Role of the Anteroventral Third Ventricle Region and the Renin Angiotensin System in Methylprednisolone Hypertension

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SUMMARY Methylprednisolone (M, 10 mg/kg/week subcutaneously) was administered to cause hypertension in rats, and the role of AV3V region was assessed before and after development of the hypertensive state. Participation of the renin angiotensin system (RAS) was evaluated by changes in mean arterial pressure (MAP) induced by administration of saralasin (S, 10 μg/kg/min i.v.) or captopril (C, 20 mg/kg/p.o). An AV3V lesion before M administration partially prevented and delayed the beginning appearance of M hypertension. Furthermore, a prior AV3V lesion abolished an angiotensin II (AII)-dependent pressor component normally identified by S and C administration in this type of hypertension. During the maintenance phase of the hypertension, an AV3V lesion caused a partial reduction in blood pressure. A spontaneous disappearance of a vasoconstrictor component mediated by AH was observed in the late phases of M hypertension. It is concluded that the AV3V region is essential to the full development and maintenance of M hypertension in the rat. Also in this model, integrity of the AV3V area is essential to the expression of the All-mediated pressor component. Finally it is apparent that M can cause hypertension even in the absence of the AV3V area or during chronic renin angiotensin blockade, indicating multiple pathogenetic mechanisms in this experimental model.

KEY WORDS • glucocorticoid hypertension • renin angiotensin system • captopril • central nervous system

Gluocorticoid hypertension in the rat may develop even with low dietary salt intake and may involve activation of the renin angiotensin system as well as alterations in the autonomic nervous system.

Angiotensin II (All) is a potent direct vasoconstrictor agent and when given directly into the central nervous system evokes blood pressure elevation and dipsogenic responses. The central action of All in the dog is mainly through the area postrema but in the rat, the main site of All action appears to be the anteroventral third ventricle (AV3V) region, which has also been implicated in the pathogenesis of several types of experimental hypertension.

The present work was designed to test a possible role for the AV3V area in a glucocorticoid-induced hypertension in the rat and its relationship to the renin angiotensin system.

Material and Methods

Hypertension was induced by weekly subcutaneous administration of a long-acting methylprednisolone preparation (Depo-Medrol, Upjohn) given at a dose of 10 mg/kg body weight in male Wistar rats weighing from 300 to 400 g. Blood pressure was determined either by a tail arterial pressure (TAP) method or by direct continuous determination of mean arterial pressure (MAP) in unrestrained unanesthetized animals. The electrolytic AV3V lesion was performed using a technique previously described. In short, an anodal lesion using a discharge of 2 mA for 20 seconds was produced by a 24-gauge nichrome electrode according to the following coordinates: 0.0–0.5 mm caudal to the bregma to a depth of 7.5 mm from the dura, on the midline. Sham lesions were produced by positioning the electrode to a depth 6.0 mm from the dura without current being passed.

Renin Angiotensin System (RAS) Blockade

In unanesthetized unrestrained animals, saralasin (S, Eaton Laboratories) was infused intravenously (i.v.), at a dose of 10 μg/kg/min, and 2 hours after the end of this infusion, captopril (C, Squibb and Sons) was administered at a dose of 20 mg/kg by mouth.

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(p.o.). Results of RAS blockade are expressed by the lowest values of MAP observed during the 20-minute infusion period of saralasin or during the 120-minute period after captopril administration. A positive response for both agents was defined as a fall in MAP greater than 10 mm Hg. Chronic RAS blockade was accomplished by oral administration of captopril, 20 mg/kg body weight every 8 hours, for 35 days. Chronic RAS blockade was assessed weekly by determination of TAP obtained 2 hours after the morning captopril dose.

Technique for the Implantation of Intracranial Cannulas

Stainless-steel cannulas were implanted into the third ventricle according to the following coordinates: 0.0–0.5 mm posterior to the bregma, on the midline, to a depth of 6.0 mm from the dura. Cannulas were then fixed by a previously described technique and appropriately used either as a guide for AII injections into the third ventricle or for introduction of an electrode to produce a lesion of the AV3V area. The completeness of AV3V lesion was functionally assessed by the changes in dipsogenic and pressor responses to centrally administered AII. Animals with a previously implanted cannula had their MAP continuously recorded in the unanesthetized state, through a carotid catheter. After a 30-minute control period, 20 ng of AII (Hypertensin, Ciba) was directly injected into the third ventricle as a bolus using a 10 μl Hamilton syringe; the total injected volume did not exceed 1 μl. Results are expressed as total volume of water ingested over a 20-minute period, and blood pressure elevations as the maximal increases in MAP 2–5 minutes after AII injection.

Experimental Groups

To verify the functional completeness of the AV3V lesion, five rats (Group 1) had central dipsogenic and pressor effects of AII tested before and after 56 days of AV3V lesion.

In 11 rats (Group 2) the AV3V region was lesioned. After a 15-day recovery period, methylprednisolone (M) treatment was initiated and TAP values recorded every week for 35 days. After this, acute blockade of RAS with S and C was carried out. Eight animals (Group 3) with sham AV3V lesions were prepared and studied similarly to those of Group 2. In 14 rats (Group 4) submitted to AV3V lesion and allowed 15 days to recover, captopril and M were concomitantly administered for a 35-day period and TAP measured every week. These animals were then submitted to S infusion. In 15 rats (Group 5), M was given for 56 days. On the 28th day of treatment, when animals were hypertensive, AV3V lesions were performed. TAP was determined weekly, and after 56 days, acute RAS blockade with S and C was performed in seven rats. A group of 11 animals (Group 6) was treated identically to Group 5 except that no AV3V lesion but rather a sham lesion was performed at the 28th day. To evaluate establishment of the lesion, the 24 hours water intake was registered for 2 days before and for 15 days after AV3V or sham lesion in Groups 2–6. Animals were considered adipsic when daily water intake for 1 or more days was reduced to less than 6 SD below the mean obtained in the control period for all animals (mean –6 SD = 12.4 ml). Mortality rates were also registered in these animals.

Histology

Histological examinations were performed in all animals at the end of their experimental period to confirm the intended lesions. Rats were decapitated and their brains removed and stored in 10% formalin. After 7–10 days, frozen sections were taken through the extent of the lesion and examined using light microscopy.

Statistics

Values are expressed as means ± SEM. Levels of significance were assessed by either paired t test or analysis of variance when appropriate.

Results

Table 1 shows individual elevations in MAP (ΔMAP) and water intake following AII injections in the rats of Group 1 before and 56 days after AV3V lesion. It is evident that, following AV3V lesion, central pressor responses to AII were remarkably diminished (32.6 ± 1.9 and 7.2 ± 0.4 mm Hg, respectively, p < 0.05) and a sharp reduction in AII-induced water intake was observed (9.0 ± 0.4 vs 1.4 ± 0.2 ml/20 min, p < 0.05). Thus, AV3V lesion effectively reduced two central actions of AII functionally attesting to the completeness of the lesion for the 56-day period.

Figure 1 summarizes TAP values for Groups 2, 3, and 4. As can be seen, TAP values rose earlier and to higher levels in animals with sham lesion (Group 3) as compared to those with AV3V lesion (Group 2). After 35 days, the total increase was significantly greater in Group 3 than in Group 2 (38 ± 2 vs 29 ± 2 mm Hg, p < 0.05). TAP values in animals with AV3V lesions were also significantly different from those of Group 2 (8 ± 2 vs 8 ± 1 mm Hg, p < 0.05) and Group 3 (9 ± 1 vs 8 ± 1 mm Hg, p < 0.05). After 35 days, the total increase was significantly greater in Group 3 than in Group 2 (38 ± 2 vs 29 ± 2 mm Hg, p < 0.05). TAP values in animals with AV3V lesions were also significantly different from those of Group 2 (8 ± 2 vs 8 ± 1 mm Hg, p < 0.05) and Group 3 (9 ± 1 vs 8 ± 1 mm Hg, p < 0.05).

Table 1. Effect of AV3V Lesion Upon Central Actions of Angiotensin II (AI) (Group 1)

<table>
<thead>
<tr>
<th>Control</th>
<th>AV3V lesion</th>
<th>Control</th>
<th>AV3V lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔMAP (mm Hg)</td>
<td>ΔMAP (mm Hg)</td>
<td>water intake (ml)</td>
<td>water intake (ml)</td>
</tr>
<tr>
<td>26</td>
<td>8</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>8</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>32</td>
<td>6</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>38</td>
<td>8</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>32</td>
<td>6</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td>32.6</td>
<td>7.2</td>
<td>9.0</td>
</tr>
<tr>
<td>SEM ± 1.9</td>
<td>± 0.4</td>
<td>± 0.4</td>
<td>± 0.2</td>
</tr>
</tbody>
</table>

Elevations in Mean Arterial Pressure (ΔMAP) represent maximal increases 2–5 minutes after AII administration. Water intake values represent total volume of water ingested during 20 minutes after AII. AI was given directly into the third ventricle at a single dose of 20 ng.
that were chronically treated with captopril (Group 4) were similar to those of Group 2 up to the 14th day of M treatment. After this period, TAP values in Group 4 increased more slowly than those of Group 2. After 35 days the total increase for Group 4 was 23 ± 2 mm Hg, which was lower than that observed in Group 2 (29 ± 2 mm Hg, p < 0.05). The hypertensive effect of M was therefore delayed and diminished by the AV3V lesion. Captopril administration caused a further diminution in TAP of AV3V lesioned animals.

Figure 2 depicts values of TAP observed in Groups 5 and 6. Methylprednisolone administration during the initial 28 days caused progressive and similar rises in TAP values for both groups (ΔTAP: Group 5 = +47 ± 2 mm Hg and Group 6 = +45 ± 2, p > 0.05). After this period, AV3V lesion in Group 5 caused a sharp and sustained drop in TAP values for the subsequent 28 days (ΔTAP = -28 ± 2 mm Hg, p < 0.05). However, TAP in this group did not return to pretreatment values, remaining significantly higher at the end of the study (138 ± 1 vs 118 ± 2 mm Hg, p < 0.05). In contrast, sham lesion did not affect the evolution of hypertension (Group 6). Thus, AV3V lesion in the established phase of hypertension induced by M caused a sharp and sustained reduction in TAP values without, however, normalizing arterial pressure.

Water intake values in animals of the sham groups diminished by 33%, none was found adipsic, and no deaths occurred. Lesioned animals presented a 56.5% reduction in water intake, 68% became adipsic, and mortality rate was 38% among the adipsic animals.

Changes in ΔMAP with saralasin (S) infusion (Groups 2 to 6) and after captopril (C) administration (Groups 2, 3, 5, and 6) are shown in Table 2. A positive response (see methods) to both S and C was obtained only in animals submitted to sham lesion and subsequently treated with M (Group 3). Thus, for up to 35 days the angiotensin component detectable in M-treated rats (Group 3) was abolished by an AV3V lesion (Group 2). In Group 4, prepared as Group 2 but

**Table 2. Changes in Mean Arterial Pressure (MAP) During Renin Angiotensin System (RAS) Blockade**

<table>
<thead>
<tr>
<th>Groups</th>
<th>(n)</th>
<th>Control MAP (mm Hg)</th>
<th>Saralasin ΔMAP (mm Hg)</th>
<th>Captopril ΔMAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>(11)</td>
<td>149 ± 3</td>
<td>-2 ± 1</td>
<td>-1 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>(8)</td>
<td>161 ± 3</td>
<td>-14 ± 3</td>
<td>-34 ± 3</td>
</tr>
<tr>
<td>4</td>
<td>(14)</td>
<td>143 ± 2</td>
<td>-3 ± 1</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>(7)</td>
<td>148 ± 5</td>
<td>-2 ± 2</td>
<td>-2 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>(10)</td>
<td>158 ± 3</td>
<td>-3 ± 2</td>
<td>-3 ± 1</td>
</tr>
</tbody>
</table>

MAP values immediately before (control) and during saralasin infusion (maximal decrease in 20 minutes) and after captopril administration (maximal decrease in 120 minutes).
FIGURE 2. Tail arterial pressure (TAP) values in Groups 5 and 6. AV3V or sham lesion was performed 28 days after methylprednisolone administration.

receiving chronic C administration, saralasin was unable to cause further drops in MAP. After 56 days, both AV3V-lesioned and sham animals (Groups 5 and 6) failed to respond, with diminutions in MAP following S and C.

Histological analysis performed in all animals of Group 1 showed a common zone of tissue damage over the optic chiasm and below the tip of the cannula, including the periventricular tissue surrounding the optic recess of the third ventricle (fig. 3). Similar findings were found in preoptic-anterior hypothalamic ependyma in Groups 2, 4, and 5 (fig. 4), but no significant lesion was detected in the same area for Groups 3 and 6.

Discussion

In the present study, destruction of the AV3V region caused significant diminution of centrally mediated pressor and dipsogenic actions of All that
lasted for 56 days after the lesion. These alterations were previously reported and are in agreement with the areas of anatomical destruction, as observed in histological analyses, and attest to the functional completeness of the destruction of the AV3V area in our experimental groups. In those groups not submitted to functional assessment of AV3V lesion water intake, adipsia and mortality rates as well as histologic findings were compatible with a complete AV3V lesion.

Administration of M causes an arterial hypertension that is accompanied by an increase in the activity of the RAS. Our observations confirm these findings since elevations of arterial pressure in the group which received only M were accompanied by positive responses to both S and C up to the 35th day of the hypertensive state. Destruction of the AV3V area caused a delay and partial prevention of M hypertension (Group 2 contrasted with Group 3). Since no significant decreases in MAP were obtained with administration of S and C to AV3V-lesioned animals 35 days after M treatment (Group 2), it is apparent that destruction of the AV3V area completely abolished the renin angiotensin component that is normally found in this group. Thus, lesion of AV3V caused both quantitative and qualitative differences in the hypertension. The quantitative modification induced by an AV3V lesion occurred even during chronic RAS blockade with C (Group 4).

From these data it is apparent that integrity of AV3V is essential for the full expression of the renin angiotensin component of M-hypertension. This observation can be interpreted in different ways. The integrity of AV3V may be necessary for renin liberation by the kidney either directly or indirectly through AV3V-dependent alterations in salt and water balance. The pressor effect of AII, in this model, could be more dependent on its central rather than its peripheral pressor action and, finally, M could possibly act through a selective activation of the cerebral RAS whose expression could be blunted by the AV3V lesion.

Our data, however, do not allow conclusions as to which mechanism(s) were operative. Regardless, the lack of an angiotensin component may help to explain the lower levels of arterial pressure observed in AV3V lesioned groups. However, levels of arterial pressure in these groups at the 4th week were within the hypertensive range, showing that M can cause hypertension by mechanisms that are independent of the integrity of AV3V and RAS. On the other hand, we demonstrated that the administration of M to intact animals caused hypertension and that the integrity of the AV3V area was clearly necessary for its maintenance. Accordingly, the destruction of this area on the 28th day caused a sharp and sustained reduction in blood pressure as opposed to the sham-lesioned rats, in which blood pressure was not reduced. The AV3V-induced reduction in TAP (Group 5) was not sufficient, however, to completely normalize arterial pressure levels. In this latter group, as in Groups 2 and 4, S and C were not effective in further lowering arterial pressure. Thus, M hypertension can be partially maintained after the destruction of AV3V area and during chronic RAS blockade. It is interesting to observe that on the 56th day S and C caused no more reductions in MAP in sham-lesioned M hypertensive rats (Group 6) than those with lesions (Group 5). Similar findings were previously reported in another experimental model of hypertension in which an angiotensin-dependent phase was also spontaneously followed by an angiotensin-independent one.

In conclusion, our data show that AV3V destruction before the initiation of methylprednisolone hypertension partially prevented the arterial pressure rise and abolished an angiotensin component normally detectable in this type of hypertension. Once hypertension is already established, AV3V integrity is essential for maintenance of the full expression of the hypertension. However, methylprednisolone can cause hypertension by mechanism(s) that do not depend on the integrity of the AV3V or the renin angiotensin system.

References

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