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Research Article

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Intracellular and Nuclear Binding of [^{3}H]Dihydrotestosterone in Cultured Genital Skin Fibroblasts of Patients with Severe Hypospadias

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Abstract

Androgens stimulate the development and growth of the male external genitalia. Because hypospadias is the most common congenital defect of the male urethra and because in most cases the cause of this malformation is unknown, we examined the hypothesis that the etiology of the severe forms of this disorder, which is frequently associated with other genital anomalies, might be explained by receptor abnormalities. Intracellular and nuclear binding of androgens were determined in cultured genital skin fibroblasts from 10 males who underwent circumcision for phimosis (controls A), 2 patients with 5α-reductase deficiency (controls B), and 11 patients with severe forms of hypospadias of unknown etiology.

Genital skin fibroblast monolayers were incubated for 60 min at 37°C with varying concentrations of [^{3}H]-dihydrotestosterone ([^{3}H]DHT), and specific binding in whole cells and nuclei was measured. Maximum binding (B_{max}) in the whole cell assay averaged 0.88±0.15 fmol · μg DNA^{-1} (mean±SD) in the control group (controls A, 0.89±0.16 fmol · μg DNA^{-1}; controls B, 0.85 fmol · μg DNA^{-1}) and 0.73±0.25 fmol · μg DNA^{-1} in the patients with hypospadias. In the latter group, B_{max} in six patients was below the minimum values determined in the controls. Maximum specific nuclear binding in the control groups averaged 43% (range, 30–55%) of the corresponding intracellular binding. In contrast, nuclear binding in strains from patients with hypospadias was lower (range, 0–12% of whole cell B_{max}). In particular, no high affinity saturable nuclear [^{3}H]DHT binding could be measured in 6 of the 11 patients. We interpret these data to suggest that defective intracellular and/or nuclear binding might be the cause of defective genital development in some patients with severe hypospadias.

Introduction

The conversion of the indifferent urogenital tract into the male phenotype is mediated by testicular hormones, principally androgens, secreted by the fetal testis during a critical period of embryogenesis. In accordance with this concept, several syndromes of hereditary male pseudohermaphroditism result either from deficient testicular testosterone secretion or from resistance of target organs to the action of androgen. For example, the autosomal recessive disorder pseudovaginal perineoscrotal hypospadias (5α-reductase deficiency) results from a defective 5α-reduction of testosterone to dihydrotestosterone (1, 2). Other disorders such as testicular feminization or the Reifenstein syndrome, which are inherited in an X-linked fashion, result from abnormalities of the high-affinity intracellular androgen receptor protein (1–3). These disorders are, however, rare; for example, testicular feminization, the most common of these disorders, occurs at an incidence of ~ 1 in 20,000 to 1 in 60,000 male births.

In contrast to these rare but well-characterized forms of male pseudohermaphroditism, the etiology in many instances of incomplete virilization, particularily hypospadias, the most common congenital defect in males with an estimated incidence of 0.1–0.8% (4–6), is less well understood. We have therefore examined the hypothesis that the etiology of severe forms of hypospadias, which are frequently associated with other anomalies of the external genitalia, might be explained by receptor abnormalities. Intracellular and nuclear binding were assessed in cultured genital fibroblasts in normal males (controls A), two patients with 5α-reductase deficiency (controls B), and patients with severe forms of hypospadias of unknown etiology.

Methods

Materials. Silica gel TLC sheets with plastic back (Polygram Sil G-Hy) were from Macherey and Nagel, Düren, FRG. [1,2,4,5,6,7-^{3}H]-Dihydrotestosterone ([^{3}H]DHT[1]; 5,217 GBq/mmol), [1,2-^{3}H]-testosterone ([^{3}H]T; 1,813 GBq/mmol), and Riafluor scintillation fluid were from New England Nuclear, Dreieich, FRG. [^{3}H]DHT and [^{3}H]T were purified, if necessary, by TLC with the system dichloromethane/ethyl acetate/methanol (85:15:2; vol/vol/vol) to assure that the [^{3}H]-DHT then was > 95% pure. Nonradioactive dihydrotestosterone and sucrose were from Merck, Darmstadt, FRG. Eagle's minimum essential medium (MEM), penicillin and streptomycin solution (10,000 U of penicillin and 10 mg streptomycin/ml), trypsin-EDTA solution (1×), and nonessential amino acids were from Gibco Laboratories, Karlsruhe, FRG.

FCS and PBS were from Seromed, Berlin, FRG. Falcon dishes (100 × 20 mm) were from Becton-Dickinson & Co., Heidelberg, FRG. Bovine albumine powder fraction V was from Boehringer, Mannheim, FRG; diaminobenzoic acid dihydrochloride was from Aldrich-Chemie, Steinheim, FRG and desoxyribonucleic acid from herring sperm was from Carl Roth, Karlsruhe, FRG. Dextran T 70 was from Pharmacia Fine Chemicals, Freiburg, FRG, and Norit A was from Serva Feinbiochemica, Heidelberg, FRG. All other chemicals were reagent grade or better and used as supplied by the manufacturer.

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1. *Abbreviations used in this paper:* DHT, 17β-hydroxy-5α-androstan-3-one; 5α-reductase, NADPH: Δ^4-3-ketosteroid-5α-oxidoreductase.

Source of tissue. The fibroblast strains used in this study were derived from explants of genital skin (scrotum or foreskin) from 10 normal males, 2 patients with 5α-reductase deficiency and 11 patients with severe forms of hypospadias (Tables I and II). The tissue was obtained either at surgery for circumcision or repair of developmental defects of the urogenital tract. Biopsies of minors were performed with the consent from the parents. The biopsies were obtained from various university and city hospitals. Immediately after biopsy, the tissue was brought into a cryotube (1.8 ml volume) containing MEM fortified with 20% FCS and mailed to our laboratory. Detailed clinical information on each patient (karyotype, history, drug intake by the patient's mother during the first trimester of pregnancy, penile size, description of the genitalia, and the results of serum testosterone determination before and after human chorionic gonadotropin stimulation) were provided by the referring physicians and are listed in Table II. A description of one patient with 5α-reductase deficiency (cell strain GS 180) has been published previously (7).

Cell culture and incubation of cells with [^{3}H]DHT. The skin fibroblasts were established from the genital biopsies as described (8, 9) and were used between passages 5 and 12. Cells from stock dishes (100 × 20 mm) were dissociated with trypsin-EDTA and seeded on day 0 at a concentration of ~ 200,000 cells in 100 × 20 mm dishes (Falcon Labware, Oxnard, CA). On day 3 the medium was replaced with fresh growth medium (MEM plus 10% FCS). On day 6 the medium was removed, the cells were washed twice with 4 ml of PBS, and 8 ml of MEM without FCS were added to each dish. On day 7 the medium was removed again and the monolayers were incubated in duplicate with varying concentrations of [^{3}H]DHT (0.25–2.0 nM) in MEM with or without a 500-fold excess of nonradioactive DHT for 60 min in a humified incubator at 37°C in the presence of 5% CO_2.

Whole cell binding assay. Binding was assessed using (with minor modifications) the methods described by Brown et al. (10, 11). In brief, after incubation, the medium was removed, and the monolayers were washed three times with 5 ml ice-cold Tris-NaCl buffer (0.9% NaCl, 20 mM Tris-HCl, pH 7.4). All subsequent procedures were conducted at 0–4°C. Cells were harvested in 5 ml Tris-EDTA-glycerol (TEG) buffer (20 mM Tris-HCl, 1.5 mM EDTA, and 10% [vol/vol] glycerol, pH 7.4) by scraping with a rubber policeman. Cell suspensions from each of the two dishes containing corresponding steroid concentrations were combined (10 ml), and a 2-ml aliquot of the suspension was used for the whole cell binding assay, whereas in the remainder (8 ml) the nuclear uptake was assessed.

Centrifugation of the cell suspension for 10 min at 800 *g* yielded a pellet of whole cells. After the supernatant was aspirated, the pellet was resuspended in 0.9 ml Tris-EDTA-KCl (TEK) buffer (20 mM Tris-HCl, 1.5 mM EDTA, and 0.5 M KCl, pH 7.4) and subjected to sonic disruption using an ultrasonic system (model 1510, Labsonic; Braun Melsungen, FRG) (20,000 Hz, 300 W maximum power). Samples were exposed to two 5-s periods of sonication at 50 W power using a needle probe (4 mm diam). The cell lysates were centrifuged at 1,600 *g*, and two aliquots of the supernatant (300 μl each) were taken for the determination of total or nonspecific binding, and the DNA content was measured in a 100-μl aliquot by the method described by Collier et al. (12).

Adsorption of free steroid was performed by adding 600 μl of dextran-coated charcoal suspension (20 mM Tris-HCl, 1.5 mM EDTA, 0.1% gelatine, 0.05% dextrane T 70, and 0.5% activated charcoal) to each tube, which was then stirred several times over a 10-min period. The charcoal was pelleted by centrifugation at 2,500 *g* for 15 min, and the radioactivity was counted in a liquid scintillation counter (model 300C; Packard Instrument Co., Downers Grove, IL) in a 500-μl aliquot of the supernatant pipetted into 10 ml Riafluor. Specific receptor bound steroid was calculated as the difference between total and nonspecific binding and corrected for DNA content. The maximum binding capacity (B_{max}) and the apparent dissociation constant (K_d) of the androgen receptor were determined from Scatchard plots (13) analyzed by a linear regression program using a calculator (TI 59; Texas Instruments, Inc., Dallas, TX).

Nuclear [^{3}H]DHT binding. An 8-ml aliquot of the cell suspension saved for preparation of purified nuclei from intact fibroblasts was removed and nuclear binding was performed as described by Collier et al. (12), except that the final purification step by ultracentrifugation was modified to our laboratory conditions. For this purpose the nuclear pellet was suspended in 6 ml Tris-sucrose buffer by vortexing for 1 min. 5.8 ml of this nuclear suspension were then layered over 5.8 ml hypertonic buffer solution, and the mixture was centrifuged at 100,000 *g* for 75 min at 1°C in an ultracentrifuge (model TGA-50, Kontron Analytical, Everett, MA) using a fixed angle TFT 50.13 rotor. The supernatant was aspirated and the cellulose-acetobutyrate tubes were cut ~ 1.5 cm above the bottom. The nuclear pellet was suspended in

Table I. Clinical Data, Results of 5α-Reductase Activity in Fibroblast Homogenates, and [^{3}H]DHT Binding in Intact Fibroblasts and Fibroblasts Nuclei from Males with Normal Genital Development (Controls A) and Two Patients with 5α-Reductase Deficiency (Controls B)

Site of skin biopsy/cell strain	Diagnosis	Age	5α-Reductase activity	Androgen binding		Percent of whole cell binding
				Intact cells	Nuclei	
		yr	*pmol · mg protein^{-1} · h^{-1}*	*fmol · μg DNA^{-1}*		
Controls A: Foreskin	Normal males					
GS-58	(Phimosis)	3	232	1.19	0.45	(38)
GS-60		7	164	0.84	0.45	(46)
GS-62		7	195	0.76	0.42	(55)
GS-63		4	376	1.08	0.41	(38)
GS-64		5	135	0.70	0.21	(30)
GS-65		3	294	0.78	0.43	(55)
GS-66		3	99	0.93	0.50	(53)
GS-70		3	294	0.79	0.38	(48)
GS-184		4	467	0.78	0.38	(49)
GS-213		4	377	1.00	0.42	(42)
Controls B: Foreskin	5α-Reductase					
GS-180	deficiency	12	0.3	0.76	0.27	(35)
GS-199		3	0	0.94	0.28	(30)

Table II. Clinical, Endocrine, and Biochemical Profile of Patients with Hypospadias

Cell strain	Age	History	Karyotype	Degree of hypospadias	Testosterone; Post hCG	5α-reductase	Androgen binding		Remarks
							Intact cells	Nuclei	
	yr				*ng/dl*	*pmol · mg protein^{-1} · h^{-1}*	*fmol · μg DNA^{-1}*		
GS-24	2	+	46,XY	scrotal	307	167	0.98	0	Microphallus, chordee, bifid scrotum
GS-41	1	−	46,XY	scrotal	60	108	1.01	0.03	Microphallus, bifid scrotum, rudimentary vagina
GS-42	26	−	n.d.	perineal	basal 600	219	0.55	0	Microphallus, rudimentary vagina
GS-72	10	−	46,XY	penoscrotal	256	219	0.98	0.12	Partial penoscrotal transposition
GS-78	10	−	n.d.	penile	81	434	0.50	0.02	Microphallus
GS-114	4	−	46,XY	perineal	436	0·84; 0	1.12	0	Microphallus, chordee
GS-115	4	−	46,XY	penile	440	179	0.60	0	Bifid scrotum
GS-142	1	−	46,XY	penoscrotal	249	4	0.45	0	Microphallus
GS-149	2	−	46,XY	perineal	810	191	0.55	0.05	Microphallus, rudimentary vagina
GS-161	6	Brother: perineal hypospadias	46,XY	perineal	730	878	0.80	0.10	Microphallus, penoscrotal transposition
GS-168	9	−	46,XY	perineal	120	788	0.48	0	

GS, genital skin; −, negative; +, mother received gestagen-estrogen injections during second and third month of pregnancy.

1.0 ml TEK buffer followed by one passage through a 25-gauge needle. An 0.5-ml aliquot was then put into 10 ml Riafluor for determination of radioactivity, and two aliquots of 0.2 ml each were used for DNA measurement as described by Collier et al. (12). Specific binding, B_{max}, and K_d were determined as described above for whole cell androgen binding. The curves had a correlation > 0.8 within the Scatchard analysis.

To investigate if the results were affected by the type of rotor used, in one experiment, identical aliquots of nuclear pellets from two cell strains (GS 79 [phimosis] and GS 72 [hypospadias]) were subjected to ultracentrifugation at 100,000 *g* with a fixed angle rotor (TFT 50.13; Kontron) in the ultracentrifuge and in a swinging bucket rotor in a ultracentrifuge (SW 27 and L265 B, respectively; Beckman Instruments, Inc., Fullerton, CA) (Table III).

5α-reductase assay. 5α-reductase was determined in cell sonicates of genital skin fibroblasts at pH 5.5 with 100 nM [^{3}H]T essentially as described by Leshin et al. (14) but with these modifications: cell sonicates were incubated for 60 min at 37°C in the presence of 1 mM NADPH and ~ 20 μg fibroblast protein.

Under these conditions rates of enzyme activity were proportional to the time of incubation and to the amount of protein (sonicate) added.

Statistical analysis. Statistical analysis of the data was performed using the *t* test and the Wilcoxon U test.

Table III. Reproducibility of Androgen Binding Measurements in Intact Fibroblasts and Fibroblast Nuclei

	Exp. no.	DHT binding		r values
		Intact cells	Nuclei	
		fmol · μg DNA^{-1}		
Cell strain GS-70 phimosis, foreskin	1	0.79 (−0.96)	0.38	(−0.86)
	2	0.73 (−0.83)	0.39	(−0.90)
	3	0.82 (−0.96)	0.35; 0.37*	(−0.97); (−0.92)
Cell strain GS-72 hypospadias, foreskin	1	1.09 (−0.97)	0.13	(−0.82)
	2	0.98 (−0.96)	0.12	(−0.92)
	3	1.11 (−0.98)	0.20; 0.16*	(−0.97); (−0.94)

* Values determined by ultracentrifugation of nuclei with swinging bucket. Values in parentheses, Scatchard analysis, *r* value of the regression line.

Results

Karyotypes were obtained in 9 of the 11 patients and were found to be 46,XY (Table II). Of the two patients in which this parameter was not assessed, a 26-yr-old man had undergone orchidopexy for cryptorchidism of the right testis and a 10-yr-old boy had penile hypospadias. The human chorionic gonadotropin (hCG) stimulation tests were performed in all subjects except a 26-yr-old patient who had basal testosterone serum levels within the normal male range (Table II).

A normal response of serum testosterone to hCG was found in nine patients, whereas in one boy, serum testosterone after hCG stimulation (0.6 ng/ml) remained below the lowest values measured (0.83 ng/ml) in a control cohort of 30 boys with normal penile development. 5α-reductase activity was within the normal range (> 1 pmol · mg protein^{-1} · h^{-1}) in all but one patient with hypospadias of unknown etiology. As expected, 5α-reductase activity was < 1 pmol · mg protein^{-1} · h^{-1} in both 5α-reductase deficiency patients.

The methods that have been used for measurement of androgen binding in intact fibroblasts and isolated nuclei are illustrated diagrammatically in Fig. 1. When purified nuclei were examined by phase contrast microscopy, they were found

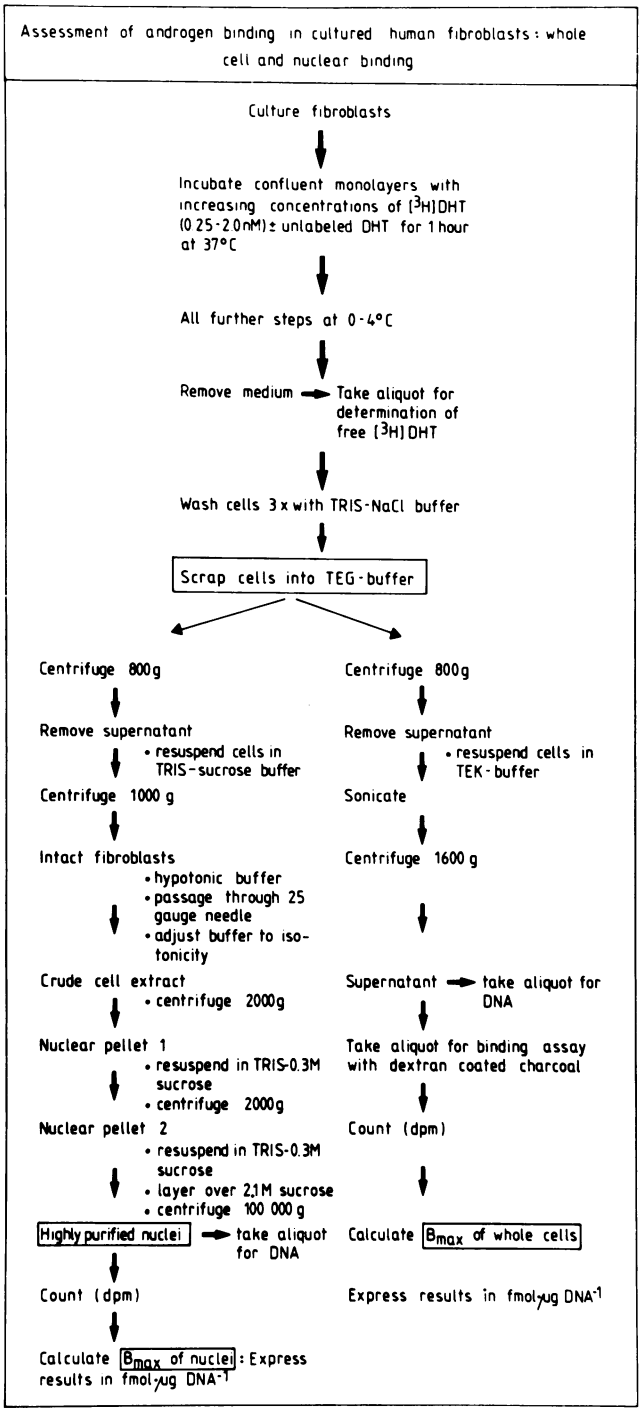

Figure 1. Diagram of methods.

to be free of cytoplasmic contamination, as has been demonstrated before (12).

To determine the reproducibility of our procedures, total cellular and nuclear binding were measured in two cell strains on three occasions. In one experiment in two cell strains similar results were also obtained with a fixed angle rotor and a rotor equipped with a swinging bucket (Table III).

Representative binding curves from cell strains derived from the genital skin of a boy with normal male genitalia and from two patients with hypospadias of unknown etiology are shown in Fig. 2 *A–C*. Nuclear binding in these two patients with hypospadias differed from that seen in males with normal genital development; namely, nuclear binding was reduced or absent. High-affinity androgen binding was then assessed in intact fibroblasts and nuclei in a total of 23 cell strains (Tables I and II and Fig. 3) that were derived from 10 males with normal genital development (controls A), 2 patients with 5α-reductase deficiency (controls B) and 11 patients with severe forms of hypospadias.

Maximum specific binding in the intact cell assay averaged 0.88±0.15 fmol·μg DNA^{-1} (mean±SD) in the control group (controls A, 0.89±0.16 fmol·μg DNA^{-1}; controls B, 0.85 fmol·μg DNA^{-1}). In controls A the range of binding in intact cells and in isolated nuclei was similiar to that reported previously (12); on average, 45% of the binding was recovered in the nuclei (range, 30–55%). Similar binding was observed in cells from patients with 5α-reductase deficiency (controls B).

Maximum specific binding in the cells from the patients with hypospadias averaged 0.73±0.25 mol·μg DNA^{-1}, although binding in intact cells from a subset of six of the patients was below the minimal values determined in the controls. In contrast, nuclear binding in fibroblasts from the patients with hypospadias of unknown etiology was uniformly ($P < 0.01$) lower, and 0–12% of the binding observed in the corresponding intact cells was found. Indeed, in 6 out of 11 patients no high-affinity saturable nuclear binding could be measured.

Discussion

This study has examined androgen binding in cultured human genital skin fibroblasts from males with incomplete virilization of the external genitalia. Among the 11 patients studied, 10 had severe hypospadias, in some instances associated with other genital abnormalities. We selected these patients in an attempt to avoid subtle abnormalities of the external genitalia. Skin fibroblasts were chosen because these cells retain the biochemical properties of genital skin with regard to androgen binding and metabolism. This model also allows investigation under defined chemical conditions and is not affected by a limitation of tissue.

Two major findings emerge from our study. First, in a subgroup of patients with hypospadias (6 out of 11), androgen uptake in intact genital fibroblasts was below the minimum values determined in control groups. These results are in accordance with several previous studies. Svensson and Snochowsky (15) were the first who reported a lower binding of methyltrienolone to receptor proteins of foreskin homogenates of boys with hypospadias compared with appropriate controls with normal genital development. Later, Keenan et al. (16) reported a lower mean [^{3}H]DHT binding in fibroblasts cultured from foreskin of 28 infants with simple idiopathic hypospadias as compared with a control group. Similar results have also been observed in our laboratory (17, 18): in a group of 10 patients with idiopathic hypospadias, mean maximum specific binding of methyltrienolone in cultured genital skin fibroblasts was lower than in 8 males with normal penile development.

Second, nuclear binding in cell strains from patients with hypospadias of unknown etiology was in all instances lower than in the control groups. In 6 of 11 patients no nuclear binding was found. From a theoretical standpoint, several possible biochemical mechanisms may be responsible for these

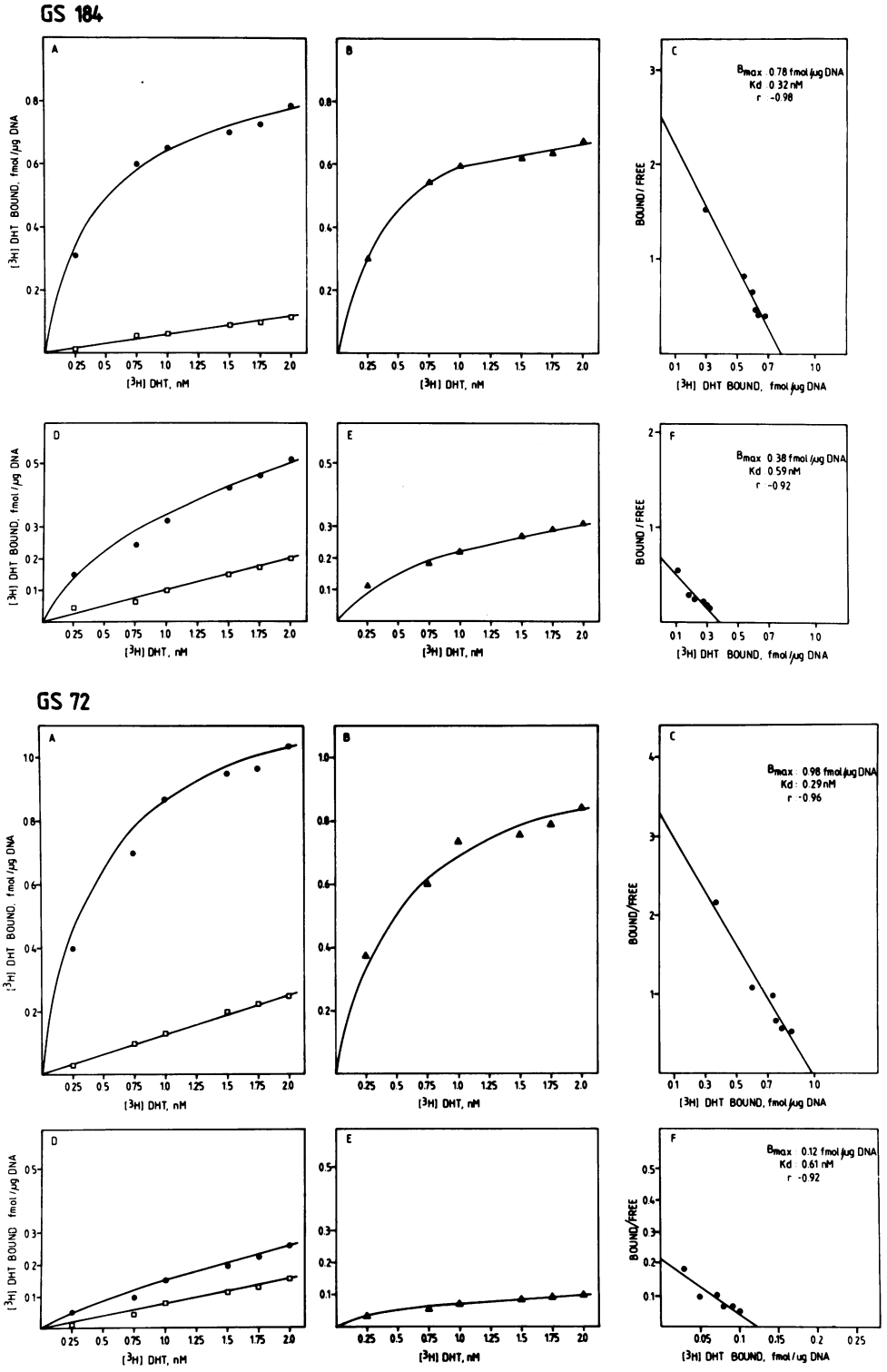

Figure 2. Binding of [^{3}H]-DHT by cultured fibroblasts from three different cell strains as a function of steroid concentrations. (*Cell strain GS 184*) Normal genital development; (*Cell strain GS 72*) hypospadias; (*Cell strain GS 115, depicted on next page*) hypospadias. Cells were grown under standard conditions as outlined in the text. On day 7, the medium was removed and the monolayers were incubated with increasing concentrations of [^{3}H]DHT (0.25–2 nM) in MEM with or without a 500-fold excess of nonradioactive DHT for 60 min at 37°C. On each piece, *A* depicts (● —— ●) total and (□ —— □) nonspecific binding in fibroblasts; *B* depicts the respective specific binding, whereas *C* illustrates the Scatchard analysis of the data shown in *B*. [^{3}H]DHT binding data obtained in the nuclei are depicted in *D–F;* the data illustrated are analogous to those shown in *A–C*.

results, either impaired transformation of the hormone-receptor complex to the DNA-binding state or defective translocation of the hormone-receptor complex into the nucleus.

This interpretation is based on current concepts of androgen action. It is thought that the androgen receptor complexes of human fibroblast cytosol acquire the capacity to bind to DNA by a process termed "transformation." The chemical nature of this process is unclear, but it is assumed that a conformational change in the receptor protein results in an alteration of the charge that then permits the hormone-receptor complex to be translocated into the nucleus and to bind DNA. There are only a few reports addressed to these processes in males with incomplete virilization of the external genitalia. Kovacs et al. (19) identified an impairment of receptor transformation in genital skin fibroblasts of two cousins with severe hypospadias as the underlying biochemical defect of the observed androgen resistance.

Interestingly, Gyorki et al. (20) presented data of impaired nuclear compartimentalization of androgen binding ([^{3}H]-methyltrienolone binding) in three patients with different de-

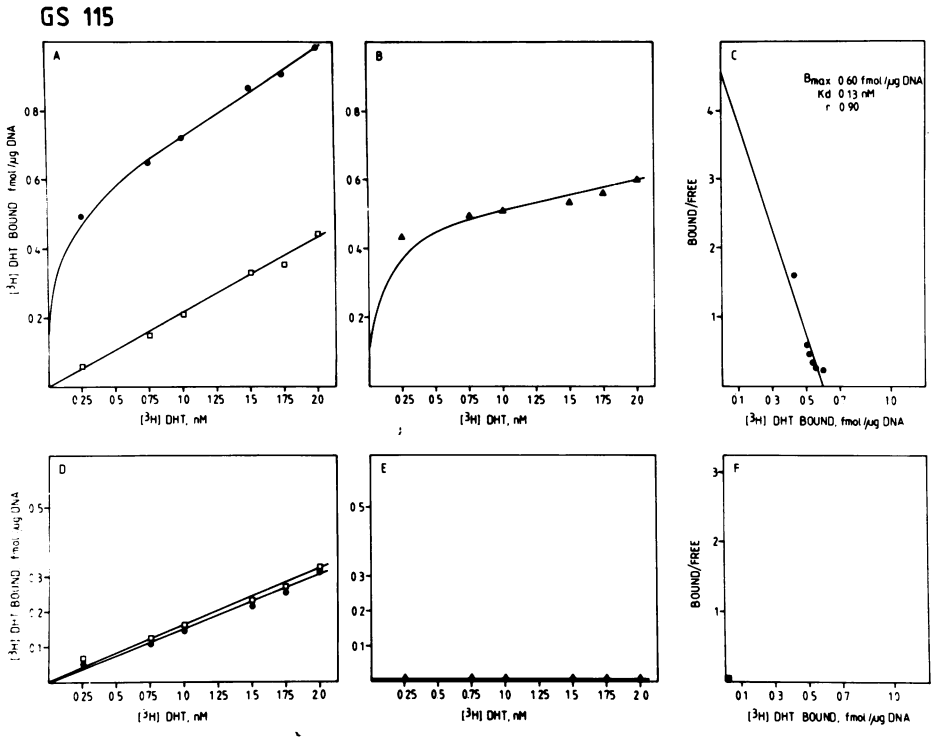

Figure 2 (Continued)

grees of hypospadias (penile, perineoscrotal, and perineal), two of whom also had microphallus. Reconstitution experiments in which cytosol from normal fibroblasts and from these patients were mixed revealed that nuclei from each patient bound cytoplasmic androgen receptors to a lower degree than controls, regardless whether the source was from affected or normal males.

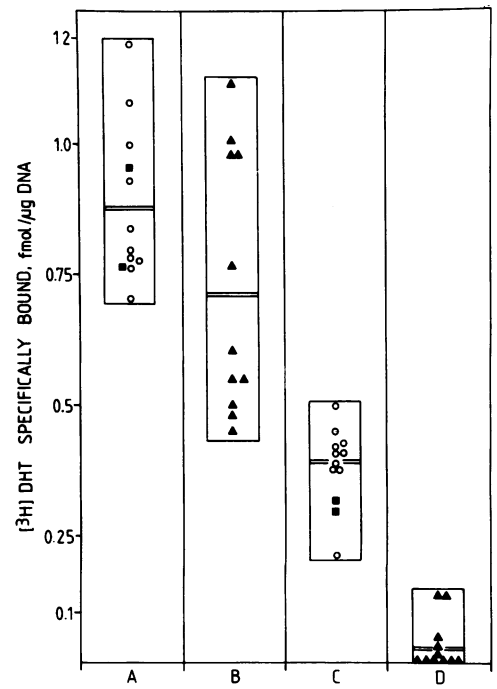

Figure 3. High-affinity binding of [^{3}H]DHT by intact fibroblasts (columns *A* and *B*) and fibroblasts nuclei (columns *C* and *D*) from the genital skin of 10 males with (○) normal genital development (controls A), two patients with (■) 5α-reductase deficiency (controls B), and 11 patients with (▲) severe hypospadias.

At variance with these results and our present findings are data presented by Eil et al. (21) who measured [^{3}H]DHT binding in both intact fibroblasts and isolated crude fibroblasts nuclei from patients with various genitourinary anomalies including hypospadias and normal controls and did not observe differences in either type of androgen binding.

The reason for these differences are unclear, but some might be different patient populations or different methodology. For example, Eil et al. incubated their cells at a lower temperature (22°C). In analogy to previous observations that some patients with androgen resistance syndromes have thermolabile fibroblast receptors which bind normally at 26°C, but have decreased binding at 37°C, it is thus conceivable that the higher incubation temperature used by Keenan et al. (16) and by us (17, 18) might have influenced the measured B_{max} values in intact cells. As for the different results obtained in isolated nuclei, it again appears conceivable that different patient populations and methodology might explain these discrepancies. For example, in contrast to Eil et al. (21), who used crude nuclear preparations, we have applied a more vigorous method to yield nuclei that are essentially free of endoplasmic reticulum (12).

Several other aspects of this study deserve comment. Most of the studies in patients with hypospadias have addressed only narrow etiological parameters. To provide a more comprehensive study, we obtained genetic, clinical, endocrine, and biochemical profiles of the patients. Aside from the described receptor abnormalities, we ascertained in some of the patients the following anomalies. First, the history of one patient revealed that a progestin-estrogen preparation was administered to the mother during the second and third month of gestation, a time that coincides with the critical period of genital develop-

ment. If Aarskog's hypothesis (22) that maternal ingestion of estrogen-gestagen preparation during the first trimester of pregnancy may cause hypospadias is correct, it is conceivable that this factor, in addition to the defective nuclear androgen uptake observed in this patient, had a negative synergistic effect on penile development; however, up to now, Aarskoog's hypothesis has not been substantiated.

Second, in one patient a subnormal testosterone response to hCG stimulation was observed. As it is well documented that any of at least five enzymatic errors that can cause defective androgen synthesis result in incomplete virilization of the male during embryogenesis, it is conceivable that the impaired testosterone secretion observed in this patient has also contributed to their genital malformation. Indeed, Knorr et al. (23) have demonstrated a subnormal testosterone response to hCG stimulation in 36 boys with hypospadias, whereas Walsh et al. (24) were unable to document such a defect in 11 prepuberal boys with hypospadias.

Third, in one patient with defective nuclear androgen uptake deficient activity of the enzyme 5α-reductase was found. In analogy with previous studies by Jukier et al. (25), it is conceivable that 5α-reductase deficiency in this patient was secondary to the androgen receptor defect determined in his genital fibroblasts.

In conclusion, we have examined clinical, endocrine, and biochemical parameters relevant to the etiology of hypospadias in patients with severe forms of this disorder. The most consistent finding in these studies was defective intracellular and/or nuclear binding in some of the patients. We interpret these data to suggest that defective intracellular and/or nuclear binding in some patients with severe hypospadias might be the cause of the defective genital development.

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