SHORT COMMUNICATION

A COMPARISON OF DOUBLE-ISOTOPE DERIVATIVE AND RADIOIMMUNOLOGICAL ESTIMATION OF PLASMA ALDOSTERONE CONCENTRATION IN MAN

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SUMMARY

1. Two techniques for estimating plasma aldosterone concentration are compared by means of repeated assays of a plasma pool and also by analysis of a wide range of plasma samples.

2. No significant difference was found in the results obtained by the methods. Radioimmunoassay required only one tenth of the volume of plasma needed for the double-isotope derivative method.

3. Its rapidity and relative inexpensiveness makes radioimmunoassay at present the most suitable technique for large-scale population screening.

Key words: aldosterone, plasma, radioimmunoassay, double-isotope derivative assay.

Radioimmunoassay techniques for steroid assay are considerably more sensitive than the majority of physiochemical methods but may be less specific. One means of assessing such a quality is to compare the technique with another which has already been extensively validated. A range of plasma samples varying widely in source and in aldosterone concentration were therefore assayed both by a simple radioimmunoassay method and by a double-isotope derivative method (see below) and the results compared.

MATERIALS

Solvents and reagents for the double-isotope derivative assay were as described by Fraser & James (1968). Glass-fibre paper impregnated with silica gel (I.T.L.C. Type SAF) was obtained from Gelman Instrument Co. and used without further purification. For radioimmunoassay, all solvents were of 'Nanograde' quality (Mallinkrodt) and chromatography paper (Whatman no. 2) was washed with methanol in a Soxhlet apparatus before use.

Radioactively labelled aldosterone (New England Nuclear Corp.) was purified before use and stored at 4°C in methanol solution. Dextran T80 (Pharmacia) was used as supplied and Norit A charcoal (Hopkin and Williams Ltd) was washed thoroughly with methanol.

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Antiserum to aldosterone-21-hemisuccinate, conjugated with bovine serum albumin, was raised in the sheep and was a gift from the National Institutes of Health, Bethesda, U.S.A.

METHODS

Double-isotope derivative assay

The method of Fraser & James (1968) was used with two major modifications. After acetylation of the residue from the plasma neutral extract, excess of reagent was removed under high vacuum and the residue redissolved in methanol (0.2 ml) which was also removed in vacuo. The one-dimensional thin-layer-chromatographic (t.l.c.) system was replaced by two-dimensional t.l.c. on glass-fibre paper impregnated with silica gel.

Radioimmunoassay

Neutral extracts of 2 ml of plasma containing 25 000 c.p.m. of 3H-labelled aldosterone (50 Ci/mmol) were chromatographed on the Bush B5-type paper chromatography system of Mayes, Furuyama, Kem & Nugent (1970). The aldosterone region was located by means of isotope scanning equipment and was eluted dropwise with methanol (5 ml). Portions of the eluate were assayed for 3H recovery and by using this information, duplicate samples containing 5000 c.p.m. of 3H were removed for radioimmunoassay. Ethylene glycol (5%, v/v; 0.1 ml) in methanol was added to the samples and standards at this point.

The samples, together with standards ranging from 0 to 200 pg of aldosterone, each containing 5000 c.p.m. of 3H-labelled aldosterone, were evaporated to dryness at 30°C in a vacuum oven. The residues were then dissolved in antiserum solution [0.5 ml; 1:500 000 in 0.05 mol/l borate buffer, pH 7.5, containing 2% (v/v) methanol and 0.5% (w/v) bovine gamma-globulin] and incubated at room temperature for 1 h and then at 4°C for at least 1 h. Free steroid was absorbed by means of dextran-coated charcoal solution (0.5 ml; 0.25%, w/v, Norit A charcoal and Dextran T80) and after centrifugation at 4°C, 0.5 ml of the supernatant was assayed for 3H content by accumulating 10 000 counts.

RESULTS

Control data

(a) Double-isotope derivative assay. Plasma (20 ml) containing 14C-labelled aldosterone equivalent to 1.21 ng was used for each assay and duplicate samples of water and of a normal plasma pool were included in each batch of assays. During the period of this study, the gross blank was 1.43 ng±0.26 (SD) (n = 20) which, correcting for added 14C-labelled aldosterone, gives a mean net blank of 0.22 ng/sample. The mean concentration (ng/100 ml) of aldosterone in the plasma pool was 10.7±1.25 (SD) (n = 20).

(b) Radioimmunoassay. Over the period of this study, no measurable blank occurred when 2 ml of water was assayed. The normal plasma pool gave a mean concentration of 9.8±0.53 (SD) ng/100 ml (n = 16).

Comparison of the two methods

In addition to the comparison of results with a normal pool of plasma (see above), seventy-four plasma samples from a variety of normal subjects, resting and during such manoeuvres as sodium deprivation and angiotensin II infusion, from subjects with hypertension of various
Plasma aldosterone in man

FIG. 1. Correlation of plasma aldosterone concentrations measured by double-isotope derivative assay and by radioimmunoassay. ○, Samples from two pregnant subjects, for details see the text; ●, plasma from other subjects.

Aetiology and from pregnant women were analysed by both methods. The results are compared in Fig. 1. With the exception of the two results (○), the correlation coefficient \( r \) for the series was 0.97. The mean concentration by using double-isotope derivative assay (\( x \)) was 23.25 ng/100 ml ± 81.70 (SD) and for radioimmunoassay (\( y \)) was 21.57 ng/100 ml ± 18.19 (SD). The linear regression of radioimmunoassay on double-isotope derivative assay was: \( y = -0.34 + 0.94x \). The standard error was 4.53. The slope of the regression did not differ significantly from unity and had upper and lower confidence limits of 1.00 and 0.89 respectively (\( t = 1.96 \)).

Of this series of samples, thirty-one gave values of 15 ng/100 ml or less by both methods with mean concentrations for radioimmunoassay and double-isotope derivative assay of 7.46 ng/100 ml ± 3.81 (SD) and 7.92 ng/100 ml ± 4.31 (SD) respectively. Agreement within this group was slightly less satisfactory. The correlation coefficient \( r \) was 0.85, the linear regression: \( y = 1.55 + 0.75x \) with upper and lower confidence limits for the slope of 0.92 and 0.59 respectively (\( t = 1.96 \)) and a standard error of 2.05.

Taking the complete range of seventy-four comparisons, forty agreed within 2 ng/100 ml and a total of fifty-seven within 5 ng/100 ml but in the remaining seventeen a discrepancy greater than 5 ng/100 ml occurred. Of these seventeen, two (○) are discussed in more detail.
below and five were in the high concentration range where such a discrepancy is relatively unimportant. However, the remaining ten were within the normal range, three being classified as normal by one technique and above the upper limit of normal by the other.

Two results were widely discrepant, giving values by radioimmunoassay which were much lower than those by the physicochemical method. Both samples were obtained from women in the last trimester of pregnancy and the possibility of the presence of some factor in this condition which interferes with radioimmunoassay was considered. However, when five further subjects in late pregnancy were studied (x and y co-ordinates: 41.9, 42.1; 58.0, 59.6; 62.8, 61.0; 45.0, 46.8; 46.7, 46.1) no such discrepancy occurred.

DISCUSSION

The technique of radioimmunoassay, because it is highly sensitive and relatively simple, has been applied widely to the measurement of plasma aldosterone concentration. However, the absolute specificity of the technique is less easy to establish than that of physicochemical methods. Radioimmunoassay methods consist of simple extraction of plasma with organic solvent followed by minimal purification of the residue before assay. The 'purification' may consist of chromatography on paper (Mayes et al., 1970; Bayard, Beitins, Kowarski & Migeon, 1970), Sephadex LH 20 (Ito, Woo, Haning & Horton, 1972) or thin layers of silica gel (Banks, Ekins & Slater, 1971). Such procedures do not achieve total purification and methodological specificity may depend largely on the quality of the antiserum employed in the assay.

For this reason, assessment of specificity is frequently restricted to measurement of cross-reaction of the antiserum with steroids considered likely to interfere. Such an approach ignores the possible interference of unknown steroids and drug compounds and their metabolites, as yet incompletely understood. Metabolites of spironolactone, for example, cross-react with some aldosterone antisera (P. Vecsei & C. Joumaah, personal communication). Since specificity is a function both of the preliminary purification and the quality of the antiserum, an attempt has been made to assess the overall specificity of a simple radioimmunoassay by comparing its performance with that of a double-isotope derivative assay which has been tested exhaustively over a period of 6 years. The antiserum (NIH 088) is in use in many countries. This comparison may therefore be of wide interest.

Over the complete range of assays (0–80 ng/100 ml) there was a slight tendency by the radioimmunoassay method to underestimate as compared with the physicochemical method and this tendency was somewhat greater within the normal range (0–15 ng/100 ml). In a small series of six comparisons, Ito et al. (1972) found a similar discrepancy when paper chromatography was used but not when purification was accomplished by gel filtration. Although great care was taken in cleaning chromatography paper, some interference may be derived from this source. No evidence has been obtained that radioimmunoassay is less specific than the physicochemical method for the cases examined here. The radioimmunoassay technique is ideally suited to large-scale screening arising from hypertension surveys in the population.

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REFERENCES


