Production Rates of Testosterone in Patients With Cushing's Syndrome

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Testosterone production rates were determined in 16 patients with Cushing's syndrome (4 men and 12 women) using the stable-isotope dilution technique and mass spectrometry. 1α,2α-D-Testosterone was infused for 10 hours at a dose of 20 μg/h (men) and 0.4 μg/h (women) and blood samples were obtained at 20-minute intervals during the last 4 hours of the observation period. Estimated production rates in men with Cushing's syndrome were 27, 73, 150, and 180 μg/h (mean, 106 ± 70 μg/h; healthy men [n = 12], 210 ± 70 μg/h). In the 12 women with Cushing's syndrome, testosterone production rates were 0.3 to 22.3 μg/h (healthy women [n = 5], 4.3 ± 1.9 μg/h). There was no difference in testosterone production rates in female patients with central (n = 8) versus adrenal (n = 4) Cushing's syndrome. In summary, testosterone production rates are subnormal or low-normal in male patients with endogenous hypercortisolism, but not in female patients with the same disorder. We conclude that testosterone production in men, but not in women, is predominantly of gonadal origin and hence susceptible to a glucocorticoid-induced suppression of gonadotropin secretion.

EXOGENOUS GLUCOCORTICOIDS suppress plasma testosterone concentrations in men by inhibiting luteinizing hormone–releasing hormone secretion.[1,2] The suppressive effect of corticotropin (ACTH) on gonadotropin secretion[3] is absent in patients with adrenocortical insufficiency[4] and is therefore also mediated by glucocorticoids. In male patients with Cushing's syndrome, hypogonadotropic hypogonadism is induced by an elevated plasma concentration of cortisol independently of the presence of space-occupying pituitary lesions.[5]

Although these effects of glucocorticoids on plasma testosterone concentrations have been well characterized, their impact on testosterone production rates has not been directly evaluated. This is of particular interest in women, in whom testosterone is primarily of adrenal origin and hence independent of the hypothalamus-pituitary-gonadal axis. Using the stable-lable isotope dilution technique, we have therefore examined testosterone production rates in male and female patients with Cushing's syndrome.

SUBJECTS AND METHODS

Experimental Protocol

Sixteen patients with Cushing's syndrome (12 women and 4 men) were included in the study. Informed consent was obtained in each case. Eleven patients (8 women and 3 men) had pituitary disease (Table 1). An adrenal adenoma was found in 3 women, and 1 woman had an adrenal carcinoma. Finally, a ACTH-producing carcinoid tumor of the lung was found in 1 man. In each patient, the biochemical diagnosis was based on the results of dexamethasone-suppression tests and ACTH-releasing factor–stimulation tests. Each case was subsequently cured by the appropriate surgical procedure.

On the day of the experiments, an indwelling catheter was inserted into an antecubital vein. A constant (40 mL/h) intravenous infusion of 1α,2α-D-testosterone (0.25 mg for men and 0.01 mg for women in 500 mL 0.9% saline also containing 2 mL of the individual's own blood) was started at 8 AM. At the beginning and end of each infusion, a sample of the infusate from the end of the infusion line was obtained to determine loss of stable testosterone by adsorption. Hence, actual individual infusion rates were determined retrospectively. After an equilibration period of 6 hours (at 2 PM), a second indwelling catheter was inserted in the contralateral arm and blood samples were obtained from 2 to 6 PM at 20-minute intervals. These blood samples were subsequently pooled, and the pooled samples were used for analysis. The results were compared with data previously obtained in 12 healthy men aged 22 to 34 years and five healthy women aged 19 to 32 years.[6,7]

Materials

All organic solvents were of high-performance liquid chromatography grade and were purchased from Baker Chemicals (Phillipsburg, NJ). Nonactive testosterone (17β-hydroxy-4-androsten-3-one) was obtained from Steraloids (Wilton, NH). Radioactive [^{3}H]1,2,6,7-testosterone (specific activity, 100 Ci/mmol) and stable-labeled 1α,2α-D-testosterone (isotopic enrichment 99.0%) were purchased from New England Nuclear (Boston, MA) and CDN Isotopes (Andover, MA), respectively.

Sample Preparation and Analysis by Gas Chromatography–Mass Spectrometry

Plasma samples (5.0 mL) supplemented with 20,000 dpm ^{3}H-testosterone for later control of recovery and with 20 mL 0.5% trifluoroacetic acid (TFA) were applied to Sep-Pak C-18 cartridges (500 mg; Waters/Millipore, Milford, MA) pretreated with successive application of 5.0 mL methanol, 5.0 mL ethyl acetate, 20 mL water, and 5.0 mL TFA (0.5% wt/vol). Following sample application, the cartridges were first treated with 3 × 5.0 mL TFA (0.5% wt/vol). Testosterone was subsequently eluted by ethyl acetate (2 × 1.0 mL), dried under a stream of nitrogen at 37°C, reconstituted in 100 μL CH_2Cl_2, and further prepurified by thin-layer chromatography (chloroform:acetone 70:30). The zone containing testosterone was eluted (2 × 2.5 mL methanol) and supplemented with 10 ng dehydrotestosterone (1,4-androstadien-17β-ol-3-one) as an internal standard for gas chromatography—mass spectrometry (GC-MS) analysis. Derivatization was subsequently performed with heptafluorobutyric anhydride:acetone (1:4, t = 60 minutes) at room temperature. Analysis by GC-MS (Finnigan MAT95 equipped with a 25-m CB5 fused silica column) was then performed using the selective ion monitoring mode and electric ionization (resolution 6,000). The tracer ions were *m/e* 678 (dehydrotestosterone, internal standard, *m/e* 680 (native testosterone), and *m/e* 682 (1,2D-testosterone). The sensitivity at a peak to noise ratio of 10:1 was less than 100 fg.[6,7]

Calculation of Testosterone Production Rates

Production rates of testosterone (PR[T]) were calculated from the product of the known infusion rate (Rt) and the ratio of tracer infusate

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Table 1. Plasma Concentration of Native Testosterone and Infused 1α,2α-D-Testosterone as Determined by GC-MS and Calculated Production Rate of Testosterone (2 to 6 PM) in 16 Patients With Cushing's Syndrome)

Patient No.	Age (yr)	Menses	Diagnosis	BS (m^2)	Infusate (ng/dL)	T (ng/dL)	dT (ng/dL)	PR T (μg/h)	PR T/m^2 (μg/m^2 · h)
Women									
1	38	Irreg	PA	1.70	2,250	5.1	16.2	0.3	0.2
2	24	Normal	PA	2.35	2,370	23.6	1.5	15.5	6.6
3	31	Irreg	PA	2.20	1,165	22.7	2.9	3.7	1.7
4	37	Irreg	PA	2.00	2,370	58.7	2.5	22.3	10.1
5	53	Menop	PA	2.10	2,246	7.3	5.2	1.3	0.6
6	35	Sec am	PA	1.70	1,060	21.2	2.4	3.7	2.2
7	20	Sec am	PA	1.75	1,785	34.3	1.6	15.3	8.7
8	48	Irreg	PA	1.85	2,481	27.0	1.7	15.9	8.6
9	64	Menop	AA	1.95	1,050	7.8	1.6	2.1	1.1
10	33	Sec am	AA	1.96	2,108	15.0	4.4	2.9	1.5
11	22	Sec am	AA	2.09	2,470	4.9	1.1	4.5	2.2
12	38	Sec am	AC	1.88	1,789	41.0	3.2	9.1	0.5
Healthy women (n = 5, mean ± SD)								4.3 ± 1.9	
Men									
13	28		PA	2.15	35,600	92.9	7.4	180.0	83.7
14	50		PA	1.88	32,460	171.7	81.3	27.0	14.4
15	27		PA	1.75	62,015	131.0	21.6	150.0	85.7
16	26		Ect ACTH	2.15	38,260	58.4	12.1	73.0	40.0
Health men (n = 12, mean ± SD)								210.0 ± 70.0	

Abbreviations: BS, body surface; T, testosterone; dT, stable-labeled testosterone; PR, production rate; Irreg, irregular; Menop, menopause; Sec am, secondary amenorrhea; PA, pituitary adenoma; AA, adrenal adenoma; AC, adrenal carcinoma; Ect ACTH, ectopic ACTH syndrome.

enrichment (Et) to tracer dilution in the plasma (Es): PR[T] = Rt × (Et/Es-1).[8]

Statistical Analysis

The data are expressed as the mean ± SD. Mean values were compared using Student's *t* test for unmatched pairs. *P* values less than .05 were considered statistically significant.

RESULTS

The plasma concentration of native and stable-labeled testosterone and the production rate of testosterone calculated in the 16 patients are shown in Table 1. In the female patients, the plasma concentration of endogenous testosterone was within the normal range (<60 ng/dL). The steady-state concentration of exogenous (labeled) testosterone was 2.6 ± 1.3 ng/dL in 11 patients; however, in 1 female patient, steady-state labeled testosterone was markedly higher (16.2 ng/dL). In this patient, the concentration of testosterone in the infusate was similar to that of the other female patients (2,250 ng/dL; other female patients, 1,899 ± 571 ng/dL). Plasma testosterone in this patient as determined by radioimmunoassay was 22.9 ng/dL, which is equivalent to the sum of labeled and unlabeled plasma testosterone determined by GC-MS (21.3 ng/dL). While the values for testosterone for this patient in Table 1 are therefore analytically correct, they resulted in a very low calculated production rate of testosterone. The production rate of testosterone in the remaining 11 female patients was 1.3 to 22.3 μg/h (healthy women, 4.3 ± 1.9 μg/h,[6] $P > .05$). Among both women with pituitary and with adrenal Cushing's disease, there were single patients with below-normal, normal, or above-normal production rates of testosterone. In contrast, none of the 4 male patients presented an above-normal production rate of testosterone (mean, 108 ± 70 μg/h; healthy men,[6,7] 210 ± 70 μg/h, $P < .05$).

DISCUSSION

The low-normal or below-normal production rates of testosterone in 4 male patients with Cushing's syndrome in this study is in keeping with the well-documented suppressive action of glucocorticoids on gonadotropin secretion,[5] which in men exceeds any potential stimulatory effect of ACTH on adrenal and/or gonadal[9] androgen synthesis. This is demonstrated by the suppressed testosterone production in the 1 male patient with ectopic ACTH syndrome.

In women, the adrenal's contribution to overall androgen production is more important than in men. Hence, androgen production in women is less susceptible to the suppressive effect of glucocorticoids on gonadotropin secretion, and suppressed testosterone production rates, as a rule, are not to be expected in response to increased cortisol production. Conversely, testosterone production in women is much more ACTH-dependent than in men. The normal or below-normal testosterone production rates in the 3 women with benign cortisol-producing adrenal tumors are thus explained by the parallel suppression of ACTH secretion in this form of Cushing's syndrome. Given the variability in the steroid production of adrenocortical carcinomas, the elevated testosterone production found in 1 woman with this diagnosis does not argue against this explanation.

The larger impact of ACTH on testosterone production in females also explains the elevated testosterone production rates in 4 of 8 women with central, ie, ACTH-dependent, Cushing's syndrome. Based on results obtained in a preponderantly female group of patient's with Cushing's syndrome, it has been reported that serum concentrations of the most abundant adrenal androgen, dehydroepiandrosterone sulfate (DHEA-S), are normal or increased in patients with the pituitary form of the disease but are suppressed in patients with benign (but not

malignant) adrenocortical tumors,[10] suggesting an important if not major role of ACTH in the regulation of adrenal DHEA-S secretion. Although the relative contribution of DHEA to the plasma testosterone concentration has been estimated to be only about 15% in healthy women[11] and may not be of major importance in hirsute women,[12] it is of note that the highest production rates of testosterone among our female patients were found in those with central disease. Plasma concentrations of DHEA and DHEA-S were not determined in our patients. However, it is likely that in central Cushing's syndrome the contribution of ACTH to adrenal androgen synthesis is larger than in healthy women or in idiopathic hirsutism. However, among the 8 women with ACTH-dependent Cushing's syndrome, there were also 2 patients with normal and 2 with suppressed production rates of testosterone. This heterogeneity of testosterone production in women with ACTH-dependent Cushing's syndrome is difficult to explain. In contrast to the female control group studied in the follicular phase of the menstrual cycle, the timing of the investigation with regard to the menses could not be standardized because menstrual bleeding was irregular or absent in all but 1 patient (Table 1). In the patients with regular or irregular menses, we therefore cannot exclude a possible influence of the individual's menstrual cycle phase on testosterone production rates. However, the heterogeneity in testosterone production did not relate to the presence or absence of a menstrual cycle. In one female patient with a very low calculated production rate of testosterone, the substantially higher steady-state concentrations of infused (stable-labeled) testosterone may have interfered with endogenous production of testosterone. Whether elevated endogenous glucocorticoids in the other patients of this subgroup may have directly inhibited ovarian androgen production[13] remains speculation at this point.

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