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Note

Estimation of free urinary aldosterone and 18-hydroxycorticosterone by a combination of automatic high-performance liquid chromatography and radioimmunoassay

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Among the adrenal steroids, aldosterone and 18-hydroxycorticosterone (18-OH-corticosterone) are the only ones that are effectively regulated by the renal renin—angiotensin system [1, 2]. Disorders of this system, such as in primary hyperaldosteronism [3], or of adrenal biosynthesis, such as in type 2 methyl oxidase deficiency [4], are therefore signalled by altered secretion of these steroids. Consequently, the ratio of plasma 18-OH-corticosterone to aldosterone has recently been established as a potent marker of primary hyperaldosteronism [3]. Here, we present a method which allows the simultaneous estimation of the free urinary fractions of both steroids from a single urine sample using automatic high-performance liquid chromatography (HPLC) with subsequent radioimmunological quantitation.

EXPERIMENTAL

Materials and instrumentation

Solvents, reagents, extraction devices, non-labelled and labelled steroids were

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as previously described [5]. The aldosterone antiserum was a gift of the National Institute of Health, Bethesda, MD, U.S.A. The characteristics of the corresponding aldosterone radioimmunoassay (RIA) have been published by Haning et al. [6]. The features of the 18-OH-corticosterone RIA have been described previously [7].

A Hewlett-Packard high-performance liquid chromatograph (Model 1084 B), equipped with a variable injector, a UV detector at 254 nm, and an automatic sampler was used. The HPLC system used [column 250 × 4.5 mm I.D., with a polar-coated silica (DIOL®, particle size 5 μm, Knauer, Berlin, G.F.R.), *n*-hexane—isopropanol and gradient elution] was the same as already described for the estimation of free urinary cortisol [8].

Analytical procedure

Urine (2 ml) traced with ^{3}H-labelled aldosterone and 18-OH-corticosterone (about 75 Bq of each) was extracted with 20 ml of diethyl ether using a solid-phase technique [9]. The evaporated residues were redissolved in 150 μl of *n*-hexane—isopropanol (85:15, v/v) and subjected to HPLC. Sampling, chromatography and collection of the steroid-containing fractions were achieved automatically [5]. Retention times of steroid fractions were calibrated before each batch of samples using UV-visible amounts of steroid standards (about 500 ng). The coefficient of variation of retention times was less than 1% [8]. The organic solvents of the eluted fractions were evaporated, and the residues were redissolved in 180 μl of ethanol—water (50:50, v/v). An aliquot of 50 μl was counted for ^{3}H-radioactivity in order to monitor procedural losses. A further 50 μl in duplicate were subjected to RIA. Evaluation of the data in final terms of excretion rates per day was done by a computer program using "spline-approximation" as standard curve model [10].

RESULTS AND DISCUSSION

Fig. 1a demonstrates the chromatogram with UV detection of a steroid mixture chromatographed by the HPLC system described. It is seen that aldosterone and 18-OH-corticosterone are distinctly separated both from each other and from the major endogenous steroid hormones. The chromatogram with UV detection of the organic extract of a normal urine sample is shown in Fig. 1b. This demonstrates that considerable amounts of non-specific, UV-absorbing compounds prevail in the areas of aldosterone and 18-OH-corticosterone. Still more clearly than for the free urinary cortisol estimation [8], it is evident that quantitation of aldosterone and 18-OH-corticosterone solely by UV detection is not possible if one considers the normal ranges of both urinary steroid excretions, which are about ten to fifty times lower than those of urinary free cortisol [8, 11—14].

The chromatograms of aldosterone and 18-OH-corticosterone immunoreactivities arising in 1-min fractions eluted by HPLC of normal urine samples are outlined in Fig. 2a and b. Although the antisera used are described as specific ones [6, 7], it is clear that considerable amounts of non-specific immunoreactivities arise in the organic extract of normal urine.

Intra-assay variability in terms of the coefficient of variation was found to be

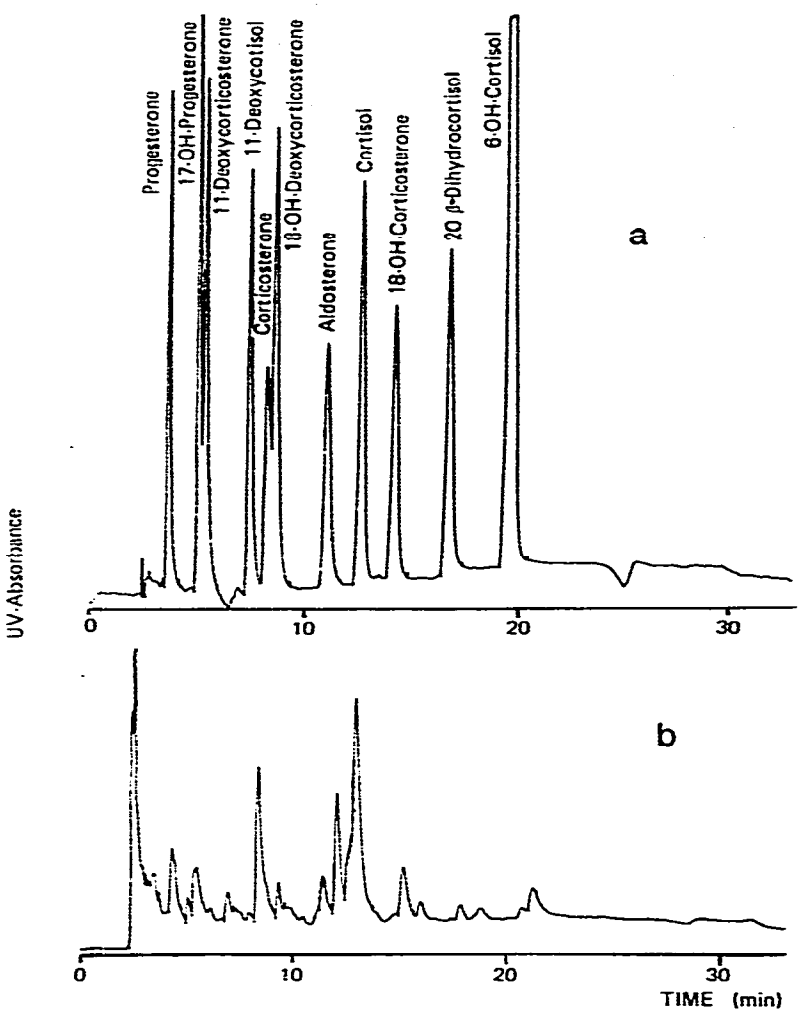

Fig. 1. HPLC of a mixture of steroid standards (a) and of the organic extract of a normal urine specimen (b). The amount of each steroid was 500 ng (a). HPLC system used: column, DIOL®; solvent A, *n*-hexane; solvent B, *n*-hexane—isopropanol (75:25); stepwise linear gradient from 50 to 100% solvent B; flow-rate, 1.3 ml/min; temperature of column, 40°C; detection at 254 nm.

9.4% for aldosterone and 15.9% for 18-OH-corticosterone ($n = 12$). The corresponding data on inter-assay variability were 13.5% for aldosterone and 19.2% for 18-OH-corticosterone ($n = 8$).

The reference ranges of the 24-h excretion rates were established in normal subjects who were on free diet and were following their normal daily activities. The upper and the lower limits, if calculated according to a logarithmic distribution, were found to be 0.39—1.09 nmol per 24 h for aldosterone ($n = 86$) and 3.79—9.17 nmol per 24 h for 18-OH-corticosterone ($n = 42$). The data on free aldosterone excretion are similar or slightly higher than those published in the literature [11—14], the differences potentially being caused by different dietary conditions. The data on 18-OH-corticosterone excretion accord well with those previously published [12]. Furthermore, the data also confirm

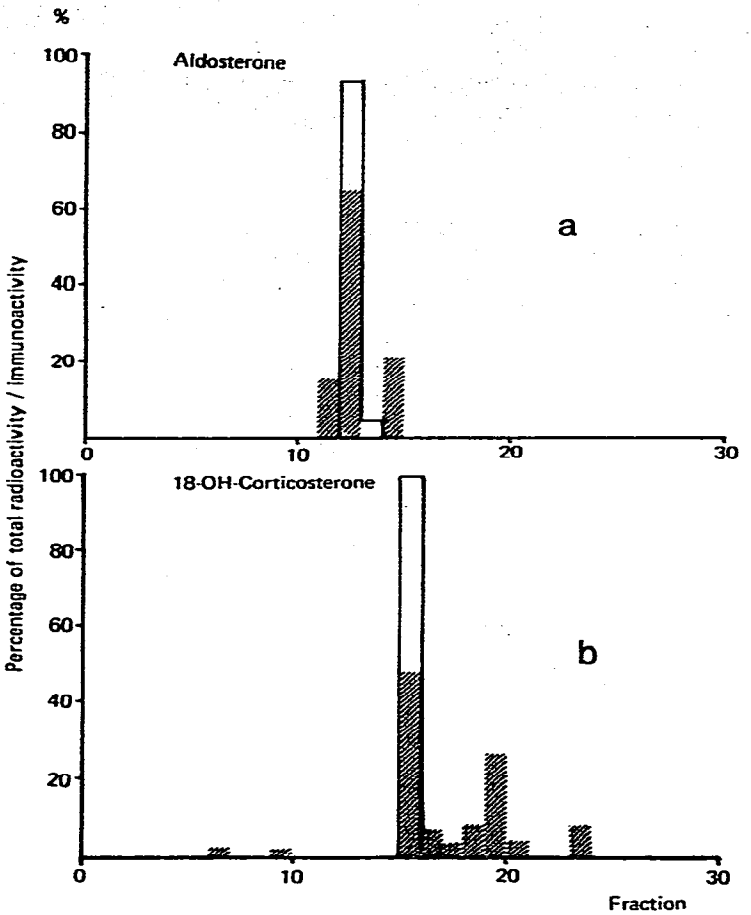

Fig. 2. Profiles of aldosterone and 18-OH-corticosterone immunoreactivities (hatched areas) and ^{3}H-radioactivities (open areas) in 1-min fractions eluted by HPLC. Values are plotted as percentage of total immunoreactivity and radioactivity, respectively.

earlier findings [12] that the ratio of the excretion rates of free urinary 18-OH-corticosterone to aldosterone (about 10) is significantly higher than the ratio of the serum levels of these steroids (about 3) [5].

If compared with the classical techniques of chromatography, the automatic version of the present HPLC technique renders this method considerably practicable. Thus, by applying automatic overnight chromatography, the processing of about 40 samples requires only one day of manual operations.

In conclusion, the present data on considerable unspecific immunoreactivities both of aldosterone and 18-OH-corticosterone document that specific estimation of these low-concentration steroids in urine can not be realized by exclusive immunological quantitation, but requires efficient, preceding purification. Thus, the present method, involving automatic HPLC, represents a significant advantage over conventional chromatographic techniques.

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