Area Postrema Is Critical for Angiotensin-Induced Hypertension in Rats

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SUMMARY The effect of surgical ablation of the area postrema on acute (5–10 minutes) and chronic (5–10 days) increases in mean arterial pressure produced by intravenous infusion of angiotensin II in conscious, instrumented rats was studied. In agreement with previous studies, pressor responses of area postrema-ablated rats (n = 11) to acute angiotensin II infusion were identical to those of control sham-lesioned rats (n = 13). In these same rats, however, a 5-day infusion of angiotensin II produced a sustained hypertension in the sham-lesioned group whereas mean arterial pressure was increased only transiently (1–3 days) in the area postrema-ablated rats. No differences before infusion of arterial pressure, heart rate, water intake, urinary sodium excretion, and urinary potassium excretion were observed between sham-lesioned and area postrema-ablated rats; only arterial pressure was changed significantly during angiotensin II infusion in either group. Twenty-four hours after terminating angiotensin II infusion, mean arterial pressure was within the normotensive range in both sham-lesioned and area postrema-ablated rats. In a separate group of sham-lesioned (n = 13) and area postrema-ablated (n = 12) rats, angiotensin II was infused intravenously for a 10-day period; mean arterial pressure was increased significantly over the entire 10-day infusion in sham-lesioned rats, but for only 1 day in area postrema-ablated rats. An intact area postrema appears necessary for the development of chronic, but not acute, hypertension during intravenous infusion of angiotensin II in the rat. (Hypertension 9: 355-361, 1987)

KEY WORDS • central nervous system • renin-angiotensin system • arterial pressure • sodium and water balance • circumventricular organs

THERE is little doubt that angiotensin II (ANG II) is an important pathogenic factor in some forms of clinical and experimental hypertension. The utility of reducing plasma ANG II levels, primarily with angiotensin converting enzyme inhibitors, in the treatment of human hypertension has renewed interest in the mechanisms by which ANG II causes a sustained increase in arterial pressure. Chronic (days to weeks) intravenous administration of ANG II to experimental animals and to humans has been used to produce a relatively “pure” angiotensin-dependent hypertension, uncomplicated by other physiological interventions commonly employed to create experimental hypertension. A detailed analysis of many of these studies led to the conclusion that ANG II does not cause chronic hypertension by direct vascular constriction, but rather by an ability to stimulate a more slowly developing (hours to days) pressor mechanism: a mechanism whose sensitivity to ANG II increases with prolonged exposure to the hormone. Actions of ANG II on aldosterone secretion, renal handling of salt and water, and the central nervous system all have been proposed to be responsible for this chronic pressor effect.

In a recent series of experiments, the role of the central nervous system in chronic ANG II-induced hypertension was examined in rats. Sustained blockade of periventricular ANG II receptors in brain, using long-term intracerebroventricular infusion of an ANG II receptor antagonist, failed to affect the development of hypertension during a 5-day i.v. infusion of ANG II. Although this result brought into question the existence of an important contribution of circumventricular ANG II receptors to the pressor actions of circulating ANG II, other recent work demonstrated that ANG II antagonists delivered intracerebroventricular-

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ly readily penetrated some circumventricular structures, such as the organum vasculosum of the lamina terminalis, but were relatively ineffective in displacing blood-borne ANG II from two of the circumventricular organs where the peptide is presumed to act: 1) the subfornical organ and 2) the area postrema (AP). Thus, if circulating ANG II acts in the brain to bring about a maintained pressor response, these two structures seemed likely sites of action. Subsequently, we demonstrated that electrolytic ablation of the subfornical organ in rats did not attenuate hypertension development during 5-day i.v. ANG II infusion. The current studies, therefore, examined the contribution of the AP to chronic ANG II–induced hypertension.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 250 to 400 g were used in these studies. Before lesion surgery all rats were housed in large plastic cages in temperature-controlled and light-cycled rooms. They received tap water and standard rat chow (Purina, St. Louis, MO, USA) ad libitum throughout this period.

Ablation of the Area Postrema

Immediately before the lesion procedure, all rats were anesthetized with a pentobarbital–chloral hydrate mixture and then immobilized in a stereotaxic instrument (David Kopf, Tujunga, CA, USA). The atlantooccipital membrane then was exposed through a dorsal midline incision. In AP-lesioned rats, an incision was made in this membrane that allowed access to the dorsal medulla in the region of the AP. With the help of a dissecting microscope, the AP was clearly visible beneath the surface of the medulla. A tungsten microelectrode (Micro Probe, Clarksburg, MD, USA) was used to make electrolytic lesions of the AP. The tip had been cut from the electrode, leaving a 127-μm (outside diameter) shaft. No insulation was removed from the shaft; therefore, only the cross-sectional diameter of the tip itself (where the cut had been made) was uninsulated. This electrode was positioned on the surface of the AP (i.e., the electrode was not sunk into the subsurface body of the AP). The AP was ablated by passing a 700-μA anodal current through this electrode for a total period of 9 to 12 seconds (6.3–8.4 mC). The current was generated by a Grass DC Constant Current Lesion Maker (Quincy, MA, USA). In most rats, the current was passed for 9 seconds, as follows: on the center of the AP surface for 3 seconds; on the anterior left surface for 2 seconds; on the anterior right surface for 2 seconds; and on the posterior central surface (near calamus scriptorius) for 2 seconds. In several rats, however, it was clearly visible that a subtotal lesion had been made with this procedure; in these rats, an additional 2 to 3 seconds of current was passed where AP tissue still remained intact.

Since the AP was clearly visible, this technique allowed us to pass only the minimum current necessary to destroy the AP. At the same time, the technique made possible a small and specific lesion and ensured that a complete lesion was produced in virtually every rat. Skoog and Mangiapane have shown that this technique results in 1) little visible damage to the nearby nucleus of the solitary tract, 2) no evidence of functional impairment to the baroreceptor reflex, and 3) no increase in lability of mean arterial pressure (MAP). Sham-operated rats were given the same surgical treatment given to the AP-lesioned rats, except that a lesion was not made. After the operation, all rats were given 100,000 U of procaine penicillin i.m. and returned to their cages.

Lesions were produced in all rats in the laboratory of one author (M.L.M.), after which the rats were sent to another laboratory (G.D.F.) for ANG II infusion. At the end of the infusion protocol, each rat was perfused intracardially with buffered formalin. Its brain was then removed, coded, and returned to the original laboratory for a blind histological analysis of lesion size and location. Serial frozen sections (30 μm thick) were cut through the region of the caudal medulla, slide-mounted, and then stained for Nissl substance (cresyl violet stain). The sections were then carefully examined by light microscopy to determine the lesion location. The examiner had no knowledge of the data for any particular animal. Rats whose brains had no discernable damage were assigned to the sham classification (n = 26). Rats that sustained more than 80% destruction of the AP were assigned to the APX classification (n = 26). Twenty-one of the 26 APX rats sustained minor damage to the commissural subnucleus of the nucleus tractus solitarii (NTS), which is directly subjacent to the entire extent of the AP. In no rat was more than 10% of the commissural subnucleus destroyed. No other NTS structures were damaged. Thus, no rats showed any damage to the medial subnucleus or the substantia gelatinosa of the NTS. Visible NTS damage was therefore practically nil. In addition, the dorsal motor nucleus of the vagus was undamaged in all rats. Photomicrographs of representative lesions from each group are shown in Figure 1.

Chronic Infusion Technique

Two to 5 weeks after lesion surgery, polyvinyl-silicone rubber catheters were inserted in the abdominal aorta and vena cava of each rat through the left femoral vessels; a metal spring tether was attached to the skull with small screws and dental acrylic during the same operation. These procedures were performed in rats anesthetized with sodium pentobarbital (50 mg/kg i.p.). Throughout the remainder of the study rats were housed in standard metal metabolism cages. The tether was attached to a plastic hydraulic swivel that was mounted above the metabolic cage, and the venous catheter was connected to a syringe pump (Model 975; Harvard, Millis, MA, USA) through the swivel. The arterial catheter, which exited the cage inside the protective tether, was filled with heparinized solution and plugged when not in use.

The rats received 15 to 20 g of sodium-deficient rat
chow (Na+, 0.002 mEq/g; K+, 0.3 mEq/g) daily and free access to distilled water in calibrated bottles. Isotonic saline (0.9% NaCl in water) was infused continuously (24 hr/day) intravenously throughout the study at a rate of 1.66 ml/hr to provide a fluid intake of 40 ml/day and a fixed sodium intake of 6.2 mEq/day. High sodium intake (two to three times normal) accelerates and potentiates hypertension development during ANG II infusion. Each rat also received twice-daily injections of ampicillin (10 mg i.v.) after being placed in a metabolism cage.

The experimental protocols were started 3 days after catheterization of the rats and their housing in metabolism cages. Two days of control measurements were followed by 5 or 10 days of i.v. infusion of ANG II amide (Hypertensin, CIBA-Geigy, Summit, NJ, USA) at 10 ng/min (added fresh each day to the saline infusate); 2 days of postinfusion recovery values then were obtained. All measurements were performed in the morning (0800-1100). Arterial blood pressure was recorded by connecting the aortic catheter to a low volume displacement pressure transducer (Model P50; Statham, Oxnard, CA, USA) for 10 to 30 minutes. The lowest stable pressure was used to calculate MAP using the formula $MAP = (2 \times diastolic \, pressure + systolic \, pressure)/3$. Heart rate (HR) was determined by counting the arterial pressure pulses recorded on a Grass polygraph (Model 7). Water intake was calculated as the sum of voluntary drinking volume and the 40 ml/day infused volume. Urine volume was determined by 24-hour collection into calibrated tubes placed below the metabolic cages. Urinary sodium and potassium excretions were calculated by multiplying daily urine volume by the appropriate electrolyte concentration measured in an aliquot of the daily urine (flame photometry).

**Acute Pressor Effects of ANG II**

In some rats the changes in MAP and HR produced by acute (5–10 minutes) i.v. infusions of ANG II were examined. These tests were performed 2 to 3 days after termination of the chronic ANG II infusion protocol already described, and while the rats remained tethered in metabolism cages and on fixed sodium intake. The arterial catheter was attached to a pressure transducer (Statham P50) and the venous catheter to a syringe-type infusion pump (Harvard, Model 975). After obtaining stable values of MAP and HR (determined as already described), ANG II was infused at doses of 3, 10, and 30 ng/min for 5 to 10 minutes or until new steady state values of MAP and HR were reached. Doses were infused in a sequential fashion and in ascending order.

**Experimental Groups**

This study consisted of two separate, but related, experiments on two different groups of rats. The first experiment used 13 sham-lesioned rats and 14 rats with histologically verified ablation of the AP (APX). These animals received ANG II (10 ng/min i.v.) for 5 days; measured variables included MAP, HR, water intake, calculated water balance (daily water intake minus urine volume), and urinary sodium and potassium excretion. In all sham-lesioned rats in this group, and in 11 out of 14 APX rats, acute pressor activity of ANG II also was assessed.

The second experiment was performed in 13 sham-lesioned and 12 APX rats. These animals received ANG II (10 ng/min i.v.) for 10 days; measured variables included MAP, HR, water intake, and calculated water balance only. These rats were not tested for acute pressor responsiveness to ANG II.

**Statistical Analyses**

The data were analyzed using a mixed design (split-plot) analysis of variance followed by the "protected" least significant difference test for individual comparisons over time. Comparisons between sham-lesioned and APX rats at any given time were performed using Tukey’s test for nonconfounded comparisons. A p value of less than 0.05 was the criterion for statistical significance.

**Results**

The results of 5-day ANG II infusion into conscious sham-lesioned ($n = 13$) and APX ($n = 14$) rats are illustrated in Figure 2. During the control preinfusion period, there were no significant differences between sham-lesioned and APX rats in any measured variable. When ANG II infusion (10 ng/min i.v.) was begun, MAP rose significantly within 24 hours in both groups of animals. In sham-lesioned rats MAP was maintained 20 to 25 mm Hg above control values throughout the 5-day infusion period. In APX rats MAP was significantly elevated on the first 3 days of ANG II administration, but by Days 4 and 5 MAP had returned to a level not significantly above control values. After
5 days of ANG II infusion, MAP was significantly lower in APX rats than in sham-lesioned animals. During the postinfusion recovery period MAP was similar in both groups of rats and was not significantly different from the control period measurements. No change in HR, water intake, water balance, or urinary sodium or potassium excretion was observed in either sham-lesioned or APX rats during ANG II infusion.

The effect on MAP and HR of short-term (5–10 minutes) ANG II infusion in sham-lesioned \((n = 13)\) and APX \((n = 11)\) rats is shown in Figure 3. Graded increments in ANG II infusion rate produced dose-dependent increases in MAP; these increases were identical in sham-lesioned and APX rats. The bradycardia accompanying the pressor effects of ANG II was consistently larger in APX than in sham-lesioned rats, but this difference did not achieve statistical significance at any infusion rate.

The data from 10-day ANG II infusion in sham-lesioned \((n = 13)\) and APX \((n = 12)\) rats are illustrated in Figure 4. In the preinfusion period there were no significant differences between sham-lesioned and APX rats in MAP, HR, water intake, or water balance. In sham-operated rats ANG II caused a progressive rise in MAP over the 10-day infusion; MAP was approximately 35 mm Hg higher on Days 8 through 10 of infusion than during the control period. In contrast, in APX rats ANG II significantly increased MAP only during the first 24 hours of administration; during the remainder of the infusion MAP was not significantly increased compared with preinfusion values. On Days 4 to 10 of ANG II infusion MAP was significantly higher in sham-operated than in APX rats. During the postinfusion period MAP was the same in sham-lesioned and APX rats and was not significantly different from preinfusion control values. Neither HR, water intake, nor water balance differed in the two groups of rats throughout the experimental protocol; however, both sham-lesioned and APX rats exhibited 2 to 3 days of decreased water balance during ANG II infusion.
A critical role for the central nervous system in the acute and chronic pressor actions of ANG II was postulated many years ago (see Reference 5). Strong support for this idea was provided by the cross-circulation experiments of Bickerton and Buckley and by demonstrations that very low amounts of ANG II administered selectively into the cerebral circulation could cause a neurogenically mediated rise in MAP during both short-term and long-term infusions. Early experiments in dogs suggested that the major site of ANG II interaction with the central nervous system was the brainstem. Subsequent lesion studies established the AP — a circumventricular structure with relatively high blood-brain permeability — as the specific brainstem site at which circulating ANG II acted to produce neurogenic amplification of the peptide’s acute, direct pressor effects. In recent years, extensive investigation of the neurochemistry, neuroanatomy and physiology of the AP as it related to cardiovascular control in dogs has been performed by Ferrario and co-workers. Their work established, among other findings, the existence of a sympathoexcitatory neural pathway emanating from the AP in the dog, confirmed earlier reports of a substantial connectivity between the AP and the NTS, and showed marked, chronic alterations in cardiovascular regulation in dogs subjected to surgical ablation of the AP. Thus, previous work clearly suggested that an action of ANG II on the AP could be an important contributor to the hypertension produced when blood levels of the hormone are chronically elevated. Although such a possibility has been mentioned by numerous workers (see Reference 5), we believe the current study represents the first attempt to directly test this hypothesis.

Earlier investigations on the cardiovascular functions of the AP in the rat revealed that ablation of the structure neither consistently affected arterial pressure in the normotensive animal nor altered the pressor response to acute intravenous administration of ANG II. These latter findings are in marked contrast to similar studies in dog, cat, and rabbit, in which ablation of the AP was shown to significantly blunt pressor responses to brief i.v. infusions of ANG II. Despite this apparent drawback to the use of a rat model for studies on ANG II interactions with the AP, three additional considerations nevertheless encouraged our use of this species in the experiments reported here: 1) receptors for ANG II are found in high concentration in the rat AP, and intravenously infused ANG II readily penetrates the structure; 2) stimulation of the AP either electrically or with direct microinjections of ANG II causes an acute pressor response in the rat; and 3) recent work on chronic ANG II–induced hypertension in rats pointed to the AP as a likely critical site for hormone interaction with the central nervous system (see Introduction).

The results presented here confirm other reports that integrity of the AP in the rat is not necessary for a full pressor response to short-term i.v. (5–10 minutes) infusions of ANG II. The results also support the notion that the mechanism underlying the chronic hypertension produced by ANG II differs from that responsible for its short-term pressor effect, since AP ablation eliminated chronic ANG II–induced hypertension in the same rats in which it failed to affect the pressor response to acute hormone infusion. In animals with AP lesions ANG II infused at 10 ng/min caused a significant increase in MAP of about 15 mm Hg; this increase occurred within minutes of starting the infusion and was maintained for 1 to 3 days before pressure returned toward control levels despite continued hormone infusion. In the absence of conclusive proof, we speculate that this early increase in MAP represents primarily the direct vasoconstrictor action of ANG II, as well as some indirect central and peripheral neurogenic amplification of the direct pressor response. This latter conclusion is based on reports that forebrain lesions and central injection of ANG II antagonists attenuate acute pressor effects of ANG II in rats and that peripheral sympathoinhibition also reduces ANG II vasoconstriction in some vascular beds. Although the AP in the rat does not appear to contribute to the acute pressor actions of blood-borne ANG II, this interpretation must be made with caution in light of recent evidence that “resting” plasma renin activity is elevated in AP-ablated rats on normal sodium intake (unpublished observations, 1986); thus, equivalent pressor responses to exogenous ANG II in normal and AP-ablated rats may be achieved only at substantially higher plasma concentrations of ANG II in rats with lesions.

The present experiments clearly demonstrate, on the other hand, that an intact AP is necessary for the production of sustained hypertension with long-term i.v. infusion of ANG II. In the initial study with 5-day peptide infusion, rats with AP lesions showed signifi-
cant elevations in MAP only on the first 3 days of infusion; pressure subsequently fell toward control levels despite continued ANG II infusion. In normal rats, MAP was elevated approximately 25 mm Hg after 1 day of infusion and remained at a similar level throughout the remainder of the infusion period. Since this first study suggested that MAP in AP-ablated rats receiving ANG II would return to control levels during more prolonged infusions, a second experiment was performed using 10-day hormone administration in a separate group of animals. In this second study, rats with AP lesions exhibited a significant elevation in MAP only on Day 1 of ANG II infusion; by Day 6 average MAP had returned to control period values and remained there throughout the final 4 days of infusion. In contrast, normal rats showed a progressive rise in MAP during the entire 10-day ANG II infusion period.

The mechanism by which destruction of the AP in rats interferes with chronic ANG II-induced hypertension is as yet unknown; this conclusion is predicated partly on existing uncertainty about the physiological processes that normally mediate angiotensin-induced hypertension. Direct vasoconstrictor effects appear important only during the first few days of ANG II infusion. In dogs chronic angiotensin infusion causes renal sodium retention2-4, 28 that is reversed only when systemic arterial pressure rises.26 The dependence of angiotensin-induced hypertension on adequate sodium intake3 further points to a possible renal mechanism. In rats, however, this laboratory18 and others27 have failed in previous studies to observe sodium or water retention during chronic i.v. ANG II infusion. The current studies also did not reveal sodium or water retention in either normal or AP-ablated rats during ANG II infusion. Abnormalities in sodium and water homeostasis have been reported, nonetheless, in rats with lesions of the AP and surrounding tissue.28 Furthermore, other brain lesions that clearly disrupt body fluid regulation (i.e., anteroventral third ventricle lesions) also attenuate hypertension development during i.v. ANG II infusion in rats.29 Thus, these factors cannot be excluded as a possible explanation for the failure of AP-ablated rats to exhibit chronic hypertension during ANG II infusion.

The AP has interconnections with the NTS17, 28 and circulating ANG II causes marked alterations in baroreceptor reflex function.30 Direct stimulation of the AP produces sympathoexcitation and pressor responses in dogs16 and rats. 21 Several reports also have indicated that ANG II can cause a slowly developing, neurogenically mediated rise in arterial pressure during long-term administration, either intravenously or into the circulation of the brainstem (see Reference 5).12, 13 More recent studies, however, have not supported increased sympathetic nervous system activity as a contributor to ANG II-induced hypertension.21, 35 It remains to be determined whether or not AP ablation prevents the chronic hypertensive effect of ANG II by interference with baroreceptor reflex or autonomic blood pressure control mechanisms, or with both.

Since the AP is in very close proximity to the NTS, possible damage to this structure must be considered as a factor contributing to the current findings with AP ablation. Careful blind histological evaluation of the NTS region in all rats used in this study indicated little, if any, visually apparent injury. In addition, AP-ablated rats showed normal cardiac slowing during acute ANG II-induced pressor responses (see Figure 3), and arterial pressure lability was not noted during daily 15- to 30-minute recordings of phasic arterial pressure. Frequency distributions of arterial pressure in AP-ablated rats in another study30 also were identical to those of normal rats, providing further evidence that removal of the AP does not attenuate baroreceptor reflex function. Finally, a previous study in dogs33 demonstrated that complete sinoaortic denervation did not affect the final blood pressure level achieved during chronic ANG II infusion. Further studies in rats are planned, nonetheless, to evaluate baroreceptor reflex mechanisms in ANG II-induced hypertension.

In summary, an intact AP is required for the normal development of hypertension during chronic intravenous ANG II infusion in the rat. The mechanisms responsible for the effects of AP ablation in this model of hypertension are as yet unknown.

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