Lateral Parabrachial Nucleus and Angiotensin II–Induced Hypertension

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The objective of this study was to determine if ablation of the lateral parabrachial nucleus (LPBN) would prevent angiotensin II–induced hypertension in rats. Thirteen male Sprague-Dawley rats were studied. Bilateral electrolytic lesions in the LPBN were produced in six rats; the remaining seven rats were subjected to sham lesion surgery only. All rats were instrumented with vascular catheters and housed in metabolism cages. Daily measurements during the 16-day protocol included arterial pressure, heart rate, water intake, urine output, and urinary sodium excretion. Periodically throughout the protocol depressor responses to ganglion blockade and to blockade of V$_2$-type vasopressin receptors also were measured. The protocol was divided into three control-period days, 10 days of continuous (24 hr/day) angiotensin II infusion (10 ng/min i.v.), and three recovery-period days. There were no significant differences between the two groups of rats for any variable during the control period. During angiotensin II infusion, sham-lesion rats exhibited a progressive increase in arterial pressure and the depressor response to ganglion blockade and a decrease in urinary sodium excretion. No other variable was significantly changed. In rats with LPBN lesions, arterial pressure was significantly increased only on days 1 and 3 of angiotensin II infusion. No other variable was affected.

It was concluded that ablation of the LPBN in rats prevented sustained hypertension during intravenous infusion of angiotensin II by interfering with neurogenic pressor mechanisms normally activated by the peptide. (Hypertension 1991;17:1177–1184)

Drugs that interfere with the renin-angiotensin II (Ang II) system have proven to be effective antihypertensive agents. This has caused renewed interest in elucidating the precise mechanism by which Ang II contributes to the pathogenesis of hypertension. The direct constrictor effect of circulating Ang II on vascular smooth muscle is unlikely to be an important factor except when plasma concentrations of the peptide are quite high.12 Since this is rarely the case in hypertensive individuals, other actions of blood-borne Ang II have been implicated, including 1) renal effects leading to sodium and water retention13,4; 2) release of other pressor hormones, such as aldosterone or vasopressin1,4,5-6; 3) augmentation of sympathetic neurotransmission5,6; and 4) an action on the brain to increase sympathetic nervous system activity.9,10 Local tissue generation of Ang II in blood vessels, kidney, adrenal gland, or brain, independent of the circulating hormone, also could play an etiologic role in some forms of hypertension.11

Recent studies from this laboratory highlighted the importance of brain mechanisms in the hypertension caused by circulating Ang II. Ablation of the area postrema, a brain stem circumventricular organ containing neurons known to be activated by blood-borne Ang II,12 was shown to prevent chronic hypertension in rats subjected to either long-term intravenous Ang II infusion13 or unilateral renal artery constriction.14 The area postrema projects to numerous brain nuclei involved in the regulation of cardiovascular function,15 and stimulation of neurons in the area postrema causes a sympathetically mediated increase in arterial pressure.16 Thus, it has been proposed that a key feature of the hypertension caused by blood-borne Ang II is an action of the peptide on the area postrema to augment sympathetic nervous system activity.13 A full understanding of the interaction of Ang II with neural blood pressure control systems, however, would require further elucidation of the pathways involved in the neural response to circulating Ang II.

The major efferent projections of the area postrema are to the adjacent nucleus of the tractus...
solitarius and to the lateral parabrachial nucleus (LPBN) in the pons. Substantial evidence already exists supporting the importance of the area postrema/nucleus tractus solitarius pathway in some of the central cardiovascular actions of Ang II. The objective of the current investigation was to determine if areas of the LPBN known to receive neuronal input from the area postrema participate in Ang II–induced hypertension. Specifically, it was hypothesized that electrolytic ablation of the lateral subnuclear region of the LPBN would prevent hypertension development during chronic intravenous infusion of Ang II in rats.

**Methods**

**Animals**

Male Sprague-Dawley rats initially weighing 300–350 g were used in these experiments. Before lesion placement in the LPBN, the animals were housed individually in hanging wire cages in temperature-controlled and light-cycled quarters. Regular rodent chow and tap water were provided ad libitum.

**Lesion Production**

This segment of the experiment was conducted in San Antonio, Tex. Rats were anesthetized with choral hydrate (300 mg/kg i.p.). The head of each rat was positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, Calif.) designed specifically for this species. The skull was exposed via a dorsal midline incision and leveled between lambda and bregma. A hole was opened through the skull at 9.3 mm posterior to the bregma and 2.4 mm lateral to the midsagittal suture. A stainless steel electrode was lowered 5.9 mm ventral to the surface of the dura, and a 1.6 mA current was passed through the electrode for a total of 4 seconds. A similar procedure was carried out on the contralateral side. Sham operation was determined from calibrated water bottles. The brains were removed, stored in a 10% formaldehyde solution, and sent to San Antonio for a blinded histological analysis. Coronal sections were cut on a freezing microtome at 40 μm intervals throughout the extent of the LPBN, mounted on slides, and stained with cresyl violet. Lesion extent was assessed using light microscopy.

**Histology**

After completion of the cardiovascular experiments, each rat was deeply anesthetized with sodium pentobarbital (40 mg/kg i.v.) and perfused transcardially with saline followed by a 10% formaldehyde solution. The brains were removed, stored in 10% formaldehyde solution, and sent to San Antonio for a blinded histological analysis. Coronal sections were cut on a freezing microtome at 40 μm intervals throughout the extent of the LPBN, mounted on slides, and stained with cresyl violet. Lesion extent was assessed using light microscopy.

**Experimental Preparation**

Rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and placed on a heated surgical table. Catheters constructed from polyvinyl chloride and silicone rubber tubing were inserted through the femoral artery and vein such that their tips lay in the abdominal aorta and vena cava, respectively. An additional catheter was positioned in the right jugular vein. The ends of the catheters were tunneled to the skull, then passed through a protective spring; the femoral venous catheter was attached to a hydraulic swivel. One end of the spring was attached to the swivel, and the other was fixed to the rat’s skull using small screws and dental acrylic. The arterial catheter and the jugular venous catheter were filled with a heparinized solution and plugged when not in use. Penicillin and dihydrostreptomycin in aqueous suspension (0.2 ml i.m., Combiotic, Pfizer, Inc., New York) was injected at the time of surgery. Each rat then was housed permanently in a separate stainless steel metabolism cage to allow quantitation of urinary volume and electrolyte excretion. Water intake was determined from calibrated water bottles. The hydraulic swivel was mounted above the cage to allow free movement within the cage at all times. The venous catheter was attached, via the swivel, to an infusion pump, allowing the rat to receive a continuous (24 hr/day) intravenous infusion of sodium chloride solution (5 ml/day) throughout the entire protocol. A sodium-deficient rat chow (0.002 meq Na+/g, 0.3 meq K+/g) was the only food made available to the rats, and sodium intake was fixed at 6 meq/day throughout the study. Arterial pressure was recorded for 10–30 minutes between 8:00 AM and noon by attaching the exteriorized arterial catheter to a pressure transducer without disturbing the rat. Heart rate was counted directly from the pulsatile pressure tracing. Urinary sodium concentration was determined in daily aliquots of urine using an electrolyte analyzer (model E2A, Beckman Instrument Co., Schiller Park, Ill.). Daily urinary sodium excretion was calculated by multiplying urinary sodium concentration by 24-hour urinary volume.

**Experimental Protocol**

At least 3 days were allowed from the time of catheter placement to the beginning of the 16-day experimental protocol. Before Ang II infusion, 3 days of control measurements were obtained, including arterial pressure and heart rate and 24-hour water intake, urine volume, and urinary sodium excretion. These measurements were continued throughout the entire protocol. On the second control-period day, arterial pressure was measured before and exactly 5 minutes after an injection of the ganglion blocker hexamethonium bromide (20 mg/kg i.v.). On the third control period day, similar measurements were taken after injection of a V1-selective vasopressin (AVP) receptor antagonist ([d(CH2)5Tyr(Me)]-AVP, 10 μg/kg i.v.). After measurements were completed on the third control-period day, Ang II ([Ile5]-Ang II, Sigma Chemical Co., St. Louis, Mo.) was added to the intravenous infusate such that the peptide was delivered continually intravenously at a rate of 10
ng/min for the next 10 days. Fresh peptide was added to the infusate on a daily basis. Finally, a 3-day recovery period was allowed during which time Ang II was no longer added to the intravenous infusate. The response of arterial pressure to ganglionic blockade was reassessed on days 4 and 8 of the Ang II-infusion period and on day 2 of the recovery period. The response to AVP receptor blockade was reassessed on days 5 and 9 of the Ang II-infusion period and on day 3 of the recovery period.

**Statistical Analyses**

Data were analyzed using a mixed design analysis of variance (one "between groups" factor and one "repeated measures" factor). Post hoc testing was performed using the protected least significant difference test. A value of $p<0.05$ was considered statistically significant.

**Results**

Animals were accepted into the lesion group only if the damage was bilateral, centered on the external lateral subnucleus of the LPBN, and extended throughout most of the rostral-caudal extent of the LPBN. The most rostral portion of the nucleus was not ablated in all cases. Other structures commonly damaged were the extreme and dorsal subnuclei of the LPBN, the Kölliker-Fuse nucleus, the lateral tip of the medial parabrachial nucleus, the ventrolateral edge of the ventral spinocerebellar tract, the dorsal edge of the supratrigeminal nucleus, and the dorsal portion of the principal sensory nucleus of the trigeminal nerve. Sham lesions were examined to ensure that the electrode did not damage target tissue (Figure 1). Based on these criteria, six rats were included in the lesion group, and seven animals comprised the sham-lesion group. Figure 2 depicts the area common to all lesions in rats used for this study.

Mean arterial pressure and heart rate data are summarized in Figure 3. Before Ang II infusion, mean arterial pressure and heart rate did not differ between the two groups. Infusion of Ang II in sham rats caused a significant increase in mean arterial pressure throughout the 10-day infusion (except on day 4 of the infusion period); mean arterial pressure was no longer significantly higher than control period values 48 hours after Ang II infusion was terminated. In rats with LPBN lesions, Ang II infusion increased mean arterial pressure significantly above control-period values only on infusate days 1 and 3. Mean arterial pressure was significantly lower in lesion rats than in sham-lesion rats on days 9 and 10 of the infusion period and days 2 and 3 of the recovery period. Sham-lesion rats tended to exhibit bradycardia during the first days of Ang II infusion, but this was significant only on day 5 of the infusion period. No change in heart rate was observed in lesion rats during Ang II infusion. As shown in Figure 4, water intake, urine output, and sodium excretion did not differ during the control period between the two groups. Infusion of Ang II did not significantly alter water intake or urine output. In sham-lesion rats, Ang II infusion resulted in small but significant decreases in urinary sodium excretion (days 1, 2, 4, 5, 7, 8, and 10); no effect was seen in rats with LPBN lesions.

Administration of the V$_2$-selective AVP receptor antagonist did not change mean arterial pressure significantly at any time during the experiment in either group of rats (data not shown). Changes in mean arterial pressure in response to ganglion blockade with hexamethonium are summarized in Figure 5. During the control period, the depressor responses in sham and lesion rats were identical. In sham-lesion rats, infusion of Ang II caused an increase in the depressor response to ganglion blockade, but this reached statistical significance only during the later stages of the infusion period (day 8). No significant changes in depressor responses were observed in lesion rats at any time. Heart rate increased slightly on the average after hexamethonium administration in both groups, and this effect was not significantly changed during Ang II infusion.

**Discussion**

This investigation was predicated on the theory that circulating Ang II in Sprague-Dawley rats causes hypertension at least in part via an action on (or through) the area postrema to augment sympathetic nervous system activity. Evidence has been presented both for and against the existence of an absolute increase in sympathetic nervous system activity in rats receiving chronic Ang II infusions. Most investigators agree, however, that the hypertension associated with increased plasma Ang II concentrations in rats involves an enhanced neurogenic pressor activity as assessed using ganglion blockade or adrenergic receptor blockade. A role for the area postrema in this model of hypertension was suggested by our finding that ablation of the area postrema prevented chronic hypertension in rats receiving long-term Ang II infusions. That study did not address, however, the potential brain pathways through which Ang II, acting on area postrema neurons, might influence sympathetic regulation of arterial pressure. The LPBN receives a major neuronal projection from the area postrema, and lesions in the LPBN have been shown to produce some of the same behavioral alterations as lesions in the area postrema. Thus, we hypothesized that ablation of LPBN tissue in subnuclear regions known to receive afferents from the area postrema would also attenuate Ang II-induced hypertension.

The results of this experiment confirm that hypothesis. Infusion of Ang II in sham-lesion rats caused a progressive rise in mean arterial pressure associated with an increased depressor response to ganglion blockade. In rats with LPBN damage, on the other hand, Ang II infusion produced only a transient increase in mean arterial pressure, possibly due to the direct vasoconstrictor effects of Ang II, and no
significant increment in the depressor response to hexamethonium. These results provide further support for the postulate that chronic hypertension during increases in circulating Ang II concentration in rats requires a central augmentation of neurogenic pressor activity. The results also suggest that the neuronal connection between the area postrema and the LPBN has a critical part in this central response. In discussing potential mechanisms for an LPBN participation in the genesis of Ang II-induced hypertension, however, several other possibilities also require consideration.

First, although area postrema and LPBN damage both caused very similar impairments in the chronic hypertensive response to Ang II infusion, the effect was not due to nonspecific brain damage per se: lesions in at least two other brain regions failed to affect the development of hypertension in this model. Second, both area postrema and LPBN damage have been reported to alter drinking behavior in rats, and potential Ang II–induced dipsogenic actions could contribute to volume-dependent hypertensive mechanisms. This and other studies, however, demonstrated that increased drinking was not required for hypertension development in this model; also, LPBN damage did not change drinking behavior in the rats used here. Third, although damage to the LPBN greatly augments the release of AVP to hemodynamic stimuli, there is no evidence that Ang II–stimulated release is altered by LPBN damage. Ang II can, however, stimulate the release of this potent vasoconstrictor and antidiuretic hormone under some conditions. The current results with a V,–selective AVP receptor antagonist indicate that the vasoconstrictor actions of AVP did not contribute to arterial pressure regulation in either
FIGURE 2. Reconstructions of area common to all lesions redrawn from Paxinos and Watson. Sections are taken −9.2 (panel A), −9.3 (panel B), −9.7 (panel C), and −9.8 (panel D) mm from bregma. bc, Brachium conjunctivum; d, dorsal lateral parabrachial nucleus; e, external lateral parabrachial nucleus; kf, Kölliker-Fuse nucleus; Mo5, motor nucleus of the trigeminal nucleus; mpbn, medial parabrachial nucleus; Pr5, principal sensory nucleus of the trigeminal nerve; v, ventral lateral parabrachial nucleus.

FIGURE 3. Line graphs show changes in mean arterial pressure and heart rate during 10-day intravenous infusion of angiotensin II (Ang II) in seven sham-operated (SHAM) rats and in six rats with bilateral lesions in the lateral parabrachial nucleus (LPBN). Bars on day 2 data points represent standard errors for within-group (over time) statistical comparisons. *Significant difference from control period values; + significant differences between SHAM and LPBN rats on a given day. Standard errors for between-group comparisons were mean arterial pressure, 18.4 mm Hg; heart rate, 36.0 beats/min.

group of rats in this experiment. Finally, studies in dogs strongly support the idea that the ability of blood-borne Ang II to decrease urinary sodium excretion is critical to the hypertensive action of the peptide. Furthermore, in the present experiments, Ang II infusion resulted in significant sodium retention in sham-lesion rats but not in rats with LPBN lesions. It is possible, therefore, that LPBN lesions attenuate hypertension development in this experiment by impairing the antinatriuretic actions of Ang II. This explanation seems less likely, however, when considering that 1) numerous previous studies of Ang
II-induced hypertension in rats, including animals with lesions of the area postrema, failed to document sodium retention during hypertension development \cite{13,25,26}; and 2) the degree of sodium retention observed in sham rats in the present experiment was quite modest.

Thus, LPBN involvement in Ang II-induced hypertension is probably related to the role of this brain region in regulating autonomic nervous system activity \cite{30}. The LPBN receives significant neuronal input not only from the area postrema \cite{15} but also from the nucleus tractus solitarius, and the paraventricular nucleus and median preoptic nucleus in the forebrain \cite{18,31}. In turn, the LPBN sends projections to the forebrain, the nucleus tractus solitarius, and the ventrolateral medulla, and also directly to the sympathetic preganglionic cell column in the spinal cord \cite{18}. Both chemical and electrical stimulation of the LPBN raise blood pressure, though the effect of glutamate is less robust \cite{30,32,33}. These findings suggest that neuronal connections in this nucleus, as well as fibers of passage, play a role in cardiovascular regu-
lation. Since the electrolytic lesion destroys both fibers of passage and cell bodies, this technique cannot distinguish between lesion effects resulting from damage to fibers or neurons. Thus, it cannot be concluded with certainty that the effects of LPBN damage observed here are directly related to interference with a neuronal pathway originating in the area postrema. Further studies are necessary to address this question.

In conclusion, this study showed that bilateral damage to the LPBN prevented the development of sustained hypertension during a 10-day intravenous infusion of Ang II in Sprague-Dawley rats. It is hypothesized that this effect resulted from an inability of lesion animals to increase neurogenic pressor activity during Ang II infusion because of interruption of a neuronal projection from the area postrema to the LPBN. This hypothesis, however, remains to be proven conclusively.

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