BRIEF COMMUNICATIONS

Subfornical Organ
Does It Protect against Angiotensin II-Induced Hypertension in the Rat?

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SUMMARY. The purpose of this study was to examine the contribution of the subfornical organ to the chronic hypertension produced by intravenous angiotensin II infusion in rats. Male rats were instrumented with permanent arterial and venous catheters and housed in metabolism cages for daily measurement of arterial pressure, heart rate, water intake, water balance, and urinary electrolyte excretion. Angiotensin II was infused intravenously at a rate of 10 ng/minute for 5 consecutive days, preceded by 2 control days, and followed by 2 recovery days. Normal rats with an intact subfornical organ (n = 7), and rats with an electrolytic lesion placed such that greater than 80% of the subfornical organ was destroyed (n = 9), were studied using this infusion protocol. An additional group of eight rats did not receive angiotensin II, and, thus, served as a time control. Both groups of rats receiving angiotensin II exhibited significant elevations in arterial pressure during the 5-day hormone infusion period, but pressure in rats with subfornical organ lesions (Δ23-29 mm Hg) was increased significantly more than that of intact rats (Δ14-20 mm Hg). Water intake was significantly increased on the 3rd, 4th, and 5th days of angiotensin II infusion only in rats with lesions in the subfornical organ. In contrast to previous studies showing that an intact subfornical organ is required for normal pressor and drinking responses to acute elevations in circulating angiotensin II in the rat, the current experiments indicate that the presence of the subfornical organ actually inhibits these same responses during more chronic increases in plasma angiotensin II levels. (Circ Res 56: 462-466, 1985)

CIRCULATING angiotensin II (All) is believed to produce several physiological effects which are mediated at least in part through a direct action of the hormone on the central nervous system (Brody and Johnson, 1980; Ramsay, 1982; Reid, 1984). Such effects include drinking, increased arterial blood pressure, release of vasopressin, and release of adrenocorticotropic hormone. The location of the receptor sites at which blood-borne All interacts with the brain to cause these physiological changes is a matter of some controversy (Hartle and Brody, 1984; Reid, 1984). The circumventricular organ(s) (CVO)—structures which lack a blood-brain barrier and are situated on the luminal surface of the cerebral ventricles—have been proposed as neural sensors for blood-borne All. The CVO include the organum vasculosum of the lamina terminalis (OVLT), the subfornical organ (SFO), and the area postrema. The median preoptic nucleus, intimately connected to the SFO and OVLT, may also be All-sensitive (Lind and Johnson, 1982; Mangiapane et al., 1983; Hartle and Brody, 1984).

Although there is evidence for all the above structures, most recent evidence strongly suggests that, in the rat, circulating All exerts many of its acute central actions via stimulation of receptor elements in the SFO. In vitro binding techniques (Sirett et al., 1977) have revealed receptors for All in high concentration in the SFO, and in vivo studies have shown high-affinity binding sites for blood-borne or cerebrospinal fluid-borne All in the SFO (Van Houten et al., 1980, 1983). Neurons within the SFO are excited by iontophoretic application of All (Phillips and Felix, 1976), and electrolytic destruction of the structure in rats blocks or reduces the dipsogenic and pressor responses to short-term (minutes to hours) intravenous infusion of All (Simpson et al., 1978; Mangiapane and Simpson, 1980a). In addition, microinjection of All directly into the SFO provokes pressor responses within seconds and water ingestion within 1 minute postinjection (Mangiapane and Simpson, 1980b). Intravenous or intracerebroventricular injection of All is a potent stimulus for vasopressin release in normal rats, but this release of vasopressin is blocked or attenuated in SFO-ablated rats (Knebel et al., 1982; Mangiapane et al., 1984). Dehydration, which causes a marked increase in plasma All concentration (Abdelaal et al., 1976; Mann et al., 1980), stimulates cellular metabolism in the SFO as determined by the 2-deoxyglucose technique (Gross et al., 1983). These studies have provided ample evidence sup-
porting the postulate that blood-borne All acts at the SFO in rats to elicit drinking behavior and bring about a centrally mediated pressor response. Nonetheless, all of the preceding studies have involved measuring physiological responses to All during rather short-term (minutes to a few hours) increases in blood levels of the hormone. The purpose of the present experiment was to investigate the contribution of the SFO to a model of hypertension involving chronic increases in plasma All concentration.

**Methods**

**Animals**

Male Sprague-Dawley rats weighing 300–400 g were studied. Before entering the experimental protocol, all rats were group housed (two rats per cage) in temperature-controlled and light-cycled quarters and were fed standard rat chow. All surgical procedures were carried out under sodium pentobarbital anesthesia (50 mg/kg, ip).

**Infusion Protocol**

In a preliminary operation, permanent polyvinyl-silicone rubber catheters were placed in the abdominal vena cava and aorta via the femoral vessels. The free ends of the catheters were tunneled subcutaneously to the skull and anchored there with dental acrylic. A flexible protective spring connected to a hydraulic swivel also was attached to the skull. Mounting the swivel above a standard metal metabolic cage allowed the rat unrestricted movement within the cage. Both catheters exited the metabolic cage inside the protective metal spring attached to the swivel. The venous catheter was attached to a Harvard infusion pump via the swivel to allow continuous (24 hr/day) iv infusion of fluids. The arterial catheter was filled with a heparinized solution and plugged when not in use. The plugged end of the arterial catheter rotated freely above the cage except during the recording of arterial pressure. The rats were fed a commercial sodium-deficient diet (12–15 g/day; 0.002 mEq Na⁺, 0.3 mEq K⁺/g) for the remainder of the experiment. The rats also received 40 ml/day of isotonic saline via intravenous infusion for the duration of the experiment (daily Na intake = 6.2 mEq). Distilled water was available ad libitum from stainless steel drinking tubes. All rats received twice daily injections of ampicillin (10 mg iv) after being housed in the metabolism cages.

Three days after placement of the rats in metabolism cages, the experimental protocol was started. This consisted of 2 control days, 5 days during which angiotensin II amide (Hypertensin) was infused continuously, iv, at a rate of 10 ng/min, then 2 postinfusion recovery days. Daily measurements included: (1) arterial pressure and heart rate obtained by connecting the arterial catheter to a low volume displacement pressure transducer (Statham P50) for 10–30 minutes each morning, (2) water intake calculated by adding the 40-ml infused volume to voluntary drinking volume obtained from a calibrated drinking tube, (3) urine volume obtained by quantitative collection from the metabolic cage, and (4) urinary sodium and potassium excretions calculated by multiplying urine volume by electrolyte concentrations (flame photometry).

**Groups**

Three groups of rats were studied in the preceding protocol. The first group (n = 7) received only saline during the 5-day "hormone infusion" period. The second group of rats (n = 8) received All (10 ng/min) during the same 5-day infusion period. A third group (n = 9) was subjected to electrolytic destruction of the SFO 2–4 weeks prior to a 5-day infusion of All (10 ng/min).

**Lesion of the SFO**

Rats were anesthetized with a pentobarbital-chloral hydrate mixture and immobilized in a Kopf stereotaxic instrument. A trephine hole was drilled in the skull dorsal to the lesion site, and a 30-gauge Teflon-insulated monopolar tungsten electrode was lowered to the lesion site. Because the anterior stalk of the SFO is quite ventral to the posterior stalk, three electrode penetrations were required. A total of 21 milliampere hours (mA) for 21 sec of anodal current was passed, with 7 mA passed per penetration. All penetrations were made in the midline after the superior sagittal sinus had been retracted. The penetration was stopped at a point 0.3 mm posterior and 0.2 mm dorsal to the preceding one. After surgery, each rat received 100,000 IU of procaine penicillin (im) and was returned to its home cage. Control rats (second and third groups) were age and weight matched and treated identically to rats with SFO lesions throughout the study, except that they did not undergo surgery 2–4 weeks prior to All infusion.

Lesions were produced in 22 rats in the laboratory of one of the authors (M.M.), after which the animals were sent to another laboratory (G.F.) for All infusion. At the end of the infusion protocol, the brain of each rat was perfused with buffered formalin, coded, and returned to the original laboratory for a blind histological analysis of lesion size and location. Frozen sections (35 μm) were cut through the lesion site and stained with cresyl violet. The sections then were examined carefully in a light microscope to determine the location of the lesion; the examiner had no knowledge of the data for any particular animal. Only after confirmation that the lesion destroyed >80% of the SFO was a given rat included in the "SFO lesion" group. Sixteen rats completed the All infusion protocol, and nine of these had >80% destruction of the SFO. These nine were included in the analysis. None of these had damage to the median preoptic nucleus (nucleus medius). As is typical of SFO lesions (Mangiapanne et al., 1984), all sustained a minor degree of damage to the fornical commissure, triangular septal nucleus, and paraventricular nucleus of the thalamus. Photomicrographs of the normal SFO and a representative SFO lesion are shown in Figure 1.

**Statistical Analyses**

The data were analyzed by a mixed design analysis-of-variance followed by the "protected" least significance difference test for individual group comparisons. A P value of less than 0.05 was the criterion for statistical significance.

**Results**

The results of 5 days of continuous All infusion (10 ng/min, iv) in the three groups of rats are illustrated in Figure 2. Mean arterial pressure (MAP), heart rate (HR), water intake (WI), urinary sodium excretion (U_NaV), and urinary potassium excretion (U_KV) were unchanged during the 5-day infusion.
period in rats receiving only saline, compared to control period values. In normal (SFO intact; SFOI) rats receiving AII, MAP was increased significantly throughout the 5 days of infusion and returned to the control range by 24 hours after termination of the infusion. In rats with SFO lesions (SFOX), MAP also was increased significantly during the AII period and returned to a normal range by 24 hours after termination of the infusion. Moreover, MAP was greater in SFOX rats than in SFOI animals throughout the AII infusion period; however, this difference was statistically significant only on days 1, 4, and 5. SFOI rats exhibited a gradual increase in HR which was statistically significant by the 5th day of AII administration, and HR remained elevated for 48 hours after the infusion was stopped. No significant change in HR was observed in SFOX rats at any time during the protocol. The administration of AII did not produce changes in WI in SFOI rats, whereas WI was significantly increased by the 3rd day in SFOX rats, and remained significantly elevated for the remainder of the AII infusion period. Water intake returned to normal within 24 hour after AII infusion in SFOX rats was stopped. In all three groups of rats, urine output paralleled water intake; therefore, water balance remained un-

Discussion

In the present experiments, the effect of chronic iv infusion of AII on arterial pressure and fluid/electrolyte changes throughout the experiment (not shown). Neither UNaV nor UKV was significantly changed by AII infusion in either SFOX or SFOI rats.

During the control and recovery periods, MAP, WI, and UKV were the same in all rats. Likewise, HR and UNaV did not differ in the three groups of rats during the control period. In SFOI rats, HR was significantly higher during the recovery period than in either saline-infused rats or SFOX rats. During the recovery period, UNaV was higher in SFOX rats than in saline-infused or SFOI rats.
electrolyte homeostasis was assessed in rats. It is important to point out that the All infusion rate utilized here (10 ng/min) has been shown previously to produce blood concentrations of All in the rat roughly equivalent to those found during 24-48 hours of water deprivation (Mann et al., 1980). Thus, these experiments explored cardiovascular changes produced by blood levels of All well within the "physiological" range.

The response of normal rats under the conditions of this study to chronic iv All infusion was largely cardiovascular. Arterial pressure was significantly elevated throughout the 5-day infusion period, and a slowly developing tachycardia occurred. The delayed increase in heart rate observed here is a common, but not invariable, finding in this model and may be related to the known ability of circulating All to interfere with baroreflex-mediated vagal activation and sympathoinhibition (Lumbers et al., 1979; Guo and Abboud, 1984). Water intake, water balance, and urinary electrolyte excretions were unchanged during All infusion. This pattern of responses to iv All is typical of many similar studies carried out in recent years in this laboratory (unpublished results). Although others have reported that chronic iv All infusion produces transient urinary sodium retention and increased drinking behavior in the dog (Cowley et al., 1981), neither phenomenon occurs consistently in the rat at the doses of All used in this experiment. Furthermore, despite the perception of All as a hormone involved in the conservation of body water, none of the published studies on chronic All infusion, including this one, have reported sustained positive water balance during All infusion.

The primary issue examined in this study was the extent to which the SFO is necessary for cardiovascular and fluid/electrolyte responses to chronically elevated blood levels of All. The large body of evidence indicating a significant contribution of the SFO to the major effects of All on the central nervous system—that is, increased arterial pressure, drinking, and vasopressin release—was outlined in the introduction. However, in the current experiment, when rats with greater than 80% of the SFO destroyed received chronic iv infusions of All, they responded with an even larger increment in MAP than did normal rats with an intact SFO. Furthermore, although normal rats did not increase water intake during the 5-day All infusion, rats with SFO lesions exhibited a significant increase in drinking in response to the same infusion. Neither SFO-ablated rats nor normal rats exhibited changes in water balance or urinary electrolyte excretion during All infusion. These results, in contrast to those obtained in studies utilizing acute All stimulation, clearly indicate that All receptors of the SFO are not required for pressor responses to circulating All in our 5-day infusion model. If anything, the opposite is true: the presence of the SFO appears to retard the increases in arterial pressure and water turnover observed in association with chronically elevated plasma All levels. Although this result appears to conflict with the finding (Buggy, 1981) that SFO-ablated rats are protected against chronic hypertension development in the presumably renin-dependent two-kidney, one-clip renal hypertension model, it should be noted that in Buggy's study, rats with lesions of the SFO had blood pressures markedly below control rats only more than 3 weeks after renal artery constriction. There is considerable dispute as to whether this model of hypertension remains "renin-dependent" after the first few weeks (Buggy and Fink, 1982).

There are a number of possible explanations for the disparities between our results and those from previous studies utilizing more short-term protocols. First, sodium intake may influence the neural response to circulating All. In the current experiments all rats were receiving a high sodium intake (6.2 mEq/day), since this has been shown to augment greatly the hypertensive response to chronic iv infusion of All (Fink et al., 1982). In fact, in this laboratory, it has proven impossible to produce sustained hypertension with iv All in rats on "normal" (1-2 mEq/day) sodium intake (Fink et al., 1982). Previous short-term studies have used rats on a "normal" sodium intake. Since some CVO (e.g., OVLT) stimulated by All also are known to be osmosensitive (Brody and Johnson, 1980; Thrasher et al., 1982) it is possible that a high sodium intake alters the relative sensitivity of the circumventricular organs to circulating All. Thus, blood-borne All may act to a greater extent at the SFO in rats on "normal" sodium intake, but preferentially at other CVO during concomitant osmotic stimulation accompanying higher sodium intakes.

A second possible explanation is that the cerebral receptors mediating thirst and the central component of the pressor response to elevated blood concentrations of All are simply different during acute vs. chronic increases in blood hormone levels. Physiological stimuli that increase All levels in blood can be either relatively acute (i.e., hemorrhage, water deprivation) or more chronic (sodium depletion, unilateral renal artery stenosis). Perhaps different cerebral mechanisms are activated, via different neural pathways, under these two different temporal conditions.

On the other hand, the finding here that SFO lesions actually potentiate the drinking and pressor response to chronic iv All also requires examination. One explanation for this finding is based on the assumption that an acute electrolytic lesion placed in a structure bordering the cerebral ventricles might disrupt the normal blood-brain barrier and allow passage of blood-borne All into sensitive brain areas to which it normally does not have access. However, this is an unlikely factor in the present experiments, since the lesions were placed 2-4 weeks prior to All infusion, thus allowing adequate time for healing. A more likely explanation can be postulated based...
on recent neuroanatomical studies of the SFO. An efferent neural pathway which exhibits ALL-like immunoreactivity has been identified (Lind and Johnson, 1982) passing from the SFO to the median preoptic region. The destruction of this pathway by SFO lesions might render ALL receptors in the median preoptic region supersensitive to the hormone. If circulating ALL exerts some of its neural actions on the median preoptic region, as has been proposed (Hartle and Brody, 1984), then receptor supersensitivity in this region could explain the augmented responses of SFO-ablated rats to chronic iv infusion of ALL.

The present chronic, and previous acute, studies are in agreement that rats with SFO destruction do not respond normally to elevated plasma ALL levels. A final possibility to be considered is in view of all studies to date is that both excitatory and inhibitory neural pathways pass through, or originate, in the SFO, and can be influenced by circulating ALL. Qualitatively different abnormalities in pressor responses and drinking behavior during acute and chronic elevations of plasma ALL in rats with SFO lesions may reflect a different temporal pattern of engagement of such excitatory and inhibitory systems.

In conclusion, although the underlying mechanisms for the results obtained in these experiments remain highly speculative, it is clear that chronic elevation of blood ALL levels, within a physiological range, can produce sustained hypertension without increasing water intake and in the absence of cerebral pressor mechanisms activated through the SFO.

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