MODIFIED RADIOIMMUNOASSAY FOR PERIPHERAL PLASMA ALDOSTERONE

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Received 1 April 1975

1. Introduction

Since a monospecific antiserum has not, as yet, been obtained for aldosterone, it is necessary to minimize the possible interference of other plasma steroids such as cortisol and cortisone by purification because of the relative amounts in which they occur physiologically. The losses of aldosterone during this purification step depends largely on the used chromatographic separation method e.g. chromatography on paper [1], Sephadex LH 20 [2] or thin layers of silica gel [3]. The plasma aldosterone concentration was measured with a modified procedure of the radioimmunoassay previously reported in [1].

2. Materials

Tritiated aldosterone (50 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, England), purified by paper chromatography and stored at 4°C in an absolute, pure ethanol solution. d-Aldosterone (crystalline) was obtained from Steraloids Inc. (Pauling, New York, USA) and a stock solution of 1 μg of d-aldosterone per ml absolute, pure ethanol was prepared every three months and also stored at 4°C. All the used solvents are spectroscopic grade. Whatman No. 1 chromatography paper has been washed continuously for 3 days with methanol in a Soxhlet extraction apparatus and dried immediately before use. Norit A charcoal (Scharz & Mann, Orangeburg, USA.) was thorougly washed with methanol. A very specific antiserum to aldosterone-21-hemisuccinate, conjugated with bovine serum albumin, was raised in the sheep (088) and was kindly given by the National Institutes of Health, Bethesda, U.S.A.

3. Methods

After the extraction of aldosterone from 4 ml peripheral plasma, which contains 3000 cpm of 1,2-[^{3}H]aldosterone, with 20 ml methylene chloride (nanograde) and removal of the plasma layer by aspiration, the organic extract was dried unter a stream of nitrogen at 37°C. The dry residue was dissolved in a few drops of a methanol–acetone solution (1:1) and purified by descending chromatography on Whatman No. 1 paper for 16 hr in a benzene–methanol–water–hexane solvent system of McCaa et al. [4]. The aldosterone region was located between the cortisol and cortisone standards, cutted out and eluted with 8 ml methanol. An aliquot (1 ml) of the eluate and 100 μl of standards ranging from 0 to 200 pg of d-aldosterone and containing 300 cpm of tritiated aldosterone, both in duplicate, were dried in siliconized, glass tubes. The dried residues were dissolved in 0.5 ml antiserum solution (dilution titer 1/500 000 in 0.05 M borate buffer pH 8.0 containing 0.5% bovine serum albumin). After mixing and preincubation at 37°C for 10 min, the tubes were incubated for 24 hr at 4°C. The free and bound aldosterone were than separated by means of 0.5 ml dextran-coated charcoal solution (0.25%, w/v, Norit A charcoal and dextran T 70) in icewater for 10 min and centrifuged at 2000 *g* for 5 min at 4°C. Five-tenth ml of the supernatant was transferred to a scintillation vial, mixed with 10 ml Corusolve (ICN,

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North-Holland Publishing Company – Amsterdam

Portland, USA) and counted for 10 min in a Packard liquid scintillation counter. An equal aliquot of the eluate was also taken for the determination of the losses of labelled aldosterone during the purification step. A standard curve was set up by plotting the percent bound aldosterone vs the added pg of aldosterone in the standards. The aldosterone content of the unknown plasma samples was extrapolated from this curve.

4. Results and discussion

The mean recovery of 1,2-[^{3}H]-aldosterone after the extraction and purification step was 65.56 ± 6.62 (SD) % (n=49). Samples with recoveries less than 40% were discarded. Before the purification step the mean recovery of aldosterone in 10 plasma samples was 95.30 ± 1.84 (SD) %. The mean plasma aldosterone concentration for a plasma pool, obtained from 10 normal subjects on an ad lib diet, was 19.67 ± 2.12 (SD) ng/100 ml (n=18) with a variation coefficient of 10.8%. The accuracy of the method was tested by estimation of the plasma aldosterone concentration in 4 aliquots of increasing volume from a plasma pool. There was a linear relationship over the range 0–120 pg of aldosterone. Redistilled water was used for the determination of the blank value. The mean aldosterone content of 10 water blanks was 0.33 ± 0.23 (SD) ng/100 ml.

With this modified radioimmunoassay it is possible to measure the plasma aldosterone concentration with a good reproducibility, precision, sensitivity and accuracy. The need for a chromatographic purification step negates however potential simplicity of the radioimmunoassay and introduces a new concept in the immunoassay of peptide hormones, namely the 'blank' due to solvent residues and chromatogram eluates. The blank value can be strongly reduced by using pre-washes chromatography paper. The purity of this paper is very important.

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