DETERMINATION OF PLASMA RENIN ACTIVITY BY RADIOIMMUNOASSAY: COMPARISON OF RESULTS FROM TWO COMMERCIAL KITS WITH BIOASSAY

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Measurement of peripheral and renal venous renin activity has achieved practical clinical importance in the evaluation of the role of the kidneys in hypertension and in the diagnosis of renovascular hypertension and of primary hyperaldosteronism (*1–5*).

The use of radioimmunoassay for the determination of plasma renin activity through the assay of generated angiotensin I (*6–8*) has several advantages over the standard bioassay procedures currently in general use (*9*). The radioimmunoassay procedure facilitates the assay of a much larger number of samples than can be handled for bioassay. It obviates the necessity of maintaining a colony of assay animals. The sensitivity and reproducibility of the method is greater. The volume of blood required for analysis by radioimmunoassay is less than one-fifth the volume required for bioassay.

Widespread application of immunoassay procedures is limited partially by the lack of general availability of the necessary reagents from commercial sources including especially monospecific antisera and iodinated angiotensin I. Recently, two radiopharmaceutical manufacturers, Squibb and Schwarz-Mann, have begun to market as test kits the angiotensin I immutope™ kit and the renin activity radioimmunoassay kit, respectively. Since the initial criteria in establishing an immunoassay for clinical use are quite stringent, careful quality control is necessary to ensure accurate results. Such problems as cross reactions of antisera and impurities of the ^{125}I-angiotensin standard can lead to serious error. It is therefore essential that the reliability and reproducibility of any commercial kit of this nature be carefully assessed before introducing it into the laboratory for routine use.

The present report describes the results of a critical comparison of the results obtained through the use of these two kits with the bioassay method of Boucher, et al (*9*) for the measurement of renin activity in human plasma samples.

MATERIALS AND METHODS

The bioassay procedure is based on the method originally developed by Boucher, et al (*9*), as modified by Blaufox, et al (*10*), and used extensively in this laboratory during the last 6 years. Plasma incubation is carried out for 3 hr at pH 5.5 in the presence of Dowex resin. The generated angiotension is eluted from the resin and is assayed in the rat by a bioassay technique. The renin activity values obtained using this procedure agree within 10% when coded and assayed blind in two separate rats blocked with pentolinium and atropine prior to assay against a standard mixture of angiotensin II. Since the angiotensin I generated in the procedure is rapidly converted to angiotensin II in the rat, the results are expressed as ng A II/100 ml/3 hr.

The preparation of reagents for the radioimmunoassay procedures followed with the two commercial kits differ slightly and are briefly described below.

Schwarz-Mann kit. Preparation of reagents for use with the Schwarz-Mann kit requires the weekly preparation of trisacetate buffer. For dilution of plasma samples and ^{125}I-angiotensin I, trisacetate buffer containing lysozyme must be prepared each day the test is run. The barbital buffer is readily diluted in water and Dextran-coated charcoal suspensions are made using this buffer. A stock suspension is prepared and a working suspension is prepared by 1–4 dilution of the stock suspension. One milliliter of active ^{125}I-angiotensin I is supplied frozen in

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the presence of stabilizing material. After thawing, the activity is distributed into four aliquots of 250 μl in plastic tubes supplied with the kit containing additional stabilizing material, and these are stored frozen until used for dilution. At the time of dilution of the activity for a test run, the contents of one tube are thawed, the stabilizer material is resuspended, and an aliquot from this lot is withdrawn and diluted with tris buffer containing fresh lysozyme. It is also recommended by the manufacturers that the standard angiotensin I and the antisera be thawed for use not more than two times.

Squibb kit. The preparation of reagents using the Squibb kit is simplified through the use of previously calibrated materials which are diluted to appropriate volumes. The trisacetate buffer solution can be stored up to 1 month. An aliquot of the active ^{125}I-angiotensin I preparation containing a scavenging resin for adsorption of any free radioiodide generated during storage is withdrawn and diluted with the trisacetate buffer containing bovine serum albumin prepared freshly every week.

Comparative procedures. The active ^{125}I-angiotensin received from both manufacturers was chromatographed on anion exchange resin paper to check the purity of the labeled compound. Blood samples for analysis were collected in ice cold stoppered tubes* containing disodium ethylenediamine tetraacetic acid (EDTA). The tubes are tipped end to end gently to ensure that all of the EDTA is dissolved after adding 5 ml of blood and are then centrifuged in the cold at 2°C to separate the plasma. Simultaneously 20 ml of blood was collected into tubes containing ammoniated EDTA for use in the bioassay. One milliliter of plasma from each clinical sample for radioimmunoassay is pipetted into a Falcon Plastics polystyrene tube (12 × 75 mm) and then 10 μl each of 8-hydroxyquinoline and dimercaprol are added (in the Schwarz-Mann kit, only 2 μl of dimercaprol is recommended). The mixture is then separated into two 500-μl aliquots, and one tube is incubated at 4°C and one at 37°C for 3 hr. After incubation, the samples are ready for immunoassay of the generated angiotensin I. If samples are not assayed immediately, they are frozen and then thawed at 4°C for assay at the time of carrying out the series.

The protocol for the radioimmunoassay test procedure is slightly different for the two kits. With the Schwarz-Mann kit, 0.9 ml of tris buffer containing lysozyme is added to each tube, followed by the addition of 5 μl (50 pg), 10 μl (100 pg), 20 μl (200 pg), 30 μl (300 pg), 50 μl (500 pg), and 70 μl (700 pg) of standard angiotensin I for calibration and 50-μl plasma samples from the 4 and 37°C incubation for clinical samples. Fifty microliters of active angiotensin I solution is aliquotted into each tube and finally 50 μl of angiotensin I antiserum is added to all of the tubes. In two tubes containing tris buffer with lysozyme, only aliquots of 50 μl of active angiotensin I are added and in two others, buffer solution, 50 μl of active angiotensin I and 50 μl of antisera added—the first two serving as controls and the second two as tracer amounts of angiotensin only.

With the Squibb kit 1 ml of diluted active angiotensin I solution is added followed by addition of 5 μl (50 pg), 10 μl (100 pg), 20 μl (200 pg), 30 μl (300 pg), 50 μl (500 pg), and 70 μl (700 pg) of standard angiotensin I for calibration and 50 μl of patient plasma samples incubated at 4 and 37°C. Two tubes containing only active angiotensin solution serve as tracers. Fifty microliters of angiotensin antisera are added to all tubes.

All the tubes in each series prepared as above are gently mixed, and incubated at 4°C for 24 hr, after which 1 ml of charcoal suspension is added into each tube, gently shaken, and centrifuged at 2,500–3,000 rpm for 5–10 min. The supernate from each tube is decanted into similarly numbered tubes and both the residue and supernate are counted in a well scintillation counter. The standards are generally done in triplicate, and the unknown plasma samples for purposes of this study were analyzed in

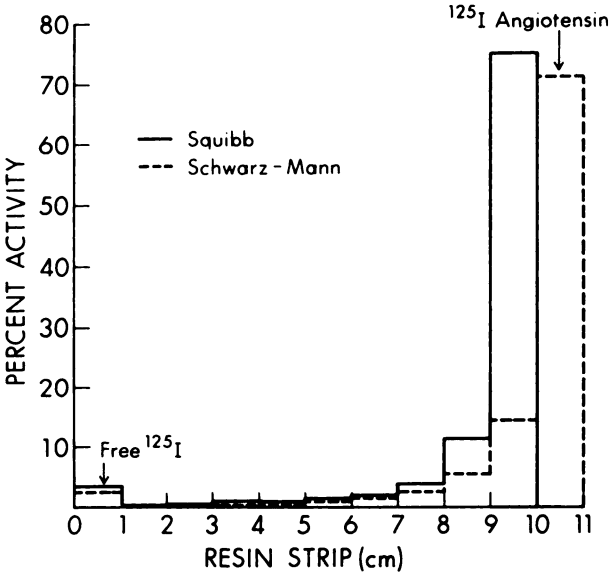

FIG. 1. Chromatographic separation of angiotensin I and free ^{125}I supplied with sample kit from each manufacturer. Each contains small amount of free radioiodide which remains at origin in chromatographic system used. This represents potentially serious source of error and should be checked frequently.

* Lavender top vacutainer tubes, Becton-Dickinson & Co., Cat. No. 4770.

triplicate with an additional value obtained for a smaller aliquot of 10 μl. Aliquots were all measured using Oxford microliter pipettes except the standard and antisera which were dispensed using Hamilton microsyringe pipettes with a repeating dispenser attachment.

A total of 40 plasma samples were obtained from patients with a wide variety of sodium intake and pathology for comparison of the three test procedures: bioassay, Squibb immunoassay, and Schwarz-Mann immunoassay. The results of all three tests were obtained blind with coded samples. Statistical comparisons were carried out later after decoding.

RESULTS

The results of sample chromatograms as shown in Fig. 1 indicate that the radioiodide remains at the point of application in the system used and the active material moves with the solvent front. About 3% free radioiodide is present in the active angiotensin I from both manufacturers.

The calibration curve may be drawn in two ways: (A) by plotting the average percent bound values against the standard amount of angiotensin I added resulting in a curvilinear plot and (B) by plotting the free/bound ratio against the standard which results in a straight line within the usual range of values encountered. The concentration of angiotensin I in the 4 and 37°C incubated plasma samples is calculated by interpolation using these standard curves. The plasma renin activity values are then calculated as angiotensin I generated per milliliter per hour from the equation

$$\text{renin activity (ng AI/ml/hr)} = \frac{(\text{ng AI}^{37^\circ} - \text{ng AI}^{4^\circ})20}{3}.$$

The renin activity values thus obtained are plotted in Figs. 2 and 3 against the corresponding bioassay values (expressed as ng A II/100 ml/3 hr) for the same plasma samples. In Fig. 4 are plotted the data obtained from the samples analyzed with both the kits against one another. The calculated regression lines are drawn with the inserts in the figures giving the equations for the lines. The correlation coefficient, r, obtained with the Squibb kit and the Schwarz-Mann kit are 0.94 and 0.91, respectively. Between the two kits a correlation coefficient of 0.94 is obtained. All three of these correlations are highly significant ($p < 0.01$).

As indicated above, the reagents supplied by Squibb are readily prepared by simple dilutions without any further weighings and the shelf life of the reagents is longer which may be advantageous. Thus for example, the trisacetate buffer with bovine serum albumin is made only once for use with the Squibb kit with a shelf life of 1 month as against the trisacetate buffer to be weighed every week for dilution and pH adjustment and daily weighing of lysozyme for preparing the buffer needed for the test procedure using the Schwarz-Mann kit. The active solution once made is stable for 1 week using the Squibb procedure which, unlike the Schwarz-Mann procedure, has to be made the day the test is run.

DISCUSSION

These results indicate that the two commercially available test kits for radioimmunoassay of angio-

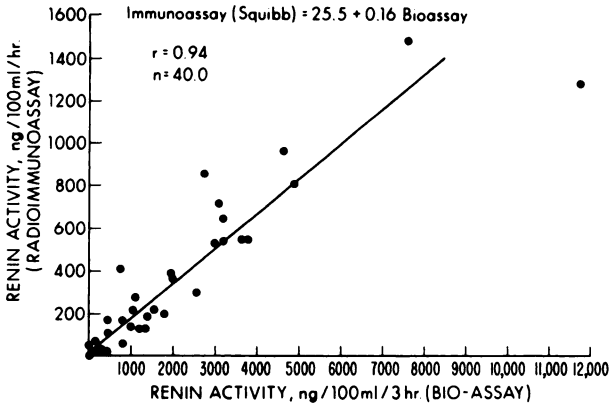

FIG. 2. Forty values of renin activity obtained from analysis with Squibb kit reagents are plotted against corresponding bioassay values. There is good correlation over very wide range of values. Regression equation is shown. Immunoassay is plotted as ng/100 ml for ease of comparison. Overlapping values are plotted as one dot.

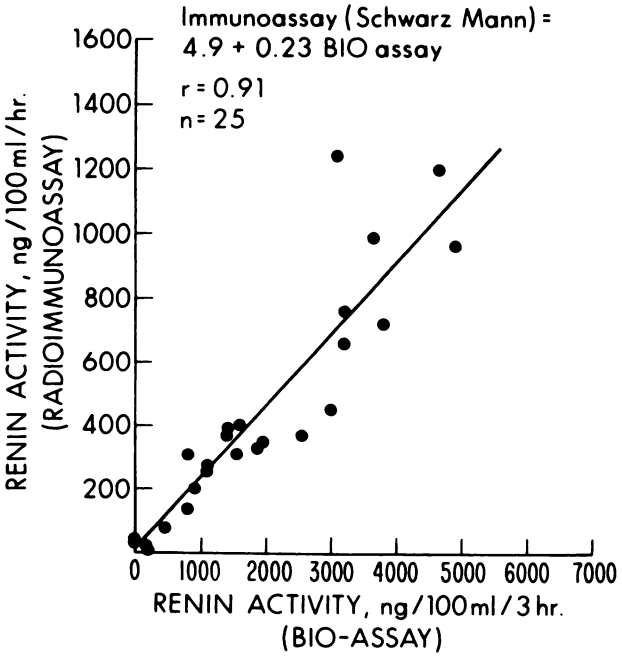

FIG. 3. Twenty-five values of renin activity obtained from analysis with Schwarz-Mann kit reagents are shown plotted against corresponding bioassay values. Regression equation is shown.

tensin I are capable of providing accurate measurements of renin activity. The data correlate to a high degree of statistical significance with the well established bioassay for renin activity. The major difference between the two kits appears to be the ease of performance rather than the reliability of the method. However, the user must constantly check the possible sources of error for variation among test lots.

In the Schwarz-Mann test procedure, repetitive pipetting of 0.9 ml of buffer is somewhat tedious, particularly when a large number of samples are to be assayed. Thus in the actual run, whereas the Squibb procedure involves four main pipetting steps, the Schwarz-Mann procedure has five such steps, and this, together with the above mentioned preparation of active reagent and buffer every day, constitutes a significant drawback for adapting the latter kit for assay of a large number of clinical samples. The 0.2 ml Biopette, available from Schwarz-Mann together with the adapter kit for dispensing different volumes, is not of much use in aliquotting the different volumes necessary for the calibration curve, and in our opinion, the Oxford microliter samplers of different capacities with disposable tips are accurate and make it easier to dispense the desired volumes including 1 ml active solution. The recommended speed at which the Falcon Plastics disposable polystyrene tubes can be centrifuged is below 3,000 rpm and in our experience several are broken at any higher speed. This is contrary to the centrifuging speed of 5,000 rpm recommended in the Schwarz-Mann procedure for packing down the charcoal in the tubes.

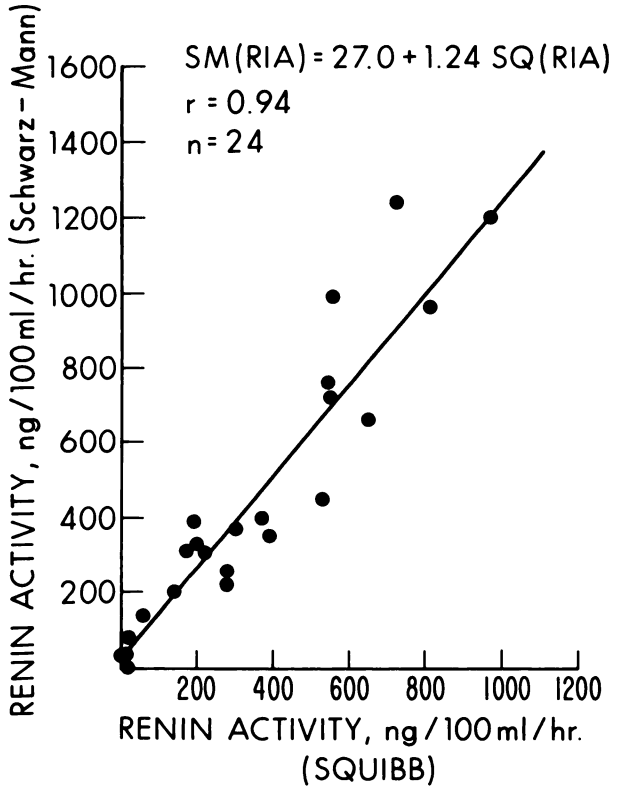

FIG. 4. Values obtained from duplicate analysis of 24 plasma samples with both commercial kits are shown. Schwarz-Mann kit yields slightly higher values than Squibb kit, but relationship between two is linear over wide range of values.

The slopes of 0.16 and 0.23 for the regression lines drawn in Fig. 2 and 3 may be compared with the value of 1.8 given by Cohen, et al (*8*) for a similar comparison of radioimmunoassay with bioassay. In the latter work, incubation of plasma is done at a pH of 5.5 at which value, generation of angiotensin I, it is reported, is approximately three times that at pH 7.5 (the incubation pH in the present study). Cohen, et al considered a pH of 5.5 as the optimum plasma incubation pH value with a shorter time of incubation for the plasma, particularly when excess substrate is not assured. This approach was not considered very suitable for routine assay using the kits on all plasma samples. However, in those clinical cases where renin activity levels are suspected to be abnormally low, as in primary aldosteronism, plasma incubation at pH 5.5 is perhaps warranted.

SUMMARY

The availability of the radioimmunoassay kits for measurement of renin activity levels through the generation of angiotensin I may prove to be of aid in the clinical evaluation of renal diseases in a number of medical institutions throughout the country. The present study has established that a high degree of correlation exists between the well established bioassay method and the radioimmunoassay results obtained using the kits. The comparative ease and accuracy with which the protocol for the radioimmunoassay procedure is followed has led to our use of immunoassay in favor of bioassay as a routine procedure.

ACKNOWLEDGMENT

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REFERENCES

1. Stockigt JR, Collins RD, Biglieri EG: Determination of plasma renin concentration by angiotensin I immunoassay. Diagnostic import of precise measurement of subnormal renin in hyperaldosteronism. *Circ Res* (Suppl II) 28, 29: 175–191, 1971

2. Bull MB, Hillman RS, Cannon PJ, et al: Renin and aldosterone secretion in man as influenced by changes in electrolyte balance and blood volume. *Circ Res* 27: 953–960, 1970

3. KOTCHEN TA, LYTTON B, MORROW LB, et al: Angiotensin and aldosterone in renovascular hypertension. *Arch Intern Med (Chicago)* 125: 265–272, 1970

4. BLAUFOX MD, FROMOWITZ A, LEE HB, et al: Renal blood flow and renin activity in renal venous blood in essential hypertension. *Circ Res* 27: 913–920, 1970

5. PAWSEY CG, VANDOGEN R, GORDON RD: Renal venous renin ratio in the diagnosis of renovascular hypertension. Measurement during active secretion of renin. *Med J Australia,* 1: 121–126, 1971

6. HABER E, KOERNER T, PAGE LB, et al: Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J Clin Endocr* 29: 1349–1355, 1969

7. GIESE J, JORGENSEN M, NIELSEN MD, et al: Plasma renin concentration measured by use of radioimmunoassay for angiotensin I. *Scan J Clin Lab Invest* 26: 355–367, 1970

8. COHEN EL, GRIM CE, CONN JW, et al: Accurate and rapid measurement of plasma renin activity by radioimmunoassay. *J Lab Clin Med* 77: 1025–1038, 1971

9. BOUCHER R, VEYRAT R, D'CHAMPLAIN J, et al: New procedures for measurements of human plasma angiotensin and renin activity levels. *J Canad Med Ass* 90: 194–201, 1964

10. BLAUFOX MD, BIRBARI A, HICKLER RB, et al: Peripheral plasma renin activity in renal homotransplant recipients. *New Eng J Med* 275: 1165–1168, 1966