Vascular Renin-like Activity and Blood Pressure Maintenance in the Rat

Studies of the Effect of Changes in Sodium Balance, Hypertension and Nephrectomy

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SUMMARY Aortic renin-like activity and plasma renin concentration were measured in rats subjected to dietary salt loading or depletion. Homogenates prepared from rat aortic tissue generated angiotensin I from plasma substrate at both pH 5.3 and pH 6.5. Aortic renin-like activity measured at incubation pH 6.5 and plasma renin concentration changed in parallel, rising with salt restriction and falling with salt loading. However, the capacity of aortic homogenate to generate angiotensin I at an incubation pH of 5.3 did not show any significant change with alteration of sodium balance. In addition, administration of the converting enzyme inhibitor (CEI) SQ20,881 produced a greater fall of blood pressure in salt-depleted than salt-loaded animals.

In order to determine whether arterial or plasma renin was the important variable in blood pressure regulation, the depressor response to CEI was studied in Goldblatt two-kidney, one clip hypertensive rats before and 1, 2, 6 and 24 hours after bilateral nephrectomy. Aortic and plasma renin concentration were measured in a second series of hypertensive rats at the same times after bilateral nephrectomy. Although plasma renin concentration showed the expected fall to basal levels within 1 hour, a significant depressor response to CEI was observed at 1 and 2 hours after bilateral nephrectomy, with a smaller fall at 6 hours. When a competitive antagonist of angiotensin II (saralasin) was infused, a significant fall in blood pressure occurred 1 hour after bilateral nephrectomy, and no further fall was induced by injection of CEI. Thus, the fall of blood pressure induced by CEI was due to renin-angiotensin blockade and not an additional hypotensive effect.

Aortic renin-like activity measured at an incubation pH of 6.5 fell slowly after bilateral nephrectomy and was significantly lower only at 6 and 24 hours. This closely parallels the reduction of depressor response observed with converting enzyme inhibitor. However aortic renin-like activity measured at pH 5.3 showed no significant change at anytime after bilateral nephrectomy except for activity after 1 hour, which was significantly elevated.

This evidence supports more indirect studies which have suggested that arterial renin-like activity derived from the kidney is important in blood pressure maintenance by the local generation of the effector hormone of the system, angiotensin II, and that this activity has a longer half-life than plasma renin. Enzymic activity detected at a low incubation pH probably represents a tissue enzyme that is not important in blood pressure maintenance.

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KEY WORDS • blood pressure • aorta • renin-like activity • plasma renin concentration • sodium balance • nephrectomy • converting enzyme inhibitor • angiotensin antagonist

The renin-angiotensin system probably plays a role in blood pressure maintenance in salt depletion and in some forms of experimental and clinical hypertension. Thus, in some situations, elevated plasma renin levels are associated with a depressor response to pharmacological antagonism of the renin-angiotensin system. The extent of this role is controversial as there are differences in the depressor action of the two most important inhibitors: converting enzyme inhibitor and the competitive antagonist of angiotensin II, Sar-Ala, angiotensin II (saralasin). The components of the renin-angiotensin system are present in the circulation, where generation of the effector hormone of the system, angiotensin II takes place. However, renin-like enzymes are also present in many other tissues and angiotensin II could therefore be generated locally, if the other components of the renin-angiotensin system were present. It has been postulated that generation of angiotensin II within the nervous system and within the arteriolar wall is of physiological and pathological importance. To confirm such a role, it is not only necessary to demonstrate that a tissue can...
generate angiotensin II in vitro, but it is also necessary to demonstrate a role in vivo that can be distinguished from the effect of circulating renin. This is difficult when both plasma and tissue renin levels change in parallel. Indirect evidence previously adduced by our group\(^1,2\) suggested that the half-life of vascular renin after bilateral nephrectomy is much longer than the half-life of plasma renin.

The present study was designed to observe the changes in rat aortic wall renin in response to manipulation of sodium balance and the production of Goldblatt two-kidney, one clip hypertension. In addition, we have also studied the sequential change in plasma and aortic renin in hypertensive rats during the first 24 hours after both kidneys have been removed. The results show that aortic renin-like activity changes in parallel with plasma renin in response to salt depletion or loading and both were markedly raised in acute Goldblatt two-kidney hypertensive rats. Following bilateral nephrectomy aortic renin fell at slower rates than plasma renin. Injection of the converting enzyme inhibitor, SQ20,881, also produced depressor responses in nephrectomized hypertensive rats, although plasma renin had fallen to a very low level. Thus the responsiveness to the inhibitor correlated with aortic rather than plasma renin, suggesting the former is of major importance in maintaining blood pressure.

**Methods**

Female white Wistar rats weighing 150-250 g were used throughout and were divided into eight groups.

**Aortic Renin Measurement**

**Normotensive Rats (Groups 1-3)**

Group 1 consisted of five rats on severe salt restriction. The rats were allowed free access to a diet containing 0.013 mmoles/g sodium and to deionized water for 2 weeks. The eight rats in Group 2, on a moderate salt diet, were treated in a similar fashion but the diet contained 0.035 mmoles/g sodium. The seven rats in Group 3, on a salt loading diet, were given chow containing 0.166 mmoles/g sodium and allowed to drink 1% saline.

**Hypertensive Rats (Groups 4-8)**

Hypertension was induced by the application of a silver clip (internal diameter 0.2 mm) to the left renal artery under ether anesthesia; the contralateral kidney was undisturbed. Blood pressures were checked by a light plethysmographic method\(^13\) and animals were selected that had a sustained indirect mean blood pressure of 150 mm Hg or more within 6 weeks of renal artery constriction. The rats were divided into the following groups:

- **Group 4:** 11 non-nephrectomized rats
- **Group 5:** eight rats 1 hour after bilateral nephrectomy
- **Group 6:** seven rats 2 hours after bilateral nephrectomy
- **Group 7:** eight rats 6 hours after bilateral nephrectomy
- **Group 8:** 10 rats 24 hours after bilateral nephrectomy

**Inhibitor Studies**

A tracheotomy was performed under intraperitoneal pentobarbitone anaesthesia and the jugular vein and carotid artery were cannulated. Arterial blood pressure was monitored with a Statham P23 gb transducer connected to a Grass polygraph recorder. Patency of the arterial and venous lines was maintained by heparinized 5% dextrose. After a steady baseline had been obtained, 250 µg of the nonapeptide converting enzyme inhibitor, SQ20,881, dissolved in 0.1 ml 5% dextrose was injected as a bolus and the blood pressure was monitored for a further 20 minutes. Infusion studies were carried out on rats from all groups except Group 2 (see table 2). In addition, to check the specificity of the response to the converting enzyme inhibitor, animals from Group 5 were infused with 10 µg/min of the angiotensin antagonist Sar\(^1\)-Ala\(^8\) angiotensin II (saralasin) for 30 minutes, and then 250 µg of the converting enzyme inhibitor was injected.

**Plasma Renin Concentration (PRC) Assay**

Blood was obtained by cardiac puncture of animals stunned by a blow on the head. The sample was immediately placed in a pre-cooled tube moistened with a drop of concentrated solution of dipotassium EDTA. Plasma was separated after spinning in a refrigerated centrifuge and frozen at \(-20°C\). The source of renin substrate was pooled plasma taken from rats 24 hours after bilateral nephrectomy. We added 500 µl of tris maleate buffer and 500 µl of a saturated ethanolic solution of phenylmethylsulphonyl fluoride (PMSF) to each 10 ml of nephrectomized rat plasma and pH adjusted to 6.5 with 1 molar hydrochloric acid. Then 400 µl of the resultant solution was added to 100 µl of the plasma sample to be assayed. The pH (6.5) was checked and 100 µl of the mixture was drawn for assay of angiotensin I (T\(_0\)). The remaining mixture was incubated at 37°C; a further 100 µl aliquot was drawn at 15 (T\(_{15}\)) and 30 (T\(_{30}\)) minutes. Samples with low renin concentration were assayed at 60 (T\(_{60}\)) and 180 (T\(_{180}\)) minutes as well as at T\(_0\). Angiotensin I was measured by radioimmunoassay\(^14\) and renin concentration was calculated as nanograms of angiotensin I/ml/hr.

**Aortic Renin Concentration (ARC)**

After blood for PRC had been taken, the abdomen was opened and the aorta dissected to the bifurcation; the diaphragm was opened and the descending thoracic aorta dissected out as well. The aorta was removed as a single vessel and immediately washed in cold saline. Connective tissue and fat was removed and the vessel was cut lengthwise, washed again in cold saline, blotted and stored at \(-20°C\). The aorta
was thawed, frozen and thawed four times, weighed and homogenized in 500 µl ice-cold saline with a Teflon pestle; the homogenate was frozen at -20°C and thawed for assay. Then 100 µl of homogenate were withdrawn and assayed according to the same protocol used for the rat plasma specimens, except that each sample was assayed twice at an incubation pH of 5.3 as well as 6.5. Results were calculated as nanograms of angiotensin I per 100 mg tissue per hour of incubation.

**Recovery of Renin**

Inactivation of renin was assessed by adding 0.003 Goldblatt units of porcine renin to aortic homogenate obtained from rats nephrectomized 24 hours previously. This was incubated at pH 5.3 and 6.5 (n = 5 for each pH).

**pH Profile**

Aortic homogenate from four rats with Goldblatt two-kidney hypertension was assayed in duplicate at different pHs between 4.5 and 7.

Paired or unpaired t tests were used to compare groups where appropriate.

### Results

#### Aortic and Plasma Renin

Salt restriction raised PRC and salt loading reduced it. Aortic renin concentration measured at an incubation pH of 6.5 showed parallel changes (table 1). Thus, ARC of the severely salt-restricted (Group 1) rats was significantly higher than the ARC of the salt-loaded rats (Group 3) (p < 0.01), and was also significantly higher than the ARC of rats receiving a moderate salt intake (Group 2) (p < 0.05). By contrast, ARC was still not significantly different from the initial value, although at 6 and 24 hours it was significantly less (p < 0.05) (fig. 2). The difference between the values obtained at 6 and 24 hours was not significant (p > 0.1). Aortic renin concentration incubated at pH 5.3 showed a different pattern of results. At 1 hour ARC was significantly elevated (p < 0.05) but throughout the rest of the study it was not significantly different from the initial value (fig. 2).

**Recovery of Added Renin**

Recovery of porcine renin added to aortic homogenate was 96.5 ± 5.09% at pH 5.3 and 124.1 ± 1.48% at pH 6.5

**pH Profile**

Maximum generation of angiotensin I by plasma occurred between pH 6.6 and pH 7.0. By contrast, the aortic homogenate generated substantial amounts of angiotensin I over the whole pH range with maximal generation at pH 4.5 (the lowest pH studies) with a second peak occurring at pH 6.5 (fig. 3).

**Inhibitor Studies**

Converting enzyme inhibitor produced a 90% fall in the pressor response to 50 ng test dose of angiotensin I. Converting enzyme inhibition produced a significantly greater fall in blood pressure of the severely salt depleted (Group 1) compared with the salt-loaded rats (Group 3, table 2). Blood pressure of rats with Goldblatt two-kidney hypertension (Group 4) fell significantly. Blood pressure also fell at 1 and 2 hours after bilateral nephrectomy (Groups 5 and 6). A small change of pressure at 6 hours (Group 7) and 24 hours (Group 8) was not statistically significant (p > 0.01). Infusion of saralasin produced a significant fall in blood pressure of rats 1 hour after bilateral nephrectomy. Injection of converting enzyme inhibitor into these animals during saralasin infusion produced no significant change in blood pressure (p > 0.1) (table 2).

**Discussion**

The present work was designed to assess the relevance of vascular renin-like activity to blood pressure control in response to changes in sodium imbalance and in experimental renovascular hypertension. Aortic enzyme activity was used as a reflection of changes in the inaccessible resistance vessels where generation of angiotensin may be of more profound importance in circulatory homeostasis. This is possible because, at least in hog aorta, renin is located primarily within the adventitia and outer media, which is the site of the vasa vasorum and in the uterus of the rabbit the highest concentration of renin was found within the smallest arterioles and venules that could be isolated.

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**Table 1. The Effect of Varying Dietary Salt Intake on Plasma (PRC) and Aortic Renin Concentrations (ARC) in Normal Rats**

<table>
<thead>
<tr>
<th>Diet</th>
<th>PRC</th>
<th>ARC (5.3)</th>
<th>ARC (6.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low salt</td>
<td>262.6 ± 29.99</td>
<td>2.41 ± 0.71</td>
<td>0.72 ± 0.127</td>
</tr>
<tr>
<td>Moderate</td>
<td>109.2 ± 13.17</td>
<td>2.56 ± 0.88</td>
<td>0.22 ± 0.13</td>
</tr>
<tr>
<td>High salt</td>
<td>14.5 ± 1.67</td>
<td>1.9 ± 0.18</td>
<td>0.03 ± 0.019</td>
</tr>
</tbody>
</table>
The presence in arterial tissue of an enzyme that releases a pressor substance from plasma has long been known. Kinetic studies of aortic renin by Gould et al. in the dog and more recently by Barrett et al. in the rat indicate that the enzyme present generates angiotensin and has a similar pH optimum and $K_m$ to renal renin. The present work confirms the presence of such an enzyme in aortic wall preparation. However, until the pure enzyme can be prepared, its identity or lack of identity with renal renin cannot be finally established.

In addition to generating angiotensin I at a physiological pH (6.5), aortic homogenate also proved to be active in this respect at pH values as low as 4.5 (fig. 3). This wide pH range is quite different from that observed with plasma renin (fig. 3) and suggests that several enzymes are participating in the generation of angiotensin. There are at least two possibilities to explain the generation of angiotensin at a low pH. Skeggs and co-workers have described 'pseudorenin' present in high concentrations in the salivary glands, spleen and thymus with a pH optimum of about 4.5, although with a broad range of activity extending to the neutral pH band. However, 'pseudorenin' is inhibited by a naturally occurring plasma inhibitor. Since plasma from nephrectomized rats rather than synthetic tetra-decapeptide substrate was used in the present studies, 'pseudorenin' cannot be responsible for the generation of angiotensin I. Other acid proteases such as Cathepsin D and pepsin can also hydrolyze renin substrate at the same bond as renin to form angiotensin I. But neither enzyme would be expected to show significant activity at pH 6.5. Nevertheless it seems possible that such tissue acid proteases account for the angiotensin generation observed at pH 5.3. If such enzymes have a local, rather than a renal origin, this would explain the apparent failure of vascular 'renin-like' activity to fall after bilateral nephrectomy when a low incubation pH was used to assay renin.

In our studies the response to changes in sodium balance and nephrectomy was quite different when renin-like activity was measured at pH 5.3 and then at pH 6.5. These distinctive responses indicate that at least two different enzyme systems are involved. The only significant change in renin-like activity measured at pH 5.3 was the rise observed 1 hour after bilateral nephrectomy (fig. 2). This rise was not associated with a change in blood pressure and appears to be a response to the surgical procedure. By contrast renin-like activity measured at pH 6.5 showed significant changes with modification of salt balance and after bilateral nephrectomy. Thus, this component of aortic renin activity rose with salt depletion and was lowered by salt loading. Furthermore, in animals with intact kidneys, there was a correlation between PRC and aortic renin-like activity at pH 6.5 ($r = 0.71$). This is consistent with the view that renin-like activity measured at pH 6.5 is derived from plasma renin. However, the present experiments do not delineate a role for aortic renin as opposed to plasma renin in Groups 1 to 3. Thus, although the salt-depleted animals (Group 1) showed a greater fall in
blood pressure with converting enzyme inhibitor than salt-loaded animals (Group 3), this could equally well be due to a greater dependency of blood pressure of salt-depleted rats upon plasma or upon aortic renin. The alternative view is that bradykinin potentiation (a known property of converting enzyme inhibitor) accounts for the difference in response between animals of Groups 1 and 3. However similar differences in blood pressure response between salt-loaded and salt-depleted rats can be demonstrated with the competitive antagonist of angiotensin II, Sar⁻¹-Ala⁸ angiotensin II, which has no bradykinin potentiating action. It is therefore reasonable to assume that increased aortic or plasma renin is important in maintaining blood pressure in the salt-depleted animals of these experiments.

The difficulty in discriminating between the part played by aortic and plasma renin can be resolved by studying the effect of bilateral nephrectomy in situations where plasma renin is very high. Indirect evidence suggested that plasma and arterial wall renin concentrations diverged after bilateral nephrectomy, with arterial renin having a much longer half-life. Thus it has been postulated with considerable experimental support, that the pressor response to exogenous angiotensin II largely reflects the degree of occupancy of vascular receptors by endogenous angiotensin II. The greater the proportion of occupied receptors, the fewer free receptors and the smaller the response to exogenous angiotensin II. We have postulated also that the volume of angiotensin II antiserum needed to block the pressor response to a fixed dose of angiotensin II is also inversely proportional to the degree of receptor occupancy. Bilateral nephrectomy predictably causes an increase in both the pressor response to angiotensin II and in angiotensin II antiserum blocking requirement. Blockade of endogenous angiotensin by converting enzyme inhibitor has a similar effect upon pressor responsiveness to angiotensin II, although not to other pressor agents. However, the change in both pressor responsiveness and antiserum blocking requirement takes more than 6 hours to reach a maximum, while the fall in plasma renin (measured as activity or concentration) occurs with a half-life of 10 to 15 minutes. Furthermore, despite the fact that changes in pressor responsiveness to angiotensin II appear to be largely if not entirely due to endogenous angiotensin generation, isolated rabbit strips retain such differences in responsiveness. Finally, cross circulation studies in the rat have shown that the pressor effect of endogenous renin persists well beyond the time at which pressor material can be detected in the circulation, suggesting that extra circulatory renin has a longer half-life than plasma renin. These experimental results therefore provide indirect support for the hypothesis that renin derived from the kidneys persists at an extra circulatory site and is active in generating angiotensin II for several hours. While the central nervous system may provide such a site, it seems more probable that local generation of angiotensin II within the resistance vessel walls is responsible for these phenomena.

In the present experiments, the blood pressure fall produced by converting enzyme inhibitor was observed in two-kidney Goldblatt hypertensive rats.
and 2 hours after bilateral nephrectomy; a smaller fall was observed 6 hours after nephrectomy (table 2). Such a response is quite different from that which would be predicted if plasma renin were maintaining blood pressure, since the fall in plasma renin is rapid and complete within 1 hour. There remains the previously mentioned possibility that converting enzyme inhibitor lowers blood pressure in these rats by another mechanism such as bradykinin potentiation. However, while in some situations this property of converting enzyme inhibitor may be significant, bradykinin potentiation seems to be unimportant after bilateral nephrectomy. This was confirmed by the fact that the competitive antagonist of angiotensin II, saralasin, blocked the depressor response to converting enzyme inhibitor after bilateral nephrectomy (table 2). There is no evidence that saralasin impairs the bradykinin potentiating action of converting enzyme inhibitor. It is reasonable to conclude, therefore, that in our experiments, the fall in blood pressure after nephrectomy produced by converting enzyme inhibitor was due to inhibition of the renin angiotensin system and that saralasin, by blocking the renin angiotensin system at a point distal to angiotensin I conversion, prevented a further fall in blood pressure with SQ20,881 administration.

In the present work, the long half-life of renin-like activity measured at pH 6.5 (fig. 2) was consistent with the indirect experimental evidence for persistent activity of the renin angiotensin system several hours after bilateral nephrectomy. The fact that this renin-like activity had fallen at 6 and 24 hours supports the view that it is partly derived from the kidney. The present experiments do not identify the locus of its activity. However, detection of this activity is clearly not due to plasma contamination of aortic specimens: not only were these repeatedly washed, but aortic renin at 1 and 2 hours after nephrectomy was high when plasma renin was low. Presumably the renin-like ac-

TABLE 2. Blood Pressure Measurements in Normotensive and Hypertensive Rats Before and After Bilateral Nephrectomy

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Rats</th>
<th>Procedure</th>
<th>Initial BP (mm Hg)</th>
<th>Final BP (mm Hg)</th>
<th>Change in BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>Low salt</td>
<td>117.3 ± 4.8</td>
<td>75.1 ± 5.7</td>
<td>-42.2 ± 5.8</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>High salt</td>
<td>112.0 ± 2.8</td>
<td>106.0 ± 2.4</td>
<td>-6.0 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>Hypertension (renal artery constriction)</td>
<td>160.2 ± 8.7</td>
<td>121.9 ± 10.5</td>
<td>-38.3 ± 8.4</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>Hypertension (1 hour post nephrectomy)</td>
<td>142.9 ± 6.8</td>
<td>104.9 ± 12.2</td>
<td>-38.0 ± 10.1</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>Hypertension 1 hour post nephrectomy (pre-treatment with saralasin)</td>
<td>118.1 ± 13.9</td>
<td>121.9 ± 15.3</td>
<td>+3.8 ± 3.0</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>Hypertension (2 hour post nephrectomy)</td>
<td>125.9 ± 8.1</td>
<td>93.7 ± 10.7</td>
<td>-32.1 ± 5.7</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>Hypertension (6 hours post nephrectomy)</td>
<td>140.4 ± 11.1</td>
<td>134.1 ± 10.9</td>
<td>-6.3 ± 2.7</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>Hypertension (24 hours post nephrectomy)</td>
<td>76.6 ± 8.0</td>
<td>78.8 ± 8.9</td>
<td>+0.2 ± 2.1</td>
</tr>
</tbody>
</table>
tivity lies within the extracellular fluid component of the aortic wall; whether free within this compartment or bound to receptors is uncertain. It is important to note that converting enzyme activity is also present within the peripheral vessel wall. If, as seems likely, renin substrate also has access to the same site, all the necessary components for the local generation of angiotensin II are present. It is possible that local generation may explain the high doses of saralasin needed to block endogenous as opposed to exogenous angiotensin II since the angiotensin antagonist may be competing against a local concentration at the receptor site higher than the plasma concentration of angiotensin II.

References
Vascular renin-like activity and blood pressure maintenance in the rat. Studies of the effect of changes in sodium balance, hypertension and nephrectomy.

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