Estimation of Plasma Renin Activity by
Radioimmunoassay of Angiotensin I

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Introduction

The renin-angiotensin-aldosterone hormonal system has been shown to be involved in the pathogenesis of certain hypertensive disorders, notably primary aldosteronism, malignant hypertension, renovascular hypertension and that induced by oral contraceptives.1-7 Plasma renin assay has become an important diagnostic tool for investigating these disorders.

Previously, plasma renin activity has been measured by a tedious, complicated bioassay. The availability of synthetic 125I-angiotensin I and its antibody has made possible the measurement of renin activity in plasma by radioimmunoassay,8-11 by utilizing the principle of saturation analysis. Normal human plasma contains angiotensinogen (a substrate) and the enzymes renin and angiotensinase. During the incubation of plasma, the decapeptide angiotensin I is generated by the action of renin on the substrate. The further conversion of angiotensin I to angiotensin II by the action of angiotensinase can be prevented by the addition of EDTA, dimercaprol and 8-hydroxyquinoline to the plasma sample. The angiotensin I generated per ml of plasma per hour (ng/ml/hr) at 37°C (plasma renin activity or PRA) is determined by radioimmunoassay.

Collection of Blood Specimens

The blood specimen should be drawn between 8:00 and 11:00 AM after the patient has been in the upright position for one hour. It should be taken into a chilled Vacutainer tube containing Na2EDTA (not the potassium salt), which is then immersed immediately in ice-water. The blood is centrifuged in the cold (2° to 4°C), the plasma separated without delay and kept frozen (−20°C) until analyzed.

Reagents

It is convenient to use the Angiotensin I Immutope™ Kit.* However, only the first three reagents are difficult to prepare. The kit consists of:

1. Angiotensin I Antiserum; Antibody can be prepared by immunizing rabbits with synthetic Asp1-Ile5-angiotensin I coupled to either poly-L-lysine or bovine serum albumin in the presence of carbodiimide. The antiserum to angiotensin I is available from a commercial source.† It is preserved frozen.

2. 125I-Asp1-Ile5-Angiotensin I; The labeled angiotensin I can be prepared by the chloramine-T method of Hunter

* E. R. Squibb & Sons, Inc., New Brunswick, NJ.
† Schwartz/Mann, Orangeburg, NY.
et al.\textsuperscript{14} and is also commercially available.\textsuperscript{f} A working solution of \textsuperscript{125}I-Asp\textsuperscript{1}-Ile\textsuperscript{8}-angiotensin I is prepared by diluting about 0.2 ml of the stock radioactive solution in BSA-Tris acetate buffer (reagent \#6)\textsuperscript{2} to a volume which contains an activity of approximately 5,000 cpm. The diluted \textsuperscript{125}I-angiotensin I should be stored at 4° to 6°C and prepared fresh each week.

3. **Stock Standard Solution of Asp\textsuperscript{1}-Ile\textsuperscript{8}-Angiotensin I** (0.1 mg per 100 ml); The angiotensin I is dissolved in water. The working standard solution is prepared by diluting 1 ml of stock standard solution to 100 ml with water. It is divided into 2 ml portions, which are kept frozen to avoid deterioration.

4. **Tris-Acetate Buffer, 0.1 M, pH 9.0;** 12.1 g of tris (hydroxymethyl) aminomethane are dissolved in 990 ml of distilled water, and the pH adjusted to 9.0 with glacial acetic acid. The solution is then diluted with water to one l. The buffer should be stored at 4° to 6°C.

5. **Barbital Buffer, 0.1 M, pH 9.0;** 20.6 g of sodium barbital are dissolved in 990 ml of distilled water. The solution is adjusted to pH 9.0 with 0.1 M HCl and then diluted with water to a volume of one l. The buffer should be stored at 4° to 6°C.

6. **BSA-Tris-Acetate Buffer, 0.1 M, pH 9.0;** 0.25 g of bovine serum albumin (BSA) powder are dissolved in 100 ml of Tris-acetate buffer. The solution should be kept at 4° to 6°C and is stable up to one month.

7. **Charcoal Suspension;** 6.25 g of activated charcoal (Norit A) are added to 1,000 ml of barbital buffer (reagent \#5), vigorous stirring being maintained with a magnetic stirrer for 5 minutes. This charcoal suspension should be kept at 4° to 6°C, and should be stirred vigorously before removal of aliquots for use.

8. **2,3-Dimercaptopropanol (Dimercaprol or BAL);** 200 mg of dimercaprol and 400 mg of benzyl benzoate are dissolved in 2 ml of peanut oil. The solution should be stored at 4° to 6°C and well shaken before use.

9. **8-Hydroxyquinoline Sulfate Solution;** 660 mg of 8-hydroxyquinoline sulfate (AR) are dissolved in 10 ml of distilled water. The solution should be stored in the dark.

### Materials

- Plastic test tubes, 12 x 75 mm with caps.\textsuperscript{*}
- Eppendorf pipettes, 5 \( \mu l \), 10 \( \mu l \), 20 \( \mu l \), 50 \( \mu l \) (or equivalent).\textsuperscript{†}
- Repipet (or equivalent) to dispense 1 ml volumes.\textsuperscript{‡}
- Counting Tubes, 16 x 125 mm with screw cap.\textsuperscript{§}
- Automatic gamma counter (or equivalent).\textsuperscript{||}

### Method

#### Generation of Angiotensin I

Tubes are set up as follows: two for each patient sample and two for the control (pooled normal plasma). To each tube are added, with mixing after each addition, 0.5 ml of plasma (control or sample), 5 \( \mu l \) of 8-hydroxyquinoline sulfate solution and 5 \( \mu l \) of 2,3-dimercaptopropanol solution. One member of each pair is incubated at 4°C for 3 hours (to determine the endogenous angiotensin I); the other member of the pair is incubated at 37°C (to determine

\footnotesize{\textsuperscript{*} #2054, Falcon Plastics, Los Angeles, CA 90054.\textsuperscript{†} Brinkman Instruments, Inc., Los Angeles, CA 90017.\textsuperscript{‡} Labindustries, Berkeley, CA.\textsuperscript{§} #2025, Falcon Plastics.\textsuperscript{||} Nuclear Chicago, Chicago, IL.}
generated angiotensin I). After incubation, the mixtures can be kept frozen until required for radioimmunoassay.

**Radioimmunoassay of Angiotensin I**

The radioimmunoassay is based on saturation analysis, in which there is competition between labeled and unlabeled antigen for a fixed number of binding sites on the antibody. After equilibrium has been reached, the free antigen is separated by adsorption onto charcoal. The free antigen and antibody-bound antigen are then determined by measurement of their radioactivity. The unlabeled antigen in the clinical sample is determined by comparing the results to a standard curve. Tubes are set up as follows, each test being performed in duplicate (A and B), and requiring two pairs of tubes, one for the mixture incubated at 37°C, the other for the mixture incubated at 4°C. To each of the appropriate tubes are added 50 µl of the 37°C mixture or the 4°C mixture, 1.0 ml of diluted 126I-angiotensin I solution and 50 µl of angiotensin I antiserum. The contents are mixed gently and the capped tubes incubated at 4°C to 6°C for 24 hours. Calibration standards are prepared at the same time, as described in section 3. At the end of the incubation period, 1 ml of charcoal suspension is added to each tube, which is then gently shaken by hand for about 15 seconds and centrifuged (r.c.f. = 4,000) to pack the charcoal firmly. The supernatant is decanted completely into another tube. As the charcoal suspension settles rapidly, the bulk solution should be kept in the Repipet container and stirred vigorously (magnetic stirrer) before use. The 1 ml volumes should be dispensed without delay. The activities of the supernatant and the residue are determined. Note: each plastic tube should be inserted into a counting tube if activities are measured in the automatic counter.

**Standard Curve**

Five µl (50 pg), 10 µl (100 pg), 20 µl (200 pg), 30 µl (300 pg), 40 µl (400 pg), 50 µl (500 pg) of the standard angiotensin I solution are added to a series of plastic test tubes and the assay procedure is carried out as described for plasma. The percent bound angiotensin I for each sample is calculated as follows:

\[
\text{Percent bound} = \frac{\text{Activity in supernatant tube (cpm)}}{\text{Activity in supernatant tube (cpm)} + \text{activity in charcoal tube (cpm)}} \times 100
\]

The average value from the duplicates is used in the final calculation. The standard curve is obtained by plotting the average percent bound values (ordinate) against the known quantities of angiotensin I standards (abscissa). The angiotensin I concentrations of the samples are obtained by interpolation in the standard curve. The plasma renin activity (PRA), as nanograms of angiotensin I generated per ml of plasma per hour of incubation at 37°C

\[
= \frac{\text{(pg Angiotensin I in 37°C sample}}{\text{— pg Angiotensin I in 4°C sample)}} \times 20 \\
= \frac{\text{3 × 1,000}}{150 \text{ ng}^*/\text{ml/hr}}
\]

**Discussion**

Essentially, the measurement of plasma renin activity is an enzyme assay using a radioisotopic method for measuring the product of the reaction. As in any enzyme assay, optimum conditions should be employed and there should be an excess of substrate. Unfortunately, the substrate (human angiotensinogen) is not available in suitable quantities in a pure form. The endogenous angiotensinogen in the plasma

* 1 pg = 10⁻¹²g; 1 ng = 10⁻⁹g.
TABLE I

<table>
<thead>
<tr>
<th>Posture</th>
<th>Diet</th>
<th>No e</th>
<th>Value</th>
<th>for</th>
<th>Angiotensin 1 in ng/ml/hr</th>
</tr>
</thead>
<tbody>
<tr>
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|         |      | a    |       |     |                          |
|         |      | l    |       |     |                          |

![Image](image.png)

TABLE I

Normal Values for Angiotensin I in ng/ml/hr

<table>
<thead>
<tr>
<th>Posture</th>
<th>Diet</th>
<th>NaCl &gt; 5 g</th>
<th>Low NaCl</th>
<th>Diuril</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 g Daily</td>
<td>for 5 Days</td>
<td>500 mg b.i.d.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Upright, 1 hr</th>
<th>mean</th>
<th>1.76</th>
<th>3.46</th>
<th>7.80</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% range</td>
<td>(0.52-3.58)</td>
<td>(0.69-6.46)</td>
<td>(2.87-15)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Supine</th>
<th>mean</th>
<th>0.77</th>
<th>1.48</th>
<th>3.46</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% range</td>
<td>(0.17-1.87)</td>
<td>(0.41-2.70)</td>
<td>(1.19-7.77)</td>
<td></td>
</tr>
</tbody>
</table>

sample is used instead. However, this is variable in quantity and is usually insufficient to provide an excess of substrate. Because of this variation in endogenous substrate from sample to sample and the presence of renin inhibitors in some sera,15 it is not always clear what is being measured in the assay. Wide variations in PRA result from different methods.16 It is essential to establish the “normal” range with the method used, as even small changes in technique will affect the answer. Measurement of plasma renin concentration (PRC) has been attempted, substrate excess being provided by angiotensinogen derived from hog plasma and other non-human sources. Unfortunately, human and non-human angiotensin I’s differ both structurally and in their reactivity towards the antibody. The PRC procedure therefore presents many problems and a satisfactory method has yet to be developed. In addition, there are other factors affecting the PRA assay. Posture of the patient,17 drugs such as methyl-dopa, L-dopa, guanethidine, diazoxide, reserpine, hydralazine and others18,20 interfere. It is therefore important that drug therapy be discontinued for at least one week prior to blood sampling; also the patient should be maintained on a salt restricted diet. Recent studies have demonstrated an angiotensin I-like protein21 and a renin inhibitor15 in plasma. Correction for the endogenous angiotensin I-like protein should be routinely made by subtraction of the value obtained from the assay of an unincubated plasma sample or sample incubated at 4°C for 3 hours. Dilution of the plasma might provide some additional evidence of the presence of inhibitors. Katz22 has shown that disodium EDTA as an anticoagulant yields considerably greater angiotensin I values than those found when tripotassium EDTA is used. The high potassium content (22 µmole per ml blood) may inhibit renin activity. Ammonium EDTA has been suggested as an alternative for collecting blood specimens. Since both N-terminal and C-5 amino acids are critical in the activity of the antibodies, Hollemans23 recently has proposed the use of human angiotensin (Asp^Ile^Angiotensin I) as an international standard. The use of this standard might enhance the specificity of an antigen-antibody reaction.

From our experience, the working range of the assay is limited to 50 pg to 300 pg of angiotensin I; above this range a slightly flattened curve is observed. Smaller volumes of incubated plasma may be used in cases of high angiotensin I concentrations. The average coefficient of variation of plasma renin activity in the 5 different standard concentrations was approximately 8 percent, comparable to the values in the literature.11,23,24 The coefficient of variation for duplicate PRA determinations was about 6 percent. The normal values by this method are given in table I. The outlined procedure, although still far from perfect, has
provided the clinician, in many cases, with useful information in the investigation of hypertension and allied conditions.

Acknowledgment

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References


