Arterial wall renin and renal venous renin in the hypertensive rat

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Summary

1. Infusion of sufficient renin to raise the blood pressure of normal rats to hypertensive levels resulted in increased renin in the arterial wall.

2. Arterial wall renin and renal venous renin were normal in younger spontaneously hypertensive rats, but in older spontaneously hypertensive rats arterial wall renin was significantly increased and renal venous renin was significantly decreased.

3. Arterial wall renin in rats with either acute or chronic two-kidney Goldblatt renal hypertension was significantly increased, whereas circulatory renin was elevated in the former, but depressed in the latter.

4. Arterial wall renin may play a role in the maintenance of acute and chronic renal hypertension and also perhaps of spontaneous hypertension of long duration in older rats.

Key words: arterial wall renin, plasma renin activity, renal venous renin, spontaneous hypertension, two-kidney Goldblatt renal hypertension.

Abbreviation: SH, spontaneously hypertensive (rat).

Introduction

The role of the renin–angiotensin system in blood pressure maintenance is still unsettled. Studies with radioactive angiotensin II indicate that this compound is bound to specific receptor sites in vascular smooth muscle (Baudoin, Meyer & Worcel, 1971). This suggests that renin, through angiotensin, has a specific function in this tissue. But in chronic renal hypertension and in spontaneous hypertension in rats (Okamoto–Aoki strain) the relationship of renin to elevated blood pressure has been questioned because of the observation that circulating renin is normal or low (Koletsky, Rivera-Velez, Marsh & Pritchard, 1967; Sokabe, 1966; Koletsky, Shook & Rivera-Velez, 1970; Sen, Smeby & Bumpus, 1972; Vincent, Dupont & Sassard, 1976). On the other hand, others (de Jong, Lovenberg & Sjoerdsma, 1972) have reported that renin increases with age in spontaneously hypertensive rats.

Renin is known to be present in the blood vessel wall of pigs and rats (Dengler, 1956; Gould, Skeggs & Kahn, 1964; Rosenthal, Boucher, Rojo-Ortega & Genest, 1969) and thus might play a role in blood pressure maintenance. Injection of anti-renin into dogs with either acute renin-induced hypertension or chronic one-kidney Goldblatt renal hypertension caused a rapid, partial drop in blood pressure during the first 20 min after infusion in the first model and a much slower drop (4–10 days) in the second. The rate of production of the hypotensive effect is consistent with the hypothesis that neutralization of circulating renin occurred in the acutely hypertensive animals and of vascular wall renin in the chronically hypertensive ones (Hill, Chester & Wisenbaugh, 1970).

In the rat, the action of an angiotensin II antagonist which can penetrate the arterial wall and
an angiotensin II antiserum which cannot sug-
gested that renin generates angiotensin at a local
vascular level in a site not readily accessible to anti-
serum (Thurston & Swales, 1974). Additional
support for this theory is that converting enzyme
inhibitor causes a fall in blood pressure for several
hours after bilateral nephrectomy, indicating that
the renin–angiotensin system maintains blood
pressure in this model even after plasma renin has
fallen to insignificant levels (Thurston & Swales,
1977). In addition, pressor action of renin is
present in nephrectomized animals without detect-
able circulating renin (Schaechtelin, Regoli &
Gross, 1964). Thus it would appear that the
amount of circulating renin may not reflect the
activity of this substance at its major site of action,
namely, the arterial wall.

In the present study we have tested the
hypothesis that significant amounts of renin are
present in the arterial wall of rats with renin-
induced hypertension, chronic renal hypertension
or spontaneous hypertension.

Methods

Infusion experiments

Normal adult male albino rats were
anaesthetized with sodium amylobarbitone, and
were vagotomized and heparinized. Both jugular
veins were cannulated, the right to obtain blood
samples and the left to connect to the infusion
pump. Pentolinium tartrate was given as a gangli-
onic blocking agent. Carotid arterial pressure was
continuously monitored. A total of 1.1 Goldblatt
units of rat renin in 0.408 ml of sodium chloride
solution (155 mmol/l: saline) was infused at the
rate of 0.0136 ml/min for 30 min into each of 20
rats. Preliminary trials had shown that this could
produce a peak blood pressure similar to that of
rats with acute renal hypertension. Twenty-one
control rats were infused with 0.408 ml of saline at
the same rate and for the same length of time. Four
other rats were subjected to the same surgery and
connected in the same way to the infusion pump
without any infusion.

Approximately 0.5 ml of blood was collected
immediately before the start of the infusion, and
after 30 min infusion and at 30 min after the end of
the infusion. The collected blood was promptly
centrifuged in the cold. The plasma was removed
and frozen until assayed for angiotensin. Im-
mediately after the third sample was taken the rat
was killed with sodium amylobarbitone and the
aorta was removed with as many branches as could
be obtained. Extraneous fat and connective tissue
were quickly removed, the artery was slit length-
wise and washed by dipping once in each of four
lots of cold saline with intervening blotting, and
quickly weighed and frozen. The homogenized
tissue was prepared by the method of Rosenthal et
al. (1969).

The amount of blood that still might be adhering
to the arterial wall samples was estimated in six
rats to evaluate the magnitude of contamination
with plasma renin. The method used depended on
the spectral absorbance of haemoglobin at the 415
nm peak. The rat's own blood was used to con-
struct a standard absorbance curve to be used to
determine the volume of blood in the supernatant
of the homogenized tissue. From this volume and the
concentration of renin in the rat's own plasma, the
amount of contamination with plasma renin, if any,
could be calculated.

Such an adjustment is an overcorrection since
the peak at 415 nm was due in part, if not largely,
to non-haemoglobin material. The absorbance
curve of the supernatant of the arterial homo-
genate was highest in the near-ultraviolet and
declined gradually without showing a peak at 415
nm. Despite this, the aforementioned correction
was applied to all values obtained for renin
concentration in the arterial wall.

Hypertensive rats

Three groups of adult male albino rats were
used: normal Wistar (Charles River); sponta-
neously hypertensive (SH) rats of the Okamoto–
Aoki strain (Kyoto–Wistar derived) from National
Institutes of Health, Bethesda, Maryland, U.S.A.;
Wistar rats with acute (ARH) or chronic (CRH)
experimental renovascular hypertension (two-
kidney Goldblatt). Renovascular hypertension was
produced by unilateral renal artery constriction as
described by Koletsky et al. (1967). The right
kidney was untouched. After obtaining the blood
samples (Koletsky et al., 1967), the animals were
killed and the entire aorta and attached major
branches was removed and prepared as described
above. The plasma obtained was frozen until used.
The samples of blood from the ARH rats were
taken from 1 to 6 days after induction of high
blood pressure and those from the CRH rats when
the blood pressure had remained elevated from 1 to
6 months. Blood and arterial wall samples also
were obtained in a similar manner from the SH
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43 rats. Environmental conditions were maintained constant throughout.

The normal and the SH rats were divided into groups according to mean age: normal rats, younger, 3-5 months, and older, 9-5 months; SH rats, younger, 6-5 months, and older, 12-0 months. All renal hypertensive rats were young (5-1 months). The young normal rats served as controls for all of the young hypertensive animals.

Assay methods

Assay of renin in a sample was done by measurement of the angiotensin produced by the action of renin on renin substrate. The renin activity of a sample depends on the amount of endogenous substrate present. Since the latter may be insufficient for the amount of renin present, determination of renin concentration in a sample requires the addition of substrate. Rat renin substrate (Boucher, Menard & Genest, 1967) was prepared from rats given a solution of 5% glucose and 1% sodium chloride solution instead of water to drink 48 h before nephrectomy. The substrate preparation was tested for endogenous renin activity by incubation and assay with no added renin. The concentration of substrate in the preparation in terms of the angiotensin I1 produced by the substrate preparation was assayed with standardized rat renin.

Renin concentration was determined by the methods of incubation and chromatography of Boucher et al. (1967) in plasma samples obtained in the infusion experiments and from the older SH rats and their normotensive controls, and also in all homogenized arterial wall samples. The following changes were made in the incubation. Only four samples were prepared at a time and they were not filtered. Either the entire arterial homogenate or 0.1 ml of plasma was incubated with prepared substrate (pH 6.5) for 16 h at 37°C in a gyratory water bath (New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.). Chromatography of the incubation mixture was done with no changes on Dowex resin 50W-X2 (NH4+) (Dow Chemical Co., Midland, Michigan, U.S.A.) in a glass column.

The angiotensin I obtained by the above methods was assayed (Skeggs, Kahn & Marsh, 1953) in a rat prepared as for the infusion experiments except that only one jugular vein was cannulated. This was used for the injection of the assay material or standard. Carotid arterial pressure was recorded directly. The volume of sample or standard plus any necessary diluent (Skeggs et al., 1953) was 0-25 ml per injection. Angiotensin II standard (research standard A/65, courtesy of the Medical Research Council, National Institute for Medical Research, Mill Hill, London, U.K.) was used to bracket aliquots of the samples assayed. The sensitivity of the bioassay allowed at least three, or more usually six to eight, aliquots of a sample to be assayed. The lower limit of sensitivity for the average test rat is 0-47 pmol of angiotensin II.

The samples of blood or tissue for analysis were not large enough to permit the use of aliquots for non-incubated control systems. The information sought was whether or not there was a significantly greater amount of renin in the blood or arterial wall of the hypertensive rats than in the normotensive control animals under the same conditions. The addition of ethylenediamine tetra-acetate ensures that the angiotensin I formed during the incubation will not be converted into any other angiotensin. The rapid pressor response when the angiotensin sample is injected is due to the instantaneous conversion of angiotensin I into angiotensin II. The results would not be affected by any angiotensin III formed in the test rat since its formation is slower than that of angiotensin II.

Renin activity in the plasma samples from the younger hypertensive rats and their control rats was determined as angiotensin I by a double-antibody radioimmunoassay with 125I-labelled angiotensin I (Clinical Assays, Cambridge, Mass., U.S.A.). The radioactivity was counted without transfer from the assay tubes in a solid crystal well counter (Picker). The highly specific antisera bound less than 1% of renin substrate and less than 0.1% of angiotensin I1. The limit of sensitivity was approximately 0-10 pmol. The radioimmunoassay could not be used for measurement of angiotensin in the arterial wall as a method in which exogenous substrate is not added does not produce enough angiotensin to be measured by this procedure.

The Mann--Whitney U test (Siegel, 1956) was used to determine the significance of the differences found because of the abnormally distributed data obtained in the study.

Results

Normotensive rats infused with renin plus saline had a significantly greater (P = 0.008) concentration of arterial wall renin than did normotensive rats infused with saline only (Fig. 1). The mean rise in blood pressure for the renin-infused rats was 85
FIG. 1. Infusion experiments with normotensive rats. Renin concentration in the arterial wall was measured as angiotensin II. Median values are indicated by lines on the graph.

FIG. 2. Endogenous renin concentration measured as angiotensin II in the arterial wall of normal (N), spontaneously hypertensive (SH) and renal hypertensive (acute, ARH and chronic, CRH) rats. Median values are indicated by lines on the graph.

FIG. 3. Endogenous renin concentration measured as angiotensin II in blood of normal (N) and spontaneously hypertensive (SH) older rats. Median values are indicated by lines on the graph.

mmHg compared with 10 mmHg for the saline-infused rats. The older SH rats showed a significant rise ($P < 0.05$) in the concentration of renin in the arterial wall, a highly significant fall ($P < 0.001$) in plasma renin in the renal vein and a small, but significant ($P < 0.05$), fall in plasma renin in the femoral artery compared with normal rats (Fig. 2 and Fig. 3). On the other hand, the younger SH rats had normal amounts of renin in the arterial wall ($P > 0.05$) and in the plasma of both the renal vein and the femoral artery ($P > 0.05$) (Fig. 2 and Fig. 4).

The acute renal hypertensive (ARH) rats showed a significant rise ($P < 0.01$) in the renin in the arterial wall and in the plasma renin in the femoral artery ($P < 0.01$), and a rise of lesser significance ($P < 0.05$) in the plasma renin in the renal vein (Fig. 2 and Fig. 4) compared with normal rats. But, although the chronic renal hypertensive (CRH) rats had normal or low plasma renin in the renal vein and femoral artery ($P > 0.05$), they had significantly elevated arterial wall renin ($P < 0.01$) (Fig. 2 and Fig. 4).
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Discussion

The concentration of circulating renin does not necessarily represent the effective concentration at the site of action, i.e. the arterial wall. We have demonstrated that renin infused into the normotensive rat in a quantity large enough to produce hypertension can at the same time raise the concentration of renin in the arterial wall. We also have shown that experimental renal hypertension in the rat is accompanied in the acute stage not only by elevated plasma renin activity, but also by an elevated concentration of renin in the arterial wall. Furthermore, our results show that as the hypertension becomes chronic, the concentration of renin in the arterial wall remains elevated as the plasma renin activity is reduced to normal or below. Thus, increased arterial wall renin is associated with elevated blood pressure in the acute stage of renal hypertension, and increased renin content may well continue to maintain hypertension in the chronic stage despite the fall in plasma renin at this stage.

Although our results for the young rats with spontaneous hypertension show no abnormal amount of renin in the circulation or in the arterial wall, the results for the older rats show that as the rats age circulating renin is depressed and arterial wall renin is elevated. The pattern of change resembles that in chronic renal hypertension. It was reported by Sweet, Columbo & Gaul (1976) that an intracerebroventricular injection of Sar\(^1\)-Ile\(^8\)-angiotensin II, an antagonist of angiotensin II, into SH rats lowered the mean arterial pressure in mature rats, but not in young rats.

It is well documented that benign experimental renal hypertension in both the rat and the dog can be divided into two phases: acute and chronic. There is general agreement that angiotensin is involved in the production of elevated blood pressure in the acute phase, but there has been considerable doubt expressed that it can be responsible for the elevated pressure in the chronic phase (Koletsky et al., 1967; Blair-West, Coghlan, Denton, Orchard, Scoggins & Wright, 1968; Pals, Masucci, Denning, Sipos & Fessler, 1971; Page, 1974; Hutchinson, Matthews, Dax & Johnston, 1975; Lohmeier & Davies, 1976; Masaki, Ferrario, Bumpus, Bravo & Khosla, 1977).

The inability of various workers to reduce elevated blood pressure in experimental chronic renal hypertension by the use of agents which block the renin–angiotensin system may lie in the length of the interval of observation after the administration of the blocking agent (cf. Pals et al., 1971, and Hill et al., 1970). Or it may be that a longer period of administration of the blocking agent is required. Riegger, Lever, Millar, Morton & Slack (1978) recently reported that prolonged infusion of either saralasin or converting enzyme inhibitor gradually corrects renal hypertension.

Our results show clearly that in acute two-kidney Goldblatt renal hypertension elevated circulating renin is accompanied by an increased concentration of renin in the arterial wall. They also show that in the chronic phase of this model circulating renin is depressed, whereas arterial wall renin is augmented as it is in the acute phase. Thus these results suggest a role of the renin–angiotensin system in the maintenance of chronic renal hypertension and possibly in spontaneous hypertension of long duration in older rats.

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References


