A CASE OF ADRENOGENITAL SYNDROME WITH ABERRANT 11β-HYDROXYLATION

By

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ABSTRACT

A 17 year old female patient with hypertension, amenorrhoea and hirsutism was found to have subnormal levels of plasma and urinary cortisol, significant plasma levels of Reichstein's compound S and 21-deoxycortisol, high urinary levels of THS and pregnanetriolone as well as elevated levels of plasma and urinary testosterone. Treatment with 0.5 mg/day of dexamethasone or 25 mg/day cortisone reduced her hypertension and restored her menstrual cycles, but also resulted in the development of moon face, body striae and a gain in weight. Lower doses of cortisone were without effect. The deficient cortisol production coupled with the presence of unusual intermediates such as Reichstein's compound S and 21-deoxycortisol can be explained by a shift in the substrate specificity of 11β-hydroxylase from C-21-hydroxylated substrates (i.e. compound S) to C-21-deoxy substrates (i.e. 17-hydroxyprogesterone).

The most common forms of adrenogenital syndromes are those with steroid hydroxylation deficiencies at C-21 (Finkelstein et al. 1968) and C-11 (Bongiovanni et al. 1967). The variant with deficiency in C-21-hydroxylation is characterized by the urinary excretion of pregnanetriolone1) (Finkelstein et al. 1953) and the variant with C-11β-hydroxylation deficiency is characterized by an increased excretion of urinary THS (Eberlein & Bongiovanni 1955; Bongiovanni et al. 1967). Usually those different forms of adrenogenital syndro-
mes can be well defined by the particular excretion of the above steroids. There has been, however, one report (Thomas & Steinbeck 1969) which has mentioned a few patients who excreted these metabolites simultaneously. Recently we have studied a patient with adrenogenital dysfunction featuring the simultaneous excretion of these steroids and we wish to discuss the implications of our observations.

**CLINICAL CASE**

J. B., a 17 year old female patient was referred to us with the following complaints: nausea, headache, syncope, hypertension, hirsutism and secondary amenorrhoea of 6 months duration. Menarche occurred at the age of 13, but her periods were always irregular, appearing every 2–8 weeks; the bleedings were of 7–14 days duration. From the age of 15 she noticed excessive hair growth on the chest, face, abdomen and extremities. According to the anamnesis there were no cases of hirsutism, sterility or hypertension in the family.

Physical examination revealed that the patient was 155 cm in height, weighing 57 kg, with masculine hair distribution and musculature. Blood pressure was 160/90 mmHg in both arms. Neurological, ophthalmological and gynaecological examinations (including laparoscopy) revealed no pathological deviations. ECG, chest and skull X-rays were normal. Kidney and liver functions were normal. Haematological analysis showed a slight leucocytosis with normal differential white cell count. Fasting glucose, electrolytes, catecholamines and BUN were normal. IVP and selected renal arteriography did not reveal any abnormality; no tumour could be visualized in the suprarenal area after presacral air insufflation. Estimations of plasma renin activity and aldosterone were within the normal range, both under basal conditions and following intravenous administration of 40 mg furosemide. Her urinary excretion of 17-ketosteroids (17-KS) was 12–14 mg/24 h which is within the upper limits of our normal values for women, but the level of urinary testosterone was elevated to 24 µg/24 h (normal range 2–7 µg/h; Horn et al. 1972).

Because the clinical observations and the steroid estimations suggested a post-pubertal type virilizing syndrome the patient was treated with dexamethasone (0.5

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1) The following abbreviations and trivial names are used:

17-Hydroxyprogesterone, 17α-hydroxy-4-pregnene-3,20-dione;
Reichstein’s compound S (S), 17α-21-dihydroxy-4-pregnene-3,20-dione;
21-Deoxycortisol, 11β,17α-dihydroxy-4-pregnene-3,20-dione;
Corticosterone, 11β,21-dihydroxy-4-pregnene-3,20-dione;
Cortisol, 11β,17α,21-trihydroxy-4-pregnene-3,20-dione;
Cortisone, 17α,21-dihydroxy-4-pregnene-3,11,20-trione;
Testosterone, 17β-hydroxyandrost-4-en-3-one;
Pregnanetriol, 5β-pregnan-3a,17a,20α-triol;
Pregnanetriolone, 3α,17α,20α-trihydroxy-5β-pregnan-11-one;
Hexahydro-S, 5β-pregnan-3a,17α,20β,21-tetrol.

2) Plasma renin activity and aldosterone values were kindly supplied by Dr. A. Rosler of the Department of Chemical Endocrinology, Hadassah University Hospital.
mg/day) for 4 months. Normal menstrual cycles commenced 1½ months after therapy was started and blood pressure decreased to 115/80 mmHg; the headache and nausea disappeared. However, symptoms characteristic of glucocorticoid excess gradually developed. The symptoms included the appearance of moon face, buffalo neck and red body striae as well as a gain of 7 kg in weight; the somatic changes were accompanied by mental depression. Dexamethasone treatment was discontinued and when examined 1½ months later, the patient's weight had decreased from 64 to 60 kg and both the moon face and striae had disappeared. Blood pressure, however, had increased to 155/90 mmHg, the expected menstruation did not occur and the patient once again complained of headache and nausea. The patient's status was again evaluated at this stage (5 months after treatment with dexamethasone was discontinued) and dynamic tests were carried out which involved adrenal stimulation and suppression. The stimulation with ACTH (80 IU/day for 3 days) had an adverse effect on the patient; on the second day of ACTH treatment her face became reddish, rounded and Cushing-like. This effect was even more pronounced on the third day of treatment. Replacement therapy was again attempted two weeks later and the patient was treated with cortisone (25 mg/day) for 4 months. Under this treatment the response was similar to the dexamethasone therapy and the dosage of cortisone was reduced to 12½ mg/day for a 4 months period. With this dose the Cushing-like symptoms gradually disappeared, but blood pressure increased to 160/90 mmHg, headache and nausea reappeared, and the patient became amenorrhoeic. At this stage contact with the patient was lost.

**MATERIALS AND METHODS**

**Dynamic tests**

a) Adrenal stimulation. ACTH (80 IU/day) was administered im for 3 days. Blood was collected on the two days prior to and on the second and third day of ACTH administration. Twenty four h urine specimens were also collected on the two days before and during the second and third day of ACTH treatment. b) Adrenal suppression. Dexamethasone was given orally in a dose of 2 mg/day for 3 days. Urine was collected on the second and third day of treatment.

The following steroids were estimated: cortisol, corticosterone, testosterone, Reichenstein's compound S (11-deoxycortisol), 21-deoxycortisol, pregnanetriol, pregnanetriolone, tetrahydro-S and 17-ketosteroids. Since it was possible that the patient's sensitivity to small doses of corticoids was linked with a decreased cortisol binding capacity, plasma transcortin was also estimated.

**Plasma steroids and transcortin**

Plasma cortisol, corticosterone, compound S and 21-deoxycortisol were estimated by the competitive binding assay method using dog plasma CBG and [3H]corticosterone as tracer (*Murphy* 1967, 1969). 21-Deoxycortisol, estimation of which by competitive binding was not described hitherto, renders a curve similar to that of cortisol. Twenty ml of plasma, to which 10,000 cpm of each of the appropriate [3H]steroid had been added, were extracted with ether and the relevant steroids separated by paper and thin layer chromatography. Aliquots of the eluates were taken for the binding assay and for recovery estimation. In all cases the recoveries were between 45–55% and adjustments were made accordingly. Plasma testosterone was estimated according to *Horn et al.* (1972). Plasma transcortin was estimated according to *Doe et al.* (1964).
**Table 1.**

Plasma and urinary steroids in patient J. B. under basal conditions and after ACTH stimulation or dexamethasone suppression.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Blood μg/100 ml</th>
<th>Urine μg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ACTH</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt; 2.0 (7–10)&lt;sup&gt;1,10&lt;/sup&gt;</td>
<td>3.0</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>0.05 (0.15–0.4)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.2</td>
</tr>
<tr>
<td>21-Deoxycortisol</td>
<td>3.3 (&lt;0.3)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.3</td>
</tr>
<tr>
<td>Reichstein's compound S</td>
<td>0.2 (0.01–0.07)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.7</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.26 (&lt;0.1)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.2</td>
</tr>
<tr>
<td>Pregnantriol</td>
<td>150 (100–800)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>600</td>
</tr>
<tr>
<td>Pregnantriolone</td>
<td>80 (&lt;2)&lt;sup&gt;8&lt;/sup&gt;</td>
<td>375</td>
</tr>
<tr>
<td>THS</td>
<td>80 (&lt;30)&lt;sup&gt;9&lt;/sup&gt;</td>
<td>900</td>
</tr>
<tr>
<td>17-KS</td>
<td>13.8 (mg) (7–14)&lt;sup&gt;10&lt;/sup&gt;</td>
<td>5.4 (mg)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Basal values reported for normal women are given in parenthesis.

<sup>1</sup>Oddie et al. (1972); Klein et al. (1973).

<sup>2</sup>Loriaux et al. (1974); Franks (1974).

<sup>3</sup>Oddie et al. (1972); Brown et al. (1972); own unpublished results.

<sup>4</sup>Horn et al. (1966b).

<sup>5</sup>Pal (1966); Espiner (1966); Pinser et al. (1968).

<sup>6</sup>Horn et al. (1972).

<sup>7</sup>Finkelstein et al. (1968) and unpublished.

<sup>8</sup>Finkelstein (1968).

<sup>9</sup>Touchstone et al. (1957) and own unpublished results.

<sup>10</sup>Own unpublished results.
Urinary steroids

Urinary pregnantriol, pregnantriolone, THS and cortisol were estimated according to Finkelstein (1968). Final identification of pregnantriolone and THS (as its hexahydro-S derivative) was done by gas chromatography-mass spectrometry on a CH5 Varian MAT gas chromatograph-mass spectrometer using 1.5% SE-30 (column length 121.9 cm, diameter 0.6 cm). The conditions were those described by Halperin et al. (1973). Urinary testosterone was estimated according to Horn et al. (1972) and 17-KS according to Holtorff & Koch (1940).

RESULTS

The results of the plasma and urinary steroid estimations are summarized in Table 1. The basal values are the average of 2 estimations carried out on consecutive days; the results did not differ from one another by more than 20%. The results after ACTH represent the higher of the values found for the second or third day of treatment, and the results after dexamethasone represent the lower of the values obtained for those days of treatment.

The following were the most significant deviations from normal:

a) Plasma cortisol was low, even after ACTH stimulation. Urinary cortisol was below detectable levels both under basal conditions and following stimulation with ACTH.

b) Levels of both plasma and urinary testosterone were elevated.

c) Plasma levels of Reichstein's compound S were elevated as was urinary THS; after ACTH stimulation THS level was 900 μg/24 h, a 10-fold increase from basal levels. The identity of urinary THS (as a hexahydro-S derivative) was confirmed by gas chromatography-mass spectrometry in the specimens collected both before and after ACTH stimulation.

d) 21-deoxycortisol was present at detectable levels in plasma; its metabolite, pregnantriolone which was increased 5-fold following ACTH stimulation (from 80 to 375 μg/24 h), was positively identified in the urine by gas chromatography-mass spectrometry.

DISCUSSION

It is customary to classify the various forms of congenital adrenal hyperplasia by the types of defects in enzymes participating in the biosynthesis of steroid hormones. The most common types are those with a deficiency either in C-21-hydroxylation or in C-11/β-hydroxylation. The first one is characterized by readily detectable levels of plasma 21-deoxycortisol (Wieland et al. 1965; Franks 1974; Loriaux et al. 1974) and urinary pregnantriolone (Finkelstein et al. 1953) and the second by increased plasma compound S and urinary THS.
(Eberlein & Bongiovanni 1955; Bongiovanni et al. 1967). In both types medication with cortisol or its analogues ameliorates most of the symptoms and restores certain functions to normal.

In the present case the low cortisol and corticosterone values are in agreement with the usual findings in the above variants of congenital adrenal hyperplasia. However, the concomitant increased levels of both 21-deoxycortisol and compound S are difficult to reconcile with the concept of single enzymic deficiencies – either in C-11β or C-21-hydroxylases – as are believed to be present in the variants of congenital adrenal hyperplasia. Thus, by assuming a deficiency in 11β-hydroxylase an increase in compound S could be explained, but such an assumption would be untenable with the elevated presence of 21-deoxycortisol. On the other hand by postulating a 21-hydroxylase deficiency the elevated 21-deoxycortisol could be accounted for, but such a deficiency would be not compatible with the observed increase in compound S.

A more tenable explanation may be offered by postulating the presence of an aberrant 11β-hydroxylase, preferentially acting on 21-deoxysteroids instead of the regular enzyme, which hydroxylates mainly the 21-hydroxylated substrates. This 11β-hydroxylase may cause a shift in the pathway of cortisol biosynthesis by stimulating the conversion of 17-hydroxyprogesterone to 21-deoxycortisol. The latter is a poor substrate for a further hydroxylation at C-21 (Finkelstein et al. 1968 and unpublished). That portion of 17-hydroxyprogesterone which does not undergo 11β-hydroxylation, may be hydroxylated at C-21 and converted into compound S. Because of the deficiency in the normal 11β-hydroxylase, compound S can not be efficiently converted to cortisol, resulting in a partially unopposed secretion of ACTH. The increased stimulation by ACTH would cause an increase in the production of adrenal steroids, which, in the present case, is reflected by the high values of testosterone, 21-deoxycortisol and compound S. Indeed, exogenous ACTH caused a further increase in 21-deoxycortisol and compound S. This increase was accompanied by a decrease in testosterone level, a paradoxical result which has been occasionally observed by several authors (Sorcini et al. 1963, 1968; Korenman et al. 1965; Rivarola et al. 1966; Horn et al. 1966a).

The possibility of a "mixed" syndrome, which would ensue from a single defect in 11β-hydroxylase by a shift in its activity from 21-hydroxy to 21-deoxysteroids, is supported by the results of Thomas & Steinbeck (1969). The latter detected both pregnanetriolone and THS in the urine of 1 case with polycystic ovary syndrome, 2 cases of congenital adrenal hyperplasia and 2 cases of adrenocortical hyperfunction due to adrenal carcinoma. However, the compounds were not fully identified. In the present case and in one additional case of post-pubertal virilizing syndrome (unpublished), where both pregnanetriolone and THS were excreted simultaneously, the identity of these
metabolites was proven unequivocally by gas chromatography-mass spectrometry.

Although the postulated enzymic abnormality may explain the adreno-genital syndrome-like features, the patient's response to corticoid therapy is puzzling. Administration of corticoids produced the anticipated amelioration of some of the patient's primary symptoms, probably by a suppression of the increased synthesis of adrenal intermediates (such as DOC), but at the same time her peripheral response was exaggerated – possibly due to an increased sensitivity to glucocorticoids. Our initial suspicion was that the hypersensitivity might be due to low plasma transcortin (Doe et al. 1960). The plasma transcortin value, however, was 32 mg/l, which is within the normal range (Doe et al. 1964). Further possible investigations of the basis for the differential effect of the corticoids was not attempted, since contact with the patient was lost, but the fact that such an effect did occur was deemed worth reporting.

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REFERENCES


838

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