Journal of Chromatography, 310 (1984) 386—389
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2217

Note

Simultaneous determination of fifteen steroid hormones from a single serum sample by high-performance liquid chromatography and radioimmunoassay

G. EIBS

Department of Pediatrics, Division of Pediatric Endocrinology, Klinikum Charlottenburg, Free University Berlin, Heubnerweg 6, D-1000 Berlin 19 (F.R.G.)

and

M. SCHÖNESHÖFER*

Institute for Clinical Chemistry and Clinical Biochemistry, Klinikum Charlottenburg, Free University Berlin, Spandauer Damm 130, D-1000 Berlin 19 (F.R.G.)

(First received February 16th, 1984; revised manuscript received May 4th, 1984)

The precise biochemical diagnosis of inborn errors of steroid biosynthesis requires the estimation of the steroidal substrates and products of all the enzymes involved in steroid biosynthesis [1, 2]. Generally, the diagnosis of those inborn defects is the subject of paediatric endocrinology, and therefore analytical methods have to provide estimates of multiple steroids from small samples [3, 4].

In the present communication, we describe the evaluation of a routine-suited method, which allows the simultaneous estimation of fifteen steroid hormones from a single, small-volume sample. These steroid hormones are progesterone (P), androstenedione (AD), pregnenolone (PL), 5α-dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), testosterone (T), 11-deoxycorticosterone (DOC), 17-hydroxyprogesterone (17-OHP), 17-hydroxypregnenolone (17-PL), 11-deoxycortisol (S), 18-hydroxy-11-deoxycorticosterone (18-OH-DOC), corticosterone (B), aldosterone (Aldo), cortisol (F), and 18-hydroxycorticosterone (18-OH-B). In principle, the procedure involves high-performance liquid chromatography (HPLC) followed by radioimmunological quantitation [4]. Using the present method, the reference ranges of all steroid hormones from children 4—10 years' old were determined.

0378-4347/84/$03.00

EXPERIMENTAL

Materials

Reagent-grade chemicals and solvents were used throughout. Extrelut® was purchased from E. Merck (Darmstadt, F.R.G.); plastic syringes (20 ml) used as extraction columns were from Pharmaseal Labs. (Glendale, CA, U.S.A.). Radioactive steroids were from New England Nuclear (Boston, MA, U.S.A.). Other solvents, reagents and accessories used for radioimmunoassay (RIA) were as previously described [4].

High-performance liquid chromatography

The high-performance liquid chromatograph was from Hewlett-Packard (Model 1084 B). The polar bonded phase (DIOL®, 5 μm, 250 × 4.6 mm) was from Knauer, Berlin, F.R.G. Gradient mode was used for elution.

HPLC system I. Solvent A = *n*-hexane; solvent B = *n*-hexane—isopropanol (75:25, v/v). The gradient was run from 20% to 100% of B within 40 min; flow-rate 1.3 ml/min. The temperature of the column oven was 40°C. Ultraviolet (UV) detection was at 254 nm.

HPLC system II. Solvent A = *n*-hexane; solvent B = *n*-hexane—isopropanol (85:15, v/v). The gradient was run from 15% to 100% of B within 30 min; other conditions were as in system I.

Methods

Blood was drawn by venipuncture from nineteen children aged 4—10 years, who had no signs of endocrine diseases. Serum was stored until analysis.

Serum samples (1 ml) were spiked with trace amounts of ^{3}H-labelled isotopes of all steroids. Steroids were extracted from serum samples into 5 ml of diethyl ether using the solid-phase (Extrelut) technique [5]. The evaporated organic extracts were redissolved in *n*-hexane—isopropanol (85:15, v/v) and subjected to automatic HPLC using system I. The times for collecting individual steroids were calibrated by a run of UV—visible amounts or of tritiated steroids prior to each assay [4]. In a first run, fractions were collected containing the steroids P, AD, 17-PL, S, 18-OH-DOC, B, Aldo, F and 18-OH-B. The steroids PL, DHT, DHEA, T and DOC were collected in a common fraction. This latter fraction was evaporated, redissolved in *n*-hexane—isopropanol (95:5, v/v) and again subjected to HPLC using system II.

Quantitation of steroids from the individual organic fractions by RIA and computer evaluation have been described previously [4].

RESULTS AND DISCUSSION

The efficiency of the solid-phase extraction step was better than 95% for all steroids when checked with ^{3}H-recovery measurement.

The HPLC system I (upper chromatogram of Fig. 1) had been successfully applied for the efficient separation of eleven steroid hormones in a single run [4]. From the chromatogram of all the fifteen steroids under study, however, it is evident that the more unpolar steroids PL, DHT, DHEA, T and DOC are not sufficiently resolved from each other by HPLC system I. Since the amount

of serum may become problematic in the paediatric laboratory, it was necessary to cumulatively collect the unresolved non-polar steroids from the first run and to subject them to a second system which provides sufficient resolution of these steroids (lower chromatogram of Fig. 1).

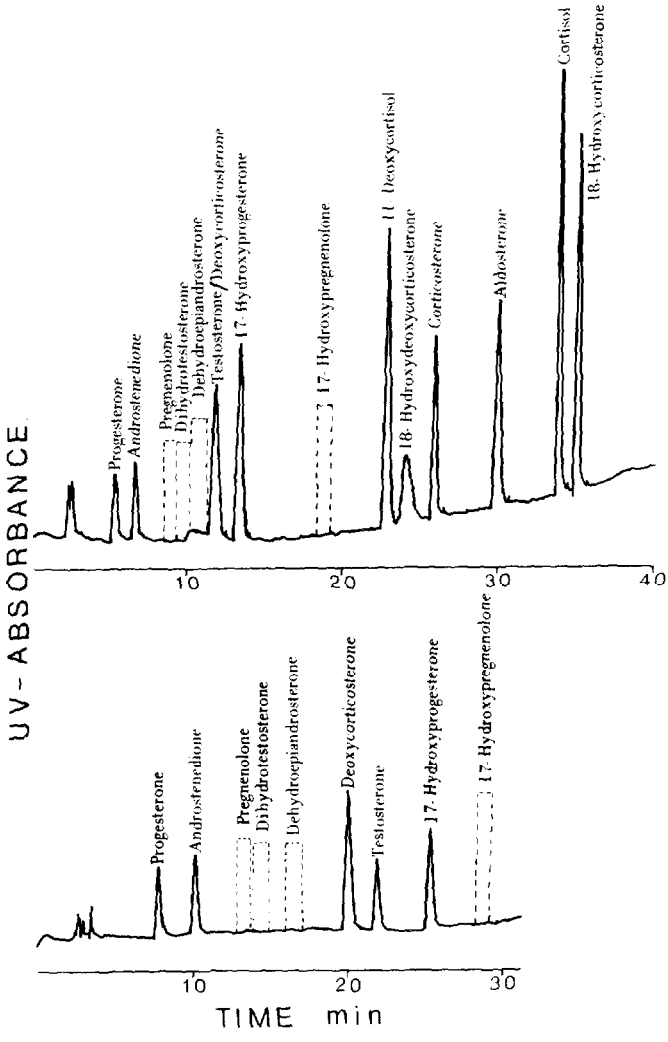

Fig. 1. Chromatograms of steroid standards. Amount of each steroid injected was 750 ng. Steroids not detectable by UV absorbance were localized by ^{3}H-labelled steroid measurement (dotted lines). Upper chromatogram with HPLC system I. Lower chromatogram with HPLC system II.

The analytical variables, such as recovery, sensitivity, specificity, precision, accuracy and practicability, were comparable to those obtained in the eleven-steroid assay [4]. Due to the double chromatography, the overall recoveries of the non-polar steroids PL, DHT, DHEA, T and DOC were slightly lower than those of the other steroids.

The reference ranges of steroid concentrations in serum from 4—10-year-old children are listed in Table I.

TABLE I

CONCENTRATIONS OF FIFTEEN STEROID HORMONES IN SERUM SAMPLES FROM NINETEEN CHILDREN (THIRTEEN GIRLS, SIX BOYS) AGED 4—10 YEARS

Steroid	Median (nmol/l)	Range (nmol/l)
Progesterone	1.29	1.00—1.50
Androstenedione	1.13	0.76—1.34
17-Hydroxyprogesterone	0.57	0.43—0.78
17-Hydroxypregnenolone	3.61	1.76—4.45
11-Deoxycortisol	0.89	0.72—1.46
18-Hydroxydeoxycorticosterone	0.07	N.D.*—0.92
Corticosterone	9.47	6.74—33.77
Aldosterone	0.28	0.14—0.46
Cortisol	167.74	117.25—215.74
18-Hydroxycorticosterone	0.67	0.09—1.03
Pregnenolone	2.30	1.30—3.60
5α-Dihydrotestosterone	0.7	N.D.—1.74
Dehydroepiandrosterone	2.33	0.33—3.57
11-Deoxycorticosterone	0.16	0.03—0.38
Testosterone	0.16	N.D.—2.47

*N.D. = not detectable due to insufficient assay sensitivity.

All subjects were on a random diet and had no signs of endocrine or systematic diseases. The median as well as the lower and upper limits have been calculated according to a logarithmic distribution of values. The serum concentrations of the steroids measured are comparable with the results of previous publications [6–8].

ACKNOWLEDGEMENTS

The skillful technical assistance of Mrs. A. Fenner and B. Weber is gratefully acknowledged.

REFERENCES

1 M. Finkelstein and J.M. Schäfer, Physiol. Rev., 59 (1979) 353.
2 M.I. New and L.S. Levine, Clin. Biochem., 14 (1981) 258.
3 W.G. Sippell, F. Bidlingmaier, H. Becker, T. Brunig, H. Dörr, H. Hahn, W. Golder, G. Hollmann and D. Knorr, J. Steroid Biochem., 9 (1981) 63.
4 M. Schöneshöfer, A. Fenner and H.J. Dulce, J. Steroid Biochem., 14 (1981) 377.
5 M. Schöneshöfer and A. Fenner, J. Clin. Chem. Clin. Biochem., 19 (1981) 71.
6 P.A. Lee and C.J. Migeon, J. Clin. Endocrinol. Metab., 41 (1975) 556.
7 W.G. Sippell, H.G. Dörr, F. Bidlingmaier and D. Knorr, Pediatr. Res., 14 (1980) 39.
8 P.A. Lee, T. Xenakis, J. Winer and S. Matsenbaugh, J. Clin. Endocrinol. Metab., 43 (1976) 775.