Anteroventral Third Ventricle and Renin-Angiotensin System Interaction in the Two-Kidney, One Clip Hypertensive Rat

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SUMMARY To test the peripheral mechanisms of prevention and reversal of two-kidney, one clip (2K1C) hypertension in the rat by lesion of the anteroventral third ventricle (AV3V) region, we studied blood pressure responses in rats to AV3V lesion produced before (n = 8) or after (n = 8) clipping the left renal artery. Two groups of sham-lesioned, clipped rats (n = 9 each) served as controls. At the end of the experiments, saralasin and captopril were given to evaluate the angiotensin-dependent component of blood pressure. To study the influence of the procedures on plasma renin activity (PRA), two parallel groups of rats (n = 26 and 24, respectively) were submitted to similar surgical protocols. We observed that increases in blood pressure were significantly smaller in the previously lesioned compared to previously sham-lesioned animals (ΔBP = 21.5 ± 3.7 vs 32.9 ± 2.5 mm Hg, p < 0.01); also, AV3V lesion almost completely reversed hypertension (BP from 167.5 ± 2.9 to 136.0 ± 4.1 mm Hg, p < 0.001), which was not observed in the sham-lesioned animals (BP from 172.0 ± 2.8 to 168 ± 2.7 mm Hg, NS).

Saralasin produced a significantly smaller decrease in BP in the lesioned animals compared to those with sham lesions during both prevention and reversal experiments. Similar results were observed with captopril. Previous AV3V lesion did not significantly affect PRA with clipping of the renal artery, but AV3V destruction after hypertension had been established resulted in significantly lower PRA compared to sham-lesioned animals (4.58 ± 0.72 vs 8.38 ± 1.79, respectively, p < 0.001). It is concluded that, besides being an important central site of angiotensin action, the AV3V area is also important in the pathophysiology of 2K1C hypertension because it acts in the regulation of the peripheral production and action of angiotensin II in this model.

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KEY WORDS • anteroventral third ventricle area • central lesions • renovascular hypertension • Goldblatt hypertension • captopril • saralasin • peripheral renin action • renin production

Lesions of the anteroventral third ventricle (AV3V) interfere with the development and maintenance of high blood pressure in several models of hypertension. Those models in which the renin-angiotensin system plays a central pathophysiologic role are especially susceptible to prevention or reversal by discrete lesions of the AV3V region. Interruption of a central action of peripherally generated angiotensin II (All) is generally accepted as the underlying mechanism because the AV3V region is recognized to be the main central nervous system site of action of that hormone in the control of blood pressure. Nevertheless, evidence in support of this hypothesis is scarce and indirect, and further elucidation is needed.

We have previously demonstrated in glucocorticoid-induced hypertension in the rat, which is a renin-dependent model, that pharmacologic blockade of the renin-angiotensin system resulted in a diminution of the depressor response after lesions of AV3V tissue. Although this fact does not rule out the possibility of interruption of the central action of All, it could also be interpreted as the result of a diminished secretion of renin or of an impaired sensitivity of the peripheral mechanisms of angiotensin action. We thus studied the...
Peripheral mechanisms of blood pressure attenuation in the two-kidney, one clip hypertensive rat. We analyzed simultaneously the angiotensin-dependent component of blood pressure (by pharmacological blockade) and plasma renin activity (PRA) during prevention and reversal of this hypertension by lesion of AV3V tissue.

Material and Methods
Prevention of Hypertension
Seventeen male Sprague-Dawley rats were studied to establish the mechanism of AV3V lesion in the prevention of 2K1C hypertension. In eight rats, an actual AV3V lesion was produced by standard stereotaxic technique. In short, lesions were produced by introducing an electrode into the third ventricle according to the following coordinates in a stereotaxic apparatus (David Kopf, Model 900): 0.0–0.5 mm posterior to the bregma, in the midline, to a depth of 7.5 mm from the dura. The lesioning current consisted of 20 mA sustained for 20 seconds.

Histologic examination of lesioned areas revealed destruction of the anterior portion of the third ventricle, between the anterior commissure and optic chiasm, including the organum vasculosum of the lamina terminalis, and extending posteriorly to the periventricular preoptic area and the medial portions of the medial preoptic nucleus. These results are similar to those published previously using the same technique. In nine rats, the same technique was used to produce sham AV3V lesions, but no electric current was passed. In the sham-lesioned animals, histologic examination showed the integrity of the above-mentioned third ventricular area. Two weeks after these manipulations, a silver clip was placed on the left renal artery of all 17 rats. Blood pressure (by the tail-cuff method) was determined at weekly intervals from the time of the central lesions to 4 weeks after placement of the renal artery clips. At the end of this time, all 17 rats were catheterized through the femoral artery with an Intramedic PE-50 catheter (Clay-Adams, New Jersey), and tests were done 24 hours after catheterization to determine the angiotensin-dependent component of blood pressure. These tests consisted of i.v. infusion of saralasin (Eaton Laboratories) at a rate of 10 \( \mu \text{g/kg/min} \) for 10 minutes and captopril administration (10 mg/kg) by gavage 1 hour after recovery from the infusion. Blood pressure was continuously measured throughout the tests using a Statham P23ld pressure transducer (Gould-Statham Instruments, Cleveland, Ohio) connected to a Gould-Brush recorder 2200S (Gould Instruments, Cleveland, Ohio). The lowest levels of BP registered after about 10 minutes of saralasin infusion and 30 minutes of captopril administration were considered as maximal blood pressure responses to the drugs. The efficacy of angiotensin blockade was assessed by injection of 10 ng of AI in all animals at the end of the experiments with either blocker.

In a parallel group of rats (n = 26) in which the same protocol was observed, we studied the influence of the surgical procedures on PRA. Two weeks after AV3V manipulations, the rats with actual (n = 6) or sham lesions (n = 6) were sacrificed by decapitation, and blood was collected for PRA determination. Four weeks after the renal artery clipping, rats with previous AV3V lesions (n = 8) or sham lesions (n = 6) were also decapitated for the same purposes. Plasma renin activity was determined using an Al [I125]-RIA Kit (New England Nuclear, Boston, Massachusetts). Normal values for decapitated animals with this method are 2.3 ± 1.5 ng/ml/hr.

Reversal of Hypertension
Another 17 rats were used to study the mechanism of AV3V lesions in the reversal of 2K1C hypertension. Clips were placed on the left renal arteries and, 4 weeks later, an actual (n = 8) or sham lesion (n = 9) was produced in the AV3V region. Weekly tail artery pressure was determined from before renal artery clipping to 4 weeks after central manipulations. At the end of this period, the rats were catheterized for blood pressure determinations during saralasin infusion and captopril administration, as described above. AI injections were also made to attest the efficacy of blockade at the end of experiments.

A parallel group of 24 rats was used to study the influence of the lesions on PRA. Twelve rats were sacrificed 4 weeks after renal artery clipping; animals with clipped renal arteries and sham (n = 6) or actual lesions (n = 6) were sacrificed 4 weeks after the manipulations in the central nervous system.

Statistical Analysis
Student’s t test for unpaired data were used to determine the significance of the differences between groups. Analysis of variance was used to determine the significance of responses within groups. Results are expressed as means ± standard errors of the mean (SEM).

Results
Prevention of Hypertension
AV3V manipulations did not significantly alter tail arterial pressure (TAP) in the rats with either actual (from 125.3 ± 1.4 to 119.8 ± 1.8 mm Hg, NS) or sham lesions (from 120.9 ± 0.9 to 125.3 ± 1.6 mm Hg, NS). Renal artery clipping increased TAP in both groups significantly (to 141.3 ± 2.4 and to 158.7 ± 1.7 mm Hg respectively; p < 0.001 for both) (fig. 1).

However, the increments in blood pressure (BP) observed in the group with actual AV3V lesion were significantly smaller than in the sham-operated group (ΔBP = 21.5 ± 3.7, vs 32.9 ± 2.5 mm Hg, p < 0.01).

Two weeks after the AV3V lesion, PRA was not significantly different from that observed in the group sacrificed 4 weeks after lesions plus renal artery clip (5.18 ± 0.44 vs 5.32 ± 0.62 ng/ml/hr, NS). Also,
Reversal of Hypertension

Control TAP was similar between the two groups studied for the reversal of hypertension (122.8 ± 1.0 vs 125.6 ± 2.2 mm Hg, NS). Four weeks after clipping, TAP had risen to similar levels in the two groups (167.5 ± 2.9 vs 172.0 ± 2.8 mm Hg, respectively, NS). The production of an AV3V lesion produced a sharp decrease in TAP (fig. 2), to levels of 136.0 ± 4.1 mm Hg (p < 0.001), while in the sham-lesioned group it remained elevated (168.4 ± 2.7 mm Hg, NS, compared to prelesion levels). This difference persisted until the end of the study period (135.5 ± 1.7 vs 165.3 ± 4.1 mm Hg, respectively, p < 0.001). No significant differences in PRA were observed in the two groups with renal clip before manipulations of the central nervous system (PRA = 5.63 ± 0.47 vs 6.25 ± 0.99 ng/ml/hr, NS), while after the AV3V lesion, PRA remained at levels not statistically different (PRA = 4.58 ± 0.72, NS), but it increased in the group with sham AV3V lesions (8.38 ± 1.79, p < 0.01).

The response of mean arterial pressure (MAP) to angiotensin antagonists was significantly smaller in the group with actual lesion compared to their controls with either blocker, saralasin (AMAP = -1.3 ± 0.8 vs -15.8 ± 1.7 mm Hg, p < 0.001) or captopril (AMAP = -7.8 ± 1.0 vs -29.4 ± 5.3 mm Hg, p < 0.001).

Discussion

Evidence that the peripherally administered All has blunted pressor effects in animals with lesions in the AV3V area has led to the concept that this area may act as a central site of action of peripherally generated All in the "renin-dependent" models of hypertension. Although this may help to explain the importance of the AV3V region in the mediation of pressor responses to All, alternative possibilities must be investigated because blood-borne All has not been demonstrated to cross the blood-brain barrier. Thus, the purpose of our study was to evaluate the peripheral mechanisms of prevention and reversal of 2K1C hypertension by lesions of the AV3V region.

A marked attenuation of the blood pressure rise was observed in the group of animals with AV3V lesion preceding the clipping of renal arteries as compared to their sham-lesioned controls. Also, the hypertensive levels of blood pressure were promptly reversed when AV3V lesion was produced after 4 weeks of established hypertension; this reversal was sustained for a 4-week observation period. These results are similar to those obtained in other renin-dependent models and almost completely reproduce those of Haywood et al. in the same model of hypertension in the rat.

Although pharmacologic tests for the assessment of the angiotensin components of blood pressure have limitations, we tried to overcome this difficulty by use of two different angiotensin blockers, saralasin and captopril. We observed that administration of both blockers resulted in almost no blood pressure reduction in the lesioned groups, compared to the appropriate controls both when AV3V lesion preceded or followed
renal artery clipping. This clearly shows that the component of blood pressure that could be linked to angiotensin activity is negligible after AV3V lesion in this form of hypertension, and reflects a lack of peripheral activity of the renin angiotensin system. These observations suggest two distinct possible effects of AV3V lesion: 1) inhibition of renin production by the kidney; or 2) a diminished sensitivity of angiotensin receptors or peripheral effector mechanisms.

The first hypothesis was confirmed by analysis of the PRA behavior in the group in which AV3V lesion reversed hypertension. In this situation, the AV3V lesion blunted a further rise of PRA with time, which could be observed in the sham-lesioned animals. This observation supports previous studies that demonstrated the necessity of the AV3V region remaining intact for appropriate renin release by the kidney either directly or indirectly through AV3V-dependent regulation of salt and water balance.

However, when AV3V lesion preceded the renal artery clipping, the same mechanism cannot be implicated because no difference in PRA between the lesioned and the sham-lesioned animals accompanied the diminution in response to angiotensin blockade. In this instance, a reduction in the sensitivity of vascular angiotensin effector mechanisms must be assumed. The etiology of this diminished sensitivity to angiotensin cannot be clarified from analysis of our data. Possible mechanisms involving AV3V-mediated shifts in vascular Na\(^+\) pump activity\(^{20}\) might help to explain this phenomenon, but they still remain controversial and difficult to interpret.\(^{21,22}\)

In summary, the importance of the AV3V area as a central site of action of angiotensin is affirmed because its integrity is necessary to fully express even the acute pressor effects of AII.\(^{3,13}\) Our results also show that AV3V is important to the full development of the peripheral actions of blood-borne AII.

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References

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