DETERMINATION OF PLASMA RENIN ACTIVITY BY RADIOIMMUNOASSAY OF ANGIOTENSIN I

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SUMMARY

1. A simple, rapid radioimmunoassay of angiotensin I has been applied to the measurement of plasma renin activity.
2. Antibody to angiotensin I was raised in rabbits by injecting angiotensin I conjugated with rabbit serum albumin.
3. Angiotensin I was generated in plasma by 3 h incubation at 37°C and pH 5.5 after adding EDTA and di-isopropylfluorophosphate (DFP).
4. The simple procedure of boiling for 10 min was performed to eliminate the inhibitory effect of plasma protein on immunoassay. After centrifugation, the supernatant was incubated for 18 h with 131I-labelled angiotensin I and antiserum. Free fractions of 131I-labelled angiotensin I were separated using dextran-coated charcoal, and compared with the standard curve.
5. Mean recovery of renin through the method was 91.8%; mean recovery of angiotensin I was 87.0%.
6. Normal values for plasma renin activity (estimated as the rate of generation of angiotensin I) was 1.17 + 0.90 ng ml⁻¹ h⁻¹; n = 21. Plasma renin activity was normal in essential hypertension; high in chronic glomerulonephritis with oedema; often high in renovascular hypertension; and low in primary aldosteronism.

Key words: renin, radioimmunoassay, angiotensin I.

The renin–angiotensin system probably plays an important role in the regulation of body-fluid and electrolytes in health and disease. However, there have been for many years difficulties in assaying renin. Bioassay lacked sensitivity, specificity and reproducibility, partly because of the unstable pressor response in animals. Recently a number of investigators have attempted to apply the technique of radioimmunoassay of angiotensin I to the problem, but the methods are relatively complicated, and, in particular, need extraction from fuller's earth before immuno-
assay (Boyd, Adamson, Fitz & Peart, 1969), estimation on samples both before and after incubation for the generation of angiotensin I (Haber, Koerner, Page, Kliman & Purnode, 1969), or estimation after incubation of plasma with an excess of renin substrate (Stockigt, Collins & Biglieri, 1971). In none of the methods described hitherto has renin recovery been assessed.

In this report the details of a simple, rapid radioimmunoassay without extraction of angiotensin or addition of renin substrate are described.

**METHODS**

*Preparation of angiotensin antibodies*

[Asp\(^1\)-Ile\(^5\)]-angiotensin I (10 mg) purchased from the Protein Research Foundation (Osaka) and rabbit serum albumin (10 mg) were dissolved in 0·5 ml of water. Then 200 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide hydrochloride in 0·25 ml of water was added and the mixture was stirred at room temperature for 30 min. The mixture was dialysed against water at 4°C for 18 h, with one change of the dialysis fluid. The conjugate of angiotensin I with rabbit serum albumin was emulsified with an equal volume of Freund's complete adjuvant by vigorous agitation, and administered to three rabbits by intramuscular injections at intervals of 2 weeks.

*Preparation of tracer \(^{131}\)I-labelled angiotensin I*

Iodination of angiotensin I was performed by the chloramine T method (Hunter & Greenwood, 1962). In a small test tube 10 \(\mu\)l (containing 5 \(\mu\)g) of synthetic [Asp\(^1\)-Ile\(^5\)]-angiotensin I was added to 20 \(\mu\)l of 0·05 M-phosphate buffer, pH 7·4. With a microsyringe 2–3 mCi of carrier-free \(^{131}\)I were transferred to the test tube. Then, 25 \(\mu\)l of the oxidizing agent chloramine T (0·8 mg/ml in 0·05 M-phosphate buffer) was added, and the solution was mixed quickly. After 15 s, 50 \(\mu\)l of sodium metabisulphite (1·2 mg/ml in 0·05 M-phosphate buffer) was added to stop the reaction. To protect the angiotensin from excess radiation damage or loss on the glassware, 10 \(\mu\)l of normal human serum was added, and was then diluted with 300 \(\mu\)l of phosphate buffer.

The iodination mixture was applied to a small (70 mm x 5 mm) column of Amberlite CG-400 (Rohm and Haas), equilibrated with 0·05 M-sodium acetate-HCl, pH 5·0, and eluted with the same buffer. Each three drops of unabsorbed radioactivity were collected into a test tube containing 1·0 ml of 0·1% lysozyme in 0·05 M-phosphate buffer, pH 7·4 (lysozyme diluent). The fraction with the highest radioactivity was divided into five tubes and stored frozen.

*Generation of angiotensin I in plasma*

Each 8 ml of blood was collected with a syringe containing 0·1 ml of 8% EDTA (disodium salt) and promptly chilled in ice. The plasma was removed, separated into aliquots, and stored at \(-20^\circ\)C until analysed. No plasma sample was stored for longer than 2 weeks during which time no detectable decrease in renin activity was observed. The storage of plasma samples for longer than 4 weeks after sampling caused some depletion in plasma renin activity.

Plasma samples were thawed on ice and quantities of 1 ml were adjusted to pH 5·5 with 0·5 M-HCl after the addition of 1 drop of 5% DFP and 1 drop of 20% dihydrostreptomycin sulphate. Without the antibiotics, a slightly lower value was obtained in plasma renin activity, and this was probably caused by some degradation of the renin or renin substrate during the
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incubation. The mixture was incubated in a shaking water-bath at 37°C for 3 h. After the incubation, the pH was adjusted to 7.4 with 0.1 m-NaOH, and the volume to 2 ml with phosphate buffer (pH 7.4, 0.05 M). The sample was then heated in a boiling water-bath for 10 min, cooled and centrifuged at 3000 rev./min for 10 min. Each 0.1 ml of the supernatant was added to 0.9 ml of lysozyme diluent and the angiotensin I content was measured by radio-immunoassay.

![Graph](image)

**Fig. 1.** Standard curve of angiotensin I constructed with $^{131}$I-labelled angiotensin I and diluted antibody. Final antibody dilution, 1 : 600 000.

**Radioimmunoassay**

To small glass tubes were added 1 ml of unknown or standard angiotensin I in lysozyme diluent, 0.1 ml of $^{131}$I-labelled angiotensin I in lysozyme diluent (about 5000 c.p.m.), and 0.1 ml of antiserum diluted with lysozyme diluent (1 : 10 000–100 000).

This gave a total volume of 1.2 ml in each tube. The tubes were shaken and stored at 4°C for 18 h. Then 0.2 ml of dextran-coated charcoal was added to each of the tubes which were then shaken for 15 min, and centrifuged at 3000 rev./min for 3 min. The dextran-coated charcoal was diluted 5 times by lysozyme diluent from the stock solution which had been made previously by mixing an equal volume of 0.5%, dextran-80 and 5% charcoal. The supernatant was discarded and the precipitate was counted in a well-type scintillation counter. Since the dextran-coated charcoal binds 'free' angiotensin but not 'antibody-bound' angiotensin, the
charcoal count or ‘percentage free radioactivity’ rises with the addition of increasing quantities of angiotensin (Fig. 1). The results were divided by three, which gave the amount of angiotensin generated in 1 ml of plasma in 1 h.

**Bioassay method**

Pressor bioassay of angiotensin produced by the 16 h incubation of 2.0–3.0 ml of plasma was performed in duplicate in two rats which had been treated with pentobarbital and hexamethonium as reported by Miura (1969). Bioassay as well as immunoassay was carried out with an angiotensin I standard. This avoided the problem which was often encountered when angiotensin II amide was used as a standard for bioassay of angiotensin I samples, namely, the variation in the apparent converting enzyme activity in different rat preparations, and the corresponding variation in the apparent potency of the unknown sample. The pressor activity of angiotensin I in the rat-bioassay preparation, when compared with angiotensin II amide, was 60% on a weight basis (mean of five assays).

**Preparation of renin**

Purification of human renin from cadaver kidneys was performed in four steps; initial extraction and salt precipitation, then application of DEAE cellulose, CM-Sephadex and finally Sephadex G-200, as previously described (Lucas, Fukuchi, Conn, Berlinger, Waldhausl, Cohen & Rovner, 1970). The final product was reasonably stable when stored at pH 7.5 and \(-20^\circ\text{C}\). Incubation of this purified renin with human renin substrate produced a pressor substance which behaved like angiotensin I or II in the rat bioassay. The renin preparation, with a specific activity of 1.0–1.3 Goldblatt units/mg of protein, was purified approximately 600-fold on a large scale.

**Preparation of renin substrate**

The method of Skinner (1967) was followed, using human plasma taken from anephric patients. Substrate content was determined by incubating 0.2 and 0.5 ml of a 1 : 100 dilution of this preparation with excess human renin, 0.025 Goldblatt unit/ml. Substrate concentrations of between 1800 and 2800 ng equivalent/ml were obtained in various preparations.

Plasma angiotensin II was assayed by the method by Fukuchi & Katsushima (1971).

**Patients and control subjects**

Twenty-one normotensive subjects, between 18 and 62 years of age, with no cardiovascular or renal abnormalities, were studied.

Nineteen patients had essential hypertension with blood pressure greater than 140/90 mmHg, serum electrolytes within normal range, normal renal function as assessed by serum creatinine and creatinine clearance, and no evidence of any renovascular or endocrine abnormality.

Twenty-three patients had chronic glomerulonephritis, with marked albuminuria and haematuria. Three of this group had oedema. Renal biopsy, where performed, showed a patchy glomerulitis, with focal thickening of glomerular loops, proliferation of the capsular epithelium, and some hyalinized glomeruli.

Sixteen patients had renovascular hypertension with arteriographic evidence of either fibromuscular dysplasia or arteriosclerotic narrowing of one or both of the major renal arteries. Intravenous injection of contrast medium revealed a delay in appearance in the affected
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Kidney in every patient and a discrepancy in renal size over 1.5 cm in pole-to-pole diameter in nine of them. A marked difference in the function of the separate kidney in urine volume, and sodium and creatinine concentrations in urine obtained separately from both kidneys were detected in every patient. After surgical repair of the renal artery stenosis, a reduction in systolic pressure of at least 40 mmHg and a reduction in diastolic pressure to less than 100 mmHg were observed in eleven of them, without antihypertensive agents or sodium restriction. In five of six patients with bilateral renal artery stenosis the blood pressure was lowered, but still remained abnormally high after surgery.

Seven patients had clinical features of malignant hypertension, defined as rapid development of severe hypertension with diastolic pressure above 120 mmHg, renal impairment with serum creatinine above 2 mg/100 ml and creatinine clearance below 30 ml/min, bilateral refined haemorrhages and papilloedema, and high aldosterone secretion.

Fifteen patients diagnosed as primary aldosteronism had persistent and sustained hypertension, hypokalaemia, increased aldosterone production, no suppression of aldosterone secretion by deoxycorticosterone acetate, and when assays were practicable, reduced plasma renin activity measured by bioassay. Adrenal exploration revealed an adrenocortical adenoma in every patient, and unilateral adrenalectomy was carried out.

In all studies, diet, posture, and time of sampling were strictly controlled. Before any experiment, each subject was maintained for at least 5 days on a constant diet containing 250 mmol of sodium per day which is the usual intake in Japan. Blood samples were obtained early in the morning from fasting recumbent subjects taking no special medications which might influence body water or electrolyte balance.

RESULTS

Production of antibodies

Antibody to angiotensin I was detectable after about 3 months and became useful for radioimmunoassay 6–10 months after the first injection, with a final antiserum concentration of 1:10 000–100 000. The usual dilution of the antiserum was 1:20 000, giving a sensitivity of 10 pg for each assay. Further injections of angiotensin I-rabbit serum albumin did not produce any rise in anti-angiotensin titre. No cross-reactivity to angiotensin II amide was detected.

\[^{131}I\]-labelled angiotensin I

A simple method using an Amberlite CG-400 column was useful for the purification of \[^{131}I\]-labelled angiotensin I. The best tracer was obtained from the fraction with the highest radioactivity which probably represents monoiodoangiotensin I. The tracer \[^{131}I\]-labelled angiotensin I could be used for as long as 10 days after iodination, if stored in a deep-freeze. The mean specific radio activity of the iodinated angiotensin was approximately 200 mCi/mg.

Specificity

The high specificity of this immunoassay was demonstrated by the fact that large excess of bradykinin, vasopressin and purified renin substrate did not displace labelled angiotensin I from the antibody. Experiments were also carried out to examine the possibility that degradation products of angiotensin I present in the plasma or formed during the incubation
period might contribute significant interference in the system. Histidyl-leucine dipeptide (Sigma), released from angiotensin I by converting enzyme in plasma, showed very little cross-reaction in the assay, possessing only 2.3% of the immunological potency of angiotensin I. Angiotensin II and its degradation compounds produced by angiotensinase in plasma during a 1 h incubation, had less than 0.1% of the competitive effect of the decapeptide to the binding of labelled angiotensin I to antibody. Heptapeptide and hexapeptide fragments (given by the courtesy of Dr B. Riniker of Ciba, Basle) formed by the removal of amino acid from the N-terminal end of angiotensin II and purified by thin-layer chromatography, also failed to inhibit the binding of labelled angiotensin I to antibody. The 3 h incubation product of 0.1 ml of plasma from an anephric patient added to 1.0 ml of purified human renin substrate did not cause significant displacement of the tracer.

These results suggest that no significant interference with the immunoassay by the degradation products of angiotensin I, II or renin substrate during the 3 h incubation of plasma occurred.

Sensitivity
The lower limit of sensitivity of the assay was related to the amount of labelled peptide used. For example, by using 5 pg of labelled peptide, it was possible to detect about 10 pg. Therefore, the final sensitivity in measurement of plasma renin activity was 0.07 ng ml\(^{-1}\) h\(^{-1}\).

Recoveries and reproducibility
Some 20 ng of purified human renin (Lucas et al., 1970) yielded an equivalent of 4.17–4.87 [mean, 4.54 ± 0.18 (SD), \(n = 6\)] ng ml\(^{-1}\) h\(^{-1}\) of angiotensin I with 1.0 ml of purified human renin substrate during a 3 h incubation as assayed by radioimmunoassay. Recovery rate was checked in two ways: by the addition of 20.0 ng of the purified human renin and the addition of 10.0 ng of angiotensin I, to blood taken with a syringe containing EDTA from an anephric patient. Plasma was separated from both blood samples, stored for 2 weeks in deep-freeze, and then assayed, after adjusting the pH to 5.5 and adding DFP, by the present method. Estimated values in the case in which purified renin was added, ranged from 3.77 to 4.43 [mean, 4.17 ± 0.26 (SD), \(n = 8\)] ng ml\(^{-1}\) h\(^{-1}\), which indicated a mean renin recovery of 91.8%. The mean and standard deviation of recovery of angiotensin I added, was 87.0 ± 7.6%, which implied some degradation during the incubation.

Repeated measurements on the same plasma sample showed that the values ranged from 1.87 to 2.27 [mean, 2.08 ± 0.12 (SD), \(n = 10\)] ng ml\(^{-1}\) h\(^{-1}\) after 3 h of incubation.

The amount of angiotensin I generated during a 3 h incubation of a standard human renin preparation with plasma from an anephric patient did not significantly change after the further addition of a purified human renin substrate. Therefore, under conditions of substrate excess, contained in normal plasma (Pickens, Bumpus, Lloyd, Smeby & Page, 1965), the rate of formation of angiotensin I is proportional to the amount of renin present.

Factors affecting the standard curve
It was observed that the presence of serum protein in the incubation mixture exerted a significant influence on the binding of angiotensin I by its homologous antibody, as described in the case of angiotensin II. Even the presence of dialysed pooled plasma in a concentration of only 1-0% (in addition to 0.1% of lysozyme) resulted in some interference with the binding of
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antigen and antibody. Gocke, Gerten, Sherwood & Laragh (1969) and Haber et al. (1969) avoided the interfering effect of plasma protein by comparison against a standard curve prepared with the same amount of dialysed pooled plasma. However, differences in the interfering effect were found among individual plasmas. The boiling procedure was therefore used for elimination of the effect of plasma protein. Bovine serum albumin, used in the radioimmunoassay of angiotensin II (Fukuchi & Katsushima, 1971), also markedly decreased sensitivity in the standard curve of angiotensin I.

**pH at incubation**

The production of angiotensin I by incubation of plasma was tested at different pH values. The highest value of angiotensin I production was detected between pH 5.5 and 6.0, as shown in Fig. 2.

**Time-course of angiotensin I production**

The time-course of angiotensin yield was studied at various lengths of incubation time up to 24 h. The amount of angiotensin I in the incubation mixture increased linearly with time up to 10 h and then declined gradually, as shown in Fig. 3. An incubation time of 3 h was normally used for the generation of angiotensin I.

**Inhibition of angiotensinase activity**

Inhibitory effects of pH, EDTA and DFP against angiotensinase activity were studied from the recovery rate of angiotensin I, which had been added to the plasma at the time of blood sampling. The angiotensinase activity was inhibited most effectively with the addition of EDTA (disodium salt) at the sampling and DFP before incubation. Further addition of 8-hydroxyquinoline sulphate or dimercaprol showed no effect on the inhibition of angiotensinase activity. That is to say, no marked difference in results was found between EDTA–DFP, EDTA–DFP–8-hydroxyquinoline sulphate and EDTA–DFP–8-hydroxyquinoline sulphate–dimercaprol. However, the inhibition of angiotensinase activity with EDTA and DFP was not necessarily complete, because samples in which there was haemolysis showed lower recovery.

**Effect of EDTA and DFP on converting enzyme**

To 1.0 ml samples of plasma containing EDTA and DFP, 200 ng of angiotensin I were added to test the effect of EDTA and DFP on the plasma converting enzyme. One of each pair was assayed immediately, and the other was incubated for 3 h at 37°C. A third 1.0 ml sample was incubated for 3 h without added angiotensin. The plasma samples were all subjected to radioimmunoassay of angiotensin I and II.

The recovery of angiotensin I after incubation was 85–95% of the control tube and the angiotensin II was 7–13% of the angiotensin I value. The results show that a small amount of angiotensin I was converted into II during the incubation even in the presence of EDTA and DFP.

The addition of EDTA and DFP did not inhibit the reaction between renin and renin substrate. Using both bioassay and radioimmunoassay, there was no detectable difference in the rates of generation of angiotensin I when purified human renin was added to angiotensinase-free human renin substrate with or without the usual concentrations of EDTA and DFP.
Fig. 2. Angiotensin I generation by three unknown samples incubated for 3 h at pH 4.5-7.5.

Fig. 3. Time-course of angiotensin I generation. Angiotensin I was generated with renin contained in 1 ml of plasma with EDTA and DFP at 37°C and pH 5.5.
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Correlation of immunoassay to bioassay

Angiotensin generated by incubation at 37°C for 16 h with endogenous renin, in the presence of EDTA and DFP in 1 ml of plasma to inhibit angiotensinase, was assayed both biologically and immunologically. In general, the values observed with both types of assay were comparable, although the immunoassay results tended to be somewhat lower. Excellent correlation between bioassay and immunoassay determination was obtained, as shown in Fig. 4 ($r = 0.97$). When the radioimmunoassay of angiotensin II was applied to the measurement of angiotensin generated during plasma incubation, a value only one tenth of the bioassay results was obtained.

![Graph](image)

**Fig. 4.** Comparison of immunoassay and bioassay measurements of plasma renin activity. Plasma was incubated with endogenous renin for 16 h with EDTA and DFP at pH 5.5 and determinations were carried out on portions of the same sample. Immunoassay = $0.92 \times$ bioassay $- 0.71$. Good correlation was found between values obtained by the two methods ($r = 0.97$).

Clinical observations

The renin activity in plasma, drawn in the morning for twenty-one normal subjects aged 18–62 years, thirteen of whom were male, ranged from 0.13 to 3.00 ng ml$^{-1}$ h$^{-1}$, average 1.17 ng ml$^{-1}$ h$^{-1}$, with a standard deviation of 0.90.

Plasma renin activity in essential hypertension was within the normal range, but showed a tendency to a low average.

Renin activity was within the normal range in a majority of twenty-three cases of chronic glomerulonephritis. Three patients with oedema had high values of 4.17–8.63 ng ml$^{-1}$ h$^{-1}$.

Plasma renin activity was raised to 3.83–9.30 ng ml$^{-1}$ h$^{-1}$ in nine of ten cases of unilateral renal artery stenosis, but was normal in five of six cases of bilateral renal artery stenosis.

In seven cases of malignant hypertension plasma renin activity was 0.72–10.0 ng ml$^{-1}$ h$^{-1}$, being elevated in five cases.

Plasma renin activity in eleven of fifteen cases of primary aldosteronism was suppressed to
Normal subjects

Essential hypertension

Chronic glomerulonephritis

Renovascular hypertension

Malignant hypertension

Primary aldosteronism

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<th>Plasma renin activity (ng ml(^{-1}) h(^{-1}))</th>
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<td>Normal subjects</td>
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<td>Essential hypertension</td>
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DisCUSSION

Assay of plasma renin has become an important technique in the diagnosis of hypertension, but most methods lack sufficient sensitivity to determine subnormal values accurately. In order to define those conditions characterized by suppressed renin and to look for diagnostic distinctions, a sensitive and rapid workable renin assay was developed, using radioimmunoassay of generated angiotensin I after incubation of plasma at pH 5.5. This assay enables the precise measurement of markedly subnormal plasma renin concentrations.

Methods of production of antibody to angiotensin I have been reported by Boyd et al. (1969), Haber et al. (1969) and Goodfriend, Ball & Farley (1968). Boyd et al. (1969) used intra-lymph-node and intra-splenic injection of angiotensin absorbed on to macroparticles of carbon black. Haber et al. (1969) administered the conjugate of angiotensin I and poly-L-lysine to rabbits. However, these two methods resulted in poor titres in spite of a very long immunization period. Injection of conjugates of angiotensin I and rabbit serum albumin made by carbodi-imide condensation, on the other hand, produced highly specific antibody with a high titre. The good antibody enabled a standard curve of high sensitivity to be achieved. Therefore, extraction procedures, such as the use of fuller's earth by Boyd et al. (1969), were not needed.

Radio-iodinated angiotensin I was purified by the simple method using an Amberlite CG-400 column, which can be completed within a few minutes. The purified tracer was found to have a single band on paper chromatoelectrophoresis with a barbital buffer of pH 8-0. This was the most effective labelled angiotensin I and probably represented monoiodoangiotensin I. Cohen, Grim, Conn, Blough, Guyer, Kem & Lucas (1971) and Haber et al. (1969) used a complicated method of polyacrylamide-gel electrophoresis or paper chromatoelectrophoresis for purification. Their tracer angiotensin I showed two distinct antigenically active peaks.
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In the present method, the pH at the incubation of plasma was 5.5. Haber et al. (1969) incubated plasma without adjustment of pH after the addition of EDTA. The pH was found to change to acidity from pH 7.4 after EDTA. However, a pH of 5.5 resulted in the highest conversion into angiotensin I. As the radioimmunoassay was required to be performed at pH 7.4, it was necessary in their procedure to adjust the pH again to 7.4 after incubation.

With the present technique, the angiotensin I generated during incubation increased linearly up to 10 h and then declined gradually. Four possible explanations are given as to the cause of the decline in the amount of angiotensin generated on prolonged incubation: (1) exhaustion of renin substrate contained in plasma, (2) degradation of renin in plasma, (3) conversion from angiotensin I into II, and (4) incompleteness in angiotensinase inhibition. The first is considered unlikely since the amount of renin substrate contained in human plasma is stated to be plentiful (Pickens et al., 1965). The latter three possibilities are more probable; for example, storage of plasma with EDTA for longer than 5 h at 4°C causes some depletion in plasma renin activity, radioimmunoassay of the pressor fraction generated on prolonged incubation reveals that it contains about nine parts of angiotensin I to every one part of angiotensin II by weight, and about 7% of angiotensin I in plasma is destroyed during 24 h even when stored at 4°C in the presence of EDTA and DFP.

It was found that plasma protein inhibited the reaction of antibody and 131I-labelled angiotensin I. Therefore, the method without the elimination of plasma protein gave falsely higher values on radioimmunoassay than were found on bioassay. Even bovine serum albumin, which was used in radioimmunoassay of angiotensin II (Fukuchi & Katsushima, 1971) and which was also used in radioimmunoassay of angiotensin I by Cohen et al. (1971), showed some interference effect on radioimmunoassay. The simple procedure of boiling was used to eliminate the inhibitory effect of plasma protein. The very small amount of plasma protein contained in 10 µl of plasma for immunoassay, which was used by Cohen et al. (1971), also had some inhibitory effects. The results obtained by measurement in plasma from an anephric patient with a known amount of angiotensin I added before incubation, showed very good agreement with the amount calculated theoretically. L. B. Page (personal communication) stated that the molecular weight of plasma protein with immunogenically angiotensin-like effect was 118 000 in the case of radioimmunoassay of angiotensin I and 140 000 in the case of angiotensin II, which was found by using Sephadex-column chromatography. Haber et al. (1969) estimated values directly without extraction or boiling on samples both before and after incubation, and calculated plasma renin activity by the subtraction of the results after incubation from that before incubation.

The duration of incubation for plasma was 3 h using the present method. Cohen et al. (1971) used a short (15 or 30 min) incubation of plasma, during which the linear generation of angiotensin I in plasma was demonstrated. The short incubation minimized the possibility of renin degradation. They mentioned that if excess of substrate was not ensured, the incubation time became particularly critical to ensure accuracy in the measurement of angiotensin production rate. However, since human plasma is believed usually to contain a large amount of renin substrate and also since it is difficult to incubate every plasma sample accurately for 30 min, a 3 h incubation was used in the present method.

The specificity of the assay was supported by the excellent correlation between immunoassay and bioassay measurements of angiotensin I concentration in the sample.

The results obtained in normal subjects (expressed as the rate of angiotensin I generation)
were somewhat lower in this method than in those reported by others (Boyd et al., 1969; Haber et al., 1969; Stockigt, et al., 1971; Cohen et al., 1971). This may be caused by the larger amounts of sodium intake contained in a usual diet in Japan (220–300 mmol/day). However, since not all of these methods were calibrated against standard renin preparations, such comparisons may well be invalid. Definitely low but easily measureable values were obtained in patients with proven primary aldosteronism. Conversely, high values were obtained from patients with renovascular hypertension with radiologic evidence of renal artery stenosis. These results show that the method is clinically applicable to diagnostic procedures.

REFERENCES


