimulus to the AP in six dogs. Onset of the pressor response occurred within 2 seconds after application of the stimulus, and a hypertensive plateau was reached between 6 and 8 seconds after initiation of the stimulus. On the average, mean arterial pressure rose from 129 ± 1 to 161 ± 3 mm Hg \((p < 0.05)\), accompanied by transient increases in heart rate and cardiac output. The peak change in heart rate \((20 ± 6\%)\) and cardiac output \((10 ± 4\%)\) occurred within 2 to 4 seconds after initiation of the stimulus; neither tachycardia nor increased cardiac output were present at the peak of the pressor response. On the other hand, rises in total peripheral resistance \((29 ± 6\%)\) paralleled the increases in mean arterial pressure.

The hemodynamic changes following stimulus offset are also illustrated in figure 1. Within seconds after interruption of the stimulus there was a gradual decrease in mean arterial pressure to below control values, accompanied by bradycardia and decreased total peripheral resistance. The poststimulus response persisted for about 20 to 30 seconds, after which time all variables returned to control levels.

Specificity of the Pressor Response

To ascertain that the pressor responses originated within the anatomical boundaries of the AP, small electrolytic marking lesions were produced at the tip of the electrode for later histological verification. An illustrative example is shown in figure 2. In this example, a maximal pressure increase of 40 mm Hg (stimulus 50 \(\mu\)A, 50 Hz) was obtained 0.2 mm below the surface. At 1 mm below the ependymal surface the pressor response had decreased to 32 mm Hg. No response was obtained when the electrode passed out of the AP and into the dorsal motor nucleus of the vagus. Similar confirmation was made in all other animals.

Autonomic Pathways Involved in the Expression of the Pressor Response due to Electrical Stimulation of the AP

The contribution of the sympathetic and parasympathetic efferent nerve pathways to the cardiovascular changes produced by electrical stimulation of the AP were investigated in nine additional dogs. The data are...
FIGURE 1. Time course of the changes in mean arterial pressure, heart rate, cardiac output and peripheral resistance due to electrical stimulation of the area postrema at 4.5 times threshold. The latter is defined as the amount of current needed to raise mean arterial pressure by 5 mm Hg (17 ± 2 μA). Values are means ± SE of 13 pressor responses in six anesthetized dogs averaged at 2-second intervals. Black area denotes length of stimulation (10 sec). For this group of dogs control values are: 129 ± 7 mm Hg for mean arterial pressure, 78 ± 1 beats/min for heart rate, 1474 ± 17 ml/min for cardiac output and 9.06 ± 0.08 units for total peripheral resistance.

FIGURE 2. Changes in mean arterial pressure due to electrical stimulation (50 μA, 50 Hz) at three selected depths (black and white dots) along an electrode tract passing through the area postrema. Maximal pressor response was obtained at 0.2 mm below the brain-stem surface (first dot); when electrode was lowered 0.8 mm the response decreased to 32 mm Hg (second dot); no response could be obtained at depths below 1.5 mm (bottom dot). Histological section 1 mm anterior to the obex. AP = area postrema; DMV = dorsal motor nucleus of the vagus; NTS = solitary tract nucleus; TS = solitary tract; and 12 = hypoglossal nucleus.
summarized in table 1. To determine whether the onset of transient increases in heart rate was due to withdrawal of parasympathetic tone, the response to AP stimulation was studied before and after unilateral and bilateral vagotomy. Both the peak change in arterial pressure and the increases in heart rate were still present after unilateral or bilateral cervical vagotomy, indicating that withdrawal of vagal tone to the heart did not contribute to the tachycardia. Administration of propranolol at a dose that prevented tachycardia in response to I.V. isoproterenol abolished the increases in heart rate but had no effect on the magnitude of the increase in mean arterial pressure. These results suggested that activation of cardiac sympathetic nerves to the heart mediated the tachycardia associated with the early rise in blood pressure during AP stimulation. However tachycardia itself did not contribute essentially to the peak change in blood pressure.

The failure of either vagotomy or beta-adrenergic blockade to affect significantly the magnitude of the pressor response suggested that activation of peripheral sympathetic vasoconstrictor pathways was responsible for the increased blood pressure. This was confirmed in four other experiments by investigating the magnitude of the pressor response both before and after administration of pentolinium. The effectiveness of sympathetic blockade was demonstrated by abolition of the reflex pressor responses due to bilateral occlusion of the carotid arteries. In bilaterally vagotomized animals, administration of pentolinium completely abolished the elevation in mean arterial pressure produced during electrical stimulation of the AP (table 1).

**Effect of Ablation of the AP on Arterial Blood Pressure, Cardiac Output and Peripheral Resistance**

Continuous measurements of arterial pressure and cardiac output lasting from 1 to 3 hours were obtained in seven awake dogs for 15 to 42 days before ablation of the AP. Similar hemodynamic measurements were obtained in three other dogs before and after sham operation. During hemodynamic monitoring all dogs rested quietly inside a pen in a darkened laboratory and were shielded from ambient disturbances. Under these strictly standardized conditions, pre-lesion group mean arterial pressure averaged $96 \pm 1$ mm Hg, and the dogs displayed relatively low heart rates $(88 \pm 2$ beats/min). Cardiac output averaged $2264 \pm 49$ ml/min, and peripheral resistance was $4.4 \pm 0.1$ units.

Within 1 hour after ablation of the AP and recovery from gas anesthesia, four animals had significant ($p < 0.05$) increases in mean arterial pressure $(40 \pm 1\%)$, tachycardia $(34 \pm 14\%)$ and increased cardiac output $(15 \pm 6\%)$. In one of the seven animals monitored for several hours after ablation of the AP (fig. 3), the increase in cardiac output was present only during the first 3 to 4 hours after AP ablation; thereafter cardiac output decreased while peripheral resistance rose further.

**Table 1. Effects of Bilateral Vagotomy and Sympathetic Blockade on the Pressor Response Due to Electrical Stimulation of the Area Postrema**

<table>
<thead>
<tr>
<th>Table 1. Effects of Bilateral Vagotomy and Sympathetic Blockade on the Pressor Response Due to Electrical Stimulation of the Area Postrema</th>
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<tbody>
<tr>
<td><strong>Mean arterial pressure (mm Hg)</strong></td>
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<tr>
<td>------------------------------------</td>
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<tr>
<td>Before vagotomy</td>
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<tr>
<td>After vagotomy</td>
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<tr>
<td>After propranolol</td>
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<tr>
<td>Before pentolinium</td>
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<td>After pentolinium</td>
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</table>

**Heart rate (beats/min)**

| Before vagotomy | $44 \pm 7$ | $63 \pm 6$ | $19 \pm 6$ |
| After vagotomy  | $151 \pm 10$ | $175 \pm 10$ | $24 \pm 10$ |
| After propranolol | $127 \pm 8$ | $130 \pm 8$ | $3 \pm 1$ |
| Before pentolinium | $153 \pm 13$ | $164 \pm 12$ | $12 \pm 3$ |
| After pentolinium | $113 \pm 8$ | $113 \pm 8$ | $0 \pm 0^*$ |

Values are means ± SE.  
*p < 0.05.

**Figure 3. Average hemodynamic changes obtained in a representative dog during the first 7 hours (shaded area) and 7 days after area postrema ablation. Values are means ± SE during each denoted time interval.**
Figure 4. Average changes in mean arterial pressure, heart rate, cardiac output and peripheral resistance 1 hour (4 dogs), and 1 day (7 dogs) to 5 weeks after bilateral ablation of the area postrema. Values are expressed as percent of each dog’s control values, which for this group of seven dogs averaged 96 ± 1 mm Hg for mean arterial pressure, 88 ± 2 beats/min for heart rate, 2264 ± 49 ml/min for cardiac output and 4.4 ± 0.1 units for peripheral resistance.

Figure 4 summarizes the average percent changes in mean arterial pressure, heart rate, cardiac output and peripheral resistance 1 to 5 weeks after AP ablation. One experiment was terminated 7 days after operation and three others during the third and fifth weeks. Only one lesioned animal died overnight from an undetermined cause; in the three other dogs, experiments were discontinued because of premature failure of the electromagnetic flowmeter. After the initial hypertensive response lasting no more than 4 days there was a tendency for the mean arterial pressure to remain below control values. Slight hypotension was accompanied by tachycardia and reduced cardiac output. Peripheral resistance progressively decreased to control values. None of these changes was present in sham-operated controls.

Changes in Hemodynamic Variability after AP Ablation

Beat-by-beat computer analysis of arterial pressure, heart rate, cardiac output, and peripheral resistance enabled us to characterize in detail the stability of hemodynamic variables before and after ablation of the AP. Histograms for one sham-operated and one AP-ablated dog are shown in figures 5 and 6, respectively. Each distribution curve represents the total accumulation of values obtained during a recording session at the designated time interval.

Examination of figure 5 clearly shows that there were no significant changes in hemodynamic variability after sham operation. At each of the illustrated time periods (control, 1 and 5 weeks after sham operation) the distribution curves for each of the pertinent hemodynamic variables have essentially the same shape and tend to overlap each other. In this sham-operated animal, control pressures were distributed around a mean of 83 ± 3 (se) mm Hg with an average intra-session variability (sd) of ± 7 mm Hg. One month after sham operation, average blood pressure was 85 ± 3 (se) mm Hg and intra-session variability increased to ± 11 mm Hg. After sham operation there was no significant change in the distribution curves of heart rate and cardiac output, both of which are broader than the mean arterial pressure curves and tend to show a bimodal distribution, consistently more pronounced for cardiac output. Bimodal distribution is due to beat-by-beat spontaneous variations in both heart rate and stroke volume, reflecting the presence of sinus respiratory arrhythmia. Mean heart rate averaged 70 ± 3 (se) and 68 ± 2 (se) beats/min before and 1 month after sham operation. During the same time, intra-session variability was ± 16 beats/min during the control and rose to ± 22 beats/min on the fifth week of measurement.

Figure 6 is an overlay of the frequency distribution curves for one lesioned animal. During the control period, average pressures were distributed around a mean of 97 ± 2 (se) mm Hg and fell to 75 ± 4 (se) mm Hg during the fifth week. Average heart rate increased from 76 ± 4 to 90 ± 10 (se) beats/min, while cardiac output was 2371 ± 68 before and 2037 ± 159 (se) ml/min 1 month after ablation of the AP. Compared to the control histograms there is a significant decrease in intra-session variability of arterial pressure, heart rate, cardiac output, and total peripheral resistance as denoted by both increases in peak amplitude and the narrow base of the curves 1 week and 1 month after AP ablation. Compared to control values intra-session variability (sd) of mean arterial pressure and heart rate were reduced by 43 ± 2% and 45 ± 7% (se), and that of cardiac output and peripheral resistance by 59 ± 5% and 43 ± 10% (se), respectively.

Table 2 shows the group average percent changes in intra-session variability 2 to 5 weeks after ablation of the AP. Reduced variability was consistently present for stroke volume, heart rate and cardiac output. The decrease in the variability of mean arterial pressure and peripheral resistance was not statistically significant for the group as a whole.

Changes in the Dose-Response Curve to I.V. Norepinephrine and Angiotensin II after AP Ablation

Pressor doses of angiotensin II and norepinephrine were given before and after ablation of the AP. The
Figure 5. Overlay of frequency distribution curves for mean arterial pressure, heart rate, cardiac output, and peripheral resistance before (control) and after sham operation (1 week, and 1 month).

Figure 6. Examples of the change in the frequency distribution curves for arterial pressure, heart rate, cardiac output, and peripheral resistance 1 week and 1 month after ablation of the area postrema (AP). During Week 1 after AP ablation the average arterial pressure is only once transiently elevated during the recording session 1 day after placement of lesion (rightmost histogram on Week 1 panel). Blood pressure remains decreased below control values 1 month after operation (22 to 35 days). The change in the shape of the histogram denotes a decrease in variability of spontaneous hemodynamic fluctuation. The bimodal distribution of heart rate due to sinus-respiratory arrhythmia disappears after AP ablation.
TABLE 2. Percent Changes in Intra-Session Variability (SD) After Area Postrema Ablation

<table>
<thead>
<tr>
<th>Week</th>
<th>Mean pressure (Δ %)</th>
<th>Stroke volume (Δ %)</th>
<th>Heart rate (Δ %)</th>
<th>Cardiac output (Δ %)</th>
<th>Peripheral resistance (Δ %)</th>
<th>No. of dogs</th>
<th>No. of recording sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2 ± 9</td>
<td>-44 ± 3*</td>
<td>-42 ± 4*</td>
<td>-45 ± 5*</td>
<td>-20 ± 10</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>-28 ± 6*</td>
<td>-50 ± 5*</td>
<td>-47 ± 6*</td>
<td>-58 ± 4*</td>
<td>-40 ± 8*</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>-6 ± 10</td>
<td>-37 ± 7*</td>
<td>-41 ± 8*</td>
<td>-49 ± 6*</td>
<td>-13 ± 9</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>-5 ± 24</td>
<td>-45 ± 7*</td>
<td>-47 ± 4*</td>
<td>-55 ± 5*</td>
<td>-29 ± 8*</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Percent changes ± 1 SEM of intra-session standard deviations for all recording sessions 2 to 5 weeks after ablation of the area postrema.

*p = < 0.05.

Arterial pressure increases attained during injection of angiotensin II (0.5–5 μg) were significantly blunted after ablation of the AP (fig. 7). For an arbitrary 50-mm Hg pressure rise an additional 3 μg of angiotensin was needed after AP ablation to elicit the same increases in blood pressure obtained in the control period. On the other hand, the dose-response curve to norepinephrine was significantly shifted to the left but remained parallel to the control curve. Again, using the arbitrary figure of 50 mm Hg, 13.6 instead of 18.7 μg of norepinephrine was needed after ablation of the AP to elicit the same rise in arterial blood pressure obtained before the lesion. No changes in the dose-response curve to either norepinephrine or angiotensin were observed in the sham-operated controls (fig. 7).

Histological Verification of Lesions

Figures 8a and 8b illustrate cross sections of the medulla from one sham-operated dog and one lesioned animal. In both cases detailed examination of each brain stem confirmed complete ablation of the AP. However, lesions were usually accompanied by some gliosis and other degenerative changes in adjacent structures (nucleus gracilis, medial nucleus tractus solitarii, dorsal motor nucleus of the vagus and rarely hypoglossal nucleus), but both the location and the extent of damage of neighboring structures did not correlate with the magnitude of the hemodynamic changes.

Discussion

Previous experiments in the dog have implicated the AP as a site mediating the central cardiovascular effects due to infusion of angiotensin II into the vertebral artery.11,12 While there has now been significant progress in deciphering the multiple effects and sites of action of angiotensin in other circumventricular organs,14,16 further investigation of the contribution of the AP to the control of cardiovascular function has lain dormant. This is surprising since it was the discovery of the action of angiotensin on the AP that first renewed an interest in the central neurogenic actions of this hormone.13,18 The evidence
collected in both acute and chronic experiments separately and together indicates that the AP may be active in the central regulatory mechanisms of cardiovascular control.

Both the increases in mean arterial pressure and the hemodynamic characteristics of the onset and offset transients obtained during monopolar low-intensity stimulation of the AP revealed that this structure contains a previously unrecognized neural pressor pathway mediating an increase of central sympathetic vasomotor activity. This was further corroborated by the combined physiological and pharmacological dissection of the efferent pathways of the cardiovascular response to AP stimulation. These experiments revealed that facilitation of cardiac accelerator and peripheral sympathetic vasoconstrictor nerves is the predominant feature of the cardiovascular response to AP stimulation, since bilateral vagotomy had no effect on the response. Since blockade of cardiac sympathetic chronotropic activity did not attenuate the magnitude of the increases in blood pressure, activation of peripheral sympathetic vasoconstrictor pathways appears to be essential for the expression of the pressor response. Disappearance of the pressor response after sympathetic ganglionic blockade confirmed this possibility.

It is of interest to note that both the hemodynamic characteristics and efferent pathways of the pressor response produced by electrical stimulation of the AP were essentially the same as those reported for the action of angiotensin in the vertebral artery territory of the morphine-chloralose anesthetized dog. In previous experiments, Ferrario et al. determined that the cardiovascular response due to infusion of small quantities of angiotensin II into the vertebral arteries of the mongrel dog was due to vasoconstriction, as indicated by increases in mean sympathetic nerve activity in fiber twigs of the splanchnic nerve. The pressor response could also be abolished by either high spinal cord transection (C-2) or administration of bretylium tosylate. Although we are not committing ourselves to the concept that chemical stimulation of the AP with angiotensin II is equivalent to stimulation of the same area electrically, the similarity of the effects produced by the two different kinds of stimuli is remarkable. On the other hand, in dealing with sensory receptor mechanisms it is common knowledge that the effects of the natural stimuli can be mimicked electrically. For example, variations in endosinus pressure as a natural stimulus to study the characteristics of the baroreceptor reflex can be replaced equally well by electrical stimulation of the central end of the carotid sinus nerve. Therefore, the similarity of the effects obtained with either chemical or electrical stimulation of the AP suggests, but obviously does not prove, the existence of a receptor mechanism in the AP. This possibility is also compatible with the kind of neuronal arrangement described by Chernicky et al. in the dog AP.

In animals with inactivated AP there were strong indications that removal of this structure produces a significant selective decrease in natural hemodynamic lability, despite the fact that the procedure did not cause large changes in resting hemodynamics. Intraindividual hemodynamic variability was characterized in awake dogs as suggested by Ito and Scher by the magnitude of the standard deviation around the mean value of the corresponding variable. Since trained dogs were accustomed to rest quietly throughout the length of each recording session, unremitting spontaneous hemodynamic fluctuations contributing to the magnitude of the standard deviations were in the most part produced by sinus respiratory arrhythmia. The decrease in natural hemodynamic variability after AP ablation, most pronounced for heart rate and cardiac output, was due in part to disappearance of sinus respiratory arrhythmia. In other words, inactivation of the area postrema produced an increase in the regularity of cardiac rate, which is reflected as a decreased variability of heart rate and cardiac output.
and a narrowing of the histogram. The increased regularity of the cardiac rhythm was not solely due to the presence of tachycardia. The phenomenon appears to be more complex in nature, since decreased variability was found even in those animals that did not show tachycardia consistently after AP ablation.

An increase in hemodynamic lability, notably of arterial blood pressure, follows procedures that affect the baroreceptor reflex. The present findings of decreased hemodynamic variability after AP ablation may be construed as evidence for the existence of additional mechanisms involved in the regulation of resting cardiovascular stability. In this respect the AP may be part of an integrative mechanism acting to modulate the sensitivity of the central baroreceptor synapse to small and otherwise normal fluctuations in cardiac rhythm and arterial pressure. Fukiyama suggested that angiotensin may modulate baroreceptor impulse traffic in the region of the nucleus tractus solitarius (NTS). Reis et al. have shown that the catecholamine innervation of NTS seems to modulate baroreceptor reflexes. They showed that selective destruction of the noradrenergic input of NTS in the rat results in the development of persistent blood pressure lability without changes in its average level. It is notable that the NTS is richly innervated by terminals of catecholamine-containing neurons from cell groups arising elsewhere, particularly the A5 and A1 areas. Torack et al. have further shown that in the rat the AP contains numerous noradrenergic fibers from cell bodies located on the lateral (A5) and ventral surface of the structure. Detailed examination of the structure and synaptic arrangement of neurons in the rat AP led Torack et al. to conclude that the neural architecture of the AP is consistent with a chemosensing function and with a transfer of neurogenic stimuli to the medulla. Therefore, we believe that the reduced lability after AP ablation could indicate that the receptor pathway in the AP may act to oppose functionally the restraining influences of the NTS inhibitory neurons.

The changes in resting hemodynamic values recorded in trained, conscious dogs after selective destruction of the AP also suggest that this structure plays a tonic role. The slight fall in average blood pressure observed 2 to 5 weeks after ablation of the AP was accompanied by moderate tachycardia and a tendency for both cardiac output and peripheral resistance to be reduced with respect to control values. The mechanism underlying these hemodynamic changes is not yet known, but it may depend largely on depressed activity of the central sympathetic nervous system. The increased pressor responsiveness to injected norepinephrine favors this possibility.

The effects of AP ablation have been studied previously in the rat but not the dog. Zandberg et al. observed no significant differences in blood pressure, heart rate and body weight of rats with and without an excised AP. Ylitalo et al. observed the presence of chronic labile hypertension after thermocoagulation of the AP in the rat. We do not agree with Ylitalo's findings, since the area postrema appears to contain a pressor pathway; therefore, abolition of the pressor influence should produce, as demonstrated in our experiments, a decrease rather than an increase in blood pressure. It is possible that in their study the lesion extended to the NTS and produced a kind of hypertension similar to that described after NTS destruction. We would agree with Zandberg et al. that the anatomical proximity of the AP to the nucleus tractus solitarii makes it difficult to ablate one area without causing damage to the other one. We attempted to avoid this possibility and were reasonably successful in not causing obvious histological damage to the NTS, but it is still possible that destruction of the connections between these two areas affected the structural integrity of the NTS. With regard to the fact that Zandberg et al. did not find any significant change in blood pressure after AP ablation, the method used by the authors to monitor blood pressure in the rat (tail-cuff) is relatively insensitive to the small changes in blood pressure that we have observed. Finally, it should be emphasized, as reviewed by Brody et al., that unlike the AP in the dog, the AP of the rat is not involved in the central pressor action of angiotensin; a species difference that could well extend to the role of this region in regulation of cardiovascular activity.

The blood pressure responses to I.V. administration of vasoconstrictor doses of angiotensin were significantly reduced after AP ablation. These findings provide additional evidence that the pressor response to systemic administration of angiotensin in normal animals is in part mediated by central neurogenic mechanisms. The associated increase in the magnitude of the pressor response to vasoconstrictor doses of norepinephrine excluded the possibility that blunting of the pressor response to angiotensin II was due to depressed baroreceptor function.

In summary, the present study demonstrates the effects of either electrical stimulation or ablation of the area postrema on the cardiovascular function of the dog. The hemodynamic changes following inactivation of the AP appear to result from removal of a system facilitating central sympathetic efferent activity. We advance the hypothesis that under normal conditions the bulbar cardiovascular neurons may be under the influence of reciprocal activity from a facilitative (area postrema) and inhibitory (nucleus tractus solitarii) system. After inactivation of the facilitative system there may be an increase in inhibitory activity, which results in a decrease in tonic central sympathetic efferent vasomotor discharges.

**References**

Physiological and pharmacological characterization of the area postrema pressor pathways in the normal dog.

C M Ferrario, K L Barnes, J E Szilagyi and K B Brosnihan

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