High Dose Androgen Therapy in Male Pseudohermaphroditism Due to 5α-Reductase Deficiency and Disorders of the Androgen Receptor

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Abstract. We describe the clinical and biochemical features of six men with male pseudohermaphroditism due to androgen resistance. Each of the subjects had male-gender behavior but incomplete virilization. The underlying defects in androgen metabolism were defined by studies of the 5α-reductase enzyme and the androgen receptor in fibroblasts cultured from biopsies of genital skin. Four of the six have 5α-reductase deficiency, and two have defects of the androgen receptor (the Reifenstein syndrome).

The responses of these men to androgen treatment were assessed by monitoring nitrogen balance, plasma luteinizing hormone (LH) values, and clinical parameters of virilization including penile growth, potency and ejaculatory volume, muscle bulk, and growth of body and facial hair. In all of the subjects with 5α-reductase deficiency and one man with the Reifenstein syndrome significant response occurred, as evidenced by nitrogen retention, lowered plasma LH levels, and improved virilization, with doses of parenteral testosterone esters that raised plasma testosterone levels above the normal male range and brought plasma dihydrotestosterone levels into the normal male range. The subject who did not respond with clinical virilization nevertheless showed nitrogen retention in response to acute testosterone administration. This patient had a profound deficiency of the androgen receptor, whereas the man with a receptor defect who did respond clinically to therapy had normal amounts of a qualitatively abnormal receptor.

We conclude that high dose androgen therapy may be of benefit in improving virilization, self-image, and sexual performance in subjects with 5α-reductase deficiency who have male-gender behavior and in some subjects with defects of the androgen receptor.

Introduction

At least three categories of defects can result in hereditary resistance to androgen action—deficiency of the 5α-reductase enzyme that converts testosterone to dihydrotestosterone in androgen target tissues, disorders of the high affinity androgen receptor protein, and abnormalities that are presumed to reside at some subsequent step in androgen action (1). The first two categories of androgen resistance are the best understood.

5α-Reductase deficiency is an autosomal-recessive trait in which affected 46,XY individuals have normal testosterone-mediated virilization of Wolffian ducts during embryogenesis but defective virilization of the external genitalia, a process that is mediated in the normal male by dihydrotestosterone. Such subjects are usually regarded as female at birth and are raised as such. At the time of expected puberty these individuals virilize partially; however, phallic enlargement, development of body and facial hair, and temporal hair regression are usually less than in normal men, ejaculatory volumes are small, and they are uniformly infertile. Before or after the commencement of virilization, affected individuals commonly undergo a change of gender role from female to male; subjects who assume the male role usually complain of incomplete virilization, once surgical revision of the external genitalia, if

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practical, is accomplished. Other patients are castrated in infancy and raised successfully as women (1–5).

Disorders of the androgen receptor are inherited as X-linked recessive traits and result in a spectrum of phenotypic manifestations, depending on the severity of impairment of receptor function. They range from 46,XY phenotypic women (complete testicular feminization) to men with severe hypospadias, infertility, and gynecomastia, commonly termed the Reifenstein syndrome, to otherwise normal men with infertility due to severe oligospermia and/or azoospermia (1). Those subjects at the male end of this spectrum also complain of incomplete virilization, particularly of the paucity of facial and body hair and small ejaculatory volume.

Plasma testosterone levels are normal or elevated in patients with 5α-reductase deficiency and disorders of the androgen receptor (1). Most previous studies of the effects of supraphysiological doses of androgen in subjects with disorders of the androgen receptor have provided inconsistent evidence of either biochemical or clinical benefit (6–10), and the effects of supplemental androgen in 5α-reductase deficiency have not been examined systematically. We reasoned that treatment with large amounts of testosterone might result in significant acceleration of virilization in individuals with 5α-reductase deficiency who wish to function as men. Such virilization might occur on the basis of one or both of two mechanisms. First, all patients with 5α-reductase deficiency characterized to date have some residual 5α-reductase activity measurable either in tissues or in cultured fibroblasts (1, 2, 11–13), and all have some dihydrotestosterone detectable in the circulation, albeit in subnormal levels (1–5); as a consequence, raising the plasma testosterone above the normal range might result in an increase in dihydrotestosterone formation and/or blood levels to the normal range. Second, if as has been postulated, dihydrotestosterone formation functions in the normal male only as a magnifier of a weak hormonal signal (14), a supraphysiological level of plasma testosterone might in and of itself, by mass action, act to promote virilization that could ordinarily be accomplished by much lower levels of dihydrotestosterone.

Therefore, we studied the effects of two high dose regimens of testosterone administration on nitrogen balance in four subjects with 5α-reductase deficiency and then monitored their clinical responses to long-term high dose therapy. Treatment with dihydrotestosterone itself is not practicable because it is not available in a suitable preparation. These results have been compared with similar studies in two men with incomplete male pseudohermaphroditism associated with abnormalities of the androgen receptor.

Methods

Patients. The clinical features of the six men are summarized in Table I. C.P. and P.P. have been described previously (15), and the characteristics of the 5α-reductase in M.M. have been described in brief (16). The four men with 5α-reductase deficiency were reared as girls but had disturbances of gender role and identity during childhood. They all exhibited tomboyish behavior, and at some stage between ages four and ten each came to feel that he was a male but nevertheless continued to play a female role for several years. Eventually, each of the four changed gender role to that of men and claimed unequivocal male gender identity. Each of the men has heterosexual orientation. C.P. and P.P. are brothers, and another (N.A.) has two younger siblings with similar phenotypes and 5α-reductase deficiency (see Table II). The two men with androgen receptor defects had male gender assignment and roles from birth (S.C.) or age 6 wk (B.P.).

All six subjects have a 46,XY karyotype and were born with perineoscrotal hypospadias and a bifid scrotum. In five, a urogenital sinus was present with a blind ending vagina leading into a female bulbar urethra. The exact genital anatomy of N.A. is uncertain although that of his two affected siblings is known and is characteristic of 5α-reductase deficiency. Absence of Müllerian duct structures has been demonstrated in five of the six at laparotomy. Each of the subjects has had operations to correct hypospadias and/or other genital abnormalities.

After completion of the nitrogen balance studies the subjects were given long-term replacement therapy with 500 mg/wk of intramuscular testosterone esters either in the form of testosterone enanthate or of a mixture of testosterone propionate, 60 mg; testosterone phenylpropionate, 120 mg; testosterone isohexanoate, 120 mg; and testosterone decanoate, 200 mg, for 9–36 mo and then were changed to oral testosterone undecanoate, 80 mg, three times daily. These regimens are described in Table IV. All six subjects were subjected to all four regimens, but plasma hormone values were not measured in all periods. Their clinical progress was assessed over 30–86 mo on treatment.

Materials. Materials for cell culture have been described (17, 18). [1,2,4,5,6,7-^{3}H]Dihydrotestosterone (123 Ci/mmol), [1,2-^{3}H]testosterone (40 Ci/mmol), and [4-^{14}C]dihydrotestosterone (57 mCi/mmol) were from New England Nuclear (Boston, MA). Sodium molybdate was from Sigma Chemical Co. (St. Louis, MO).

Cell culture. The fibroblast strains used in these experiments were established and propagated from explants of foreskin or scrotum as described (17). The controls include normal subjects, patients with developmental defects of the external genitalia (hypospadias, microphallus, cryptorchidism), and a 46,XY individual with pseudohermaphroditism due to deficiency of the enzyme 17β-hydroxysteroid dehydrogenase. All fibroblasts were utilized before the 20th transfer.

5α-Reductase assay. To grow fibroblasts for 5α-reductase assays, cells from stock flasks were dissociated with 0.05% trypsin and 0.02% EDTA, and 5×10^5 cells were seeded (day 0) into Petri dishes (diameter 15 cm) in 25 ml of Eagle's minimum essential medium containing 10% newborn calf serum. On days 3 and 6 the medium was removed, and the same volume of fresh growth medium was added. On day 7 cell-free extracts were prepared at 4°C. Each monolayer was rinsed twice with 10 ml of Tris-saline (50 mM Tris Cl, pH 7.4, and 150 mM NaCl) and harvested by scraping into 5 ml of the same buffer. The cell suspension was centrifuged at 800 *g* for 10 min, and the pellet was twice resuspended in 5 ml of Tris-saline and recentrifuged. The pellet was finally resuspended in 10 mM Tris Cl, pH 7.4, and subjected to sonic disruption. The standard assay mixture for 5α-reductase contained 50 nM [1,2-^{3}H]testosterone (4.4×10^5 dpm), 0.8 μM [4-^{14}C]dihydrotestosterone (10,000 dpm), 3 mM NADPH, 0.1 M Na citrate, 0.1 M Tris Cl, pH 5.5, and enzyme (10–300 μg protein) in a total volume of 0.1 ml. Samples were incubated 1 h at 25°C. To estimate Michaelis constant (K_m) values for the substrate, concentrations of testosterone were varied from 0.01 to 2.5 μM; for measurement of

Table 1. Clinical Features of the Subjects before Treatment with Testosterone Esters

Diagnosis:	5α-reductase deficiency				Defects of the androgen receptor	
Subjects:	C.P.	P.P.	N.A.	M.M.	B.P.	S.C.
Age at time of study (*yr*)	17	19	23	26	32	23
Country of origin	Cyprus	Cyprus	Pakistan	Malta	United Kingdom	United Kingdom
Gender assignment in infancy	Female	Female	Female	Female	Male	Male
Cognitive gender identity in childhood before change of gender role to male	Male from age 8	Male from age 4	Male from age 5	Male from age 10	NA	NA
Age of onset of virilization/change of gender role to male (*yr*)	13/14	12/16	13/14	10/24	13/NA	12/NA
Family history	C.P. and P.P. are brothers; a maternal third cousin is probably a male pseudohermaphrodite		Parents are first cousins; two 46,XY siblings have 5α-reductase deficiency	Uninformative	Uninformative	Uninformative
External genitalia	Perineoscrotal hypospadias with a urogenital sinus and blind ending vaginal pouch on the posterior surface of the urethra; partial labioscrotal fusion; small glans; penis stretched length 4.9 cm in C.P. and 4.5 in P.P.		Perineoscrotal hypospadias with a bifid scrotum, small glans with a stretched penis length of 4 cm	Perineoscrotal hypospadias with a bifid scrotum; blind ending vaginal pouch opening into the bulbar urethra; small glans with a stretched penis length of 5 cm	Perineoscrotal hypospadias; vagina and female urethra; small glans with a stretched penis length of 5 cm	Perineoscrotal hypospadias; vaginal pouch leading into urethra; small glans with a stretched penis length of 4 cm

Epididymis present	Yes	Yes	?	?	Yes	?
Müllerian duct derivatives	No	No	?	No	No	No
Site and size of testis	Scrotum after orchiopexy; 15 and 12 ml	Scrotum after orchiopexy; 15 ml bilaterally	Scrotum; 12 and 10 ml	Right-sided orchiopexy; 4 ml on left	Inguinal canal after orchiopexy; size undetermined	External inguinal ring, 1–2 ml; orchidectomy at age 23
Prostate by palpation	Small	Small	Small	Not palpable	Not palpable	Not palpable
Habitus	Male; good muscle development	Male; good muscle development	Male; fair muscle development	Male; fair muscle development; female fat distribution	Male; poor muscle development	Female fat distribution
Facial and body hair	Scant	Scant	Scant	Scant	Moderate beard and pubic hair	Scant
Gynecomastia	None	Minimal unilateral	None	None	Unilateral	Bilateral; marked
Acne	Mild	Mild	None	None	None	None
Sperm count	Small volume ejaculate (0.4 ml) containing 128,000 sperm	Small volume ejaculate containing 32,000 immotile sperm	Azoospermia	Small volume ejaculate; azoospermia	Small volume ejaculate; azoospermia	Trace ejaculate; azoospermia
Sexual activity	Masturbation or intercourse three times weekly	Masturbation three times weekly	Occasional masturbation	Occasional masturbation	Regular sexual intercourse	Occasional masturbation

apparent K_m for cofactor, concentrations of NADPH were varied from 0.0025 to 2.5 mM, and the concentration of testosterone was held constant at 0.25 or 0.5 μM. Apparent K_m values were determined from double-reciprocal plots using the method of least squares. At the end of the incubations, reactions were stopped by the addition of 5 vol of chloroform/methanol (2:1). Steroids were then extracted and prepared for chromatography. 10 μg of authentic dihydrotestosterone was added to the extracts, which were then taken to dryness, reconstituted in 20 μl chloroform, and applied to plastic sheets (20 × 20 cm) precoated with silica gel. After chromatography in dichloromethane/ethyl acetate/methanol (85:15:3) the steroids were visualized by spraying with water. The zone corresponding to dihydrotestosterone was cut and assayed for ^{3}H and ^{14}C after the addition of 10 ml of 0.4% 2,4-diphenyloxazole in toluene/methanol (10:1). 5α-Reductase activity (picomoles per hour) was calculated from the fraction of total ^{3}H-radioactivity recovered in the dihydrotestosterone area corrected for the recovery of the [^{14}C]dihydrotestosterone.

To assess the stability of 5α-reductase after exposure to cycloheximide, confluent monolayers of fibroblasts were incubated with 1 mM cycloheximide for 24 h, and 5α-reductase was assayed in the standard broken cell preparation. The enzyme activity in cycloheximide-treated cells was expressed as the percentage of the activity in untreated cells of the same strain assayed at the same time.

Dihydrotestosterone binding. Cells from stock flasks were dissociated with 0.05% trypsin-0.02% EDTA at 37°C for 3 min and seeded (day 0) in 60-mm diam wells in Linbro multiwell plates at a concentration of ~150,000 cells in 8 ml of medium containing 10% newborn calf serum. On day 3 the medium was removed and replaced with the same volume of fresh medium with serum. On day 6 the monolayers were rinsed with 2 ml of phosphate-buffered saline, and 8 ml of medium without serum were added. On day 7 the medium was removed, and the monolayers were rinsed once with 2 ml of medium without serum and then incubated with various concentrations of [^{3}H]dihydrotestosterone (0.2–3 nM) in medium without serum with or without a 250-fold excess of nonradioactive dihydrotestosterone. The standard assay was carried out at 37°C in a 5% CO_2 incubator for 60 min. In experiments at 42°C the monolayers were preincubated at 42°C for 30 min in medium without serum; the medium was removed, and medium containing [^{3}H]dihydrotestosterone (also warmed at 42°C) was added. The monolayers were then incubated for 60 min in the CO_2 incubator at 42°C. After incubations at either 37° or 42°C the medium was removed, the monolayers were rinsed, the cells were harvested with trypsin-EDTA, and aliquots were taken for measurement of radioactivity and protein after sonication as described (19, 20). The amount of high affinity binding (B_{max}) was calculated from linear regression of the plot of total dihydrotestosterone binding as a function of the concentration of steroid incubated with the cells, using the values obtained at higher steroid concentrations (i.e., above 1 nM) and extrapolating the line back to the ordinate as described (19). Comparisons of binding at the two temperatures were always made in the same experiment for a given cell strain. A >40% decrease in B_{max} at 42°C compared with that obtained at 37°C was considered evidence for thermolability (20).

Density gradient centrifugation. Cells from stock flasks were dissociated with trypsin-EDTA and seeded (day 0) in 15-cm Petri dishes as for the studies of 5α-reductase activity. The medium was changed on days 3 and 6 as described for monolayer binding. On day 7 the cells were scraped from each dish into Tris-saline (50 mM Tris Cl, 150 mM NaCl, pH 7.4) and kept at 4°C. The cells were pelleted by centrifugation at 800 *g*, rinsed twice by resuspension in an equal volume of TESH[1] buffer (10 mM Tris-Cl, 1 mM EDTA, and 12 mM monothioglycerol, pH 7.4) with or without 10 mM sodium molybdate. The cell suspension was subjected to sonic disruption (18) using six 10-s bursts, and the sonicate was centrifuged at 100,000 *g* for 1 h. The resultant supernatant was added to glass tubes in which an appropriate amount of [^{3}H]dihydrotestosterone had been evaporated to dryness from organic solvent. After incubation of the tubes in melting ice for 3 h, one-fourth volume of a suspension containing charcoal (50 mg/ml) and dextran (5 mg/ml) in 10 mM Tris-Cl, pH 7.4, containing 1 mM EDTA was added, and the tubes were mixed. The dextran-coated charcoal was immediately removed by centrifugation at 4,800 *g* for 10 min. 200 μl of the supernatant was then layered on the top of 5.3 ml of 5–20% sucrose gradients prepared in TESH containing 10% glycerol. [^{14}C]Albumin was added to the top of the gradient as an internal marker. Density gradients were centrifuged in polyallomer tubes for 18 h in an SW 50.1 rotor at 50,000 rpm (250,000 *g*) at 0°C in an ultracentrifuge (Beckman Instruments, Inc., Fullerton, CA). Four-drop fractions were collected from the top of the tube with an ISCO gradient fractionator model 640 (ISCO, Lincoln, NE) and assayed for radioactivity in a Packard 2650 liquid scintillation counter (Packard Instrument Co., Downers Grove, IL).

Nitrogen balance. Identical diets were prepared daily from a single batch of food. Paired meals were obtained; one was eaten, and the other was saved for analysis. After a 3-d equilibration period, the start of a 3-d control balance was marked by ingestion of a blue or red marker. After the control period, two further 3-d balance periods were measured; during the first, testosterone propionate was given daily at a dose of 1 mg/kg body weight per d by intramuscular injection, and during the second testosterone propionate was given at a dose of 5 mg/kg body weight per d intramuscularly. The paired food eaten for 3 d, and the pooled urine and feces were analyzed for their nitrogen content (21). In patient S.C. the lower dose testosterone balance study was carried out before orchidectomy, and the higher dose study was performed at a later date after orchidectomy.

Hormone assays. Dihydrotestosterone (DHT), testosterone (T), and estradiol (E) were assayed in isolated serum samples by using a modification of the multiple-steroid fractionation and radioimmunoassay method with correction for procedural losses as previously described (22). In brief, steroids were separated on ethylene glycol/celite minicolumns under positive-pressure nitrogen by eluting in sequence with isooctane (10 ml), 97:3 cyclohexane/benzene (8 ml), 15% ethyl acetate in isooctane (5 ml), and 30% ethyl acetate in isooctane (5 ml). The following 1-ml fractions inclusive were collected: 4–7 for DHT; 13–17 for T; and 24–28 for E. After evaporation and reconstitution in gelatin assay buffer (1 ml for DHT and T, 0.6 ml for E), aliquots were counted for recovery, and 100-μl duplicates were assayed using highly specific antisera. Blanks were not significantly different from zero; intraassay variation was <12.5%, and interassay variation was <10% for pools of male serum. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by specific radioimmunoassay in the same samples described above using Medical Research Council (Britain) standards 68/40 for LH and 69/104 for FSH.

1. *Abbreviations used in this paper:* DHT, dihydrotestosterone; E, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LHRH, LH releasing hormone; TESH, 10 mM Tris-Cl, 1 mM EDTA, and 12 mM monothioglycerol, pH 7.4.

Results

Enzyme and receptor studies (Table II)

Studies of 5α-reductase activity and the high affinity androgen receptors in fibroblasts cultured from biopsies of the scrotum from the four men with 5α-reductase deficiency, the affected siblings of N.A., and the two individuals with defects of the androgen receptor are summarized in Table II. In two of the families with 5α-reductase deficiency (C.P.-P.P. and N.A.-B.A.-S.A.), the findings are similar to those reported in the original Dallas and Dominican Republic patients with the disorder (11–13, 16, 23), i.e., enzyme activity in the standard assay is low, and the residual activity exhibits a high apparent K_m for testosterone and a normal apparent K_m for NADPH. In the subject from the third family (M.M.), the 5α-reductase exhibited features indicative of a qualitatively abnormal enzyme—namely, low but measurable activity that exhibits abnormal apparent K_m's for both testosterone and NADPH and is unstable in the presence of cycloheximide; these findings are similar to those previously observed in another family with 5α-reductase deficiency (24). In all subjects with 5α-reductase deficiency the amounts of high affinity androgen receptor were normal.

The androgen receptor defects in the other two subjects were of two types. One (S.C.) had a diminished amount of androgen receptor, a finding similar to that observed in other patients with the Reifenstein phenotype (1, 17, 19–20); the amount of receptor was too low to be characterized further. In this patient, 5α-reductase activity was also lower than average, a finding that has been observed on occasion in subjects with androgen-receptor defects (25) and in fibroblasts cultured from the subjects (12). In the other man with an androgen receptor defect (B.P.), the androgen receptor was normal in amount but qualitatively abnormal, in that it was unstable to ultracentrifugation, a phenomenon observed in some other subjects with androgen resistance (18).

Nitrogen balance response to testosterone

All six of the subjects demonstrated retention of nitrogen during the short-term periods of testosterone treatment (Table III) and developed greater degrees of nitrogen retention on the higher dose of testosterone than on the lower dose. This group included the four men with 5α-reductase deficiency and both men with abnormalities of the androgen receptor.

Endocrine investigations

Basal plasma testosterone concentrations were in the normal range in the four subjects with 5α-reductase deficiency, and plasma dihydrotestosterone levels were in the low normal range (Table IV). B.P. had low basal testosterone levels thought to be secondary to previous orchiopexy and episodes of

Table II. Characterization of 5α-Reductase and Androgen Receptor in Genital Skin Fibroblasts from Controls, Five Subjects with 5α-Reductase Deficiency, and Two Subjects with Defects of the Androgen Receptor

	5α-Reductase				Androgen receptor		
Group	pH 5.5 activity	Apparent K_m for testosterone	Apparent K_m for NADPH	Cycloheximide stability	Amount	Labile at 42°C	Stabilization on density gradients by molybdate
	pmol·h⁻¹·mg protein⁻¹	*μM*	*μM*		*fmol/mg protein*		
Controls, mean (range)	31 (1.4–220)	0.08 (0.06–0.17)	40 (15–65)	>95%	30 (14–60)	No	Yes
(*n*)	(41)	(9)	(9)		(33)		
5α-Reductase deficiency							
C.P.	<0.2	3.5	53	>95%	38		
P.P.	<0.2	3.5	40	>95%	16		
N.A.	<0.2	4.5	29	52%	19		
B.A.*	0.2	—	—	—	27		
S.A.*	0.2	—	—	—	40		
M.M.	0.6	1.1	780	68%	50		
Androgen receptor defects							
B.P.	220	—	—	—	22	No	No
S.C.	0.8	0.16	16	95%	2.2		

The 5α-reductase and androgen receptor assays are described in the text; B.A.* and S.A.* are younger sibs of N.A.

Table III. Nitrogen Balance Expressed as Difference between Mean Nitrogen Balance during the Control and Treatment Periods

Patient	Net nitrogen balance	
	Testosterone propionate 1 mg/kg/d by intramuscular injection	Testosterone propionate 5 mg/kg/d by intramuscular injection
	g N/d	*g N/d*
5α-Reductase deficiency		
C.P.	+1.2	+2.5
P.P.	+3.8	+5.7
N.A.	+1.3	+2.6
M.M.	+3.2	+4.1
Androgen receptor defect		
B.P.	+3.4	+6.0
S.C.	+1.1	+3.1

epididymo-orchitis. S.C. had had a bilateral orchidectomy at age 23. However, before orchidectomy, normal levels of plasma 17β-hydroxy androgens (which measures testosterone predominantly, using competitive protein binding) had been recorded on several occasions (10.2–14 ng/ml; normal adult male 4.9–21 ng/ml). Dihydrotestosterone levels were low in the two patients with receptor defects, in part at least because of the low levels of plasma testosterone. On therapy with high doses of testosterone, dihydrotestosterone levels in all six subjects were in or above the normal range.

Basal gonadotropin levels were elevated in two of the subjects with 5α-reductase deficiency, and the response of plasma LH to 100 μg intravenous luteinizing hormone releasing hormone (LHRH) was amplified in one of the two subjects tested. Basal levels of LH suppressed in the first few days of testosterone therapy in all but one (N.A.) of the patients with 5α-reductase deficiency and was suppressed in all four on long-term, high dose testosterone therapy. Subject S.C. had LH levels in the upper normal range before orchidectomy (mean±SE 9.5±0.4 U/liter, $n = 12$) and an exaggerated response of plasma LH to intravenous LHRH; LH levels rose after orchidectomy and did not decrease during the acute studies with testosterone or on long-term therapy with high doses of testosterone. In B.P. plasma LH levels were elevated during the acute studies with both dose levels of testosterone propionate but fell to the normal range after long-term testosterone ester therapy.

Basal plasma estradiol levels were normal in three of the subjects with 5α-reductase deficiency but high in N.A. Plasma estradiol levels rose and were above the normal range in all patients on parenteral testosterone therapy but returned to normal during testosterone undecanoate treatment.

Seminal fluid analysis after 3 d abstinence. Four patients were azoospermic at the time of study. The exceptions were C.P. and P.P. who had total counts of 132,000 and 32,000 spermatozoa per ejaculate with 93 and 80% abnormal forms respectively.

Clinical responses to long-term testosterone therapy (see Table V).

All four subjects with 5α-reductase deficiency had beneficial clinical responses to long-term high dose parenteral androgen therapy as evidenced by improvement in virilization. Enlargement of the penile shaft was recorded with an increase both in length and circumference although the glans did not enlarge in any patient. Erectile potency improved in each, and an increase in ejaculatory volume was reported by three subjects. Muscle bulk increased, and facial and body hair were more masculine (Fig. 1). All of the men with 5α-reductase deficiency developed transient acne, two developed mild gynecomastia while on long-term treatment, and all reported an improved feeling of well-being. Similar changes were noted in one man (B.P.) with a receptor defect. S.C. noted improved well-being and muscle strength but achieved insignificant virilization of the genitalia and sexual hair and only a slight increase in potency; these inadequate responses occurred despite having similar circulating plasma testosterone and dihydrotestosterone levels and equally positive nitrogen retention as the other subjects. There was no improvement in sperm count in any of the six patients while on testosterone therapy.

Improved erectile potency and secondary sexual characteristics were maintained in the four patients with 5α-reductase deficiency on oral testosterone undecanoate, although testosterone levels were not as high on this regimen and LH levels were elevated as compared with those while on long-term parenteral testosterone ester therapy. Nevertheless the circulating dihydrotestosterone levels on testosterone undecanoate were maintained within or above the normal range.

Discussion

Our six patients with male pseudohermaphroditism fulfill the phenotypic and endocrine criteria for the diagnosis of androgen resistance (1). The clinical features of the four men with 5α-reductase deficiency (and of two siblings of one of the subjects) conform to those reported elsewhere, namely a disorder in which there is normal mullerian duct regression, normal virilization of Wolffian duct structures, and incomplete virilization of the external genitalia and urogential sinus (1–5). As in the other reported cases, significant virilization of the external genitalia as well as development of some secondary sexual characteristics occurred at the time of expected puberty. Seminal fluid analyses revealed severe oligospermia or azoospermia. The diagnosis of 5α-reductase deficiency can be

made by measuring the ratio of plasma testosterone to dihydrotestosterone or the ratio of urinary etiocholanolone to androsterone (1–5). Low-normal plasma dihydrotestosterone levels (in the presence of a normal plasma testosterone) were present in the subjects with 5α-reductase deficiency, and the ratios of plasma testosterone to dihydrotestosterone were higher than in controls. The diagnosis can also be established, as was done in our subjects, by characterizing the level and qualitative features of the enzyme in fibroblasts cultured from genital skin. Two of our three families with 5α-reductase deficiency have very low activities of 5α-reductase, similar to the findings observed in the Dallas and the Dominican Republic families (23). M.M. had low but measurable 5α-reductase activity and exhibited abnormal apparent K_m's for both testosterone and NADPH and instability in the presence of cycloheximide, a qualitative defect of the enzyme similar to that reported in a previous family with 5α-reductase deficiency (23). Thus, the subjects reported here fulfill phenotypic, endocrine, and biochemical features of 5α-reductase deficiency (1–5).

The clinical features of patients B.P. and S.C. are characteristic of androgen receptor abnormalities (1). Studies of the androgen receptor in genital skin fibroblasts from B.P. revealed normal amounts of a qualitatively abnormal receptor whereas S.C. has a deficiency of androgen receptors in cultured skin fibroblasts.

High dose testosterone administration, sufficient to raise plasma testosterone concentrations to levels above the upper limit of the normal adult male range, resulted in short-term positive nitrogen balance and a long-term enhancement of virilization in all four subjects with 5α-reductase deficiency. We believe that this is the first time the effects of such a therapeutic maneuver have been systematically investigated in this condition. The modest phallic growth, the development of hair on the trunk, face, and extremities, and the increased potency and ejaculatory volume have been gratifying to these subjects, and each has reported a more satisfactory sexual performance and a marked improvement in their self-images as men. The latter features of course are difficult to assess in quantitative terms, and indeed a placebo effect may play a major role in such phenomena, as evidenced by the fact that subject S.C. who had no clear-cut objective evidence of increased virilization reported an increased frequency of masturbation. Virilization has been maintained with amounts of oral testosterone undecanoate that result in lower plasma testosterone levels than on high dose testosterone therapy but normal or elevated dihydrotestosterone concentrations. The discrepancy between plasma testosterone and plasma dihydrotestosterone levels has been described previously in men receiving testosterone undecanoate (26, 27).

The mechanism by which testosterone promotes nitrogen balance and virilization in subjects with 5α-reductase deficiency is not resolved by the present study. The effect may be mediated by dihydrotestosterone formed by the small amount of residual 5α-reductase in such subjects (1, 11–13) since plasma dihydrotestosterone levels rose into the normal range on treatment. Alternatively, massive plasma and tissue levels of testosterone may saturate the androgen receptor (14) and thereby promote androgen action directly. The androgen receptor binds both testosterone and dihydrotestosterone; however, the affinity for dihydrotestosterone is much greater than for testosterone (14). It is of interest that the degree of positive nitrogen balance achieved in these subjects is of approximately the same magnitude as has previously been observed in normal men given large doses of testosterone propionate (28–31). In normal men the positive nitrogen balance after supplemental androgen therapy is temporary (28–30). We do not know whether the positive balance was temporary or sustained in our subjects; however, the growth of facial and body hair has been progressive during the course of these studies, and the improvements in phallic size and muscle bulk have been maintained.

The finding in the present study that high dose testosterone therapy resulted in positive nitrogen balance in the two men with receptor abnormalities was unexpected. Most previous studies in patients with complete testicular feminization have failed to show nitrogen retention or clinical evidence of virilization when supplemental androgen therapy was administered (6–9). However, Rosenfield et al. (10) described one subject with incomplete male pseudohermaphroditism and androgen resistance who developed positive urinary nitrogen retention when plasma androgens were raised above the physiological range. The development of short-term positive nitrogen balance in S.C., even though long-term improvement in male secondary sex characteristics failed to occur on exogenous androgens, indicates a dissociation of effects that are not explained by current knowledge. Since the outcome in these patients cannot be predicted by short-term studies of nitrogen balance, treatment with supraphysiological doses of androgens should be considered in all men with androgen receptor defects who exhibit inadequate virilization.

Basal LH levels are sometimes elevated and sometimes normal in 5α-reductase deficiency, suggesting that dihydrotestosterone may mediate in part the negative feedback of testosterone at the hypothalamic-pituitary level (1–5). The exaggerated response of plasma LH to the administration of LHRH in one of the men with 5α-reductase deficiency is in keeping with this interpretation. As would be expected, basal plasma LH levels and the response to LHRH are usually elevated in subjects with male pseudohermaphroditism due to receptor defects (7).

The response in these subjects of plasma gonadotropins to both short- and long-term testosterone therapy deserves comment. In all five subjects who responded to androgen therapy by clinical and laboratory criteria, plasma LH and FSH levels were eventually suppressed to normal or subnormal levels. This response may therefore be used as a predictor of subsequent responsiveness to testosterone therapy. In each instance, this fall was accompanied by and/or preceded by increases in

Table IV. Plasma Hormone Levels before and after Androgen Therapy

	Testosterone (T)	Dihydro-testosterone (D)	T/D Ratio	LH	LH 20 min after 100 μg LHRH	FSH	Estradiol
Normal range	10–28	1–5	6.43±SD 1.48	1–10	13–58	1–7	55–165
	mmol/liter	*mmol/liter*		*U/liter*	*U/liter*	*U/liter*	*pmol/liter*
5α-Reductase deficiency							
Subject C.P. basal levels	19.1±1.1 (6)	1.2±0.1 (6)	15.1±1.8 (6)	6.9 (1)		7.2 (1)	40 (1)
Regimen A	42.4±8.5 (4)	4.6±0.4 (5)		—		—	—
Regimen B	172±20 (3)	7.4±1.6 (4)		—		—	—
Regimen C	72.5±20 (3)	2.6±0.4 (3)		3.5 (1)		1.2 (1)	—
Regimen D	24.9±3.1 (3)	7.1 (1)		11.0 (1)		14.6 (1)	105 (1)
Subject P.P. basal levels	28±1.1 (6)	1.6±0.2 (6)	17.8±1.5 (6)	13.7 (1)		18.8 (1)	40 (1)
Regimen A	104±1.1 (5)	3.2±0.3 (5)		—		—	—
Regimen B	261±18 (5)	4.5±0.3 (5)		—		—	—
Regimen C	82±4.1 (3)	3.8±0.7 (3)		1.2 (1)		0.5 (1)	—
Regimen D	28±2.7	2.5 (1)		12.5 (1)		22.6 (1)	135 (2)
Subject N.A. basal levels	29±1.1 (6)	1.2±0.1 (6)	23.8±1.4 (6)	14.0±1.4 (4)	85.3 (1)	16.7±0.5 (4)	268±31 (6)
Regimen A	96±9.7 (5)	2.4±0.3 (5)		6.3±1.0 (5)		5.3±0.8 (5)	497±85 (5)
Regimen B	540±95 (5)	7.8±1.1		11.4±2.0 (3)		3.8±0.4 (3)	700±71 (4)
Regimen D	15.3 (1)	1.5 (1)		23.5 (1)		22.5 (1)	105 (1)
Subject M.M. basal levels	19.2±2.1 (6)	1.5±0.2 (3)	11.3±1.0 (3)	9.9±1.5 (7)	29.6 (1)	28.5±2.7 (7)	156±1.0 (3)
Regimen A	188±17 (5)	1.9 (1)		4.5±0.7 (5)		12.3±3.5 (5)	222±18 (5)
Regimen B	462±20 (5)	5.3 (1)		2.8±0.6 (5)		3.8±0.8 (5)	343±22 (4)
Regimen C	65.9±7.9 (14)	3.1±0.4 (14)		—		—	—
Regimen D	7.8 (1)	5.2 (1)		—		—	—

Table IV. (Continued)

	Testosterone (T)	Dihydro-testosterone (D)	T/D Ratio	LH	LH 20 min after 100 μg LHRH	FSH	Estradiol
Normal range	10–28	1–5	6.43±SD 1.48	1–10	13–58	1–7	55–165
	mmol/liter	*mmol/liter*		*U/liter*	*U/liter*	*U/liter*	*pmol/liter*
Androgen receptor defects							
Subject B.P. basal levels	4.3±0.3	0.9±0.1	5.0±0.5	31.3±1.2	>50	>25	78±2.4
	(6)	(6)	(5)	(6)	(1)	(6)	(6)
Regimen A	80.3±7.4	3.3±7.4		19.8±4.4		23.7±1.7	248±12
	(5)	(5)		(5)		(5)	(5)
Regimen B	200±10.7	7.8±1.4		29.4±4.1		19.4±2.0	339±19.6
	(5)	(4)		(3)		(3)	(4)
Regimen C	—	—		2.4		0.4	—
				(1)		(1)	
Subject S.C. basal levels*	0.6±0.1	0.4±0.1	2.2±0.8	30.5±0.7	61.8	52.4±1.6	48.8
	(6)	(6)	(6)	(6)	(1)	(6)	(1)
Regimen B	307±398	9.4±0.9		23.3±1.2		27.8±3.9	302±409
	(5)	(5)		(4)		(4)	(4)
Regimen C	120	—		33.5		>25	260
	(1)			(1)		(1)	(1)
Regimen D	11	6.9		>50		>25	120
	(1)	(1)		(1)		(1)	(1)

* Basal Levels in subject S.C. were measured after castration. Values are given as the result of single assays or as means±SEM; numbers in parentheses indicate the number of samples pooled for each assay. Regimen A: Testosterone propionate 1 mg/kg body weight per d for 3 d. Regimen B: Testosterone propionate 5 mg/kg body weight per d for 3 d. Regimen C: Long-term parenteral testosterone ester therapy, 500 mg/wk. Regimen D: Long-term oral testosterone undecanoate, 240 mg/d.

plasma testosterone, dihydrotestosterone, and estradiol. It has been the interpretation of most previous workers that the suppression of plasma gonadotropins by exogenous testosterone in subjects with androgen resistance is the consequence of the aromatization of testosterone to estradiol in peripheral tissues and the consequent rise in plasma estradiol (32, 33). However, our observations suggest that this is not necessarily so since plasma estradiol rose similarly in S.C. and in B.P.; yet plasma gonadotropins were not suppressed in S.C. It is possible that gonadotropin secretion may become autonomous or semiautonomous in some subjects with defects of the androgen receptor, a phenomenon that has previously been described in the Klinefelter syndrome (34), and that the failure of plasma gonadotropins in S.C. to decrease after the rise in plasma estradiol is a secondary consequence of such autonomy.

The change of gender-role behavior in the subjects with 5α-reductase deficiency has previously been described in subjects with 5α-reductase deficiency and other forms of male pseudohermaphroditism (4, 35). All four of the men with 5α-reductase deficiency were identified as females at birth but described an early change in gender identity before noticing virilization at the time of expected puberty. All four lived in communities where rigorous conformity with a prescribed gender role was expected and gender change difficult. However, these men, who were reared and lived as females until they were between 14 and 24 yr, were able to overcome both the social and legal barriers to such a change and make successful adjustments to roles that conform to their genetic, endocrine, and psychological sex. It is not known whether this change in gender role is the result of a true change in gender identity or merely the resolution of a confusion as to gender identity consequent to the genital ambiguity that is present from birth (35). An equally attractive possibility is that male gender identity might be imprinted before or immediately after birth by androgens themselves (35).

The findings in the present studies indicate that long-term treatment with supraphysiological doses of testosterone may be of benefit in partially repairing the incomplete virilization, and in improving the self-image of masculinity in men with 5α-reductase deficiency and, in selected instances, of men with defects of the androgen receptor. To date, no adverse effects of this regimen have been noted, but it is important to follow

Table V. Clinical Response to Long-term Treatment with High Dose Testosterone

Patient	Size of penis	Size of glans	Frequency of erection	Sexual activity	Prostate size	Muscle bulk	Facial hair	Body hair	Acne	Gynecomastia
C.P.	Longer and thicker (6 cm stretched)	Unchanged	Markedly increased	Increased ejaculatory volume	Unchanged	Markedly increased	Markedly increased	Markedly increased	++	1.5 cm bilaterally
P.P.	Longer and thicker (6 cm stretched)	Unchanged	Markedly increased	Increased ejaculatory volume	Unchanged	Markedly increased	Markedly increased	Increased	++	1–1.5 cm
N.A.	Longer and thicker (4.5 cm stretched)	Unchanged	Increased	Sexual intercourse for first time	Unchanged	Unchanged	Markedly increased	Increased	++	None
M.M.	Longer*	Unchanged	Increased	More frequent masturbation	Just palpable	Increased	Increased	Increased	++	None
B.P.	Longer and thicker	Unchanged	Increased	More frequent sexual intercourse with increased ejaculatory volume	Just palpable	Increased	Increased	Increased	++	None
S.C.	No change (4.5 cm stretched)	Unchanged	Increased	Masturbates more frequently, slight ejaculate	Not palpable	Unchanged	Unchanged	Slight increase in body hair	None	Previously excised

* Patient had surgical correction of chordee during this treatment period.

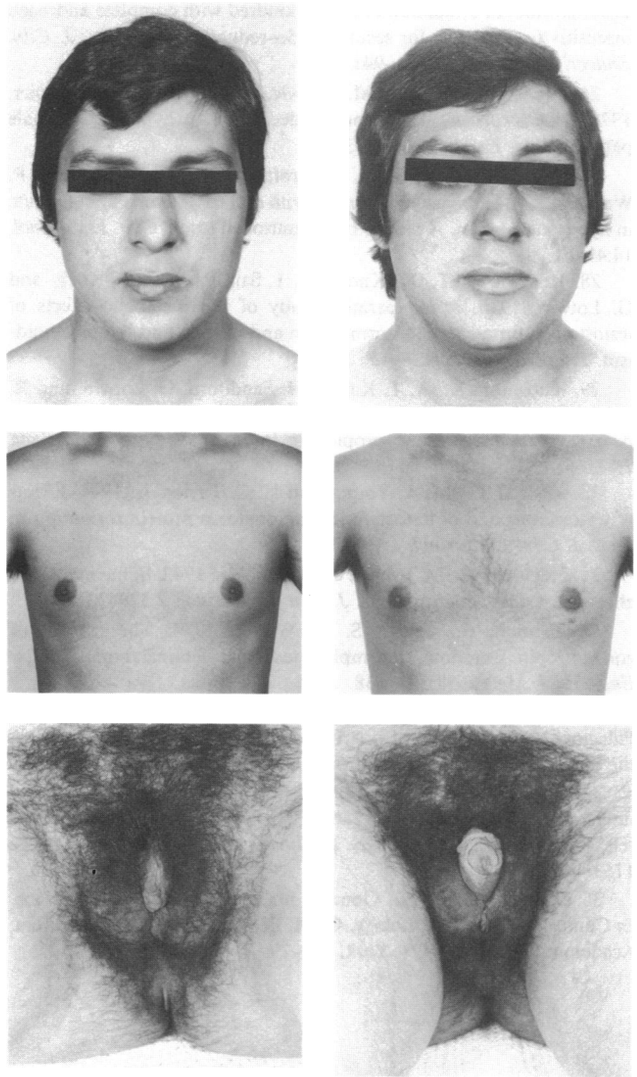

Figure 1. Photographs of patient M.M. On the left he is shown before treatment (June 1976), and the photographs on the right were taken after treatment (March 1980) with high dose androgen therapy (Sustanon, 500 mg i.m., weekly). Correction of the chordee was performed between these dates.

such individuals closely as long as they are on such a regimen. Androgen therapy is not appropriate in individuals with either syndrome who have female behavior patterns.

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