Radioimmunoassay for plasma renin activity

Manjula S. Kumar, Ph.D.*

Division of Research

Sharad D. Deodhar, M.D., Ph.D.

Section on Immunopathology

The role of the kidney in the regulation of blood pressure through the secretion of proteolytic enzyme renin was first suggested by Goldblatt et al[1] in 1934. Since then, the interrelations of the different components of the renin-angiotensin system have become known, and it is now clear that renin is not only involved in homeostatic control mechanisms of blood pressure, but also plays an important role in the pathogenesis of certain hypertensive diseases. As a result of more recent observations, it also appears that the renin-angiotensin system is one of the important regulators of aldosterone secretion. Because of this, quantitative laboratory renin determinations of peripheral and renal venous blood have become important in the diagnosis of hypertension. The biological activity of this system involves the enzymatic cleavage of a plasma substrate protein by renin to release a decapeptide angiotensin I, which is converted to octapeptide angiotensin II by the "converting" enzyme during the pulmonary circulation. The latter is not only a highly active pressor agent, but also a major stimulus to aldosterone secretion and, as such, is thought to be the mediator of most of the biologic properties of renin. In the past, quantitative renin determinations were performed only by bioassay procedures. Renin was allowed to interact with its substrate to produce angiotensin II, which then was

* *Fellow, Division of Research.*

assayed through its pressor effect in an experimental animal. These bioassays are cumbersome, technically difficult, time-consuming, and relatively insensitive. Also, variations in each experimental animal and in the ability of the technologists to handle these animals have often made these procedures difficult to reproduce. Radioimmunoassay procedures have been applied to this problem, and the recent development of radioimmunoassay for angiotensin I[2-4] allows the direct measurement of renin activity. The purpose of this study was to compare the bioassay and radioimmunoassay for renin in a large number of samples, and to study the various conditions in the radioimmunoassay and its application to clinical studies of hypertensive patients.

Materials and methods

Antiserum to angiotensin I was produced in rabbits by first coupling it to a carrier protein, bovine serum albumin (BSA), by the carbodiimide reaction and then injecting 2 mg of the antigen mixed with complete Freund's adjuvant.[5] The rabbits were given booster injections of the antigen every 15 days until a suitable antibody titer developed. Angiotensin I antiserum can also be produced by coupling it to poly-L-lysine HCl via its carboxyl terminal phenylalanine.[6] More recently antisera produced by the latter method have been obtained from other sources* and gave similar results.

The purity of labeled angiotensin I, ^{125}I (Schwarz/Mann) was checked by paper electrophoresis which showed a single peak.

* Schwarz/Mann, Orangeburg, New York, and E. R. Squibb & Sons, New Brunswick, New Jersey.

Bioassay. A modified method by Pickens et al[7] was used for bioassay. Ten to 20 ml of blood was drawn with di-sodium ethylenediamine tetraacetic acid (EDTA) (1 mg/ml), and the plasma was dialyzed against distilled water for 24 hours at 4 C and then incubated for 4 hours with di-isopropylfluorophosphate (1 drop/5 ml) at pH 5.5. The mixture was heated to boiling for 10 minutes to precipitate the proteins, then centrifuged, and then supernatant was separated and evaporated to dryness. The residue was dissolved in saline solution to 1/20th of volume before evaporation. Its pressor activity was assayed in pentolinium-treated rats by intravenous injection and observing the rise in mean arterial pressure.

Radioimmunoassay. Five to 10 ml of blood was drawn into a prechilled vacutainer containing EDTA (1 mg/ml) and immediately centrifuged at 2 C. To 2 ml of this plasma were added 5 μl of dimercaptol in peanut oil (0.8 M) and 20 μl of 8-hydroxyquinolin sulfate solution (0.34 M) to inhibit the converting enzyme. Plasma was frozen until used. Each sample was thawed on ice or at 4 C and separated into two equal parts. One part was refrigerated and used as a control for endogenous angiotensin; the other part was incubated at 37 C for 3 hours to allow the renin to react on plasma substrate to produce angiotensin I. Fifty microliters of each sample was added to 0.9 ml of tris acetate buffer (0.1 M, pH 7.5 with BSA 2.5 mg/ml). Fifty microliters of diluted antiserum and 50 μl of diluted labeled angiotensin I (5,000 cpm, specific activity 400–500 mc/mg) were added with the dilutions, suitable to bind approximately 50% of the labeled antigen. A

separate set of standards of angiotensin I with the same incubation volume was prepared, varying from 0.05 ng/ml to 0.6 ng/ml. Each sample was tested in duplicate and incubated for 24 hours at 4 C. Plastic tubes (12 × 75 mm) were used in the assay since in glass tubes there can be absorption of the angiotensin on the wall.

The separation of bound and unbound labeled angiotensin I was achieved by the use of dextran-coated charcoal solution.[8] Dextran T-70* 0.5 g was dissolved in 100 ml of barbital buffer (0.1 M, pH 7.4) and 5 g of charcoal† suspended in 100 ml of barbital buffer were mixed together. One milliliter of this solution was added to each tube, then immediately mixed on a vortex mixer and centrifuged at 4 C for 10 minutes at 2,000 to 3,000 rpm. The supernatant was transferred to counting vials and both the charcoal residue and the supernatant were counted in a β/γ liquimat scintillation counter.‡ The ratio of free and bound counts (F/B) was calculated for each tube. The values of the unknown were read on a standard curve, the corrections were made by subtracting the value of the serum held at the 4 C incubation from that of the 37 C, and the renin activity was calculated as angiotensin I released per 1 ml/hr.

Renin activity

$$= \frac{(\text{ng } 37\text{ C} - \text{ng } 4\text{ C})}{3} 20$$

$$= \text{ng/ml/hr}$$

* *Pharmacia, Uppsala, Sweden.*
† *Norit "A", Neutral, Amend Drug and Chemical Company, New York, New York.*
‡ *Picker Nuclear, White Plains, New York.*

Results

Immunization. Two rabbits were immunized. Both produced antisera of good potency, and after seven injections (14 weeks) the antiserum at dilution 1:2,000 was able to bind 45% and 50% of 20 pg of labeled antigen. On further immunization (16 weeks), the antiserum from rabbit 2 at a dilution of 1:8,000 was able to bind 40% of 20 pg of labeled angiotensin I (*Fig. 1*).

Specificity of antiserum. The specificity of the antiserum was tested against octapeptide angiotensin II for its ability to compete with labeled angiotensin I for binding with the antibody. No cross reaction was noted (*Fig. 2*).

Reproducibility. Reproducibility of the radioimmunoassay was tested. A

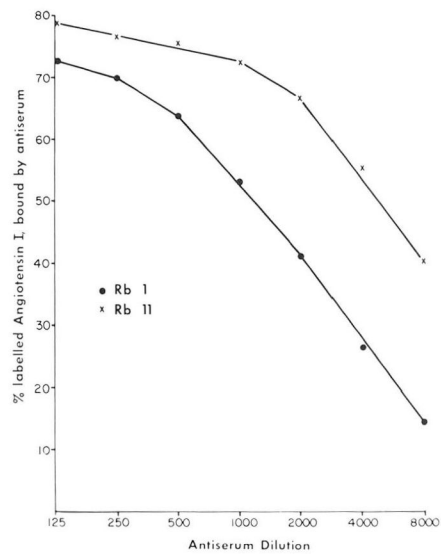

Fig. 1. Antibody titration curves. Serial dilutions of antisera from rabbits immunized with angiotensin I (Isoleucine-5) were incubated at 4 C for 24 hours with labeled angiotensin I (^{125}I). Binding is progressively decreased by dilution of the antiserum, with rabbit 1 sharing 40% binding at 1:2,000 and rabbit 2 at 1:8,000 dilution.

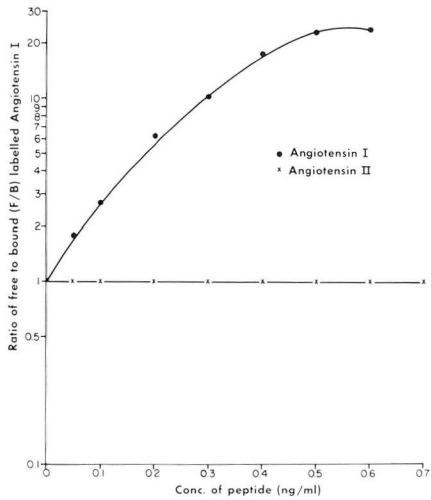

Fig. 2. Lack of cross reaction of antiserum to angiotensin I with angiotensin II. Varying amounts of unlabeled angiotensin I and angiotensin II were incubated for 24 hours at 4 C with labeled angiotensin I and antiserum to angiotensin I. Angiotensin II shows no cross reaction with the antiserum.

low renin level plasma and a high renin level plasma were tested 16 different times. *Table 1* shows the actual values obtained and the standard deviation.

Effect of plasma proteins. To rule out the possibility that plasma proteins may interfere in the radioimmunoassay, two standard curves were run, one in tris buffer only, and the second in normal human plasma at 1:20 dilution as used in the radioimmunoassay. The two curves were similar (*Fig. 3*), indicating that plasma proteins at 1:20 dilution do not interfere in the assay; thus avoiding the need for separation of angiotensin I from plasma proteins. If known amounts of angiotensin I were added to plasma samples, recovery was 90% to 100%, indicating lack of interference from plasma components under the conditions of the assay. Also, correction was made for each sample by subtracting the value obtained for the unincubated sample from that for the incubated sample.

Table 1. Reproducibility of measurements of low renin level and high renin level plasma

Low renin level plasma angiotensin I, ng/ml/hr	High renin level plasma angiotensin I, ng/ml/hr
0.99	32.5
1.2	31.5
1.0	33
1.3	31.3
1.5	29.3
2.0	31.5
1.8	33.3
1.7	35.3
1.5	28.8
1.1	29.2
1.2	27.3
1.9	28.1
2.0	26.4
2.1	28.4
2.0	28.9
1.4	28.0
Mean = 1.54 ± 0.39 (S.D.)	Mean = 30.1 ± 2.5 (S.D.)

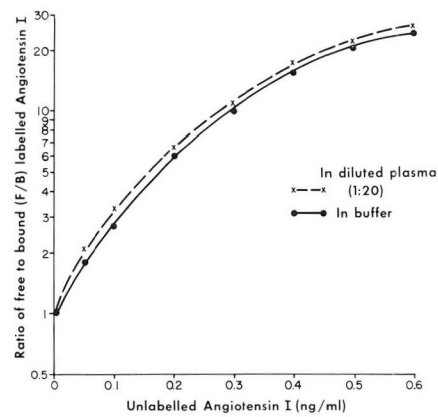

Fig. 3. Standard curves for radioimmunoassay of angiotensin I in buffer only and in diluted plasma.

Rate of formation of angiotensin I. Plasma samples from a normal person, from hypertensive patients, one with renal artery disease, and a patient with primary hyperaldosteronism, were incubated at 37 C for 1 to 8 hours to study the rate of generation of angiotensin I. The generation curve for angiotensin I in normal plasma was linear up to 8 hours, but in the plasma of the hypertensive patient with renal artery disease, generation of angiotensin I was linear only up to 4 hours (*Fig. 4*). In the plasma of the patient with primary hyperaldosteronism, no generation of angiotensin I was detectable.

Effect of pH on generation of angiotensin I. The maximum generation of angiotensin I was noted at pH 5.5 (*Fig. 5*). Generation of angiotensin I at pH 5.5 was studied in four normal persons and in four patients with primary hyperaldosteronism. In normal persons the generation was linear, but in patients with primary hyperaldosteronism the maximum angiotensin was generated only within 15 minutes and no linear relationship was observed (*Fig. 6*). This observation has practical significance in the distinction of patients with primary hyperaldosteronism from patients with low normal renin values.

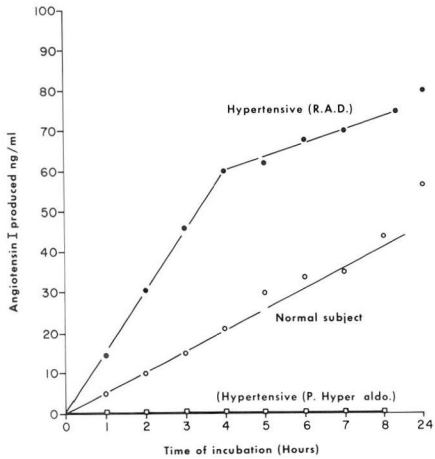

Fig. 4. Rate of angiotensin I production in normotensive and hypertensive subjects (R.A.D. = renal artery disease, P. Hyperaldo. = primary hyperaldosteronism). Plasma samples were incubated for 24 hours and aliquots were taken out for radioimmunoassay at 1 hour intervals up to 8 hours.

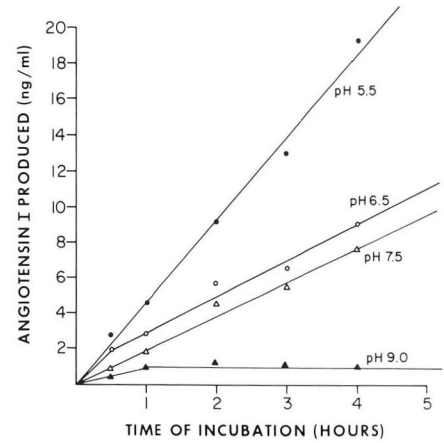

Fig. 5. Effects of pH on generation of angiotensin I in normal plasma.

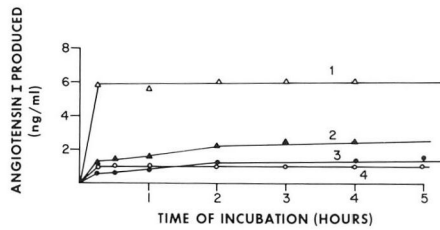

Fig. 6. Generation of angiotensin I at pH 5.5 in 4 patients with primary hyperaldosteronism.

Comparison of bioassay and radioimmunoassay. A total of 103 plasma samples from different patients were assayed by bioassay as well as by radioimmunoassay. Plasma was divided into two parts and was tested separately by the two methods. In this study the value of t for 103 samples was 0.3381, indicating that there is no significant

difference in the values obtained from the two methods. *Figure 7* shows the relationship between the values obtained from bioassay and radioimmunoassay, and it is apparent that for most samples the points representing the relationship between the results of the two assays are within close range of the line of identity.

Normal values. Plasma renin levels were determined on 31 normal subjects (8 men and 23 women) on unrestricted diets. The women were not taking oral contraceptives. Blood was drawn in the morning, after overnight fasting and 2 hours of upright activity. The plasma renin activity ranged from 0.1 ng/ml/hr to 3.5 ng/ml/hr, with an average level of 1.35 ± 0.91 ng/ml/hr. In 20 normal persons (6 men and 14 women) renin activity in supine position after 10 minutes rest was 0.82 ± 0.69 and after 1 hour rest, 0.53 ± 0.403 ng/ml/hr.

Renin levels in patients with various kinds of hypertension. In 10 hypertensive patients whose renal angiographic studies showed significant renal artery disease, renal venous blood samples (right and left) were studied for renin activity. As shown in *Table 2,* significant levels were noted in all these cases and in four cases (cases 2, 3, 5, and 9), the renin levels on the side of the more severe renal artery lesion were significantly greater than levels from samples on the opposite side with less severe or no disease.

In 16 patients with essential hypertension, the plasma renin activity ranged from 0.06 to 0.7 ng/ml/hr with a mean value of 0.27 ± 0.18 ng/ml/hr; whereas in five patients with primary hyperaldosteronism, it ranged from

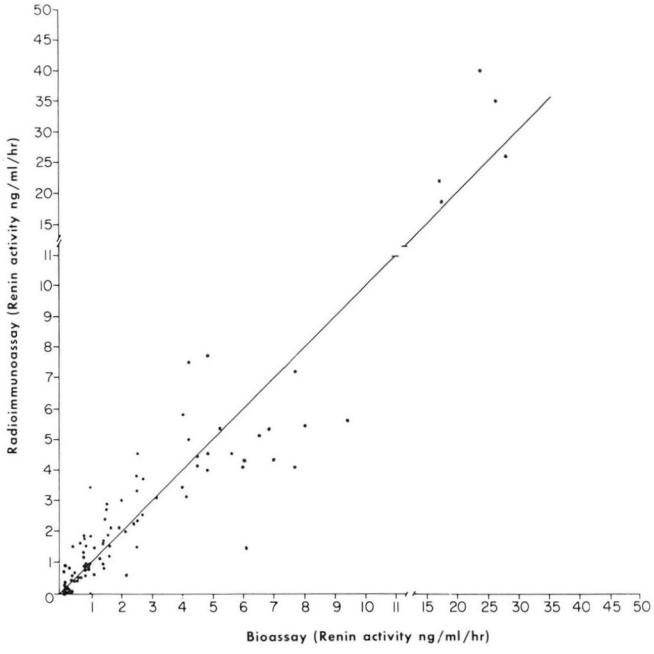

Fig. 7. Relationship of renin activity as measured by radioimmunoassay and bioassay in 103 specimens.

Table 2. Results on patients with renal artery stenosis

Case no.	Clinical data	Plasma renin activity—angiotensin I, ng/ml/hr	
		Right renal vein	Left renal vein
1	90% stenosis—right renal artery; narrowing of multiple branches involving left kidney	40.0	35.0
2	95% stenosis—left renal artery; 50% stenosis—right renal artery	14.2	23.3
3	30% stenosis—right renal artery; 95% stenosis—left renal artery	13.5	25.9
4	Aneurysm in right renal artery	19.3	11.8
5	Renal artery sclerosis, left greater than right	13.3	19.6
6	Left main renal artery—90% stenosis; right main artery—25%; right posterior branch—25%	47.45	36.8
7	Slight atherosclerotic disease of the abdominal aorta; minimal bilateral medial fibroplasia of renal arteries	10.6	11.0
8	Bilateral medial fibroplasia of renal arteries	15.0	14.6
9	Bilateral medial fibroplasia more severe on right than left	160.0	38.7
10	Renal artery stenosis left greater than right	10.3	16.0

less than .06 to 0.5 ng/ml/hr with an average level of 0.17 ± 0.18 ng/ml/hr.

Discussion

It is generally accepted that the renin angiotensin system plays an important part in renal hypertension in experimental animals and also in some forms of human hypertension, especially that associated with renal arterial disease. It also plays a key role in the normal regulation of salt and water metabolism through its control of aldosterone secretion by the adrenal cortex. Thus, on a clinical level, the laboratory determinations of renin have become important in studying patients with hypertension due to renal artery disease or aldosterone hypersecretion. The clinical distinction between primary and secondary hyperaldosteronism often depends on renin determinations. Both entities are characterized by elevated aldosterone levels. In primary hyperaldosteronism caused by adrenal cortical adenoma or hyperplasia, renin activity is depressed, but in secondary hyperaldosteronism caused by renal artery stenosis, elevated plasma renin activity is observed. In the study of patients with known renal artery disease, renin determinations of both renal venous samples are often useful and, in the small number of patients we studied, there was a good correlation between renin level and the degree of vascular disease. In patients with essential hypertension, renin levels were significantly lower ($p < .005$) than those in normotensive individuals.

Page et al[9] have identified two proteins in human plasma which interfere in the immunoassay of angiotensin I and angiotensin II. But for the determination of renin activity by the immunoassay of angiotensin I, generated at 37 C incubation, plasma was used at 1:20 or more dilution, and at this dilution we found that plasma pro-

teins did not interfere. Also, correction for the presence of "angiotensin I-like protein" was made by subtracting the value obtained in the unincubated plasma from that for the incubated sample. The 90% to 100% recovery of added angiotensin I in plasma samples also indicates the specificity of the system and lack of interference from plasma components under the assay conditions.

Sealey et al[10] reported significantly higher results by radioimmunoassay than those obtained by bioassay using angiotensin I or angiotensin II as standards. In contrast, in our studies excellent correlation was observed in the values obtained by the two methods. The main difference in their technique and the technique we used was the incubation pH for the radioimmunoassay. We used plasma pH 7.4 for incubation; they used pH 5.7. At the lower pH of incubation, more angiotensin I is generated and at a more rapid rate, than is generated at pH 7.4 (*Fig. 5*). This may explain the difference between our results and those of Sealey et al.[10]

This radioimmunoassay procedure is a significant advance over the previous bioassay procedures for renin determination. The advantages are apparent in sensitivity, specificity, reproducibility of technique, objectivity, and performance of large numbers of tests. The good correlation that we observed between radioimmunoassay and bioassay provides additional assurance that the results by the former procedure are also physiologically significant.

Summary

Our experience with the radioimmunoassay of angiotensin I for the measurement of plasma renin activity was found to be sensitive and reproducible, and required only 50 μl of plasma. At the dilution used in the assay, plasma proteins did not interfere. Comparable values were obtained by radioimmunoassay and bioassay. In 10 patients with hypertension due to renal artery stenosis, a good correlation was observed between renin levels and the degree of vascular disease indicated by renal angiography.

Acknowledgments

Synthetic angiotensin I (Isoleucine[5]) was supplied by Dr. F. Merlin Bumpus of the Research Division. We thank Mr. James Kolar for his technical assistance.

References

1. Goldblatt H, Lynch J, Hanzal RF, et al: Studies on experimental hypertension; production of persistent elevation of systolic blood pressure by means of renal ischemia. J Exp Med **59:** 347–379, 1934.
2. Boyd GW, Adamson AR, Fitz AE, et al: Radioimmunoassay determination of plasma renin activity. Lancet **1:** 213–218, 1969.
3. Goodfriend TL, Ball D: Radioimmunoassay of angiotensin and measurement of renin activity. (Abstr) J Lab Clin Med **70:** 884, 1967.
4. Haber E, Kroener T, Page LB: Immunoassay for angiotensin I and its application to a determination of renin activity. (Abstr) Circulation **38:** (suppl VI): 91, 1968.
5. Goodfriend TL, Levine L, Fasman GD: Antibodies to bradykinin and angiotensin; a use of carbodiimides in immunology. Science **144:** 1344–1346, 1964.
6. Haber E, Page LB, Jacoby GA: Synthesis of antigenic branch chain copolymers of angiotensin and poly-L-lysine. Biochemistry **4:** 693–698, 1965.
7. Pickens PT, Bumpus FM, Lloyd AM, et al: Measurement of renin activity in human plasma. Circ Res **17:** 438–448, 1965.

8. Herbert V, Lau KS, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol Metab **25:** 1375–1384, 1965.
9. Page LB, Dessaulles E, Lagg S, et al: Interference with immunoassays of angiotensin I and II by proteins in human plasma. Clin Chim Acta **34:** 55–62, 1971.
10. Sealey JE, Gerten-Banes J, Laragh JH: The renin system: variations in man measured by radioimmunoassay or bioassay. Kidney Int **1:** 240–253, 1972.