Forebrain Contributions to One-Kidney Renal Hypertension in the Rabbit

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SUMMARY Electrolytic lesions were placed along the anteroventral wall of the third cerebral ventricle (AV3V region) in 10 albino rabbits (AV3V-X), and sham lesions were produced in 10 additional rabbits (SHAM). Two to 3 weeks later, all rabbits underwent unilateral nephrectomy and renal artery stenosis (clip I.D. = 0.508 mm). During a 1-week control period, and for 4 weeks after renal artery stenosis, measurements were made of mean arterial pressure (MAP), heart rate, body fluid compartment volumes, plasma electrolytes, and daily sodium, potassium, and water balances. Four weeks after renal artery stenosis (RAS), cardiovascular responses to norepinephrine (NE), angiotensin II (AII), saralasin, and autonomic blockade were obtained in the conscious animals. In SHAM rabbits, MAP rose from 77 to 117 mm Hg 4 weeks after RAS. In AV3V-X rabbits, MAP rose from 77 to only 92 mm Hg 4 weeks after RAS. Body fluid compartment volumes, plasma electrolytes, and fluid, sodium, and potassium balances showed similar modest changes in both groups of rabbits. Neither saralasin infusion nor autonomic blockade caused significantly different changes in MAP between SHAM and AV3V-X rabbits 4 weeks after RAS. However, pressor responses to both NE and AII were significantly less in AV3V-X rabbits at this time. It is concluded that one-kidney, one clip renal hypertension involves activation of neurohormonal pressor mechanisms originating in the forebrain, and that the expression of these pressor mechanisms in part includes an increase in cardiovascular reactivity. (Hypertension 5: 900-907, 1983)

KEY WORDS • hypothalamus • sodium balance • water balance • arterial pressure • kidney • vascular reactivity

CONSIDERABLE evidence exists that central nervous system mechanisms controlling arterial blood pressure make a critical contribution to the development and maintenance of experimental renal hypertension. An important part of this evidence is the demonstration that disruption of various brain regions involved in cardiovascular regulation, either with electrolytic lesions or neurotoxins, prevents or retards the development of experimental renal hypertension in several different animal models. Recent studies of this type in the rat have centered upon brain structures of the anteroventral wall of the third cerebral ventricle (AV3V), a forebrain area known to contain neuronal elements sensitive to blood and cerebral fluid osmolality, and AII concentration. Ablation of the AV3V region can prevent or reverse one-kidney, one clip Grollman hypertension, and reduce the severity of aortic coarctation and two-kidney, one clip renal hypertension in the rat. Although the known ability of AV3V lesions in the rat to reduce or abolish many physiological responses to AII and osmotic stimulation suggest an appealing link between renal manipulations and abnormal neural cardiovascular control in renal hypertension, a somewhat atypical distribution of AII-sensitive neurons in the rat brain raises the question of the applicability to other species of conclusions obtained in experiments on rats alone. Thus, we have undertaken an examination of the influence of AV3V lesions on renal hypertension development in another common experimental animal — the rabbit. In a prior report, we showed that AV3V lesions in the rabbit did not alter the pathogenesis of two-kidney, one clip renal hypertension. In the current study, we assessed the effect of AV3V lesions on one-kidney, one clip renal hypertension in the rabbit.

Materials and Methods

Male albino rabbits weighing 2.3 to 3.1 kg were housed in standard metal metabolism cages in air-conditioned, light-cycled quarters and offered free access to tap water throughout the study. A diet of 100 g of high-fiber rabbit chow (Purina HF rabbit chow) containing 11 mEq of sodium and 40 mEq of potassium per 100 g (by analysis) was offered daily. Measurements of food and water intake, urine volume, and
fecal weight were performed between 8:00 and 11:00 am every day. Rabbits were housed in the cages and subjected to routine care as outlined above for 1 to 2 weeks before the start of the metabolic balance studies.

Brain Lesions

Electrolytic lesions of the AV3V region were produced several weeks prior to renal artery stenosis using techniques described in detail previously. Briefly, rabbits were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and a burr hole was made in the skull 4.0 to 4.5 mm anterior to the bregma. A hollow stainless steel electrode was lowered stereotactically into the optic recess of the third ventricle, using aspiration of cerebrospinal fluid as confirmation of placement. Lesions were produced by passing 3.0 to 3.5 mA of anodal current through the 1 mm uninsulated tip of the electrode for 15 seconds. The electrode was raised 1.5 mm, and another lesion was produced in the same manner. Sham lesioning involved only lowering the electrode and aspirating cerebrospinal fluid. A permanent 23-gauge stainless steel cannula also was implanted in the left lateral ventricle. Each rabbit received 100,000 U of procaine penicillin G and 125 mg of dihydrostreptomycin i.m. postoperatively.

Renal Artery Stenosis

Rabbits were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and a laparotomy was performed under sterile conditions. After removal of the right kidney, a solid silver clip (I.D. = 0.508 mm) was placed around the left renal artery as close as possible to the aorta. A single postoperative injection of penicillin and dihydrostreptomycin was administered as described above.

Cardiovascular Measurements

Arterial pressure was measured by percutaneous puncture of a dilated central ear artery with a 23-gauge needle connected to a Statham P23AC pressure transducer. Heart rate was obtained by counting pressure pulses on a paper chart record (Grass model 7 polygraph). Plasma volume and extracellular fluid volume were estimated using the 10-minute distribution space of Evan's Blue dye and thiocyanate concentrations in plasma. Hematocrits were determined by microcentrifugation. Plasma, urine, food, and fecal electrolyte concentrations were determined by flame photometry. Food and feces were ashed for several weeks in concentrated nitric acid prior to measurement of electrolyte content. Plasma osmolality was determined on 50 μl samples by freezing point depression (Micro-Osmette, Precision Systems, Inc., Sudbury, Massachusetts). Blood urea nitrogen was measured using a urease method.

Analytical Procedures

Brain sections were prepared as 40 μ paraffin embedded sections stained with cresyl violet, and were examined under low power light microscopy. Evan’s Blue dye and thiocyanate concentrations in plasma were measured spectrophotometrically using standard techniques. Hematocrits were determined by microcentrifugation. Plasma, urine, food, and fecal electrolyte concentrations were determined by flame photometry. Food and feces were ashed for several weeks in concentrated nitric acid prior to measurement of electrolyte content. Plasma osmolality was determined on 50 μl samples by freezing point depression (Micro-Osmette, Precision Systems, Inc., Sudbury, Massachusetts). Blood urea nitrogen was measured using a urease method.

General Protocol

Two to 3 weeks after lesion surgery, the rabbits were adapted to the metabolism cages, then the first series of cardiovascular and fluid volume measurements were performed. For the remainder of the experiment, food and water intake, feces weight, and urine volume were determined daily in each rabbit. Seven days after the first cardiovascular measurements, repeat control measurements were obtained and renal artery stenosis plus nephrectomy performed. Measurements were repeated every 7th day for 28 days, at which time the drug infusion protocol was performed and the experiment terminated.

Statistical Analysis

All variables measured repeatedly over time were analyzed using a mixed design (split-plot) analysis-of-variance followed by Dunnett’s test for comparisons over time, and the least significant difference for comparison between sham-lesioned and AV3V-lesioned
rabbits at individual time points. The Student’s $t$ test was used for comparison of single variables between groups. A probability level of less than 5% was considered significant.

**Results**

The criteria for an acceptable AV3V lesion in these experiments were: 1) a symmetrical lesion around the midline on the anterior wall of the third cerebral ventricle extending from the optic chiasm to the anterior commissure; and 2) a pressor response to intracerebroventricular AN more than 2 standard deviations below the mean response of the 10 control rabbits. All 10 rabbits with AV3V lesions reported on here met these criteria.

Before renal artery stenosis, there were no differences between AV3V-lesioned rabbits (AV3V-X) and sham-lesioned rabbits (SHAM) in mean arterial pressure, heart rate, body weight, extracellular fluid volume, plasma potassium concentration, hematocrit, water intake, sodium balance, or potassium balance. Plasma volume, plasma sodium concentration, and plasma osmolality all tended to be higher in AV3V-X rabbits in the control period, but only the plasma sodium concentration difference was statistically significant.

After renal artery stenosis, mean arterial pressure rose from 77 to 117 mm Hg over 4 weeks in SHAM rabbits, but went from 77 to only 92 mm Hg over the same period in AV3V-X rabbits (fig. 1). Mean arterial pressure was significantly higher in SHAM rabbits than in AV3V-X rabbits at all times after renal artery stenosis. Heart rate was unchanged after renal artery stenosis in both groups of rabbits (fig. 1). Also shown in figure 1 is the finding that plasma volume was unchanged in either group of rabbits by renal artery stenosis, whereas extracellular fluid volume was increased modestly in all rabbits, achieving statistical significance only in SHAM animals at Weeks 1, 2, and 3 after stenosis. Body weight was decreased after renal

![Figure 1](image1.png)

**Figure 1.** Changes in mean arterial pressure (MAP), heart rate (HR), plasma volume (PV), extracellular fluid volume (ECFV), and body weight (BW) in rabbits subjected to unilateral nephrectomy (UNILAT. NX) and renal artery stenosis. C = control week; P = poststenosis weeks. Bars on C2 points are standard errors for within groups (over time) comparisons. Asterisks indicate a significant difference compared to the C2 measurement. Bars at right of figure are standard errors for between-groups comparisons (SHAM vs AV3V-X at a fixed time period). Crosses indicate a significant difference between groups.

![Figure 2](image2.png)

**Figure 2.** Changes in plasma sodium concentration ($[\text{Na}^+]_p$), plasma potassium concentration ($[\text{K}^+]_p$), plasma osmolality ($P_{\text{osm}}$), and hematocrit (HCT) in rabbits after unilateral nephrectomy and renal artery stenosis. Symbols and abbreviations are the same as in figure 1.
artery stenosis in all rabbits, but there were no significant differences in body weight between SHAM and AV3V-X rabbits at any time.

Figure 2 shows that plasma sodium concentration, plasma potassium concentration, and plasma osmolality were all unchanged by renal artery stenosis, whereas hematocrit fell identically in SHAM and AV3V-X rabbits during the first 2 weeks of hypertension development.

Figures 3 and 4 illustrate water, sodium, and potassium balance in SHAM and AV3V-X rabbits prior to, and following, renal artery stenosis. In SHAM rabbits, modest water loss (−179 ml on Days 1–3) and sodium retention occurred during the first few days, but all balances were normal throughout the remainder of the experiment. Water intake was significantly increased in SHAM rabbits during the final week of the study. In AV3V-X rabbits, a significantly greater amount of water was lost in the first few days (−216 ml on Days 1–3) than that observed in SHAM animals, but less sodium was retained. Water intake did not increase significantly after renal artery stenosis in AV3V-X rabbits.

Pressor responses and heart rate changes to i.v. infusion of norepinephrine (NE) and All are shown in figure 5. Pressor responses to both NE and All were significantly less in AV3V-X rabbits than in SHAM rabbits. On the other hand, AV3V-X rabbits exhibited no reflex slowing of heart rate during NE infusion, but showed greater reflex bradycardia during All infusion than did SHAM rabbits.

Figure 6 illustrates changes in mean arterial pressure produced by i.v. saralasin, autonomic blockade and intracerebroventricular All. Responses to i.v. saralasin infusion and autonomic blockade were identical in SHAM and AV3V-X rabbits. The pressor effect of centrally administered All was significantly lower in AV3V-X rabbits when compared to that in SHAM rabbits (by definition).

BUN concentration in SHAM rabbits was 14.5 ± 1.6 mg% (mean ± sem) on the day of renal artery stenosis and rose significantly to 22.9 ± 3.2 mg% after 28 days. In AV3V-X rabbits, BUN concentration was 17.5 ± 3.8 mg% prior to stenosis and rose significantly to 26.3 ± 2.6 mg% after 28 days. In SHAM rabbits, kidney weight (9.8 ± 1.9 g) and trans-clip pressure gradient (22 ± 5 mm Hg) were not significantly different from kidney weight (8.2 ± 1.5 g) and pressure gradient (20 ± 7 mm Hg) in AV3V-X rabbits.
FIGURE 4. Changes in water turnover, sodium balance, and potassium balance in AV3V-X rabbits after unilater- 
al nephrectomy and renal artery stenosis. Symbols and abbreviations are the same as in figure 3.

FIGURE 5. Changes in mean arterial pressure (MAP), heart rate (HR) in response to i.v. infusion of nor epineph- 
rine and angiotensin II. Bars represent standard errors of the individual groups, and asterisks indicate a 
significant difference between overall responses of AV3V-X and SHAM rabbits.
DISCUSSION

The results presented here demonstrate that destruction of forebrain areas known to be sensitive to osmotic stimulation and All in the rabbit will significantly attenuate the development of one-kidney, one clip renal hypertension in this species. Since kidney weights, pressure gradients across the renal artery stenosis, and increases in BUN were the same in SHAM and AV3V-X rabbits, the results observed were not due simply to disparities in the stenosis procedure between SHAM and AV3V-X rabbits. Furthermore, the lesions produced in the AV3V-X rabbits studied here were not only anatomically similar to those of our previous work, but also produced physiological changes consistent with the known effects of the lesion in rabbits (i.e., decreased pressor responses to central All, and modest plasma volume expansion and hypernatremia). These data offer additional evidence then of the protective effect of the lesion. However, most studies have shown suppressed or normal plasma renin activity in chronic stages of one-kidney, one clip renal hypertension. Thus, it is important to consider now the mechanisms by which the brain may contribute to hypertension development following renal artery stenosis.

Both blood-borne All and volume expansion have been postulated to contribute to the pathogenesis of one-kidney, one clip renal hypertension. The ability of the AV3V lesion to disrupt both neural angiotensin sensitivity and fluid volume regulation suggests consideration of these actions as possible mechanisms for the protective effect of the lesion. However, most studies have shown suppressed or normal plasma renin activity in chronic stages of one-kidney, one clip, renal hypertension in the rabbit. In addition, in our study and others, i.v. infusion of the competitive All antagonist saralasin did not lower arterial pressure, however, argues against an exclusive role for increased pressor activity in the maintenance of the hypertensive state. Nonetheless, it is interesting to note that: 1) chronic intracerebroventricular infusion of All in rabbits also will produce hypertension associated with increased pressor reactivity to NE; and 2) lesion of the AV3V region will prevent the development of this type of hypertension. Since AV3V lesions both attenuate the hypertension and the pressor reactivity to norepinephrine in one-kidney, one clip rabbits in the present study, it is possible to speculate that one-kidney, one clip renal hypertension in the rabbit may involve an activation of neurohormonal pressor mechanisms in the forebrain by either circulating or endogenous brain All. This hypothesis has been advanced previously to explain the protective effect of AV3V lesions on certain models of renal hypertension in the rat.

Thus, a reduced effect of blood-borne All is not likely to explain the action of the AV3V lesion demonstrated here.

Although considerable literature indicates that renal sodium and water retention and subsequent volume expansion contribute to hypertension in one-kidney models (see ref. 15 for review), recent studies in the rabbit failed to show increased plasma volume in the one-kidney, one clip animal. Our own experiments demonstrated only modest, transient sodium retention after renal artery stenosis, accompanied by actual loss of body water (by balance measures), no expansion of plasma volume, and only slight increases in extracellular fluid volume. These changes were very similar in the hypertensive SHAM rabbits and in the mostly normotensive AV3V-X rabbits, making it unlikely that such factors contribute directly to the pathogenesis of hypertension after renal artery stenosis in the rabbit. In addition, although osmotic stimulation of the brain can produce an acute increase in arterial pressure, which is attenuated by AV3V lesions in the rabbit, plasma osmolality and sodium concentration were not changed by renal artery stenosis in either group of rabbits in the present study. Thus, diminished direct osmotically stimulated pressor activity also does not appear to be a critical effect of the lesion in protecting against renal hypertension.

Several recently published studies have implicated increased cardiovascular reactivity to NE (and other vasoactive substances) in the pathogenesis of renal hypertension in the rabbit. Increased pressor responsiveness of conscious rabbits to NE, or vasopressin infused i.v. was observed both in the prehypertensive period after clipping, and 30 days after the onset of one-kidney, one clip renal hypertension. Thus, the authors postulated that this increased responsiveness was a contributor to the development of hypertension, not merely an end result of hypertensive vascular abnormalities. Furthermore, it was demonstrated that infusion of a competitive All antagonist could reverse the increased cardiovascular reactivity to NE particularly in the early stages of hypertension development. The failure of the antagonist to acutely normalize arterial pressure, however, argues against an exclusive role for increased pressor reactivity in the maintenance of the hypertensive state. Nonetheless, it is interesting to note that: 1) chronic intracerebroventricular infusion of All in rabbits also will produce hypertension associated with increased pressor reactivity to NE; and 2) lesion of the AV3V region will prevent the development of this type of hypertension. Since AV3V lesions both attenuate the hypertension and the pressor reactivity to norepinephrine in one-kidney, one clip rabbits in the present study, it is possible to speculate that one-kidney, one clip renal hypertension in the rabbit may involve an activation of neurohormonal pressor mechanisms in the forebrain by either circulating or endogenous brain All. This hypothesis has been advanced previously to explain the protective effect of AV3V lesions on certain models of renal hypertension in the rat.
The mechanism of forebrain stimulation in one-kidney models of renal hypertension, and the nature of the resulting pressor influence, is controversial. Since circulating levels of All are presumably quite low in one-kidney renal hypertension, \(^1\) pressor effects emanating from the AV3V region are not likely to be stimulated by systemic All. However, there is strong evidence that the brain renin-angiotensin system is activated by relative sodium retention, \(^2\) such as occurs in one-kidney, one clip renal hypertension. It is possible, then, that renal artery constriction activates neurohormonal pressor systems emanating from, or passing through, the AV3V region by mediation of endogenously generated All within the brain. Part of this neurohormonal pressor effect is expressed as an increased cardiovascular reactivity, and both the increased arterial pressure and reactivity are interrupted by damage of the AV3V region.

The nature of the central neurohormonal pressor influence in one-kidney renal hypertension in unknown. There is evidence supporting a centrally driven increase in peripheral sympathetic nervous system activity in this model, \(^3\) which may be augmented by high sodium intake, \(^4\) although other studies do not support these findings. \(^5\) The results of the present experiments do not support a reduction in neurogenic vasoconstrictor activity as the major mechanism by which AV3V lesions reduce the severity of one-kidney, one clip renal hypertension, since blockade of autonomic neurotransmitters produced similar depressor responses in SHAM and AV3V-X rabbits.

The cause of the lower pressor responsiveness in AV3V-X rabbits compared to SHAM rabbits may be related to a mechanism involving AV3V, as discussed above, but again the exact reason for this defect is unclear. Increased baroreflex buffering could decrease pressor responses to exogenous agonists, but in the present study baroreflex heart rate slowing during NE infusion were less in AV3V-X rabbits, making this an unlikely explanation for the decreased pressor effects obtained. Interestingly, baroreflex cardiac slowing during angiotensin infusion was greater in AV3V-X than SHAM rabbits, and thus could have played a part in the smaller pressor actions of this agonist in AV3V-X rabbits.

Finally, we can speculate that the disruptive effect of AV3V lesions on fluid and electrolyte regulation, arterial pressure regulation and hypertension. In Frontiers in Neuroendocrinology, Vol. 6, edited by Martini L, Ganong WF. New York: Raven, 1980, pp 249-292

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