The Area Postrema in Deoxycorticosterone-Salt Hypertension in Rats

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SUMMARY Ablation of the area postrema in rats prevents sustained hypertension during angiotensin II infusion and after unilateral renal artery constriction (two-kidney, one clip hypertension). The current experiment was performed to determine whether an intact area postrema is required for hypertension development in a low renin model of experimental hypertension in rats. In 11 rats, the area postrema was destroyed using electrical current; the extent and specificity of each lesion was confirmed later by blind histological analysis. In 12 rats, sham operations were performed. All rats were uninephrectomized and drank saline. During once-weekly injections of deoxycorticosterone pivalate (5 mg/wk) for 4 weeks, sham-operated rats (n = 10) showed a significant increase in mean arterial pressure (Days 6–28) and saline intake (Days 12–28), but no significant increase in sodium or water retention. Deoxycorticosterone-treated rats with area postrema ablation (n = 9) exhibited no change in arterial pressure, sodium retention, or water retention, but a significant increase in saline intake (Days 17–28). Plasma renin activity was equally suppressed in both groups of rats. The depressor response to ganglion blockade with hexamethonium (20 mg/kg i.v.) was significantly increased during the 2nd, 3rd, and 4th weeks of steroid treatment in sham-operated, but not area postrema-ablated, rats. Four rats (2 sham-operated; 2 ablated) showed no change in any variable over 28 days in the absence of steroid treatment. It is concluded that the area postrema may be important in some non-angiotensin-dependent forms of experimental hypertension, possibly by affecting neurogenic control mechanisms. (Hypertension 9 [Suppl III]: III-206–III-209, 1987)

KEY WORDS • area postrema • mineralocorticoid hypertension • arterial pressure • rat

The area postrema (AP) is a small, highly vascular structure at the caudal end of the fourth cerebral ventricle on the dorsal medulla. The permeability of this brain structure to blood-borne substances and its substantial connectivity to other brain regions known to be important in neural cardiovascular regulation make it a likely site for integration of hormonal and neural signals involved in maintaining cardiovascular homeostasis. Detailed studies by Barnes and colleagues have established that the AP in the dog can be activated by acute increases in circulating angiotensin II (ANG II) so as to cause a sympathetically mediated increase in arterial pressure. In the rat, on the other hand, it was reported that the AP did not participate in the pressor response to acute (5–10 minutes) increases in plasma ANG II levels. Recently, we have confirmed these earlier results in rats and have also shown that ablation of the AP in this species prevents sustained hypertension during long-term (5–10 days) intravenous infusion of ANG II. Additional studies revealed that the renin-dependent, two-clip model of experimental hypertension in rats also was attenuated by lesions of the AP. The purpose of the current experiment was to determine whether AP ablation would affect the development of a clearly non-angiotensin-dependent form of experimental hypertension.

Materials and Methods

Male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 300 to 400 g were used in these studies. Care of the rats was strictly in accordance with institutional (Michigan State University) guidelines and those of the American Physiological Society. All rats underwent surgery for electrolytic destruction of the AP. Briefly, the rats were anesthe-
tized with a pentobarbital–chloral hydrate mixture and placed in a stereotaxic device (David Kopf, Tujunga, CA, USA). After exposure of the AP on the surface of the medulla 700 μA of anodal current were delivered by a tungsten electrode for 9 to 12 seconds (6.3–8.4 mC). In sham-operated rats, the electrode was placed on the AP, but no current was passed. After surgery all rats were given 100,000 U of procaine penicillin, i.m., and returned to their cages. A more detailed description of the lesion procedure is available elsewhere."}

Lesions were produced in Rochester, New York, and then all rats were sent to East Lansing, Michigan, for the remainder of the study. At the end of the study, each rat was perfused intracardially with buffered formalin while under deep sodium pentobarbital anesthesia (40 mg/kg i.v.). The brains were removed, coded, and sent back to Rochester for histological analysis of lesion extent. Serial frozen sections (30 μm thick) were cut through the caudal medulla, slide mounted, and stained with cresyl violet. Sections were examined by light microscopy to assess lesion location and completeness. Only rats with more than 90% of the AP destroyed and with little or no destruction of surrounding brain tissue were included in the AP-ablated (APX) group (n = 12). Rats with no discernible damage to the AP were included in the sham-operated (sham) group (n = 12).

For the hypertension experiment, rats were unilaterally nephrectomized through a flank incision under sodium pentobarbital anesthesia (50 mg/kg i.p.). At least 1 week later, catheters were inserted into the aorta and vena cava through the femoral vessels under pentobarbital anesthesia, and a flexible, coiled-spring tether was attached to the skull of each rat with jeweler's screws and dental acrylic. For the remainder of the experiment, rats were housed individually in metal metabolism cages with free access to 0.9% NaCl drinking solution and sodium-deficient rat chow. Details of these procedures have been published previously. After 3 days of recovery from surgery, experiments were started. Daily measurements included mean arterial pressure (MAP), heart rate (HR), saline intake, urine volume, and electrolyte excretion. Sodium balance was estimated as the difference between sodium intake (saline drinking solution) and urinary sodium excretion. Fecal sodium excretion is minimal in rats fed sodium-deficient chow.) Three days of control measurements were followed by a 4-week period of treatment with deoxycorticosterone pivalate (DOC; 5 mg per rat, s.c., once weekly). Four rats did not receive steroid and served as time controls. A 1.0-ml blood sample was drawn on control day 1 and on treatment days 7, 14, 21, and 28 for assay of plasma renin activity, as previously described. On control day 2 and on treatment days 3, 10, 17, and 24, hexamethonium bromide was injected intravenously (20 mg/kg), and exactly 5 minutes later the fall in MAP was recorded.

Data were analyzed using a two-factor mixed design analysis of variance. Factor 1 was a repeated-measure factor (changes in a variable within a group over time), while factor 2 was a random factor (sham-operated group vs APX group). Homogeneity of variance was tested using the F test. Individual post hoc comparisons of means were performed using the "protected" least significance difference test or Dunnett's test. A probability level of less than 0.05 was considered significant.

**Results**

Both sham and APX rats gained weight normally and significantly during the 4-week steroid treatment protocol. Weight of the sham rats (n = 10) increased from 363 ± 20 to 409 ± 15 g; that of the APX rats (n = 9) from 368 ± 15 to 419 ± 17 g. Changes in MAP and HR during DOC treatment are illustrated in Figure 1. In sham rats, DOC treatment caused a rise in MAP that was significantly greater than control period levels from treatment day 6 on. In APX rats, DOC injection caused no significant change in MAP throughout the study. Arterial pressure was significantly less in APX than in sham rats from day 6 of DOC treatment on. Control period HR was significantly lower in APX than in sham rats, and this difference was maintained throughout DOC treatment. No significant change in HR was observed in either group of rats during DOC treatment.

Changes in fluid intake, calculated water balance, and sodium balance are shown in Figure 2. Both sham and APX rats increased their saline intake during DOC.
Changes in water intake (as saline; WI), water balance (intake minus urine volume; WB) and sodium balance (intake minus urinary excretion; Na⁺B) during DOC treatment in area postrema-ablated (APX) and sham-operated (SHAM) rats. Drinking was significantly increased over control period values in sham rats (days 12–28) and APX rats (days 16–28, except days 18, 19, and 21). Throughout the study, drinking by APX rats was significantly greater than that by sham rats. No significant changes in WB or Na⁺B were observed within or between groups.

Plasma renin activity (PRA) in area postrema-ablated (APX) and sham-operated (SHAM) rats during DOC treatment. C = control period; P = days after initiation of DOC treatment; AI = angiotensin I. Asterisks indicate a significant difference from control period values. No significant between-group differences were found.

Changes in mean arterial pressure (MAP) and heart rate (HR) during ganglion blockade with hexamethonium. Sham = sham-operated rats; Apx = area postrema-ablated rats. Asterisks indicate a significant difference from control period values. No significant between-group differences were found.

Depressor responses to ganglion blockade in DOC-treated rats are shown in Figure 4. In the control period, depressor responses were slightly (but not significantly) greater in APX than in sham rats. During DOC treatment, sham rats exhibited significantly greater depressor responses in the 2nd, 3rd, and 4th weeks compared to responses during the control period. No significant changes in response to ganglion blockade were observed in APX rats during the DOC period. Nonetheless, depressor responses to hexamethonium were not significantly different between APX and sham rats at any time. No significant differences in HR in response to hexamethonium were found within or between groups during the experiment. In four uninephrectomized rats drinking saline but not treated with DOC (two sham; two APX), no significant change in any variable was found over the 28-day "treatment" period (data not shown).

Discussion
The rise in arterial pressure typically observed in uninephrectomized, saline-drinking rats receiving the mineralocorticoid DOC was not seen over a 28-day treatment period in rats in which the AP was ablated. The expected increase in saline drinking, however,
was seen in both sham and APX rats. Although AP ablation has been shown to prevent two different forms of hypertension associated with increased circulating ANG II in rats, this factor is not likely to be involved in the DOC model, since plasma renin activity was equally suppressed by DOC in hypertensive sham rats and normotensive APX rats.

Differences in fluid volume regulation between sham and APX rats are not likely to explain their differing responses to DOC, since neither group exhibited significant sodium or water retention during DOC treatment, and APX rats actually had higher salt intakes. Our failure to observe the expected transient sodium retention on initiation of DOC treatment is probably the result of the technical difficulty of measuring small changes in urinary sodium excretion in 24-hour urine collections during variable intake of high amounts of sodium. It is interesting, however, that a sharp increase in sodium retention occurred in sham and APX rats during the 4th week of the DOC period, at the precise time when glomerular damage becomes readily apparent in this model. A reduced creatinine clearance also has been reported in DOC-salt–treated rats after 5 weeks of treatment. Although actual body fluid volumes were not compared directly in sham and APX rats given DOC, these measurements are not in any case reliable predictors of hypertension in this model. It is not known whether APX rats would become hypertensive after longer periods of DOC treatment, when the model presumably becomes more “volume-dependent” as a result of progressive renal damage.

There is substantial evidence that an increase in sympathetic nervous system activity contributes to the pathogenesis of DOC-salt hypertension in rats, but there is also evidence that refutes this mechanism. One method of assessing overall autonomic cardiovascular control is to block ganglionic transmission and measure the resulting change in MAP before compensatorypressor systems are activated. It is known that rats with established DOC-salt hypertension exhibit augmented depressor responses to ganglion blockade compared to those of normotensive rats. This may be the result of reduced baroreceptor reflex function in DOC-salt hypertensive rats. In the present study, serial measurement of depressor responses to ganglion blockade revealed a progressive increase in sham rats during DOC treatment; no such change was observed in APX rats. This finding is not definitive evidence of increased neurogenic cardiovascular activity in sham versus APX rats because greater depressor responses could result simply from the higher initial MAP in sham rats, but the data are consistent with such an increase. Many of the sham rats treated with DOC exhibited enhanced depressor responses to hexamethonium prior to significant rises in “ basal” MAP. The finding that many neuronal projections extend from the AP to the nucleus of the tractus solitarii lends credibility to the notion that disruption of AP tissue may alter hormonal modulation of sympathetic outflow. This notion remains to be tested more directly.

In summary, we found that ablation of the AP prevented hypertension development during 28 days of DOC treatment in uninephrectomized, saline-drinking rats. This effect clearly was not due to interference with the actions of blood-borne ANG II and was not a nonspecific effect of the lesion on arterial pressure control, since at least one other model of experimental hypertension (one-clip, one kidney) is not affected by AP ablation. Hormonal interactions with cardiovascular control mechanisms in the brainstem are likely to be impaired by AP ablation, which may explain the effect of AP removal on DOC-salt hypertension.

References
The area postrema in deoxycorticosterone-salt hypertension in rats.
G D Fink, C M Pawloski, M L Blair and M L Mangiappe

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