Hypertension

Laboratory Studies

Arterial Wall Uptake of Renal Renin and Blood Pressure Control

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SUMMARY We have studied the contribution of circulating renin of renal origin to renin-like activity within the arterial wall and to blood pressure. Bolus injections of renin sufficient to elevate blood pressure by 44.7 mm Hg caused aortic renin to rise from 0.13 to 1.48 ng angiotensin I/100 mg/hr in nephrectomized rats. Elevation of aortic renin was still present at 6 hours, and this was associated with significant blood pressure elevation (p < 0.05) which could be reversed by infusion of sarcosine,1 alanine,8 angiotensin II (saralasin). Prevention of the pressor effect by pretreatment with the converting enzyme inhibitor captopril did not reduce renin uptake. When kidneys were left in situ, although significant uptake of renin could be demonstrated 1 hour after injection, the increase at 3 hours was no longer significant (p > 0.05) and blood pressure returned to normal by V/4 hours. This change in blood pressure may be related to the much more rapid clearance of circulating renin in the presence of normal kidneys or to other renal factors influencing the blood pressure response. The present studies demonstrate therefore that most of the renin-like activity within the aortic wall is derived from plasma renin and it seems probable that this component of the renin-angiotensin system plays an important role in blood pressure maintenance in the nephrectomized rats injected with renin. The relationship is less obvious in the presence of normal kidneys where additional influences may come into play. (Hypertension 5: 629-634, 1983)

KEY WORDS • vascular renin • saralasin • arterial renin-like activity • vascular reactivity • local angiotensin II • bilateral nephrectomy

The role of the renin-angiotensin system in renovascular hypertension is still debatable. Thus, plasma renin levels are high in the two-kidney, one clip model in most species during the early phase of hypertension but they usually become normal during the chronic phase of blood pressure elevation.1-3 While other mechanisms could maintain blood pressure that had initially been raised by renin hypersecretion, it is still theoretically possible for renin to be responsible for blood pressure maintenance despite normal plasma renin levels. This could occur if, for instance, the renin-angiotensin system were active in strategic tissues such as the central nervous system,4 or the resistance vessel wall,5-8 independently of plasma renin. Thus, it has been shown that the other essential components of the renin-angiotensin system, such as substrate9 and converting enzyme activity,10,11 are present in the arterial wall.

Renin could be present in the vessel wall either as a result of local synthesis or alternatively as a result of uptake of renal renin from plasma; there is evidence for both these processes. Thus, vascular smooth muscle cells in tissue culture synthesize a renin-like enzyme.12 While aortic renin-like activity declines after bilateral nephrectomy although much more slowly than plasma renin,13 In other studies, however, arterial wall renin has persisted despite bilateral nephrectomy.14-16 Persistent locally produced renin has been implicated in the maintenance of blood pressure in some forms of hypertension when plasma renin is normal,6,7 and also in the depressor response to converting enzyme inhibitor in the presence of a normal or low plasma renin.16 On the other hand, we have failed to demonstrate a discrepancy between plasma and arterial renin in a variety of conditions in which a steady state prevails and we have previously concluded that vascular renin is only disproportionately elevated in relation to plasma renin when the latter is changing rapidly.17 In an attempt to evaluate uptake of renal renin from the plasma and its role in blood pressure control, we have studied the
changes in plasma and aortic renin after the injection of renin and correlated these with blood pressure responsiveness to angiotensin blockade. To avoid the confounding effects of changes in endogenous renin, most of our studies have been carried out in nephrectomized animals.

Methods

All studies were carried out on female Wistar rats weighing 180 to 200 g.

Surgical Procedures

Animals were anesthetized with ether, and a jugular vein and carotid artery cannulated; the cannulas were led around subcutaneously to the animal's back where they were led out through the skin. Bilateral loin incisions were made, the kidney pedicle ligated, and bilateral nephrectomy performed. After the wounds had been sutured, animals regained consciousness. Slack on the cannulas was taken up by slight tension maintained by a counterbalanced arm. A sufficient length of the cannula was left free to allow unrestricted movement within a normal-sized cage. Access to water but not food was allowed throughout the experiments. Blood pressure was monitored continuously during the experiments using a Statham P23 gb transducer and Grass polygraph paper recorder. Patency of the arterial and venous lines was maintained by heparinized 5% dextrose.

Plasma Renin Concentrations

All blood samples were collected from the carotid cannulas into a precooled tube and moistened with 100 μl of a concentrated solution of dipotassium EDTA. Plasma was separated by spinning in a refrigerated centrifuge at 2000 g for 7 minutes and frozen at -20°C. The source of renin substrate was pooled plasma specimens. Incubation periods of 6, 16, and 24 hours after nephrectomy were studied (n = 8) in which blood pressure was followed for 9 hours after injection of heparinized dextrose without renin.

Aortic Renin Concentration

Animals were killed by a blow on the head and the aorta rapidly dissected free down to its bifurcation. The aorta was washed in cold saline, connective tissue and fat were removed, and the vessel was cut lengthwise, blotted, and stored at -20°C. The aorta was thawed, frozen, and thawed four times, washed four times in normal saline, blotted, weighed, and homogenized in ice-cold saline (10 μl/mg wet weight) with a Teflon pestle. The homogenate was spun at 36,000 g for 30 minutes, frozen at -20°C, and thawed for assay. Then 100 μl of homogenate was withdrawn and assayed according to the same protocol used for the rat plasma specimens. Incubation periods of 6, 16, and 24 hours were used. A control was also incubated and assayed, using an equal volume of normal saline instead of aortic homogenate. Results were calculated as nanograms of angiotensin I per 100 mg tissue per hour of incubation.

Experimental Groups

Group 1: Nephrectomy and Renin Injection

On the day after cannulation and nephrectomy, animals were injected with 100 μl solution of semipurified rat renin equivalent to approximately 0.6 Goldblatt units dissolved in heparinized dextrose solution. Blood and aortic samples were taken at 1 hour (n = 9), 3 hours (n = 10), 6 hours (n = 10), and 9 hours (n = 9) after injection and also from one group that had not been injected with renin (n = 10). An additional group had plasma only sampled 10 minutes after injection (n = 10). Timing of renin injection was adjusted so that sampling in injected animals always took place 24 hours after nephrectomy. An additional group was studied (n = 8) in which blood pressure was followed for 9 hours after injection of heparinized dextrose without renin.

Group 2: Nephrectomy, Renin Injection and Saralasin Infusion

Two groups were studied as above (n = 10 each) until 2.5 or 5.5 hours after renin injections when a solution of saralasin (sarcosine, alanine, angiotensin II, Beckman) in 5% dextrose was infused at 10 μg/min/kg for 30 minutes while the blood pressure was monitored.

Group 3: Nephrectomy, Renin Injection and Captopril Pretreatment

To determine how far uptake of injected renin by the aorta was facilitated by the concomitant rise in pressure, the latter was blocked by a 500 μg i.v. injection of the converting-enzyme inhibitor, captopril, dissolved in dextrose (5 g/100 ml). Renin (0.6 Goldblatt units) was injected and an infusion of captopril (8.3 μg/kg/min) was continued for 3 hours. Plasma and aortic renin samples were then taken. The rats (n = 11) were otherwise treated as those in Group 1.

Group 4: Nephrectomy, and 125I Albumin Injection

To assess the amount of assayed renin-like activity due to the presence of renin in plasma contaminating the aortic sample, bilaterally nephrectomized rats (n = 10) were treated as in Group 1 except that no renin was administered and 100 μl of 125I-labeled albumin was injected intravenously (500,000 cpm/100 μl, Radiochemical Centre, Amersham, England). Blood and...
aortas were sampled after 30 minutes. Plasma (100 μl) was counted for radioactivity for 5 minutes, and aortas after washing four times with 0.9% NaCl were counted for 10 minutes.

**Group 5: Normal Rats, Renin Injection**

Rats were cannulated without bilateral nephrectomy. The next day, samples for plasma and aortic renin were taken from one group, and in further groups at 1 and 3 hours after renin injection (n = 9 for each group).

**Statistical Analysis**

Results are expressed as means ± standard error of the mean. Since plasma and aortic renin are not normally distributed, a nonparametric (Mann-Witney) test of significance was used for these. Blood pressure differences were assessed by Student’s t test.

**Results**

**Nephrectomized Rats (Groups 1-4)**

Renin injection produced an immediate rise in blood pressure, which remained significantly elevated at 6 hours (p < 0.05) but was not significantly different from baseline values at 9 hours after injection; control animals, on the other hand, maintained a steady blood pressure throughout the period of observation (fig. 1). Saralasin infusion reversed the renin-induced rise in blood pressure from a value of +20.6 ± 3.3 to +4.2 ± 2.4 mm Hg at 3 hours. An elevation of blood pressure of +18.5 ± 4.9 mm Hg was observed at 5½ hours, and this was reduced to +4.9 ± 4.4 mm Hg by saralasin infusion. Blood pressures before and after saralasin administration were significantly different both at 3 and 6 hours (p < 0.05; fig. 2).

Plasma renin was maximal in the specimen taken 10 minutes after injection (1115.2 ± 159.4 ng AI/ml/hr). This fell rapidly by 1 hour, with an initial half-life of 32 minutes; a slower fall occurred over the next 2 hours, with a half-life of 66 minutes (fig. 3). Aortic renin-like activity rose from initially low levels (0.13 ± 0.03 ng AI/100 mg/hr) to 1.48 ± 0.29 ng AI/100
mg/hr at 1 hour and remained significantly elevated at 3 and 6 hours (0.61 ± 0.15 and 0.65 ± 0.2 ng AI/100 mg/hr, respect.\(p  < 0.05; fig. 4\)). The volume of distribution of \(^{125}\)I-albumin was 2.29 ± 0.39 µl/100 mg aortic tissue. Aortic renin values were corrected for the renin content of contaminating plasma (\(^{125}\)I-albumin distribution space × simultaneous plasma renin concentration). Corrected and uncorrected aortic renin-like activity declined much more slowly than plasma renin concentration. Both corrected and uncorrected aortic renin took 6 to 9 hours to fall to less than half the values observed at 1 hour. Aortic renin was slightly although nonsignificantly elevated compared with aortic renin values in normal animals at 9 hours, but significantly elevated compared with nephrectomized animals (\(p  < 0.05\)).

Pretreatment with captopril prevented the pressor response to renin (fig. 5). At 3 hours, plasma renin was nearly identical in this group compared with noncaptopril-treated animals (47.7 ± 8.6 compared with 46.4 ± 7.7 ng/ml/hr). Aortic renin-like activity was also similar in the two groups (0.71 ± 0.32 and 0.61 ± 0.16 ng AI/100 mg/hr, respectively; \(p  > 0.05\)).

Normal Rats (Group 5)

Initial blood pressure was similar to that observed in nephrectomized rats (\(p  > 0.05\) between the two groups). The acute rise in pressure with renin injection was also similar (\(p  > 0.05\)), but was of much shorter duration, and blood pressure returned to baseline values by 1½ hours (fig. 6). Plasma renin 10 minutes after injection was substantially lower than in nephrectomized rats injected with renin (\(p  < 0.01; fig. 3\)). One hour after injection, plasma renin had returned to normal levels. Aortic renin-like activity rose from 0.20 ± 0.05 to 1.23 ± 0.32 ng/100 mg/hr at 1 hour after injection, and had fallen to 0.41 ± 0.10 mg/100 mg/hr at 3 hours after injection. Although the rise in activity...
at 1 hour was less compared with nephrectomized rats, the difference was not significant \( (p > 0.05) \), and after 3 hours, values were not significantly different from baseline \( (p > 0.05) \).

Discussion

Our previous studies of angiotensin II responsiveness\(^ {19, 20} \) and our more recent ones of direct measurement and aortic renin\(^ {8, 11} \) suggested that locally generated angiotensin II within the resistance vessel wall played a role in blood pressure control. Both direct\(^ {13} \) and indirect\(^ {20} \) evidence suggested that this angiotensin II was largely derived from renal renin taken up from the plasma, although the clearance of renin from this site after bilateral nephrectomy was much slower than the clearance of plasma renin. However, other groups have demonstrated persistence of renin-like activity after bilateral nephrectomy\(^ {14-16} \) and have concluded that renin is synthesized locally. The present experiments demonstrate substantial uptake of exogenous renin by the aorta and a subsequent rate of decline in nephrectomized animals similar to that observed for endogenous renin-like activity after bilateral nephrectomy and much slower than the fall of plasma renin concentration. Our findings are consistent with earlier studies of Schaectelin et al.\(^ {21} \) who demonstrated, using a cross-circulation model, that the pressor effects of renin persisted even when it had been cleared from the circulation.

We examined two possible experimental artifacts. First, there was the possibility that the renin-like activity detected was due to plasma contamination not removed by the washing procedures. However, correction for renin contained within the I\(^ {128} \)-albumin accessible space only produced a substantial decrease in apparent aortic renin-like activity at 1 hour after injection when plasma renin was high (fig. 4). Correcting for plasma contamination thus prolonged the apparent half-life of vascular renin-like activity. Even if albumin space measurements underestimate plasma contamination, the contribution of this confounding factor to observed persistent aortic renin-like activity must be negligible since plasma renin had returned to normal levels at 3 hours and beyond after injection (fig. 3). The second possibility that we examined was that the acute rise in blood pressure produced by renin injection resulted in abnormal uptake of renin by the aorta. This was not the case since we observed an equally significant uptake of renin by the aorta when the rise in blood pressure was prevented by converting enzyme inhibition.

In our study, renin-like activity was defined by the capacity of aortic homogenate to generate angiotensin I when incubated at pH 6.5 with renin substrate. The latter pH was selected as it is the optimal pH for the rat renin-renin substrate reaction. Other tissue proteases generate angiotensin I in this system at other pHs, as is shown by greater activity in more acid incubation media.\(^ {13} \) We have previously demonstrated that activity measured at pH 5.3 does not reflect the changes observed in activity measured at pH 6.5 when the renin angiotensin system is activated or suppressed.\(^ {13} \) This may explain the persistence of renin after bilateral nephrectomy where a low incubation pH was used.\(^ {15} \) In other studies, samples were acidified before assay, which may result in in vitro activation of renin that is inactive in vivo.\(^ {14} \) On the other hand, aortic renin-like activity measured with an incubation pH of 7.4 has been demonstrated in spontaneously hypertensive rats 24 hours after bilateral nephrectomy.\(^ {16} \) Whether the different incubation pH or strain of rat can account for this discrepancy remains uncertain. In our study, the minor degree of activity present 18 hours after nephrectomy in animals not injected with renin was at the lower-most limits of the sensitivity of the assay and may indeed be due to a minor contribution of other tissue proteases to substrate-splitting activity at pH 6.5. The present studies do not, therefore, convincingly demonstrate any aortic renin-like activity 18 hours after bilateral nephrectomy.

Renin produced a prolonged pressor effect in bilaterally nephrectomized rats which was not seen when the kidneys remained in situ. Saralasin infusion studies confirm that the blood pressure elevation at 3 and 6 hours after renin injection was indeed angiotensin-induced (fig. 2). Although the clearance of circulating renin was reduced in nephrectomized rats, as has previously been demonstrated,\(^ {22, 23} \) plasma renin had declined from its peak to low levels after 3 hours. At these values for plasma renin, saralasin normally produces a pressor action.\(^ {24} \) The persistent pressor effect of renin in nephrectomized rats despite clearance of plasma renin has been previously demonstrated by Bing and Nielsen,\(^ {25} \) who attributed it to renin uptake by the resistance vessel wall. An alternative hypothesis is that the prolonged pressor effect of renin after nephrectomy may be due to an alteration in angiotensin II pressor dose-response curve.\(^ {25} \) This would be consistent with the much shorter duration of the pressor response to renin in our normal rats. Under these circumstances, however, it would not be anticipated that the relationship between plasma renin and blood pressure would be lost, as it was in our nephrectomized animals, unless sensitivity to angiotensin were progressively to increase in the hours after renin injection, which is unlikely at this stage after nephrectomy.

By contrast with nephrectomized animals, normal rats showed a much lower initial peak plasma renin value after injection and plasma renin had returned to normal by 1 hour. Aortic renin was elevated at 1 hour but not significantly different from baseline values at 3 hours. Blood pressure returned to baseline values by 1½ hours after injection. Thus, although a differential clearance of plasma and aortic renin was again demonstrated, the effect was less clearcut, and it is not possible to relate blood pressure as convincingly to aortic renin rather than to plasma renin. Whether this is due to the much more rapid clearance of plasma renin in normal animals after a single injection so that arterial tissue is exposed for much shorter periods of time is uncertain. An alternative possibility is that other renal factors play a role in blood pressure maintenance and
that these influence the relationship between vascular renin and blood pressure. The elucidation of these problems requires further study.

Thus, although the present studies do not exclude a contribution from locally synthesized renin, they suggest that the greater part of aortic renin-like activity demonstrated here is renal renin derived from the plasma. Further, our results are consistent with the view that renin at this site plays a role in blood pressure control in some situations.

References


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M Loudon, R F Bing, H Thurston and J D Swales

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