Localization of the Anterior Hypothalamic Angiotensin II Pressor System

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SUMMARY Previous studies by this laboratory have shown that an electrolytic lesion of tissue surrounding the anterorentral third cerebral ventricle (AV3V) produces pressor deficits to both intravenously (i.v.) and intracerebroventricularly (i.c.v.) administered angiotensin II (All). These studies were designed to identify the neural substrates critical to the central All pressor response. The All pressor system was mapped employing a spectrum of overlapping electrolytic lesions within the medial preoptic-anterior hypothalamic area. The effect of each lesion on the pressor response to All (i.c.v.) was tested in each animal, which was then grouped as a responder (R) or nonresponder (NR). The extent of damage produced by lesions that abolished the All response was mapped. Bilateral destruction of tissue along the lamina terminalis (LT) either below or at the level of the anterior commissure eliminated the All pressor response as did destruction of tissue near the margin of the preoptic and anterior hypothalamic nuclei. These data suggested that an AH pressor pathway originating in the ventral AV3V region ascends along the LT to the level of the anterior commissure and then descends through the anterior hypothalamus. The path of the descending projection through the anterior hypothalamus was ascertained by making a series of horizontal knife cuts. Transections were found that effectively eliminated the central All pressor response without impinging upon the LT. It is concluded that the anterior hypothalamus contains an efferent pathway from the AV3V region associated with the central All pressor response. (Hypertension 4 (suppl II): II-159-II-165, 1982)

KEY WORDS • angiotensin II • anterior hypothalamus • anteroventral third ventricle (AV3V) • hypertension • central nervous system

VENTRICULAR plugging1 and electrolytic lesioning studies2-4 have shown that the anteroventral third cerebral ventricle (AV3V) region is involved in the responses to centrally and peripherally administered angiotensin II (All). It thus appears that the area contains neural substrates responsive to All which promote an increase in blood pressure. In distinction to the sites involved in the pressor response to centrally administered All, the area postrema in the dog and other species5 and the subfornical organ in the rat6 have been demonstrated to respond to blood-borne All but not cerebrospinal fluid (CSF)-borne All. The present study focuses on the pathway involved in the increase in blood pressure elicited by intracerebroventricular All.

There appear to be two mechanisms for the pressor response. In addition to neurogenically-mediated vasoconstriction, All (i.c.v.) stimulates release of vasopressin.7 Injection of as little as 50 fg of All into the ventricle at the optic recess adjacent to the organum vasculosum of the lamina terminalis (OVLT) causes an increase in blood pressure and an increased release of antidiuretic hormone (ADH).8,9 If the anterior third ventricle is plugged with cold cream, centrally delivered All produces no pressor response.1 Additional evidence that the AV3V region contains neural substrates for the All pressor response is provided by electrolytic lesion experiments that produce deficits in the response to All. AV3V lesions eliminate the pressor response to i.c.v. All5 and attenuate the response to systemic All.1 The release of vasopressin in response to central administration of All is blunted in AV3V lesion rats.5,10 Specific binding of radiolabelled-AII to receptors in the AV3V region has been demonstrated by autoradiography. The All antagonist, saralasin, effectively competed for the same sites.11 Excitation of cells in the

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The pressor response to centrally administered All was blocked by an electrolytic lesion in the ventromedial hypothalamic-median eminence (VMH-ME) region. Also, the VMH-ME lesion significantly attenuated vasoconstriction in the mesenteric and renal vasculatures observed during electrical stimulation of the AV3V region. These results suggested that the VMH-ME lesion eliminates both the vasopressin-related and sympathetic components of the central All pressor response.

The present studies were designed to map functionally the preoptic-anterior hypothalamic route of an All pressor pathway from the AV3V region. The results suggest that a pathway critical for the central All pressor response projects dorsally along the lamina terminalis (LT) and then descends through the anterior hypothalamus.

Methods
Preparation for Sterotaxic Surgery

Male Sprague-Dawley rats (300-500 g) were anesthetized with pentobarbital (35 mg/kg, i.p.) and treated with atropine (1.3 mg/kg, i.p.) to reduce secretions in the respiratory tract. Each animal was placed in a Kopf stereotaxic apparatus. The skull was exposed and then leveled between lambda and bregma. A 3 mm diameter hole was drilled through the skull, and the superior sagittal sinus was deflected to one side.

Electrolytic Lesions

The lesion electrode was fashioned from a 3 cm length of 25-gauge insulated nichrome wire with the insulation removed to expose 0.3 mm of the tip. The cathode of the lesion maker (Grass constant current lesion maker, Model DC, LM5) was inserted rectally to the shaft of the knife produced a horizontal semicircular cut of reproducible radius. The clamp was tightened so that the shaft of the knife could be visualized while it was in position in the brain. The shaft of the knife was held in place on the stereotaxic apparatus by means of a square clamp. The clamp was tightened so that the shaft of the knife was unable to slide vertically but it could be rotated by moving the handle of the knife in the horizontal plane. The knife was positioned with its shaft 0.3 mm caudal to bregma. The blade was centered on or near the midline and projected posteriorly from the shaft. The knife was then lowered to various coordinates ranging from -5.8 to -7.9 mm from the dura. Once in position the knife was rotated 90° to the right and then 90° to the left from midline. The knife was removed from the brain along its pathway of descent. Rotation of the knife produced a horizontal semicircular cut of reproducible radius.

Since the blade was rigid, it also produced a vertical cut 1.25 mm in length down the midline as it was lowered into the anterior hypothalamus. Two control experiments were performed to check the effects on central All pressor responsiveness due to damage in this midline area. In the first group, the knife was lowered only 5.5 mm from the dura. This was the limit of vertical descent for control knife cuts since studies using electrolytic lesions suggested that midline tissue ventral to this depth was critical for All pressor responses. This brought the knife through midline structures dorsal to and sometimes including the subfornical organ. In a second group, knife cuts were done similarly to the midline knife cuts except that a 2.5 mm knife was lowered 1 mm off midline in an anteroposterior orientation. Once in position, the blade was orientated sideways 90° so that the blade would span 1.25 mm on each side of the third ventricle. The knife was then moved in a posterior direction 1.25 mm to produce a horizontal knife cut. The knife was returned to its anterior location and the blade extruded through the initial path of descent.

Lateral Ventricle Cannulation

Cannulas were implanted in the lateral ventricle at stereotaxic coordinates: -0.8 mm from bregma, +1.1 mm from midline and -6.0 mm from the top of the skull.

Histological Procedures

After completion of an experiment, the animals were anesthetized and the patency of the lateral ventricle cannula was determined by injecting 2 μl of a 1:2 dilution of a saturated solution of Pontamine sky blue dye into the cannula. Immediately following injection of the dye, the animal was perfused intracardially with isotonic saline followed by 10% formalin. Patency of
the cannula was determined by the presence or absence of blue dye in the AV3V region.

The brain was frozen and then sectioned into 40 μ slices using a Leitz microtome apparatus with a remote Histofreeze connected to the microtome stage. Brains with midline lesions were cut in horizontal section. Alternate slices from the most ventral third ventricle at the base of the brain to the level of the subfor- nical organ in the septal region were examined. If brains were sliced in coronal section, alternate slices were examined throughout the extent of the knife cut. Each slice was numbered so that distances between slices could be determined for mapping purposes. The tissues were then stained for Nissl substances with cresyl violet.

Mapping Procedure for Lesions

Each set of brain sections was examined under the light microscope. Tissue sections were selected which corresponded to four different horizontal planes through the AV3V region. The first plane was at the level of the ventral portion of the anterior com- missure. The other three planes were −760 μ (at the level of the paraventricular nuclei), −1200 μ (at the level of the OVLT), and −1840 μ (at the level of the optic tract) from the first plane.

Normal tissue sections representative of these four planes were projected and outlined on tracing paper. These sketches were used as templates for mapping the extent of damage. Appropriate sections corresponding to these same four planes were selected from each lesion brain and projected at the same magnification. A profile of the section and the extent of the damage was drawn on the paper.

Overlap analysis for damage at these four levels in the AV3V area was performed. First, a normal rat brain was profiled at each of the four planes. This template (on tracing paper) was placed on top of each lesion animal profile, fitted to various landmarks along the anterior midline, and the area of lesion damage traced onto the template. This same procedure was repeated for each lesion animal at each of the four horizontal planes.

Mapping Procedure for Knife Cuts

Histological analysis was performed similarly to the lesion brains except that brains with horizontal knife cuts were cut in coronal section. The extent of each knife cut was mapped. Its position relative to the anterior commissure and the base of the brain in a dorsal-ventral plane at the level of the paraventricular nuclei was determined. The distance of the onset of damage from a vertical plane just posterior to the anterior commissure was also determined. The caudal extent of the cut was judged by the distance of the most caudal damage from a vertical plane through the most anterior section of the paraventricular nuclei. In this way, a two-dimensional sagittal reconstruction of the relative position of several knife cuts was mapped.

Central AII Pressor Response

Each animal was instrumented with a carotid catheter (PE-50) for arterial pressure determination (Ailtech pressure transducer) 24–28 hours before the experiment. A 10 μ Hamilton syringe filled with AII (100 μg/ml) was attached to a lateral ventricle injector with a length of PE-10 tubing. The injector (30 gauge stainless steel hypodermic tubing) was fashioned so that its tip would extend approximately 0.2 mm beyond the intracranial tip of the lateral ventricle can- nula. Once the injector was in place, a 2 μl injection from the syringe delivered 200 ng AII in a 2 μl volume into the lateral ventricle. Each animal was tested 3–5 times with 200 ng of AII. The maximum pressor responses obtained from animals during the first 2 minutes after each injection were recorded and averaged. Control vehicle injections of 2 μl isotonic saline were injected similarly. These injections did not alter blood pressure.

Intact animals typically responded to 200 ng AII (i.c.v) with mean arterial blood pressure increases ranging from 10–35 mm Hg. An operated animal was defined as a NR only if it responded to this dose of AII with a 0–5 mm Hg increase.

Results

Lesion Mapping

Maps of eight lesions representative of the range and extent of 44 lesions that eliminated the pressor response to central AII are shown in figures 1 and 2. Lesion A and Lesion B (fig. 1) were very large lesions that covered extensive areas in each of the four panels. Lesion C and Lesion D were smaller lesions that appear in only Panels 2 and 3. Overlap analysis revealed areas common to all four lesions in Panels 2 and 3 in the AV3V area at the level of the paraventricular nuclei and the OVLT. Lesion E (fig. 2) was similar to Lesions A–D in that damage occurred bilaterally along the LT in the AV3V region. Lesions F and G were dorsally placed lesions that caused no damage in the AV3V region but did damage structures along the LT at and above the anterior commissure. Lesion H produced no damage to either the AV3V region or the LT at any point. Areas of overlap in groups of these lesions identified regions of the preoptic area and hypothalamus that appeared to be necessary for the AII-induced pressor response.

Figure 3 shows the location of four lesions within the region which did not block the central pressor response to AII. Lesion 1 (fig. 3, Panels 1 and 2) destroyed tissue at the anterior margin of the brain along the midline. This result suggested that tissue along the rostral border of the LT was not involved in the AII pressor response.

Lesion J of figure 3 was located in about the same region as Lesion H of figure 2. The latter lesion blocked the central AII pressor response. Since Les-

ion J was a unilateral lesion, it appears that destruc-
Attention was focused again on Lesion H. A photograph of a horizontal section through this lesion is shown in figure 4; this section is at the level of the OVLT. This lesion destroyed periventricular tissue in an area of the hypothalamus that was removed from the structures associated with the LT. Nevertheless, it blocked the central AII pressor response. It was hypothesized that an AII pressor pathway from the AV3V traveled through this region. Figure 5 represents a hypothetical map of the AII pressor region of the anterior hypothalamus based on information obtained from the lesion experiments. The stippled area represents the proposed route for an AII pressor pathway that originates in the AV3V region and ascends along the ventricular border of the LT to the area of the anterior commissure. The critical area then descends through the anterior hypothalamus in the region anterior to the paraventricular nuclei.

Knife Cut Mapping Analysis

An alternative test of the location of the AII pressor pathway was made on the descending limb of the pathway. Horizontal knife cuts were made in 40 rats. Figure 6 represents a composite map of 14 anterior
hypothalamic knife cuts representative of the entire group. Knife cuts that blocked the pressor response to central AII are indicated by solid lines. Hatched lines represent cuts that did not block the response, and the stippled area represents a common region transected by each of the knife cuts that blocked the pressor response. This route correlates well with that indicated by the lesion studies (fig. 5).

In two control experiments (see Methods), rats with midline knife punctures (5.5 mm from the dura) had a normal pressor response to AII (i.c.v.) while four rats with horizontal anterior hypothalamic knife cuts produced when the knife was lowered deliberately off the midline showed no pressor response to AII (i.c.v.). In addition, the path of the knife in several of the animals with effective horizontal cuts was slightly lateral, sparing midline structures above the level of the horizontal cut. These experiments suggest that the anterior hypothalamic knife cut blocked the AII pressor response as a result of the horizontal cut and not because of a common area damaged on the midline by lowering the knife.

A group of 15 rats with anterior hypothalamic knife cuts was tested for pressor responsiveness to 200 ng AII (i.c.v.). Thirteen of the animals did not respond to AII. The average mean arterial pressure increase for the knife cut group was 2.9 ± 2.0 mm Hg compared with a group of 15 control animals which averaged 23.7 ± 1.6 mm Hg (p < 0.001, group comparison Student's t test). In the two knife-cut animals that did respond normally to central AII, the knife cuts did not destroy the critical tissue bilaterally.

**FIGURE 5.** Schematic diagram of a midsagittal section through the rat brain. Various hypothalamic nuclei are represented as they appear in a sagittal plane 500 μ from midline. The stippled area represents the region that must remain intact for pressor responsiveness to centrally administered angiotensin II (200 ng). AC = anterior commissure; AH = anterior hypothalamic nucleus; AV3V = anteroventral third cerebral ventricle; F = fornix; M1 = massa intermedia; MPO = medial preoptic nucleus; OVLT = organum vasculosum of the lamina terminalis; SFO = subfornical organ; IIIV = third cerebral ventricle; PVN = paraventricular nucleus.
FIGURE 6. Schematic diagram of a midsagittal cut through the anterior hypothalamic region of the rat brain. The horizontal scale represents the distance in $\mu$m from the anterior commissure (AC). The vertical scale represents the distance in $\mu$m from the base of the brain on a vertical plane at the most anterior aspect of the paraventricular nuclei (PVN). The area studied by these knife cuts is outside the area typically destroyed by an anteroventral third ventricle (AV3V) lesion. The solid lines represent the location of seven knife cuts that eliminated the pressor response to AI1 (200 ng, i.c.v.). The hatched lines represent the location of seven knife cuts that did not affect the pressor response to the same dose of AI1. The stippled area represents the proposed route of the anterior hypothalamic AI1 pressor pathway.

The pressor response to intracranial AI1 is mediated in part by the release of vasopressin and partly by an increase in sympathetic nervous system activity. The AV3V region in the rat contains receptors that interact with CSF-borne AI1 to cause an increase in blood pressure. The major goal of these experiments was to identify pathways from the AV3V region involved in this pressor response. The studies employed electrolytic lesions and knife cuts distributed throughout the preoptic and anterior hypothalamic areas. Mapping analysis identified several regions critical for the central AI1 pressor response.

The results of these studies indicate that the tissue at the anterior border of the third ventricle along the LT is involved in the pressor response to centrally administered AI1. If this region was destroyed bilaterally at any point between the OVLT and the region of the nucleus medianus above the anterior commissure, the response to central AI1 was eliminated. A lesion was also found in the anterior hypothalamus that effectively eliminated the central AI1 pressor response but did not damage the LT. This finding suggested that this region of hypothalamus might contain efferents from the AV3V region that were involved in the response. This hypothesis was confirmed by data obtained from transecting the region with horizontal knife cuts. Knife cuts that were effective in eliminating the central AI1 pressor response were in the hypothalamus anterior to the paraventricular nuclei. Ineffective knife cuts were caudal to this region.

Integrity of the paraventricular nuclei appears not to be necessary for the pressor response to centrally administered AI1 since neither total electrolytic destruction of these nuclei (fig. 3) nor knife transection (fig. 6) diminished the response. This is surprising since the magnocellular elements of these nuclei as well as the supraoptic nuclei are major sites of vasopressin synthesis. Both the supraoptic nuclei and paraventricular nuclei have been shown to respond to AI1 (i.c.v.) by increased firing activity of neurosecretory cells as well as vasopressin release.

Furthermore, if the paraventricular nuclei are ordinarily involved in the response, the supraoptic nuclei can compensate for the absence, or the sympathetic component of the response may dominate under these conditions.

Recent neuroanatomical data indicate that neural circuitry exists within the region that could be consistent with the route of the AI1 pressor system. Neural projections have been described from the medial preoptic area to the medial basal hypothalamus and to the periventricular region to the posterior hypothalamus and then to the mesencephalic central gray. This pathway has been proposed to be involved in the sympathetic vasconstrictor activity elicited by electrical stimulation in the AV3V region. Recent studies have shown that the anterior hypothalamic AI1 pressor pathway does not block this sympathetic excitation. Therefore, the AI1 pressor pathway appears to be identical to the vasconstrictor pathway activated during electrical stimulation of the AV3V region.

The entire medial preoptic area is richly innervated by neurons originating in the OVLT. Neurons in both nucleus medianus (NM) and OVLT were labelled by retrograde transport of horseradish peroxide injected into the ventromedial supraoptic nuclei. Tissue damage along the LT would probably destroy these connections.

In summary, the present studies provide functional and anatomic evidence that a pathway, mediating the central AI1 pressor response, originates within the AV3V region along the LT. Efferents from this region appear to project dorsally along the LT to the vicinity of the dorsal NM above the anterior commissure. The pathway then descends medially through the hypo-
thalamus near the margin of the preoptic and anterior hypothalamic nuclei. The route of this proposed pathway is consistent with known neural projections within the region. Preliminary studies indicate that this pathway is also involved in the pressor response to blood-borne All and in the development of renin-dependent hypertension but is not involved in drinking responses elicited by All (i.c.v.). We conclude that the prevention of renin-dependent renal hypertension by AV3V lesions probably depends in part upon interruption of the pathway critical for the central pressor action of All.

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