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Analysis of the Androgen Receptor in Isolated Testicular Cell Types with a Microassay that Uses an Affinity Ligand*

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ABSTRACT. A microassay for the androgen receptor was developed to investigate the cellular distribution of receptor in freshly isolated testicular cell types. The microassay uses an androgen affinity ligand, 17β-dihydrotestosterone bromoacetate. Binding of this ligand by the androgen receptor is rapid and irreversible, which permits the development of a highly sensitive assay. The androgen receptor microassay is completed within 4 h and detects receptor in as little as 0.5 μg cellular protein. There was no detectable binding of the affinity label by albumin or Sertoli cell-secreted proteins, including androgen-binding protein. Androgen receptor was found in cellular sonicates of human foreskin fibroblast, rat ventral prostate, rat kidney, and rat liver. Although the relative distribution of receptor was similar to that obtained using a traditional equilibrium binding assay, the levels of receptor were significantly higher using the microassay. The androgen receptor microassay was subsequently used to investigate the receptor in isolated testicular cell types. Androgen receptor was detected in freshly isolated peritubular myoid cells (80 fmol/μg DNA), Sertoli cells (88 fmol/μg DNA), and Leydig cells (35 fmol/μg DNA). No androgen receptor was detected in a mixed population of germ cells. Hormones were not found to influence androgen receptor levels in cultured peritubular cells or Sertoli cells. Electrophoretic analysis of androgen receptor radiolabeled with the affinity ligand demonstrates a single 52-kDa form of the receptor in peritubular cells, Sertoli cells, and Leydig cells. The size of the androgen receptor species detected in the rat testicular cell types was slightly smaller than the 56-kDa protein detected in a human fibroblast cell line. The current study demonstrates the utility of the microassay and affinity ligand to investigate androgen receptor biology. Data indicate that androgen receptors are present in several testicular cell types and suggest that the control of testicular function by androgens probably involves actions on multiple cell types. (*Endocrinology* **125:** 2628–2635,1989)

ANALYSIS of the cellular distribution of a specific receptor is an important initial requirement in understanding the mode of hormone action. The testis is the principal site of androgen production as well as a critical site of androgen action. Potential cellular sites of androgen action in the testis include Leydig cells (1, 2), peritubular (myoid) cells (1, 3), Sertoli cells (1, 4, 5), and germinal cells (1, 6). Many studies to localize the androgen receptor have used cultured cells, which may not reflect the *in vivo* expression of the receptor protein. The sensitivity of conventional assays and the limited amounts of specific cell types available have hindered a detailed analysis of the distribution of the androgen receptor in freshly isolated cell types.

Traditional biochemical assays for the androgen receptor generally rely on reversible steroid binding by the receptor. This type of assay requires the establishment of equilibrium binding conditions that are time consuming and often tedious in the separation of bound from free steroid (7). Factors that disrupt the binding activity decrease the sensitivity of the assay. Receptor protein instability and low receptor concentrations are also problems with the assay. An alternative method for detecting the androgen receptor is the use of an assay based on an affinity ligand which binds irreversibly to the receptor.

17β-Dihydrotestosterone bromacetate (DHT-BA) is an affinity probe for the androgen receptor that is derived from the natural androgen receptor ligand dihydrotestosterone (DHT) (8). Previous studies indicate that DHT-BA appears to bind with high affinity and specificity to the human, bovine, and rat androgen receptor (9–12). Several biochemical properties of the protein that binds DHT-BA are identical to those of the androgen receptor observed with the reversible binding assay (12). Therefore, the androgen receptor affinity ligand DHT-

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BA was considered useful for the development of a convenient assay for the androgen receptor.

The current study describes the development of a microassay for the androgen receptor which was used to quantitate androgen receptor levels in freshly isolated testicular cell types. In addition, the affinity ligand was used to determine the mol wt of the receptor species labeled in each cell type. This study provides a technical advance in the analysis of androgen receptor biology, as well as develops a better understanding of androgen actions in the testis.

Materials and Methods

Cell isolation and culture

Human genital skin fibroblast cell-lines established from explants and shown to contain the androgen receptor were cultured as previously described (13). Cells were grown in medium with 10% fetal calf serum; 24 h before cell harvest the medium was aspirated, the cells were washed with PBS, and serum-free Minimum Essential Medium with 500 ng/ml BSA was added to the plates.

Sertoli cells were isolated from the testes of 20-day old rats by sequential enzymatic digestion (13), using a procedure that has been described previously (14). The cells were plated at approximately 5×10^5 cells/well and maintained at 32 C in a 5% CO_2 atmosphere in Ham's F-12 medium with medium changes every 48 h. In some experiments the cells were cultured with one or more regulatory agents (FSH, Pituitary Agency, Baltimore, MD; 100 ng/ml; insulin, 5 μg/ml; retinol, 0.35 μM; testosterone, 1 μM). Treatments were initiated at the time of plating and maintained throughout the culture period. Sertoli cells were shown to be 98–99% pure by morphological and biochemical criteria (15).

Peritubular cells were obtained from the collagenase digestion supernatant of the testicular cell preparation after tubule fragments had sedimented, as previously described (16). The cells were collected by low speed centrifugation, resuspended, and plated at approximately $0.5–1.0 \times 10^5$ cells/2 cm^2 and were maintained at 32 C in a 5% CO_2 atmosphere in Ham's F-12 medium with medium changes every 48 h. Peritubular cells were plated and maintained in 10% newborn calf serum (Hazelton, PA) that had been charcoal treated to remove steroids. In some experiments cells were cultured with testosterone (1 μM). Treatments were initiated at the time of plating and maintained throughout the culture period. Peritubular cells were shown to be greater than 95% pure by morphological appearance and judged to be essentially free of Sertoli cell contamination by both histochemical and biochemical criteria (15).

Leydig cells were partially purified by a modified procedure previously described (17). Cells obtained from the supernatants of the trypsin digest washes of testes from 20-day-old rats were separated by Percoll gradient (10–90%) centrifugation. Leydig cells were obtained between a 1.08–1.09 g/ml density in the gradient. Cells obtained by this method were reported to be greater than 95% pure (17).

Germ cells were prepared by a modified procedure previously described (18). Cells obtained from the collagenase washes of the Sertoli cell preparation were separated by Percoll gradient (20–45%) centrifugation. Germ cells were recovered from the Percoll gradient between a density of 1.04–1.06 g/ml. The germ cells obtained by this method were reported to be at least 95% pure (18).

Isolated cells were either immediately sonicated and assayed for androgen receptor content or plated for culture. When designated, cells were frozen (−70 C) and assayed at a later date. Tissue (ventral prostate, kidney, and liver) was obtained from adult intact male Sprague-Dawley rats, placed on ice, cleaned, blotted, and weighed. Minced tissue was homogenized in buffer A (20 mM Tris, pH 7.4, with 1 mM EDTA, 12 mM monothioglycerol, and 10 mM Na_2MoO_4) at 1 g tissue/ml buffer and sonicated before assay. In some experiments a cytosol preparation was obtained by centrifugation of the cellular sonicates at $100,000 \times g$ for 1 h at 40 C. Unless otherwise designated, all chemicals used in these experiments were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of the affinity ligand

The nonradioactive DHT-BA was prepared by a method previously described (9, 11). DHT and bromacetic acid were dissolved in tetrahydrofuran, and the sample was chilled to −20 C. Dicyclohexyl-carbodiimide and pyridine were added, and the sample was incubated on ice for 4.5 h. The reaction mixture was then filtered through glass wool to remove precipitated dicyclohexylurea, and the dissolved steroid was taken to dryness under a nitrogen stream. The residue was redissolved in chloroform and chromatographed on a silica gel column. The eluted peak of DHT-BA was again dried under a nitrogen stream. The structure of the purified product was confirmed with nuclear magnetic resonance spectroscopy, as previously described (11). Radioactive DHT-BA was synthesized by the method of Chang *et al.* (10). Bromacetyl bromide was reacted with [1,2,4,5,6,7-^{3}H]DHT 140 Ci/mmol (Amersham, Arlighton Heights, IL) in the presence of diisopropylethylamine at room temperature. After 4.5 h the solvent was evaporated, and fresh reactants were again added. After 18 h the solvent was evaporated, and the residue was dissolved in hexane-ether (6:4) and purified by chromatography on a silica gel column. Eluted radiolabel was further purified as necessary on TLC plates developed in chloroform-acetone (9:1). The resulting compound was greater than 95% pure, as determined by TLC with purified nonradioactive DHT-BA as an internal marker.

Equilibrium binding assay for quantitation of the androgen receptor

The equilibrium binding assay used was similar to a method previously reported (19). Aliquots (100 μl) of cytosol were incubated for 12–14 h at 0-C with 3 nM [^{3}H]mibolerone (Amersham). To estimate the nonspecific binding, parallel incubations were performed which included radioactive ligand in the absence and presence of nonradioactive mibolerone (1.5 nM). At the end of the incubation period hydroxylapatite [500 μl of a 50% (wt/vol) slurry in buffer A] was added to each tube. The tubes were incubated at O C for 30 min and stirred every 10 min. The hydroxylapatite was pelleted by centrifugation at $800 \times g$ for 3 min, and the supernatant was discarded. The pellet was washed three times with 2 ml buffer A containing 0.3 M

NaCl and 0.5% (vol/vol) Triton X-100. The washed pellet was extracted with 1 ml absolute ethanol for 30 min, and the radioactivity in 500 μl of that extract was quantitated by liquid scintillation counting in 10 ml ACS (Amersham).

Microassay for the quantitation of the androgen receptor

Cultured cells were washed four times with ice-cold Tris-saline (50 mM Tris, pH 7.5, and 0.15 mM NaCl) and sonicated in the plate. The cells were sonicated in a homogenizing buffer which contained 20 mM Tris (pH 8.5), 1 mM EDTA, 10 mM Na_2MoO_4, 0.5 M NaCl, 50 nM leupeptin, 100 mM benzamidine, and 50 mM phenylmethylsulfonylfluoride. A portion of the cell sonicate was preincubated with nonradioactive DHT-BA (2 μM) for 30 min on ice to determine nonspecific binding. Aliquots (100 μl) of the cellular sonicates (either with or without nonradioactive DHT-BA) were then incubated with buffer containing the radioactive ligand for 10 min (150-μl aliquot prepared in homogenizing buffer with 1 mg/ml gelatin; final concentration, 2 nM [^{3}H]DHT-BA). The incubation was terminated by the addition of dextran-coated charcoal [250 μl; 0.5% activated charcoal and 0.05% dextran (mol wt, 72,000) suspended in a 10-mM Tris buffer, pH 7.5, with 1 mM EDTA] and incubated for 2 h at 4 C with shaking. The extraction was terminated by centrifugation of the samples at 13,000 × *g* for 15 min at 40 C. Radioactivity in an aliquot of the supernatant (250 μl) was quantitated by liquid scintillation counting in 5 ml ACS.

Androgen receptor content was calculated from the specific binding obtained in the assay and the specific activity of the radioligand. Specific binding was obtained by subtracting the binding obtained in aliquots containing nonradiolabeled ligand from binding obtained in parallel incubations containing only the radiolabeled ligand. In some experiments data are normalized to cellular DNA content determined at the time of the assay.

Gel electrophoresis and fluorography

Cytosol preparations were prepared from the cellular soni cates, and aliquots were incubated with 5 nM [^{3}H]DHT-BA for 15 min. The incubations were terminated with the addition of trichloroacetic acid (10%, wt/vol). Precipitated proteins were pelleted by centrifugation (2800 × *g*) and rinsed twice with 95% ethanol and once with acetone-ether (50:50). The radiolabeled proteins were electrophoretically analyzed on sodium dodecyl sulfate-5–15% polyacrylamide gradient slab gels under reducing conditions with the Laemmli (20) buffer system. The gels were fluorographed with diphenyloxazole in acetic acid as previously described (21).

DNA and protein assays

DNA was measured fluorometrically with ethidium bromide (22) as previously described (16). An aliquot of the sonicated cell suspension was added to an equal volume of ethidium bromide solution containing 0.25 mM ethidium bromide, 100 U/ml heparin in ethidium bromide buffer (EBB; 20 mM sodium chloride, 5 mM EDTA, and 10 mM Tris, pH 7.8; Sigma), diluted 1:2 with EBB buffer, and allowed to incubate at room temperature for 30 min. Fluorescent emission at 585 nm with 350-nM excitation was then monitored. A standard curve with calf thymus DNA was used to quantitate DNA levels in the culture wells. This assay has a sensitivity of approximately 0.1 ng DNA and is linear up to 2.5 ng DNA. The total protein concentration was measured according to the method of Bradford (23).

Results

Androgen receptor microassay

Experiments were performed to determine the optimal conditions for the androgen receptor microassay. Aliquots of the fibroblast cellular sonicates were incubated with the affinity ligand, and the assay was terminated at various time points by the addition of dextran-coated charcoal (Fig. 1). Binding of the ligand was rapid, with detectable levels of specific binding found within 1 min, and binding was complete within 30 min. Nonspecific binding increased significantly after 15 min of incubation. To obtain optimal binding with the least amount of nonspecific binding an incubation period of 10 min was chosen. Linear binding with respect to protein or DNA concentration could be demonstrated when aliquots of fibroblast sonicate were within 0.1–0.3 μg DNA or 1.0–2.0 μg protein (Fig. 2). In all subsequent experiments cellular sonicates were diluted so that binding was within the linear range for the assay.

The specificity of the androgen receptor microassay was investigated. Specific binding of the radiolabeled ligand by BSA (20–200 μg/100 μl) was not detectable with an assay sensitivity of 3 fmol. Concentrated (100-fold) Sertoli cell-secreted proteins that contained approximately 200 ng androgen-binding protein (ABP) were also not found to have detectable specific binding in the androgen receptor microassay (data not shown). Steroid specificity was evaluated by the ability of various steroids to compete for binding of radioactive affinity

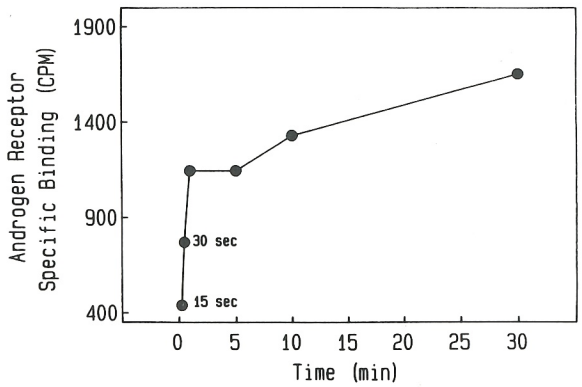

FIG. 1. Effect of incubation time on binding of the affinity ligand by the androgen receptor. Aliquots of fibroblast cellular sonicates were prepared and incubated with [^{3}H]DHT-BA, as indicated in *Materials and Methods*. Parallel incubations included nonradiolabeled DHT-BA for the determination of specific binding. Incubations were terminated at the indicated time points by the addition of dextran-coated charcoal. The data presented are from an experiment performed in triplicate which is representative of three experiments using different cell preparations.

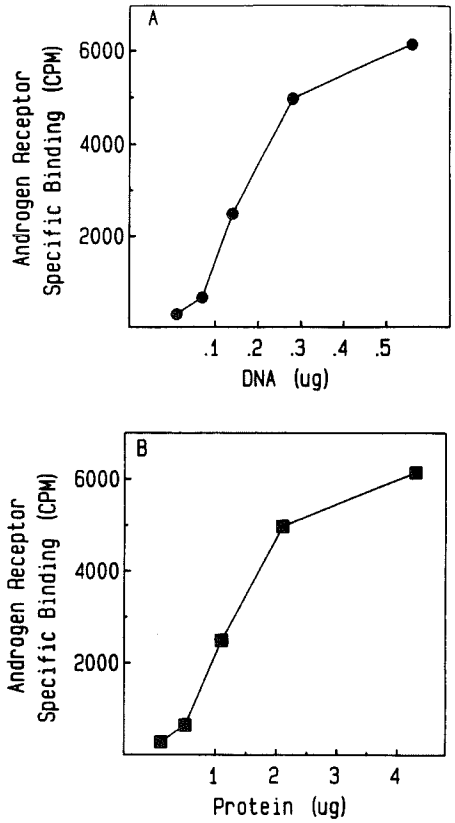

FIG. 2. Effects of DNA and protein concentration on binding of the affinity ligand by the androgen receptor. Fibroblast sonicates were diluted and assayed for specific binding by the androgen receptor and for DNA (A) and protein (B) content as outlined in *Materials and Methods*. The data presented are the results of one experiment performed in triplicate, with a coefficient of variation of approximately 10%, which is representative of three experiments using different cell preparations.

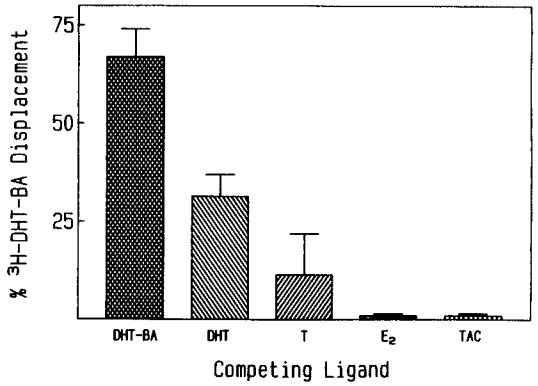

FIG. 3. Steroid specificity of the binding of the affinity ligand by the androgen receptor. Aliquots of the fibroblast sonicate were incubated overnight with the various steroids, as outlined in *Results*. The data are presented as [^{3}H]DHT-BA binding that was displaced during a 5-min incubation. The final concentration of nonradioactive steroid was 4 nM for DHT-BA, DHT, testosterone (T), E_2, and triamcinolone acetonide (TAC). The data presented are the results of two experiments performed in triplicate, with the mean and SEM indicated.

TABLE 1. Androgen receptor levels determined with the microassay

Tissue/cell	Androgen receptor (fmol/μg DNA)
Fibroblast	103 ± 28
Ventral prostate	172 ± 20
Kidney	205 ± 38
Liver	50 ± 13

Data are presented as the mean ± SEM duplicate samples from three different experiments. Ventral prostate androgen receptor levels determined by the conventional equilibrium binding assay performed on the same samples averaged 0.2–0.3 fmol/μg DNA.

ligand ([^{3}H]DHT-BA). To assess the ability of other steroids to compete for the specific binding site, cellular homogenates were preincubated overnight with excess nonradioactive hormones [DHT, testosterone, estradiol (E_2), and triamcinolone] before incubation with [^{3}H] DHT-BA. The data indicate that nonradioactive DHT-BA was the best binding competitor and that the androgens DHT and testosterone also competed for binding to the protein (Fig. 3). E_2 and triamcinolone acetonide, which binds to both the glucocorticoid and progestin receptors, showed no detectable competition in the assay (Fig. 3).

The androgen receptor content of a number of tissues was quantitated by the microassay. The range for linear binding in the microassay with the tissue sonicates was determined to be similar to that obtained for the fibroblasts (0.1–0.3 μg DNA/aliquot). High levels of specific binding were found in sonicates of cultured fibroblasts, ventral prostate, and kidney. Low but detectable levels of specific binding were found in the liver (Table 1). For comparison, a conventional equilibrium binding assay was performed using an overnight incubation with [^{3}H] mibolerone and separation of bound ligand by adsorption to hydroxylapatite. Additional data (not shown) indicated that the two assay methods were similar in the relative distribution of receptor found in these tissues, but the microassay detected significantly higher levels of androgen receptor. The levels of androgen receptor detected with the microassay were 1–2 orders of magnitude higher than levels determined using the traditional assay method. Comparison of the sensitivities of the assays demonstrated that the traditional assay required milligram quantities of cellular protein, while the microassay was routinely performed using 5×10^5 cells or approximately 2 μg protein.

Cellular distribution of the androgen receptor in the testis

Androgen receptor levels were determined on sonicates of freshly isolated peritubular cells, Sertoli cells, Leydig cells, and germ cells (Fig. 4). Although cell counts were not performed routinely, the starting material was equivalent to approximately 5×10^5 cells. Androgen receptor was detected in peritubular cells, Sertoli cells, and Leydig cells, but not in germ cells. When normalized for DNA, receptor levels were similar in Sertoli and peritubular

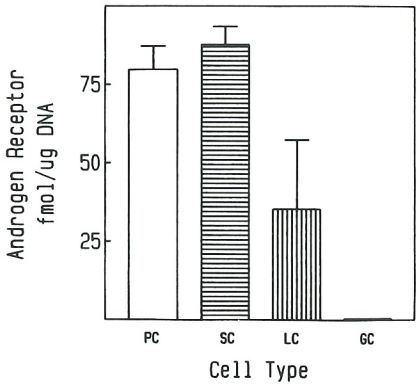

FIG. 4. Quantitation of the androgen receptor in different testicular cellular fractions. Fresh or frozen cells were prepared and assayed for specific binding as outlined in *Materials and Methods*. PC, Peritubular cells; SC, Sertoli cells; LC, Leydig cells; GC, germ cells. The data presented are the average ± SEM from four different cell preparations and experiments assayed in triplicate. Undetectable levels were found in the germ cells.

cells, and these levels were higher than those found in Leydig cells (Fig. 4). Androgen receptor levels were also determined at various times during the culture of peritubular and Sertoli cells. Although a small rise was observed on day 6 of culture, no statistically significant change ($P < 0.01$) in receptor levels was detected during the culture of either peritubular cells or Sertoli cells (Fig. 5). The effects of regulatory agents known to stimulate cellular functions in these cells were also evaluated. The addition of testosterone to peritubular cells or a combination of regulatory agents (FSH, insulin, retinol, and testosterone) to Sertoli cells did not alter receptor levels in cultured cells.

Electrophoretic analysis of the androgen receptor

Proteins radiolabeled with [^{3}H]DHT-BA from cytosol preparations of fibroblasts, Leydig cells, peritubular cells, and Sertoli cells were separated by sodium dodecyl sulfate-gel electrophoresis. The electrophoretic analysis showed a major radiolabeled protein of approximately 56 kDa from human fibroblasts, while in the rat Sertoli cell, Leydig cell, and peritubular cell preparations a single protein with a 52 kDa molecular mass was found (Fig. 6). Affinity radiolabeling carried out in the presence of excess nonradioactive DHT-BA resulted in no detectable radiolabeled band (data not shown). Radiolabeled proteins comigrating with ABP were not detected in any Sertoli cell preparation. Additional bands of radiolabeled protein with lower apparent mol wt were noted in the fibroblast and peritubular cell preparations with greater than 15-min radiolabeling periods.

Discussion

A variety of methodologies are available that can identify and quantitate the androgen receptor. Androgen

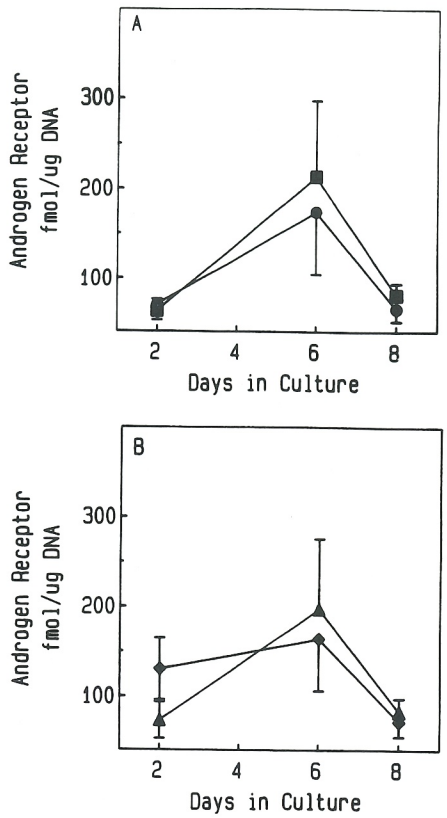

FIG. 5. Effect of culture duration on androgen receptor content in peritubular and Sertoli cells. Specific binding was assayed in peritubular cells (A) and Sertoli cells (B) that had been cultured for 2–8 days. Peritubular cells were plated and cultured in the presence of 10% newborn calf serum that had been extracted with charcoal to remove steroids. The peritubular cells were either cultured with no further additions to the medium as a control (●) or with the addition of testosterone (■). Sertoli cells were either plated and cultured with no additives to the culture medium as a control (♦) or with a combination of regulatory agents FSH, insulin, retinol, and testosterone, FIRT (▲). The data are averages ± SEM of duplicate wells assayed in duplicate from four different cell preparations and experiments.

receptors can be detected in tissue with autoradiographic techniques using radiolabeled steroids, but specific binding by the receptor is often difficult to establish, and the method is only semiquantitative. Recombinant DNA technology recently has made available molecular probes that can identify and quantitate androgen receptor gene expression (24–27). These probes are useful in determining androgen receptor gene expression in isolated cells. For example, recent experiments indicate that mRNA levels for the androgen receptor in tissue from castrate animals are higher than those in normal untreated animals (27). mRNA levels, however, do not necessarily reflect the levels of androgen receptor protein. Traditional equilibrium binding assays for the androgen receptor protein are quantitative and specific, but are lengthy procedures which require relatively large amounts of cells. This conventional assay is dependent on the stability of the protein during the procedure. The displacement of bound steroid during the procedure reduces the

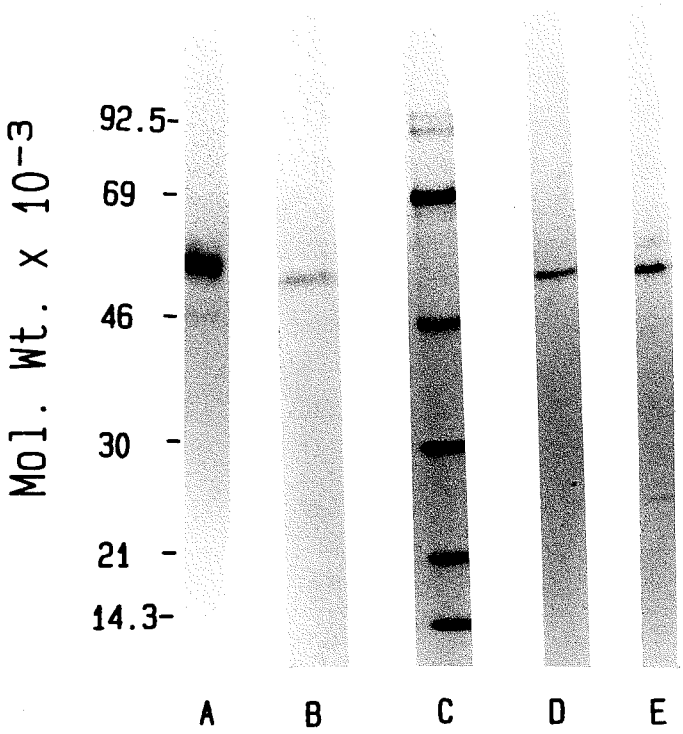

FIG. 6. [^{3}H]DHT-BA radiolabelled proteins from human fibroblast and rat testicular cells. Cytosol preparations from confluent cultures of human fibroblasts (A) and freshly isolated peritubular cells (B), Sertoli cells (D), and Leydig cells (E) were radiolabeled and analyzed by sodium dodecyl sulfate-gel electrophoresis and flurography. Mol wt were determined from separation of ^{14}C-methylated mol wt marker proteins (C). Data are representative of three different experiments.

amount of androgen receptor detected. Identification and quantitation of the androgen receptor protein would, therefore, be aided by a more direct, rapid, and sensitive assay.

The steroid DHT-BA is a chemically reactive androgen affinity ligand which has been reported to covalently bind to purified androgen receptor (9, 10). In human foreskin fibroblasts DHT-BA was found to displace DHT from the androgen receptor and bind with high affinity and specificity to a single saturable protein of 58 kDa that was absent in cells with no detectable androgen receptor (11). The protein that binds the DHT-BA ligand was compared to the authentic androgen receptor protein and found to be indistinguishable by gel filtration chromatography, hydrophobic interaction chromatography, sucrose density gradient centrifugation, and chromatofusing (12). Combined data indicate that the affinity ligand DHT-BA provides an efficient affinity ligand for the androgen receptor which can be used in the characterization and quantitation of the receptor protein. The microassay using DHT-BA developed in the current study is rapid and specific, with no sample purification required. The microassay can be completed in 4 h compared to the 2 days required for the equilibrium binding assay. BSA and ABP are proteins known to bind most androgens, but did not efficiently bind DHT-BA or interfere with the microassay. The inability of DHT-BA to affinity label BSA and ABP implies that a reactive nucleophile may not be present in the binding sites of these proteins as it is in the androgen receptor. While the rapid covalent binding of a ligand such as [^{3}H]DHT-BA does not permit conventional determinations of the relative binding affinities of the various natural noncovalently binding ligands by equilibrium competitive experiments, the data of Fig. 3 do indicate that the steroid specificity of the binding site is characteristic of an androgen receptor.

The levels of androgen receptor measured by the microassay in the rat tissue sonicates were substantially higher than those obtained using a conventional assay method based on a noncovalent ligand. We found no difference between the total androgen receptor content of cell sonicates and that of cytosol preparations. Since unoccupied receptors are thought to be less stable, degradation of the receptor protein over the prolonged incubation period of the traditional assay may result in a significant loss in receptor-binding sites. The problem of receptor protein instability is greatly reduced by the shorter incubation period used in the microassay and the irreversible covalent binding of the affinity ligand. Therefore, the high levels of specific binding detected with the microassay may be more representative of the actual androgen receptor concentration. Alternatively, the affinity ligand may recognize an abundant stable degradation product of the androgen receptor that does not bind the natural ligand.

Binding of the affinity ligand to a protein unrelated to the androgen receptor does not appear to be probable, due to the specificity of binding and the biochemical characterstics previously reported (12).

The microassay was used to determine the cellular distribution and levels of the androgen receptor in freshly isolated testicular cell types. Androgen receptors were detected in freshly isolated Leydig cells, confirming previous reports of the presence of the receptor in Leydig cells (1, 2). The present study supports the possibility of an autocrine regulation of androgens on Leydig cell androgen synthesis in the testis (28). A mixed population of germ cells was not found to contain detectable levels of androgen receptor. This observation supports previous studies that indicate that the androgen regulation of spermatogenesis is mediated by the somatic cells and not the germ cells (29).

The two somatic cell types in the seminiferous tubule that provide potential sites of androgen action are the Sertoli cell and peritubular (myoid) cell. Sertoli cells have previously been shown to contain the androgen receptor (1, 4, 5) and have been postulated to be a major site for androgen action in the regulation and maintenance of spermatogenesis. The purity of the Sertoli cell

preparations used for most of these previous studies, however, was not generally quantitated and proves a limitation to data interpretation. The Sertoli cell preparation used in the current study was determined to be greater than 98% pure (15). The androgen receptor microassay detected a relatively high level of receptor in this Sertoli cell preparation. This observation confirms that Sertoli cells provide a potential site of action for androgens. Peritubular cells have also previously been shown to contain the androgen receptor (1, 3), and differentiation of peritubular cells is postulated to be under androgen regulation (30). The androgen receptor microassay detected receptor levels in peritubular cells similar to those found in Sertoli cells. Therefore, the current study confirms that peritubular cells also provide a potential site of androgen action in the seminiferous tubule.

Previously, the levels of androgen receptor in cultured peritubular cells were reported to be stimulated by androgens (3), and receptor levels in cultured Sertoli cells stimulated by androgens and FSH (4). In the current study the androgen receptor microassay did not detect any significant effects of hormones on receptor levels in the cultured cells. The traditional equilibrium binding assay does not distinguish between the stabilization of the receptor binding and increased expression of the androgen receptor gene product. The inability of the microassay to detect any effects of hormones on the cultured cells suggests that stabilization of receptor binding may be a more significant effect than alterations in androgen receptor production. Further investigations will be required to elucidate the hormonal regulation of androgen receptor expression by Sertoli cells and peritubular cells.

The androgen dependence of spermatogenesis may be mediated by Sertoli and peritubular cells. Purified Sertoli cell preparations have been shown to have negligible responses to androgens, as assessed by a number of functional parameters (31). Therefore, androgens do not appear to have a major role in the control of Sertoli cell function and differentiation *in vitro*. The detection of a relatively high level of androgen receptor, however, suggests that androgens may have a function in the control of Sertoli cell differentiation *in vivo*. Elucidation of the direct actions of androgens on Sertoli cells will require further investigation of both androgen receptor biology and Sertoli cell function. Interestingly, peritubular cells produce a paracrine factor termed PModS, which has dramatic stimulatory effects on Sertoli cell function and differentiation *in vitro* (16, 32, 33). The apparent production of PModS can be stimulated by androgens (16, 34). Therefore, the indirect mode of androgen action is proposed in which Leydig cells produce androgens that act on peritubular cells to stimulate PModS production, which, in turn, can act on Sertoli cells to regulate functions important for the maintenance of spermatogenesis (32, 34). Previously, peritubular cells have been shown to augment the actions of androgens on Sertoli cells (35), and the current study confirms that peritubular cells provide a site for androgen action. Peritubular cell-Sertoli cell interactions mediated via PModS are postulated to be an important indirect mode of androgen action in the control of testis function and the process of spermatogenesis.

The current study also provides an initial characterization of the androgen receptor through determination of the mol wt of an apparently stable processed form of the receptor. Electrophoretic analysis of the radiolabeled receptor confirmed the presence of a specifically labeled 56-kDa form of the androgen receptor in a human foreskin fibroblast line (12). Binding of the ligand to this 56-kDa protein has previously shown to be specific for androgens (12). Freshly isolated Leydig cells, peritubular cells, and Sertoli cells from rat testis contain a 52-kDa form of the androgen receptor. The similarity in the size of the androgen receptor in the three rat testicular cell types implies that the difference in size between rat and human fibroblast receptors (56 *vs.* 52 kDa) may be a species difference in the structure and/or processing of the protein. The identification of a single protein binding the DHT-BA ligand implies that the assay is specific for the androgen receptor and does not detect other proteins. The apparent mol wt of this species, however, is lower than that predicted from the full-length cDNA (27) and higher mol wt species previously observed. The affinity-labeled moiety is postulated to represent a stable degradation product of the native protein. The inability to affinity label higher mol wt species may reflect the relative distribution of the different forms of the receptor and/or the steroid-binding capacity. Studies have been initiated to purify and further characterize the affinity-labeled form of the receptor as well as compare the immunological properties of the native androgen receptor and the affinity-labeled form of the receptor. Information currently available, however, indicates that the physiochemical properties of the affinity-labeled form of the receptor are indistinguishable from those of the androgen receptor detected with the noncovalent androgen ligand (12). The data presented indicate the utility of DHT-BA affinity ligand in characterization of the androgen receptor and imply that this electrophorectic analysis is a useful procedure to confirm the specificity of the androgen receptor microassay.

The microassay described provides a rapid and sensitive procedure for the quantitation of the androgen receptor. This microassay is anticipated to provide a technical advance which will develop insight into androgen receptor biology for many tissues. This was demonstrated through an investigation of the cellular distribution and quantitation of the androgen receptor in isolated testicular cell types. This analysis demonstrates the presence

of androgen receptors in multiple cell types, suggesting both direct and indirect modes of action for androgens in the regulation of testicular function.

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