Role of persistent vascular renin after bilateral nephrectomy in Goldblatt-two kidney hypertension

H. THURSTON, B. C. HURST, R. F. BING AND J. D. SWALES

Department of Medicine, Leicester General Hospital, Gwendolen Road, Leicester, U.K.

Summary

1. Aortic homogenate contains renin-like activity which on incubation generates angiotensin I over a wide pH range.

2. Rat aortic renin measured at an incubation pH of 6-5 rose and fell in parallel to plasma renin with salt depletion and salt-loading respectively. Renin measured at an incubation pH of 5-3 showed little relationship with plasma renin.

3. Aortic renin (pH 6-5) was elevated in Goldblatt-two kidney hypertension and slowly fell for 24 h after bilateral nephrectomy whereas the fall in plasma renin was complete by the first hour. Aortic renin (pH 5-3) was also high, but did not fall after bilateral nephrectomy.

4. Aortic renin (pH 6-5) is probably derived from plasma renin whereas renin measured at pH 5-3 is probably a tissue renin.

5. The prolonged half-life of aortic renin (pH 6-5) explains the observation that the renin–angiotensin system appears to be active in maintaining blood pressure for several hours after bilateral nephrectomy whereas the decline in plasma renin is rapid and does not continue significantly beyond 1 h.

Key words: nephrectomy, renin, sodium balance, vascular renin.

Introduction

Studies with angiotensin II antagonists support the view that the renin–angiotensin system plays a major role in hypertension produced by renal artery constriction in the rat with the opposite kidney undisturbed (Brunner, Kirshman, Sealey & Laragh, 1971; Thurston & Swales, 1974a, Mac-Donald, Boyd & Peart, 1975). Until recently, it has been generally assumed that renin maintains blood pressure by angiotensin II formed within the circulation. However, renin is found in arterial walls (Gould, Skeggs & Kahn, 1964) and suggestions have been made that it could exert a major vasoconstrictor effect by means of angiotensin II generated within resistance vessel walls (Daum, Uehleke & Klaus, 1966; Rosenthal, Boucher, Rojo-Ortega & Genest, 1969; Hayduk, Ganten, Boucher & Genest, 1972).

We have recently suggested that vascular generation of angiotensin II is an important determinant of the pressor response to exogenous angiotensin II (Swales & Thurston, 1973) and maintains the blood pressure in Goldblatt-two kidney hypertension (Thurston & Swales, 1974b). Indirect studies of vascular responsiveness (Swales, Tange & Thurston, 1975) suggest that the half-life of vascular renin is considerably longer than plasma renin. In addition studies of blood pressure responsiveness to renin–angiotensin inhibition suggest that the renin–angiotensin system maintains blood pressure even after plasma renin has fallen to a very low level (Thurston & Swales, 1977).

Direct studies of arterial renin have yielded somewhat conflicting results (Rosenthal et al., 1969; Hayduk et al., 1972) which may partly depend on the incubation pH used for the renin assay system. We have therefore examined changes in plasma and aortic renin concentration in Goldblatt-two kidney hypertensive rats for 24 h after bilateral nephrectomy, to test the hypothesis that preservation of the blood pressure response to inhibition of the renin–angiotensin system is due to persistence of vascular renin when plasma renin has fallen to a low level.
Materials and methods

Female, white Wistar rats weighing 150–250 g were used throughout. After dietary or operative treatment as detailed below, animals in each group were killed by a blow on the head, a blood sample taken, the aorta dissected free down to the bifurcation and removed.

Normotensive rats (diet study)

Twenty-three animals were given one of the following regimens for 2 weeks before study.

- Group 1 (low salt) received a diet containing 0.013 mmol of sodium/g with free access to deionized water.
- Group 2 (moderate salt) were given a diet containing 0.035 mmol of sodium/g and deionized water to drink.
- Group 3 (high salt) received normal rat chow containing 0.099 mmol of sodium/g but in addition were given 1% saline as drinking fluid.

Hypertensive rats (nephrectomy study)

Hypertension was produced by the application of a silver clip (internal diameter 0.2 mm) to the left renal artery under ether anaesthesia: the opposite kidney was not disturbed. After surgery the animals received normal rat chow (0.099 mmol of sodium/g) and tap water to drink. Hypertensive animals (defined as having a sustained blood pressure > 140 mmHg) were selected on the basis of indirect blood pressure readings (Swales & Tange, 1970) within 28 days of renal artery clipping and with hypertension of less than 2 weeks duration.

Hypertensive rats were divided into five groups. four groups were studied after bilateral nephrectomy. The rats received water but no food after surgery. The animals were studied before (Group 4), 1 h (Group 5), 2 h (Group 6), 6 h (Group 7) and 24 h (Group 8) after nephrectomy.

Plasma renin concentration

Blood samples were taken into pre-chilled tubes containing potassium EDTA as anticoagulant. Plasma samples were stored at -20°C before assay. A 100 µl sample of plasma was incubated with 400 µl of nephrectomized rat plasma as substrate at pH 6.5 and the generated angiotensin I measured by radioimmunoassay. The method used was similar to that of Sealey, Laragh, Gertn-Banes & Aceto (1974) except that phenylmethyl-sulphonyl fluoride was used as an angiotensinase inhibitor.

Aortic renin concentration

After removal, each aorta was washed in cold saline, trimmed free of all connective tissue and cut length-wise before storage at -20°C. The whole aorta was thawed, frozen–thawed four times, weighed and then homogenized in 500 µl of ice-cold saline with a Teflon pestle. The homogenate was frozen at -20°C before thawing for assay, which was performed on 100 µl samples as above, except that each sample was incubated at both pH 5.3 and 6.5. In preliminary experiments aortic homogenate from rats with Goldblatt-two kidney hypertension was incubated with substrate over a pH range of 4.5 to 7.0. All results are expressed as a mean ± SEM and statistical significance determined by the use of a Student 't' test.

Results

Angiotensin I was generated at all pH values studied. Renin-like activity was particularly evident between pH 5.3 and pH 6.5. By contrast, the plasma pH profile was narrow with an optimum of pH 6.5.

The aortic and plasma renin concentrations of both the normal and hypertensive rats are shown in Table 1. In the normotensive rats plasma renin rose with salt restriction and fell with salt loading. Aortic renin concentration was dependent on the pH of incubation: thus at pH 5.3 more activity was present than at pH 6.5. At the latter pH, aortic renin activity changed in parallel with plasma renin (Table 1).

Aortic renin was markedly elevated in the hypertensive rats compared with normal animals (P < 0.01) and showed a slowly progressive fall after bilateral nephrectomy. This contrasted sharply with the plasma renin concentration which showed a marked immediate fall which was completed by the end of the 1 h. Aortic renin measurement at pH 5.3 and 6.5 showed a markedly divergent pattern after nephrectomy. At 24 h, aortic renin activity measured at pH 5.3 was not significantly lower than the initial value (P > 0.1) whereas at pH 6.5 activity was at its lowest.

Discussion

Our results demonstrate that changes in plasma and aortic renin occur in parallel with alteration of
sodium balance: on the other hand the half-life of aortic renin is substantially longer than that of plasma renin as previously reported (Rosenthal et al., 1969). By contrast, dog mesenteric arterial wall renin activity persisted indefinitely after bilateral nephrectomy (Hayduk et al., 1972) and it has been suggested that renin may be synthesized within the blood vessel wall. These discrepancies may represent species or tissue differences but could result directly from the choice of pH used for the incubation stage of the renin assay. Thus Hayduk et al. (1972) incubated arterial homogenate at pH 5-0 which is somewhat below the optimum (5.5-6.5) for dog renal renin. It is noteworthy however that Ganten, Schelling, Vecsei & Ganten (1976) concluded that arterial wall renin showed an identical optimum pH to that of renal renin. In our studies, simultaneous assays at a low (5.3) and high (6.5) pH revealed a marked divergence in aortic renin-like activity after bilateral nephrectomy. This may reflect the presence of both a tissue isorenin and absorbed renal renin in aortic tissue. Since tissue isorenin is generally thought to be synthesized locally, bilateral nephrectomy would not be expected to influence that system, and apart from an initial post-operative rise, no consistent change was noted. However, when aortic renin measurements were made utilizing the higher pH (the optimum for rat renal renin) a slow steady decline was observed which continued long after plasma renin had fallen: this supports the hypothesis that activity at this pH represents a tissue bound renin of renal origin with a much longer half-life than plasma renin. The long half-life of vascular renin is consistent with other evidence supporting that view that vascular, rather than circulating renin is important in blood pressure maintenance in high renin states. The evidence is

### References


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