0022-202X/85/8401-0062$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 84:62–65, 1985

Vol. 84, No. 1
Printed in U.S.A.

Androgen Receptor Characteristics in Skin Fibroblasts from Hirsute Women*

CHARLES EIL, M.D., PH.D.,† GORDON B. CUTLER, JR., M.D., AND D. LYNN LORIAUX, M.D., PH.D.

Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, U.S.A.

Hormonal measurements in some women with hirsutism often reveal little or no elevation in androgen levels to explain the disorder. Thus, it has been postulated that increased sensitivity of the hair follicle to androgen may contribute to the development of hirsutism in such patients. We, therefore, sought androgen receptor abnormalities in skin fibroblasts cultured from 10 hirsute women (ages 17–43) and normal or mildly elevated plasma testosterone levels (28–82 ng/dl). Androgen receptor content (R_0) and binding affinity (K_d) in cultured pubic skin fibroblasts were measured using a dispersed, whole cell assay. Ten such cell lines from these women were compared with 19 pubic skin cell lines from 9 normal volunteers (6 males and 3 females) and from 10 other subjects (males with gynecomastia or hypospadias). There was no statistically significant difference in the mean androgen receptor content (11,600 ± 2700 (SE) sites/cell fibroblasts vs 7900 ± 700 sites/cell or binding affinity (2.0 ± 0.3 (SE) × 10^{-9} M vs 1.5 ± 0.2 × 10^{-9} M, respectively) between the patients' fibroblasts and those of the controls.

We conclude that hirsutism cannot be explained by abnormalities in fibroblast androgen receptor number or affinity. These observations do not exclude the possibility that other mechanisms might lead to increased peripheral androgen sensitivity in such patients.

Hirsutism in women who have normal or mildly elevated plasma testosterone levels, and who have no known etiology to account for elevated androgen production, is often termed idiopathic hirsutism (IH). Such patients may or may not have associated menstrual abnormalities. Several lines of evidence suggest that IH is an androgen-dependent disorder. First, the usual onset of the disorder is at puberty, when rising androgen levels stimulate pubic and axillary hair development. Second, the distribution of hair parallels that of androgen-dependent hair in males, which includes areas such as the upper lip, chin, sideburns, and chest [1]. Third, women with IH, as a group, have elevated levels of free plasma testosterone and elevated testosterone blood production rates compared to normal women [2,3]. Fourth, therapeutic agents that suppress adrenal or ovarian androgen production, or that block the action of testosterone on the hair follicle, reduce the rate of hair growth in many hirsute women [4–12].

Despite this evidence, several observations have cast doubt on whether IH can be explained entirely on the basis of elevated androgen production. Some women with IH have hirsutism despite normal circulating levels of free and total testosterone. Other women have high androgen levels, similar to the levels usually seen in IH, but do not have hirsutism [5]. These findings have suggested that heightened end-organ sensitivity to androgen might play an important role in the pathogenesis of hirsutism [13]. Three mechanisms that might cause such an increase in sensitivity are an increase in the activity of peripheral 5α-reductase, which converts testosterone to the more active dihydrotestosterone, an increase in androgen receptor number (R_0) or binding affinity (K_d), or an increase in the efficiency of processes subsequent to receptor binding. Since 5α-reductase activity in the pubic hair follicles of hirsute women has been shown to be similar to that of normal women [14], we sought to test the hypothesis that hirsutism may result from enhanced androgen receptor activity by measuring androgen R_0 and K_d in pubic skin fibroblasts grown from 10 hirsute women and from 19 control subjects.

MATERIALS AND METHODS

Subjects

All patients included in this study showed moderate to severe hirsutism according to the criteria of Lorenzo [1] and presented for evaluation and treatment of this condition. Eight of the 10 patients had terminal hair in the midsternal region which occurs in less than 3% of normal women [15]. The patients had no evidence of the following disorders: adrenal or ovarian tumors, Cushing's syndrome, or congenital adrenal hyperplasia (either the classic or attenuated forms). None showed evidence of virilization such as clitoromegaly, deepening of the voice, or a male muscular habitus. Many were obese and only 4 had normal menses. The remaining 6 patients had oligomenorrhea or amenorrhea. Two patients had enlarged or polycystic ovaries. Further clinical and laboratory features of these patients are provided in Table I.

Normal control subjects consisted of men and women volunteers between the ages of 18–24 who consented to skin biopsy and several men with gynecomastia or hypospadias who also consented to biopsy. Since the pubic skin fibroblast androgen receptor characteristics from these latter subjects have been shown to be normal in earlier studies [17], these data were pooled with those for the normal volunteers. Additionally, since our data and that of several published studies showed no difference in fibroblast androgen receptor number or affinity between normal men and women [18–21], we pooled male and female control data.

Materials

Collagenase (Type I from Cl. histolyticum) and Tricene (N-Tris[hydroxymethyl]methyl glycine) were purchased from Sigma Chemical Co. (St. Louis, Missouri). Fetal calf serum (mycoplasma and virus screened) was obtained from Grand Island Biological Co. (Grand Island, New York). Tris (Tris[hydroxymethyl]aminomethane) was obtained from Bethesda Research Laboratories, Inc. (Rockville, Maryland). [1,2,4,5,6,7-^{3}H(N)]dihydrotestosterone (DHT), 123–133 Ci/mmol, and liquid scintillation fluid (Aquasol) were from New England Nuclear (Boston, Massachusetts). Insulin (Iletin, U-100) was from Eli Lilly and Company (Indianapolis, Indiana). Tissue culture flasks (75 cm^2 and 150 cm^2) were purchased from Costar (Cambridge, Massachu-

Manuscript received April 2, 1984; accepted for publication July 10, 1984.

* Portions of this work were presented in abstract form at the 62nd Annual Meeting of the Endocrine Society, Washington, D.C., June 1980.

† Present address: Box 396, National Naval Medical Center, Bethesda, Maryland 20814.

Reprint requests to: Charles Eil, M.D., Box 396, National Naval Medical Center, Bethesda, Maryland 20814.

Abbreviations:
DHT: [1,2,4,5,6,7-^{3}H(N)]dihydrotestosterone
IH: idiopathic hirsutism
IMEM: improved Eagle's minimal essential medium
K_d: binding affinity
PBS: phosphate-buffered saline
R_0: receptor content

TABLE I. *Clinical and laboratory features of women with hirsutism*

Patient no.	Age (years)	Percent desirable weight[a]	Menses[b]	Duration of hirsutism (years)	Total testosterone (ng/dl)	LH (mIU/ml)	FSH (mIU/ml)	Prolactin (ng/ml)	DHAS[c] (μg/dl)	24-h Urine 17-ketosteroids (mg)	Comments
1	36	149	N	24	36	17	13	15	121	10	
2	29	147	N	10	50	10	6	12	303	16	Acne
3	26	145	N	13	28	10	13	8	349	14	Polycystic ovaries[d]
4	26	230	A	12	38	14	11	11	234	13	Acne
5	24	106	0	7	78	20	11	23	280	14	
6	33	193	0	20	82	17	10	7	132	10	
7	17	203	0	3	73	20	17	21	186	10	Generalized scalp hair loss
8	31	149	0	16	75	22	8	6	237	ND[e]	
9	43	141	N	16	46	36	14	9	15	5	
10	34	100	0	18	52	57	14	7	36	6	Bilaterally enlarged ovaries[f]
Mean ± SEM	30 ± 3	156 ± 12		14 ± 3	56 ± 6	22 ± 4	12 ± 1	12 ± 2	189 ± 34	11 ± 1	
Normal range					20–60	5–25	5–25	<30	60–380	6–15	

[a] Derived from the medium-frame ideal body weight estimates of the Society of Actuaries [16].
[b] N = normal; 0 = oligomenorrhea; A = amenorrhea (no menses within 12 months).
[c] DHAS, dehydroepiandrosterone sulfate.
[d] Enlarged cystic ovaries by physical examination and ultrasound.
[e] ND, not determined.
[f] Enlarged ovaries by physical examination.

setts), Falcon (Oxnard, California), and Corning Glass Works (Corning, New York). Petri dishes (60 mm) were also from Falcon. Nonradioactive methyltrienolone (R1881) was a gift from Dr. J. P. Raynaud, Roussel UCLAF (Romainville, France). All tissue culture media, trypsin-EDTA solutions, and phosphate-buffered saline (PBS) were prepared and supplied by the National Institutes of Health Media Unit.

Cell Culture

Fibroblast strains were established from pubic skin specimens obtained by punch biopsy. The specimens were finely minced in 60-mm Petri dishes containing 4–5 ml of Medium A consisting of improved Eagle's minimal essential medium (IMEM) supplemented with 10% fetal calf serum, 10^{-7} M insulin, collagenase (2 mg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and glutamine (0.584 g/liter). This medium was freshly prepared for each sample and filtered through a Swinnex-13 Millipore filter (Bedford, Massachusetts) immediately prior to use. After 48 h at 37°C in the presence of 5% CO_2 in a humidified incubator, the medium was changed to a growth medium (Medium B) consisting of Medium A without collagenase. When grown to confluence (usually within 3–4 weeks), the cells were disrupted with 0.05% trypsin-0.02% EDTA in saline at 37°C and passed serially into larger flasks (75 cm^2 and 150 cm^2).

Whole Cell [^{3}H]DHT Binding Assay

Fibroblasts were grown to confluence in 5 or 6 150-cm^2 tissue culture flasks for routine assay. This usually required 5–6 weeks from the time of the initial seeding of the cell line. Two days prior to assay the medium was changed to Medium C (Medium B without fetal calf serum). This was repeated again 24 h prior to assay. The remainder of the procedure has been previously described [18]. The results were calculated by Scatchard analyses and binding capacity was expressed as binding sites/cell [22].

Hormone Assays

Serum testosterone LH, FSH, prolactin, and dehydroepiandrosterone sulfate were measured by radioimmunoassay as previously described [23–27]. Urine 17-ketosteroids were measured by Bioscience Laboratories (Columbia, Maryland).

Statistical Analysis

Statistical analysis of the data was performed using Student's *t*-test and the Wilcoxon rank sum test.

RESULTS

Clinical Data

The pertinent clinical characteristics and laboratory data of the patients are shown in Table I. The ages of the patients ranged from 17–43 with a mean of 30 years. Hirsutism was present an average of 14 years. Many had irregular menses and most were above ideal body weight. Serum testosterone was 56 ± 6 ng/dl (mean ± SE). Four of the patients had serum testosterone levels slightly above the normal range (<60 ng/dl).

TABLE II. *Androgen receptor characteristics of cultured skin fibroblasts*

Cell line	R_0 (sites/cell)	K_d (× 10^{-9} M)
Hirsute women		
1	6,610	1.71
2	4,510	0.57
3	8,590	3.00
4	10,160	2.36
5	4,500	1.97
6	15,450	0.96
7	29,110	2.50
8	25,220	3.83
9	6,860	1.10
10	5,390	2.25
Mean ± SEM	11,600 ± 2,700[a]	2.0 ± 0.3[a]
Controls (n = 19)		
Mean ± SEM	7,900 ± 700	1.5 ± 0.2

[a] Not significantly different from normal controls.

Androgen Receptor Data

The mean [^{3}H]DHT binding capacity (R_0, sites/cell) and mean dissociation constants (K_d) in the fibroblasts from pubic skin of the women with hirsutism and the controls is shown in Table II. There were no statistically significant differences for either binding parameter in the patients' cells when compared to those for the cells from the control subjects. The individual R_0 values for all of the fibroblast strains assayed are shown in Fig 1. The data from both groups of subjects overlapped, although the binding capacities for 2 of the patient cell lines (Nos. 7 and 8) were higher than for any of the control cells. These patients did not differ in any apparent way from the other hirsute women.

DISCUSSION

Normal androgen action is a multistep process that depends on both normal androgen biosynthesis and normal end-organ sensitivity to androgen. In patients with male pseudohermaphroditism in whom blood levels of androgens are normal or elevated, in vitro studies with fibroblasts cultured from human skin have been particularly useful in elucidating the mecha-

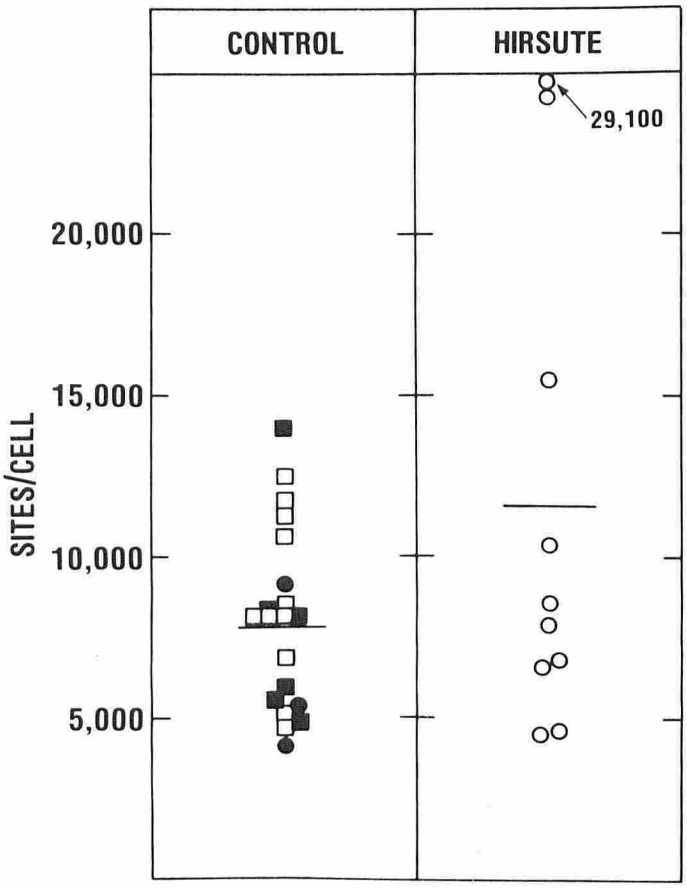

FIG 1. Scattergram of binding capacities for whole cell binding of [^{3}H]DHT in dispersed fibroblasts cultured from pubic skin in normal men (■), normal women (●), men with gynecomastia or hypospadias (□), or women with hirsutism (○).

nisms of the impairment in androgen action [19,28–30]. Although many women with hirsutism have been shown to have increased androgen production [3,31–33], it has been postulated that increased end-organ androgen sensitivity at the hair follicle may also contribute to hirsutism in these women. Moreover, such increased sensitivity might be the sole pathogenic mechanism in women who have no detectable abnormality of androgen production.

To test this hypothesis we measured androgen binding in pubic skin fibroblasts from hirsute and control subjects. Our results did not indicate any increase in the number or affinity of fibroblast androgen receptors in hirsute women. Furthermore, while these studies were in progress Mowszowicz et al [21], using a monolayer technique, also showed normal androgen receptors in pubic skin fibroblasts from hirsute women. Additionally, Sultan et al have shown that patients with adolescent hirsutism have normal fibroblast androgen receptors [34]. Thus, all of the available data suggest that hirsute women do not have heightened androgen sensitivity on the basis of altered androgen receptor number or affinity. These observations do not exclude the possibility, however, of increased hair follicle sensitivity to androgen based upon other mechanisms, such as increased local production of androgens from plasma precursors [21,35] or increased efficiency of the steps distal to androgen binding to receptor. Additional in vitro studies with skin or cultured cells from hirsute women on the factor(s) responsible for the striking elevation of plasma 3α,17β-androstanediol glucuronide, a marker of increased peripheral androgen action in these women [32], may shed some light in this regard.

The authors are grateful to Mr. Grant Coffman for technical assistance, to Dr. Robert A. Vigersky for referring patient 10, to Dr. Marc E. Lippman for laboratory support, and to Ms. Cathy Morris for preparing the manuscript.

REFERENCES

1. Lorenzo EM: Familial study of hirsutism. J Clin Endocrinol Metab 31:556–564, 1970
2. Rosenfield RL: Plasma testosterone binding globulin and indexes of the concentration of unbound plasma androgens in normal and hirsute subjects. J Clin Endocrinol Metab 32:717–728, 1971
3. Bardin CW, Lipsett MB: Testosterone and androstenedione blood production rates in normal women and women with idiopathic hirsutism or polycystic ovaries. J Clin Invest 46:891–902, 1967
4. Casey JH, Burger HG, Kent JR, Kellie AE, Moxham A, Nabarro J, Nabarro JDN: Treatment of hirsutism by adrenal and ovarian suppression. J Clin Endocrinol Metab 26:1370–1374, 1966
5. Hosseinian AH, Kim MH, Rosenfield RL: Obesity and oligomenorrhea are associated with hyperandrogenism independent of hirsutism. J Clin Endocrinol Metab 42:765–769, 1976
6. Ismail AAA, Davidson DW, Souka AR, Barnes EW, Irvine WJ, Kilimnik H, Vanderbecken Y: The evaluation of the role of androgens in hirsutism and the use of a new anti-androgen "cyproterone acetate" for therapy. J Clin Endocrinol Metab 39:81–95, 1974
7. Boisselle A, Tremblay RR: New therapeutic approach to the hirsute patient. Fertil Steril 32:276–279, 1979
8. Shapiro G, Evron S: A novel use of spironolactone: treatment of hirsutism. J Clin Endocrinol Metab 54:429–432, 1980
9. Vigersky RA, Mehlman I, Glass AR, Smith CE: Treatment of hirsute women with cimetidine: a preliminary report. N Engl J Med 303:1042, 1980
10. Ekoe JM, Burckhardt P, Ruedi B: Treatment of hirsutism, acne, and alopecia with cyproterone acetate. Dermatologia 160:398–404, 1980
11. Cumming DC, Yang JC, Rebar RW, Yen SSC: Treatment of hirsutism with spironolactone. JAMA 247:1295–1298, 1982
12. Milewicz A, Silber D, Kirschner MA: Therapeutic effects of spironolactone in polycystic ovary syndrome. Obstet Gynecol 61:429–432, 1983
13. Brooksbank BWL: Endocrinologic aspects of hirsutism. Physiol Rev 41:623–676, 1961
14. Miyazaki M, Takayasu S, Karakawa T, Aono T, Kurachi K, Matsumoto K: Activity and testosterone 5α-reductase in the hair follicle of women with polycystic ovaries. J Endocrinol 78:445–446, 1978
15. Ferriman D, Gallwey JD: Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 21:1440–1447, 1961
16. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039–1057, 1979
17. Eil C, Crawford JD, Donahoe PK, Johnsonbaugh RE, Loriaux DL: Fibroblast androgen receptors in males with genito-urinary anomalies. J Andrology 5:313–320, 1984
18. Eil C, Lippman ME, Loriaux DL: A dispersed-whole cell method for the determination of androgen receptors in human skin fibroblasts. Steroids 35:389–404, 1980
19. Griffin JE, Punyashthiti K, Wilson JD: Dihydrotestosterone binding by cultured human fibroblasts. Comparison of cells from control subjects and from patients with hereditary male pseudohermaphroditism due to androgen resistance. J Clin Invest 57:1342–1351, 1976
20. Amrhein JA, Meyer WJ III, Danish RK, Migeon CJ: Studies of androgen production and binding in 13 male pseudohermaphrodites and 13 males with micropenis. J Clin Endocrinol Metab 45:732–738, 1977
21. Mowszowicz I, Melanitou E, Doukani A, Wright F, Kuttenn F, Mauvais-Jarvis P: Androgen binding capacity and 5α-reductase activity in pubic skin fibroblasts from hirsute patients. J Clin Endocrinol Metab 56:1209–1213, 1983
22. Aitken S, Lippman ME: A simple computer program for quantitation and Scatchard analysis of steroid receptor proteins. J Steroid Biochem 8:77–93, 1977
23. Neischlag E, Loriaux DL: Radioimmunoassay for plasma testosterone. Z Klin Chem Klin Biochem 10:164–168, 1972
24. Odell WD, Ross GT, Rayford PL: Radioimmunoassay for luteinizing hormone in human plasma or serum: physiological studies. J Clin Invest 46:248–255, 1967
25. Cargille CM, Rayford PL: Characterization of antisera for follicle-stimulating hormone radioimmunoassay. J Lab Clin Med 75:1030–1040, 1970
26. Aubert ML, Becker RL, Saxena BB, Raiti S: Report of the National Pituitary Agency: a collaborative study of the radioimmunoassay of human prolactin. J Clin Endocrinol Metab 38:1115–1120, 1974
27. Cutler GB Jr, Glenn M, Bush M, Hodgen GD, Graham CH, Loriaux DL: Adrenarche: a survey of rodents, domestic animals, and primates. Endocrinology 103:2112–2118, 1978
28. Keenan BS, Meyer WJ III, Hadjian AJ, Jones HW, Migeon CJ: Syndrome of androgen insensitivity in men: absence of 5α-

dihydrotestosterone binding protein in skin fibroblasts. J Clin Endocrinol Metab 38:1143–1146, 1974
29. Kaufman M, Straisfeld C, Pinsky L: Male pseudohermaphroditism presumably due to target organ unresponsiveness to androgens: deficient 5α-dihydrotestosterone binding in cultured fibroblasts. J Clin Invest 58:345–350, 1976
30. Eil C: Familial incomplete male pseudohermaphroditism associated with impaired nuclear androgen retention: studies in cultured fibroblasts. J Clin Invest 71:850–858, 1983
31. Kirschner MA, Bardin CW: Androgen production and metabolism in normal and virilized women. Metabolism 21:667–668, 1972
32. Horton R, Hawks D, Lobo R: 3α,17β-Androstanediol glucuronide in plasma: a marker of androgen action in idiopathic hirsutism. J Clin Invest 69:1203–1206, 1982
33. Wild RA, Umstot ES, Andersen RN, Renney GB, Givens JR: Androgen parameters and their correlation with body weight in one hundred thirty-eight women thought to have hyperandrogenism. Am J Obstet Gynecol 146:602–606, 1983
34. Sultan C, Terraza A, Devillier C, Michell B, Descomps B, Jean R: Androgen receptors in cultured human skin fibroblasts. Br J Dermatol 107 (suppl 23):40–46, 1982
35. Kuttenn F, Mowszowicz I, Schaison C, Mauvais-Jarvis P: Androgen production and skin metabolism in hirsutism. J Endocrinol 75:83–91, 1977