Defective Nuclear Accumulation of Androgen Receptors in Disorders of Sexual Differentiation

SUSANNE GYORKI and G. L. WARNE, *Endocrine Clinic, Royal Children's Hospital, Parkville 3052 Australia*

B. A. K. KHALID and J. W. FUNDER, *Medical Research Centre, Prince Henry's Hospital, Melbourne 3004, Australia*

ABSTRACT Nuclear transfer of androgen receptors (AR) and glucocorticoid receptors (GR) was determined in cultured genital skin fibroblasts from 10 normal controls and eight patients with abnormalities of the external genitalia. In whole cell studies, cultures were incubated for 20 min at 37°C with [^{3}H]methyltrienolone (^{3}H-R1881) or tritiated dexamethasone, and specific binding was determined in whole cell, cytoplasmic, and crude nuclear fractions. Between normal and affected fibroblasts no difference was seen in cellular levels of GR, or in cytoplasmic and nuclear distribution of GR. In normal fibroblasts, cytoplasmic binding of ^{3}H-R1881 represented 56%, and crude nuclear binding 44%, of total binding; in fibroblasts from five of the eight patients similar values (cytoplasmic 55% and nuclear 44%) were seen for ^{3}H-R1881 binding. In fibroblasts from the other three patients no decrease in total cellular levels of AR were seen; nuclear compartmentalization, however, was much lower (~20%) than in other cultures. In vitro reconstitution studies, combining ^{3}H-R1881-loaded cytosol with naive nuclei, lead us to suggest that the defect in nuclear compartmentalization lies at the level of the nuclear acceptor site rather than the cytoplasmic binder in affected cells. We interpret the data to suggest that defective nuclear binding of AR complexes may be involved in a proportion of cases of abnormal development of the external genitalia.

INTRODUCTION

The currently accepted model of steroid hormone action includes an initial step of binding of the hormone to specific cytoplasmic receptors, followed by chromatin binding of these activated steroid-receptor complexes, which in turn results in the DNA-directed, RNA-mediated synthesis of specific proteins mediating or constituting the target tissue response (1).

In testicular feminization, the best-documented clinical disorder of the androgen receptor (AR),[1] levels of receptor in cultured genital fibroblast cells are usually very low (~10% of normal) or below detection limits (2–5). More recently, however, a subgroup of patients with the clinical features of androgen insensitivity has been described, in which group cellular AR levels are normal (6, 7). Cells from such patients may show absent (6) or normal (7) levels of nuclear accumulation of AR.

The spectrum of clinical disorders in which androgen insensitivity is implicated is now known to be much broader than was previously appreciated. This spectrum includes patients with gynecomastia, infertility, hypospadias, micropenis, and pseudovagina (8, 9).

In this study, we established genital skin fibroblast cultures from 10 normal controls, and from eight patients with disorders of the external genitalia. In these cultures we determined total receptor levels, and the extent of cytoplasmic and nuclear localization of receptors, after incubation under defined conditions (20 min, 37°C) with near-saturating concentrations of androgen ([^{3}H]methyltrienolone [R1881]) or glucocorticoid ([^{3}H]dexamethasone [DM]).

METHODS

^{3}H-R1881, 87 Ci/mmol, and [^{3}H]DM, 19 Ci/mmol, were from New England Nuclear, Boston, MA. Nonradiolabeled

Address all correspondence to Dr. Funder.

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[1] *Abbreviations used in this paper:* AR, androgen receptor; DHEA, dehydroepiandrosterone; DHT, dihydrosterone; DM, dexamethasone; GR, glucocorticoid receptor; HCG, human chorionic gonadotropin; ^{3}H-R1881, [^{3}H]methyltrienolone; TEDG buffer, 10 mM Tris, 2 mM EDTA, 1.5 mM dithiothreitol, 10% (vol/vol) glycerol.

DM was the gift of Merck, Sharp & Dohme, Sydney, Australia, and dihydrotestosterone (DHT) was from Steraloids (Wilton, NH). Versene, Eagle's modified basal medium, and fetal calf serum were from Commonwealth Serum Laboratories, Parkville, Australia. Trypsin was purchased from Flow Laboratories, North Ryde, N. S. W., Australia; roller bottles, Bellco Glass Inc., Vineland, NJ; Hepes buffer, Calbiochem Australia, Sydney, N. S. W., Australia; and Tris, Sigma Chemical Co., St. Louis, MO. All other chemicals and solvents used were reagent grade.

Fibroblast strains in these studies were established from genital skin explants obtained from normal patients (6 mo–9 yr) and affected patients (6 mo–16 yr). Procedures for the maintenance and storage of these strains were as described by Griffin et al. (4). All fibroblasts were used between the 6th and 15th transfer.

For receptor studies, cells growing in stock flasks were dissociated in 0.02% versene–0.025% trypsin and transferred to roller bottles (285-mm diam) containing 100 ml Eagle's modified basal medium supplemented with 1 M Hepes buffer (2% vol/vol) and 10% fetal calf serum. Fibroblasts grew to confluence in ~7 d, at which time the medium was replaced with a medium containing charcoal-stripped fetal calf serum, to allow detection of total receptor levels. Cells were detached for binding studies with trypsin/versene, and placed in iced buffer (5 mM glucose, 6 mM K_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Tris-HCl, 133 mM NaCl, pH 7.3 at 4°C) at a concentration of 4–8 × 10^6 cells/ml.

Aliquots (0.5 ml) of cell suspensions were incubated with ^{3}H-R1881 (5 nM) or [^{3}H]DM (40 nM) in an atmosphere of 95% air/5% CO_2 at 37°C for 20 min, shown in preliminary studies to achieve plateau levels of binding. Nonspecific binding was determined by the addition of 100-fold DHT or DM to parallel sets of tubes. At the end of the period of incubation, cells were washed three times in iced buffer (5 mM Tris-HCl, 5 mM glucose, 133 mM NaCl, pH 7.3 at 4°C) and resuspended in 0.5 ml of the same buffer, thoroughly mixed, and an aliquot (0.2 ml) added to 0.8 ml of deionized water in 10 ml of aqueous scintillant (670 ml toluene, 33 ml Teric X-10, 5.5 g/liter PPO, 0.1 g/liter POPOP). Samples were vortexed, chilled, and counted in a Packard Liquid Scintillation Counter (model 3375, Packard Instrument Co., Inc., Downers Grove, IL) to determine total and nonspecific cellular binding of ^{3}H-R1881 and [^{3}H]DM.

The remaining cell suspension (0.3 ml) was centrifuged and the cells lysed by the addition of 0.7 ml hypotonic buffer (10 mM Tris-HCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, pH 8.0 at 4°C). The resultant 1-ml cell suspension was shaken at 37°C for two 20-min periods, with a 10-min interval between shakings, to lyse the cells; the nuclei (recovery ~85%) were pelleted by centrifugation at 1,500 *g* for 10 min, and 0.8 ml of the supernatant added to 0.2 ml to deionized water in 10 ml of aqueous scintillant. To the nuclear pellet (and the remaining 0.2 ml of supernatant) 1 ml of buffer was added, the pellet resuspended by vortexing, and a 1-ml aliquot taken into aqueous scintillant. The extent of binding in the crude nuclear preparation so obtained was estimated by subtracting radioactivity accounted for by the residual supernatant; binding in both cytoplasmic and crude nuclear fractions was expressed as a percentage of total cellular binding determined independently. A worked example of actual data with raw counts is shown in Table I (A and B). For this reason the sum of cytoplasmic and crude nuclear binding so expressed does not automatically equal total cellular binding; for ^{3}H-R1881 the sum was equal to 102±1.8% (mean±SEM, n = 18) of total binding, and for [^{3}H]DM the equivalent figure was 98±2% (mean±SEM, n = 18). In parallel studies, equivalent nuclear recovery and nuclear/cytoplasmic compartmentalization of AR were seen when cells were lysed by freeze-thawing in dry ice-ethanol.

For reconstitution studies, cells were suspended, an aliquot counted, and the remainder disrupted by dry ice-ethanol freeze-thawing in 1 ml TEDG buffer (10 mM Tris, 2 mM EDTA, 1.5 mM dithiothreitol, 10% vol/vol glycerol, pH 7.4 at 0°C). Nuclei were pelleted by centrifugation at 600 *g* for 10 minutes. The supernatant cytoplasmic fraction was diluted to 3 ml with TEDG buffer, and aliquots incubated with 5 nM ^{3}H-R1881 for 4 h at 4°C, in the presence and absence of 100-fold DHT. Residual free ^{3}H-R1881 was removed with charcoal, and the ^{3}H-R1881-receptor complexes activated by heating for 15 min at 25°C.The cytosol was cooled to 4°C, and 200-μl aliquots of naive nuclei (~2–4 × 10^6 nuclei/

TABLE I
Worked Example of Whole Cell Binding Study

	Whole cells	Cytosol	Nucleus + $\frac{\text{Cytosol}}{4}$	Nucleus
A NC-8 (control) cells, 1.5 × 10^6/500 μl				
Cell Equivalents	0.6 × 10^6	0.72 × 10^6	—	0.9 × 10^6
Total binding (*cpm*)	686,746	603,547	549,454	—
Nonspecific (*cpm*)	359,358	351,298	286,217	—
Specific (*cpm*)	338	251	250	187
Specific (*cpm/10^6 cells*)	563	348	—	208
Percentage	100	62	—	37
B XY-6a (low nuclear transfer), 2.37 × 10^6/500 μl				
Cell equivalents	0.94 × 10^6	1.14 × 10^6	—	1.48 × 10^6
Total binding (*cpm*)	797,868	1213,1004	646,584	—
Nonspecific (*cpm*)	534,443	713,822	394,446	—
Specific (*cpm*)	343	342	195	109
Specific (*cpm/10^6 cells*)	365	300	—	77
Percentage	100	82	—	18

ml) from control and affected cells added to 0.5 ml of cytosol and allowed to incubate for 1 h. A 200-μl aliquot was taken to determine residual cytosol binding, 500 μl of TEDG buffer added, the mixture vortexed and centrifuged, and 800 μl of supernatant discarded. The residual nuclear pellet, plus the equivalent of 100 μl cytosol, was counted for calculation of nuclear uptake. A worked example of actual data with raw counts is shown as Table II. In both whole cell and reconstitution studies, values quoted are for specific binding, and are derived from duplicate determinations of both total and nonspecific binding.

Brief clinical details of each of the eight affected patients studied are in Results, as parts of Tables IV and V. Of the eight patients, six were seen at the Royal Children's Hospital,

TABLE II
Worked Example of Reconstitution Study

	Cytosol	Nuclei + $\frac{\text{Cytosol}}{2}$	Nuclei
A NC-4 (control) cytosol + NC-4 nuclei			
Cell Equivalents	2.4×10^6	—	1.24×10^6
Measured binding			
Total (*cpm*)	689,779	893,842	—
Nonspecific (*cpm*)	395,325	455,312	—
Specific (*cpm*)	374	483	—
Calculated specific (*cpm*)	1,309	—	296
Calculated specific (*fmol*)	22.5	—	5.10
Calculated specific (*fmol/10^6 cells*)	9.4	—	4.1
Percentage	69	—	31
B XY-6a (affected) cytosol + XY-6a nuclei			
Cell equivalents	1.1×10^6	—	0.56×10^6
Measured binding			
Total (*cpm*)	895,815	432,442	—
Nonspecific (*cpm*)	508,603	304,212	—
Specific (*cpm*)	299	179	—
Calculated specific (*cpm*)	1047	—	29
Calculated specific (*fmol*)	18	—	0.5
Calculated specific (*fmol/10^6 cells*)	16.4	—	0.9
Percentage	95	—	5
C NC-4 cytosol + XY-6a nuclei			
Cell equivalents	1.8×10^6	—	1.36×10^6
Measured binding			
Total (*cpm*)	661,602	604,518	—
Nonspecific (*cpm*)	250,276	329,346	—
Specific (*cpm*)	368	223	—
Calculated specific (*cpm*)	1,288	—	39
Calculated specific (*fmol*)	22.2	—	0.7
Calculated specific (*fmol/10^6 cells*)	12.3	—	0.5
Percentage	92	—	8
D XY-6a cytosol and NC-4 nuclei			
Cell equivalents	1.3×10^6	—	1.04×10^6
Measured binding			
Total (*cpm*)	765,647	715,991	—
Nonspecific (*cpm*)	455,517	303,339	—
Specific (*cpm*)	220	535	—
Calculated specific (*cpm*)	770	—	425
Calculated specific (*fmol*)	13.3	—	7.3
Calculated specific (*fmol/10^6 cells*)	10.2	—	7.0
Percentage	59	—	41

Melbourne; skin biopsies from the other patients were provided by Dr. Martin Silink, Sydney (HS-36), and Dr. R. J. Maneshka, Bombay, India (Ep-1a).

In the patients found to have abnormal nuclear compartmentalization of androgen receptors, details of clinical and laboratory investigations were as follows. Patient XY-6a was 15 mo old and temporarily in Australia at the time of the study, having been delivered in Canada by Cesarian section for fetal distress at 41 wk gestation after an uneventful pregnancy. At birth, perineoscrotal hypospadias and a very small phallus were noted; testes (1 ml) were palpable at the level of the pubic tubercle; endoscopy showed no vagina. Laboratory investigations showed a 46 XY karyotype; on human chorionic gonadotropin (HCG) stimulation test (5,000 U of HCG, single dose) serum testosterone rose slightly from 1.6 nM^{-1} to the low post stimulation level of 2.5 nM at 72 h, with no change in serum levels of dehydroepiandrosterone (DHEA), DHEA sulfate, or androstenedione. On the basis of such a response post-HCG, abnormalities in androgen biosynthesis cannot be excluded in this patient. Subsequent attempts to locate the patient for a repeat HCG test have been unsuccessful. Treatment with 25 mg testosterone enanthate i.m. was given on two occasions and produced an increase (from ~1 to 2 cm) in penile length over 2 mo.

Patient HS-36, aged 36 mo at time of referral, was born in Vietnam, also with perineal hypospadias and micropenis; in addition, the scrotum extended above the level of the phallus. On examination, testes of volume 1.5 ml could be felt; the phallus itself measured 2 × 1.2 cm. Investigations were carried out at the Royal Alexandra Hospital for Children, Sydney. Urethroscopy showed a prostatic utricle (1–1.5 cm long) with no cervix or uterus. The child's karyotype was 46 XY. The level of plasma androgens (testosterone plus DHT) showed a brisk response (0.3–4.6 nM) to 3,000 U HCG. Serum DHT post-HCG was 1.7 nM (normal < 2 nM), suggesting no impairment of 5α-reductase activity. Testosterone enanthate (25 mg every 3 wk) was given four times, and resulted in an enlargement of the phallus to 3 × 1.4 cm.

The penile skin biopsy from patient HS-35b was obtained at the time of hypospadias repair. The only abnormality noted was a coronal hypospadias; the phallus was measured and was normal in size (5 × 1.8 cm), as were the testes. The patient had no previous investigations, and was lost to follow-up for 2 yr. Recently the patient was found, and a blood sample taken 72 h after 5,000 U HCG showed a testosterone level (10.9 nM) in the normal adult range.

RESULTS

Total cellular AR and glucocorticoid receptor (GR) levels, and the distribution between cytoplasmic and nuclear compartments are shown in Table III. For normal foreskin fibroblasts in culture, the concentration of AR was 9.8±1.0 fmol per 10^6 cells (41 + 4 fmol/mg protein; mean±SEM, n = 10), and of GR 8.4±1.3 pmol per 10^6 cells (n = 10). As shown in Table IV, comparable total cellular receptor levels were found in five of eight affected patients examined (AR, 8.6±1.0, range 5.8–9.2; GR, 7.2±1.7, range 5.1–11.4). The extent of nuclear transfer of [^{3}H]DM and [^{3}H]DHT was similarly indistinguishable between these five patients and normal controls. Similar concentrations of total, cytoplasmic and crude nuclear androgen receptors were found in cells from a further 21 patients with hypospadias, in whom [^{3}H]DM studies were not performed. In controls, 52±2.8% of bound [^{3}H]DM and 56±2.6% of bound ^{3}H-R1881 was found in the cytoplasm, with 48±2.8 and 44±2.3% in the nuclear compartment; in the five patients, corresponding values for [^{3}H]DM were 49±3.1 and 51±3.6%, and for ^{3}H-R1881, 55±5.4 and 44±4.5%.

In three of the eight patients, however, a clear discrepancy was seen between [^{3}H]DM and ^{3}H-R1881 in terms of nuclear compartmentalization (Table V). These three patients had normal total cellular levels of bound [^{3}H]DM, and normal nuclear transfer of [^{3}H]DM; in contrast, they showed consistently lower

TABLE III

Androgen Receptor Levels, and Cytoplasmic/Nuclear Compartmentalization of Androgen Receptors and Glucocorticoid Receptors, in Fibroblasts from 10 Normal Control Patients

Cell line	Age	Clinical diagnosis	Passage number	Androgen binding	Nuclear transfer			
					R1881		DM	
					Cyt	Nuc	Cyt	Nuc
	yr			*fmol/10⁶ cells*	*%*			
NC-26	4	Circumcision	12	6.3	60	42	38	59
NC-8	9	Circumcision	13	9.7	62	37	40	60
NC-4	6/12	Circumcision	12	11.1	60	40	68	30
NC-3	4	Circumcision	12	8.6	56	38	36	65
NC-16	6/12	Circumcision	8	8.9	60	34	53	50
NC-20	10	Circumcision	8	9.3	59	40	49	54
NC-17	3	Circumcision	12	14.3	63	40	48	50
NC-15	11/12	Circumcision	12	10.1	50	50	49	45
NC-6	7	Circumcision	8	11.5	59	43	66	40
NC-22	9/12	Circumcision	15	10.5	42	60	50	50

TABLE IV

Androgen Receptor Levels, and Cytoplasmic/Nuclear Compartmentalization of Androgen Receptors and Glucocorticoid Receptors, in Fibroblasts from Five Patients with Incomplete Androgenization

Cell line	Age	Clinical diagnosis	Passage number	Karyotype	Androgen binding	Nuclear transfer			
						R1881		DM	
						Cyt	Nuc	Cyt	Nuc
	yr				*fmol/10⁶ cells*			%	
EP-1/a	3	Epispadias	6	XY	6.3	40	54	68	40
HS-40/a	3	Penile hypospadias	9	ND	9.2	66	36	38	60
MG-8	16	Micropenis, 3 ml testes, Short stature	10	XY	6.9	67	30	48	55
XY-2	3/12	Clitoral enlargement and partial fusion of labioscrotal folds	13	XO/XY	5.8	63	40	43	58
XY-5	4	20,22 desmolase deficiency: normal female phenotype	13	XY	8.2	60	40	57	40

ND, not determined.

percentage levels of nuclear transfer of ^{3}H-R1881 than the 15 cell lines detailed in Tables III and IV. Patient 2 (HS-36) is perhaps worthy of special attention, in that total cellular levels of androgen receptor (11.9 fmol/10^6 cells) were the second-highest recorded in all 18 cell lines detailed; despite this fact, the percentage transfer of ^{3}H-R1881 in this patient was consistently below the normal range.

The results of a limited series of in vitro reconstitution experiments are shown in Table VI. Cytosol from normal fibroblasts (NC26, NC4, NC17) and from the three patients with low percentage nuclear com-

TABLE V

Androgen Receptor Levels, and Cytoplasmic/Nuclear Compartmentalization of Androgen Receptors and Glucocorticoid Receptors, in Fibroblasts from Three Patients with Incomplete Androgenization

Cell line	Age	Clinical diagnosis	Passage number	Karyotype	Androgen binding	Nuclear transfer			
						R1881		DM	
						Cyt	Nuc	Cyt	Nuc
	yr				*fmol/10⁶ cells*			%	
HS-35	3	Simple penile hypospadias	8	ND	6.92	85	19	51	48
			8		9.8	78	20	60	36
			9		7.5	76	22	55	45
					—	74	23	—	—
XY-6/A	3/12	Perineoscrotal hypospadias, micropenis with chordee, no vagina	10	XY	6.3	82	21	53	50
			10		7.1	82	20	60	36
			9		6.0	78	21	55	42
					—	88	10	—	—
					—	76	20	—	—
HS-36	3	Large prostatic utricle, micropenis, perineal hypospadias, shawl scrotum	9	XY	8.4	90	12	60	38
			8		11.9	88	15	55	40
					—	80	22	—	—
					—	80	18	—	—

TABLE VI

Reconstitution Studies ([^{3}H]Methyltrienolone-loaded Cytosol, Naive Nuclei) in Which Cytosol and Nuclei from Affected and Normal Control (NC) Fibroblasts Were Mixed

	CYT	NUC
NC-26 Cyt + NC-26 nuclei	66	34
HS-35 Cyt + HS-35 nuclei	85	15
NC-26 Cyt + HS-35 nuclei	89	11
HS-35 Cyt + NC-26 nuclei	55	45
NC-4 Cyt + NC-4 nuclei	69	31
XY-6/a Cyt + XY-6/a nuclei	95	5
NC-4 Cyt + XY-6/a nuclei	92	8
XY-6/a Cyt + NC-4 nuclei	59	41
NC-17 Cyt + NC-17 nuclei	59	41
HS-36 Cyt + HS-36 nuclei	91	9
NC-17 Cyt + HS-36 nuclei	98	2
HS-36 Cyt + NC-17 nuclei	65	35

Figures shown represent percentages of specific binding in each compartment, determined as previously shown in Table II.

partmentalization was found to be equivalent as a source of AR, in that nuclei from normal, steroid unexposed cells bound equivalent levels. In contrast, however, nuclei from all three patients bound much lower levels of cytoplasmic AR, whether the source was their own or normal cytoplasm.

DISCUSSION

The findings described in this paper appear worthy of discussion under several headings. First, the disorder at a subcellular level appears not to be one of an abnormal receptor, but of a lowered number of nuclear binding sites for androgen receptors. In contrast, the primary defect in most patients with testicular feminization appears to reside at the level of the cytoplasmic androgen receptor, in that minimal or absent androgen binding is found.

In a minority of cases of incomplete male pseudohermaphroditism, normal androgen receptor levels with normal (7) or impaired (6) nuclear transfer have been reported. The impairment of nuclear transfer in the latter instance has been suggested to be a reflection of a receptor abnormality, rather than abnormal nuclear acceptor sites. Similarly, of the many S-49 lymphoma cell line mutants resistant to DM, and with decreased (or increased) nuclear localization, the defect has always appeared to be present in the receptor and not in the nuclear acceptor sites (10). One interpretation of this finding is that the selection system used for such mutants (growth in 100 nM DM) imposes conditions under which only certain classes of steroid-unresponsive variants survive.

Consistent with the clinical presentation of incomplete androgenization, rather than complete androgen insensitivity, cells from the patients described in this study showed consistently and significantly lower, but not absent, nuclear binding of AR complexes. Under the conditions of assay, cytoplasmic/nuclear compartmentalization of AR in normal cells is at equilibrium after 20 min incubation. There are two possible interpretations of this ~60% cytoplasmic/40% nuclear localization of androgen receptors, at near-saturating ^{3}H-R1881 concentrations. The first is that the affinity with which androgen-receptor complexes are bound to acceptor sites is relatively weak, so that the bulk of the complexes remain unbound in the cytoplasm. The second possibility is that the nuclear acceptor sites have a high affinity, but limited capacity, for androgen-receptor complexes, and that the nuclear acceptor sites are all essentially occupied at this concentration of ^{3}H-R1881 under the incubation conditions used.

The interpretation of an altered ratio of cytoplasmic to nuclear localization is dependent on whether one or both of the above mechanisms is limiting. If the affinity with which nuclear acceptor sites bind AR complexes is modest, then an overall fall in the affinity of the whole population of nuclear acceptor sites would be reflected in an overall fall in nuclear localization, as was seen in these three patients. Conversely, if the affinity of the acceptor site-receptor complex interaction is high, and the capacity of the nuclear acceptor system limiting, a similar fall in overall acceptor affinity would minimally affect compartmentalization; in contrast, a fall in the number of nuclear acceptor sites would be reflected very closely by lowered nuclear accumulation, as was seen in these studies.

On the basis of the evidence available from the present studies it is not possible to select between these possibilities. While intuitively the latter may sound more plausible, and is strongly supported by the comparable nuclear accumulation by normal nuclei in whole cell and in reconstitution studies, the two possibilities outlined above are in fact nonexclusive. In normal cells, acceptor site capacity may be limiting; in affected cells a substantial reduction in affinity of all acceptor sites for AR complexes may occur, so that both the altered affinity and an unchanged, limited capacity become determinants of nuclear localization. Two of the three patients with defective nuclear transfer were given testosterone, and both responded positively in terms of penile growth. Though in neither case was the response particularly marked, we interpret this response to indicate that high ambient testosterone concentrations can be followed by increased expression of androgen-inducible genes in these patients.

Whatever the molecular mechanism of the impaired nuclear binding of AR complexes, it does not appear

to affect nuclear binding of GR complexes, which was equivalent in all cells tested. Secondly, though glucocorticoid transfer studies were performed on only eight patients with genital abnormalities, normal AR levels and compartmentalization were found in a further 21. The incidence of defective nuclear acceptor sites, as a presumptive cause of incomplete androgenization, is therefore more likely to be of the order of 10% than the ~40% that might otherwise be assumed.

Currently, studies are directed at establishing the incidence of defective nuclear transfer of AR complexes in a range of disorders of partial androgen insensitivity, and to determine the molecular mechanisms involved in such defective nuclear binding.

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