Hypertensive Vascular Disease Induced by Heterologous Renin

By Georges M. C. Masson, Ph.D., Chujiro Kashii, M.D., Masato Matsunaga, M.D., and Irvine H. Page, M.D.

Crude rat renin administered to uninephrectomized rats caused hypertensive vascular disease, while semipurified hog renin in equipressor doses, as determined by bio-assay, was ineffective. To explain this difference, the following three possibilities were considered. (1) The lack of activity of hog renin in rats is due to its heterologous nature. (2) Crude rat renin contains an active principle absent in the semipurified hog renin. Or (3), the impurities in crude preparations retard renin resorption from the subcutaneous injection sites and thereby prolong its effects. In the present paper we demonstrate that the third possibility provides the most likely explanation.

Methods

Female Sprague Dawley rats weighing 130 to 160 g were fed Purina fox chow and given tap water. They were individually housed in metabolism cages. Following a period of two to four days for control studies, they were uninephrectomized and treatment was started three to four hours later. Unless stated otherwise, materials under test were administered subcutaneously three times daily every eight hours. Blood pressure, body weight, and urine output were measured daily. Systolic blood pressure was determined by tail sphygmography once or twice a day prior to injections. Animals were killed on the twelfth day of treatment. From previous observations this period is amply sufficient to permit development of hypertension and vascular disease. Blood was drawn under ether anesthesia, ethylenediaminetetraacetic acid (EDTA), 3 to 5 x 10^-3 M, being added as an anticoagulant and angiotensinase inhibitor. After centrifugation in the cold, the cell-free plasma was kept in the freezer, then incubated at 37°C for two hours and assayed for pressor activity in pentolinium-treated rats anesthetized with amobarbitral (Sodium Amytal). Both incubated and nonincubated plasma samples were assayed against angiotensin standard and results expressed in nanograms (ng) per ml. At autopsy tissues were removed, weighed, examined for gross lesions, fixed in 10% formalin or in Zenker's fluid and stained with hematoxylin eosin, periodic acid fuchsin, or Sudan IV. Before fixation, a part of each kidney was kept frozen until determination of pressor activity. Saline extracts were prepared and assayed in rats which had been bilaterally nephrectomized 24 hours previously. The height of the pressure response was referred to a standard curve obtained with hog renin and results expressed in Goldblatt units per gram of tissue. The width of the zona glomerulosa was measured in micra with a contour projector from hematoxylin eosin sections passing through the center of the adrenals. Each value represents the average of four readings, one in each quadrant.

Preparations tested consisted of rat and hog renin of various degrees of purity. One renin preparation of porcine origin was obtained commercially from Princeton Laboratories, Inc., Princeton, New Jersey. It was prepared by alcohol and ammonium sulfate fractionation and contained 0.8 unit per mg. It will be referred to as semipurified hog renin. It was administered as a solution in saline, an oil suspension, a gel, or in the form of pellets. The oil suspension was prepared in a Potter homogenizer with peanut oil at the concentration of 100 mg per ml. The gel was prepared by dissolving renin in a saline solution containing 7% gelatin. Pellets were prepared with a hydraulic press following addition of 20 mg cholesterol per 100 mg of renin. The purpose of these three preparations was to delay the rate of absorption of renin.

Crude renin was extracted from rat and hog kidneys according to the procedure of Haas et al. Frozen kidneys were homogenized in a Waring blender; following acidification and centrifugation, the supernatant was fractionated with 2.2 M ammonium sulfate at pH 4.3. The resultant precipitate was suspended in distilled water, dialyzed for a period not exceeding 24 hours.
hours, centrifuged, and concentrated. All these operations were done at 0°C. Extraction of 20 kilos of rat kidneys yielded 2.3 units per gram of tissue as compared with 4.6 units for hog kidneys. All the renin preparations, including those obtained commercially, were assayed according to the same method used for determination of pressor activity in kidneys.5

Results

1. COMPARATIVE EFFECTS OF CRUDE HOG RENIN, CRUDE RAT RENIN, AND SEMIPURIFIED HOG RENIN

Rats were divided in four groups of twelve animals each and treated as follows: group 1 received crude hog renin; group 2, semipurified hog renin, and group 3, crude rat renin. Group 4 received injections of physiologic saline and was used as control. Renin was administered at the daily dose of about 80 units. From preliminary observations, the dose of 80 units was chosen as the one which would regularly cause severe hypertension within a period of 10 to 14 days. This short period of time seemed important if interference from antirenin formed against hog renin was to be avoided. Results are summarized in table 1.

Normal growth was slightly inhibited in the three experimental groups. The course of arterial pressure was similar in groups 1 and 3 treated with crude renin (fig. 1). Starting on the third day, systolic pressure rose gradually until the end of the experiment to reach values of 204 and 206 mm Hg, respectively; all rats except one had pressures of 200 mm Hg or more. In group 2, which re-

![Graph](image-url)

**Figure 1**

Curves of systolic blood pressures of rats injected with crude hog renin (1), semipurified hog renin (2), crude rat renin (3), and saline (4).

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>Crude hog renin</th>
<th>Semipurified hog renin</th>
<th>Crude rat renin</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>141 ± 8</td>
<td>139 ± 7</td>
<td>135 ± 15</td>
<td>141 ± 12</td>
</tr>
<tr>
<td>Initial</td>
<td>150 ± 11</td>
<td>163 ± 11</td>
<td>151 ± 11</td>
<td>179 ± 7</td>
</tr>
<tr>
<td>Final</td>
<td>404 ± 54</td>
<td>378 ± 21</td>
<td>406 ± 22</td>
<td>316 ± 12</td>
</tr>
<tr>
<td>Heart weight, mg/100 g body wt</td>
<td>41 ± 9</td>
<td>30 ± 18</td>
<td>32 ± 9</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>Urine flow, ml/24 hr</td>
<td>72 ± 7</td>
<td>59 ± 10</td>
<td>65 ± 7</td>
<td>44 ± 2</td>
</tr>
<tr>
<td>Zona glomerulosa, width in μ</td>
<td>0.9 ± 0.4</td>
<td>1.5 ± 0.8</td>
<td>0.69 ± 0.2</td>
<td>7.5 ± 3.4</td>
</tr>
<tr>
<td>Renal renin, units/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressor activity in plasma, ng/ml</td>
<td>1.52 ± 0.5</td>
<td>1.17 ± 0.84</td>
<td>1.35 ± 0.34</td>
<td>0.97 ± 0.09</td>
</tr>
<tr>
<td>Incubated</td>
<td>0.59 ± 0.25</td>
<td>0.38 ± 0.1</td>
<td>0.55 ± 0.32</td>
<td>0.47 ± 0.07</td>
</tr>
<tr>
<td>Nonincubated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>0.93 ± 0.43</td>
<td>0.78 ± 0.31</td>
<td>0.80 ± 0.12</td>
<td>0.49 ± 0.11</td>
</tr>
<tr>
<td>Vascular lesions, % incidence</td>
<td>66</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

*Results are expressed as mean values with standard deviations.

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received semipurified renin, arterial pressure remained below 120 mm Hg during the first five days, then varied between 120 and 137 mm Hg. These values are slightly, but significantly, above those of the control group. All these determinations were made six to eight hours after a renin injection. When the interval between injections and pressure measurements was lengthened to about ten hours, values were lower by about 30 mm Hg, thus indicating the importance of time in taking pressures and the adequacy of the eight-hour schedule of injections to produce a sustained pressor effect. Urine flow was increased to about the same degree in the three experimental groups: it averaged around 35 ml on the fourth day and stabilized around this level until the end. Heart weights paralleled changes in arterial pressure; they were markedly increased following treatment with crude renins, but less so following semipurified renin. P values were less than 0.01 in the three experimental groups.

The width of the zona glomerulosa was increased in the three experimental groups treated with renin. The difference between control and groups 1 and 3 treated with crude renins was highly significant (P < 0.01) and at the limit of significance (P = 0.05), for the groups on semipurified hog renin. Histologically the zona glomerulosa of the control group consisted of closely packed cells with little cytoplasm and few lipids; in the three renin-treated groups cells were enlarged, arranged in well-defined clusters, and loaded with lipids. Renal renin was significantly depressed in the three experimental groups. Pressor activity in plasma was about doubled by incubation; although values in rats treated with renin (groups 1 to 3) were slightly elevated over control values, differences were not significant except in group 1 where P was equal to 0.05, the limit of significance.

At autopsy, lesions were observed only in rats treated with crude hog and rat renins. Some of the kidneys were mottled with petechiae. Hearts had areas of necrosis and sometimes hemorrhages, chiefly in the right ventricular wall. Hepatic arteries where vascular lesions occur initially, were enlarged and covered with a gelatinous sleeve; occasionally, definite nodules of arteritis could be observed. Histologically the kidneys showed diffuse or granular deposits of hyalin material in the glomerular tuft, with occasional thrombi, thickening of membranes, tubular casts and arteriolar necrosis. Hearts showed focal necrosis of small arteries and large arterioles, and complete necrosis of arterioles of capillary size associated with extravasation and diffusion of plasma in surrounding tissues, necrosis of cardiac muscle fibers, hemorrhages and/or myocarditis. The branches of the hepatic arteries showed lesions of periarteritis nodosa at various stages of evolution, characteristic of hypertensive vascular disease in the rat. Clearly defined vascular lesions were observed in all 12 rats treated with crude rat renin and in 8 of 12 rats treated with crude hog renin; there were none in those which received semipurified hog renin.

II. EFFECTS OF LONG-ACTING RENIN PREPARATIONS

By demonstrating that crude hog renin was as effective as crude rat renin in causing hypertensive vascular disease, we eliminated the species-specific immunological reactions as the immediate cause for the relative lack of activity of semipurified hog renin. Thus, the two remaining possible explanations were: presence of another principle in crude extracts or retarded absorption of renin because of inactive proteins. Although rats treated with semipurified hog renin did not show vascular lesions, there was a slight and sustained rise in pressure. Therefore, instead of looking for a hypothetical substance, we decided to see whether this pressor effect could be intensified by addition of nonspecific materials, such as rat proteins, gelatin, oil, or cholesterol which would be expected to retard absorption from subcutaneous injection sites.

Rats were divided into five groups of six animals each and treated with semipurified hog renin modified as follows: group 1, hog renin plus rat liver proteins; group 2, hog renin in a gel; group 3, hog renin suspended
TABLE 2

Comparative Effects of Various Long-Acting Renins*

<table>
<thead>
<tr>
<th>Group and treatment</th>
<th>Renin and liver extracts</th>
<th>Renin in gelatin</th>
<th>Renin in oil</th>
<th>Renin pellets</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>143 ± 8</td>
<td>144 ± 9</td>
<td>145 ± 6</td>
<td>146 ± 6</td>
<td>144 ± 8</td>
</tr>
<tr>
<td>Final</td>
<td>138 ± 14</td>
<td>132 ± 8</td>
<td>131 ± 9</td>
<td>118 ± 9</td>
<td>179 ± 11</td>
</tr>
<tr>
<td>Heart weight, mg/100 g body wt</td>
<td>428 ± 21</td>
<td>516 ± 18</td>
<td>464 ± 41</td>
<td>551 ± 13</td>
<td>333 ± 13</td>
</tr>
<tr>
<td>Urine flow, ml/24 hr</td>
<td>51 ± 22</td>
<td>45 ± 7</td>
<td>26 ± 6</td>
<td>62 ± 12</td>
<td>10 ± 1.4</td>
</tr>
<tr>
<td>Zona glomerulosa, width in μ</td>
<td>71 ± 8</td>
<td>73 ± 8</td>
<td>70 ± 12</td>
<td>53 ± 6</td>
<td></td>
</tr>
<tr>
<td>Kidney weight, mg/100 g body wt</td>
<td>654 ± 24</td>
<td>651 ± 25</td>
<td>658 ± 25</td>
<td>757 ± 59</td>
<td>506 ± 41</td>
</tr>
<tr>
<td>Renal renin, units/g</td>
<td>1.6 ± .7</td>
<td>1.7 ± .8</td>
<td>1.0 ± .4</td>
<td>1.1 ± .2</td>
<td>5.8 ± 1.1</td>
</tr>
<tr>
<td>Pressor activity in plasma, ng/ml</td>
<td>12.6 ± 2.4</td>
<td>37.8 ± 37</td>
<td>18.7 ± 4.9</td>
<td>18.8 ± 10.8</td>
<td>6.9 ± .7</td>
</tr>
<tr>
<td>Incubated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonincubated</td>
<td>4.1 ± 1.7</td>
<td>7.8 ± 3.2</td>
<td>6.1 ± 1.2</td>
<td>9.2 ± 5.6</td>
<td>1.9 ± .7</td>
</tr>
<tr>
<td>Difference</td>
<td>8.5 ± 2.2</td>
<td>30 ± 26.1</td>
<td>12.6 ± 3.9</td>
<td>11.4 ± 3.4</td>
<td>5.0 ± .6</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>% incidence</td>
<td>83</td>
<td>83</td>
<td>66</td>
<td>83</td>
</tr>
</tbody>
</table>

*Results are expressed as mean values with standard deviations.

FIGURE 2

Curves of systolic blood pressures of rats injected with semipurified hog renin plus liver proteins (1), semipurified hog renin in a gel (2), suspended in oil (3), and in the form of pellets (4). Control rats received only liver proteins (5).

in oil; group 4, hog renin plus cholesterol in the form of pellets; group 5, which received only liver proteins, was used as control. The daily dose of renin, including that in pellets, was 80 units. A saline extract of rat liver was added to a solution of semipurified hog renin so that protein concentration, as in crude hog renin preparations, was equal to 3 g per 100 ml. Before injections, the renin gel was liquefied by heating at a temperature below 40°C. Pellets were implanted subcutaneously once a day under ether anesthesia. The animals were killed on the 12th day. Results are summarized in table 2.

Renin treatment caused loss of body weight in all groups. The progressive and severe cachexia in rats of group 4 which received implants can be attributed to the procedure, which produced an intense subcutaneous tissue reaction, as well as to the severity of hypertension. Diuresis occurred in all experimental groups and was associated with the known proteinuric effect of renin. Urinary proteins averaged about 2 g/liter as compared with 0.4 g/liter in the controls. Blood pressure changes are presented in figure 2. Again, after an initial period of two to three days, during which pressure showed variable changes, there was a steady increase to averages around 190 mm Hg. The subsequent decrease in groups...
renin and hypertension

1 and 2 which started on the 8th day, if real, may reflect antirenin formation. During the whole experiment, arterial pressure in the control group remained below 126 mm Hg. Heart weights were increased significantly in the experimental groups; the largest increase was found in the pellet-treated rats, which also showed the greatest rise of pressure. Kidneys were enlarged and almost depleted of renin. The width of the zona glomerulosa was increased. Pressor activity in plasma was at least doubled by incubation. Before as well as after incubation, values of pressor activity were larger in the four experimental groups than in the control group; the greatest difference was in group 1 and the smallest in group 2. One rat in group 2 had a value of 112 ng/ml after incubation; this rat had the highest pressure and the most extensive cardiac lesions. Vascular disease was present in all experimental groups. Incidence and severity were somewhat less in the group which received renin in oil suspension than in the other groups. The lesions, (fig. 3), being similar to those reported above there is no need to describe them.

Discussion

These experiments demonstrate that exogenous renin simulates the endocrine function of a kidney perfused under reduced pressure. When given to uninephrectomized rats, it causes not only hypertension and vascular disease, but also the specific changes seen in rats with unilateral clipping of the renal artery, i.e., nephrosclerosis and renin depletion in the remaining kidney as in the kidney contralateral to the clipped kidney,\textsuperscript{7} and stimulation of the zona glomerulosa.\textsuperscript{8}

They demonstrate further that hog renin is as active as rat renin. The absence of incompatibility is not unexpected, since hog renin in vitro reacts with rat renin-substrate and in vivo elicits most, if not all, the known pharmacologic effects (pressor, diuretic, proteinuric, corticotropic) of renin. One can assume, therefore, that renin from any origin will cause hypertension in a given animal species as long as an enzymatic reaction takes place between exogenous renin and the renin-substrate of the recipient animal. It is likely, however, that under continued treatment with a heterologous renin, the duration of hypertension will be limited by the formation of antirenin.\textsuperscript{9}

Another point apparent from these experiments is that a slow and sustained release of renin is necessary for the development of hypertension. Subcutaneous, as compared with intravenous, administration of semipurified renin, causes a sustained pressor effect,\textsuperscript{10} which is further amplified by addition of retarding substances. The significance of such a mechanism is supported by previous observations showing that bilateral nephrectomy or treatment with adrenal steroids, that are known to intensify and prolong the pressor effect of renin,\textsuperscript{10} permits the development of malignant hypertension in rats treated with semipurified hog renin.\textsuperscript{11,12} Thus, whatever the way by which it is produced, prolongation of action seems to be the factor which, under these experimental conditions, determines the ability of renin to cause hypertension.

The dose of renin injected may appear enormous when compared with the intravenous dose necessary to produce the same pressor response. However, if the daily dose of 80 units, usually administered subcutaneously, was infused over a 24-hour period, it would correspond to a rate of only about 0.05 unit per minute. Since some local inactivation may take place, the actual amounts released into the circulation are likely to be less than this value. Only a moderate increase in pressor activity was found in the plasma of renin-treated rats. Although a slow and sustained resorption of renin from subcutaneous tissue is equivalent to an intravenous infusion, the latter method of administration was only partially successful in reproducing hypertensive disease with homologous renin;\textsuperscript{13} the rise in pressure was moderate and not associated with vascular lesions. The use of normal, instead of uninephrectomized animals and the presence of vasoactive impurities in extracts may account for this discrepancy.

Whether hypertension and vascular disease
Vascular lesions in rats treated with semipurified hog renin in the form of pellets (A and B) and in a gel (C and D). PAS stain. A: renal glomerulus showing simplification and homogenisation of the glomerular tuft, thickening of membranes and thrombi. B: renal arteriole near its...
are due to renin or to some other renal principles has been a much debated subject, since Winternitz postulated the existence of pressor, necrotic, and hemorrhagic principles. More recently the possibility of a permeability factor different from renin has also been raised. With the availability of pure angiotensin, a growing body of evidence suggests that most, if not all, the pharmacologic properties of renin, including its ability to cause hypertensive vascular disease, are effected through angiotensin. Angiotensin elicits hypertension and stimulation of the zona glomerulosa in normal rats, rabbits, and dogs; and hypertensive vascular disease in desoxycorticosterone-treated or bilaterally nephrectomized rats, as well as in normal rats. Thus, one can assume that the effects described in this paper are due to renin and not to extra factors or impurities.

In attempting to define the mechanism involved in the development of renin-induced hypertension, it is logical to try to relate it first to the acute pressor effect of angiotensin; after an initial rise in pressure, each subsequent injection would contribute a small increment until hypertension becomes established. This simple explanation is not, however, supported by facts. In the present experiments, there was regularly a latent period of a few days during which time blood pressure remained within the normal range without any definite trend, then a gradual rise to hypertensive levels. Similar observations have been made in rats and rabbits treated with angiotensin. Because of the short half-life of angiotensin of less than 30 minutes, it is unlikely that there is a gradual accumulation of pressor materials. If the initial period of vascular refractoriness is due to renin tachyphylaxis, we have no explanation for its temporary nature in the presence of continued treatment, unless some superimposed factors intervene.

As an alternative, the existence of other mechanisms either endocrine or nervous has to be considered. Endocrine participation would occur through the adrenals. Renin or angiotensin, even in suppressor doses, is associated with an increase of aldosterone secretion; aldosterone causes hypertension; aldosterone and renin are elevated during the malignant phase of clinical hypertension.

In spite of this supporting evidence, there is, however, some doubt that aldosterone is a determining factor, because rats that were treated with semipurified renin, and that remained normotensive, showed stimulation of the zona glomerulosa like those which became hypertensive.

Apart from its direct vasoconstrictor effect, angiotensin elicits vascular effects which are mediated through the nervous system. Of particular significance, is the observation that dogs with early renal hypertension, like dogs infused with angiotensin, show an enhanced response to tyramine and that this response is not altered by angiotensin tachyphylaxis.

Consistent with these facts, we would like to suggest the following working hypothesis. Angiotensin elicits two types of pressor effects: one direct and the other nervously mediated. The first effect occurs immediately and requires relatively high doses of angiotensin; the second is delayed and requires smaller doses which are not necessarily acutely pressor. The first effect is not a prerequisite for the second. On this basis the development of renal hypertension would proceed as follows: after compression of the renal artery there is an almost instantaneous increase of renin secretion and an acute elevation of arterial pressure, which subsides partially when renal perfusion pressure tends to increase and renin secretion to decrease. From the time of clamping, the angiotensin liberated initiates nervous mechanisms, which gradually become sufficiently intense to raise the blood pressure further and to establish hypertension. Thus, we would have an acute component and a delayed component. A small but sustained
of renin secretion sufficient to initiate the delayed component would cause benign renal hypertension. On the other hand, if a strong acute pressor component adds its effects to those of the delayed component, malignant hypertension would ensue.

By postulating the existence of two separate pressor components acting through different mechanisms, one can explain why tachyphylaxis prevents the acute pressor effects which occur normally during renin infusion or after release of a complete obstruction of a renal artery, but does not affect blood pressure in hypertensive animals while antirenin, which blocks all the renin effects, lowers blood pressure to normal levels. This hypothesis is presented not as a substitute but as a complement to others. Were the mechanisms suggested proved to be real, they would represent another facet of a pathogenesis which is not only complex but also changing as hypertension develops.

Summary

Chronic treatment with crude hog, or rat, renin administered subcutaneously to uninephrectomized rats elicits hypertension, renal and vascular lesions, hypertrophy of the zona glomerulosa of the adrenals, and renin depletion in the remaining kidney. Semipurified hog renin given in equipressor doses also causes renin depletion and stimulation of the zona glomerulosa, but no hypertension nor vascular disease. However, semipurified hog renin becomes as effective as the crude preparation after addition of retarding substances such as gelatin, oil, or cholesterol. Thus, a prolonged and sustained resorption of renin seems to be a prerequisite for the development of hypertension. Since hypertension takes place only after a latent period during which arterial pressure remains within the normal range, it does not appear that hypertension can be attributed to the acute pressor effect of renin. It is proposed as a working hypothesis that renin, through angiotensin, initiates nervous mechanisms which are responsible for the development of benign hypertension and that the acute pressor effect of angiotensin is more specifically associated with the malignant phase of hypertension.

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