Studies on Renin Antibodies

By Oscar M. Helmer, Ph.D.

Intramuscular injection of various kidney extracts containing renin in several animal species caused the appearance in sera of a substance which neutralized renin. The antirenin titers of the antisera were determined by assaying the quantity of unneutralized renin. The assay was based on the production of angiotonin. The dog differs from the other species studied in that heterologous kidney extracts cause a production of antibodies against the treated animal's own renin.

Our interest in antirenin came about because of studies on the nature of the sustained pressor substance.\(^1\) We found this substance was derived from the kidney and was a protein. Consequently, we attempted to prepare antibodies to it in the same manner that Wakerlin and his associates\(^4\) had done with renin. We found that the sera of rabbits that had been injected intraperitoneally with a cat kidney extract over a period of weeks would neutralize the sustained pressor material\(^5\) in vitro or in vivo.

The antisera contained antibodies for renin as well as for sustained pressor substance and in approximately equal amounts. Since it appeared that it might be difficult to quantitate the titer of the antisera against sustained pressor principle, the antirenin titers were determined by a quantitative method based upon the production of angiotonin by the unneutralized renin.

An outline of the procedure is shown in table 1. A hog renin powder assayed by Goldblatt's method\(^6\) was used as a standard. Renins prepared from other species were also compared to this standard, although it was realized that there are quantitative differences between the renins obtained from different species when reacting with heterologous renin substrates (hypertensinogen). For example, cat renin incubated with cat plasma will form 3 times as much angiotonin as it does when it is allowed to react with hog or dog plasma. Hog and dog renin produce about the same amount of angiotonin whether incubated with hog or dog substrate. The angiotonin was assayed in pithed cats that had been nephrectomized 1 or 2 days previously. Before the antisera were assayed, angiotonase was destroyed in the serum by subjecting it to a pH of 3.9 at 37 C. for 20 minutes. The antirenin titer was expressed in Goldblatt units per milliliter of plasma.

We agree with Lamfrom, Hass, and Goldblatt\(^7\) who state: "Aside from its possible significance as a possible therapeutic method for the treatment of hypertension, the renin-antirenin reaction seems well suited to serve as a model for the study of immunological phenomena in general." For example, it is possible to study the time required for the combination of the antibody with the renin. As shown in figure 1, when an amount of antirenin in excess of what is needed to completely inactivate a standard amount of renin is used, the neutralization occurs almost instantly. When less than maximal amounts are used the reaction requires 30 to 40 minutes for completion. We have checked the method discussed in this paper with the direct determination of the pressor activity of the unneutralized renin and have found good agreement.

Production of Antirenin in Dogs with Experimental Hypertension

Five dogs with hypertension of the Goldblatt type were injected with kidney extracts intramuscularly 3 to 4 times a week for a period of a few weeks to 5 years. Different renin preparations were used. In the 2 dogs

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Part of the work presented in this paper was done with my colleagues, Drs. Shipley and Kohlstaedt.
STUDIES ON RENIN ANTIBODIES

<table>
<thead>
<tr>
<th>Renin—0.07 G.U.</th>
<th>Inub.</th>
<th>Renin</th>
<th>Incub.</th>
<th>Acidify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Serum</td>
<td>Room temperature—30'</td>
<td>+ Substrate</td>
<td>37°—15'</td>
<td>Boil</td>
</tr>
<tr>
<td>Anti-Serum</td>
<td>Room temperature—30'</td>
<td>+ Substrate</td>
<td>37°—15'</td>
<td>Boil</td>
</tr>
</tbody>
</table>

TABLE 1.—Outline of Antirenin Assay

in which antirenin was produced, the kidney extracts averaged about 8 Goldblatt units per milligram of nitrogen. In both of these dogs, mean blood pressures were reduced to normal levels. In the hypertensive dogs in which antirenin could not be demonstrated in the plasma, no fall in blood pressure occurred.

Figure 2 shows in detail the effect of injecting kidney extracts containing renin into a dog with experimental hypertension produced by wrapping both kidneys in dry silk by the method described by Page. This dog had mean arterial pressures in the hypertensive range for 4 months before injection of the renin preparation began. After 4 weeks of treatment with kidney extract, antibodies to renin appeared in the plasma. The lowest blood pressure reading occurred at 7 months, a drop in average mean arterial pressures of 58 mm. Hg. The highest antirenin titer in the first course of treatment was 84 Goldblatt units per 100 ml. When the injections were stopped, the antirenin titer dropped to 7 Goldblatt units per 100 ml. with a gradual rise to an average mean pressure of 180 mm. over a period of 5½ months.

A second course of kidney extract containing renin was now started. In contrast to the first course, the blood pressure dropped precipitately, reaching an average level of 143 mm. in 2 weeks and 134 mm. in the next 2 weeks. There was also a correspondingly greater elevation in the antirenin titer. Since our interest was in correlating blood pressure changes with antirenin titer rather than obtaining the highest titers possible, the injections were again discontinued when the blood pressure had stabilized at near normotensive levels for 2½ months and when the antirenin titer had reached 336 Goldblatt units per 100 ml. Further observations in this experiment are shown in figure 2. Six months after stopping the third course of kidney extract injections, the mean pressure rose suddenly to levels of over 200 mm. Hg. After 4 months of elevated pressure, the animal died, more than 5 years after kidney extract was first injected. Much of the time its blood pressure had been maintained at near normal levels by means of the injections.

With a second dog, the results were so similar that the details of its course will not be discussed. Kidney extracts were started in this animal 22 months after the development of hypertension. The monthly average of mean blood pressures varied between 200 and 255 mm. before treatment with a renin preparation was started. The lowest pressure obtained during the first course of injections was an average of 156 mm. over a 2 week period with a range of 145 to 175. This was accompanied by an antirenin titer of 180

Fig. 1. Rate of neutralization of renin by antirenin; ordinate, unneutralized renin; abscissa, time in minutes.
Goldblatt units per 100 ml. During a second course of kidney extract injections, the mean blood pressure dropped to an average of 133 mm. Hg. over a 2 week period. Blood pressure values were maintained near this level for 10 months. When the treatment was stopped, the mean arterial pressure gradually rose; the level being 170 mm. when the experiment was terminated, 5 years after its start.

**Treatment of Hypertensive Rats with Kidney Extracts Containing Renin**

Hypertension was produced by constricting both renal arteries of rats of the Long-Evans strain with silk thread. For the production of renin antibodies, 8 rats were injected subcutaneously 3 times a week with a kidney extract equivalent to 14 to 58 Gm. of hog kidney. The shortest time for which an extract was injected was 15 weeks, the longest 30 weeks. For the assay of the renin antibodies, blood was obtained from the tail vein. The lowest antirenin titer was 70 Goldblatt units per 100 ml. against hog renin, the highest 890 Goldblatt units. The antisera also neutralized dog, cat, and rabbit renin. With one antisera, there was an insignificant antirenin titer of 3 Goldblatt units per 100 ml. against rat renin. The others showed no antibody against rat renin. In none of the 8 hypertensive rats was there a significant lowering of blood pressure.

This is in direct contrast, as shown in figure 3, to the lowering in blood pressure produced in 9 hypertensive rats by the daily subcutaneous injection of kidney extract derived from 150 Gm. of hog kidney. There was a prompt fall in pressure which was maintained for 30 days, and a prompt rise in pressure when the kidney extract was stopped.
TABLE 2.—Summary of Antirenin Studies in Various Species

<table>
<thead>
<tr>
<th>Renin into animal</th>
<th>Hog</th>
<th>Dog</th>
<th>Rabbit</th>
<th>Cat</th>
<th>Sheep</th>
<th>Horse</th>
<th>Rat</th>
<th>Human</th>
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</thead>
<tbody>
<tr>
<td>Hog → human</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Hog → dog</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cat → rabbit</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rat → rabbit</td>
<td>+</td>
<td>+</td>
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The fall was not due to the production of rat antirenin. No antirenin was found in the plasma of 2 of the rats after 7 days of treatment. When compared to the dog data, the lowering of blood pressure caused by the in vivo formation of antirenin requires a longer time and the elevation of pressure after the cessation of the injections occurs gradually. The chief difference in the kidney extracts administered to the 2 groups of rats aside from the greater dose in terms of original kidney tissue was that in the antirenin experiments the proteins containing the angiotonase (hypertensinase) were removed by a treatment with trichloracetic acid at pH 3.9 in the presence of 5 per cent NaCl. These data indicate that the kidney has a factor or factors, albeit in small quantities, that can lower the blood pressure in hypertensive animals.

Formation of Antirenin in Man

Hog renin was injected intramuscularly 3 times a week in 11 patients with hypertension. The total weekly dose was 400 Goldblatt units. All patients developed plasma antirenin to hog renin. As in the rats, there was a considerable variation in these patients. The antirenin titers varied from 70 to 1050 Goldblatt units per 100 ml. As in the dog experiments, the highest titers were obtained when a second course of kidney extract was given to 3 patients who had been treated with kidney extracts 5 to 6 years before. In all of these patients plasma still contained antirenin to hog renin. The exact titers were not determined, but were greater than 14 Goldblatt units per 100 ml. In contrast to the slow increase in antirenin titer in the other 8 patients, the titer in these 3 patients showed a sharp rise in the first assay done after the beginning of treatment (3 to 4 weeks). Continued administration of the renin preparation did not increase the titer. Even though human antisera with high titers neutralized renins from all animals investigated—hog, dog, rabbit, cat, sheep, horse, and rat—no antibodies could be detected to human renin. There was no significant lowering of blood pressure in any of the patients. These data are in agreement with those reported by Goldblatt, Hass, and Lam from* for the injection of hog renin into patients with hypertension.
In table 2 is summarized the results of injecting resin preparations in man, rat, dog, and rabbit. Of the species studied, only in the dog was it possible by the injection of heterologous resin to produce antirenin that neutralized its own renin. In no species were antibodies to human renin induced.

SUMMARY
With the appearance of antirenin to dog reninin the sera of dogs with experimental hypertension of the Goldblatt type, there was a coincidental lowering of arterial pressure in both the acute and chronic stages, indicating that renin plays an important role in both early and late experimental hypertension.

REFERENCES
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