Circadian Changes in Plasma Renin Activity and Plasma Aldosterone Concentration in Two-Kidney Hypertensive Rats

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SUMMARY Circadian changes in plasma renin activity (PRA) and plasma aldosterone concentration (PAC) in normal and hypertensive rats were determined by measurements at 8 a.m., 4 p.m. and 12 midnight (MN). For the normals, PRA and PAC were highest at 4 p.m. Animals made hypertensive by constricting one renal artery with the other kidney intact were studied after 4, 5, 7 and 10 weeks; the clear-cut circadian rhythm for PRA in normals had disappeared but for PAC the circadian rhythm was present in the 4-, 5- and 10-week groups. Both PRA and PAC were elevated in all four hypertensive groups compared with the normal controls and there was a highly significant correlation between PRA and PAC. The 4 p.m. peak value for PAC was much higher in relation to the 8 a.m. and 12 MN values in the hypertensive animals than in the normals. Sodium balance studies failed to demonstrate any appreciable differences among the groups. When the hypertensive animals were divided into two groups on the basis of the level of hypertension, the rats with moderate hypertension showed an average elevation in PRA which was significant in only the 4- and 7-week groups whereas PRA was elevated in all four groups with severe hypertension. Thus, the present data help to define the activity of the renin-angiotensin-aldosterone system in two-kidney, one clip hypertension in the rat.

KEY WORDS • renin-angiotensin-aldosterone system • hypertension • aldosterone secretion • circadian rhythm of plasma renin activity • experimental renin hypertension • renin secretion • high blood pressure

BEFORE the role of the renin-angiotensin-aldosterone system can be adequately evaluated in two-kidney, one clip hypertension in the rat, any circadian rhythm of this hormonal system must be known. Leenen et al.1 and Gomez-Sanchez et al.2 have previously described circadian changes for both renin and aldosterone in the normal rat, but no comparable studies have been reported for the renal hypertensive rat.

The findings from studies of the role of the renin-angiotensin-aldosterone system in the two-kidney hypertensive rat have not always agreed. Possible explanations for these variations include the methods of blood sampling, whether the animals were anesthetized or conscious, and, as has been recently demonstrated, the severity of hypertension.3 Another possible explanation might be variations in circadian changes in the plasma levels of renin and aldosterone in the hypertensive rat. The purpose of this study was threefold: 1) to redefine the circadian changes in plasma renin activity (PRA) and plasma aldosterone concentration in normal rats; 2) to examine any changes in the circadian pattern for PRA and plasma aldosterone concentration in the two-kidney hypertensive rat; and 3) to determine if PRA and the plasma aldosterone level return to normal in hypertensive rats studied for 10 weeks.

Methods

All rats (Sprague-Dawley) were housed individually in stainless steel metabolic cages and kept in temperature-controlled rooms that were maintained on a 12-hour light-dark cycle beginning at 5 a.m.
Distilled water and powdered rat food (Purina) were available ad libitum. Sodium balances were measured before sacrifice from tri-weekly observations of ingested sodium and sodium excreted by the kidney; three 2-day sodium balance periods were obtained. Systolic blood pressure was determined by the tail artery occlusion method; the value recorded was the average of three determinations which were made twice weekly. Plasma renin activity was determined from blood collected during the first 5 seconds following decapitation, while plasma aldosterone concentration (PAC) was measured from a second blood sample collected during the next 15 seconds. Blood samples (5 ml) were collected in 0.1 ml of 10% ethylenediaminetetraacetate; the plasma was frozen until subsequent analyses were made by radioimmunoassay. For plasma sodium and potassium concentrations, 1 ml of blood was collected during ether anesthesia from normal, as well as from 4-, 5-, 7- and 10-week hypertensive rats by cardiac puncture at each of the 8 a.m., 4 p.m. and 12 midnight (MN) times. Following recovery from the anesthesia, the normal animals were decapitated and blood again collected so that plasma sodium and potassium concentrations by this collection procedure could be compared with blood collected by cardiac puncture. Hypertensive Studies

Eight separate groups with 16–18 male rats in each group were used in this study. Systolic pressure was measured twice prior to placing a 0.2-mm silver clip on the left renal artery; the body weight was 130–150 g. The contralateral kidney was left untouched. After 4 weeks blood pressure was determined on two of the groups; these groups were then randomly subdivided into three smaller groups of 5–6 rats each for subsequent sacrifice for collection of blood at 8 a.m., 4 p.m. or 12 MN. This protocol was also followed at the end of Weeks 5, 7 and 10 for the remaining groups of rats.

Sham Control Studies

Four groups of 18 normal male rats per group were studied after 4 weeks (average body weight = 250 g). Three groups were examined during a 2-month period (May–June), while the fourth group was studied several months later (January). Systolic blood pressure was determined during the week before sacrifice. As with the hypertension studies, each control group was then subdivided into three groups so that equal numbers could be sacrificed to obtain blood at 8 a.m., 4 p.m. or 12 MN.

Analytical Techniques and Statistics

The radioimmunoassay techniques of Sealey et al. and Buhler et al. were utilized for analyzing PRA and plasma aldosterone concentration, respectively. Urine and plasma electrolytes were measured by flame photometry. Statistical significance was determined between groups by Student's group or unpaired t test.

Results

Systolic blood pressure was 106 ± 1.4 SEM mm Hg for the sham operated control group (fig. 1). Placing a 0.2-mm clip on the left renal artery increased blood pressure from pre-clip control values of 107 to 188 mm Hg, from 104 to 180 mm Hg, from 116 to 210 mm Hg, and from 107 to 192 mm Hg, for the 4-, 5-, 7- and 10-week hypertensive groups, respectively (fig. 1) (p < 0.01 for all four groups). No significant differences were observed in the mean values for pressures recorded on rats sacrificed at 8 a.m., 4 p.m. and 12 MN within any group (p > 0.05). The death rate of each group due to factors other than anesthesia or surgery was also presented; more rats died in the 10-week hypertensive group than in any other group. None of the control rats died during the study. During the week before sacrifice, the average value for the sodium excreted by the kidney was approximately 82% of the ingested sodium for all rats studied (fig. 2). The ratio of excreted to ingested sodium was quite variable within each group but there were no obvious differences in sodium balance between any of the experimental groups and the normal controls. Of all the hypertensive animals, only five demonstrated a negative sodium balance; these rats were probably in the malignant phase of hypertension. These five animals showed a mean systolic pressure of 228 ± 5 mm Hg, a PRA of 7.1 ± 1.1 ng angiotensin I/ml/hr, and a mean plasma aldosterone concentration of 86 ± 26 ng%. These values for arterial pressure, PRA and plasma aldosterone concentration are very high compared with those recorded in normal rats (figs. 1–3).

Plasma renin activity and plasma aldosterone concentration exhibited a reproducible circadian change in the normal rats. As seen in figure 3, PRA of rats sacrificed during May–June, 1977 was significantly higher at 4 p.m. than at 12 midnight (p < 0.05). Plasma aldosterone concentration in the same rats followed a similar rhythm; the 4 p.m. values were significantly higher than either the 8 a.m. or 12 midnight values (p < 0.05 for both comparisons). Several months later (January, 1978), this experiment was repeated with similar results; PRA was significantly higher at 4 p.m. than at 8 a.m. or 12 MN. Plasma aldosterone concentration also appeared to be higher at 4 p.m. than 8 a.m. or 12 MN but the differences were not statistically significant. Data from these two groups were added together for both renin and aldosterone and subsequently referred to as data from the control group. Within this combined group (figs. 4, 5), PRA rose significantly from 0.72 ± 0.06 ng angiotensin I/ml/hr at 8 a.m. to 0.97 ± 0.01 ng angiotensin I/ml/hr at 4 p.m., and then fell to 0.58 ± 0.06 ng angiotensin I/ml/hr by midnight (p < 0.05 for both comparisons). The concentration of plasma aldosterone at 4 p.m. was 16.1 ± 1.8 ng% compared with 11.6 ± 1.4 ng% at 8 a.m. and 11.4 ± 1.4 ng% at midnight (p < 0.05 for both comparisons).
Figure 1. Systolic arterial pressure measurements in normal controls and hypertensive (HT) rats. Determinations were made at 8 a.m., 4 p.m., and 12 midnight. Each group of hypertensives had measurements made before and at 4, 5, 7 and 10 weeks after renal artery constriction.

Figure 2. Daily sodium balances measured on the fourth, the second and zero days before decapitation. $E_{Na}$ is the abbreviation for daily sodium excretion. The symbols for the 8 a.m., 4 p.m., and 12 midnight groups are for animals sacrificed at these times for blood collection and each animal had three 2-day balance periods studied.
Plasma renin activity of the hypertensive groups was not significantly elevated at 4 p.m. in comparison with the 8 a.m. and 12 MN values (fig. 4). The only statistical difference within a group existed in the 7-week hypertensive animals in which PRA was significantly greater at 8 a.m. than at midnight ($p < 0.05$). Except for the 8 a.m. value of the 4-week group, PRA of all hypertensive groups was significantly greater than in the controls ($p < 0.05$). Also, the 8 a.m. value for PRA at 7 weeks was significantly higher than the PRA values at 4 and 5 weeks ($p < 0.05$ for both comparisons). As presented in figure 4, PRA appears to increase progressively from the 4-week hypertensive group through the 7-week hypertensive group. The PRA of the 10-week hypertensive group appears to be lower than that of the 7-week hypertensive group, suggesting that PRA was returning toward the control level.

In contrast, plasma aldosterone concentration appears to have a circadian change within three of the four hypertensive groups (fig. 5). In the 4-week hypertensive animals, the plasma aldosterone level was $16.0 \pm 1.0 \text{ ng}\%$ at 8 a.m. and $63.2 \pm 27 \text{ ng}\%$ at 4 p.m. ($p > 0.05$), but fell to $13.9 \pm 3.0 \text{ ng}\%$ by midnight ($p < 0.05$). The 5-week hypertensive animals followed a similar pattern as the level of plasma aldosterone was $19.5 \pm 5.5 \text{ ng}\%$ at 8 a.m. and $42.8 \pm 10.8 \text{ ng}\%$ at 4 p.m. ($p < 0.05$), and then fell to...
23.9 ± 5.5 ng% by midnight (p > 0.05). The concentration of aldosterone in plasma for the 10-week hypertensive group was 54.0 ± 28.5 ng% at 8 a.m. compared with 90 ± 58 ng% at 4 p.m. (p < 0.05); aldosterone fell significantly at 12 MN to a value indistinguishable from the control (p > 0.05). Whereas the plasma aldosterone level in the hypertensive rats was significantly higher than control at all times during Weeks 5 and 7, only the 4 p.m. values of the 4-week group, and the 8 a.m. and 4 p.m. values of the 10-week hypertensive group were greater than control (p < 0.05).

The relationship between PRA and the plasma aldosterone level of each group was examined by linear regression analysis. Whereas no significant correlation existed between these functions at 8 a.m., 4 p.m., or 12 MN in the control group, a significant correlation was present at each of these times within the four hypertensive groups of rats (p < 0.01). A plot of PRA against plasma aldosterone concentration for all rats in the hypertensive study revealed a high significant correlation (p < 0.001).

The mean plasma sodium concentration was 138.5 ± 0.4 mEq/liter and the mean plasma potassium concentration was 4.05 ± 0.10 mEq/liter for the control group in blood collected by cardiac puncture (table 1). Plasma sodium and potassium concentrations from separate 8 a.m., 4 p.m. and 12 MN groups of normal as well as 4-, 5-, 7- and 10-week hypertensive rats did not vary significantly among the groups. However, blood collected by decapitation from the same control rats contained significantly higher plasma potassium levels (4.77 ± 0.09 mEq/liter at 0-5 seconds after decapitation and 5.40 ± 0.19 mEq/liter at 5-15 seconds after decapitation; p < 0.05 for both values compared with 4.05 ± 0.10 mEq/liter for the control values).

To evaluate more fully the role of the renin-angiotensin-aldosterone system in two-kidney, one

![Figure 5. Circadian changes in plasma aldosterone concentration for normal controls and for 4-, 5-, 7- and 10-week hypertensive (HT) rats.](image-url)
clip hypertension in the rat, all rats sacrificed at 4 p.m. were divided into two groups, a moderately hypertensive group (arterial pressure < 180 mm Hg) and a severely hypertensive group (arterial pressure > 180 mm Hg). As presented in table 2, PRA of the moderately hypertensive group was significantly elevated above control during Weeks 4 and 7, while the group with severe hypertension had a significantly higher PRA throughout the study. In the group with moderate hypertension, plasma aldosterone concentration was elevated during Weeks 4, 5 and 10, while in the group with severe hypertension plasma aldosterone was elevated in all four hypertensive groups.

Discussion

The present study is concerned with the circadian changes in PRA and plasma aldosterone concentration in two-kidney, one clip hypertension in the rat. There has been considerable difficulty in assessing the function of the renin-angiotensin-aldosterone system in the rat and many conflicting reports have appeared. It is clear that anesthesia increases PRA and it is almost impossible to obtain reproducible basal plasma levels of renin and aldosterone consistently even in conscious animals with chronic, indwelling intravascular catheters. This problem is evident from the extensive studies in the spontaneously hypertensive rat in which there is no unanimity of results on the circadian changes in PRA and plasma aldosterone concentration in experimental renal hypertension. Although PRA was elevated in hypertensive animals from 4 to 10 weeks, the circadian variation present in normals with a peak value at 4 p.m. had disappeared. It is noteworthy that the circadian periodicity in PRA also disappeared during sodium depletion in the rat. The excessive stimulation of important control mechanisms for renin release such as the renal vascular receptor and the macula densa might explain the loss of the circadian rhythm for PRA in hypertensive animals.

There is a paucity of data on aldosterone secretion or plasma aldosterone concentration in two-kidney hypertension in the rat. In 1960, Müller and Gross reported that adrenal tissue from 4-week two-kidney hypertensive rats produced more aldosterone in vitro than adrenal tissue from normal animals. In 1963, Singer and associates found that aldosterone secretion was increased in anesthetized two-kidney rats with hypertension of 5.5–12 weeks duration; corticosterone was not detectably changed. The present

<table>
<thead>
<tr>
<th>Group</th>
<th>Arterial pressure (mm Hg)</th>
<th>PRA (ng ang/mL/hr)</th>
<th>PAC (ng%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>24 106 ± 1.4</td>
<td>0.97 ± .10</td>
<td>16.1 ± 1.8</td>
</tr>
<tr>
<td>Hypertensives with systolic pressure below 180 mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 wks HT 7</td>
<td>166 ± 2</td>
<td>2.2 ± 0.8*</td>
<td>68 ± 38†</td>
</tr>
<tr>
<td>5 wks HT 8</td>
<td>180 ± 5</td>
<td>1.2 ± 0.3</td>
<td>25 ± 4†</td>
</tr>
<tr>
<td>7 wks HT 4</td>
<td>172 ± 10</td>
<td>6.7 ± 6.2†</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>10 wks HT 4</td>
<td>144 ± 3</td>
<td>1.2 ± 0.6</td>
<td>70 ± 53†</td>
</tr>
<tr>
<td>Hypertensives with systolic pressure above 180 mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 wks HT 5</td>
<td>199 ± 7</td>
<td>3.2 ± 1.1†</td>
<td>57 ± 42†</td>
</tr>
<tr>
<td>5 wks HT 8</td>
<td>218 ± 8</td>
<td>4.3 ± 1.3†</td>
<td>69 ± 21†</td>
</tr>
<tr>
<td>7 wks HT 7</td>
<td>228 ± 4</td>
<td>5.8 ± 1.9†</td>
<td>102 ± 40†</td>
</tr>
<tr>
<td>10 wks HT 4</td>
<td>209 ± 8</td>
<td>6.3 ± 2.0†</td>
<td>111 ± 45†</td>
</tr>
</tbody>
</table>

*p < 0.025.
†p < 0.01.

Abbreviations: HT = hypertension; PRA = plasma renin activity; PAC = plasma aldosterone concentration; ang = angiotensin I.
observations demonstrate that the plasma aldosterone concentration was elevated after 4-, 5-, 7- and 10 weeks of hypertension. Furthermore, the circadian periodicity for aldosterone was not only sustained in the two-kidney hypertensive rats, but the peak value at 4 p.m. appeared to be more exaggerated in the 4-, 5-, and 10-week groups than in normal animals.

The present findings suggest that factors other than PRA might have contributed to the elevated 4 p.m. values for plasma aldosterone concentration since the circadian rhythm for PRA was lost in the hypertensive animals. Plasma electrolyte concentrations were studied in an attempt to explain the circadian changes in aldosterone. When blood was collected by decapitation, the values for plasma potassium concentration were strikingly elevated; apparently, however, this elevation resulted from changes associated with the procedure for collection of blood. When blood was collected by cardiac puncture, both plasma potassium and sodium concentrations showed no evidence of circadian changes and were not altered in hypertensive animals. It is possible that changes in plasma ACTH contributed to the circadian rhythm in plasma aldosterone but no attempt was made to evaluate a role for ACTH. Although factors other than PRA could be involved in the control of plasma aldosterone concentration, it is clear from the present high correlation coefficient between the plasma levels for renin and aldosterone that PRA is a very important determinant of the plasma aldosterone concentration in the two-kidney hypertensive rat.

There is considerable confusion in the literature on the role of the renin-angiotensin system in the pathogenesis of two-kidney hypertension in the rat. Evidence gathered from studies with either angiotensin II blocking agents or actual measurements of PRA have indicated that the renin-angiotensin system is hyperactive during the acute phase (1-6 weeks) of hypertension but normal during the chronic phase (15-16 weeks) of the disease. However, in two studies in anesthetized rats, it has been reported that drops in blood pressure occurred with angiotensin II analogues after 4 months of hypertension. The present data on the circadian periodicity of both renin and aldosterone indicate that an evaluation of the renin-angiotensin-aldosterone system might be more complete if measurements were made at least three times during the day. Also, the present results point to the desirability of making studies in control and experimental animals at the same time of day. Failure of various workers to do this, along with the use of anesthesia which increases PRA in the rat, may account for much of the variation in reported results.

In the present study, PRA had not returned to normal by 10 weeks after clipping the renal artery. Carretero and Gulati have recently suggested that activation of the renin-angiotensin system in two-kidney hypertensive rats is not only dependent on the time period following renal artery constriction, but also on the severity of the hypertension. It has previously been demonstrated that placing a 0.2-mm clip on the renal artery will produce both benign and so-called malignant hypertension in two-kidney rats. Whereas the benign hypertensive animals had a high PRA only during the initial phase of hypertension, the rats with severe hypertension had an elevated PRA in association with a negative sodium balance and a very high blood pressure during the chronic stage of the disease. The present findings revealed that rats with moderate hypertension of 5- and 10-weeks duration had a normal PRA whereas at 4 and 7 weeks PRA was elevated; the rats with severe hypertension had an elevated PRA throughout the study. Only five rats in the group with severe hypertension were in a negative sodium balance. These considerations suggest, therefore, that the role of the renin-angiotensin system in the pathogenesis of two-kidney hypertension in the rat is related to both the duration and the severity of the hypertension.

References

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