The Angiotensin II Pressor System of the Rat Forebrain

Diane K. Hartle and Michael J. Brody

From the Department of Pharmacology, and the Cardiovascular Center, University of Iowa, Iowa City, Iowa

SUMMARY. An anterior hypothalamic knife cut that leaves intact two central sites of action of angiotensin II produces the same deficits in the pressor responses to angiotensin II that have been attributed to destruction of two circumventricular organs (the subfornical organ and the organum vasculosum of the lamina terminalis). The central pressor actions of angiotensin II are necessary for the full expression of renin-dependent renal hypertension. The anterior hypothalamic knife cut attenuates renin-dependent aortic ligation hypertension. It has been shown that electrolytic destruction more anterior to this knife cut, in the anterodorsal 3rd ventricle region, also attenuates two forms of renin-dependent hypertension, aortic ligation hypertension and two-kidney Goldblatt hypertension in the rat. Electrolytic lesions in the subfornical organ also reduce renin-dependent, two-kidney Goldblatt hypertension. These data are consistent with the hypothesis that a common efferent system from the organum vasculosum of the lamina terminalis and subfornical organ mediates the central pressor response to angiotensin II and is involved in the development of renin-dependent hypertension. A model is proposed for the circuitry in the rat forebrain that is involved in the pressor response to angiotensin II.

PREVIOUSLY, we reported the localization of an efferent angiotensin II pressor system from the anterodorsal 3rd ventricle (AV3V) region of the hypothalamus (Hartle et al., 1982). Bilateral destruction of the tissue along the lamina terminalis (LT), either below or at the level of the anterior commissure (AC), blocked the pressor response to intracerebroventricular (icv) administration of angiotensin II. Damage along the ventral LT that blocked the pressor response to angiotensin II suggested that the tissue in the region that interacted with angiotensin via this route of administration was located at or within diffusional radius of the organum vasculosum of the lamina terminalis (OVLT). The pathway appeared to project dorsally along the LT toward the AC, and then descended through the anterior periventricular hypothalamus. Both electrolytic lesions and knife-cuts in the region of the descending pathway eliminated the pressor response to angiotensin (administered icv). These lesions and knife cuts did not encroach upon the LT.

The drinking responses to angiotensin II (icv) in these lesion animals remained intact (Lind et al., 1981).

The role of the subfornical organ in the dipsogenic responses to angiotensin II has been well established (Abdellal et al., 1974; Lind and Johnson, 1982; Simpson, 1981; Simpson et al., 1979; Simpson and Routtenberg, 1973, 1975). Recently, it has been reported that the subfornical organ (SFO) is also a site of action for the pressor effects of angiotensin II (Mangiapane and Simpson, 1980a), and SFO lesions have been shown to attenuate the pressor response to intravenously administered angiotensin II (Mangiapane and Simpson, 1980b) and to attenuate renin-dependent renal hypertension (Buggy et al., 1984).

The present studies were designed to determine whether knife cuts through the proposed angiotensin II pressor pathway from the AV3V region would produce some of the other deficits typical of an AV3V lesion. Specifically, would a horizontal anterior hypothalamic knife cut (AHKC) affect the central pressor response to angiotensin II (administered either intravenously or intracerebroventricularly) and reduce the severity of a highly renin-dependent form of hypertension. Our results indicate that—
like an electrolytic lesion in the AV3V region—an AHKC eliminates the pressor response to centrally administered angiotensin II, eliminates a centrally mediated component of the pressor response to intravenously administered angiotensin II, and attenuates aortic ligation hypertension.

We present a model that is based on the cumulative information concerning the pressor actions of angiotensin II in the rat forebrain. This model represents a functional and conceptual framework for the organization of a forebrain angiotensin II pressor system involved in the development and maintenance of renin-dependent forms of hypertension.

Methods

Anterior Hypothalamic Knife Cuts (AHKC)

The procedure for producing the AHKC is described in detail elsewhere (Hartle and Brody, 1982a; Hartle et al., 1982). Briefly, a 1.25-mm knife was lowered into position ~7.6 mm from the dura. The knife was rotated 90° in either direction from the midline. This maneuver produces a semicircular cut in a horizontal plane spanning periventricular and the medial hypothalamus caudal to the AV3V region, but anterior to the paraventricular nuclei. Figure 1 shows schematically in horizontal section the procedure for producing the AHKC. The tissue that must be transected bilaterally to produce a functionally effective AHKC is indicated by the small rectangle along the midline. The critical area incorporates periventricular hypothalamus.

![Figure 1. Procedure for anterior hypothalamic knife cut (horizontal schematic). The knife was lowered into position with the shaft anterior to the blade along the midline. A semi-circular cut was produced by rotating the knife 90° to the right and back to midline, then 90° to the left and back to midline. The critical tissue to be transected to produce the angiotensin II pressor deficits is indicated by the periventricular rectangle.](image)

The plane of the knife is demonstrated in sagittal section in Figure 2. Figure 3 shows a coronal section of brain from an animal with the cut.

Lateral Cerebral Ventricle (LV) Cannulation

Guide tubes (23-gauge stainless steel hypodermic tubing) for injection cannulas were implanted into a lateral cerebral ventricle at coordinates: ~0.8 from bregma, ~1.1 mm from midline, and ~5.5 mm from the top of the skull. The skull was level between lambda and bregma for this procedure. Injection cannulas were custom-fitted to extend 0.2 mm beyond the intracranial tip of the guide tube. A 32-gauge stainless steel wire was kept in the guide tube between the time of instrumentation and the time of the injections. The guide tube and its plug were cemented into place on the skull with dental acrylic.

Angiotensin II Pressor Challenges

Each rat was instrumented with a carotid catheter for blood pressure determination, and a jugular cannula was inserted for angiotensin II administration. After 24 hours, each animal was placed in a cage and acclimated to the new environment for 1 hour. Water tubes were removed from the cage so that the pressor responses recorded during the angiotensin II challenges would not be complicated by alterations in blood pressure due to drinking behavior. The carotid catheter was connected via polyethylene tubing (PE50, Clay Adams) to an Ailtech pressure transducer. Voltage displacement at the preamplifier per mm Hg pressure change at the transducer was calibrated by a mercury manometer before the start of each experiment. Blood pressures were recorded on a Beckman type R dynograph. Zero pressure was calibrated by placing the
tip of the saline-filled line from the transducer at the level of the rat’s heart.

The jugular line was connected via PE10 tubing to a three-way stopcock. Two syringes were attached to the stopcock; one contained the desired dilution of angiotensin II to be injected and the other contained isotonic saline for washing the line. Doses of angiotensin II (75, 150, and 300 ng/kg) were administered in 0.1-ml volume, followed immediately by a 0.2-ml wash with saline. The estimated volume in the injection line was 50 μl. Each injection was duplicated and the pressor responses were averaged. Injections proceeded from lowest dose to highest dose to minimize the complication of contamination of a low dose of angiotensin II with residual from a previously administered high dose of angiotensin II. We observed no tachyphylaxis from these doses of angiotensin when they were administered in this manner.

After the dose-response relationship to peripherally administered angiotensin II was completed, a 10-μl Hamilton syringe filled with angiotensin II (100 μl/ml) was attached to the lateral ventricular cannula by fitting an injector to a length of PE10 tubing connected to the 10-μl syringe. The injector was fashioned so that its intracranial tip would extend approximately 0.2 mm beyond the tip of the guide tube. The entire injector and PE10 line attached to the syringe were filled with the angiotensin II solution. Once the injector was in place, a 2-μl injection via the syringe advanced the solution in the injector line so that 200 ng of angiotensin II would be administered in a 2-μl volume into the lateral cerebral ventricle. Each animal was tested 3–5 times with 200 ng of angiotensin II. The maximum pressor responses obtained from animals during the first 2 minutes after each injection were recorded and averaged for each animal. All data reported were from pressor events measured in quiescent animals. If the animal displayed any exploratory behavior during the pressor challenge, the response was not used. This precaution removes the complication of variability of arterial pressure associated with movement. Control vehicle injections of 2-μl isotonic saline made in each animal did not alter blood pressure.

Aortic Ligation

Aortic ligation surgery to produce hypertension was performed with ether anesthesia. An off-midline incision was made in the left side of the abdomen. The aorta was exposed by deflecting the viscera to one side. The aorta was then ligated with surgical silk between the renal arteries below the branching of the superior mesenteric artery. Sham surgery was performed identically, except no tie was placed on the aorta.

Histological Procedures

After completion of the central angiotensin II pressor responses, the rats were anesthetized with pentobarbital (35 mg/kg, ip) and 2 μl of a 1:2 dilution of a saturated
solution of Pontamine sky blue dye was injected into the lateral ventricle to determine the cannula patency and the accessibility of the AV3V region to the injectates. Cannulas were considered patent if blue dye was present in the AV3V region.

After injection of the dye, the brain was perfused intracranially, first with isotonic saline, then with buffered (phosphate) 10% formalin. The fixed brain was frozen and sectioned into 40-μm coronal slices, using a Leitz microtome apparatus with a remote Histofreeze connected to the microtome stage. Alternate coronal slices from 1 mm rostral to the anterior commissure through the region of the paraventricular nuclei were stained for Nissl substances with cresyl violet and examined microscopically.

Knife Cut Mapping Analysis

The position of each knife cut was determined by noting the first and last coronal sections in which damage appeared bilaterally across the midline. The horizontal plane of the knife cut was determined by measuring the distances between the knife cut, the AC, and the base of the brain in a coronal section across a plane that includes the most caudal section through the AC. The position of each knife cut was compared with the map of effective and ineffective knife cuts that has been published recently elsewhere (Hartle et al., 1982). A knife cut was considered to be effective if (1) the animal did not respond to icv angiotensin II with an increase in blood pressure and (2) the icv cannula was patent, as judged by the presence of the blue dye in the anteroventral 3rd ventricle region after delivery via the LV cannula.

Results

Experiment 1: The Effect of AHKC on the Central Pressor Response to Angiotensin II

This experiment was designed to determine whether a horizontal anterior hypothalamic knife cut that transects the route of the previously mapped angiotensin II pressor pathway (Hartle et al., 1982) would mimic the effect of AV3V lesion by producing deficits in the pressor responses to angiotensin II (both icv and iv). Figure 4 shows the results of central and peripheral pressor challenges with angiotensin II in two groups of animals, one group with sham brain surgery (n = 36) and the other with AHKCs (n = 33). In response to an icv injection of 200 ng All, the AHKC caused a significantly attenuated pressor response (P < 0.01, group Student’s t-test). The effect of AHKC on pressor responsiveness to three intravenous bolus injections of angiotensin II is depicted in the second panel of Figure 4. A central contribution to the pressor effect of All was demonstrated, since AHKC significantly attenuated the pressor response to each of the four intravenous doses of angiotensin II. The magnitude of the functional pressor deficits produced by AHKC is strikingly similar to those produced by AV3V lesion (Buggy et al., 1977). The results of this experiment are consistent with the hypothesis that the AHKC interrupts a pathway necessary for the central pressor effects of angiotensin II.

Figure 3 is a photograph of a coronal section of a rat brain with an AHKC that produced functional angiotensin II pressor deficits to both centrally and peripherally administered angiotensin II. The knife cut spans the medial hypothalamus in the region caudal to the AV3V region but rostral to the paraventricular nuclei. The knife was lowered down the midline, causing some damage to the main body of the SFO during the descent. In this animal, the ventral stalk of the SFO and the NM above the AC remained intact. In several of the AHKC animals, the path of descent was slightly off midline so damage to the main body of the SFO was partial.

Experiment 2: The Effect of AHKC on Aortic Ligation Hypertension

AV3V lesion attenuates aortic ligation hypertension (Hartle, 1981; Hartle et al., 1979). This experi-
ment was designed to test whether AHKC also affected the severity of this highly renin-dependent form of hypertension. A group of 39 rats received AHKCs. Another group of 34 rats underwent a sham surgical procedure. Several days later, each animal in these two groups underwent aortic ligation. After 5 days, 24 of the 39 AHKC group and 18 of the 34 sham group had survived. All surviving rats were instrumented with carotid catheters under ether anesthesia. The animals then were allowed to recover from the ether for 30 minutes, or until the animal exhibited no ataxia and had stopped exploring its cage. Mean arterial blood pressure then was recorded via the carotid catheter. Because we found that ether anesthesia suppresses blood pressure in rats with hypertension supported by non-renin-dependent mechanisms (unpublished results in one-kidney Grollman and steroid/salt models of hypertension), the mean arterial blood pressures of these animals were also recorded the following day. Figure 5 summarizes the results of this experiment. AHKC animals had significantly lower mean arterial pressures than the sham group on both day 5 and day 6 after aortic ligation (day 5: 152 ± 4 vs. 173 ± 5 mm Hg, P < 0.01 and day 6: 171 ± 3 vs. 188 ± 5 mm Hg, P < 0.01). Previous experience with this form of hypertension by us and others (Carretero et al., 1971; Chatelain and Ferrario, 1978; Chatelain et al., 1980, 1983; Fernandes et al., 1976; Hartle, 1981; Hartle et al., 1979; Sweet et al., 1976) indicates that blood pressures are usually at their maximum by 5–12 days after aortic ligation. The lower blood pressures on day 5 are consistent with either a depressant effect of the anesthetic, or the possibility that, in this particular group of animals, the pressures had not reached their maximum by day 5 (Chatelain and Ferrario, 1978). The magnitude of the protective effect of the AHKC during the renin-dependent stage of aortic ligation hypertension is consistent with its ability to block the central pressor effects of either blood-borne or CSF-borne angiotensin II (see Fig. 4).

Discussion

It has been demonstrated previously that either an AHKC or AV3V lesion will block the pressor response to angiotensin II (administered via the cerebroventricular system) (Buggy et al., 1977; Hartle, 1981; Hartle et al., 1982; Johnson et al., 1978). Both AV3V and SFO lesions attenuate the pressor response to intravenously administered injections or infusions of angiotensin II (Buggy et al., 1977; Hartle, 1981; Mangiapane and Simpson, 1980b). Both AV3V and SFO lesions can attenuate the development of renin-dependent renal hypertension in the rat (Buggy et al., 1984; Hartle et al., 1979; Haywood et al., 1983). The present experiments demonstrate that the AHKC can also produce the angiotensin II pressor deficits found with either SFO or AV3V lesions. We believe that these results indicate that the AHKC effectively eliminates an efferent angiotensin II pressor system originating at the lamina terminals in the forebrain of the rat.

Model of the Angiotensin II Pressor System on the Rat Forebrain

Based on the present studies, as well as on previous work by our laboratory on functional mapping of the efferent angiotensin II pressor system from the AV3V region (Hartle, 1981; Hartle and Brody, 1982a, 1982b; Hartle et al., 1982), and the studies of other investigators, we propose the following model of the angiotensin II pressor system in the rat forebrain. The functional neural circuitry for this system is presented diagramatically in Figure 6. In this scheme, A represents angiotensin II-sensitive neural substrates in the AV3V region that are accessible to either blood-borne or CSF-borne hormone. Currently available evidence does not allow us to state whether the same or separate receptor fields are reached by these two routes of administration, although recent evidence from studies using angiotensin II binding indicates that OVLT is reached by both routes (Van Houten et al., 1983). Saralasin (administered iv) effectively competes for 125I-angiotensin II binding within the OVLT. The 125I-angiotensin II was administered intravenously in these experiments (Van Houten et al., 1983). These results indicate that the diffusional distance for large doses of angiotensin II from the cerebral ventricle may be great enough to include the OVLT. However, since tight junctions occur between ependymal cells lining the 3rd ventricle at the OVLT, the access route taken by saralasin from CSF to OVLT is most certainly through adjacent tissues that are separated from the ventricle by ciliated ependymal cells that are not connected by tight junctions e.g., the ventral median preoptic nucleus. Obviously, if a molecule...
of saralasin can move from CSF through brain intercellular spaces and reach the OVLT, then the converse probably is equally true. Namely, angiotensin II should have access to the intercellular compartment immediately surrounding the OVLT. Therefore, the present experimental evidence suggests that binding sites in either OVLT or within adjacent structures may be involved in the pressor response to angiotensin II (either icv or iv).

The model includes another angiotensin II-sensitive receptor field, in the SFO (designated B). It is activated functionally by blood-borne angiotensin II or microinjections of angiotensin II into the SFO (Mangiapane and Simpson, 1980a, 1980b) but not via CSF-borne angiotensin II (Hoffman and Phillips, 1976), even though CSF-borne saralasin appears to reach SFO (Van Houten et al., 1983). These investigators demonstrated that uptake of labeled angiotensin II (administered intravenously) was significantly blocked in the peripheral SFO, but not in the central SFO, by previous intracerebroventricular treatment with saralasin. Thus, it is possible that the portion of the SFO that is involved in the pressor effects of angiotensin II may be the portion least accessible to CSF-borne angiotensin. This relative inaccessibility may explain the inability of some to demonstrate a role for the SFO in the pressor response to centrally administered angiotensin II (Hoffman and Phillips, 1976), whereas the SFO is clearly sensitive to humorally delivered angiotensin II (Mangiapane and Simpson, 1980b).

A and B send projections along the lamina terminalis to the median nucleus of the preoptic region, designated NM, or nucleus medianus. The nucleus medianus spans the distance between the SFO and the OVLT along the lamina terminalis. The projections from the angiotensin II-sensitive cells, A and B, may synapse with cells in the NM, designated C in the model. The angiotensin II pressor projections from A and B, and/or secondary projections from C, then descend through the periventricular anterior hypothalamus at the approximate margins of the medial preoptic and anterior hypothalamic nuclei. The AHKC interrupts all efferents from AV3V and SFO that are involved in the central pressor effects of either blood-borne or CSF-delivered All because it severs all projections from A, B, and C. Because the AHKC prevents a centrally mediated pressor response to angiotensin II, these projections must mediate both the increased sympathetic activity and the release of pressor quantities of vasopressin that normally characterize the central angiotensin II pressor event (Hoffman et al., 1977; Keil et al., 1975; Severs et al., 1970).
The model also accounts for the existence within the AV3V region of vasoconstrictor mechanisms that are not part of the central angiotensin II pressor system. These mechanisms (designated D) are involved in the increased sympathetic activity and vasopressin release associated with several non-renin-dependent forms of hypertension. At least one of these vasoconstrictor mechanisms projects caudally from the lamina terminalis in the region of the ventral NM. It does not, however, project via the same periventricular route as the angiotensin II pressor pathway; rather, it courses caudally through the medial forebrain bundle (Hartle and Brody, 1982a).

Experimental Evidence for Site A

Angiotensin II increases blood pressure of the rat when administered into the ventricular system of the forebrain (Hoffman et al., 1977; Keil et al., 1975; Severs et al., 1970). Attempts to localize the receptor region for CSF-borne angiotensin II indicate that the hormone acts upon periventricular tissue bordering the optic recess of the 3rd ventricle, the so-called AV3V region. If the ventricle in the AV3V region is plugged with cold cream, intracerebroventricularly delivered angiotensin II elicits no pressor response (Hoffman and Phillips, 1976). Electrolytic destruction of the periventricular structures within the AV3V region also blocks the pressor response to angiotensin II (iv) (Buggy et al., 1977; Hartle, 1981; Hartle et al., 1979, 1982; Johnson et al, 1978). Injection of minute (50 fg) quantities of angiotensin II into the 3rd ventricle at the optic recess causes an increase in blood pressure and an increase in antidiuretic hormone (ADH) release (Phillips, 1978). This amount of angiotensin II would not elicit a pressor response if injected into the lateral ventricle. This suggests that angiotensin II receptors are located very close to the site of injection. Electrolytic destruction in the AV3V region significantly attenuates the pressor response to intravenously administered angiotensin II (Buggy et al., 1977; Hartle, 1981). The same lesion has no effect on the responses to another systemic pressor agent, norepinephrine (Buggy et al., 1977).

There is autoradiographic evidence that bloodborne angiotensin II binds specifically within the OVLT (Van Houten et al., 1980, 1983). Well-localized parenchymal injections of angiotensin II into the medial preoptic nucleus adjacent to both OVLT and NM elicit drinking behavior (Swanson et al., 1978). Although blood pressure responses during these injections were not recorded, these investigators injected labeled amino acids at the sites from which drinking responses were elicited with angiotensin II. Two projection pathways were described from the region (Swanson et al., 1978). A lateral hypothalamic projection system was associated with the drinking behavior elicited by angiotensin II, whereas a periventricular pathway, not involved in the drinking behavior, was also found. The angiotensin II pressor pathway appears to project via this medial route (Hartle et al., 1982). Single units in the medial preoptic region were shown to be excited by direct microiontophoretic application of angiotensin II (Gronan and York, 1976, 1978). Other investigators have demonstrated that microiontophoretic injection of angiotensin II into in vitro brain slice preparations excites cells within the AV3V region (Knowles and Phillips, 1980; Nelson, 1983). Single units within both the OVLT and the NM have been shown to be responsive to angiotensin II (Phillips et al., 1979).

All of these putative angiotensin II-sensitive sites within the AV3V region are within the known diffusionary radius of the OVLT. Horseradish peroxidase stains the OVLT, the ventral NM, and much of the medial preoptic area shortly after an intravenous injection (Broadwell and Brightman, 1976). Presumably, a small peptide such as angiotensin II would easily reach—not only the parenchymal cells of the OVLT—but also the ventral NM and portions of the medial preoptic nucleus.

Experimental Evidence for Site B

Like the AV3V lesion, electrolytic destruction of the SFO has been shown to significantly attenuate the pressor response to intravenously delivered angiotensin II but has no effect on the pressor response to intravenously administered phenylephrine (Mangiapane and Simpson, 1980b). Blood-borne angiotensin II has been shown to bind to receptors within the SFO (Van Houten et al., 1980, 1983). Direct injection of angiotensin II into the SFO produces a pressor response that can be blocked with saralasin (Mangiapane and Simpson, 1980a) and is associated with the release of ADH (Simpson et al., 1979). Electrical stimulation of the SFO in anesthetized rats also increases blood pressure (Ishibashi and Nicolaidis, 1981) and regional vascular resistances (Mangiapane and Brody, 1983). Angiotensin II has been shown to excite units located within the SFO (Felix and Schlegel, 1978).

In conclusion, the AV3V region and the SFO both appear to be involved in the pressor response to intravenously administered angiotensin II, since both SFO lesion (Mangiapane and Simpson, 1980b) and AV3V lesion (Buggy et al., 1977) reduce this pressor response. The lesions in each study caused a specific reduction in the pressor response to angiotensin II because pressor responsiveness to systematically delivered phenylephrine (SFO study) or norepinephrine (AV3V study) was not affected.

The AHKC does not destroy either the SFO or the AV3V region, yet it totally blocks the pressor response to centrally administered All and attenuates the pressor response to peripherally administered angiotensin II by a decrement similar to that seen with either the AV3V or SFO lesion. These results suggest that the AHKC is severing efferent projections from both the AV3V region and the SFO that are involved functionally in the forebrain pressor effects of angiotensin II.
Experimental Evidence for Site C

Because of the fenestrated vasculature in both the OVLT and the SFO, these circumventricular organs lack a blood-brain barrier. Blood-borne angiotensin II has complete access to interstitial compartments of A and B and also C, if C is within the diffusional radius of the OVLT. What accounts for the fact that CSF-borne angiotensin II appears to act within the AV3V region and not at the SFO? Because tight junctions exist between the ependymal cells of both the OVLT and the SFO, these organs have a highly developed CSF-brain barrier. Therefore, angiotensin II in the CSF does not have direct access to the OVLT or the SFO. When horseradish peroxidase is injected intracerebroventricularly, these tight junctions deny access of this substance to the parenchyma of the circumventricular organs (Brightman et al., 1975; Mitchel and Card, 1978; Zimmerman et al., 1975). Neuropile adjacent to the OVLT, A and/or C is therefore accessible to either blood-borne or CSF-borne angiotensin II, while B is relatively protected from CSF-delivered hormone by virtue of its CSF-brain barrier.

There may, however, be mechanisms within both the AV3V region and the SFO to circumvent the CSF-brain barrier. For example, tanyocyte transport of many substances has been described across ependymal barriers (Dellmann and Simpson, 1974; Mitchell and Card, 1978; Zimmerman et al., 1975). There is also the possibility that angiotensin II-sensitive supraependymal neurons exist in the AV3V region or at the SFO. Supraependymal neurons have been described in the ventricular system and at the circumventricular organs (Chatelain et al., 1983; Zimmerman et al., 1975). No function is presently known for these neurons. Also, neuronal processes (from angiotensin II-sensitive neurons) may insinuate through the ventricular ependyma and thereby maintain direct contact with the CSF (Chatelain et al., 1983; Van Houten et al., 1983). Such mechanisms would allow A, B, or C to be direct targets of CSF-borne angiotensin II.

Whatever the mechanism, neural substrate within the AV3V region is able to sense CSF-borne angiotensin II and respond with dose-dependent increases in blood pressure and drinking (Buggy et al., 1977). Others have found cells located in both the OVLT and within the ventral NM that are excited by microiontophoretically applied angiotensin II (Phillips et al., 1979). These angiotensin-sensitive cells may well correspond to A and/or C in the model. Despite recent evidence that CSF-borne angiotensin II might bind to portions of SFO (Van Houten et al., 1983), the mechanisms underlying failure to demonstrate its pressor effects via SFO may involve differential anatomical sublocalization of angiotensin II-binding sites involved in different functions (pressor vs. dipsogenic). For example, if the SFO sites involved in the pressor effects of blood-borne angiotensin II were located in the central core of SFO, they would be relatively inaccessible to icv angiotensin II (Van Houten et al., 1983). We have just found additional functional evidence for site C. Using microinjection of lidocaine into NM of conscious rats, we have been able to reversibly block the pressor effect of angiotensin II given by the cerebroventricular route and attenuate the pressor action of angiotensin II produced by intravenous administration (O’Neill and Brody, 1984). The same site of lidocaine administration that blocks the centrally mediated pressor action of angiotensin II is not responsive to local microinjection of angiotensin II (O’Neill and Brody, 1984).

The Central Angiotensin II Pressor System and Experimental Renal Hypertension

The central angiotensin II pressor system appears to be activated during the development of renin-dependent hypertension in the rat and contributes significantly to the pressor effects of circulating angiotensin II in renin-dependent hypertension. Intraventricular administration of a competitive angiotensin II blocker causes a large decrease in blood pressure in aortic-ligated animals (Sweet et al., 1976). The likely site of action of the blocker when administered via the CSF is in the AV3V region, since intracerebroventricular angiotensin II has not been shown to interact functionally with the receptor region within the SFO that transduces a pressor event. Since the SFO responds to blood-borne angiotensin II, these receptors are presumably activated by the large increases in plasma angiotensin II concentrations during the development of renin-dependent renal hypertension. It would therefore be useful to know whether direct microinjection of an angiotensin II antagonist directly into the SFO would have an effect on blood pressure in a renin-dependent form of hypertension and whether this effect would be additive, with the depressor effect of the antagonist presumably acting within the AV3V region in the same model of hypertension. If the effect of the antagonist at both sites is additive, then the relative contribution of the central pressor effects of angiotensin II has been underestimated in renin-dependent hypertension by either AV3V lesion alone or SFO lesion alone.

Destruction of the AV3V region interferes with the development of renin-dependent, two-kidney Goldblatt (Haywood et al., 1983) and aortic ligation hypertension (Hartle et al., 1979), and SFO lesion attenuates the development of two-kidney Goldblatt hypertension in the rat (Buggy et al., 1984). The present results indicate that AHKC also reduces the severity of aortic ligation hypertension. Our laboratory has recently found that the AHKC attenuates...
development of acute renin-dependent renal hypertension produced in the conscious two-kidney rat by partial occlusion of one renal artery (Faber and Brody, in press). The knife cut therefore interrupts an efferent system that is not only involved in mediating the central effects of angiotensin II found in normotensive rats, but is also able to interrupt the central component involved in the elevation of blood pressure in renin-dependent hypertension.

**Experimental Evidence for Site D**

The AV3V region apparently contains alternate systems involved in other forms of experimental hypertension. The AV3V lesion prevents several non-renin-dependent forms of hypertension including one-kidney Grollman (Buggy et al., 1977), DOCA/salt (Brody et al., 1978), methyl-prednisolone (Kohlmann et al., 1981), and neurogenic hypertension caused by sinoaortic deafferentation (Brody et al., 1978) or bilateral lesion of the nucleus tractus solitarius (Brody et al., 1978), and partially prevents expression of Dahl strain salt-sensitive genetic hypertension (Goto et al., 1981). These other forms of hypertension probably are not dependent upon the central angiotensin II pressor pathway that is involved in renin-dependent forms of hypertension. These forms are interacting with some other neural or humoral vasoconstrictor system dependent upon integrity of the AV3V region but distinct from the angiotensin II pressor system. These other systems are collectively represented by D in the model.

Evidence that these forms of hypertension do not require the central angiotensin II pressor system stems from reports that SFO lesion does not prevent or attenuate either one-kidney Grollman (Knuepfer et al., in press) or DOCA/salt hypertension (Buggy et al., 1984). In addition, the AHKC does not influence the development of either of these non-renin-dependent forms of hypertension (Hartle and Brody, 1982). Taken together, these data provide evidence that distinct central mechanisms are involved in the development of renin-dependent and non-renin-dependent forms of hypertension. Indeed, there appears to be more than one mechanism involved in the non-renin-dependent category. Medial forebrain knife cuts that do not encroach upon any structure associated with the lamina terminalis prevent one-kidney Grollman hypertension, but have no effect on DOCA/salt hypertension (Hartle and Brody, 1982). These results suggest at least three different physiological systems involved in hypertension are transected by AV3V lesion.

**Neuroanatomical Evidence**

The proposed model is consistent with current neuroanatomical evidence. Lesions produced around the anterior commissure which damage the NM result in fiber degeneration in both the SFO and OVLT (Hernesniemi et al., 1972). AV3V lesions cause retrograde degenerative changes of neuronal somas located in the SFO (Carithers et al., 1980). The SFO densely innervates structures ventral to it all along the median plane at the lamina terminalis. Both the OVLT and the NM are labeled by anterograde transport of tritiated l-leucine injected into the SFO (Miselis et al., 1979). These connections are not reciprocal. Little evidence exists for innervation from the AV3V region to the main body of the SFO. After lesions in the AV3V region, degeneration of the axonal terminations within the SFO are rare (Carithers et al., 1980; Hernesniemi et al., 1972). Indeed, there are differences in the innervations of the dorsal and ventral NM. When labeled amino acid is injected into the dorsal NM, no label is found in either the ventral NM, the SFO, or the OVLT (Miselis et al., 1979). This study is in contrast to the apparent connections of the ventral circumcommissural NM with the SFO and OVLT (Carithers et al., 1980; Hernesniemi et al., 1972). The fact that the NM region is a critical component in the neural circuitry involved in the central blood pressure effects of angiotensin emphasizes the need for the further study of both the afferent and the efferent connections of the ventral NM, the circumcommissural NM, and the dorsal NM. The interconnectivity of the NM with the initiating sites for the pressor action of angiotensin II in both the SFO and the OVLT needs further elucidation. Unfortunately, the efferent projections from the NM have so far not been well characterized by anterograde transport techniques, perhaps due to the difficulties inherent in injecting anterograde markers selectively into a thin nucleus that borders the anterior wall of the 3rd ventricle for most of its extent.

Recently it was reported that knife cuts that would sever all connections between the SFO and the ventral and circumcommissural portions of the NM did not affect the pressor response to intravenously or intracerebroventricularly administered angiotensin II (Lind and Johnson, 1982). These knife cuts would eliminate the projections from B in the model but would not affect A or C. These data indicate that, even when efferents from one of the primary receptor regions for angiotensin is eliminated, the system is still responsive to the hormone. Obviously angiotensin could still exert its pressor actions through A. Recently it was reported that angiotensin-immunoreactive fibers project from the SFO to the ventral NM (Gray et al., 1982). The ventral NM may therefore be a projection field for neurons releasing angiotensin as either a transmitter or a co-transmitter. This finding correlates well with the studies of others demonstrating that angiotensin excites single units within the NM (Phillips et al., 1979) and suggests that the ventral NM (area C in model) may indeed be sensitive to angiotensin. It is easy to predict from the model that destruction of B alone would not eliminate the ability of the system to respond to angiotensin since A and C would remain unaffected. This interpretation does not di-
minimize the significance of the role of the SFO in the normal pressor response to angiotensin. It underscores, however, the need for precise definition of the boundaries of lesions that are produced to destroy the SFO. Multiple penetrations are required to destroy the SFO. In an effort to destroy the ventral stalk of the SFO, electrolytic damage may be incurred in the dorsal and circumcommissural NM. It may be predicted that any damage to C would surely diminish the central response to angiotensin.

The medial preoptic nucleus (MPO) lies adjacent to both the OVLT and the ventral NM. The most medial portion of this nucleus is well within the diffusional radius of the OVLT as indicated by the spread of horseradish peroxidase (Broadwell and Brightman, 1976). Drinking behavior has been elicited with small localized injections into MPO indicating the presence of receptors to angiotensin II (Swanson et al., 1978). Projections from this region follow two routes, periventricular and lateral hypothalamic. The former are damaged by the AHKC, which transects the caudal half of the medial preoptic nucleus in the horizontal plane. Dense neural and vascular interconnections exist between the MPO and OVLT (Palkovits et al., 1978; Palkovits and Zaborszky, 1979).

Retrograde labeling studies have provided anatomical evidence of afferent projections to the median preoptic nucleus from brain regions that are known to be involved in cardiovascular regulation (Saper et al., 1983; Saper and Levisohn, 1983). In these studies, direct input into the ventral median preoptic (area C in model) was demonstrated from the subformical organ, the paraventricular hypothalamic nucleus (from the parvocellular periventricular, medial, dorsal, ventral, and posterior subnuclei), anterior and preoptic periventricular nucleus, cells in the ventrolateral preoptic area, parabrachial nucleus, the nucleus of the solitary tract and the ventrolateral medulla. Significantly, the median preoptic region is innervated directly by catecholaminergic cell groups in the ventrolateral medulla and the nucleus of the solitary tract. These include the A1 and A2 noradrenergic cell groups and the C1 and C2 adrenergic cell groups (Saper et al., 1983).

This vast array of afferent projections to the NM suggests that descending cardiovascular information from the NM is highly integrated with ascending information from other cardiovascular centers. The NM is strategically positioned to integrate afferent humoral and neural signals that are involved with cardiovascular regulation. The NM may therefore be an important modulatory center involved with cardiovascular reflex activity.

Finally, the AHKC apparently severs all functional efferents from A, B, and C that carry angiotensin II pressor information, because the knife cut totally eliminates the centrally mediated pressor response to angiotensin. Although the efferent connections of the NM have not yet been precisely described, the model predicts that efferents from C follow a periventricular route to the medial hypothalamic region. Destruction of the medial periventricular hypothalamus eliminates the pressor response to centrally administered angiotensin (Fink et al., 1978). Direct connections exist between the dorsal preoptic area and the median eminence (Weigand and Price, 1980). Many cells in the periventricular stratum are labeled retrogradely from the medial basal hypothalamus (Conrad and Pfaff, 1976a; Palkovits and Zaborszky, 1979). The density of these labeled cells is greatest between the caudal anterior commissure and the paraventricular nuclei (Weigand and Price, 1980). These connections are transected by the AHKC. Some cells in the nucleus medianus are also labeled (Conrad and Pfaff, 1976a, 1976b; Weigand and Price, 1980). The medial preoptic nucleus is also heavily labeled retrogradely by horseradish peroxidase injections in the ventromedial hypothalamic region. All of these studies describe connections that could conceivably be carrying neural information resulting from activation of the SFO or OVLT by angiotensin II. A recent report indicated that ventral bilateral coronal knife cuts in a plane just posterior to the anterior commissure were able to reduce but not totally eliminate the pressor response to intracerebroventricularly administered angiotensin II (Bealer, 1982). The knife cuts would partially sever the projection from C to the medial basal hypothalamic region. The projection pathway is almost vertical in this plane, as mapped by us in a previous study using lesions and knife cuts (Hartle et al., 1982). Horizontal knife cuts in the present study produced a total blockade in 30/33 animals tested. Previous experience in our laboratory indicates that only 3/30 animals tested, with vertical knife cuts in the anterior hypothalamic region, were unresponsive to centrally administered angiotensin II (Hartle, 1981). Horizontal knife cuts in the medial preoptic and anterior hypothalamic regions are therefore more uniformly effective in severing the connections from the lamina terminalis that project ventrally and caudally to the medial basal hypothalamus that mediate the central pressor responses to angiotensin II.

Conclusion

In previous studies, we functionally mapped the forebrain pressor pathway activated by intracerebroventricular administration of angiotensin II (Hartle et al., 1982). In the present studies, we extend those studies and demonstrate that destruction of the angiotensin II pressor efferent projection from the AV3V region not only produces the same angiotensin II pressor deficits attributed to destruction of either AV3V region or the SFO, but also attenuates renin-dependent aortic ligation hypertension. The model we propose is of the rat forebrain angiotensin II pressor system only. Other functions of the AV3V region and of the SFO (e.g., drinking behavior or gonadotrophic functions) appear to depend upon entirely different circuitry.
The model we propose is a hypothetical scheme, supported by current experimental evidence, that can be tested by future anatomical, pharmacological, and electrophysiological studies of the circuitry of the angiotensin II pressor system of the rat forebrain. The functional significance of the angiotensin II pressor system is apparent in renin-dependent renal hypertension.

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Dr. Michael Brody's present address is: Department of Physiology, Emory University School of Medicine, Atlanta, Georgia 30322.

Address for reprints: Michael J. Brody, Ph.D., Department of Pharmacology, Bowman Science Building, The University of Iowa, Iowa City, Iowa 52242.

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INDEX TERMS: Median preoptic nucleus • Nucleus medianus • Organum vasculosum lamina terminalis • Subfornical organ • Anteroventral 3rd ventricle region • Angiotensin II • Renin-dependent hypertension • Aortic ligation hypertension • Blood pressure regulation

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D K Hartle and M J Brody

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