Effect of Lesions of the Anteroventral Third Ventricle (AV3V) on the Development of Hypertension in Spontaneously Hypertensive Rats

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SUMMARY Lesions of the anteroventral third ventricle (AV3V), an angiotensin and osmosensitive region of the anterior hypothalamus, prevent or abort hypertension in a number of rat models. To determine if AV3V lesions alter hypertension in spontaneously hypertensive rats (SHR), lesions and control sham lesions were made in young SHR at 28 days of age. AV3V lesions had no effect on the development of hypertension in SHR. However, lesioned rats demonstrated significantly reduced pressor responses to intracerebroventricular injections of angiotensin II (All) and hypertonic NaCl, and drinking produced by centrally administered All. The depressor effect of central All receptor blockade was also significantly attenuated in lesioned SHR. These effects appeared to be of central origin since the lesion did not affect the pressor action of intravenous All or norepinephrine (NE). It is concluded that unlike other models of experimental hypertension (steroid-salt, one- and two-kidney renal, neurogenic) the development of hypertension in SHR does not depend upon the integrity of the AV3V region. (Hypertension 4: 387-393, 1982)

KEY WORDS • spontaneously hypertensive rats • anteroventral third ventricle (AV3V) • brain lesions • angiotensin • saralasin • hypertension • central nervous system

It has been suggested that the central nervous system may participate in the development and/or maintenance of hypertension in the spontaneously hypertensive rat (SHR). For example, hypertension in this model can be prevented by cerebral ventricular injection of the catecholaminergic neurotoxin, 6-hydroxydopamine,1,2 and the centrally acting antihypertensive drug, alpha-methyldopa.3 Moreover, surgical transection of the brain or spinal cord lowers blood pressure to a greater extent in the SHR than in normotensive Wistar-Kyoto (WKY) control animals.4,4 Recent evidence has pointed toward a role for the brain renin-angiotensin system in the hypertension of SHR5 since these rats show greater pressor responses to centrally administered All when compared to WKY controls;6 also, central pharmacologic blockade of All receptors7,8 or All-converting enzyme9 lowers arterial pressure in SHR but not WKY.

Previous studies from this laboratory have shown that small preoptic hypothalamic lesions that destroy tissue surrounding the anteroventral third cerebral ventricle (AV3V) can prevent, ameliorate, or reverse hypertension in a number of rat models including one- and two-kidney renal, deoxycorticosterone-salt, and neurogenic hypertension.10 AV3V lesions also attenuate or abolish both the central pressor and behavioral effects of All.11 If the AV3V region is involved in the pathogenesis of hypertension in SHR, then destruction of this brain region would be expected to alter the course of the development of hypertension.

The purpose of our present study was to examine the effect of AV3V ablation upon the development of hypertension in SHR. This investigation was also predicated upon our previous finding12 that AV3V lesions in adult SHR failed to alter the level of arterial pressure in these rats even though they demonstrated all other characteristics of rats bearing AV3V lesions (e.g., thirst and central pressor deficits). This result raised the possibility that structural vascular alterations,13 abnormalities in vascular smooth muscle,14,15 or other factors might maintain elevated...
arterial pressure in this model once hypertension was established. These considerations prompted an analysis of the effect of AV3V lesions in weanling SHR to determine if the AV3V region might play a role in the developmental phase of hypertension.

Methods

Animals

Weanling SHR were obtained from an inbred colony maintained at The University of Iowa. These animals were direct descendants of the SHR strain isolated by Okamoto and Aoki in which the development of hypertension is maintained by exclusive brother-sister breeding. The original stock for these animals was supplied by the National Institutes of Health, Bethesda, Maryland.

All animals were separated from the mother and individually housed in hanging wire mesh cages for 3 days prior to surgery. Lab chow pellets (Purina) were available ad libitum on the cage floor, and tap water and 10% sucrose solution were supplied in 100 ml graduated cylinders fitted with drinking spouts. The sucrose solution was provided for 2 weeks post lesion to encourage consumption of large amounts of fluid, since it has been found that both adult and weanling rats refuse to drink water for several days following ablation of the AV3V but will often consume more palatable liquids, thus maintaining voluntary hydration after AV3V lesions. Lighting was maintained on a 12-hour light and 12-hour dark cycle; ambient temperature was 22° ± 2° C.

AV3V Lesions

Male SHR aged 28 days were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and positioned in a stereotaxic apparatus (David Kopf) with the skull level between bregma and lambda. A midline incision of the scalp was made and the skin retracted laterally to expose the dorsal surface of the skull. A 2 mm diameter hole was then trephined over bregma and the bone flap removed. The midsagittal sinus was retracted, and a 0.4 mm diameter nichrome wire, Teflon insulated except at the tip, was positioned on the midline 0.2 mm anterior to bregma and then lowered 7.5 mm from dura. Electrolytic lesions of the AV3V were made by passing 1.0 mA direct anodal current through the lesioning electrode for 25 seconds to a rectal cathode. The electrode was then withdrawn, the trephine hole filled with Gelfoam, and the incision closed with wound clips. All rats were treated identically except that the electrode was lowered 5.5 mm, and no current was passed.

Blood Pressure Determination

Beginning with rats at 9 weeks of age and continuing for 7 weeks, we measured systolic blood pressure of conscious, restrained SHR three times weekly by photoelectric sensing of tail artery blood flow during slow deflation of an occluding tail cuff (IITC). Daily blood pressure was recorded as the mean of three to seven measurements, and a single weekly value obtained for individual animals by averaging the three pressure determinations recorded during that week.

Drinking Test to Centrally Administered Angiotensin

At 18 weeks of age, AV3V-lesioned and sham-lesioned SHR under ether or pentobarbital anesthesia had cannulas implanted stereotaxically in the right lateral cerebral ventricle. The surgical procedure was the same as that described for lesions except that a 23-gauge stainless steel guide cannula was lowered 6.0 mm from the top of the skull, with the other coordinates being 0.8 mm posterior to bregma and 1.3 mm lateral to the midsagittal suture. The guide cannula was cemented into place with methyl methacrylate cement anchored by jewelers screws placed in the skull. A 30-gauge obturator cut flush to terminate at the tip of the guide cannula was inserted, and the animals were injected i.m. with 60,000 U procaine penicillin G.

Seven days following cannula implantation, SHR were taken from their home cage, the obturator removed from the guide cannula, and replaced with a 30 g injector connected by PE 10 polyethylene tubing to a remote 10 µl syringe filled with All (50 ng/µl) or artificial cerebrospinal fluid vehicle (ACSF).17 A 2.0 µl injection of All (100 ng) or ACSF was then delivered over 20 seconds into the lateral ventricle, with the injector remaining in place for an additional 30 seconds to allow for drug diffusion into the ventricular space. The injector was then removed, obturator replaced, and the rats returned to their cages. The amount of tap water consumed in the 30 minutes following intraventricular (IVT) injection was recorded to the nearest 0.1 ml from graduated burettes.

Central and Peripheral Pressor Tests

Several days after the All drinking test, SHR were anesthetized with ether and a heparin-filled PE 50 catheter was placed in the abdominal aorta via the left femoral artery for direct recording of arterial pressure. The right jugular vein was also catheterized at this time for peripheral infusion of vasoactive drugs. The catheters were tunneled subcutaneously to exit from the skin between the scapulae, the rats placed in a 20 X 20 X 30 cm testing chamber and allowed to recover from the surgery for at least 2 hours prior to the start of experimentation. At this time, the arterial catheter was connected to a low volume pressure transducer (Ailtech MS-10) and blood pressure recorded on a Beckman RM-2 type dynograph.

Angiotensin II (2, 10, 50, 250 ng) dissolved in 2 µl ACSF, or 600 mOsm/liter hypertonic ACSF (4 µl) made hyperosmolar by adding NaCl, was then injected over a period of 10–20 seconds through the guide cannula into the lateral cerebral ventricle of conscious freely moving SHR. Ventricular injections of 2.0 and 4.0 µl isotonic ACSF were employed as vehicle injection controls, and all drugs were administered in random order. Repeated injections were not made until...
blood pressure had returned to baseline, usually within 15–45 minutes.

Upon completion of the central pressor tests, SHR were infused intravenously with 33, 100, 300, and 900 ng/kg/min AII and 300 and 900 ng/kg/min NE dissolved in isotonic saline. The agents were administered in random order and the infusion rate held constant at 100 μl/min for all doses. Arterial pressure was recorded in conscious SHR as previously described. Following these experiments, the arterial and venous catheters were filled with heparinized saline, plugged with a short piece of occluded 23-gauge stainless steel tubing, and the animals returned to their home cage. The next day SHR were again connected to the arterial pressure recording apparatus, and 10 or 20 ng of the AII antagonist sar'-ala'-AII (saralasin acetate, Norwich) dissolved in 2 or 4 μl of ACSF (5 ng/μl) was injected into the lateral cerebral ventricle, and blood pressure responses recorded.

Histology

At the conclusion of these experiments, SHR were perfused through the left heart ventricle with isotonic saline followed by 10% buffered formalin, and the brains removed and stored in 10% buffered formalin. At 1 to 6 weeks later, 50 μm frozen sections were cut and stained with cresyl violet and examined microscopically for histological verification of the location of the brain lesions.

Statistical Analysis

The data from all experiments were analyzed by analysis of variance. Multiple pairwise comparisons among means were made by follow-up test procedures utilizing the error mean square terms from the overall analysis. Planned orthogonal comparisons were made by t test and a posteriori nonorthogonal comparisons by Dunnett's test. The criterion for statistical significance was set at p < 0.05 level, two-tailed.

Results

Development of Hypertension

As shown in figure 1, both sham and AV3V-lesioned SHR developed hypertension. Lesioned SHR tended to have slightly lower arterial pressure, but there was no statistically significant difference between the two groups at any time, nor was there any difference in the rate at which hypertension developed or the variability in blood pressure between groups. When blood pressure was measured directly by intraarterial catheter in 20-week-old lesioned and sham-lesioned SHR, no difference was observed for either systolic (190 ± 10 vs 191 ± 10 mm Hg) or diastolic (147 ± 10 vs 149 ± 5 mm Hg) arterial pressure between the two groups.

Central Angiotensin Drinking

Figure 2 shows 30-minute water consumption following i.v.t. injection of 2 μl ACSF or ACSF containing 100 ng AII. Little or no drinking was produced by ACSF injection in either group, nor was there any significant difference in drinking between them. Intraventricularly injected AII produced drinking in both groups of SHR. AV3V-lesioned SHR showed a substantial (52%) and significant (p < 0.001) reduction in the behavioral response to central AII stimulation.

Pressor Tests

When the pressor response to central AII was determined (fig. 3), sham-lesioned SHR demonstrated a dose-dependent increase in arterial pressure. The AV3V lesion significantly reduced the central pressor activity of AII. Post-hoc comparison of the individual means comprising the dose-response curves of the two groups (Dunnett's test) indicated that, for sham-lesioned SHR, a pressor response significantly different (p < 0.05) from control ACSF injection first occurred at a dose of 10 ng. For AV3V-lesioned SHR, a statistically reliable increase in arterial pressure was found only at the highest dose of AII (250 ng). Additionally, lesions of the AV3V significantly reduced the pressor response to central osmotic stimulation produced by i.v.t. injection of hypertonic ACSF.

Figure 4 illustrates the pressor response of lesioned and sham-lesioned SHR to intravenous infusion of various doses of AII and NE. Pressor responses for the two groups were not different for either AII or NE.
Central All Blockade

Figure 5 shows blood pressure responses of conscious AV3V-lesioned and sham-lesioned SHR to central injections of the All antagonist saralasin acetate. Blood pressure responses to 10 μg of centrally injected saralasin were quite variable in both groups. Some animals showed pressor responses and others depressor responses to this dose of the drug. Analysis of these data indicated that the blood pressure of both groups was not significantly changed by this dose of saralasin. However, when 20 μg of saralasin was injected i.v.t., all sham-lesioned SHR uniformly exhibited a depressor response averaging -8 mm Hg. The time to the peak response ranged from 5-30 seconds, with a return to baseline arterial pressure in 3-10 minutes. The depressor responses of AV3V-lesioned SHR to central saralasin were significantly attenuated (p < 0.05). Further analysis of these data (Dunnett's test) indicated that whereas 20 μg of saralasin significantly (p < 0.05) reduced arterial pressure in neurologically intact SHR, no statistically reliable effect of the drug could be detected for AV3V-lesioned rats at any dose level.

Histology

Histologic examination of the hypothalamus of AV3V-lesioned SHR indicated that the extent of the periventricular damage from the lesion was similar to that reported previously. The brain lesion was confined to the immediate periventricular strata of the preoptic and anterior hypothalamus including the medial portions of the medial preoptic nuclei.
**Figure 4.** Pressor responses produced in conscious rats by intravenous infusion of various doses of angiotensin II and norepinephrine. The peripheral pressor activity of both agents was equivalent for lesioned and sham-lesioned SHR.

**Figure 5.** Blood pressure changes of AV3V-lesioned and sham-lesioned SHR following intraventricular injection of artificial cerebrospinal fluid (ACSF) and 10 and 20 µg saralasin. AV3V lesions significantly reduced the depressor action of central saralasin at the 20 µg dose when compared to the sham-lesioned control group (*p < 0.05, t-test). Saralasin did not significantly change arterial pressure in lesioned SHR at either dose.

**Figure 6.** Representative coronal section through the brain of an AV3V-lesioned SHR at the level of the optic chiasm (OC) and anterior commissure (AC) illustrating the mediolateral extent of a typical lesion located between these two structures.
The AV3V region, an AII and osmosensitive area whose integrity is required for the development and/or maintenance of hypertension in other experimental models, might play a role in the development of hypertension in SHR. Thus, the AV3V region appears not to play a major role in the development or maintenance of hypertension. This finding does not rule out a contribution of the CNS to hypertension in SHR, but demonstrates only that the AV3V is not a critical central locus required for its expression. Additionally, it should be emphasized that, since lesions of the AV3V did not affect hypertension in SHR, its preventive action in other models is probably not due to some generalized or nonspecific (e.g., hypotensive) effect of AV3V tissue destruction, but, instead, the lesion must exert its protective effect through mechanisms perhaps common to other forms of hypertension that are not operative in SHR.

Because the development of hypertension in SHR was not affected by AV3V lesions, several tests of the functional efficacy of the lesion were performed. These tests involved measuring centrally evoked drinking and pressor responses produced by angiotensin and osmotic stimulation. When AII was administered centrally, lesioned rats showed a marked reduction in the behavioral response to this stimulus, confirming previous observations that an intact AV3V region is required for the full expression of the drinking response to central AII. Central AII pressor responses were virtually abolished by AV3V lesions, and those produced by hypertonic NaCl were substantially reduced.

It is interesting to note that, although AV3V-lesioned SHR showed residual drinking responses to central AII, pressor responses were eliminated. These results suggest that some of the lesions might have interrupted neural pathways responsible for pressor effects while partially sparing those that participate in drinking behavior. This supposition has recently been confirmed by studies showing that discrete lesions or knife cuts within the larger region defined as the AV3V can selectively alter drinking or pressor responses produced by centrally administered AII.

Several recent reports have suggested that the central action of humoral substances (AII, sodium) may contribute to hypertension in SHR. Centrally administered AII or sodium produces a greater pressor response in SHR than Wistar-Kyoto rats. The AII antagonist, saralasin, has also been shown to lower arterial pressure when injected centrally but not peripherally in SHR. Since the central pressor activity of AII was virtually eliminated by AV3V lesions placed in weanling or adult SHR, the integrity of central AII pressor mechanisms that contribute to the maintenance of blood pressure in neurally intact SHR appear not to be an absolute requirement for the development or maintenance of hypertension.

To study the effects of the AV3V lesion were confined to the CNS and did not result from alterations in peripheral cardiovascular reactivity, AII and NE were infused intravenously. Pressor responses of lesioned and sham-lesioned SHR were identical. Thus, the decreased pressor responsiveness observed following central drug injection was not due to peripheral factors. In a previous study on normotensive Sprague-Dawley rats, lesions of the AV3V reduced the pressor response to peripherally administered AII without affecting pressor responsiveness to NE. This effect was not observed in the AV3V-lesioned hypertensive SHR examined in the present study. It is possible that enhanced vascular reactivity or structural vascular alterations associated with hypertension in the SHR masked the contribution of central AII pressor mechanisms in these rats.

To evaluate the hypothesis that the depressor action in SHR of central AII blockade might be mediated via AII receptors or pathways involving the AV3V region, two doses of the AII antagonist, saralasin, were injected ivt. At the larger dose (20 μg), a small but significant fall in arterial pressure similar in magnitude to previously published reports was observed in sham-lesioned rats, confirming that the central action of AII contributes to the maintenance of blood pressure in intact SHR. However, i.v.t. saralasin administered to AV3V-lesioned SHR failed to significantly alter arterial pressure. The depressor effect of saralasin in SHR and its abolition by AV3V lesions was most probably due to an action on central rather than peripheral AII receptors. Using test procedures similar to ours, Mann et al. showed that peripherally administered saralasin increased rather than decreased arterial pressure in SHR. In other experiments, we have found essentially the same result in Sprague-Dawley rats treated identically to the SHR used in these studies. Intravenous saralasin (20 μg) produced a slight increase (2.6 ± 1.8 mm Hg) in arterial pressure. These results indicate that the depressor action of centrally administered saralasin and its blockade by AV3V lesions in SHR was probably not due to leakage of the drug into the peripheral circulation.

These data indicate that central AII mechanisms contribute to the maintenance of blood pressure in SHR. However, since the depressor activity of the AII antagonist as well as the pressor activity of AII itself were both reduced by AV3V lesions, yet hypertension developed normally, central AII pressor influences on arterial pressure must be compensated for by other mechanisms during the development and maintenance of spontaneous hypertension, indicating that central...
All mechanisms participate in, but are not a necessary requirement for, the development or maintenance of hypertension in SHR.

In summary, these data indicate that the central neural mechanisms that contribute to hypertension in SHR differ from those in other models of experimental hypertension since lesions of the AV3V have no effect upon the development or maintenance of hypertension in SHR, but can completely prevent hypertension in several other animal models. However, like all other strains of rats thus far tested, all as well as osmosensitive neurons appear to be contained within, or send fibers of passage through, the region of the AV3V, since both the centrally mediated pressor and behavioral responses to these stimuli were greatly reduced in SHR by AV3V ablation.

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References

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