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Human Minimal Androgen Insensitivity with Normal Dihydrotestosterone-Binding Capacity in Cultured Genital Skin Fibroblasts: Evidence for an Androgen-Selective Qualitative Abnormality of the Receptor

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SUMMARY

We have studied a kindred in which two pairs of siblings, maternal first cousins, have a form of "minimal" androgen insensitivity that permits morphogenesis of unambiguous male external genitalia, but interferes with normal virilization at puberty. All four had gynecomastia that required reductive surgery. Apart from this common phenotype, they varied considerably in the temporal and regional aspects of their subvirilization and appreciably in their androgenic responsiveness to pharmacological doses of testosterone. The cultured genital skin fibroblasts from one set of siblings have an androgen-receptor activity with the following properties: (1) a normal maximum-binding capacity (B_{max}) with 5α-dihydrotestosterone (DHT), or the synthetic androgen, methyltrienolone (MT; R1881) as ligand; (2) a higher than normal apparent equilibrium dissociation constant (K_d) for DHT (0.77 nM) but not for MT; and (3) an elevated dissociation rate (k) of DHT-receptor (0.013 min^{-1}, 37°C), but not MT-receptor, complexes within intact cells. In addition, prolonged incubation with MT, but not DHT, augments the specific androgen-binding activity of the mutant cells as much as that of the controls. Normal cells yield lower values of apparent K_d for DHT (0.1–0.3 nM) after 2- than after 0.5-hr incubation (0.3–1.8 nM), and 1-hr values are intermediate. This occurs despite concurrent catabolic consumption of

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DHT from the medium and is considered to reflect transformation of initial, low-affinity DHT-receptor complexes to subsequent, higher-affinity states by a process that depends on time and initial ligand concentration. The mutant complexes described here can readily attain the highest state of affinity with MT, but have an impeded, variably expressed ability to do so with DHT. These findings suggest that a structural mutation at the X-linked locus that encodes the androgen-receptor protein is responsible for its androgen-selective dysfunction. Synthetic, nonhepatotoxic androgens, with corrective effects in vitro comparable to those of MT, may be therapeutically useful for these subjects.

INTRODUCTION

Androgen insensitivity (resistance), not due to 5α-reductase* deficiency, can be divided into three classes of clinical severity [1]. Subjects in the complete class (so-called testicular feminization syndrome) are born with female external genitalia and become feminine at puberty except for absent or scanty sexual hair. The partial class consists of those with variable degrees of congenital external genital undermasculinization and pubertal subvirilization that may differ markedly within a kindred [2, 3]; they almost always develop gynecomastia. The mildest class contains those with male external genitalia, who experience normal or appreciable virilization at puberty. They may [4] or may not [5] develop gynecomastia, and, if not, oligo- or azospermia may be their sole abnormality [6]. Each of the classes is heterogeneous etiologically, as reflected in the quantity and quality of the specific androgen receptor-binding activity in cultured genital skin fibroblasts [6–9]. Here, we define a type of familial minimal androgen insensitivity associated with an androgen receptor that is qualitatively defective as expressed by its abnormal properties with DHT, but not with MT, as ligand.

MATERIALS AND METHODS

Hormone Assays

Testosterone [10], androstenedione [11, 12], estrone, and estradiol [13, 14] were assayed as described. Assay materials, including the LER 907 reference for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) radioimmunoassays, were provided by the National Hormone and Pituitary Program of the National Institutes of Health [15].

Cell Culture

The fibroblast lines were developed, as described [16], from small pieces of prepuce or scrotum skin obtained with informed consent. The medium used was Eagle's MEM

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* Trivial names and abbreviations used: methyltrienolone, MT, R1881, 17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one; dexamethasone, 9α-fluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione; 5α-dihydrotestosterone, DHT, 17β-hydroxy-5α-androstan-3-one; 5α-reductase, NADPH: Δ^4-3-ketosteroid-5α-oxidoreductase; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; androstenedione, androst-4-ene-3,17,dione; estrone, 3-hydroxy-estra-1,3,5(10)-triene-17-one; estradiol, estra-1,3,5(10)-triene-3,17β-diol.

with Earle's salts supplemented by 10% (v/v) fetal calf serum, 2 mM glutamine, 1 mM pyruvate, 1% (v/v) nonessential amino acids, and 60 mg/l each of penicillin G and streptomycin sulfate.

Androgen-Receptor Assay

Specific binding of androgen to its receptor was measured essentially as described [17]. Briefly, replicate confluent monolayers in 5-cm plastic petri dishes were incubated in 3 ml of serum-free (s-f) medium (buffered to pH 7.4 in the presence of 15 mM HEPES) with varying concentrations (0.05–3 nM) of [1,2,4,5,6,7-^{3}H]DHT (120 Ci/mmol) or [17α-methyl-^{3}H]MT (87 Ci/mmol) (both from New England Nuclear, Boston, Mass.) to measure "total" binding, or together with a 200-fold excess of radioinert ligand to measure "nonspecific" binding. After 30, 60, or 120 min at 37°C, the dishes were placed on a bed of ice, the medium was removed and sampled for free radioactivity, and the monolayers were washed thrice with 5 ml of ice-cold 20 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl and 0.3% bovine serum albumin (BSA), and then twice more with the same buffer minus BSA. The monolayers were then treated for 5 min with 2 ml of 0.1% trypsin, the loosely adherent cells were scraped with a rubber policeman, and the suspension was centrifuged twice (200 g, 5 min, 4°C). Cell pellets were dissolved in 0.5 N NaOH and sampled for protein by the Lowry technique [18] and for radioactivity in 10 ml of toluene solution containing Omnifluor (4 gm/1; New England Nuclear) by liquid scintillation spectrometry at an efficiency of 44%. Specific binding (total minus nonspecific) was plotted as a function of free androgen concentration, and maximum-binding capacity (B_{max}) and the apparent equilibrium dissociation constant (K_d) of the androgen receptor for DHT or MT were derived from Scatchard plots [19] fitted by linear regression. Only lines with an $r \geq -0.88$ and a $P < .01$ were considered. The significance of the difference among mean K_d values was evaluated by analysis of variance.

The 2-hr assay we have used to help us characterize the mutant receptor yields normal, equivalent, B_{max} values for DHT and the nonmetabolizable androgenic ligand MT (10–40 fmol/mg protein) that are identical to those from 45- to 60-min assays [6]. In contrast, it yields normal, equivalent, apparent K_d values for DHT and MT (0.1–0.3 nM) that are lower than those we (for DHT, [9]) and others (for both ligands [20]) have reported from 30- to 45-min assays performed on cells not incubated in s-f medium for 24 hrs before assay. This is true despite the fact that their receptor activity is saturated within 20–30 min of incubation with a sufficient concentration of either androgen at 37°C [20–22].

Dissociation Rates

To determine the rate of dissociation of the [^{3}H]androgen-receptor complexes formed within cells that had been incubated with ≥ 2 nM [^{3}H]androgen for 1 or 2 hrs, the assay medium in some dishes was replaced by a "chase" medium containing 0.6 μM radioinert androgen, and the percent original complexes remaining after various periods at 37°C or 40°C were plotted semilogarithmically. Replicate dishes allowed to incubate unchased, in the presence of saturating concentrations of androgen, maintained their specific receptor activity [9], indicating no net receptor degradation during the period of dissociation. The *t*-test was used to evaluate the significance of difference between mean values of dissociation rates.

DHT Metabolism

To evaluate the extent and quality of [^{3}H]DHT catabolism during 0.5–2-hr incubation at 37°C, the medium from 5-cm dishes that contained confluent monolayers was supplemented with 4×10^4 dpm of [^{14}C]DHT and 10 μg each of testosterone (T) and androstenedione, and then extracted twice with diethyl ether. The 5α-catabolites of DHT [3α(3β)-androstenediol, androstenedione, and androsterone] were separated from DHT and each other by thin-layer chromatography using two or three ascents in 99.5:0.5 chloroform:methanol.

T and androstenedione were visualized under shortwave ultraviolet light; DHT was visualized by radioautography. [^{14}C]DHT was used to monitor recovery (> 90%) and to estimate purity of the [^{3}H]DHT (> 90%) spot by recrystallization to constant isotopic ratio. The nonlinearity of DHT metabolism with time in these assays reflects the fact that the initial concentrations of DHT employed (4–6 nM) are subsaturating for the various DHT-catabolizing enzymes. This is borne out by the lesser degrees of DHT catabolism reported by Griffin [23] with 1 nM of substrate in a 45-min assay. Genital skin fibroblasts do not catabolize MT for at least 72 hrs at 37°C [24, 25].

Prolonged Incubation with DHT or MT

We assessed the ability of the mutant cells to augment their specific androgen-receptor activity in response to prolonged exposure to either androgen at 37°C as described in detail [24–26]. Briefly, three groups of replicate cultures were incubated at zero-time in s-f medium alone (medium I) or in s-f medium with 3 nM [^{3}H]DHT plus (medium II) or minus (medium III) 0.6 μM radioinert DHT. After 15 or 19 hrs, medium I was replaced by medium II or III to measure specific DHT-binding in assays lasting 5 and 1 hrs, respectively. After 19 hrs, spent media in some cultures that began with II and III were replaced by fresh supplies of the appropriate media ("19 + 1") to compare specific DHT-binding with replicates incubated continuously for 20 hrs. For MT, replicate cultures were incubated in s-f medium alone or with 2-3 nM [^{3}H]MT plus or minus 200-fold radioinert MT, and specific MT-receptor activity was assayed at intervals up to 72 hrs, without replacement of spent media 1 hr before assay. As pointed out [27], basal-specific androgen-receptor activity is stable in genital skin fibroblasts preincubated in serum- or androgen-free medium for up to 72 hrs.

5α-Reductase Activity

This was determined according to the method of Wilson [28]. The substrate was [1,2,6,7,16,17-^{3}H]testosterone (138 Ci/mmol; New England Nuclear), and DHT, the only 5α-reduced metabolite generated to an appreciable extent, was identified, recovered, and measured as described above for the assessment of DHT catabolism.

CLINICAL SUMMARIES

The propositus (III-6, fig. 1; cell line D3295) had a puberty characterized by breast development, deepening of his voice, hair growth on the lower extremities, and "some

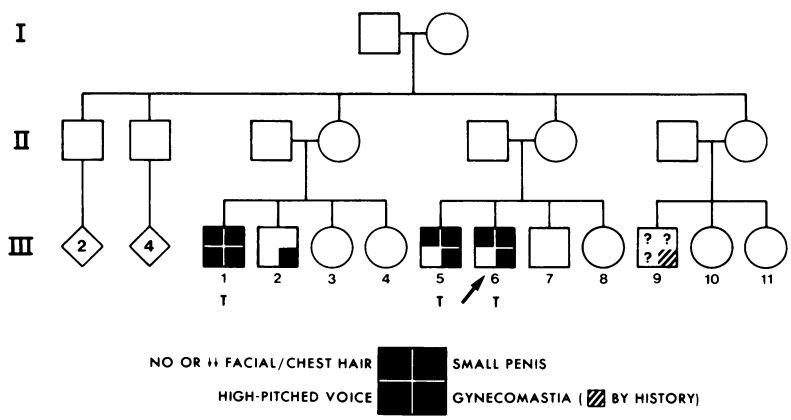

FIG. 1.—The pedigree. The propositus is identified by *an arrow*

increase in penis size," but no facial hair. Gynecomastia was corrected surgically at age 16 when a random serum testosterone (T) level was 930 ng/dl (normal: 300–1,000), and the urinary 17-ketosteroids and 17-ketogenic steroids were normal (6–21 mg/day). At that time, the basal serum levels of LH and FSH were 11.4 (normal: 5–23) and 7.2 (normal: 5–16) IU/l, respectively, and following LH-releasing hormone (LHRH) stimulation (100 μg by IV bolus), they peaked at 44.7 and 11.5, respectively. At age 18, no sperm were seen in an ejaculate of 1.5 ml. At age 21, the testes were small (L, 4 × 3 cm; R, 3 × 2 cm), the penis was 4 × 1.5 cm, and the pubic hair was Tanner stage 3. Another assay of serum T yielded a value of 459 ng/dl, at which time his basal concentrations of LH (5.1 and 4.8 IU/l, in duplicate) and FSH (1.4, 1.3 IU/l, in duplicate) rose to 22.2 and 2.2, respectively, following LHRH stimulation. The karyotype is 46,XY. After treatment for 14 months with T enanthate, up to 200 mg/week, his weight remained unchanged, he still had no facial or chest hair, and arm hair was sparse, but axillary, pubic, and leg hair were normal. His penis increased to 6 × 2 cm, but his testes remained small (L, 10 ml; R, 12 ml). After a further 18-month course of therapy, up to 400 mg/week, no objective signs changed, but he reported shaving once a week.

Subject III-5 (fig. 1; cell line B1016) had pubertal development very similar to his brother; gynecomastia required surgery at age 17. At that time, there was some hair on the lower extremeties, but little body and no facial hair. His serum T and androstenedione (normal: 60–230) concentrations varied from 350–930 and 128–216 ng/dl, respectively, on three occasions. In concert with the lowest value of T, the levels of LH and FSH were 6 and 0.4 IU/l, respectively. After 6 months of T enanthate up to 200 mg/week, some facial hair appeared. Six months after increasing the dose to 300 mg/week, he began to shave once a week and was able to cultivate a slight mustache. Hence, his dose was increased to 400 mg/week. Six months later, he still had no chest hair and "only moderate" axillary hair, but his pubic and leg hair were normal and he reported shaving twice weekly. At various times during treatment, his serum T concentration exceeded 2,500 ng/dl. One year after stopping treatment, his serum levels of T, androstenedione (630, 109 ng/dl), estradiol, estrone (40, 59 pg/ml), and LH and FSH (14 and 5 IU/l, respectively) were all within normal limits, and an ejaculate of 7 ml had a sperm concentration of 0.25×10^6/ml; 40% had normal morphology and 13% were motile.

Subject III-I (fig. 1) first noted excess breast size at 13 years of age. In his 15th year, he acquired some axillary and pubic hair, but neither voice change nor body hair. At age 20, his external genitalia remained "poorly developed," his voice high-pitched, and his chin hair sparse; he had minimal facial acne. The basal urinary 17-ketosteroid and 17-hydroxysteroid levels were normal, dexamethasone suppressible, and human chorionic gonadotropin (hCG) stimulable. The karyotype is 46,XY. He was given T cyclopentylproprionate, 300 mg/month. One year later (in 1969), on readmission for reduction mammoplasty, he had gained 14 kg and developed "considerable" facial, lower extremity, and pubic hair, but the penis remained small (fig. 2, left). No measurements or further information are available except that his children are adopted.

Subject III-2 (fig. 1) noted the onset of breast swelling at 11 years of age. At 14 years (in 1969), he was admitted for reduction mammoplasty. At that time, he had "nearly fully developed secondary-sex characteristics, with facial hair and acne, pubic and axillary hair of male type, and normal postpubertal gonads" (fig. 2, right). The basal urinary 17-ketosteroid and 17-hydroxysteroid levels were normal. Breast histology showed ductal and connective but no acinar tissue. No further information is available except that his children are adopted.

There is no evidence for consanguinity among the parents of the affected individuals.

RESULTS

Androgen Receptor-binding Activity

It is evident from table 1 that the cells of subjects III-6 (D3259) and III-5 (B1016) have mean 2-hr B_{max} values for DHT that are in the normal range, but

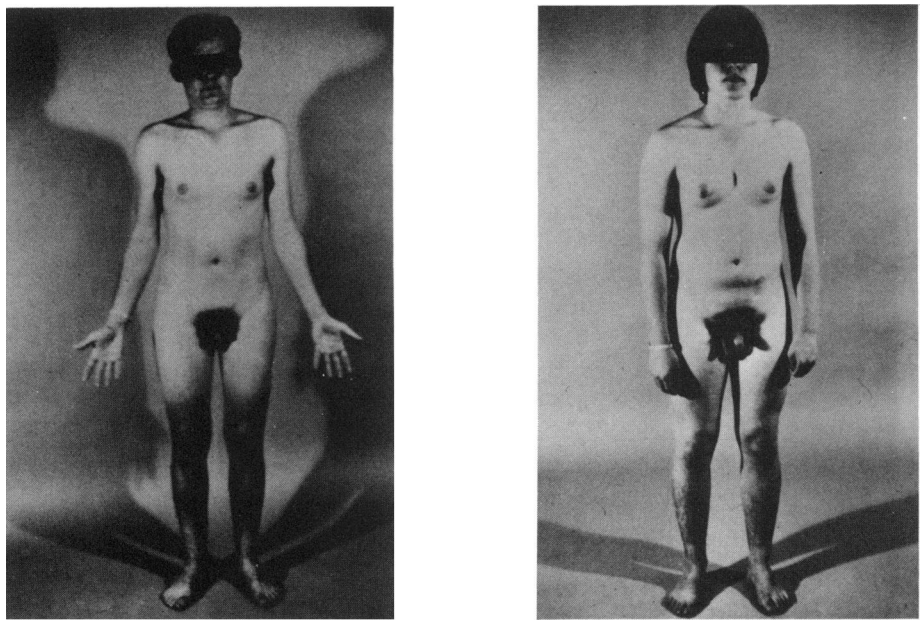

FIG. 2.—*Left,* pedigree no. III-1, age 20. *Right,* pedigree no. III-2, age 14. III-2 had more gynecomastia, a larger penis, and more distal limb hair than III-1. Both had a male habitus and abundant pubic hair with a female distribution.

mean 2-hr apparent K_d values for DHT that are significantly above normal, despite their considerable variance. In contrast, as demonstrated with B1016, the mutant receptor has 2-hr B_{max} and K_d values for MT in the normal range (table 2). These results are exemplified in figure 3. The difference in the apparent K_d of the androgen-binding activity for the two ligands could have been due to the fact that DHT is metabolizable, while MT is not.

In figure 4, the individual 2-hr K_d values for DHT of both subjects are displayed in juxtaposition with those of controls determined from 0.5-, 1-, and 2-hr assays.

TABLE 1

PROPERTIES OF NORMAL AND MUTANT ANDROGEN-RECEPTOR ACTIVITIES WITH 5α-DIHYDROTESTOSTERONE IN A 2-HR ASSAY

	B_{max} (fmol/mg PROTEIN)	No.	K_d (nM)	$k^{37°}$* (10^{-3} min^{-1})	No.	$k^{40°}$* (10^{-3} min^{-1})	No.
D3295	19 ± 4.6†	(7)	0.76 ± 0.56‡	13	(2)‖	18	(1)
B1016	35 ± 15	(4)	0.78 ± 0.75§	13	(2)‖	23	(1)
Normal	28 ± 8	(32)	0.22 ± 0.09	6 ± 1.2	(15)#	10 ± 1.4	(7)

* $k^{37°}$, $k^{40°}$: rate constants of dissociation at 37°C and 40°C, respectively.
† ± SD.
‡ $F = 12.6$; $P < .01$ vs. normal.
§ $F = 8.5$; $P < .05$ vs. normal. F (combined) = 5.7; $P < .02$ vs. normal.
‖ One of each of these dissociation rates was determined after a 1-hr assay with unchanged results.
After 1-hr assay, $k^{37°} = 5.6 \pm 1.2$ (no. = 13).

TABLE 2

PROPERTIES OF NORMAL AND MUTANT ANDROGEN-RECEPTOR ACTIVITIES WITH METHYLTRIENOLONE IN A 2-HR ASSAY

	B_{max} ± SD (fmol/mg protein)	No.	K_d ± SD (nM)	$k^{37°}$* ± SD (10^{-3} min^{-1})	No.
B1016	25	(2)	0.13	15.6 ± 2.7†	(5)
Normal	31 ± 12	(8)	0.16 ± 0.08	12.3 ± 3.4‡	(16)

* k^{37}, rate constant of dissociation at 37°C.
† $P > .05$, by unpaired, two-tailed t-test.
‡ After 1-hr assay, $k^{37°} = 11.9 \pm 5.1$ (no. = 7).

It is clear that controls yield lower apparent K_d values from 2-hr than from 0.5-hr assays, that their 1-hr values are intermediate, and that the mutant 2-hr K_d values have a distribution identical to those of controls at 0.5 hr.

The paradox of decreasing apparent K_d of the androgen-binding activity following progressively longer assay with a consumable ligand like DHT implied, at least, that concurrent DHT metabolism was not influencing the determination of apparent

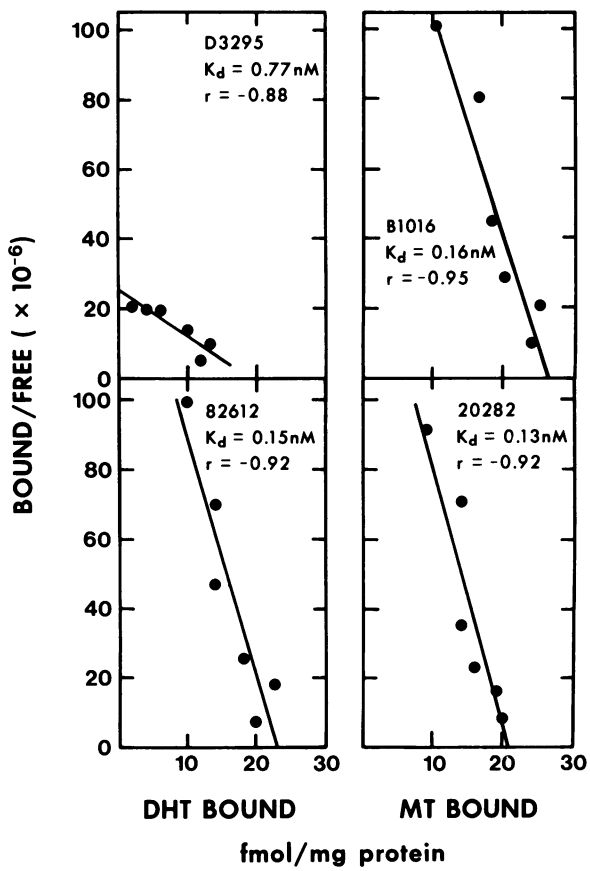

FIG. 3.—Scatchard plots of representative saturation analyses performed on the specific androgen-binding activity for DHT or MT in the mutant (D3295; B1016) and two control lines.

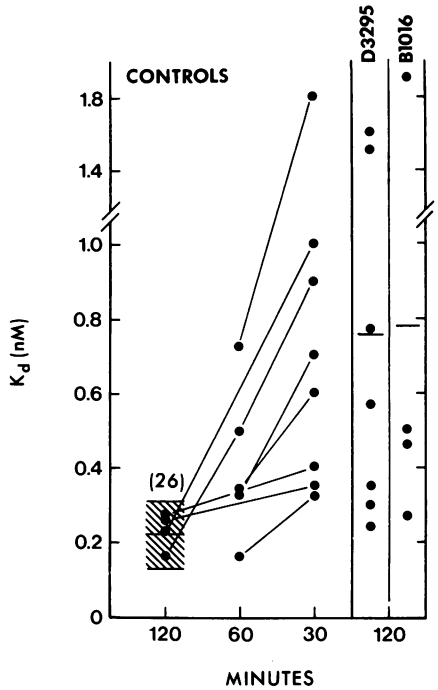

FIG. 4.—The distribution of K_d values for DHT of the androgen receptor in controls and each of the mutant lines at various times of assay. The linked K_d values were obtained at different times of assay within single experiments. *Hatched area* represents the mean ± SD of 26 control cell lines. *Bars* indicate the mean K_d value of either mutant.

K_d at any of the times. Table 3 reveals that this is true; thus, there is no relation between apparent K_d and the extent of DHT catabolism at 0.5 or 2 hrs, a control line (78516) consumed up to 80% of its initial DHT concentration and still yielded a normal 2-hr K_d, and only the mutant, B1016, did not attain a normal K_d by 2 hrs, despite DHT catabolism in the normal range.

Dissociation of Androgen-Receptor Complexes

The stability of DHT-receptor complexes, formed in genital skin fibroblasts during incubation for 1 or 2 hrs with ≥ 2 nM [^{3}H]DHT at 37°C, was assessed by

TABLE 3

THE RELATIONS BETWEEN APPARENT K_d AND DHT CATABOLISM IN 0.5- AND 2-HR ASSAYS

		0.5 HR		2 HRS	
LINE	INITIAL DHT (nM)	K_d (nM)	DHT recovered (%)	K_d (nM)	DHT recovered (%)
B1016	4	1.0	64	0.70	30
2036	4	1.0	83	0.23	40
3334	4	0.9	76	0.16	52
44218	6	0.4	87	0.27	67
78516	6	0.35	53	0.26	20

chasing them with 0.6 μM radioinert DHT at 37°C or 40°C. Table 1 and figure 5 reveal that the mutant DHT-receptor complexes dissociate at each temperature with a rate about twice normal. In contrast, the MT-receptor complexes formed within the mutant (B1016) cells dissociate at a normal rate (table 2).

Prolonged Incubation with Androgen

To identify a functional difference between the androgen-receptor complexes formed by the mutant with DHT or MT as ligands, incubations were carried out for periods beyond the time necessary to saturate the basal receptor-binding capacity at 37°C. Figure 6 demonstrates that the mutant cells could augment their binding activity normally during prolonged incubation with MT, but not with DHT.

5α-Reductase Activity

Mutant line B1016 reduced T to DHT at a rate of 2.4 pmol/mg protein per hr. This is a normal value [28].

DISCUSSION

The four affected men of the kindred described in this report share a clinical phenotype of androgen insensitivity (AI) that overlaps one extreme of the variable expressivity that is observed within certain families [2, 3] labeled as having the Reifenstein syndrome [3, 29]. The present family, and one other in the literature [4], illustrates that some mutant genes that interdict normal androgen responsiveness can yield the phenotype of minimal AI as their clinical prototype.

It is also important to realize that elevated levels of T and LH, indicative of hypothalamic-pituitary insensitivity to androgen, are not always found in random blood samples, even among affected members of a single family with complete

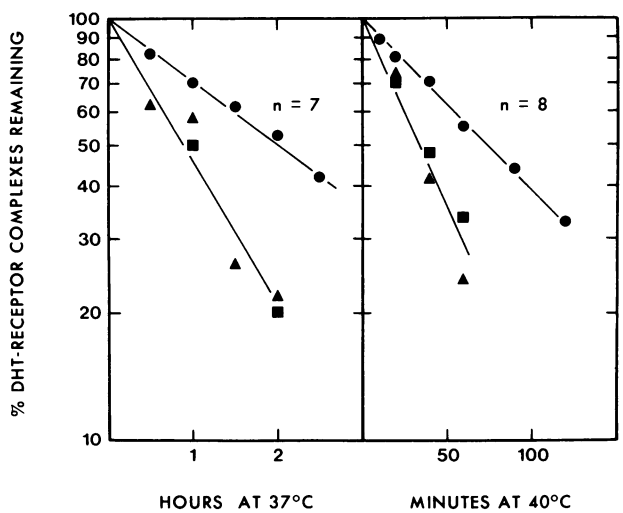

FIG. 5.—The rate of dissociation at 37°C and 40°C of the DHT-receptor complexes in control cells (●) and in those of the mutants (■, D3295; ▲, B1016).

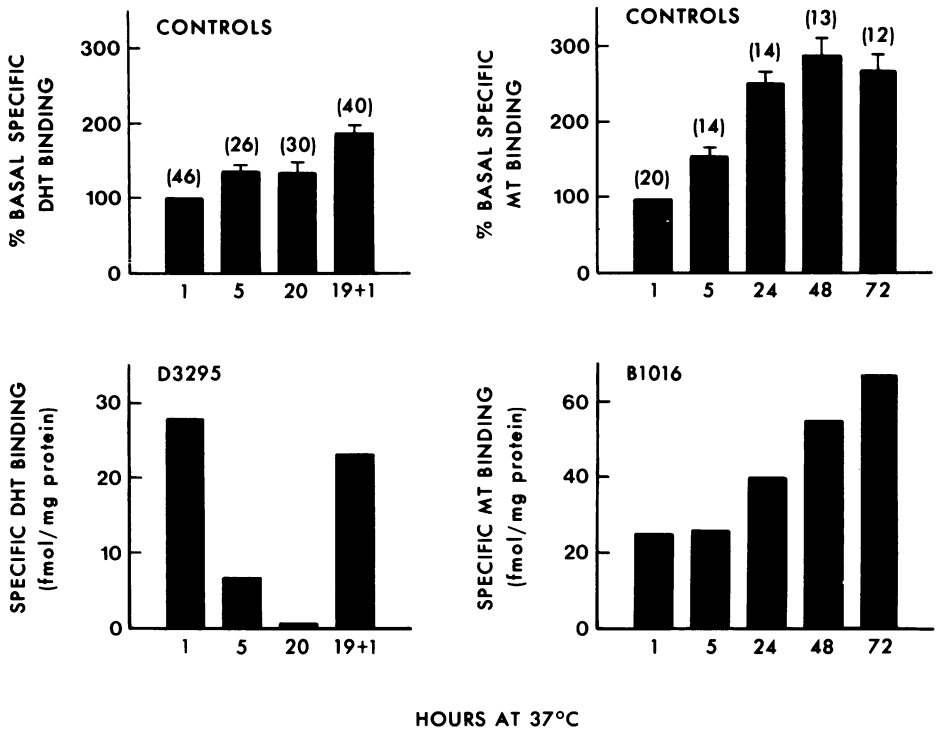

FIG. 6.—The effect of prolonged incubation with DHT or MT at 37°C on the specific androgen-binding activity in cells of the mutants compared to controls. *Values in parentheses* represent no. experiments. Basal (100%) DHT, MT binding = 25, 28 fmol/mg protein, respectively.

AI [30]. It is thus apparent that clinical-endocrine diagnosis of AI may be insecure, until definitive studies can be performed on genital or pubic [8] skin fibroblasts.

Our studies on the androgen-receptor activity in cells from two of the four affecteds have revealed that it has an abnormally high 2-hr apparent K_d for the natural androgen, DHT, but not for the nonmetabolizable ligand, MT, and that its level is augmented (up-regulated) during prolonged incubation with MT but not with DHT. Such a combination of results might be attributable to hypercatabolism of DHT in the presence of a normal androgen receptor. But we have shown that the apparent K_d for DHT in normal cells decreases (affinity increases) during incubations lasting from 0.5 to 2 hrs, despite considerable DHT catabolism in the interval. We interpret this to mean that transformation of initial, low-affinity, DHT-receptor complexes to serial higher-affinity states must override whatever tendency there is for concurrent DHT catabolism to yield artificially low estimates of its affinity for the androgen receptor. Furthermore, we know that the apparent K_d for MT also decreases between 0.5 and 2 hrs of incubation [31], despite the fact that MT is not metabolizable. In addition, we have shown not only that the mutant cells do not hypercatabolize DHT, but also that their DHT-receptor complexes dissociate abnormally quickly (and equally so whether formed during a 1- or 2-hr incubation) when this behavior is assessed by a "chase"

protocol that is immune to any confounding influence of DHT metabolism. In contrast, their MT-receptor complexes dissociate at a normal rate. These data permit us to conclude that the mutation responsible for AI in the present family is associated with an abnormal androgen receptor that expresses its dysfunction in an androgen-selective way.

This conclusion is affirmed by our recent experience [31] with another family in which partial AI is associated with a mutant androgen receptor that has consistently high 2-hr K_d (1.4 nM) and k values for both DHT and MT and that up-regulates in response to prolonged incubation with neither ligand.

We do not know why the 2-hr K_d values for DHT in the mutant cells vary as much as they do, but that variation is certainly biological rather than technical in origin. It may be akin to the comparable degree of variation observed in the 0.5-hr K_d values for DHT within and among normal cell lines, or it may reflect the expression of a "leaky" mutant. It is noteworthy that Brown et al. [32] also observed appreciable variation of the abnormally high K_d values for DHT among three members of a family with complete AI.

Notwithstanding such variation, the simplest interpretation of the androgen-receptor dysfunction in the present family is that it permits the formation of DHT-receptor complexes that do not readily transform from their initial low-affinity state to their derivative high-affinity states. In contrast, with MT as ligand, the process of transformation proceeds normally. Such an interpretation is consistent with a previous postulate [31] that androgen-receptor complexes must attain their highest-affinity state in order to function as an effective signal for up-regulation.

Transformation from low-affinity to serial higher-affinity states is a property of many [33–35], if not all, steroid-receptor complexes, and it is considered an essential step in steroid hormone action. It should not be surprising that the process of transformation should be vulnerable to mutational disruption of various types. The present family exemplifies this vulnerability.

The family with minimal AI described here deserves comparison with the one reported by Larrea et al. [4]. In theirs, two pairs of siblings, maternal first cousins, had a uniform phenotype consisting of gynecomastia, absent axillary but normal pubic hair, normal external genital and musculoskeletal virilization, and reduced ejaculate volume but normal sperm concentration, morphology, and motility. Endocrine investigations on serum revealed normal to elevated basal levels of testosterone, an exaggerated elevation of LH in response to LHRH, and nonsuppressibility of LH even after 12 weeks of T in pharmacological doses. The maternal grandfather of the two sets of siblings had post-pubertal bilateral gynecomastia, and had nine children, three of whom were unaffected males. This indicated that the mutation was probably X-linked and did not cause infertility.

Our family had an inconstant clinical phenotype, infertility, and apparently normal hypothalamic-pituitary sensitivity to androgen. Thus, it differed substantially from the one of Larrea et al. [4]. On the other hand, the pattern of inheritance in our family is also consistent with X-linkage, and very likely because of a structural mutation at the locus on the X chromosome that encodes the androgen-receptor protein in man and the mouse [36].

Minimal AI is also exemplified by some men in whom the sole abnormality is infertility due to oligo- or azospermia, and who generally do not express their androgen resistance at the hypothalamic-pituitary level. In one series, nine of 22 such men had reduced androgen receptor-binding activity in their genital skin fibroblasts, using 12 fmol/mg protein as the lower limit of normal [6]. The genetic aspects are unknown, since all the subjects identified so far have had negative family histories [6]. Nevertheless, as suggested [6], subtle qualitative defects of the androgen receptor, comparable to the one described in this paper, may account for some men with idiopathic oligo- or azospermia in whom the amount of androgen-binding activity is normal.

The ability of MT to normalize the mutant androgen receptor in the present family implies that androgens with such corrective effects in vivo may be useful prophylactically or therapeutically for subjects with this type of androgen resistance. MT itself is hepatotoxic in man, at least when taken orally [37]; therefore, other synthetic androgens will have to be screened in vitro, and evaluated toxicologically in vivo, before their therapeutic potential can be assessed.

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