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Hypertensive congenital adrenal enzymatic defects detected by high-performance liquid chromatography of corticosteroids

G. CARPENÈ*, A. VETTORETTI, F. PEDINI and S. ROCCO
Institute of Semeiotica Medica, University of Padova, Via Ospedale 105, 35128 Padova (Italy)
F. MANTERO
Institute of Clinica Medica III, University of Catania, Catania (Italy)
and
G. OPOCHER
Institute of Semeiotica Medica, University of Padova, Via Ospedale 105, 35128 Padova (Italy)

ABSTRACT

The simultaneous measurement of the adrenal deoxycorticosterone (DOC), 18-OH-DOC, corticosterone (B), 18-OH-B, 11-deoxycortisol (S) and cortisol (F) present in human plasma in cases of adrenal dysfunction was accomplished using a high-performance liquid chromatographic (HPLC) system with a UV detector and with a radioimmunoassay (RIA). After a solid-phase extraction, plasma samples were separated by HPLC using a gradient of water–acetonitrile–ethanol on a radial compressed reversed-phase column. In a 70-min cycle, a complete separation of adrenal steroids was accomplished. The UV detector allowed direct measurement of F in each plasma sample while in selected cases B and S were directly determined. It was therefore possible quickly to identify patients with hypertensive congenital adrenal enzymatic defects with this method: the 17-α-hydroxylase deficiency characterized by the absence of measurable levels of F with an evident peak corresponding to B and the 11-β-hydroxylase deficiency in which high levels of S without F are detected. The RIA of DOC, B, 18-OH-DOC and 18-OH-B complete the characterization of the adrenal defect. Therefore, with this HPLC method it is possible to recognize the major hypertensive adrenal enzymatic deficiencies such as the defect of 17-α-hydroxylase or 11-β-hydroxylase. With "RIA" detectors an almost complete spectrum of adrenal steroid secretion can be obtained.

INTRODUCTION

The investigation of adrenal enzymatic defects requires the specific determination of all major intermediates and end products of adrenal steroidogenesis. High-performance liquid chromatography (HPLC) offers many advantages over older types of chromatography such as higher resolution, a higher degree of reproducibility and, as has been proposed in recent years, the possibility of full automation [1,2] for the separation of a number of corticosteroids [3–5]. Therefore, it has been applied to identify and determine different hormones such as corticosteroids, glucocorticoids, estrogens and a variety of "non-classic" or so-called "minor" steroids and steroid conjugates in several conditions in plasma, urine and various tissue samples.

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An efficient separation and simultaneous measurement of corticosteroids in human plasma, particularly those of early step steroids, were recently achieved by reversed-phase HPLC and radioimmunoassay (RIA) [6]. In this paper, we describe the usefulness of this HPLC method as a diagnostic tool for patients with adrenal enzymatic defect.

EXPERIMENTAL

The HPLC–RIA determination of the adrenal steroids was performed as described previously [6]. Briefly, after the solid-phase extraction of plasma with Chem-Elut cartridges using methylene chloride as solvent, the samples were separated by HPLC using a ternary gradient (water–acetonitrile–ethanol) on a radial compressed reversed-phase column. The recovery was calculated by adding cortisone (E) and testosterone as internal standards and a tritiated aliquot of each steroid was analysed.

The sensitivity of the on-line spectrophotometer allows the direct assay of the cortisol (F) concentration and also, in some instances, that of corticosterone (B) and 11-deoxycortisol (S). For the other steroids, the selected fractions were collected, extracted and aliquots submitted to RIA/ or to recovery counting. RIA of the single steroids were performed with tritiated tracers and antibodies kindly provided by Professor P. Vecsei (Heidelberg, Germany) [7].

RESULTS

We studied six patients, phenotypically female (among which three were sisters), with 17-α-hydroxylase deficiency; when the diagnosis was made, they presented as young adult females with primary amenorrhea, hypertension and hypokalaemia. The two brothers, affected by 11-β-hydroxylase deficiency, presented hypertension, mild hypokalaemia and pseudoprecocious puberty.

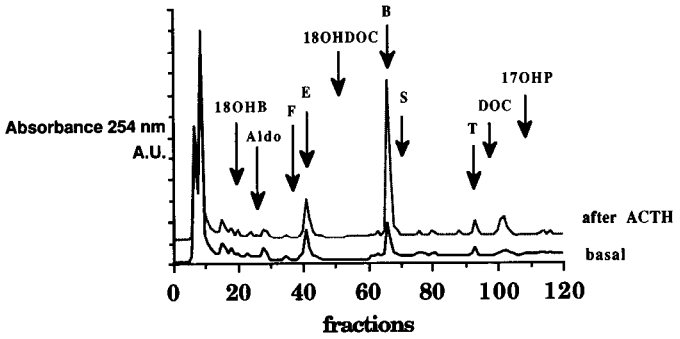

Fig. 1. Chromatograms of a plasma sample from a patient affected by 17-OHDS before and after ACTH administration. The arrow indicates the retention times of the steroids. Cortisone (E) and testosterone (T) are internal standards. Each fraction is 25 s. Column, RP C_{18} (4 μm), radial compressed. Solvent A, acetonitrile–ethanol (99:1); solvent B, water–ethanol (99:1); gradient profile, three linear steps from 28% to 44% of solvent A.

The concentrations of F, B and S were determined using the ratio of the peak area for the steroid to that of 1.14 μmol/l of cortisone (E) at a wavelength of 254 nm. The detection limit was 0.02 μmol/l. In the patients with 17-α-hydroxylase deficiency syndrome (17-OHDS) and 11-β-hydroxylase deficiency syndrome (11-OHDS) F is undetectable whereas B in the first case or S in the latter is very high, being more than ten times the upper limit of the normal range.

In all the patients the concentrations of B, deoxycorticosterone (DOC), 18-OH-DOC and 18-OH-B were also measured after off-line single-steroid RIA. In the 17-OHDS case, B, DOC, 18-OH-DOC and 18-OH-B are very high whereas F is very low. The cases of 11-OHDS have high DOC concentrations and reduced levels of F, B, 18-OH-DOC and 18-OH-B.

DISCUSSION

The adrenal gland plays a prominent role in the regulation of blood pressure. The hypersecretion of the most potent mineralocorticoid hormone, aldosterone, is responsible for the syndrome of primary aldosteronism which is characterized by hypertension, suppression of the renin–angiotensin system and hypokalaemia. Less frequently, adrenal hypertension is produced by other mineralocorticoid hormones (MCH) as in the DOC-secreting form of congenital adrenal hyperplasia, 11-β- and 17-α-hydroxylase deficiency [8,9]. In cases of 11-OHDS, the enzymatic system is missing in the mineralocorticoid and in the glucocorticoid pathways of the adrenal cortex, leading to impaired production of F and B. As a consequence, patients with 11-OHDS have high ACTH levels with high concentrations of all steroids proximal to the defect, *i.e.*, progesterone, 17-OH-progesterone, androgens, DOC and S. In cases of 17-OHDS, all steroids requiring hydroxylation in position 17 (glucocorticoids, androgens and estrogens) are very low whereas the MCH pathway, which does not require 17-α-hydroxylation, is stimulated by the excessive ACTH drive, due to the absence of F; excessive amounts of DOC, B, 18-OH-DOC and 18-OH-B are produced.

In the plasma of patients affected by adrenal enzymatic defects, with the "on-line" photometer, B or S was directly measured, whereas the peak of F, which is easily detectable in normal samples, was almost absent. Using two key steroids, F and B or F and S, the 17-OHDS and 11-OHDS cases are immediately identified. This assay seems to be very specific as this HPLC method allows a complete separation of the adrenal steroids (see the retention times of standards in Fig. 1) and the mean relative standard deviation (R.S.D.) of the retention times of individual steroids is very low, ranging between 1.9 and 0.8%; the intra-assay R.S.D.s for S, B and F are 8.1, 8.7 and 8.0%, respectively. The assay is rapid, requiring a few hours for twelve samples, as only the first two steps of the entire procedure are needed, namely extraction and HPLC separation; moreover, only a limited volume of plasma (1 ml) is required.

The concentrations of 18-OH-B, DOC and 18-OH-DOC are below the sensitivity of the photometer and cannot be measured by this method. Obviously, with the addition of the post-HPLC off-line single-steroid RIA this can be achieved and a more complete biochemical characterization can be obtained. This is required for a more sophisticated evaluation of patients. Nevertheless, when a rapid and specific diagnostic tool is needed, HPLC with direct assay of F and B or S is useful. Additional diagnostic information can be achieved after ACTH stimulation; in fact, as in the

example of 17-OHDS in Fig. 1, ACTH greatly stimulate B secretion (before the 17-α-hydroxylase) but failed to stimulate F (after the 17-α-hydroxylase) with a magnification of the enzymatic block.

In conclusion, this HPLC method can be used as a diagnostic tool in cases of 17-OHDS and 11-OHDS; even if limited to the direct assay of F, B and S, a biochemical pattern suggestive of an adrenal enzymatic defect can be obtained. Owing to its specificity and simplicity, this method can be proposed for screening programmes or large-scale assays.

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