SUMMARY Ablation of tissue surrounding the anteroventral third cerebral ventricle (AV3V) has been demonstrated to prevent and reverse renal hypertension in the rat. The contribution of this brain area to the maintenance of hypertension in other species has not been examined. In the present investigation, an attempt was made to produce two-kidney, one clip renal hypertension in rabbits with histologically and functionally defined AV3V destruction. Electrolytic lesion of the AV3V in rabbits produced effects closely resembling those previously seen in rats: increased plasma volume and plasma sodium, temporary adipsia, no change in resting arterial pressure or heart rate, and significant attenuation of pressor responsiveness to angiotensin II (AII) delivered intracranially. However, the increase in arterial pressure observed over a 4-week period following the application of a 0.5 mm silver clip to the left renal artery (opposite kidney intact) was identical in 12 AV3V-lesioned and 12 sham-operated rabbits. Hypertension development was not accompanied by significant sodium retention, water retention, or plasma/extracellular fluid volume expansion in either group of rabbits. Pressor responses to intravenous infusions of AII and norepinephrine were identical in sham and AV3V-X rabbits. Thus, destruction of the AV3V, and the attendant reduction in the central pressor action of AII, does not alter the pattern of development of two-kidney, one clip renal hypertension in the rabbit. The contrasting results in rats and rabbits could be explained by the differing contribution of the area postrema to the pressor action of AII in the two species. (Hypertension 4: 155-160, 1982)

KEY WORDS • angiotensin II • hypertension • hypothalamus • rabbit

A DISCRETE periventricular brain region in the anterior ventral hypothalamus (AV3V) is involved in two physiological systems (probably linked) which are widely believed to be important in the pathogenesis of renal hypertension. First, a series of lesion studies in the rat1 and rabbit2 have established a role for the AV3V in the regulation of body fluid balance and distribution. Specifically, destruction of the AV3V causes plasma volume expansion, hypernatremia, temporarily altered drinking behavior, and permanent disruption of the lesioned animal's ability to excrete an acute salt and water load. Second, the AV3V has been shown to be a crucial link in the responses of the central nervous system (CNS) to the hormone angiotensin II (AII). AII can initiate CNS responses either by entering select areas of the brain from the bloodstream, or by direct addition to brain extracellular fluid via an endogenous brain renin-angiotensin system. Responses to the action of AII on the brain include increased blood pressure and drinking behavior,3 attenuation of baroreflexes,4 the release of vasopressin,5 natriuresis,6 and increased salt appetite.7 In the rat, destruction of the AV3V significantly attenuates most neurally-mediated responses to either blood-borne AII or to AII added directly to the brain via intracerebroventricular (i.v.t.) injection.8,9

It is thus of considerable interest that rats subjected to lesion of the AV3V are highly resistant to the development of several forms of experimental renal hypertension, including one-kidney Goldblatt hypertension, aortic coarctation hypertension and two-kidney one-clip Goldblatt (2K1C) hypertension.10 However, the mechanism by which AV3V lesions inhibit renal hypertension in the rat is not clear, particularly in that salt and water balance,11 and both endogenous brain and blood-borne AII12,13 have been implicated in the pathogenesis of these forms of hypertension. In the current studies, the influence of AV3V lesions on the development of 2K1C hypertension was examined in the rabbit, utilizing both chronic serial measurements of salt/water balance and distribution, and assessment of the contribution of the brain renin-angiotensin system to raised arterial pressure. The rabbit is a useful model for such studies since AV3V...
lesions in this species cause altered fluid homeostasis and diminished pressor responsiveness to i.v.t. AII, but do not alter pressor sensitivity to blood-borne AII.3

Methods

Animals

Male albino rabbits weighing 2.0 to 3.0 kg were used for these studies. The rabbits were housed in metal metabolic cages in a light-cycled, temperature-controlled room for the duration of the experiments. Tap water was available ad libitum from calibrated drinking bottles, and 100 g of Purina high fiber rabbit chow (Lab Rabbit Chow HF) was offered daily. Measurement of daily water intake, food intake, urine output, fecal output, and routine cage care were carried out between 8:00 and 11:00 a.m. each day.

General Protocol

Rabbits were allowed to accommodate to the metabolic cages for 4 to 10 days (fluid/electrolyte balance was determined for the final 3 to 4 days), then were brought to the laboratory for cardiovascular measurements and lesion surgery. This was followed by 7 days of fluid/electrolyte balance measurements. On Day 7 after lesion (or sham lesion), cardiovascular measurements were repeated, and the left renal artery was clipped. Fluid/electrolyte balance was measured daily for an additional month, while cardiovascular measurements were performed weekly (a total of four additional determinations postclip).

Surgical Procedures and Cardiovascular Measurements

All surgical procedures were carried out under sodium pentobarbital anesthesia (30 mg/kg body weight, i.v.). Lesion of the AV3V was performed as previously described.4 Briefly, an insulated hollow stainless-steel electrode (23 gauge) was lowered with stereotaxic guidance into the optic recess of the third ventricle and withdrawing a small quantity of cerebrospinal fluid. A 23-gauge stainless-steel cannula was permanently attached to the skull using small screws and dental cement. Each rabbit was given a single intramuscular injection of 100,000 U of procaine penicillin G and 125 mg of dihydrostreptomycin postoperatively.

To constrict the renal artery, a solid silver clip (0.508 mm ID) was applied to the left renal artery through a midline abdominal incision. This procedure was carried out using sterile technique. Arterial pressure and heart rate were determined in conscious rabbits restrained in a head stock, using direct percutaneous puncture of the central ear artery as described previously.6 Plasma volume and extracellular fluid volume were estimated using the 10-minute distribution space of Evan's blue dye and the 30-minute distribution space of sodium thiocyanate respectively.

At the end of the chronic phase of the experiment (4 weeks after clipping), arterial and venous catheters were placed in the ears of conscious rabbits under local anesthesia (1% lidocaine), and the rabbits were allowed to sit unrestrained in a large box. Alterations in mean arterial pressure and heart rate were determined to the following treatments: i.v.t. AII (200 ng in 10 µl bolus), i.v.t. saralasin (1-sarcosine-8-alanine AII) (10 µg in 10 µl bolus), i.v. AII (100, 200 and 400 ng/min by infusion), i.v. norepinephrine (.3 and 1.0 ng/min by infusion) and "total" autonomic blockade according to a previously published protocol.4 From 30 to 60 minutes were allowed to elapse between the various treatments. All drugs were administered remotely, via long connecting tubes, without disturbing the rabbit.

Finally, the rabbits were anesthetized (sodium pentobarbital, 30 mg/kg, i.v.), and the abdomen was reopened. Arterial pressure proximal and distal to the clip on the left renal artery was determined by arterial puncture with a 27-gauge needle connected to a pressure transducer. The kidneys were then removed and weighed on an analytical balance. The rabbit's head was perfused with buffered 10% formalin through a carotid artery, and the brain was removed and stored in 10% formalin for subsequent histological analysis of lesion location.

Analytical Techniques

Brain sections were prepared using 40 µ frozen sections stained with cresyl violet, and were examined visually and under low power light microscopy.

Evan’s blue dye and thiocyanate concentrations in plasma were measured spectrophotometrically according to standard techniques. Hematocrits were determined in triplicate by microcentrifugation. Plasma sodium and potassium concentrations were determined in triplicate by flame photometry. Food and feces were ashed in nitric acid, and the electrolyte content of these solutions, and urine samples, were determined by flame photometry.

Statistical Analyses

The chronic data were analyzed using a split-plot analysis of variance with individual comparisons being made by testing least significant differences both between and within groups. Single group data were analyzed using Student's t test. A probability level of less than 0.05 was the criterion of statistical significance.
Results

The criteria for an "effective" AV3V lesion in this study were a pressor response to i.v.t. All of more than one standard deviation (1 SD) below that of the group of intact rabbits, and a lesion located anatomically on the midline of the forebrain at the level of the optic recess of the third ventricle. Of a total of 17 animals in which lesions were attempted, 12 met these criteria. Four of the rabbits that were rejected had nearly normal pressor responses to i.v.t. All, and their lesions were located lateral to the midline. The fifth rabbit rejected had a lesion that appeared appropriate anatomically but exhibited a normal pressor response to i.v.t. All. Typical lesion histology was published previously.8

Effects of AV3V Lesions

The fluid/electrolyte alterations observed in the present experiments following AV3V lesioning closely resembled those previously found in rats and rabbits. Although water drinking decreased in both groups during the control week (fig. 1), drinking deficits were greater in lesioned rabbits. However, sham-lesioned rabbits maintained a normal water balance (intake-urinary output), while lesioned animals exhibited an inappropriate diuresis leading to a significant loss of body water (fig. 1). This fluid loss was apparently from intracellular stores, since extracellular fluid volume and plasma volume actually rose in AV3V-lesioned rabbits during the week after lesioning (fig. 2). Food intake was significantly decreased during the 2 weeks

![Figure 1. Fluid and sodium balance before and after renal artery constriction in sham-lesioned (SHAM) or AV3V-lesioned (LESION) rabbits. Stars (*) indicate a significant difference from preconstriction (POST) values. Crosses (†) indicate a significant difference (p < 0.05) in water balance from the prelesion (PRE) value. Brackets on preconstriction value of water balance represent SEM for within group comparisons. Statistical comparisons were made on average weekly sodium balance values (intake-output), where the SEM for within-group comparisons was 0.66 mEq/day and for between-group comparisons 1.01 mEq/day. No significant between-group or within-group differences were found (p < 0.05).](http://hyper.ahajournals.org/)

![Figure 2. Hemodynamic and volume parameters prior to, and following, unilateral renal artery constriction. Shaded region represents the period prior to renal artery constriction. PC = post-constriction; SHAM = sham-lesioned rabbits; AV3V-X = rabbits with AV3V lesions; PRE = pre-lesion; POST = 1 week after lesion or sham surgery. Stars (*) indicate a significant difference (p < 0.05) from the immediate preconstriction control value (POST). SEM for within-group comparisons is indicated by brackets on the preconstriction control values. SEM for hematocrits was too small to be illustrated. MAP = mean arterial pressure; HR = heart rate; PV = plasma volume; ECFV = extracellular fluid volume; HCT = hematocrit.)](http://hyper.ahajournals.org/)
after AV3V lesioning, probably as a result of decreased water intake. Sodium (fig. 1) and potassium (not shown) balance were not altered significantly in either group of rabbits following lesion surgery, while plasma sodium concentration rose significantly \((p < 0.05)\) in lesioned rabbits \((140 \pm 1 \text{ to } 145 \pm 1 \text{ mEq/liter})\) but not in sham animals \((143 \pm 1 \text{ to } 144 \pm 1 \text{ mEq/liter})\). However, post-lesion plasma sodium concentrations were not significantly different in the lesioned and sham rabbits. Plasma potassium was not affected by either lesion or sham lesion. Mean arterial pressure and heart rate were not changed significantly by either lesion or sham lesion (fig. 2).

**Chronic Effects of Renal Artery Clipping**

Mean arterial pressure prior to clipping was nearly identical in sham-lesioned \((70 \pm 2 \text{ mm Hg})\) and lesioned rabbits \((71 \pm 2 \text{ mm Hg})\). Constricting the left renal artery caused arterial pressure to rise in a similar fashion in both groups of rabbits, with average pressures at 1 month being \(83 \pm 2 \text{ mm Hg}\) in sham rabbits and \(85 \pm 2 \text{ mm Hg}\) in lesioned rabbits (fig. 2). In both groups of animals these average pressure values represented some rabbits that became markedly hypertensive \((mean \text{ arterial pressure}, > 100 \text{ mm Hg})\) and others that remained normotensive throughout. When “hypertension” development is arbitrarily defined as an increase in arterial pressure of more than \(10 \text{ mm Hg}\), then both groups of rabbits showed identical incidences of hypertension \((7/12 \text{ or } 58\%)\). Control mean arterial pressure was \(68 \pm 4 \text{ mm Hg}\) in the seven sham rabbits that became hypertensive following clipping, and was \(89 \pm 2 \text{ mm Hg}\) at the end of the study. Control mean arterial pressure was \(69 \pm 3 \text{ mm Hg}\) in the seven lesioned rabbits that became hypertensive following clipping, and was \(93 \pm 4 \text{ mm Hg}\) at the end of the study. Heart rate was not changed significantly by clipping in either group of rabbits (fig. 2).

Constriction of the left renal artery in these rabbits did not significantly alter plasma volume, extracellular fluid volume, hematocrit (fig. 2), sodium balance (fig. 1), potassium balance, plasma sodium concentration, and plasma potassium concentration (not shown) whether the animal was AV3V-lesioned or sham-lesioned. Water intake was increased significantly \((p < 0.05)\) following renal artery constriction in both groups of rabbits compared to preclipping control values, but neither group of rabbits exhibited net water retention \((increased \text{ water balance})\) at any time after clipping (fig. 1). Body weight increased identically over the 4 weeks after clipping in lesioned \((2.4 \text{ to } 2.6 \text{ kg})\) and sham-lesioned \((2.3 \text{ to } 2.5 \text{ kg})\) rabbits.

**Acute Cardiovascular Responses**

The pressor response to i.v.t. administration of All was significantly \((p < 0.05)\) less in AV3V-lesioned rabbits (by definition) than in sham-lesioned animals (table 1). However, depressor responses to i.v.t. admin-

**ministration of saralasin, and pressor responses to i.v. All and norepinephrine were not significantly different in the two groups of rabbits (table 1). The baroreflex curves generated by measuring heart rate slowing during the steady-state rise in pressure produced by i.v. infusion of angiotensin and norepinephrine were not significantly different in sensitivity (slope) or position on the pressure axis between lesioned and sham-lesioned rabbits. Likewise, there were no significant differences in the modest depressor response of the two groups of rabbits to “total” autonomic blockade \((sham 86 \pm 5/79 \pm 5; \text{ AV3V } 84 \pm 2/75 \pm 2 \text{ mm Hg})\).

Finally, as indicated in table 2, kidneys from both groups of rabbits exhibited a similar disparity in size between clipped and intact organs, as would be expected based on the known propensity of the clipped kidney to atrophy and of the contralateral kidney to undergo hypertrophy. Furthermore, the directly measured pressure gradient across the renal artery clip was not significantly different in the sham and AV3V-lesioned rabbits (table 2).

**Table 1. Cardiovascular Responses to Central and Peripheral Administration of Vasoactive Agents**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Sham (12)</th>
<th>AV3V-X (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta BP)-i.v.t. All</td>
<td>200 ng</td>
<td>(30 \pm 3)</td>
<td>(11 \pm 2^*)</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta BP)-i.v. All</td>
<td>100 ng/min</td>
<td>(21 \pm 4)</td>
<td>(17 \pm 4 \text{ NS})</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>200</td>
<td>(38 \pm 5)</td>
<td>(30 \pm 6 \text{ NS})</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>(44 \pm 5)</td>
<td>(39 \pm 6 \text{ NS})</td>
</tr>
<tr>
<td>(\Delta BP)-i.v.t. saralasin</td>
<td>10 (\mu g/min)</td>
<td>(-6 \pm 2)</td>
<td>(-3 \pm 1 \text{ NS})</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Delta BP)-i.v. NE</td>
<td>0.3 (\mu g/min)</td>
<td>(6 \pm 1)</td>
<td>(6 \pm 1 \text{ NS})</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>1.0</td>
<td>(20 \pm 3)</td>
<td>(16 \pm 2 \text{ NS})</td>
</tr>
</tbody>
</table>

\(\Delta BP = change \text{ in mean arterial pressure}; \text{ i.v.} = \text{ intravenous}; \text{ i.v.t.} = \text{ intracerebroventricular}; \text{ NE} = \text{ norepinephrine}; \text{ All} = \text{ angiotensin II)}.

\(\text{Results are expressed as mean} \pm \text{ SEM. Asterisk (*) indicates a significant difference (p < 0.05) between sham and lesioned (AV3V-X) rabbits. NS = nonsignificant (i.e., p > 0.005).)}\)

**Table 2. Body Weight, Kidney Weight, and Pressure Gradient Across the Clip in Renal Hypertensive Rabbits**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>AV3V-X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>(2.5 \pm 0.1)</td>
<td>(2.6 \pm 0.1)</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>(7.24 \pm 0.47)</td>
<td>(7.52 \pm 0.43)</td>
</tr>
<tr>
<td>Clipped</td>
<td>(6.46 \pm 0.30)</td>
<td>(6.94 \pm 0.24)</td>
</tr>
<tr>
<td>(\Delta\text{RAP-clip})</td>
<td>(14 \pm 3)</td>
<td>(21 \pm 4)</td>
</tr>
</tbody>
</table>

\(\text{There were no significant differences in these measures between sham and lesioned (AV3V-X) rabbits. Results are expressed as mean} \pm \text{ SEM.}\)
Discussion

A variety of factors have been implicated in the pathogenesis of two-kidney one clip Goldblatt (2K1C) hypertension including increased plasma renin activity, augmented vasopressin release, increased sympathetic nervous system activity, increased vascular reactivity to norepinephrine and other agonists, resetting of baroreflexes, and renal retention of salt and water. However, no single precipitating cause has been agreed upon as the primary element in the development of this form of hypertension. It has been reported that AV3V lesions attenuate the rise in arterial pressure in 2K1C rats. Furthermore, sodium and water retention, and central nervous system (CNS) activation by All have both recently been suggested as contributors to hypertension development in the 2K1C model. Since AV3V lesions in rats disrupt both of these latter processes, it was considered of interest to examine both factors during the onset of 2K1C hypertension in lesioned animals. The rabbit was chosen for study because the failure of AV3V lesions to alter the pressor action of circulating All in this species would allow separation of the contributions of the peripheral and brain renin-angiotensin systems to hypertension development.

Our previous results were confirmed in the present experiments when AV3V lesions significantly reduced pressor responses to i.v. All in conscious rabbits, but did not alter pressor responses to i.v. All. In addition, the rabbits subjected to AV3V lesioning here exhibited "typical" changes in body fluid homeostasis post-lesion: increased plasma and extracellular fluid volume in the face of overall reduced water balance, hypernatremia, and temporary hypodipsia. Thus, it is apparent that the rabbits utilized in the present study had adequate AV3V lesions as currently defined.

Nevertheless, the incidence and magnitude of 2K1C hypertension in rabbits with AV3V lesions were completely comparable to those observed in normal (neurally-intact) rabbits. The variable, and usually modest, hypertensive response to unilateral renal artery constriction in unselected rabbits is typical of results reported by other previous investigators. Since the magnitude of 2K1C hypertension reportedly is inversely proportional to clip diameter, it is important to note that two lines of evidence shown here (contralateral renal hypertrophy and direct trans-clip pressure gradient) indicate that the degree of renal artery constriction was similar in lesioned and sham-lesioned rabbits. Thus, purely technical differences in clip application were not likely to have produced a spurious "agreement" in hypertension development between the two groups.

It was not possible to identify significant differences in the mechanism by which arterial pressure rose following renal artery clipping in lesioned and sham-lesioned animals. Neither group of rabbits exhibited significant sodium retention, water retention, or fluid volume expansion after renal artery constriction. During the established phase of hypertension (>1 month post-clip), pressor sensitivity to norepinephrine and All was identical in AV3V-lesioned and sham-lesioned rabbits, as were baroreflex control of heart rate and the depressor effect of blockade of the autonomic nervous system. In other words, AV3V-lesioned and sham-lesioned rabbits were indistinguishable based on their physiological response to unilateral renal artery constriction. However, it was established that the lesioned animals studied here exhibited a greatly reduced pressor responsiveness to All placed directly into the brain via the cerebral ventricles. This encourages the conclusion that the neural substrates mediating the pressor actions of intracerebroventricular All (AV3V) are not an important component of 2K1C hypertension in the rabbit. Certainly the failure of the angiotensin antagonist saralasin to significantly lower arterial pressure when given into the brain ventricles of 2K1C hypertensive rabbits supports this conclusion. Therefore, to the extent that the effects of the endogenous brain renin-angiotensin system are mimicked by ventricular injection of All, one can conclude that the brain renin-angiotensin system is an unlikely pathogenetic factor in 2K1C hypertension in the rabbit.

What, then, is the explanation for the apparent difference in rats and rabbits concerning the contribution of brain angiotensin effects to 2K1C hypertension? A likely possibility is the relative role in the two species of the medullary area postrema in the neurally mediated actions of blood-borne All. Evidence exists for increased blood renin-angiotensin activity in 2K1C hypertension in both rats and rabbits. However, in most species, including rabbits, the response of the central nervous system to increased blood levels of All is mediated via structures in or near the area postrema. In the rat, the AV3V or other forebrain areas are more important in this response, while the area postrema apparently is not involved at all. Thus, if an action of circulating All on the brain contributes to 2K1C hypertension in the rabbit, then area postrema lesions and not AV3V lesions would be expected to attenuate such hypertension. This hypothesis remains to be investigated.

Acknowledgments

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