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A NON-CHROMATOGRAPHIC RADIOIMMUNOASSAY OF 18-HYDROXY-11-DEOXYCORTICOSTERONE IN HUMAN PLASMA

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SUMMARY

A method is described for a non-chromatographic assay of 18 hydroxy-11-deoxycorticosterone (18-OH-DOC) using a sensitive and specific antiserum. This direct measurement is assessed in terms of accuracy and precision. The mean 8 a.m. plasma 18-OH-DOC levels in the supine position was 10.1 ± 6.5 ng per 100 ml in 20 normal subjects and 9.4 ± 4.2 ng per 100 ml after two hours of movement. These values are correlated with those obtained in aldosterone. The ACTH-dependency of 18-OH-DOC is demonstrated by diurnal variation and treatment with dexamethasone.

INTRODUCTION

18-hydroxy-11-deoxycorticosterone (18-OH-DOC) is known to be secreted both by the animal [1–4] and human adrenal cortex [5–7], but its role in human hypertension still leads to some controversy. For instance, Melby *et al.*[5, 6] demonstrated an increased production of this steroid, whereas Ulick *et al.* found no evidence for a specific etiologic role of 18-OH-DOC in experimental and human hypertension [8, 9]. Since 18-OH-DOC levels in human plasma are low [6], only indirect or gas–liquid chromatographic methods have been employed in evaluations on biological fluids [6, 10]. Radioimmunological methods have recently been described [11, 12] using antisera with various degrees of specificity, all including a chromatographic step.

The present report describes the development and the applications of a non-chromatographic radioimmunoassay for 18-OH-DOC in human plasma in normal and physiological conditions.

List of abbreviations and trivial names: 18-hydroxy-11-deoxycorticosterone (18-OH-DOC), 18,21-dihydroxy-4-pregnene-3,20-dione; 11-deoxycorticosterone (DOC), 21-hydroxy-4-pregnene-3,20-dione; progesterone, 4-pregnene-3,20-dione; 17-hydroxyprogesterone, 17α-hydroxy-4-pregnene-3,20-dione; 11-deoxycortisol, 17α,21-dihydroxy-3,20-dione; corticosterone (B), 11β-21-dihydroxy 4-pregnene-3,20-dione,18-hydroxycorticosterone (18-OH-B), 11β, 18,21-trihydroxy 4-pregnene-3,20-dione; cortisone, 17α, 21-dihydroxy 4-pregnene-3,11,20-trione; aldosterone (aldo), 11β-21-dihydroxy-4-pregnene-3,18,20-trione; cortisol, 4-pregnene-11β,17α,21-triol-3,20-dione, testosterone. 17-hydroxy-4-androstene-3-one; prednisone, 1,4-pregnadiene-17α,21-diol-3,11,20-trione; prednisolone, 1,4-pregnadiene-11β,17α,21-triol-3,20-dione; dexamethasone, 1,4-pregnadiene-9α-fluoro-16α-methyl-11β,17α,21-triol-3,20-dione.

MATERIALS AND METHODS

Studies were carried out on normal healthy male and female volunteers on a normal sodium and potassium intake (120 and 70 mEq/day) respectively. Standard 18-OH-DOC was purchased from Searle (Mexico) and [1,2-^{3}H]-18-OH-DOC (45 Ci/mmol) from the Radiochemical Center (Amersham). Purity was checked by Sephadex LH-20 chromatography in the dichloromethane–benzene–ethanol (63:35:2, by vol.) system. Natural and synthetic steroids were obtained from Sigma Chemical Corporation. 0.1 M phosphate buffer (pH 7) containing gelatine (0.1%) was used throughout the RIA procedure. Dextran-coated-charcoal was prepared by adding 0.5 g charcoal (Norit A, Fisher Scientific Co.) and 0.05 g dextran T-70 (Pharmacia) to 100 ml. phosphate assay buffer. Instagel (Packard Instrument Co.) was used as counting solution. Radioactivity was measured using a Tri-Carb liquid scintillation spectrometer Packard (Model 3380) with 45% counting efficiency.

Antiserum

18-OH-DOC was coupled with o-carboxymethoxime-hydroxylamine according to the method of Erlanger *et al.*[13], with slight modifications. Investigations were performed at pH 8–9 in ethanol medium for 48 h at room temperature under stirring. Coupling to bovine serum albumine was achieved by the mixed anhydride method [14]. The calculated molar ratio of hapten to protein was 16:1 based on radioactivity.

1 mg of conjugate, dissolved in 0.5 ml saline solution 0.9% and emulsified with 0.5 ml Freund's adjuvant, was injected subcutaneously into three rabbits according to the following schedule: once a week for the first four weeks, every two weeks for the next

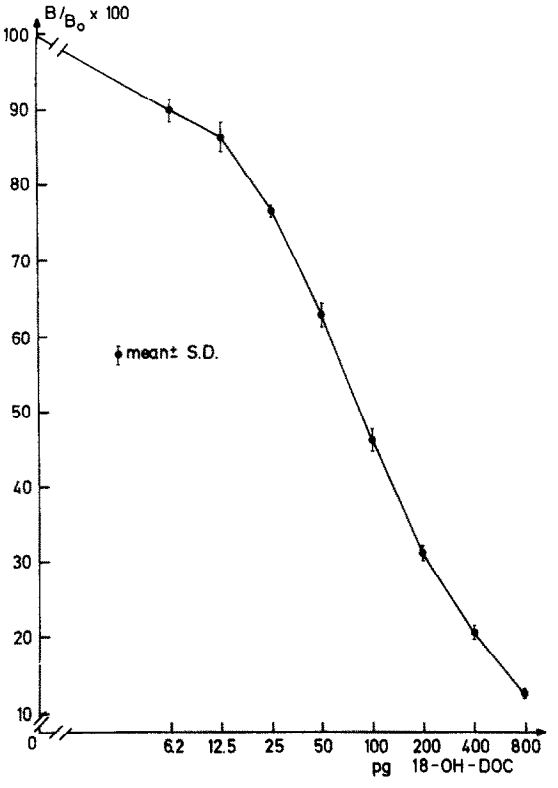

Fig. 1. Standard curve of 18-OH-DOC. Each point represents the mean (±S.D.) values calculated from six consecutive assays.

six weeks, and once a month thereafter. All three rabbits produced antibodies to 18-OH-DOC conjugate, but the antiserum described in this assay was obtained from one rabbit only, chosen on account of its specificity. The titer of the antiserum stored in a lyophylised form is 1/4000 (final dilution) at 50% binding.

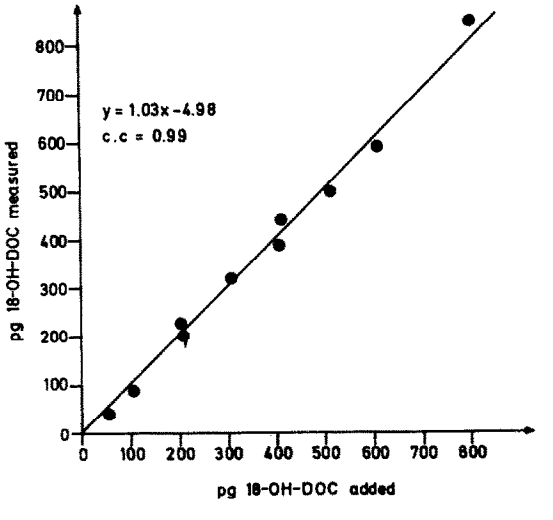

Fig. 2. Accuracy of the method. Recovery of known amounts of 18-OH-DOC added to plasma.

Procedure

1 ml of plasma is extracted with 6 ml of plasma by stirring on a vortex mixer for two min. The tubes are centrifuged and the plasma layer removed by aspiration. A 2 ml aliquot is brought to dryness in glass tubes (in duplicate). The incubation medium is prepared by adding 0.1 ml of tracer solution (containing about 3000 c.p.m.), 0.1 ml of antibody solution and phosphate buffer for an overall volume of 0.5 ml. The tubes are kept at 37°C for one h and then incubated at 4°C overnight.

At the end of the incubation time, the tubes are placed in an ice-water bath and 0.5 ml of charcoal suspension is added. The tubes are briefly vortexed and, following a ten min waiting time, are centrifuged at 2000 *g* for 10 min. The supernatant is transferred to a counting vial and 10 ml of instagel is added.

RESULTS

Standard curve

Figure 1 shows a typical standard curve obtained from six consecutive assays. The coefficient of variation determined at each point of the curve does not exceed 4%. The mean initial binding of [1, 2-^{3}H]-18-OH-DOC to the antibody is 40%. The average binding constant (K_0) and heterogeneity index of the antiserum are respectively 1.6 10^9 M^{-1} and 0.8, according to Nisonoff and Pressman[15].

Accuracy and precision

Known amounts of 18-OH-DOC were added to steroid-free plasma. The relationship between the amount of 18-OH-DOC evaluated versus added is shown in Fig. 2. The precision of the assay was estimated by multiple measurements of 18-OH-DOC in a pool of plasma. The mean values of 10 samples with normal and high concentrations of 18-OH-DOC were respectively 8.2 ± 0.9 (S.D.) and 30.7 ± 1.2 (S.D.) ng%. When different volumes of plasma were subjected to analysis, no difference was observed.

Table 1. Percent cross-reaction of various steroids with anti-18-OH-DOC-3-CMO-BSA serum

Steroids	% Cross-reaction
Progesterone	0.17
17-OH-Progesterone	0.001
11-Deoxycortisol	0.001
Cortisone	0.001
Testosterone	0.08
DOC	0.1
18-OH-DOC	100
Corticosterone	0.001
Aldosterone	0.03
Cortisol	0.001
18-OH-B	0.9
Prednisone	0.001
Prednisolone	0.001
dexamethasone	0.001

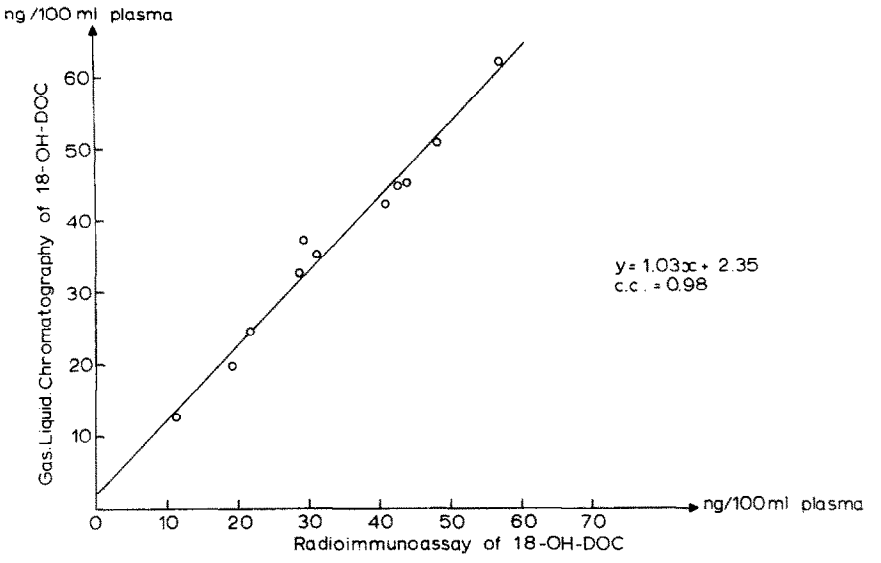

Fig. 3. Comparison of 18-OH-DOC radioimmunoassay with gas–liquid chromatographic method in plasma ($n = 12$).

Extraction of 0.5, 1 and 2 ml of a pool of plasma gave respectively 7, 8.8 and 7.9 ng%.

Specificity

The specificity of the antibody was evaluated by interference of competitive steroids. The relative activity of these compounds determined at the 50% displacement of [1, 2-^{3}H]-18-OH-DOC was negligible, as showed in Table 1. The reliability of the radioimmunoassay technique described was tested by a comparative study using a gas–liquid chromatographic method [10]. The correlation between the levels of 18-OH-DOC obtained by both methods are illustrated in Fig. 3.

Normal values

Plasma samples from twenty normal subjects (10 males and 10 females) were collected at 8 a.m. in the supine position following overnight recumbency and at 11 a.m. on the same day, after three h of movement. Subjects had received a diet containing 120 mEq of Na and 70 mEq of K for ten days before the blood samples were taken. In ten of these subjects, a further two samples were collected at 3 and 8 p.m., during a normal working day.

Mean values (± S.D.) in the supine position ($n = 20$) were 10.1 ± 6.5 ng% and 9.4 ± 4.2 ng% after movement ($n = 20$). Mean values at 3 and 8 p.m. ($n = 10$) were respectively 8.1 ± 4.0 and 5.9 ± 2.4 ng%, whereas mean values in these ten patients at 8 a.m. and 11 p.m. were 13.4 ± 7.0 and 10.9 ± 4.4 ng%. There was a significant drop in 18-OH-DOC at 3 p.m. ($P < 0.05$) and 8 p.m. ($P < 0.001$) in this group of ten subjects with respect to basal values. Mean basal values of aldosterone showed a significant increase in the upright position (from 7 ± 3 to 15 ± 4 ng%) ($n = 20$) whereas 18-OH-DOC levels remained unchanged

Treatment with dexamethasone

In a further 5 normal subjects, blood samples were drawn every two h from 8 a.m. to 8 p.m. The next day and for the following 9 days, subjects received 0.5 mg of dexamethasone every 12 h. 18-OH-DOC levels assayed in blood collected the day after termination of treatment showed a significant decrease in all subjects (Table 2).

DISCUSSION

With the specific radioimmunoassay described, it is possible to measure accurately low levels of 18-OH-DOC in human plasma. Using a 18-OH-DOC antisera chosen for its high specificity, a direct measure-

Table 2. Mean values (± S.D.) of 18-OH-DOC in five normal subjects before and after ten days of dexamethasone (0.5 mg every 12 h)

Hour	8	10	12	14	16	18	20
basal values	9.7 ± 1.7	8.2 ± 1.4	6.7 ± 1.2	5.7 ± 1.8	4.2 ± 1.3	3.5 ± 1.0	2.2 ± 0.9
after dexamethasone	3.2 ± 0.9	3.0 ± 0.8	2.5 ± 0.6	3.1 ± 1.0	1.8 ± 0.6	1.5 ± 0.7	1.0 ± 0.4

The samples were drawn every two h from 8 a.m. to 8 p.m. All values expressed in ng%.

ment of crude plasma extracts can be developed for routine clinical studies.

The basal values obtained here are in good agreement with those found by Chandler *et al.*[11] and Dale *et al.*[12] who, recently, developed a radioimmunoassay procedure for 18-OH-DOC in human plasma, including a chromatographic step. The different values previously obtained by Mason and Fraser [10] are probably due to the method employed and/or because blood samples were randomly drawn during the day.

Since 18-OH-DOC secretion has been demonstrated to be under the control of ACTH [5], the highest values of this hormone would be expected between 8 and 9 a.m. The behaviour of 18-OH-DOC observed in this study in fact confirms the ACTH-dependency and investigations will be performed to study the episodic behaviour of this steroid in order to establish whether a pulsatile activity may play a role in the wide range of values reported by Mason and Fraser.

The upright position stimuli did not affect 18-OH-DOC plasma levels, thus confirming the predominantly ACTH-dependency of this compound in normal conditions. These observations are in good agreement with those of Dale *et al.* but not with those of Chandler *et al.* who found higher values in a group of 6 subjects in the upright position than in another 6 subjects in the supine position.

The response of 18-OH-DOC to dexamethasone treatment (1 mg/day for ten days) confirms data obtained by other authors [11, 12].

In conclusion, this simple, sensitive and specific assay provides an important tool for the study of 18-OH-DOC levels and variations under physiological and pathological conditions.

A test to evaluate pulsatile activity of this steroid within a 24-h period should provide further data on the range of values in normal man.

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