Changes in blood pressure in relation to vascular and plasma renin after renin injection in rats

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Summary
1. To assess the relative importance of vascular as opposed to plasma renin, groups of conscious rats received a single intravenous injection of partially purified rat renin 18 h after bilateral nephrectomy. Blood pressure was monitored continuously and plasma and aortic renin concentrations were determined at 1, 3, 6 or 9 h after injection. In separate groups of rats the effect of the competitive angiotensin II antagonist, saralasin, on blood pressure was measured 3 or 6 h after renin injection.
2. Blood pressure remained elevated for up to 6 h after renin injection, returning to normal by 9 h. Saralasin infusion reversed the rise in blood pressure at both 3 and 6 h after injection.
3. Aortic renin concentration followed the pattern of the pressor response whereas plasma renin concentration had returned to subnormal values by 3 h.
4. Circulating renin of renal origin is taken up by aortic tissue. The pressor response to exogenous renin in rats after bilateral nephrectomy is not related to changes in plasma renin but is similar in duration to the persistence of aortic renin-like activity and can be blocked by saralasin at both 3 and 6 h after injection.

Key words: bilateral nephrectomy, exogenous renin, plasma renin, saralasin, vascular renin.

Introduction

Renin is easily measured as a constituent of plasma and most studies of the cardiovascular response to renin have assumed that generation of angiotensin II takes place within the circulation. Although this is undoubtedly true, increasing interest has been attracted recently to extracirculatory generation of angiotensin II. Our own [1-3] and other [4-6] groups have postulated that generation of angiotensin II within the resistance vessel wall is an important determinant of the pressor effect of renin. However, whereas some groups have claimed that renin is formed locally within the blood vessel wall independently of plasma renin [5, 7] we have only detected divergence between plasma and aortic renin in non-steady state conditions [3], such as the postoperative phase after bilateral nephrectomy.

In the present study we have sought to answer the question: does the arterial wall take up renin from the plasma and is such uptake associated with a pressor response? To obtain divergent changes in arterial and plasma renin, we have exploited our previous observation that arterial renin has a much longer half-life than plasma renin. Accordingly we have studied the effects of a single injection of renal renin upon the plasma and aortic renin and blood pressure in nephrectomized, conscious rats. To confirm that the pressor response was angiotensin-induced, it has been blocked by the antagonist sarcsine¹, alanine⁸-angiotensin II (saralasin).

Methods

Groups of eight to ten female Wistar rats weighing 180-200 g were used throughout.

Experimental protocol

On the first day cannulation of the left carotid artery and jugular vein [8] and bilateral nephrectomy were performed under ether anaesthesia. The animals were then allowed to recover for 18 h. On the second day continuous blood pressure monitoring was started and each animal received a single injection of 100 µl of partially purified rat renin, equivalent to approximately 0.6 Goldblatt unit. Renin for injection was prepared from 30 g
of pooled rat kidney cortex [9]. The resulting preparation with a renin activity equivalent to 800 
µg of ANGI h⁻¹ ml⁻¹ [10] was stored at -20°C 
diluted 1:4 in heparinized glucose solution before injection. Blood samples were collected 
from the carotid catheters at 1, 3, 6 or 9 h after 
renin injection. The animals were then killed by a 
blow on the head, followed by cervical dis-
location, and the aorta of each dissected free 
down to the bifurcation. Connective tissue and fat 
were removed and the vessel stored at -20°C. 
All the animals were injected so that they were 
killed 24 h after bilateral nephrectomy. A control 
group received no renin injection but was other-
wise treated in the same way.

Plasma renin concentration

Blood samples were collected in pre-cooled 
tubes containing potassium EDTA. A sample 
(100 µl) of plasma was incubated with 400 µl of 
nephrectomized rat plasma, at pH 6.5, buffered 
with Tris maleate (50 mmol/l) and with saturated 
PMSF (50 µl/ml) and 8-hydroxyquinoline (5 
mmol/l) included as inhibitors. The angiotensin I 
generated during incubation was measured by 
radioimmunoassay [11]. Plasma renin concen-
tration (PRC) was expressed as pmol of ANG I 
generated h⁻¹ of incubation ml⁻¹ of plasma at 
37°C.

Aortic renin concentration

Each aorta was frozen and thawed four times, 
flushed thoroughly in 0.9% NaCl (saline) to 
remove contaminating plasma, and homogenized in 
ice-cold saline (10 µl/mg of tissue, wet weight). 
The homogenate was spun at 36 000 g for 30 min 
at 4°C. The supernatant was assayed by the 
method described above for plasma. Aortic renin 
concentration was expressed as pmol of ANG I 
generated h⁻¹ of incubation 100 mg⁻¹ of aortic 
tissue at 37°C.

Statistical analysis

All results are expressed as means ± SEM. 
Plasma and aortic renin concentrations are not 
normally distributed and statistical analysis was 
by the Mann–Witney test.

Inhibitor studies

Two groups of animals received an infusion of 
saralasin (Sar¹, Ala⁷-angiotensin II; Beckman, 
Geneva, Switzerland, 10 µg min⁻¹ kg⁻¹) for 30 
min starting either 2.5 or 5.5 h after renin injection.

Plasma contamination

Plasma contamination of aortic samples was 
estimated by injecting bilaterally nephrectomized 
rats with ¹²⁵I-labelled albumin and measuring the 
distribution of the label in the aorta and a plasma 
sample taken at the same time. One group of rats 
received an injection of 100 µl of ¹²⁵I-labelled 
albumin (500 000 c.p.m./100 µl; The Radio-
chemical Centre, Amersham). Blood was collected 
through the aortic cannula and the animals 
were killed to remove the aorta after 30 min. 
Samples of 100 µl of plasma were counted for 
radioactivity for 5 min (Automatic gamma 
counter, LKB-Wallac 80 000). The aortae were 
cleaned, washed thoroughly in 0.9% NaCl and 
counted for 10 min. Contamination of the aorta 
by plasma not removed by the washing pro-
cedure was expressed as µl of plasma/100 mg of 
aortic tissue.

Results

Plasma and aortic renin concentrations 18 h after 
bilateral nephrectomy (3.70 ± 1.3 pmol of ANG I 
h⁻¹ ml⁻¹ and 0.08 ± 0.03 pmol of ANG I h⁻¹ 
100 mg⁻¹ respectively, mean ± SEM) were 
significantly lower than in normal rats (54.1 
± 9.5 and 0.19 ± 0.05 respectively, P < 0.05 for 
both values). Baseline mean arterial pressure was 
121.0 ± 3.3 mmHg and did not change consis-
tently or significantly over the 9 h of the study. 

The injection of renin (approximately 0.6 
Goldblatt unit) produced an immediate rise in 
blood pressure. Blood pressure remained signif-
ificantly elevated up to 6 h (P < 0.05) after 
Injection but was not significantly different from 
pre-injection values at 9 h (Fig. 1a). The 
calculated half-life of this elevation of mean blood 
pressure was 4.6 h.

Plasma renin concentration was 860 ± 123 
pmol of ANG I h⁻¹ ml⁻¹ 10 min after injection 
but fell to below the mean value for normal intact 
rats by 3 h (35.8 ± 6.0 pmol of ANG I h⁻¹ ml⁻¹). 
The half-life of plasma renin concentration was 1 
h (Fig. 1b).

Aortic renin concentration remained elevated 
above that in normal rats for up to 6 h (P < 0.05) 
after injection, returning to normal only at 9 h. 
The average value for contamination of aortic 
samples by plasma was 2.29 ± 0.39 µl of plasma/ 
100 mg of aortic tissue. Corrected aortic renin 
concentrations were then estimated for each 
separate value by subtracting the renin activity 
due to this volume of the corresponding plasma. 
The half-life of the corrected aortic renin con-
centration was calculated as 4.83 h (Fig. 1c).
Vascular renin and blood pressure control

Saralasin, infused at 10 μg min⁻¹ kg⁻¹ for 30 min reversed the pressor effect of renin injection. The blood pressure fell from +20.6 ± 3.3 to +4.2 ± 2.4 mmHg at 3 h and from +18.5 ± 4.9 to +4.9 ± 4.4 mmHg at 6 h after injection compared with baseline pressures before injection.

Discussion

The present study was designed to evaluate the role of vascular renin-like activity in blood pressure control, the source of this material and its relation to plasma renin.

Both plasma and aortic renin concentrations change in parallel in a number of experimental models [3]. However, in order to assess the role of arterial as opposed to plasma renin it is necessary to study states where the two are changing at different rates. After unclipping [12] or bilateral nephrectomy [13] in early Goldblatt two-kidney, one-clip hypertensive rats, the half-life of aortic renin is substantially longer than plasma renin. In the latter model the vasodepressor response to renin–angiotensin inhibitors falls in parallel with the aortic renin levels [2], as does the pressor effect of a single renin injection [14]. Others have found little change in arterial renin-like activity after bilateral nephrectomy in the dog [7, 15] and spontaneously hypertensive rat [16]. This suggests that renin-like material is synthesized locally or derived from some source other than the kidney [5]. These discrepancies may be due to species differences or be related to the method for measuring vascular renin-like activity. In this respect the pH of incubation is critical. At a low pH (<5.3) acid proteases such as cathepsin D and pepsin are active, whereas at near-neutral pH, such as 6-5, the activity of these is not significant. Plasma [17] and purified renal renin [18] show pH optima in the latter range, and aortic renin-like activity at pH 6-5, but not 5-3, changes with sodium balance and after bilateral nephrectomy [17]. This supports the view that tissue renin-like activity measured at pH 6-5 may be of renal origin. The fact that aortic renin-like activity measured at pH 6-5 in the normal rat 18 h after nephrectomy is very low is consistent with this.

In the present study the pressor response to renin injection was sustained for more than 6 h although plasma renin fell rapidly. On the other hand, aortic renin-like activity remained significantly elevated above normal for 6 h and, like the blood pressure, returned to normal only after 9 h. The persistence of vascular renin in this situation was similar to that measured after bilateral nephrectomy [13] and unclipping [12] in Goldblatt two-kidney, one-clip hypertensive rats. Infusions of saralasin reversed the pressor response. Blood pressure was returned to levels only slightly greater but not significantly different from baseline values. This suggests that the pressor response is angiotensin dependent.

The present results support the hypothesis that circulating renin of renal origin can be taken up by the arterial wall although local synthesis in addition to this cannot be excluded. Further, renin taken up by the artery and the resultant local generation of angiotensin II is an important determinant of the pressor response to exogenous renin.

References


