Testicular Feminization Associated with a Thermolabile Androgen Receptor in Cultured Human Fibroblasts

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ABSTRACT Evidence for a qualitative abnormality in the androgen receptor was obtained by studies of temperature sensitivity. The binding of [^{3}H]dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) was studied in monolayers of cultured genital skin fibroblasts from genetic males with abnormal sexual differentiation resulting from androgen resistance. Binding in cells from eight patients with a female phenotype (complete and incomplete testicular feminization) fell from half-normal levels at the usual assay temperature of 37°C to levels <20% of normal when cells were incubated at 42°C. This thermal inactivation was rapidly reversed when the assay temperature was lowered to 37°C, was not associated with altered dihydrotestosterone metabolism, and was also demonstrable with [^{3}H]methyltrienolone as the binding ligand. Binding increased to overlap the normal range when the assay temperature was lowered to 26°C. The patients with receptor-deficient testicular feminization include three pairs of siblings; the pedigrees in two of these families are compatible with X-linkage.

Only minor changes in the amount of binding at elevated temperatures were observed in cells from 10 control subjects and from 2 male pseudohermaphrodites with normal levels of androgen receptors. In 10 patients with androgen resistance and partial receptor deficiency associated with a predominantly male phenotype (Reifenstein syndrome and infertile men), dihydrotestosterone binding also did not change consistently with elevated temperature. Binding was approximately half-normal at 37°C and either increased or decreased slightly at 42°C.

The thermal instability in receptor-deficient testicular feminization represents a new molecular defect associated with hereditary male pseudohermaphroditism that appears to be caused by an alteration in the tertiary structure of the androgen receptor protein.

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INTRODUCTION

The action of androgen is required for the conversion of the indifferent urogenital tract into the male phenotype during embryogenesis as well as for spermatogenesis and virilization of the male at the time of puberty. Two types of syndromes of hereditary resistance to the actions of androgen have been delineated in genetic males (46,XY karyotype). One is an autosomal recessive syndrome caused by a defect in the 5α-reductase (NADPH: Δ^4-3-ketosteroid-5α-oxidoreductase) enzyme that converts testosterone (17β-hydroxy-4-androstene-3-one) to dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) (1–3); affected individuals are male pseudohermaphrodites with testes and male internal ducts but predominantly female external genitalia. The other disorder appears to be X-linked. The clinical expression of the androgen resistance in this second type of disorder varies from phenotypic women with the syndrome of complete testicular feminization through varying degrees of ambiguity of the external and internal genitalia, to infertile but otherwise normal men (4–8). The defect in these patients affects either the cytoplasmic receptor protein that binds dihydrotestosterone and testosterone or some aspect of the postreceptor mechanism that is essential for the subsequent events in androgen action. Androgen receptor concentrations in fibroblasts cultured from genital skin from such individuals vary from undetectable to detectable but quantitatively deficient to normal levels (8–13). There is no clearcut correlation between the phenotype of affected individuals and the magnitude of the receptor deficiency; for

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example, a male phenotype can be manifest in patients who have undetectable androgen receptor. Moreover, normal (or near normal) levels of the androgen receptor can also be present in phenotypic women with complete testicular feminization.

It is assumed that these disorders constitute a mixture of quantitative and qualitative abnormalities of the androgen receptor as well as abnormalities of the postreceptor, effector mechanisms for androgen action. However, no qualitative abnormalities in the receptor have been identified. In particular, in patients with androgen resistance and detectable receptors, the affinity of the receptor for androgen (14) and the capacity of the androgen receptor complex to move from cytoplasm to nucleus both appear to be normal (15).

In the present report a new technique has been used to identify qualitative changes in the androgen receptor, namely the temperature stability of the hormone-receptor complex. With this technique a hereditary qualitative abnormality of the androgen receptor has been delineated. The abnormal receptor appears to be characteristic of a subgroup of individuals with testicular feminization previously thought to have partial receptor deficiency. The abnormal receptor manifests normal dihydrotestosterone binding at 26°C, incomplete binding under the usual assay conditions at 37°C, and nearly complete inactivation of binding at 42°C.

METHODS

Materials. Materials for thin-layer chromatography and cell culture have been described (10) except that fetal calf serum was from Grand Island Biological Co. (Grand Island, N. Y.). Tissue culture plates with four 60-mm wells per plate (FB-4; Linbro Chemical Co., Hamden, Conn.) were used for the binding studies. [1,2,4,5,6,7-^{3}H]Dihydrotestosterone, 123 Ci/mmol, and [17α-*methyl*-^{3}H]methytrienolone (17β-hydroxy-17α-methylestra-4,9,11-trien-3-one) 87 Ci/mmol, were from New England Nuclear (Boston, Mass.). Nonradioactive steroids were from Steraloids, Inc. (Pauling, N. Y.) except for methyltrienolone which was from New England Nuclear. Lima bean trypsin inhibitor was from Worthington Biochemical Corp. (Freehold, N. J.).

Cell culture. The fibroblast strains used in these experiments were established from explants of genital skin (foreskin, scrotum, and labia majora) and are listed in Table I. Cell strains 144 and 151 are from two sisters with complete testicular feminization who have no other known affected family members. Cell strains 147 and 221 are from unrelated patients with complete testicular feminization who have other affected family members in several generations. Dihydrotestosterone binding has been reported in these four patients previously (10). Cell strains 91 and 105 are from previously unreported patients with complete testicular feminization. Cell strains 77 and 99 are from two phenotypic females with the incomplete form of testicular feminization to be reported in detail.[1] These sisters have the same mother but a different father, and each has clitoromegaly and posterior labial fusion characteristic of the phenotype of incomplete testicular feminization (7). Cell strain 156 is from a previously reported patient with incomplete testicular feminization (7). Cell strains 93 and 94 are from two sisters with a variant of testicular feminization associated with normal female external genitalia (i.e., no clitoromegaly or posterior labial fusion) but some Wolffian duct virilization and incomplete feminization at puberty (16). Cell strains 61 (17) and 86 and 167 (6) are from patients of reported families with the Reifenstein syndrome. Cell strain 222 is from a patient of a previously undescribed pedigree with Reifenstein syndrome. Cell strain 227 is from a patient of an Australian family with Reifenstein syndrome (18). Cell strains 37 and 107 are from patients A and C, respectively, in the report of men with infertility as the sole manifestation of androgen resistance (8), and cell strains 63, 228, and 230 are from previously unreported patients with this same syndrome. Cell strains 18 and 79 are from previously described patients with male pseudohermaphroditism associated with clinical evidence of androgen resistance but normal 5α-reductase activity and normal dihydrotestosterone binding and are termed receptor-positive resistant (15). Cell strain 65 is from a previously reported patient with 5α-reductase deficiency (1). Cell strains 196, 231, and 243 are from previously unreported patients with the syndrome of 5α-reductase deficiency. Fibroblast storage and maintenance of stock cultures have been described (10).

Monolayer binding. Cells from stock flasks were dissociated with 0.05% trypsin–0.02% EDTA at 37°C for 3 min and seeded (day 0) at a concentration of ~150,000 cells in 8 ml of medium containing 10% fetal calf serum in each well of the Linbro plate. 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 fresh medium without serum were added. In the previous method for growth of cells for monolayer binding, charcoal-treated fetal calf serum was added to the medium on day 3 and day 6 to allow detection of maximum binding capacity in the absence of endogenous steroids (10), using medium with 10% fetal calf serum for the first medium change, and medium without serum during the last 24 h before assay results in similar amounts of binding (results not shown). 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 or [^{3}H]methyltrienolone in medium without serum and with or without nonradioactive steroid (0.5 μM). The standard assay was at 37°C in a 5% CO_2 incubator for 60 min. In experiments assessing binding at 42°C the monolayers were preincubated at 42°C for 30 min in medium without serum; the medium was then removed, and medium containing the tritiated steroids (also warmed to 42°C) was added. The monolayers were then incubated for 60 min in the CO_2 incubator at 42°C. In experiments assessing binding at 26°C, medium with [^{3}H]dihydrotestosterone at room temperature was added to the monolayers, and the monolayers were incubated in a CO_2 incubator at 26°C for 16 h. The medium was removed, the monolayers were rinsed, the cells harvested with trypsin-EDTA, and aliquots were taken for measurement of radioactivity and protein after sonication as previously described (14) except that the sonication was performed in disposable plastic culture tubes using the cup horn attachment of a model W-185 Sonifier Cell Disrupter (Heat Systems-Ultrasonics, Inc., Plainview, N. Y.). Binding was expressed as femtomoles of dihydrotestosterone or methyltrienolone bound per milligram of cell protein. Binding was plotted as a function of the steroid concentration in the medium. The amount of high affinity binding in a given cell strain (B_{max})[2] was determined

[1] Griffin, James E. Manuscript in preparation.

[2] *Abbreviation used in this paper*: B_{max}, amount of high affinity binding.

TABLE I

Identification of 33 Cell Strains Obtained from Control Subjects and Patients with Androgen Resistance

	Cell strain no.	Diagnosis	Site of biopsy	Age
				yr
Controls	225	Normal	Scrotum	36
	237	Normal	Labia majora	30
	239	Normal	Scrotum	32
	242	Normal	Scrotum	49
	258	Normal	Foreskin	0.1
	284	Normal	Scrotum	3
	65	5α-Reductase deficiency	Labia majora	17
	196	5α-Reductase deficiency	Labia majora	27
	231	5α-Reductase deficiency	Scrotum	18
	243	5α-Reductase deficiency	Scrotum	0.2
Receptor-negative androgen resistance	91	Complete testicular feminization	Labia majora	11
	105	Complete testicular feminization	Labia majora	17
	221	Complete testicular feminization	Labia majora	21
Receptor-deficient androgen resistance	144	Complete testicular feminization	Labia majora	15
	147	Complete testicular feminization	Labia majora	31
	151	Complete testicular feminization	Labia majora	14
	77	Incomplete testicular feminization	Labia majora	1
	93	Incomplete testicular feminization	Labia majora	15
	94	Incomplete testicular feminization	Labia majora	16
	99	Incomplete testicular feminization	Labia majora	7
	156	Incomplete testicular feminization	Labia majora	26
	61	Reifenstein syndrome	Scrotum	21
	86	Reifenstein syndrome	Scrotum	21
	167	Reifenstein syndrome	Scrotum	28
	222	Reifenstein syndrome	Scrotum	7
	227	Reifenstein syndrome	Scrotum	2
	37	Infertile men	Scrotum	31
	63	Infertile men	Scrotum	19
	107	Infertile men	Scrotum	39
	228	Infertile men	Scrotum	28
	230	Infertile men	Scrotum	25
Receptor-positive androgen resistance	18	Male pseudohermaphroditism	Foreskin	18
	79	Male pseudohermaphroditism	Labia majora	14

by extrapolating the linear portion of the curve at higher steroid concentrations back to the ordinate using the method of least squares linear regression (14). The affinity of binding (half-maximal saturation) was determined as the concentration of dihydrotestosterone at which the amount bound was half the B_{max}. In some analyses of binding the amount of nonspecific binding (the amount bound in the presence of an added excess of nonradioactive steroid) was subtracted from the total binding to give the amount of specific binding, and this value was plotted as a function of steroid concentration. In some experiments cells were preincubated for varying times at 42°C before binding studies at 37°C.

Thin-layer chromatography. To be certain that the intracellular androgen bound in incubations at 42°C was dihydrotestosterone itself and not a metabolite, in some experiments the medium and harvested, washed cells were extracted after incubations with 1.0 nM [^{3}H]dihydrotestosterone, and thin-layer chromatography was performed as described (10).

RESULTS

When genital skin fibroblasts from a normal woman were incubated with increasing concentrations of [^{3}H]dihydrotestosterone at 37° and 42°C the B_{max} was slightly higher at 42°C. There is no change in the apparent affinity, i.e., the half-maximal saturation, of the receptor for the ligand at the higher temperature (Fig. 1A). Similar incubations with cells from three patients with complete testicular feminization are

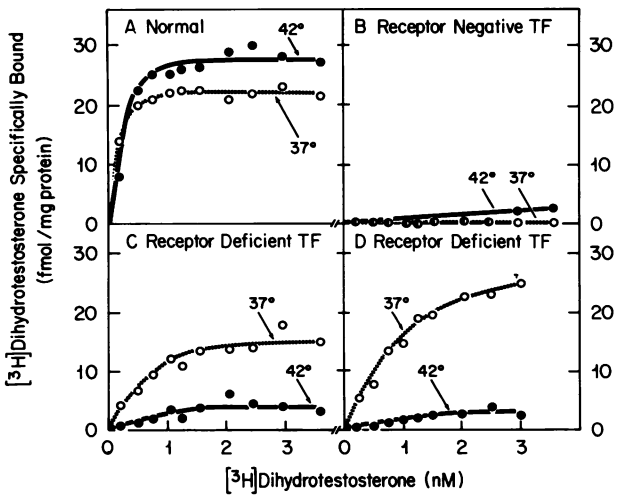

FIGURE 1 Effect of elevated temperature on dihydrotestosterone binding in fibroblast monolayers. Genital skin fibroblasts from a normal woman (strain 237) (A), a patient with receptor-negative complete testicular feminization (TF) (strain 91) (B), and two patients with receptor-deficient complete testicular feminization (strain 144 [C] and strain 147 [D]) were grown under the standard conditions. On day 7, the medium was removed and replaced with medium prewarmed to either 37° or 42°C for a 30-min preincubation followed by a 60-min incubation at the indicated temperature with various concentrations of [^{3}H]dihydrotestosterone with or without a 250-fold excess of nonradioactive dihydrotestosterone. The monolayers were rinsed and harvested with trypsin-EDTA, and the specific binding plotted as a function of dihydrotestosterone concentration.

shown in Fig. 1, B–D. In one patient with receptor-negative testicular feminization, high affinity dihydrotestosterone binding was undetectable under the usual assay conditions at 37°C and was also undetectable at 42°C (Fig. 1B). In contrast, cells from two patients with receptor-deficient testicular feminization from two different families (strains 144 and 147) showed reduced levels of dihydrotestosterone binding at 37°C and a further reduction at 42°C (Fig. 1C and D). At the higher temperature the amount of dihydrotestosterone binding was similar to that observed in the patients with receptor-negative disorder (Fig. 1B).

To be certain that the diminished dihydrotestosterone binding at 42°C was not the result of enhanced metabolism of dihydrotestosterone at the higher temperature, aliquots of medium and cells were extracted after incubation with [^{3}H]dihydrotestosterone and subjected to thin-layer chromatography (Table II). The amount of [^{3}H]dihydrotestosterone unmetabolized at the end of the incubation at 37° and 42°C was similar in medium and cells from control strains and from the patients with receptor-deficient testicular feminization.

To determine whether the diminished binding at 42°C in the cells from receptor-deficient patients is unique to dihydrotestosterone or characteristic of other steroids that bind to the androgen receptor, binding was assessed in control and receptor-deficient cells with the synthetic androgen analogue methyltrienolone (Fig. 2). The amount of high affinity [^{3}H]methyltrienolone bound in control cells was similar at 37° and 42°C and almost identical to the amount of [^{3}H]dihydrotestosterone bound at the corresponding temperatures (Fig. 2, upper panels). At 37°C the receptor-deficient cells also exhibited a similar amount of binding of [^{3}H]methyltrienolone and [^{3}H]dihydrotestosterone, and the extent of fall in B_{max} at 42°C was similar with the two steroids (Fig. 2, lower panels).

The reversibility of the effect of temperature was then examined (Table III). When control cells were incubated at 42°C with 1 nM [^{3}H]dihydrotestosterone in the presence and absence of excess nonradioactive dihydrotestosterone, specific binding decreased only 7% from the amount observed at the usual assay temperature of 37°C. Preincubation at 42°C for 45 min

TABLE II
Effect of Elevated Temperatures on Dihydrotestosterone Metabolism

Group	Strain	Component analyzed	Dihydrotestosterone unmetabolized after 45 min, 37°C	Dihydrotestosterone unmetabolized after 45 min, 42°C
			%	
Control	196	Medium	88	86
		Cells	92	92
	225	Medium	87	85
		Cells	91	91
	239	Medium	89	87
		Cells	92	92
Receptor-deficient testicular feminization	147	Medium	81	78
		Cells	81	78
	151	Medium	90	87
		Cells	90	92
	156	Medium	90	91
		Cells	85	90

Cells from three control strains and from three patients with receptor-deficient testicular feminization and thermolabile androgen binding were grown under standard conditions, and at the end of 7 d monolayers were incubated with 1.0 nM [^{3}H]dihydrotestosterone for 45 min at the indicated temperature. After the incubation aliquots of the medium and the rinsed harvested cells were extracted and subjected to thin-layer chromatography. The amount of ^{3}H that cochromatographed with authentic dihydrotesterone is indicated as the percentage of the total recovered radioactivity and represents the average of duplicates that agreed within 5%.

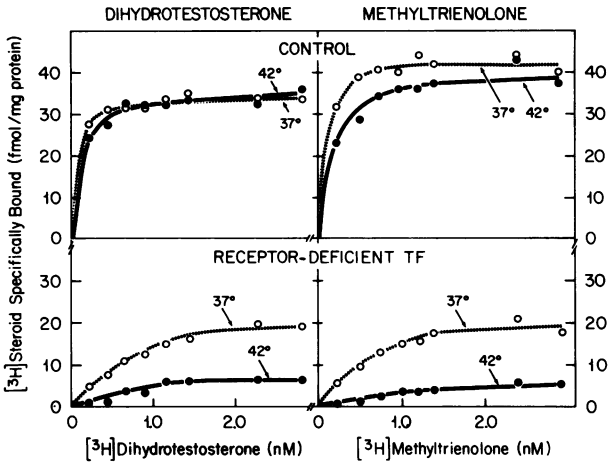

FIGURE 2 Comparison of dihydrotestosterone and methyltrienolone binding at 37° and 42°C in genital skin fibroblasts. Genital skin fibroblasts from a control subject (strain 196) (upper panels) and a patient with receptor-deficient testicular feminization (TF) (strain 144) (lower panels) were grown under the standard conditions. On day 7 after a preincubation at 37° or 42°C with plain medium for 30 min, the monolayers were incubated for 60 min with varying concentrations of [^{3}H]dihydrotestosterone (left panels) or [^{3}H]methyltrienolone (right panels) at the indicated temperatures with and without a 250-fold excess of the corresponding nonradioactive steroid. The monolayers were rinsed, the cells were harvested, and specific binding was plotted as a function of steroid concentration.

TABLE III
Reversibility of Temperature Sensitivity

Preincubation	Incubation with 1 nM [^{3}H]dihydrotestosterone	Specific binding of dihydrotestosterone	
		Control	Mutant
45 min	*45 min*	*fmol/mg protein*	
37°C	37°C	32.6	17.6
37°C	42°C	29.7	6.8
42°C	37°C	28.5	18.4
42°C	42°C	28.8	3.3

Cells from a control strain (196) and from a receptor-deficient testicular feminization patient (147) were grown under standard conditions. At the end of 7 d the medium was removed and replaced with plain medium (without serum) either at 37° or 42°C. The monolayers were then either returned to the 37°C incubator or to an incubator at 42°C. After 45 min the medium was removed and replaced with medium containing 1.0 nM [^{3}H]dihydrotestosterone with or without 0.5 μM nonradioactive dihydrotestosterone. After an additional 45-min incubation at the indicated temperature, the monolayers were rinsed and harvested with trypsin-EDTA, and binding was assessed as described in Methods. Specific binding indicates the total binding minus the amount of binding in the presence of excess, nonradioactive dihydrotestosterone and is the average of duplicate determinations.

without steroid followed by incubation with dihydrotestosterone at either 37° or 42°C resulted in little or no change in the specific binding in the control cells. In contrast, cells from one of the receptor-deficient testicular feminization patients exhibited a 62% decrease in specific dihydrotestosterone binding at an assay temperature of 42°C compared with the amount at 37°C. When these cells were preincubated at 42°C without steroid before binding with dihydrotestosterone at the same temperature the specific binding was even lower, <20% of the base-line value. However, when 42°C preincubation was followed by binding at 37°C, the specific binding was the same as when there was no 42°C preincubation, indicating that the temperature effect was promptly reversible.

The effect of elevation of incubation temperature on the amount of high affinity dihydrotestosterone binding in cells from 10 control subjects (including normal men and women and male pseudohermaphrodites with 5α-reductase deficiency), and from 23 subjects with androgen resistance is summarized in Fig. 3. B_{max} values were calculated from plots of monolayer binding curves at 37° and 42°C. The amount of binding in control cell strains changed little when the assay temperature was increased to 42°C; some

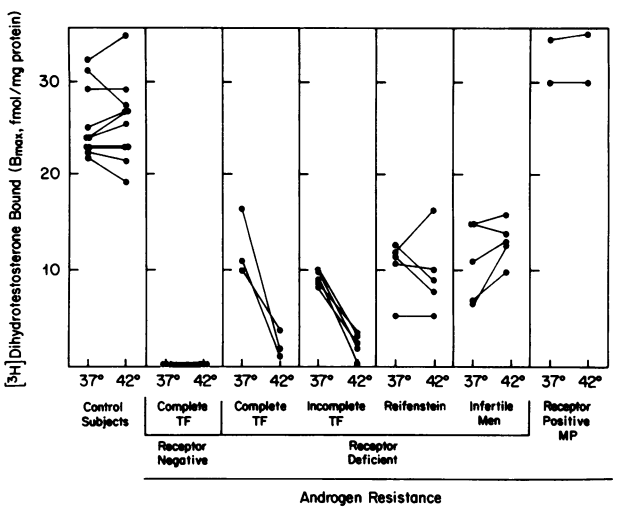

FIGURE 3 Effect of elevated temperature on the dihydrotestosterone B_{max} in fibroblasts from 10 control strains and 23 patients with androgen resistance. Monolayers of genital skin fibroblasts were grown under standard conditions. On day 7 the medium was removed, and the monolayers were incubated for 60 min with concentrations of [^{3}H]dihydrotestosterone that varied from 0.25 to 3.0 nM at the indicated temperature. The cells were rinsed and harvested, and binding was plotted as a function of dihydrotestosterone concentration. The B_{max} was calculated by least squares linear regression from the points at higher concentrations of steroid after saturation. Each point represents either an individual determination (13 strains) or the average of two to four determinations. TF, testicular feminization; MP, male pseudohermaphroditism.

cell strains had a slightly increased and others a slightly decreased B_{max}. In cells from three patients with receptor-negative, complete testicular feminization binding was undetectable both at 37° and 42°C. In fibroblasts from three patients with complete testicular feminization and partial receptor deficiency, increasing the assay temperature from 37° to 42°C resulted in a fall in the amount of binding to 4 fmol/mg protein or less. Similar results were found in cells from five patients with incomplete testicular feminization and partial receptor deficiency; in each case the amount of binding also fell to <4 fmol/mg protein at 42°C. In contrast, in fibroblasts from androgen-resistant patients with partial receptor deficiency and a predominantly male phenotype (five patients with Reifenstein syndrome and five infertile men), no or only slight temperature sensitivity was noted at 42°C. Two of the Reifenstein patients from the same family (6) showed a different pattern, one increasing and one decreasing at 42°C. Finally, in cells from two patients with receptor-positive male pseudohermaphroditism and normal 5α-reductase activity, elevated temperature did not affect the amount of binding.

The effect of lowering the temperature of the monolayer binding assay below 37°C is depicted in Fig. 4. Preliminary studies of monolayer binding at room temperature (26°C) indicated that an incubation of 16 h was necessary for binding to reach equilibrium at this temperature (results not shown). Therefore, the amount of dihydrotestosterone binding (B_{max}) is plotted

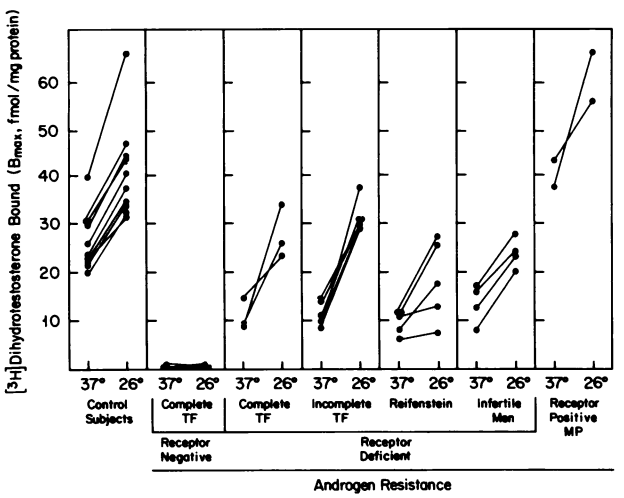

FIGURE 4 Effect of decreased temperature on the dihydrotestosterone B_{max} in fibroblasts. In the same cell strains used in Fig. 3, dihydrotestosterone binding was assessed at two temperatures without preincubation. Binding at 26°C was for 16 h, and binding at 37°C was assessed under the usual conditions for 60 min. The concentrations of dihydrotestosterone and the calculations of B_{max} are described in the legend to Fig. 3. Each point represents an individual determination. TF, testicular feminization; MP, male pseudohermaphroditism.

for cells incubated with dihydrotestosterone for 1 h at 37°C and for 16 h at 26°C. The amount of binding at 26°C increased in control cell strains to ~150% of the 37°C base-line value. Cells from patients with receptor negative testicular feminization had undetectable binding at both temperatures. Binding in cells from the receptor-deficient testicular feminization patients increased at the lower temperature to values that were often more than twice the values observed at 37°C and that overlapped the range observed in the control subjects. Binding in cells from patients with Reifenstein syndrome, men with infertility, and patients with receptor-positive androgen resistance also increased at the lower temperature to an extent similar to that of control subjects.

DISCUSSION

The data reported here identify altered binding characteristics for dihydrotestosterone in fibroblasts from certain patients with the clinical syndrome of testicular feminization. The binding of the hormone is extremely low at 42°C, is detectable but subnormal at 37°C, and is normal at 26°C. The shift from one state to another appears to occur promptly and is rapidly reversible. Because the monolayer binding studied here reflects the activity of a specific androgen receptor (10), the finding of temperature instability suggests the presence of a structural abnormality in the receptor molecule.

The phenomenon of temperature sensitivity as an indication of a structural mutation in a protein was first noted in bacterial mutants in which elevation of temperature is associated with altered growth (19). More recently, mutations have been characterized in mammalian cells in which elevated temperature inhibits the normal cell cycle (20, 21). In some of these systems a temperature-sensitive protein, usually an enzyme critical for normal cell growth and division, has been found to be structurally abnormal and responsible for the effects of elevated temperature on cell growth (22–24). Finally, temperature-sensitive mutations in some enzymes only conditionally necessary for cell growth have been characterized by other selection techniques (25–28). These include several mutations in the human X-linked enzyme, hypoxanthine-guanine phosphoribosyltransferase (26–28). In general, the level of residual enzyme activity in this class of mutants is lower than normal and is often associated with other evidence of a qualitative abnormality such as altered affinity for substrate. In one instance, the mutant hypoxanthine-guanine phosphoribosyltransferase was altered sufficiently that it did not cross-react with antibody to the normal enzyme despite the fact that some residual catalytic activity was demonstrable (27). Most of these temperature-sensitive mutations in

mammalian cells are thought to be the consequence of a "missense" substitution of an amino acid that alters the tertiary structure of the protein. Because any amino acid substitution might lead to such a change in tertiary structure, assessment of temperature stability is a sensitive means of detecting a structural abnormality in a protein.

Considered together with the previous data on temperature-sensitive mutations, the findings in the present study are compatible with the possibility that the mutation in this group of patients with testicular feminization is the result of some primary abnormality in the gene that codes for the androgen receptor. This hypothesis will require confirmation by more direct studies to characterize the receptor in broken cell preparations. Even if this supposition is correct it is unclear whether the androgen resistance in vivo is directly related to temperature instability or whether the temperature instability is simply a marker for an abnormal receptor.

Whatever the mechanism involved, these studies allow the biochemical dissection of a distinct subgroup of patients with hereditary male pseudohermaphroditism resulting from androgen resistance. Prior studies of androgen binding in cultured cells from patients with androgen resistance indicated a lack of correlation between the amount of binding and phenotypic development (8, 10–12). Although many patients with the most severe defect in virilization, i.e., complete testicular feminization, had near absence of the receptor in cultured cells, other patients with apparently identical phenotypes had normal or only partially deficient receptors (13). Likewise, intermediate states of androgen resistance with less severe defects in virilization are also characterized by variable degrees of receptor deficiency. Patients with incomplete testicular feminization and almost complete failure of virilization have half-normal levels of binding as do phenotypic men with Reifenstein syndrome or infertility (8, 10, 12). Finally, one infertile but otherwise normal man who has undetectable binding has been identified (8). The fact that fibroblast binding of dihydrotestosterone in the group of patients with testicular feminization and partial androgen receptor deficiency at 37°C is indistinguishable at 42°C from those with receptor-negative testicular feminization suggests that the failure of virilization in these patients may result from a qualitative abnormality in the androgen receptor. The fact that temperature sensitivity was demonstrable in patients with the phenotypes of incomplete as well as complete testicular feminization suggests, furthermore, that more than one mutation may be involved.

These studies do not provide insight into the nature of the defect in androgen action in the other forms of androgen resistance. Receptor deficiency associated with a male phenotype and receptor-positive resistance with a female phenotype may represent qualitative abnormalities of the receptor that are too subtle to be detected by the methods employed in this study, abnormalities that influence only receptor number, or abnormalities in the postreceptor effector mechanisms.

The fact that three pairs of siblings with receptor-deficient testicular feminization have a temperature-sensitive abnormality in the androgen receptor has major implications for the genetics of this subgroup of patients. In two of the families the pattern of inheritance is suggestive of X-linked inheritance. In the third family the two siblings are the only known affected family members. Thus, the qualitative defect is clearly hereditary and as has been demonstrated for receptor-negative testicular feminization is probably the result of an X-linked mutation (29). Whether the qualitative abnormality as demonstrated by these studies is an allele to the receptor-negative mutation is not known. In any case, profound androgen resistance, including the phenotype of complete testicular feminization, appears to result from qualitative defects as well as from absence of the androgen receptor.

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