J. Steroid Biochem. Molec. Biol. Vol. 39, No. 1, pp. 55–61, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

IS PLASMA 5α-ANDROSTANE 3α,17β-DIOL GLUCURONIDE A BIOCHEMICAL MARKER OF HIRSUTISM IN WOMEN?

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(Received 8 November 1990)

Summary—We investigated whether plasma androstanediol glucuronide (ADG) levels reflect the increased androgenicity in women with idiopathic hirsutism ($n = 24$) or hirsutism associated with polycystic ovary syndrome ($n = 10$). We also evaluated whether ADG levels parallel the clinical evolution of the hirsutism during a combined treatment, with cyproteroneacetate (2 mg) and ethinylestradiol (35 μg), of women with moderate idiopathic hirsutism. Finally, we investigated if there is evidence for increased conversion of precursors to ADG in hirsutism, by comparing the ADG levels, measured by RIA, to ADG levels obtained by applying the conversion rates of precursors obtained in non-hirsute women. ADG levels were increased in less than half of the patients with mild hirsutism. The clinical cure of hirsutism, which was obtained by the treatment in majority of patients, was accompanied by a significant decrease of plasma ADG levels, but a similar decrease was also observed in the 5 patients who did not respond to treatment. The data show that, although there is evidence for increased conversion of precursors to plasma ADG in mildly hirsute women, the latter is not a reliable parameter of androgenicity. Our data suggest, moreover, that treatment with cyproterone acetate and ethinylestradiol decreases 5α-reductase activity, as indicated by the more important decrease in ADG levels than in the precursors.

INTRODUCTION

It is generally accepted that in hirsutism, cutaneous 5α-reductase activity is increased [1–5]. Nevertheless in hirsute women, the plasma levels of the 5α-reduced testosterone metabolite, dihydrotestosterone (DHT) are often normal. This is explained by the fact that DHT, formed in peripheral tissues, is not an end metabolite of the androgens, but is further metabolized to 5α-androstane 3α,17β-diol (AD) and its glucuronide (ADG), respectively. Hence, plasma DHT is not a useful parameter of peripheral androgen formation and hirsutism [6]. On the other hand, plasma AD and ADG levels have been claimed to be end metabolites of the androgens at the target tissues level and are considered by some authors to be excellent parameters of androgenicity. It has been reported that plasma ADG levels are nearly always increased in women with idiopathic hirsutism [7–10]. Paulson *et al.* [11] observed a highly significant correlation between serum ADG levels and genital 5α-reductase. Other authors, however, could not confirm this and found that ADG levels are not more frequently increased than other androgens in idiopathic hirsutism [12–14].

High plasma ADG levels in hirsute women could be the consequence of a normal conversion of increased precursor levels [dehydroepiandrosterone sulfate (DS), dehydroepiandrosterone (D), androstenedione (A) and testosterone (T)] [15] to ADG, or of an increased conversion of normal precursor levels. In the latter circumstance ADG levels would be expected to be more frequently increased than those of their precursors.

Acne and mild hirsutism are often treated with moderate doses of an antiandrogen, usually cyproterone acetate (CPA), combined with an estrogen. The antiandrogen acts by competing with the androgens for the androgen receptor, by partially inhibiting LH levels and ovarian androgen secretion [16, 17] and, like other progestins, by inhibiting 5α-reductase. The estrogen component on the other hand increases the SHBG capacity, thus reducing the free and bioavailable androgen fraction. If plasma ADG

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is a reliable parameter of peripheral androgen formation and action, it can be expected that during treatment with such a drug combination, ADG levels should decrease and their evolution might permit prediction of the clinical outcome of treatment.

It was the purpose of this study to investigate, in patients with hirsutism, whether idiopathic or due to the polycystic ovary syndrome (PCO), if plasma ADG levels are a better parameter of androgenicity than is any other androgen level, and whether the evolution of the ADG levels permits prediction of the clinical outcome of the treatment with CPA (2 mg) and ethinylestradiol (EE) (35 μg). Moreover, we investigated if there is evidence for a reduced conversion of androgen precursors to ADG during this treatment.

MATERIALS AND METHODS

Subjects

Three groups of women were studied:

Group 1: 12 healthy, regularly cycling women, age 25–46 y (mean age 32 y), without hirsutism [Ferriman and Gallwey (FG) score <7] [18, 19] serving as controls.

Group 2: 24 women, 19–30 y, with a mild to moderate degree of hirsutism (FG score between 7 and 15) without evident ovarian or adrenal pathology (dysfunctional hirsutism). During 6 menstrual cycles these patients were given a daily dose of CPA 2 mg/day and EE 35 μg/day (Diane 35^{R}) for 21 days (from day 5 to 25 of the cycle), followed by a steroid-free interval of 1 week. After 6 months of treatment the clinical results were evaluated and the patients divided into 2 subgroups, a subgroup of cured (FG <6) or improved (FG score decreased by $>25\%$ but still above 6) patients and a therapy-resistant subgroup (decrease of FG score $<15\%$ or <2 U).

All these subjects had a normal weight (body mass index: W (kg)/H^2 (m): BMI: 20–27), were normotensive and none were diabetic; they smoked <10 cigarettes/day.

Group 3: 10 obese women with PCO (age 19–37 y, mean 25 y), defined as the combination of hirsutism, obesity (body mass index = >30) and irregular menses or amenorrhoea.

Blood samples were drawn in the morning between the 20th and 25th day of basal and treatment cycles.

Steroid assays

Specimens were processed for determination of T, A, DHT, AD and ADG, D and DS, using previously described specific RIA methods [15, 20, 21] involving chromatographic purification of the plasma extracts. SHBG and plasma levels of free T were measured after equilibrium dialysis. Non-specifically bound testosterone (NSB-T) was calculated using an association constant of albumin for testosterone of $3.6 \times 10^4\ M^{-1}$ [22].

Individual plasma levels were considered to be increased whenever they were 2 SD higher than the mean obtained in the group of healthy women of similar age.

Conversion rate (CR) of plasma precursors (except for DS) to plasma ADG, used in this study, were those obtained in an earlier study from this laboratory [15]. As to DS, after studying a larger number of subjects, the CR of DS to ADG appeared to be higher (0.068) than in our earlier study. The CR of T to ADG was adapted to NSB-T, as the latter is considered by most authors to be the biologically active fraction [23, 24].

Significance of variations of plasma concentration during the period of the study in comparison to basal levels was estimated by means of analysis of variance test (ANOVA).

RESULTS

All androgen levels in our normal healthy women (group 1) were within the normal range as previously determined in this laboratory (Table 1). Applying the CR of precursors to plasma ADG as obtained experimentally in a previous study [15] yielded calculated ADG concentrations representing $104 \pm 12\%$ of measured ADG levels (Table 2).

In group 2, mean plasma levels of AD, ADG and DS, as well as NSB-T levels in both the therapy-responsive and -resistent group, respectively, were above the upper limits of levels in normal women of a similar age; mean T, A and D levels were increased in comparison to the mean in normal women, but the means in both the successfully treated and therapy-resistant subgroup (except for T in the latter) were still within the normal range of values (Table 1). As to the levels in individual patients, T levels were increased in 9 out of 24, NSB-T in 11 out of 23 and DS in 13 out of 24 cases. AD levels were increased in 16 out of 24 cases, whereas

Table 1. Basal plasma androgen levels

	T (nmol/l)	NSB-T (nmol/l)	A (nmol/l)	D (nmol/l)	DS (μmol/l)	DHT (nmol/l)	AD (nmol/l)	ADG (nmol/l)	SHBG (nmol/l)
Normal women ($n = 12$)									
$\bar{X}$	1.07	0.208	5.56	22.05	4.38	0.62	0.103	3.76	70.5
SD	0.36	0.052	1.11	7.26	1.13	0.12	0.072	1.69	29.2
Idiopathic hirsutism ($n = 24$)									
Therapy-responsive ($n = 19$)									
$\bar{X}$	1.42	0.389	8.04	26.11	7.25	0.76	0.31	7.91	70.0
SD	0.69	0.237	3.40	8.91	3.61	0.22	0.11	5.48	30.0
Therapy-resistant ($n = 5$)									
$\bar{X}$	2.15	0.476	10.84	33.75	8.09	0.72	0.27	0.64	65.1
SD	1.07	0.166	4.84	14.36	1.92	0.12	0.10	1.41	18.1
PCO ($n = 10$)									
$\bar{X}$	2.10	0.85	11.24	20.83	5.79	0.78	0.36	7.47	49.2
SD	0.86	0.57	4.90	12.96	2.48	0.17	0.12	2.71	21.3

T: Testosterone; NSB-T: non-specifically bound testosterone; A: androstenedione; D: dehydroepiandrosterone; DS: dehydroepiandrosterone sulfate; DHT: dihydrotestosterone; AD: 5α-androstane, 3α,17β-diol; ADG: 5α-androstane, 3α,17β-diol-glucuronide; SHBG: sex hormone binding globulin.

ADG levels were increased in only 10 out of 21 cases (Fig. 1).

In group 2, no statistically significant correlation was observed between the FG score and plasma ADG or AD levels, respectively. Using the CR of plasma precursors to plasma ADG, as obtained in non-hirsute women [15], to basal plasma levels in the therapy-responsive and -resistant subgroup, respectively, we obtain a mean calculated value for plasma ADG which is clearly below the mean measured value (Table 2). When applied to the individual cases, the ADG concentrations calculated from conversion rates in non-hirsute women were below the measured ADG levels in 22 out of 24 patients.

In group 3, NSB-T levels were increased in all, whereas A levels were increased in 9, DS levels in 6 and ADG levels in 7 out of 10 subjects. Use of conversion rates, determined in non-hirsute women, yielded a mean ADG level of only 64% of measured ADG levels.

Treatment with Diane-35^R of group 2 resulted, within a month, in a highly significant decrease of NSB-T levels to about 1/3 of basal levels, of ADG levels to 40% of basal levels and of DS levels to about 2/3 of basal levels. Mean AD as well as A levels decreased slightly, whereas T and DHT levels did not vary significantly (Table 3). Applying the CR of plasma androgen precursors to plasma ADG obtained in non-hirsute women to androgen levels obtained during treatment, yielded a mean value for plasma ADG which is clearly higher than the value measured; this persisted during the whole treatment period (Table 2).

After 6 months of treatment, hirsutism was considered to be cured in 17 patients and in 2 the hirsutism was significantly improved (therapy-responsive subgroup); in 5 patients no significant effect was observed (therapy-resistent subgroup).

Table 2. Contribution of precursors (nmol/l) to plasma ADG levels using conversion rates obtained in non-hirsute women

$CR\%_{BB}^{\rightarrow ADG}$ in non-hirsute women	DS 0.068	D 1.77	A 8.8	NSB-T 40.2	Total ADG calculated from CR (nmol/l)	ADG measured (nmol/l)	% Plasma ADG accounted for by non-hirsute women
Normal women ($n = 12$)	2.98 ± 0.97	0.35 ± 0.15	0.49 ± 0.14	0.10 ± 0.03	3.98 ± 1.02	3.76 ± 1.69	104 ± 12
Hirsute women ($n = 24$)							
Therapy responsive ($n = 19$)							
Basal	4.62 ± 2.23	0.47 ± 0.16	0.69 ± 0.29	0.16 ± 0.06	5.94 ± 2.74	8.39 ± 4.27	74 ± 11
1 m	3.51 ± 2.40	0.35 ± 0.18	0.54 ± 0.26	0.02 ± 0.01	4.25 ± 2.33	3.28 ± 1.22	157 ± 19[a]
6 m	3.15 ± 1.10	0.35 ± 0.11	0.65 ± 0.23	0.03 ± 0.01	4.14 ± 1.23	3.04 ± 1.66	167 ± 17[a]
Therapy resistant ($n = 5$)							
Basal	4.43 ± 1.48	0.42 ± 0.18	0.87 ± 0.37	0.13 ± 0.06	5.85 ± 1.47	6.64 ± 1.40	86 ± 10
1 m	3.99 ± 1.42	0.28 ± 0.03	0.71 ± 0.24	0.07 ± 0.03	4.96 ± 0.62	3.32 ± 2.05	186 ± 24[a]
6 m	2.81 ± 1.73	0.27 ± 0.02	0.62 ± 0.18	0.03 ± 0.01	3.72 ± 0.79	2.77 ± 1.16	144 ± 21[a]
PCO ($n = 10$)							
Basal	3.2 ± 1.13	0.38 ± 0.22	0.97 ± 0.19	0.17 ± 0.07	4.65 ± 1.13	7.94 ± 1.23	64 ± 14

1 m, 6 m: Levels after 1 and 6 months, respectively, of treatment.
For significance of abbreviations see Table 1.
[a] $P < 0.01$ vs basal levels.
All values mean ± SD.

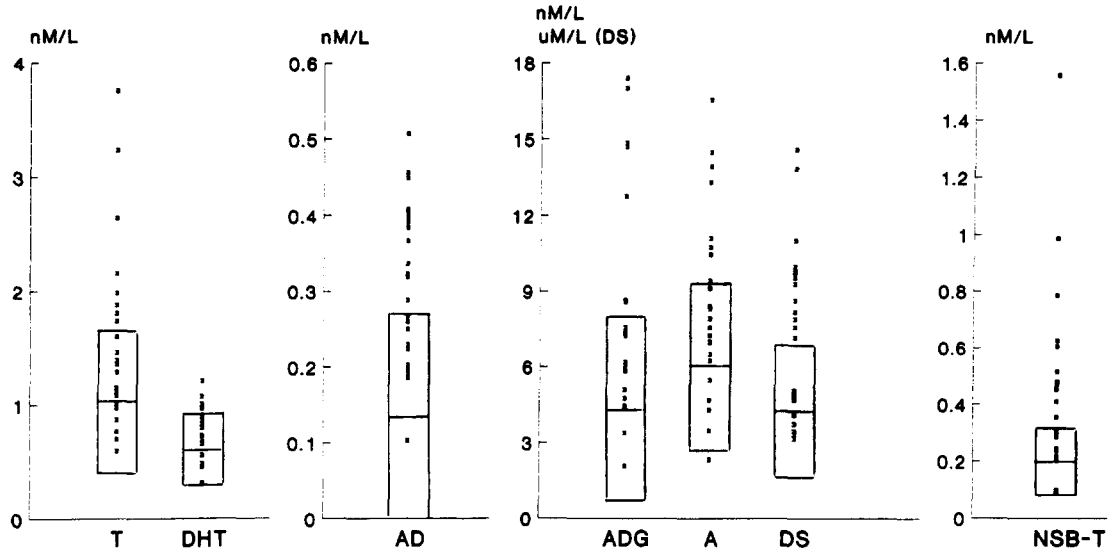

Fig. 1. Plasma androgen levels in women with mild dysfunctional hirsutism (FG score 7–15) The rectangles represent mean ± SD in normal women of similar age.

In the therapy-resistant subgroup, mean basal T, NSB-T and A levels were higher than in the responsive subgroup, but plasma levels of the other androgens, inclusive of AD and ADG, were similar to the levels in the therapy-responsive subgroup (Table 4). Moreover, evolution of plasma androgen levels during treatment in the resistant subgroup was similar to that of the successfully treated patients, except for a slower decrease of the NSB-T concentration.

DISCUSSION

Several authors have reported that ADG levels are an excellent parameter of formation of androgens and androgencity at the target tissues level [6, 25,26]. Peripheral tissues, at least under *in vitro* conditions, can form ADG from precursors [27].

Morimoto *et al.* [28], on the basis of data obtained by catheterization of the VV sushepaticae, claim that plasma ADG is not formed in the liver. Hence, ADG levels have been widely accepted as parameters of peripheral androgen formation. Nevertheless, data found in the literature are not entirely convincing. Several authors reported increased ADG levels in almost all patients with idiopathic hirsutism, but other authors could not confirm these data [12–14, 29]. Although our data show that plasma ADG levels are influenced both by the precursor levels and the 5α-reductase activity, plasma ADG levels in our patients with idiopathic hirsutism of moderate severity were increased in less than half the cases, whereas AD levels were increased in 2/3 of the cases. Even in our obese hirsute patients with PCO, ADG levels were above the upper limit of normal levels in only 7 out of 10 patients. The frequency of increased androgen levels in our patients with mild to moderate hirsutism is comparable to the frequency reported by Cummingham *et al.* [30]

Table 3. Plasma androgen levels before and during treatment with CPA (2 mg) and EE (35 μg) in the therapy-responsive group

	T (nmol/l)	NSB-T (nmol/l)	A (nmol/l)	D (nmol/l)	DS (μmol/l)	DHT (nmol/l)	AD (nmol/l)	ADG (nmol/l)	SHBG (nmol/l)
Basal									
$\bar{X}$	1.42	0.389	8.04	26.11	7.26	0.76	0.31	7.91	70.0
SD	0.69	0.233	3.40	8.91	3.61	0.22	0.11	5.48	32.0
Treatment									
1 m									
$\bar{X}$	1.25	0.111[c]	6.57[a]	21.42	5.59[a]	0.90	0.21[a]	3.08[b]	320[c]
SD	0.47	0.096	2.30	9.64	3.51	0.33	0.13	2.07	118
3 m									
$\bar{X}$	1.53	0.118[c]	6.96	22.40	5.07[b]	0.86	0.21[a]	2.81[c]	352[c]
SD	0.53	0.053	2.73	15.16	1.63	0.33	0.10	1.60	120
6 m									
$\bar{X}$	1.70	0.132[c]	7.44	20.28	4.97[c]	0.93	0.20[b]	3.05[b]	349[c]
SD	0.63	0.040	2.76	6.33	1.81	0.38	0.12	1.66	71

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ (ANOVA) vs basal values.
For significance of abbreviations see Table 1.

Table 4. Evolution of plasma levels in therapy-resistent cases ($n = 5$)

	T (nmol/l)	NSB-T (nmol/l)	A (nmol/l)	D (nmol/l)	DS (μmol/l)	DHT (nmol/l)	AD (nmol/l)	ADG (nmol/l)
Basal								
$\bar{X}$	2.15	0.69	10.84	23.58	8.09	0.76	0.27	6.64
SD	1.07	0.24	4.48	14.36	1.92	0.17	0.10	1.41
Treatment								
1 m								
$\bar{X}$	1.56	0.17	8.11	15.81	5.94	1.00	0.21	3.32
SD	0.20	0.07	2.55	6.98	2.11	0.27	0.05	2.04
3 m								
$\bar{X}$	1.53	0.215	6.99	17.22	5.55	0.65	0.24	4.04
SD	0.44	0.019	2.55	7.13	2.43	0.14	0.09	1.38
6 m								
$\bar{X}$	1.53	0.09	7.80	15.25	4.44	0.93	0.21	2.77
SD	0.27	0.02	2.22	8.12	1.83	0.26	0.06	1.18

For significance of abbreviations see Table 1.

and by Meikle *et al.* [31]. The cause of the divergent data in the literature as far as ADG levels in hirsute women are concerned, is not obvious, but in some studies the hirsutism has been more severe [10], in some the number of normal controls was rather small [7], whereas in other studies, surprisingly, ADG levels higher than in normal men have been reported in hirsute women [6].

The effects of treatment on plasma androgen levels were as expected, taking into account the effect of the estrogens on SHBG and the partial inhibition of LH levels [32] and ovarian androgen secretion by the combined treatment [16, 17, 33]. Several authors reported a decrease of D and DS levels under a combined treatment with CPA and estrogens [33–35], even at a dose as low as 2 mg [36] or 5 mg [37] of CPA together with 35 μg of EE, comparable to the decrease of DS levels observed during treatment with oral contraceptives (OCs) [38–40]. It is rather surprising that A levels decreased only to a minor extent in our study, whereas during treatment with combined OCs A levels decrease significantly. Data from this laboratory [41] have shown that after treatment with 50 mg of CPA, A levels decrease by 50%, probably as a consequence of a more complete suppression of LH levels. The decrease of plasma AD levels ($\pm$30%) was less important than the decrease of plasma ADG levels ($\pm$ 60%), which is probably largely attributable to the increase in binding capacity of SHBG, which binds AD but not ADG.

It is remarkable that, although the conversion rates have been determined experimentally in only a small number of women, they account almost exactly for ADG levels measured in our group of healthy women. DHEAS, DHEA, A and T have been found to be the major precursors of plasma ADG [15]. Also DHT and AD contribute to ADG levels, but this contribution has not been taken into account under steady-state conditions, as these steroids are not secreted as such but are derived themselves from plasma T and A. Moreover, as 50% of plasma T in women originates from plasma A, only half the plasma T has to be taken into account. Although there is some conversion of DS to D, and vice versa [42], the conversion of DS to D has not been taken into account in our calculation as the conversion rate of D to ADG and the contribution of D to plasma ADG is very low.

When comparing the CR of precursors to plasma ADG, both before and during treatment, one should take into account the effects of treatment on SHBG levels, as it is considered by most authors that only the NSB-androgen fraction is available to most tissues [23, 24]. Therefore, we used the CR for the NSB-T fraction. Application of these CRs obtained in non-hirsute women, to our mildly hirsute patients, underestimated the ADG levels in 22 out of 24 subjects and in all PCO patients. This suggests an increased 5α-reductase activity in hirsute women [43], which is more pronounced in the PCO patients than in the patients with mild dysfunctional hirsutism. On the other hand, during treatment, the CRs overestimate the ADG levels, indicating that besides precursor levels, the treatment also decreases the conversion of precursors to ADG, probably via inhibition of 5α-reductase, an enzyme which plays a major role in the pathogenesis of so-called idiopathic hirsutism [1, 4, 44]. The apparent contrast between the decrease of plasma AD and the increase of plasma DHT, steroids which have essentially the same precursors and similar affinity to SHBG, is in line with an inhibition of the 3α-hydroxysteroid dehydrogenase activity as suggested by Reed *et al.* [45] in women receiv-

ing combined OCs, although this observation requires confirmation from additional experimental data.

In the small therapy-resistant group ($n = 5$) mean basal T, NSB-T and A levels were higher than in the responsive group, but AD and ADG levels were similar. Moreover, evolution of plasma androgen levels was similar in both groups (Table 4). Hence, determination of ADG levels during treatment did not permit any prediction concerning therapy outcome.

Kirschner *et al.* [10] claim a concordant change in ADG levels and clinical response; however, inspection of their data shows that of the 6, who did not respond to treatment, ADG levels decreased in 3.

In conclusion, our data, obtained from women with mild hirsutism, whether idiopathic or due to PCO, illustrate the relative value of plasma ADG levels as a parameter of peripheral androgen activity. Our data provide evidence for an increased conversion of proandrogens to 5α-reduced plasma androgens in these mildly hirsute patients. Treatment with CPA in combination with EE, does not only reduce the levels of DS, but also inhibits 5α-reductase activity, the major antiandrogenic effect of CPA being, however, competition with androgens for the receptor. Taking into account its multiple mechanisms of action, including competition with androgens for the receptor, partial inhibition of LH and of DS secretion as well as inhibition of enzymatic conversion of DS and other proandrogens to active androgens, CPA in combination with EE might be considered to be a valuable treatment of mild hirsutism, as evident from the clinical results.

Acknowledgement—This study was partly supported by Grant No. 3.0036.86 of the FWGO research grant Belgium.

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