Comparison of the Hemodynamic Changes Produced by Electrical Stimulation of the Area Postrema and Nucleus Tractus Solitarii in the 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 further its possible role in the central control of blood pressure, stainless steel electrodes were lowered stereotaxically into the AP of morphine-chloralose-anesthetized dogs after surgical exposure of the walls of the 4th ventricle just anterior to the obex. In all experiments, large pressor responses were obtained at a relatively low stimulus strength (range: 20-80 μA, 20-60 Hz); the increases in pressure (average: 30 ± 4 mm Hg) were rapid in onset and sustained for the 10- to 20-second duration of the stimulus. Hemodynamically, the pressor response during AP stimulation was due to increases in both cardiac output (+211 ± 37 ml/min) and peripheral resistance (+0.81 ± 0.33 U). An increase in heart rate contributed to the onset but not the plateau of the pressor response. Reconstruction of electrode tracts in all experiments corroborated that these pressor responses originated in the AP. The specificity of these cardiovascular responses was confirmed further by repeating the same kind of stimuli with electrodes placed in the nucleus tractus solitarii (NTS). In contrast to the effects obtained during AP stimulation, bradycardia (-41 ± 6 beats/min) and hypotension (-29 ± 5 mm Hg) were characteristic features. The fall in blood pressure during NTS stimulation was secondary to the pronounced bradycardia and decreased cardiac output. The data suggest that the AP is part of a previously unrecognized pathway which is distinct from the primary baroreflex pathway with relays in the adjacent NTS.

THE MULTIPLICITY of actions of angiotensin II has led several investigators to explore the possibility that this hormone could have an effect within the central nervous system. This idea, first suggested by Bickerton and Buckley in 1961, lay dormant until recent years when Scroop and Lowe (1968) and Ferrario et al. (1970) showed that the infusion of angiotensin into the circulation of the dog's brain raised arterial blood pressure by a mechanism not involving direct vascular smooth muscle constriction. Since previous experiments (Ferrario et al., 1972; Gildenberg et al., 1973; Joy and Lowe, 1970) showed that the pressor effects of angiotensin in the medulla required the integrity of the area postrema (AP), a more detailed investigation of this structure was warranted. Important initial steps in this direction were: (1) to determine whether the facilitative action of angiotensin on vasomotor centers could be reproduced by means of electrical rather than chemical stimulation; and (2) to compare these hemodynamic effects with those obtained during the application of similar stimuli to the nucleus tractus solitarii (NTS), an area also involved in the central regulation of blood pressure (Doba and Reis, 1973; Nathan and Reis, 1977).

Methods

Animal Preparation

Electromagnetic flowmeters (Micron Instruments) were placed around the ascending aorta of 13 mongrel dogs (19-22 kg body weight) 1 week prior to the experiments, using a sterile technique (Ferrario et al., 1969). After anesthesia with morphine (2 mg/kg im) and chloralose (60 mg/kg, iv), catheters were placed in a femoral artery to monitor arterial pressure with a strain gauge pressure trans-
duser (Statham, P23Db) and in a femoral vein for the infusion of drugs. Body temperature was maintained at 37°C with a heating pad. The dorsal part of the brainstem was reached by fixing the dog's head in a stereotaxic head-holder with the nose tipped downward 45°, minimizing the retraction of the cerebellum necessary to expose the AP on the walls of the caudal 4th ventricle. After infiltration of the skin and deep tissues with procaine to minimize neck muscle twitches produced by cautery stimulation of muscle nerves, a midline incision was made from the crest of the occiput to the C2 region, and muscles were dissected and retracted to expose the foramen magnum. Removal of a small portion of the bone overlying the cerebellum allowed visualization of the caudal part of the 4th ventricle. With the aid of a dissecting microscope the pia was opened and, when necessary, blood vessels restricting access to the 4th ventricle were coagulated electrically.

Electrode Construction and Stimulation Procedures

In 11 of 13 dogs, monopolar stimulating electrodes mounted in a micromanipulator were inserted under visual guidance into the AP. Electrical stimulation of the NTS was performed in six of these 11 dogs after completion of the AP study and in two others that had no prior manipulation of the AP. The NTS was approached using stereotaxic coordinates determined in previous experiments on 13 other dogs. Briefly, lateral coordinates for the NTS in the vicinity of the obex (±2 mm) were determined from three histological sections cut 1.0 mm rostral, 1.0 mm caudal, and at the obex (0.0). The distance between midline and the solitary tract was measured in each of these sections and the values corrected for shrinkage of tissue during fixation. The average lateral coordinates were 1.75 ± 0.06 mm (1.0 mm rostral to obex), 1.49 ± 0.03 mm (0.0 at obex), and 1.21 ± 0.03 mm (1.0 mm caudal to obex). These measurements allowed us to position the electrode precisely within the nucleus despite variations in size of the dogs.

Monopolar electrodes were made of Teflon-coated stainless steel wire (Medwire Corp.) cut flat at the tip. The total cross-sectional diameter at the electrode tip was 0.127 mm (o.d.), with the diameter of the conducting wire being 0.076 mm. For support, the electrode was housed within 30-gauge stainless steel hypodermic tubing (i.d.: 0.152 mm) with only a 0.25-mm length protruding beyond the end. By cutting the wire straight across without stripping the Teflon insulation, it was possible to stimulate very small areas, thereby minimizing spread of current to surrounding regions. The reference electrode was a large alligator clip placed on the muscles of the neck.

Electrical stimuli were cathodal monophasic rectangular pulses of 0.2 msec in duration and constant current with 10- to 20-second train duration. The basic stimulus frequency ranged from 20 to 50 Hz. These stimuli were delivered from a Pulsar 6b generator through an RF stimulus isolation unit (Frederick Haer Co.).

With stimulus current set between 50 and 80 μA, the electrode was lowered from the surface of the brainstem in 0.1-mm increments. When a maximal response characteristic of the region being studied was obtained, threshold was determined as the amount of current required to elicit a change in mean arterial pressure equal to or greater than 5 mm Hg for 10-second duration. Then both stimulus frequency (range: 2–200 Hz) and current (10–200 μA) were plotted vs. the change in arterial pressure.

Small marking lesions, less than 200 μm in diameter (500 μA, 500 Hz or DC, 5- to 10-second duration), were placed at both the initial and final sites of stimulation for later histological reconstructions of the electrode tract. At the conclusion of each study, the brainstem was removed quickly and stored in 10% formalin for at least 2 weeks. Frozen sections were cut at 25-μm intervals and stained with cresyl violet and Luxol fast blue by the Kliver-Barrera (1953) technique. The length of the electrode tract was measured by determining the distance between the two marking lesions. The precise location of each stimulated site was determined by interpolation on serial drawings of the brainstem sections through which the tract passed.

Collection of Hemodynamic Data

Hemodynamic variables were recorded and displayed on a Brush (Gould Inc.) eight-channel recorder. Measurements included aortic blood flow, mean and pulsatile arterial blood pressure (P23Db, Gould-Statham, Inc.), heart rate, and stroke volume. Cardiac output was computed as the product of stroke volume and heart rate. The circuitry for analog computation of these variables, as used in our laboratory, has been described elsewhere (Ferrario et al., 1969). Peripheral resistance was calculated arithmetically as mean aortic pressure times 100 divided by cardiac output. Control values were always taken during the minute preceding stimulus onset, and the effects of stimulation were measured as the steady value of each hemodynamic variable just before termination of stimulation.

Statistics

The two-tailed Student's t-test for paired variates was employed for statistical analysis (Grimm, 1973).

Results

Cardiovascular Effects of Electrical Stimulation of the AP

The characteristic hemodynamic response to the application of low current electrical stimuli to the AP consisted of a rapid increase in arterial pressure,
Usually associated with modest tachycardia (Table 1). The rises in blood pressure began within 2 seconds after onset of stimulation and reached a plateau before 10 seconds had elapsed from the initiation of the stimulus. In 11 dogs the amount of current needed to raise mean blood pressure by 5 mm Hg (threshold current) averaged 17 ± 2 μA (range: 5–30 μA). Stimulation at four times threshold increased the mean arterial pressure by 30 ± 4 mm Hg (P < 0.001).

Even though the pressor response was graded (Fig. 1) and reproducible from dog to dog, there was some variability in the relative magnitude of the changes in flow and resistance among the 11 dogs.
and a greater rise in total peripheral resistance (+4.1 and +2.6 U). The reason for this contrasting change in cardiac output was not obvious; by marking lesions at the site where these pressor responses were obtained, we confirmed that the electrode tip was within the anatomical boundaries of the AP. When compared to the other nine dogs in which the pressor response was associated with tachycardia and increased cardiac output, no differences were noted in the resting values or the magnitude of the evoked pressor response.

After stimulus offset, the fall of blood pressure to control values followed a distinct pattern, suggesting that during AP stimulation peripheral baroreceptors were being impeded from opposing the rises in arterial blood pressure (Fig. 1). Both transient hypotension and bradycardia followed interruption of electrical stimulation, with the variables progressively returning thereafter to control values.

**Hemodynamic Effects of Electrical Stimulation of the NTS**

The hemodynamic response pattern characterizing electrical stimulation of the solitary tract nucleus at four times a threshold current of 13 ± 2 μA is illustrated in Figure 2 and Table 1. Mean arterial pressure fell with a latency of 2-3 seconds due to the abrupt development of bradycardia (Table 1). The decrease in heart rate began within the first second after starting stimulation and continued throughout the duration of the stimulus. Although stroke volume increased at first, presumably to compensate for the abrupt bradycardia, it returned to control values before stimulus offset, whereas heart rate and arterial pressure continued to be depressed. Both the bradycardia and the depressor response persisted after stimulus cessation, with arterial pressure often reaching its nadir several seconds after offset, lagging the profound bradycardia.

In two additional experiments the NTS was stimulated without prior study of the AP. The magnitude and characteristics of the depressor responses were identical to those from the other six dogs. In both, the decrease in blood pressure (-23 and -32 mm Hg, respectively) was associated with bradycardia (-53 and -21 beats/min) and a fall in cardiac output (-470 and -563 ml/min). These findings excluded the possibility that prior manipulation of the AP affected the responses obtained in the NTS.

**Stimulus Frequency Analysis of AP and NTS Responses**

In four of the 11 dogs undergoing AP stimulation and three of the eight dogs undergoing NTS stimulation, the stimulus frequency was varied between 10 and 200 Hz with current held constant at four times threshold (AP: 75 ± 3 μA; NTS: 60 ± 8 μA). Figure 3 relates the magnitude of the change in mean arterial pressure to the frequency of stimulation. At frequencies between 2 and 200 Hz, AP stimulation always caused pressor responses, whereas the effects of NTS stimulation were depressor. Although the directions of the change in blood pressure opposed each other, both curves demonstrate a peak change in blood pressure oc-
Figure 3  Dependency of the changes in blood pressure (mm Hg) on frequency of stimulation (Hz) plotted on a logarithmic scale. Current held constant at four times threshold (AP = 75 ± 3 μA; NTS = 60 ± 8 μA). Amplitude of the AP pressor responses (circles, n = 4 dogs) increased to a maximum of 28 mm Hg at 50 Hz. The depressor response produced by NTS stimulation (squares, n = 3 dogs) also reached its maximum of −27 mm Hg at 50 Hz. Values are means ± SE.

curring at about 50 Hz, with the magnitude of the blood pressure change falling sharply at higher stimulus frequencies.

Anatomical Verification of the Effects Obtained in the AP and NTS Regions

Figure 4 illustrates the changes in the magnitude of the pressor responses during constant stimulation (50 μA, 50 Hz) as the electrode was lowered through the AP into underlying tissue. In this representative experiment a maximal pressure increase of 60 mm Hg was obtained 0.3 mm below the surface of the brainstem. The large pressor response was maintained as the electrode was lowered another 0.5 mm; at this point the height of the pressor response was 56 mm Hg. At 1.3 mm below brainstem surface, the magnitude of the peak change in blood pressure decreased to 34 mm Hg, disappearing completely when the electrode tip entered the region of the dorsal motor nucleus of the vagus (2 mm below the brainstem surface). At this level, contiguous to but outside of the AP, arterial pressure did not change from baseline even when the current was increased to about six times threshold (100 μA).

To document the rostrocaudal and mediolateral extent of the pressor responses produced by electrical stimulation of the AP, marking lesions were mapped on a diagram of the dorsal surface of the brainstem (Fig. 5a). Figure 5a demonstrates that pressor responses were obtained only when the electrode was within the AP. In contrast, stimulation of sites no more than 1 mm away from the AP boundary did not raise blood pressure. Figure 5b documents the vertical and mediolateral extent of the AP pressor response. All stimulation points within 1.0 and 3.0 mm anterior to the obex in 11 dogs are plotted on a representative cross-section of the brainstem at 2 mm anterior to the obex, using the same symbols. Again, the pressor responses could be obtained only from the AP region; stimulation of a point less than 1 mm lateral or ventral to the AP did not evoke a pressor response. These data indicate that the pressor responses originated within the anatomical boundaries of the AP and that similar effects could not be elicited when the electrode tip was in contact with neighboring structures.

Further validation of the specificity of the pressor effects evoked during electrical stimulation of the AP was obtained by repeating the stimulus after an electrolytic lesion (500 μA DC) had been made around the tip of the electrode. In three dogs the average initial pressor response, produced 0.4 ± 0.2 mm below the ventricular surface border of the AP, averaged 59 ± 15 mm Hg at 50 μA, 50 Hz before lesioning. These rises in blood pressure were abolished when the site was restimulated after creation of the lesion which, on histological examination, was found to lie within the AP boundaries. When the electrode was lowered 0.5 mm deeper into the intact AP in one dog, a pressor response of 56 mm Hg reappeared. Abolition of the pressor response by localized destruction of AP tissue at the electrode tip confirmed that the reactive points were restricted to the AP.

Figure 6 displays the anatomical reconstruction of an electrode tract passing through the NTS, with stimulation held constant at 50 μA and 50 Hz. Variable responses were seen until the electrode reached a point 1.3 mm below the surface. At this level, blood pressure fell by 16 mm Hg and there was bradycardia (~15 beats/min). At a depth of 1.8 mm, a maximal decrease in pressure of 38 mm Hg accompanying a drop in heart rate of 30 beats/min was obtained. At 2.3 mm, the response diminished to 12 mm Hg, and below 2.8 mm no depressor response could be evoked. The histological reconstruction of this tract revealed that, at the last site
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**Figure 4** Changes in mean arterial pressure elicited by electrical stimulation (50 μA, 50 Hz) at four selected depths (black dots) along an electrode tract passing through the AP. Maximal pressor responses were obtained between 0.3 and 0.8 mm below brainstem surface (first and second dots); no pressor response could be obtained when the electrode was outside the AP (bottom dot). Histological section 1 mm anterior to obex. DMV = dorsal motor nucleus of the vagus, ION = inferior olivary nucleus, PT = pyramidal tract, TS = solitary tract, 5ST = trigeminal spinal tract, 12 = hypoglossal nucleus.

**Figure 5** Composite diagram giving the location of stimulated sites in the brainstem of 11 dogs. (a) Diagram of the dorsal surface of the dog brainstem. Point yielding pressor response marked by solid dots, and sites producing either no change in blood pressure or depressor responses are marked by open circles. Zero on the vertical scale is at the obex. (b) Cross-section of the dog brainstem 2.0 mm anterior to obex. All stimulated sites between 1.0 and 3.0 mm anterior to the obex have been plotted on this section. Same symbols as (a). In both (a) and (b), pressor points are confined to the AP, whereas nonreactive and depressor sites lie outside the AP.

Discussion

The rapidity of the rise in blood pressure and the shape of the dose-response curve during infusion of very small doses of angiotensin in the vertebral artery circulation of the anesthetized dog led Ferrario et al., (1970) to conclude that these cardiovascular effects resulted from an action of the peptide on a medullary receptor site facilitating sympathetic activity. The present experiments have confirmed this possibility by demonstrating directly that the dog's AP is the site of a previously unrecognized neural pressor pathway. On every occasion, large pressor responses with hemodynamic characteristics similar to those obtained with intravertebral angiotensin II (Ferrario et al., 1972) could be elicited during electrical stimulation of the AP. By using currents of low intensity and monopolar electrodes with minute exposure at the tip, we were able to localize pressor points precisely and map the extent of the responsive sites by repeating the stimulus along the rostrocaudal and dorsoventral anatomical boundaries of the AP. Histological verification and mapping of all sites stimulated showed that the pressor responses were elicited from stimuli applied throughout but not outside the anatomical borders of the AP. When the tip of the electrode was positioned in immediately adjacent areas outside the AP, blood pressure either did not change or it decreased.

Since various investigators (Ranson and Billing-
FIGURE 6 Changes in mean arterial pressure elicited by electrical stimulation (50 μA, 50 Hz) at four selected depths (black dots) along an electrode tract passing through the NTS at the level of the obex. Maximal depressor response obtained 1.8 mm below the brainstem surface (second dot from top). Below 1.8 mm, the depressor response progressively diminished and disappeared by 2.3 mm. Below this level, as the electrode entered the reticular formation (fourth dot), a small, delayed pressor effect was seen. Abbreviations as in Figure 4.
the pathways mediating these pressor responses remain to be identified, attention is drawn to the possibility that the AP and NTS regions participate in an integrative mechanism modulating outflow of autonomic activity to the periphery. It is also pertinent to note that modulation by angiotensin of the inhibitory activity of the first central baroreceptor synapse at the level of the NTS was previously suggested by Ferrario and McCubbin (1974) as a most likely explanation for the central actions of angiotensin in the AP. Fukiyama (1972) has provided some additional support for this possibility. On the other hand, Aars (1977) believes that the central effects of angiotensin result from the excitation of baro-independent sympathetic vasomotor neurons. The AP contains clusters of small nerve cells, a highly specialized dense, fenestrated capillary network, modified ependymal cells called tanyocytes and specialized astrocytes (Klara and Brizzee, 1977). Both its structure and its location idealize it as an interfacing site between the cerebrospinal fluid, the intravascular component, and the extracellular space of the brain. Because of its unique structure and position within the medulla oblongata, receptors in the AP could act as sensors of circulating hormones, thereby providing one arm of a renal-neurohormonal link concerned with the central regulation of blood pressure (Ferrario et al., 1972; Ferrario and McCubbin, 1974).

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