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SERUM LEVELS OF 5-ANDROSTENE-3β,17β-DIOL SULPHATE, 5α-ANDROSTANE-3α,17β-DIOL SULPHATE AND GLUCURONIDE, IN LATE ONSET 21-HYDROXYLASE DEFICIENCY

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Summary—Serum sulphates of 5-androstene-3β,17β-diol (5-ADIOL-S), 5α-androstane-3α,17β-diol (3α-DIOL-S) and dehydroepiandrosterone (DHEA-S), as well as 5α-androstane-3α,17β-diol glucuronide (3α-DIOL-G) and unconjugated androstenedione (AD) and testosterone (T), sex hormone binding globulin (SHBG), free androgen index (FAI) and 17α-hydroxyprogesterone (17OHP) were measured by specific radioimmunoassays (RIA) in 14 women with late-onset 21-hydroxylase deficiency (LOCAH), and in normal women ($n = 73$). The diagnosis of LOCAH was made on the finding of a (17OHP) response level greater than 30 nmol/l following ACTH stimulation, and/or an elevation of urinary metabolites of 17OHP. Mean values for serum concentrations of all steroids measured and the free androgen index (100 × T nmol/l ÷ SHBG nmol/l) were significantly elevated, and SHBG levels depressed in patients with LOCAH. These studies show that in LOCAH, in addition to the unconjugated steroids AD and T, the sulphoconjugated steroids DHEA-S, 5-ADIOL-S and 3α-DIOL-S are increased, as is the glucuronide conjugate 3α-DIOL-G and the index of bioavailable testosterone (FAI), and that mean SHBG levels are depressed. These data suggest that as well as AD, 5-ADIOL-S and DHEA-S may act as pro-hormones for more potent steroids (T and 5α-dihydrotestosterone) in peripheral tissues, while 3α-DIOL-S and 3α-DIOL-G may both reflect peripheral androgen metabolism in patients with LOCAH.

INTRODUCTION

Late-onset 21-hydroxylase deficiency (LOCAH) is an allelic variant of classical 21-hydroxylase deficiency [1–6]. It is most often described as a disorder of adrenal steroidogenesis with onset of virilization in late childhood, either at the time of puberty or after puberty. In the adult woman the clinical signs may be a combination of hirsutism, acne, menstrual disturbance, male pattern baldness and infertility. The reported frequency of LOCAH among women with hirsutism ranges from 1.2 to 20% [1, 7–13], by the use of an ACTH stimulation test to establish the diagnosis, with measurement of serum 17OHP before and 60 min after ACTH.

The aims of the present study were to determine by specific RIA the concentrations of the 19-carbon sulphoconjugated steroids (5-ADIOL-S and 3α-DIOL-S) of 3α-DIOL-G and of SHBG, to derive the free androgen index (FAI) in patients with LOCAH, since there are very few previous reports of such measurements, in addition to the steroids which are normally measured in LOCAH.

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EXPERIMENTAL

Patients

15 women with hirsutism as the primary complaint, age range 19–39 yr, were referred to our laboratory for steroid investigations. Our primary steroid screening procedure for the investigation of hirsutism is to profile both a basal blood sample and a 24-h urine; if the blood showed an elevated 17OHP level and/or the urine showed elevated 17OHP metabolites (pregnanetriol, pregnanetriolone, 5β, 17-hydroxypregnanolone) then an ACTH stimulation test would be recommended for 17OHP measurements for absolute confirmation of 21-hydroxylase deficiency. Blood for the determination of basal steroids was drawn between 1000 and

1600 h during either the follicular or luteal phase of the menstrual cycle and a group of normal women ($n = 73$) age range 21–40 yr, without menstrual dysfunction, hirsutism or alopecia acted as controls. An ACTH test was performed in all but one patient, and two patients were given dexamethasone the evening prior to the ACTH test. An ACTH stimulation test was also performed on 6 normal female volunteers, and for an additional comparison, the 17OHP results of ACTH tests in 133 adult premenopausal women with idiopathic hirsutism, screened for LOCAH, are included in this study. While all patients were referred from several specialist endocrinology clinics for hirsutism as the main complaint, the presence of other hyperandrogenic symptoms varied widely within the patients. 6 patients reported acne, four hair loss, four menstrual disturbances, three voice deepening and one clitoromegaly. It was not possible to make a diagnosis for LOCAH in these patients based simply on clinical observations.

Methods

Radioimmunoassays. The methods for the determination of serum DHEA-S, 5-ADIOL-S, 3α-DIOL-S, AD, T and SHBG have been described in detail previously [14] as has the method for serum 17OHP [15].

3α-DIOL-G. 3α-DIOL-G was measured in duplicate with kits purchased from Diagnostic Systems Laboratories Inc. (Webster, Tex., U.S.A.) which allow the direct assay of the steroid conjugate without necessity for prior hydrolysis. This assay measures the 3α-DIOL which is conjugated with glucuronic acid at carbon position 17 of the steroid molecule, and has very little cross-reactivity toward 3α-DIOL conjugated at carbon position 3. In our hands, the lowest detectable value was 0.5 nmol/l, and the intra-assay variation was 6.5% ($n = 12$) at a level of 23.6 nmol/l and 9.2% ($n = 12$) at 1.5 nmol/l. Cross-reactivities for the antiserum were: 5α-androstane-3α,17β-3-glucuronide 5.8%, 5α-dihydrotestosterone-17-glucuronide 11.6%, 5α-androstane-3α,17β-diol 10.7%, testosterone-17-glucuronide 1.8%, testosterone, 5α-dihydrotestosterone, androsterone glucuronide, 5α-androstane-3β,17β-diol, 5α-androstane-3, 17-dione, dehydroepiandrosterone all $< 1.0\%$.

Statistical analysis. All results are presented as mean ± SEM. The Mann–Whitney test (unpaired) for non-parametric distribution of data was used to determine statistical significance.

RESULTS

The serum 17OHP concentrations (basal and response to ACTH) are shown in Fig. 1. The mean basal 17OHP in patients with LOCAH was 22.4 ± 5.9 mmol/l (range 2.9–71.0) and the mean value 1 h following ACTH stimulation was 112 ± 35 nmol/l (range 25–543). All patients reached a post-ACTH level of greater than 30 nmol/l, which is our minimum criteria for the diagnosis of LOCAH, except for one patient who reached a level of 25 nmol/l. We elected to assign the diagnosis to this patient, however, since her urinary steroid profile showed an abnormal pattern consistent with 21-hydroxylase deficiency. In addition, 3 patients showed basal 17OHP levels within the normal range (mean ± 2SD), but their post-ACTH levels all reached greater than 30 nmol/l. The mean basal and post-ACTH 17OHP levels in 133 females with idiopathic hirsutism was 2.9 ± 0.2 and 7.2 ± 0.3 nmol/l respectively.

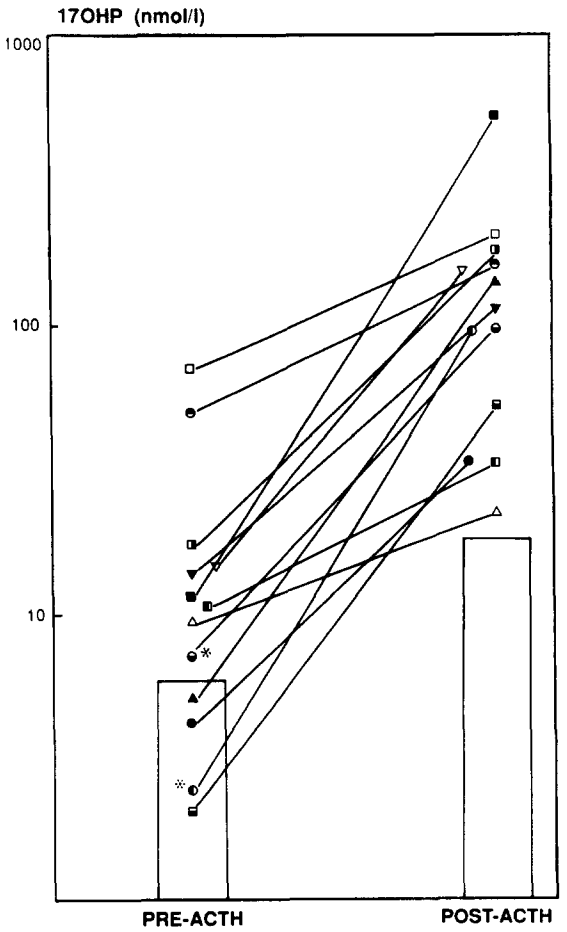

Fig. 1. Pre- and post-ACTH 17OHP levels in women with LOCAH. *Dexamethasone administered prior to ACTH stimulation. Reference range (mean ± 2 SD) for basal and post-ACTH is indicated by the bars.

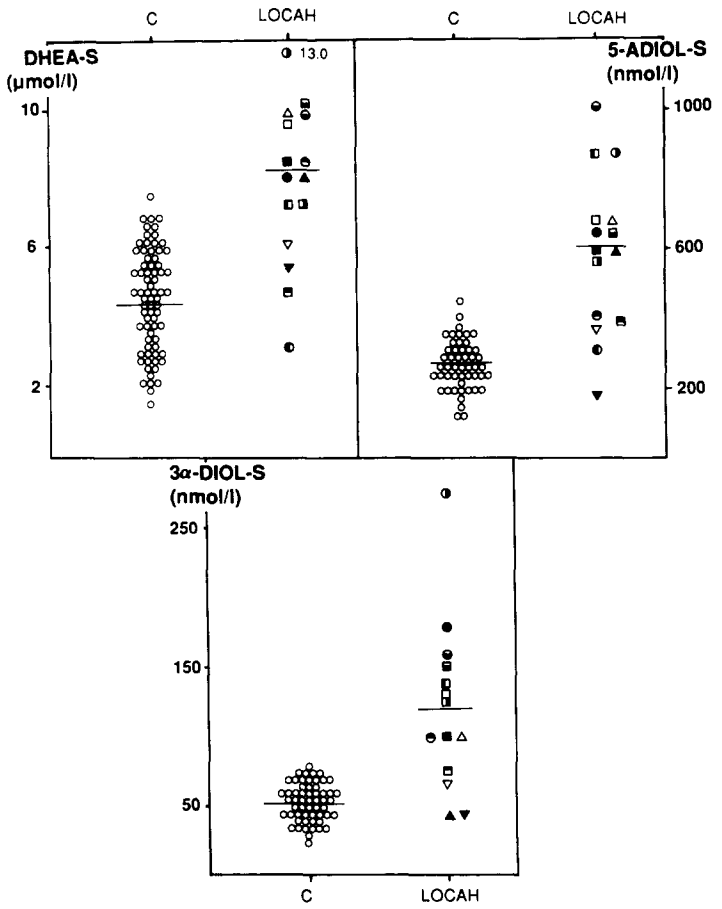

Fig. 2. Concentrations of serum DHEA-S, 5-ADIOL-S and 3α-DIOL-S in normal women (open circles) and women with LOCAH. The horizontal lines indicate the mean values.

The individual values for serum steroid sulphates DHEA-S, 5-ADIOL-S and 3α-DIOL-S are shown in Fig. 2, those for 3α-DIOL-G in Fig. 3, and those for the unconjugated steroids AD, T and SHBG and FAI in Fig. 4.

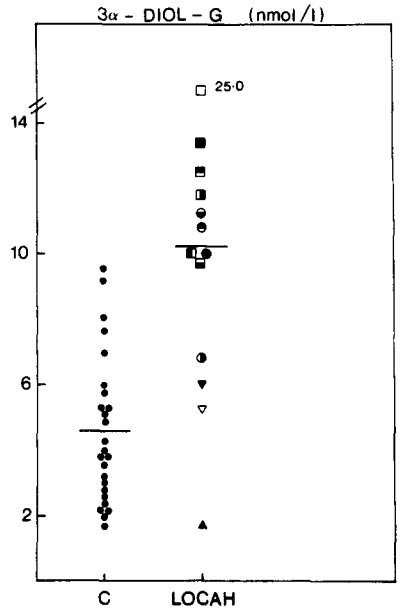

Fig. 3. Concentrations of serum 3α-DIOL-G in normal women (closed circles) and women with LOCAH. The horizontal lines indicate the mean values.

The mean values for all steroids both conjugated and unconjugated, as well as the FAI were all significantly elevated above normal, and SHBG significantly depressed in patients with LOCAH, compared to control. DHEA-S (8.3 ± 0.6 vs 4.4 ± 0.2 μmol/l), 5-ADIOL-S (603 ± 59 vs 267 ± 10 nmol/l), 3α-DIOL-S (119 ± 16 vs 52 ± 2 nmol/l), 3α-DIOL-G (10.3 ± 1.5 vs 4.6 ± 0.4 nmol/l), AD (12.8 ± 1.7 vs 3.4 ± 0.2 nmol/l), T (3.3 ± 0.4 vs 1.5 ± 0.1 nmol/l), SHBG (25 ± 5.0 vs 65 ± 2.5 nmol) and FAI (15.1 ± 3.0 vs 2.4 ± 0.1 nmol/l). All comparisons showed a statistically significant difference of $P < 0.001$. The numbers of control subjects studied for each hormone depended on sample availability and these are indicated in the figures.

DISCUSSION

The classical form of complete 21-hydroxylase deficiency causes sexual ambiguity in female

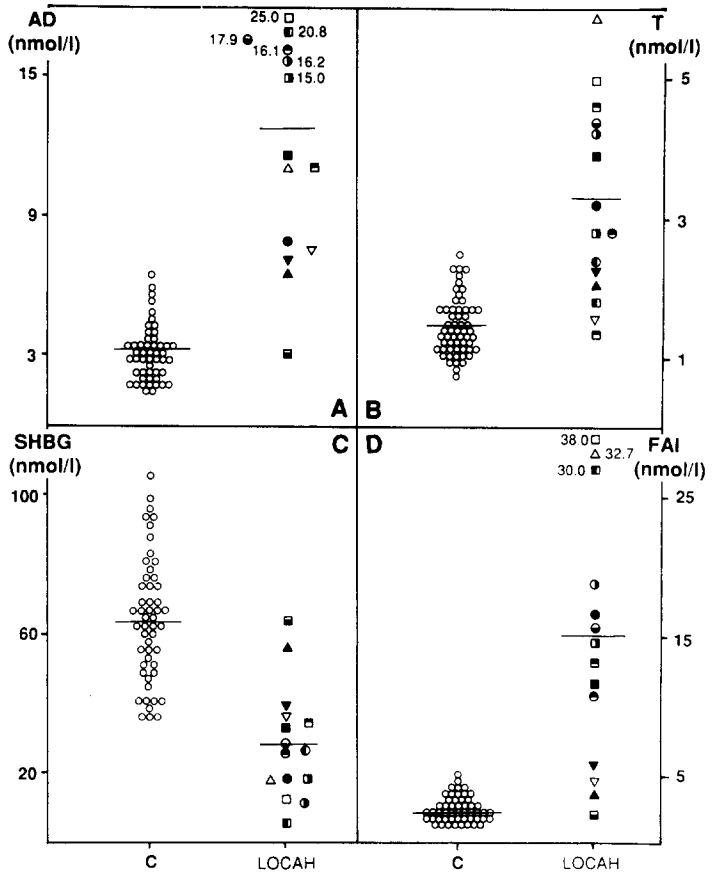

Fig. 4. Concentrations of serum AD, T, SHBG and FAI in normal women (open circles) and women with LOCAH. The horizontal lines indicate the mean values.

infants at birth. A milder form of late-onset adrenal hyperplasia due to 21-hydroxylase deficiency can occur in adult females, with mild or severe hirsutism being the most universal clinical feature, as was the case in all of our patients. In addition to hirsutism, there can be extreme variation in other clinical parameters (menstrual disturbances, acne, hair loss, clitoromegaly, increased muscularity and voice deepening). The symptoms in our group of patients range from solely the presence of chin hair to menstrual dysfunction, hair loss, increased muscularity and deepening of the voice. The distinction between classical and late onset forms of this disorder on purely clinical criteria is therefore extremely difficult, as noted in previous reports [1, 3, 8, 13, 16].

The levels of AD and T were elevated in the majority of patients with LOCAH, in agreement with previous reports [6, 8, 11, 17]. This finding was not unexpected, since AD of adrenal origin increases in LOCAH reflecting conversion of 17OHP by the enzyme 17, 20 lyase, and the increased levels of 17OHP consequent upon the mild deficiency of adrenal 21-hydroxylase. In turn, increased T-levels are most likely due to peripheral conversion of AD. Some of our patients showed very high levels of serum AD, and this may be a reflection of very efficient conversion from 17OHP by 17, 20-lyase, either intra adrenal or in peripheral tissues. It is certainly possible however, that some of these patients with LOCAH who presented in the post-pubertal period may have had clinical and biochemical symptoms in childhood, such as premature pubarche and a mild elevation of 17OHP. The mildly elevated basal 17OHP levels found in our patients with LOCAH, however, would tend to mitigate against the presence of the classical severe form of 21-hydroxylase deficiency, in whom very much higher levels of basal 17OHP would be expected.

The higher levels of adrenal DHEA-S and 5-ADIOL-S in most patients with LOCAH suggests that the adrenal androgen pathway via 17-hydroxypregnenolone, DHEA/DHEA-S and 5-ADIOL-S is also actively stimulated in this disorder, together with AD synthesis via 17OHP conversion. In addition, as our studies in children with premature adrenarche [18] previously

suggested, DHEA-S and 5-ADIOL-S may act as pro-hormones for more potent androgens in peripheral tissues such as the skin.

The glucuronide conjugate 3α-DIOL-G was determined in 13 of the patients with LOCAH and was found to be above normal limits (M ± 2SD) in 9 and normal in 4, in agreement with a recent report by Whorwood *et al.* [19]. Mauvais-Jarvis *et al.* [20] first suggested that 3α-DIOL-G might be a marker of peripheral androgen metabolism, while Horton *et al.* [21] reported elevated levels in hirsute women in whom other unconjugated steroids were not significantly elevated. Other studies [22–24] have also supported this concept, although Scanlon *et al.* [25] have recently reported normal levels of this conjugate in hirsute women with polycystic ovaries. Recent evidence suggests that androgen pro-hormones appear to be the important determinants of 3α-DIOL-G peripheral synthesis. In this respect, Rittmaster [26] found that ADIOL-G arises mainly from adrenal precursors in women, while Giagulli *et al.* [27] have recently demonstrated by infusion studies in normal men and women, that plasma DHEA-S and AD are the major precursors of plasma 3α-DIOL-G, accounting for 50 and 15% respectively of plasma 3α-DIOL-G.

The sulphate conjugate 3α-DIOL-S was found to be above normal limits (M ± SD) in 10 of our patients with LOCAH. Matteri *et al.* [28] have recently reported that 3α-DIOL-S was able to be formed directly in skin from DHT in normal men and women, and that this conversion was higher than that of DHT to 3α-DIOL-G. Furthermore, other studies have shown that C-19 steroid sulphates such as DHEA-S can be converted to DHT in skin [29–31], so that 5-ADIOL-S together with DHEA-S may be important in this respect. Matteri *et al.* [32] have recently reported studies on androgen conjugates in hirsute and non-hirsute women with polycystic ovarian syndrome (PCO) and found that 3α-DIOL-S, together with other C-19 steroids, are elevated in hirsute women with PCO, but normal in non-hirsute women with PCO, thus lending support to the view that 3α-DIOL-S may be a peripheral marker for hirsutism.

3α-DIOL-S is found as a mono-sulphate in the circulation, with the sulphate moiety attached to carbon position 3 or 17 [33, 34]. The majority is found as the 17-sulphate (75%) and the remainder (25%) as the 3-sulphate [34]. This being the case, the preferred pathway for 3α-DIOL-S formation in peripheral tissues would be from T and DHT, accounting for 3α-DIOL-S-17-sulphate formation, while the remaining 3α-DIOL-3-sulphate would be formed via androsterone metabolism.

In our study, SHBG was depressed in all but 3 patients, and serum T was elevated in 10 patients, together producing a marked elevation of free androgen index, the amount of T which is bioavailable to act on androgen sensitive tissues. This is likely to be a main contributor to androgencitiy in the patients with LOCAH.

In summary, in terms of steroid synthesis and metabolism in LOCAH, our studies suggest that: (1) Both the delta 4 (AD) and delta 5 (DHEA-S, 5-ADIOL-S) adrenal pathways are accentuated in LOCAH, with these steroids probably acting as pro-hormones for the synthesis of potent androgens in peripheral tissues. (2) The metabolism of these pro-hormones is reflected in the peripheral production of both glucuronide and sulphate conjugates of 5α-androstane-3α, 17β-diol. These conjugates are formed from DHT metabolism in peripheral target tissues, and consequently, circulating plasma DHT does not reflect its total body formation [35], and its level is often normal in hirsute women [36]. Both 3α-DIOL-G and 3α-DIOL-S would appear to be good markers of androgen metabolism, and consequently useful markers of treatment. In this respect, preliminary studies in our laboratory (unpublished observations) have indicated significant reduction of both 3α-DIOL-G and 3α-DIOL-S following treatment of patients with LOCAH with dexamethasone, and studies are continuing in this regard. (3) The bioavailable testosterone is significantly increased in LOCAH, contributing importantly to the androgenic symptoms in this disorder.

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