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RADIOIMMUNOASSAY FOR STEROID HORMONES VI. RADIOIMMUNOASSAY FOR HUMAN PLASMA DESOXYCORTICOSTERONE

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Abstract

A radioimmunoassay for measurement of human plasma desoxycorticosterone (DOC) has been developed. Antiserum was raised against DOC-21-hemisuccinate conjugated to bovine serum albumin. Plasma DOC was purified from crossreacting steroids by single column chromatography (8 mm, i. d. ×260 mm, length). The coefficients of variation for within-assay and between-assay were 13.0 % and 8.6 %, respectively.

INTRODUCTION

Desoxycorticosterone (DOC) is a powerful mineralocorticoid next to aldosterone in the human, and secreted from adrenal fascicular and glomerular zones.[1,2] However, little is known about the action of DOC in humans, and this is partly due to the lack of simple and specific methods for measurement of DOC.

In the present study, by using an antiserum raised against DOC-21-hemisuccinate BSA, a sensitive and specific radioimmunoassay was developed for measurement of DOC in human plasma.

MATERIALS AND METHODS

1. Chemicals. DOC-1,2-^{3}H (41.8 Ci/mM, New England Nuclear Corp.) was used after purification with thin-layer chromatography. Non-radioactive, standard DOC was obtained from Sigma Chemical Co. Series of steroid hormones used for cross-reactivity study of assay were kindly supplied by Teikoku Zoki Pharmaceutical Co., Japan. All other chemicals were prepared in the same way as previously reported.[3-7]
2. Antigen and antiserum. A production of DOC-21-hemisuccinate BSA and

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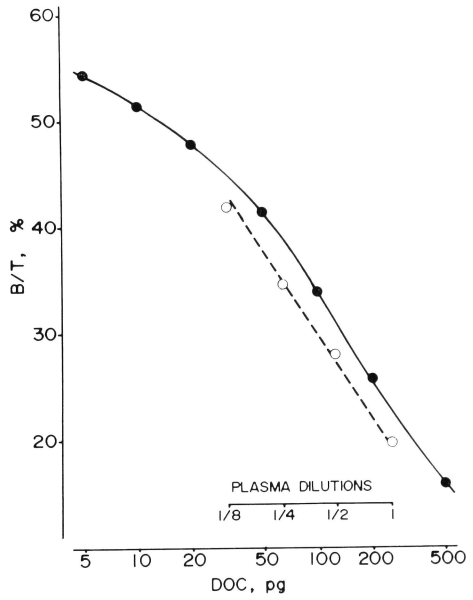

Fig. 1. A typical dose response line and dilution test on semi-logarithmic scale. Dose response line ; each closed circle represents the mean from duplicate determinations. The final dilution of anti-DOC serum was 1 : 50,000. Dilution test ; original plasma was diluted to 1 : 2, 1 : 4 and 1 : 8. Each open circle represents the mean from four determinations.

immunization of rabbits with the antigen-BSA conjugate was done according to the methods previously reported.[3]

3. Sample preparation and chromatography. DOC was extracted with 2.5 ml of ether from 0.5 ml plasma in assay tube (12 mm × 150 mm) containing dried 2,000 dpm of DOC-^{3}H. After washing with 0.5 ml of water and getting dry, DOC in the extract was separated with column chromatography (8 mm × 260 mm). A height of Sephadex LH-20 column was 210 mm. A mixture of benzene : methanol, 85 : 15 (v/v) was used for developing solvent, and the collected fractions (from 6.5 to 8.0 ml) were divided into two parts, for assay and recovery counting.
4. Radioimmunoassay and radiocounting. The entire assay after chromatographic purification and counting of the radioactivity were performed as in the previous report.[4-7] The antiserum was diluted to 1 : 50,000.

RESULTS AND DISCUSSION

1. Following an extraction and chromatographic separation, the recovery of ^{3}H-DOC was 53.5 ± 9.4 % (mean ± SD) in 50 assays.

2. A typical dose response line and sensitivity. A typical standard curve of an assay is shown in Figure 1. Bound per cent was plotted against the logarithm of the dose of DOC. Bound per cent at 10 pg differed significantly from that of 0 pg. Consequently, a sensitivity of the assay was regarded as 10 pg of DOC in the standard curve.

3. Dilution test. A result of dilution test of DOC is shown in Figure 1. Original plasma, of which concentration of DOC was 250 pg/ml , was diluted to 1 : 2, 1 : 4 and 1 : 8, and these diluted plasma showed an identical curve with authentic standard curve.

4. Within assay and between assay. The results of within assay and between assay experiments are shown in Table 1. The average coefficient of variation of within assay and between assay were 13.0 % and 3.7 %, respectively.

TABLE 1.

Precision and reproducibility of plasma DOC radioimmunoassay

Sample	DOC, pg/ml (average)	CV, %
Within assay (n=6)		
A	99.2	14.3
B	136.3	15.2
C	233.1	10.8
D	372.0	11.8
		13.0 (average)
Between assay (3 different occasions)		
E	112.3	9.9
F	158.4	8.6
G	250.8	8.1
H	376.9	7.7
		8.6 (average)

5. Accuracy of recovery. The mean recovery of added DOC from 0.5 ml plasma was 93.5 % (Table 2).

6. Specificity and chromatography. The specificity of antiserum (anti-DOC-3-1) was studied by cross reaction method with various steroids (Table 3). The antiserum showed significant crossreactivities with a lots of steroids other than DOC. Microcolumn chromatographic purification as previously reported[4-6] did not work for separating DOC from those crossreacting steroids. Although many studies have been done on the purification of DOC from peripheral plasma, they are mostly time consuming and too laborious for routine clinical

TABLE 2.

Accuracy of recovery of added DOC from plasma

DOC added (pg)	DOC determined (pg)	Recovery (%)
0	86.0	
10	92.9	96.8
20	94.9	89.5
50	114.0	83.8
100	183.3	98.5
200	278.7	97.4
500	554.7	94.7
		93.5 (average)

DOC determined ; mean from four determinations

TABLE 3.

Cross-reactivity of anti-DOC-3-1 (1 : 50,000)

Steroid	Cross-reactivity, %
Progesterone	94.0
Testosterone	54.8
Pregnenolone	35.2
Androstenedione	35.0
17-OH-progesterone	24.3
11-desoxycortisol	23.5
5β-pregnanediol	21.5
Dihydrotestosterone	21.0
5α-pregnanedione	20.1
Dehydrocorticosterone	19.1
Dehydroepiandrosterone	12.6
17-OH-pregnenolone	12.4
Cortisone	11.9
Aldosterone	10.2
Androsterone	8.5
Corticosterone	7.8
Cortisol	6.2
Etiocholanolone	3.9
Estrone	2.7
Estradiol	2.1
Epiestriol	1.9
Tetrahydrocortisol	1.9
Estriol	1.8
Pregnanetriol	1.6
Dexamethasone	<0.1

use. They include paperchromatography[8-18] or two long columns (0.9× 70 cm) with different solvents[11]. In the present study, we used mess-pipet (8 mm,i.d. ×260 mm, length) with 210 mm height of Sephadex LH-20, and favorable purification of DOC was obtained. This may be, partly, influenced by the magnitude of crossreactivity of the antiserum with various steroids.

7. Mean plasma DOC level at 9 a.m. was 103.1 ± 17.0 pg/ml (mean ± SD), and 1165.5 ± 126.0 pg/ml at 4 hrs after 500 mg metyrapone, p.o., in 10 normal subjects. These are slightly higher than previously reported values[8-11].

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