DHT-receptor in cultured human fibroblasts: binding study in a family with androgen insensitivity (complete testicular feminisation)

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SUMMARY ^3H -DHT binding was examined in cultured skin fibroblasts from a patient with complete testicular feminisation (CTF), from his heterozygote mother, and his clinically normal sister, who menstruated normally. Binding parameters were: $B_{\text{max}} < 1$ fmol/mg protein and K_D unmeasurable in CTF; $B_{\text{max}} = 24$ fmol/mg protein and $K_D = 3.63 \times 10^{-9}$ mol/l in the mother; and $B_{\text{max}} = 46$ fmol/mg protein and $K_D = 3.7 \times 10^{-9}$ mol/l in the sister. Five cultures obtained from genital and non-genital skin of normal male and female subjects were used as controls, in which B_{max} ranged from 37 to 62 fmol/mg and K_D from 2.0 to 3.6×10^{-9} mol/l.

The considerable reduction of B_{max} in the obligate heterozygote and the normal binding capacity in the sister, a probable heterozygote, suggests that it may be possible to use the DHT-receptor assay to identify carriers in families with androgen resistance.

Dihydrotestosterone, the 5α reduced form of testosterone, interacts with specific cytosol receptors in cultured human fibroblasts from normal male and female subjects.^{1,2}

The DHT-receptor protein is absent in most patients with CTF.^{1,3-5} It is believed that the synthesis of this protein is controlled by an X linked gene and it seems that such a gene locus is subject to inactivation, like other X linked loci.⁶ It remains to be clarified whether the lyonisation influences the phenotypic expression of the gene to a certain extent so that there is no discrimination between normal subjects and carriers. To this end we compared the binding capacity of cultured skin fibroblasts from an 18-year-old possible heterozygote (fig 1, IV.1), the sister of a CTF patient, with that of the 22-year-old hemizygote (IV.2), the 46-year-old obligate heterozygote mother (III.3), and a group of normal control subjects all at reproductive age.

In this way the presence of a distinct difference in B_{max} between the two female subjects of the same family examined by us was shown.

Materials and methods

Radioactive labelled steroid $1,2(n)^3\text{H}$ - 5α -dihydrotestosterone (specific activity 66 Ci/mmol) was ob-

tained from Amersham (England). Unlabelled steroid (5α -dihydrotestosterone), purchased from Sigma (USA), was recrystallised from the organic solvent before the assay. The grades of the reagents, tissue culture media, and other chemicals used were the best available from commercial sources.

FIG 1 Pedigree of family with androgen insensitivity.

CELL CULTURES

The origins of the cell cultures examined are shown in the table. Cell cultures from the CTF patient (C106, C107) were obtained from abdominal skin and testicular tissue, taken during gonadectomy. The cell cultures from his sister (C113) and mother (C114) were obtained from the skin of the forearm. The control cultures were derived as follows: C120 from the abdominal skin of a normal female; C116, C118, C119 from the skin of the forearm of three normal males; C112 from scrotal skin from one normal male.

Fibroblast cultures were propagated in Eagle's MEM supplemented with 20% fetal calf serum (FCS), vitamins, L-glutamine, and non-essential amino-acids in closed glass flasks, each having a surface area about 44 sq cm, at 37°C. All the assays were performed between the 6th and 13th subcultures.

DHT-RECEPTOR ASSAY

DHT-receptor assay was performed according to Kaufman *et al.*⁷ Confluent monolayers of fibroblasts were washed with fresh Eagle's MEM and reincubated with FCS-free medium for 24 hours. Then the MEM was removed and the monolayers were washed twice with 2 ml of FCS-free medium. The monolayers were incubated in duplicate with various concentrations (1 to 10 nmol/l) of ³H-DHT in FCS-free MEM, with or without addition of 0.5 µmol/l unlabelled DHT, for 45 minutes at 37°C.

The medium was removed and the molarity of labelled DHT was controlled by measuring the radioactivity of an aliquot. The cells were washed twice with MEM, harvested by 0.25% trypsin, and washed twice in 5 ml ice-cold buffer (0.02 mol/l Tris-HCl, pH 7.5, 0.32 mol/l sucrose, 1 g/l bovine gammaglobulin) by centrifugation at 200 g for 10

minutes at 4°C. All subsequent steps were performed at 4°C. The pellets were lysed in 0.5 ml 0.02 mol/l Tris-HCl, pH 7.5, 0.4 mol/l KCl, 1.5 mmol/l EDTA, and 2 mmol/l mercaptoethanol by an ultrasonic cleaner. After centrifugation for 20 minutes at 2000 g, the supernatants were removed and unbound steroid adsorbed with charcoal-dextran (pellets were previously prepared by centrifugation at 2000 g for 15 minutes of a mixture of 1% charcoal and 0.1% dextran T-70 in Tris-KCl, pH 7.5, buffer).

FIG 2 DHT-receptor kinetics and Scatchard plot analysis of cultures obtained from normal subjects. Total binding is dpm/mg protein bound in the presence of ³H-DHT alone; non-specific binding is dpm/mg protein in the presence of ³H-DHT and a 1000-fold excess of unlabelled DHT. The abscissa of Scatchard plots represents ³H-DHT specifically bound, the ordinate moles bound divided by concentration of free hormone. K_D is the reciprocal of the slope of the Scatchard plot.

TABLE Binding parameters of DHT-receptor and origin of cell cultures

Cell culture	Origin	K _D (×10 ⁻⁹ mol/l)	B _{max} (fmol/mg protein)
C106	Abdominal skin of CTF patient	Unmeasurable	<1
C107	Gonadal tissue of CTF patient	Unmeasurable	<1
C113	Forearm skin of sister	3.7	46
C114	Forearm skin of mother	3.6	24
C116	Forearm skin of normal male	3.6	44
C118	Forearm skin of normal male	2.2	46
C119	Forearm skin of normal male	3.2	45
C120	Abdominal skin of normal female	2.7	37
C112	Genital skin of normal male	2.0	62

DHT-receptor in cultured human fibroblasts

The cytosol-charcoal mixture was vortexed, incubated for 15 minutes, and centrifuged at 2000 *g* for 20 minutes. Portions of the supernatant were removed for protein determination⁸ and for radioactivity counting. For this purpose 150 μ l aliquots in 5 ml of Insta-gel (Packard, USA) were counted in a Tricarb (Packard, USA) scintillation spectrometer. Counting efficiency ranged from 33 to 38%, ³H-toluol serving as internal standard. The amount of specifically bound ³H-DHT was determined as the difference between the radioactivity bound in cell cultures containing a great excess of unlabelled DHT

and parallel cultures containing ³H-DHT alone. The binding capacity ($B_{\max}$) and apparent dissociation constant (K_D) were determined according to Scatchard.⁹

Results

The dissociation constants (K_D) of the hormone receptor complex and the values for the maximal binding capacities ($B_{\max}$) in the cell cultures examined are listed in the table.

The cultures derived from forearm and abdominal skin of normal subjects showed a low capacity, high affinity ³H-DHT binding, as shown in the binding curves and Scatchard's plot analysis (fig 2). The $B_{\max}$ and K_D values were similar in the cultures derived from non-genital skin, ranging respectively from 37.1 to 46.0 fmol/mg protein and from 2.2 to 3.6×10^{-9} mol/l. The non-specific binding showed no significant variation between cultures, correlating linearly with the concentration of ³H-DHT in the medium. The culture derived from genital (scrotal) skin showed a greater binding capacity (62 fmol/mg

FIG 3 Binding curves of cultured fibroblasts derived from inguinal skin (C107) and gonadal tissue (C106) of the proband compared to binding curves (mean $\pm$ SD) of normal subjects (shaded area). The absence of specifically bound ³H-DHT does not permit Scatchard's analysis.

FIG 4 Binding curve and Scatchard's analysis of cultures derived from sister's skin.

FIG 5 Binding curve and Scatchard's analysis of cultures derived from mother's skin.

protein) compared with those derived from abdominal or forearm skin (fig 2).

No appreciable DHT binding was detectable in skin or gonadal fibroblasts from the androgen insensitive patient (fig 3). The binding parameters of culture C113, derived from the patient's sister, were within the normal range (fig 4). The fibroblasts obtained from the mother's skin (C114) showed a significant reduction of binding capacity (24.8 vs 43 fmol/mg, mean $\pm$ 7.33 SD of controls), even though the affinity of the ^3H -DHT receptor complex was normal (fig 5).

Discussion

Complete testicular feminisation is a genetic disorder caused by mutation of an X linked gene.^{6 10} The corresponding phenotype is characterised by the absence of androgen receptors, leading to feminine differentiation of androgen target organs. The lack of cytosol receptor protein, responsible for the transport of DHT into the nucleus, can be shown in cultured skin fibroblasts derived from CTF patients.^{1 11 12}

The cultures derived from normal subjects showed a low capacity, high affinity ^3H -DHT binding.

Data obtained from different laboratories show considerable variation in B_{max} values in normal subjects.^{3 5 7 13} In our controls, however, the binding capacity ranged from 37 to 47 fmol/mg protein in cultures derived from non-genital skin and reached 62 fmol/mg in a culture derived from scrotal skin. Whether the reduced dispersion of the B_{max} values in our controls can be attributed to the small number of subjects examined or to the high viability of cell cultures in which the DHT-receptor assay was performed remains to be ascertained.

DHT was undetectable in cultures derived from the skin and testicular tissue of the patient with the complete form of androgen insensitivity and this is, therefore, classifiable within complete receptor negative testicular feminisation.⁵

The B_{max} of cultures obtained from the mother's skin was reduced in comparison with the controls (24 vs 43), even though DHT-receptor affinity was normal ($K_D = 3.6 \times 10^{-9}$). The reduced binding capacity of the maternal fibroblasts is in agreement with the hypothesis of inactivation of the X linked locus controlling DHT-receptor synthesis in obligate heterozygotes.⁶ In the phenotypically normal sister, binding and affinity values were within the normal range. This difference could be explained by the fact that the sister was not a carrier; this hypothesis should, however, be considered with caution. Randomised inactivation of X linked genes, according to Lyon's hypothesis,¹⁴ makes it difficult to interpret data in X linked diseases.

At present, a definite conclusion cannot be drawn from our observations. However, we believe that further studies of DHT binding parameters in the female members of receptor negative, androgen insensitive families could be useful for the identification of the heterozygote.

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