Androgen Receptor Phosphorylation, Turnover, Nuclear Transport, and Transcriptional Activation

SPECIFICITY FOR STEROIDS AND ANTIHORMONES*

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Nuclear transport, phosphorylation, ligand binding, and degradation rate of the recombinant androgen receptor (AR) were analyzed in transfected COS cells in the presence of various steroids and antiandrogens. Transcriptional activation was assessed in CV1 cells by cotransfection with an androgen-responsive chloramphenicol acetyltransferase (CAT) reporter vector. Hormone binding specificity of recombinant AR was essentially identical to endogenous AR. AR localized in the nucleus in the presence of methyltrienolone (R1881, a synthetic androgen), dihydrotestosterone, testosterone, hydroxyflutamide, cyproterone acetate, estradiol, progesterone, and RU486. In the absence of hormone or with the antiandrogen, flutamide, AR remained largely in the cytoplasm with a perinuclear distribution. AR was degraded rapidly (t1/2 = 1 h) except in the presence of androgen (t1/2 = 6 h) which accounted for an apparent 2-4-fold androgen-induced increase in AR phosphorylation, indicating that AR phosphorylation was not enhanced by androgen. CAT activity was stimulated by R1881, dihydrotestosterone, testosterone, cyproterone acetate, estradiol, progesterone, and RU486 in a dose-dependent manner. The antiandrogens, flutamide and hydroxyflutamide, lacked agonist activity and inhibited R1881-induced activation of CAT and androgen stabilization of AR. Steroids and antiandrogens with moderate to low affinity for AR promoted both nuclear transport and transcriptional activation but only at high hormone concentrations. Hydroxyflutamide acted as a true antiandrogen since it lacked agonist activity and was an inhibitor of androgen-induced transcriptional activation.

The biological effects of androgens can be inhibited by androgen antagonists known as antiandrogens. On the basis of structure, antiandrogens are subdivided into two groups, steroidal and nonsteroidal, both of which can inhibit agonist binding to the androgen receptor (AR)1 (1). Flutamide (α,α,α-trifluoro-2-methyl-4'-nitro-m-propionoluidide) is considered a pure antiandrogen because it lacks agonist activity and is specific for the AR. Administered to animals, flutamide has potent antiandrogenic effects on male reproductive tissues, decreasing organ size (2, 3) and blocking androgen effects in castrated rats. It was postulated that since flutamide shows little binding affinity for AR, its effects in vivo are mediated through a hydroxylated metabolite, hydroxyflutamide (α,α,α-trifluoro-2-methyl-4'-nitro-m-lactotoluidide) (4-7). Subsequent studies demonstrated that hydroxyflutamide administered in vivo is an effective antiandrogen (8). More recently flutamide itself was shown to inhibit receptor binding of labeled androgen but only at very high concentrations (K, 10 μM) (9).

RU486 is a synthetic progestin and glucocorticoid antagonist that binds with high affinity to glucocorticoid and progesterone receptors (10, 11) and causes efficient nuclear localization of these receptors (12-14). RU486 promotes specific binding of progesterone (15-17) and glucocorticoid receptors (18) to their hormone response elements, does not induce a transcriptional response, and blocks the constitutive transcriptional function of a truncated mutant form of the progesterone receptor (19).

This report addresses the functional activity of transiently expressed AR when bound by antiandrogens and antiglucocorticoids in comparison with biologically active androgens, testosterone and dihydrotestosterone, and the synthetic androgen, R1881. The hormone specificity of nuclear transport was investigated since previous studies demonstrated that R1881 promotes nuclear localization of AR from the perinuclear cytoplasmic region of transiently transfected COS cells (20). The effects of different steroids and antiandrogens on AR protein turnover and phosphorylation were investigated since studies on other steroid receptors (21-24) and the AR (25) provided evidence for hormone-dependent phosphorylation. Finally, steroid hormone and antihormone stimulation of AR-mediated transcriptional activation was compared using a cotransfection assay in which R1881 promoted strong

1 The abbreviations used are: AR, androgen receptor; R1881, methyltrienolone; IgG, immunoglobulin G; CAT, chloramphenicol acetyltransferase; flutamide, α,α,α-trifluoro-2-methyl-4'-nitro-m-propionoluidide, or 4'-nitro-3'-trifluoromethylisobutyr-anilide or SCH 13521; hydroxyflutamide, α,α,α-trifluoro-2-methyl-4'-nitro-m-lactotoluidide or SCH 16423; cyproterone acetate, 6a-chloro-17a-hydroxy-1a,2a-methylene-4,6-pregadien-3,20-dione-17-acetate; RU486, Roussel Uclaf list no. 36,486, mifepristone, 11β-(4-dimethylamino phenyl)-17β-hydroxy-1a-((prop-1-ynyl)-estra-4,9-diene-3-one; LNCaP, lymph node-derived human prostate carcinoma cell line; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
induction of the chloramphenicol acetyltransferase gene linked to an androgen-responsive promoter (26).

**EXPERIMENTAL PROCEDURES**

**Materials**—The following reagents were purchased: monkey kidney COS-7 and CVI cells from the American Type Culture Collection; Dulbecco's modified essential medium with high glucose and Eagle's modified essential medium without phosphate from Gibco; bovine serum from HyClone Laboratories, Inc.; p-three-[2,4,6-C]chloramphenicol, 40–60 mCi/mmol and Trans 35S-label (L-methionine, 35S), L-cysteine, 35S), 1000 Ci/ml from ICN Biomedicals, Inc.; phosphorus 32-orthophosphoric acid from Du Pont; acetyl coenzyme A and DEAE-dextran from Pharmacia LKB Biotechnology Inc.; [3H]methylthiobine (17-aza-methyl-3H) (R1881, 80 Ci/ml from Du Pont; New England Nuclear; W-OMAT-AR diagnostic x-ray film and thin layer silica gel chromatograms from Kodak; buffers and chemicals from Fisher, EM Science, and Sigma.

**Cell Transfection**—Nuclear transport, receptor turnover, and phosphorylation were determined in monkey kidney COS-7 cells transfected with 10 µg of the full-length human AR expression vector pCMVhAR (pSHbvar-A) (20, 26) using DEAE-dextran (27). Cells were maintained in 10% fetal calf serum in medium as described above. Cells were plated at 1.3 × 10^6 cells/l0-cm dish so that approximately 80–90% confluence was achieved at the time of transfection 24 h later. Cells were transfected with 1–5 µg of AR expression vector pCMVhAR and 1–5 µg of the reporter vector using the calcium phosphate precipitation method (27). The CAT reporter vector pCMVhAR (p5HBhAR-A) (20, 26) using DEAE-dextran (27). COS cells were maintained in 10% fetal calf serum in Dulbecco's minimal essential medium containing 20 mM HEPES, pH 7.2. Four ml of fresh phosphate-free or methionine-free media was added, and the cells were incubated at 37°C in a 5% CO2 incubator for 15 min. Steroids or nonsteroidal antiandrogens and 200 nM [3H]flutamide or 200 nM of Trans 35S-label were added and incubated for the indicated times at 37°C. Cells were washed twice with Dulbecco's modified Eagle's media and harvested by scraping into 1 ml of RIPA buffer (1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate, 0.15 M NaCl, 0.5 mM sodium vanadate, 5 mM EDTA, and 50 mM Tris, pH 7.4). DNA was sheared by repeated passage through a Pasteur pipette and the samples rotated for 20 min at 4°C. Supernatants of a 15-min centrifugation at 13,000 × g at 4°C were transferred to new microfuge tubes and 60 µg of AR52 AK anti-epitope IgG (28, 29) added and incubated overnight at 4°C. Pansorbin Staphylococcus aureus cells (Calbiochem Corp., La Jolla, CA) were prepared fresh by washing with six volumes of 10% SDS followed by three washes with RIPA with 1-min centrifugations to pellet the cells.

The final Pansorbin pellet was resuspended in an equal volume of RIPA and 20 µl added to each sample followed by incubation for 2 h at 4°C. After a 1-min centrifugation, a series of 0.3-ml washes of the pellets with 30 s centrifugations included twice RIPA; 0.5 M NaCl, RIPA; 1 mg/ml bovine serum albumin, RIPA; 0.5 M NaCl, RIPA; 1 mg/ml bovine serum albumin, RIPA; twice RIPA. The final pellets were resuspended in 75 µl of 2.5% SDS, 12.5% glycerol, 12.5 mM Tris, pH 6.8, and incubated at room temperature for 10 min. Following a 2-min centrifugation, the supernatants were transferred to new tubes and made 5% 2-mercaptoethanol and 0.05% bromophenol blue. Samples were heated at 55°C for 5 min, cooled to room temperature, and loaded onto 8% SDS-polyacrylamide gels prepared as described (27, 33). The extent of radiolabel incorporation was determined by computer analysis of the transferred membranes on an Ambis radioscanner. The data shown are representative of at least three independent experiments.

**RESULTS**

**Recombinant AR Steroid Binding Specificity**—In a whole cell competitive binding assay, transiently expressed AR had highest apparent binding affinity for R1881, followed by dihydrotestosterone (Fig. 1). Testosterone binding (not shown)

![Fig. 1. Steroid binding affinity of recombinant AR expressed in COS cells.](image-url)
was similar to dihydrotestosterone. Cyproterone acetate, estradiol (Fig. 1), and progesterone (not shown) competed for [3H]R1881 binding with 60–80% inhibition at a 100-fold molar excess of unlabeled hormone (Fig. 1). The antiandrogen flutamide failed to inhibit binding of [3H]R1881 in this concentration range as previously reported for the endogenous receptor (9), and hydroxyflutamide inhibited binding by about 50% at a 100-fold molar excess concentration (Fig. 1). RU486, a glucocorticoid and progesterone receptor antagonist (10, 11), inhibited binding by about 70% at a 100-fold molar excess of labeled hormone (Fig. 1). Thus, the binding specificity of recombinant AR expressed in COS cells was similar to that of the endogenous receptor (24–36) in its limited specificity at elevated steroid concentrations. Both endogenous and transiently expressed AR bind a variety of steroid hormones and antagonists with apparent moderate affinities (10^−8 Kd). How this binding effects receptor function was examined by analysis of AR nuclear localization, degradation rate, phosphorylation, and transcriptional activation.

**Hormone Specificity of AR Nuclear Transport**—Androgen-dependent transport of AR to the nucleus was previously demonstrated in transiently transfected COS cells (20) and is shown in Fig. 2, A–C. In the absence of hormone, AR is localized in the perinuclear region of the cytoplasm (Fig. 2A). Nuclear localization of AR was observed following incubations with 50 nM R1881 (B), dihydrotestosterone (C), or testosterone (not shown), or 100 nM hydroxyflutamide (E), cyproterone acetate (F), RU486 (G), estradiol (H), and progesterone (I). Hormone concentrations less than 50 nM resulted in the presence of both nuclear and perinuclear staining, suggesting that receptors were not saturated at the lower hormone concentrations. Thus nuclear localization of AR in transfected COS cells occurred with a variety of steroids and antiandrogens. AR immunostaining in the presence of 100 nM flutamide, however, was predominantly perinuclear in the cytoplasm with only minimal nuclear staining (D).

**Receptor Phosphorylation and Turnover**—Steroid effects on AR phosphorylation were investigated by incubating transfected COS cells with [32P]orthophosphate and immunoprecipitating receptor using anti-peptide AR antibody AR52 described previously (29). Transfection with the parent expression vector (P5) which lacks AR coding sequence produced no major phosphorylated products (Fig. 3A, lane 1). In the absence of hormone, AR was detected as a 114-kDa phosphoprotein (Fig. 3A, lane 2). Addition of R1881, dihydrotestosterone, or testosterone at 5–50 nM appeared to stimulate the incorporation of radiolabeled phosphate 2–4-fold following a 3-h incubation (lanes 3–5). AR phosphorylation was not significantly enhanced by treatment with 100 nM estradiol, progesterone, or the antiandrogens, cyproterone acetate, flutamide, or hydroxyflutamide (lanes 6–10). However, 100 nM RU486 caused an apparent increase in AR phosphorylation (lane 11) similar to that observed with androgens. None of the hormones altered basal receptor phosphorylation observed in the absence of androgen. Treatment with 100 nM R1881 20 min prior to harvest showed a 1.5-fold increase in apparent phosphorylation (lane 13), but no increase was observed when R1881 was added to the harvest buffer (lane 14). Thus the steroid requirements for hormone-induced phosphorylation appeared to be androgen specific with the exception of RU486.

We investigated whether the apparent increase in AR phosphorylation described above resulted from androgen-enhanced AR immunoreactivity or receptor stabilization. Parallel incubations were performed with [35S]labeled methionine and cysteine for 3- or 24-h labeling periods in the presence and absence of 10 nM androgens or 100 nM nonandrogenic steroids or antiandrogens. Androgens specifically increased 3–5-fold the amount of [35S]-labeled receptor (Fig. 3B), which was slightly greater than the apparent increase in receptor phosphorylation observed in Fig. 3A. Steroids and antiandrogens which did not enhance phosphorylation also did not increase [35S]-labeled AR. A 20-min exposure to androgen or androgen addition to the harvest medium (Fig. 3B, lanes 13 and 14) yielded a pattern similar to the phosphorylation

![Fig. 2. Immunocytochemical localization of AR in the absence or presence of steroids and antiandrogens. The pCMVhAR expression vector was transfected into COS cells using DEAE-dextran as described. Twenty-four h after transfection the cells were placed in serum-free, phenol red-free media in the absence (A) or presence of 50 nM R1881 (B), dihydrotestosterone (C), 100 nM flutamide (D), hydroxyflutamide (E), cyproterone acetate (F), RU486 (G), estradiol-17β (H), or progesterone (I). Twenty-four h later and 2 h before fixation, steroid containing media were replaced with fresh identical media. The slides were washed, fixed, and stained with the avidin biotin peroxidase method as previously described (30, 31). Magnification × 230.](image-url)
The labeling periods was immunoprecipitated using AR52 anti-peptide IgG fraction as described under "Experimental Procedures." The labeling periods were in serum-free, phosphate-free media containing the radiolabel with no addition of hormone (lane 2), or addition of 10 nM R1881 (lane 3), dihydrotestosterone (DHT, lane 4), testosterone (T, lane 5), or 100 nM estradiol (E, lane 6), progesterone (P, lane 7), cyproterone acetate (CA, lane 8), flutamide (FL, lane 9), hydroxyflutamide (OH-FL, lane 10), or RU486 (RU, lane 11). Lane 1 contains an immune extract from cells transfected with the parent pCMV expression vector (P5) which lacks AR coding sequence. Results. Thus, the apparent androgen-stimulated increase in AR phosphorylation appeared to be due to a parallel increase in AR protein. Sodium dodecyl sulfate-gel electrophoresis and immunoblot analysis of AR in whole cell extracts not immunoprecipitated (not shown) indicated that preferential immunoprecipitation of the androgen-bound receptor did not occur.

The hormone dependence of receptor turnover was examined in a pulse-chase experiment. Following a 30-min incubation with 200 μCi of [35S]methionine, cells in monolayer culture were incubated in the presence or absence of 100 nM R1881, harvested at increasing time intervals, and subjected to immunoprecipitation and SDS-gel electrophoresis. As shown in Fig. 4, a major decrease in the rate of receptor degradation occurred in the presence of androgen. AR degraded intracellularly at 37°C with a half-time of approximately 1 h in the absence of androgen and 6 h in the presence of androgen (Fig. 4, A and B). Nonandrogenic steroids included in this study had little or no effect on the degradation rate observed in the absence of androgen (not shown). The antiandrogens, hydroxyflutamide and cyproterone acetate, inhibited androgen-induced stabilization when included at a 100-fold molar excess. It is concluded that the fold enhancement of receptor stability by androgen binding could account for the apparent androgen-stimulated increase in AR phosphorylation, and therefore, androgen does not promote receptor phosphorylation in this assay system.

Transcriptional Activation—Transient cotransfection of the AR expression plasmid with a CAT reporter vector in CVI cells was performed to determine whether other steroids and antiandrogens that bind the receptor stimulate AR-mediated gene transcription. R1881, dihydrotestosterone, and testosterone each induced CAT activity in a dose-dependent manner between 0.001-1 nM (Fig. 5). Low basal activity was observed with the parent pCMV expression vector (P5) with or without 10 nM R1881 or with the AR expression vector in the absence of hormone (Figs. 5 and 6). Estradiol and progesterone stimulated CAT activity nearly equal to that of androgen but only at 1000-fold higher hormone concentrations (Fig. 5). Estradiol (1 nM) and progesterone (10 nM) produced a transcriptional response similar to that of 0.001 nM R1881 (Fig. 5) but were inactive at 0.001 nM. At 10 nM estradiol or 100 nM progesterone, CAT activity was equivalent to that induced by 0.01 nM R1881 (Fig. 5).

CAT activity induced by 100 nM cyproterone acetate or 100 nM RU486 was approximately 50 and 15-20%, respectively, of that observed with 0.01 nM R1881 (Fig. 6). In a control study not shown, RU486 failed to induce CAT activity when the full-length glucocorticoid receptor was coexpressed in CVI cells. No significant AR-induced CAT activity was detected following incubations with flutamide or hydroxyflutamide at concentrations up to 100 nM (Fig. 6).

Thus, transcriptional activation by AR was androgen specific only at hormone concentrations less than 1 nM. At higher concentrations, induction of CAT activity reflected AR bind-
Fig. 6. Transcriptional CAT activation following incubation with androgen, antiandrogens, and RU486. CAT activity was determined in CV1 cells transfected with the full-length human pCMVhAR expression vector. Immediately after transfection, cells were placed in 0.2% fetal calf serum-containing media plus the addition of hormones as indicated. The first two lanes represent the parent P5 vector lacking AR sequence and the remaining lanes are the full-length expressed AR. Fold induction of activity is indicated as a bar graph below and was determined by scintillation counting the eluted radioactivity.

Fig. 7. Inhibition of androgen-induced transcriptional CAT activity by antiandrogens. CV1 cells transfected with either parent vector (P5, lanes 1 and 2) or the full-length AR expression plasmid were placed in 0.2% serum and not further treated (no hormone addition) or treated with 0.05 μM R1881 in the presence and absence of 500 nM flutamide or hydroxyflutamide or 10 and 500 nM cyproterone acetate as indicated. Some samples as indicated received only antiandrogen treatment. The acetylated forms of [3H]chloramphenicol were separated by thin layer chromatography and the plate was exposed to x-ray film. The spots were quantitated by elution of the silica plate and radioactive scintillation counting and are shown as fold induction in the bar graph below.

**DISCUSSION**

The AR has high binding affinity and specificity for the biologically active androgens, but only at low steroid concentrations. Lack of AR steroid binding specificity at elevated steroid concentrations characterizes both the endogenous (34) and transiently expressed recombinant receptor as demonstrated in this report and shown previously for a truncated form of the expressed AR (37). A question addressed in the present study was whether the AR undergoes functional activation when bound to nonandrogenic hormones for which it has low to moderate binding affinity. The studies indicate that the antiandrogens, cyproterone acetate, estrogen, progesterone, and RU486, not only promote nuclear transport, but enhance transcriptional activation by AR. In contrast, only high affinity androgen binding stabilized the AR protein, slowing its rate of degradation and making it appear that androgen induced AR phosphorylation. Of the steroids and antihormones tested in the transient cotransfection assay, hydroxyflutamide had properties closest to a true antiandrogen since it inhibited androgen-induced transcriptional activation and did not significantly enhance AR-mediated transcriptional activation.

AR nuclear transport in transiently transfected COS cells was shown previously to be androgen-dependent (20). In the absence of hormone addition, AR displays a striking punctate perinuclear distribution in the cytoplasm, while androgen addition causes strong nuclear immunostaining. The perinuclear cytoplasmic localization of AR in the absence of androgen was not evident, however, with the endogenous receptor following androgen withdrawal by castration for reasons not fully understood (30). In tissue sections of rat ventral prostate, nuclear staining is strong in the intact animal but castration causes loss of immunoreactivity. It is conceivable that the rapid turnover of the AR protein in the absence of androgen as described in this study, albeit in transfected COS cells, contributes to the loss of immunostaining in ventral prostate tissue sections following castration. Steroids and antihormones that bind AR with moderate affinity and stimulate CAT activity at elevated hormone concentrations also promote AR nuclear transport in transfected COS cells. However, hydroxyflutamide caused strong nuclear immunostaining but failed to induce AR-mediated transactivation. It is clear, therefore, that nuclear localization *per se* is necessary but not sufficient for transcriptional activation, as suggested earlier in studies with truncated mutant forms of AR. AR deletion mutants lacking the N-terminal domain were constitutively

The antiandrogens, flutamide, hydroxyflutamide, and cyproterone acetate, were tested for their ability to inhibit androgen-induced CAT activity. As shown in Fig. 7, flutamide (500 nM) caused about a 50% inhibition of R1881- (0.05 nM) induced CAT activity. This inhibitory effect may result in part from limited metabolic conversion of flutamide to hydroxyflutamide as previously suggested from *in vivo* studies (4–7). Hydroxyflutamide- (500 nM) inhibited R1881 (0.05 nM) induced CAT activity by approximately 90%, while alone, it increased CAT activity only slightly higher than background (Fig. 7). The inhibitory activity of cyproterone acetate at 10 nM was about 50%. Higher concentrations of cyproterone acetate showed strong agonist activity as noted above. Thus all three antiandrogens inhibited androgen-induced CAT activity to different degrees, the most effective being hydroxyflutamide.

**Phosphorylation and Functional Activity**

The AR has high binding affinity and specificity for the biologically active androgens, but only at low steroid concentrations. Lack of AR steroid binding specificity at elevated steroid concentrations characterizes both the endogenous and transiently expressed recombinant receptor as demonstrated in this report and shown previously for a truncated form of the expressed AR (37). A question addressed in the present study was whether the AR undergoes functional activation when bound to nonandrogenic hormones for which it has low to moderate binding affinity. The studies indicate that the antiandrogens, cyproterone acetate, estrogen, progesterone, and RU486, not only promote nuclear transport, but enhance transcriptional activation by AR. In contrast, only high affinity androgen binding stabilized the AR protein, slowing its rate of degradation and making it appear that androgen induced AR phosphorylation. Of the steroids and antihormones tested in the transient cotransfection assay, hydroxyflutamide had properties closest to a true antiandrogen since it inhibited androgen-induced transcriptional activation and did not significantly enhance AR-mediated transcriptional activation.

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nuclear yet lacked gene transcriptional activity due to deletion of sequences critical for AR function (20).

The cell systems (CV1 and COS monkey kidney cells) used in formulating conclusions concerning hormone specificity of nuclear transport, transcriptional activity, phosphorylation, and receptor turnover, could be considered somewhat artificial since they do not express AR endogenously and may be deficient in certain transcription factors required to promote hormone-specific gene activation. However, many of the results of these transient transfection studies parallel earlier observations in vivo. For example, it was recognized that progestational steroids promote AR-mediated gene activation in vivo and stimulate growth of the male reproductive tract and virilization of the female fetus (38-40). Furthermore, progestins potentiate nuclear uptake of AR in vivo in mouse kidney (41). Cyproterone acetate, a progestational steroid (42), has not been considered a true antiandrogen because it has both agonist and antagonist activities in vivo (40, 43).

The present study supports these observations in that cyproterone acetate acts as an androgenic agonist in AR-mediated transcriptional activation as determined by CAT assay at elevated steroid concentrations (100 nM), but as an androgen antagonist at a lower hormone concentration (10 nM). On the other hand, cyproterone acetate at elevated concentrations was antagonistic to androgen-induced AR stabilization.

The parallel between endogenous and transiently expressed AR extends to the activity of the nonsteroidal antiandrogen, hydroxyflutamide. Hydroxyflutamide, unlike cyproterone acetate, lacks agonist activity in vivo yet has antiandrogenic activity nearly equivalent to that of cyproterone acetate (2). Hydroxyflutamide has therefore been considered a pure antiandrogen (2, 9). It maintains these characteristics in the in vitro system described here since it did not enhance CAT activity, yet inhibited androgen induction of AR transcriptional activation. Since hydroxyflutamide binds AR with moderate affinity and induces nuclear transport, its binding may impede an altered receptor conformation not conducive to gene activation as suggested for RU486 binding to the glucocorticoid receptor (18).

It is intriguing to note that the androgen-dependent human prostate cancer cell line, LNCaP, responds to hydroxyflutamide as well as estradiol and progesterone with an increase in cell proliferation (44, 45). Estradiol and progesterone binding was inhibited by androgen indicating that their effects were mediated by the AR (44). In the LNCaP cell line, testosterone, R1881, and cyproterone acetate each induced AR mRNA down-regulation. However, estrogen failed to down-regulate AR mRNA both in the LNCaP cell line and in normal rat prostate (46). Thus, while estrogen had agonist activity in the induction of AR gene activation, it did not mimic androgen effects on AR mRNA. The AR gene in LNCaP cells contains a single base mutation that changes amino acid residue 877 from threonine to alanine (47). In cotransfection studies with the reconstructed mutant AR, a striking increase in transcriptional activity was observed with hydroxyflutamide suggesting that an alteration of one amino acid within the steroid-binding domain allowed hydroxyflutamide to acquire agonist activity (48). The LNCaP AR also increased transcriptional activation in response to progesterone and cyproterone acetate (49).

Antiandrogen binding to steroid receptors can initiate early steps in gene activation, i.e. receptor entry to the nucleus and DNA binding. Progesterone receptor binding of RU486 promotes interaction with response element DNA but fails to stimulate transcription (15). Interestingly, although RU486 is a true antagonist for the glucocorticoid receptor, it had agonist activity when bound to AR. Furthermore, of those tested, RU486 was the only steroid that caused an increase in AR phosphorylation that was not offset by a concomitant increase in receptor protein due to stabilization. It was reported previously that RU486 binds endogenous AR of rat prostate (50) and inhibits androgen-induced prostate growth (51). Antagonistic effects of RU486 mediated through the AR were also suggested when it blocked androgen inhibition of prolactin release in vivo (52).

Another mechanism proposed for the inability of antihormones to promote receptor-mediated gene transcription involves stabilization of oligomeric receptor forms. It was reported that RU486 bound to the glucocorticoid receptor in vivo stabilizes the 8S receptor form which does not enter the nucleus (17) or interact with DNA (53, 54). The results of the present study do not support a similar effect on AR.

A question remaining from these studies is the role of AR phosphorylation in transcriptional activation by AR. The ability of AR to induce transcriptional activity in response to steroids or antiandrogens with few exceptions paralleled relative binding specificity and affinity. The apparent effect of androgen on AR phosphorylation was nullified when receptor stability was considered. Androgen increased the amount of AR phosphorylation simply by slowing the rate of degradation of the AR protein. AR stabilization by androgen was observed previously in binding studies on tissue cytosols (34). Moreover, in a ductus deferens smooth muscle tumor cell line, endogenous AR stabilization increased about 2-fold with androgen, from 1/1.2 3.1 h without androgen to 1/1.6 6.6 h with R1881 (55). The mechanism of receptor stabilization by androgen is not known but the striking specificity for androgen suggests it may be closely linked with receptor functional activity. Although other groups have reported on steroid-induced phosphorylation of AR (25) and the glucocorticoid and progesterone receptors (21-24), no androgen-dependent enhancement of recombinant AR phosphorylation was detected in the present study. While the AR is clearly a phosphoprotein, the specific role of phosphorylation in receptor function is unclear. AR sites of phosphorylation are currently being mapped, and it is conceivable that androgen binding may increase phosphorylation of a single site not detectable in our assay system.

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Antiandrogens and AR Phosphorylation and Functional Activity

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