

Glucocorticoids Manifest Androgenic Activity in a Cell Line Derived from a Metastatic Prostate Cancer¹

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ABSTRACT

The pathophysiological mechanism(s) by which androgen independence develops in prostate cancer remains to be determined. The identification in many prostate cancer specimens of a mutant androgen receptor, T877A, with altered ligand specificity has provided an explanation for some treatment failures. The T877A mutant androgen receptor recognizes a number of nonandrogenic compounds, including certain estrogens, progestins, and even antiandrogens as androgens. However, a comprehensive screen for hormonal agents which display agonist activity on this mutant has not been performed. In this study, we characterized this clinically important receptor mutant further and found that it can be activated by a wide range of compounds, including a number of endogenous glucocorticoids. Among the most clinically relevant compounds identified are DOC and corticosterone, both of which can effectively activate the mutant receptor at concentrations normally found in blood. Dexamethasone, a synthetic glucocorticoid frequently used in various contexts for prostate cancer therapy, is also recognized as an androgen by the mutant receptor. These unexpected findings suggest the need to: (a) reassess the role of adrenally derived glucocorticoids in prostate cancer disease progression; and (b) recognize the potential for iatrogenic stimulation of disease progression with certain glucocorticoid interventions.

INTRODUCTION

The most recent estimates published by the American Cancer Society predict 180,000 new cases of prostate cancer annually in the United States and that close to 32,000 men will die of this disease each year (1). Such statistics place this disease second only to lung cancer as the leading cause of mortality in United States males. Although several therapeutic options (radical surgery, external radiotherapy, interstitial brachytherapy) are available for early stage, organ-confined tumors, AA³ remains the primary, most effective mainstay therapy for advanced disease (2-4). Numerous clinical trials over the past 60 years have demonstrated that suppression of gonadal androgen production by surgical castration or medical intervention (estrogens, GnRH agonists/antagonists) can effectively induce cancer regression for substantial periods of time (reviewed in Refs. 5 and 6). However, progression eventually occurs in most patients treated in this manner. To circumvent this problem, a regimen termed TAA, which combines the use of either castration or GnRH agonists with an antiandrogen such as flutamide, has also been implemented. The effectiveness of TAA for the treatment of prostate cancer, however, remains controversial. Although some well-designed Phase III trials have shown a

modest therapeutic advantage, later studies have been unable to confirm these observations (7).

Nevertheless, even while TAA can be very effective initially in patients with advanced disease, an androgen-independent state usually develops within 1-2 years (4, 5), and survival becomes limited beyond that point. The cellular mechanisms leading to the development of androgen-independent tumors are not clear and may involve both AR-dependent and AR-independent pathways. Of particular interest is, however, a syndrome, initially termed the flutamide withdrawal syndrome, which was recognized a decade ago. In ~20-50% of patients who have failed TAA, a paradoxical drop in serum PSA levels (the marker most commonly used to monitor prostate cancer progression) or even improved symptom status was observed after cessation of flutamide (8). The duration of this withdrawal response is variable, typically lasting ~3-6 months. At this point, tumors progress despite castrate levels of androgens.

Different mechanisms have been proposed to explain the development of androgen-independent prostate cancers (reviewed in Ref. 7). Among the best described resistance mechanisms, amplification of AR, which could enhance the impact of residual androgens present in circulation, has been observed in 30% of prostate cancer specimens from patients who have failed AA (9-11). In addition, somatic mutations have been found within AR which alter its pharmacology (12-14). In particular, the T877A mutation (threonine to alanine substitution at amino acid 877), which resides within the ligand-binding domain of AR, has been frequently identified in specimens of hormone refractory metastatic prostate cancer. As a result, an apparent "promiscuous" stimulation of AR-mediated transcriptional activation by estrogens, progestins, and even synthetic antiandrogens such as flutamide has been observed in both the prostate cancer cell line LNCaP, as well as specimens harboring this mutation (15, 16). It is this mutation that is often ascribed as the underlying cause of the flutamide withdrawal syndrome (17), because its incidence is high in flutamide-treated patients and low, though clearly present, in patients who have undergone other treatments (13, 17, 18). Adding to the puzzle, however, is that a significant number of patients whose tumors were subsequently shown to have an 877 AR mutation did not show a clinical flutamide withdrawal response despite discontinuation of the drug (13). Interestingly, Zhao *et al.* (19) has recently shown that the mutations found in MDA PCa 2b cells, a cell line derived from the bone metastasis of an androgen-independent prostate cancer, harbor a double mutation L701H and T877A, which allows the mutant receptor to be activated by cortisol and cortisone; thus, these cells no longer require androgens for growth.

Taken together: (a) the observation of the flutamide withdrawal syndrome; (b) the identification of mutants of the AR that affect ligand specificity; and (c) the ability of patients failing AA monotherapy alone (*e.g.*, castration only) to exhibit frequent modest serological responses to delayed utilization of AR-directed antiandrogens suggest that at this point, such cancers are not strictly androgen independent. Indeed, the identification of receptor mutants permitting altered hormonal specificity suggests that prostate cancer cell growth might be stimulated by other endogenous-circulating hormones at certain stages of cancer progression as a result of this somatic muta-

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³ The abbreviations used are: AA, androgen ablation; TAA, total androgen ablation; AR, androgen receptor; DPA, diphenylamine; PSA, prostate-specific antigen; RT-PCR, reverse transcription-PCR; DHT, dihydrotestosterone; DOC, 11-deoxycorticosterone; Dex, dexamethasone; GR, glucocorticoid receptor; GnRH, gonadotropin-releasing hormone; OH-F, hydroxyflutamide.

tion. The latter hypothesis is supported by the fact that most flutamide-refractory cancers respond well to bicalutamide, an antiandrogen binding in the ligand-binding domain but with a somewhat different mechanism of action than flutamide (20, 21). Thus, even in flutamide-refractory cancers, it appears as if AR is still somewhat involved in prostate cell growth.

To investigate whether endogenous hormones other than androgens, estrogens, and progestins might contribute to the clinical phenomenon of treatment escape after the emergence of a mutated AR, we decided to test a wide variety of endogenous steroid hormones, as well as synthetic steroids to see if any of these compounds have the ability to activate the AR T877A mutant. It was anticipated that a screen of this nature may lead to the identification of compounds that have the potential to function as AR agonists in certain circumstances. In addition, it may also provide an explanation for the observation that AR-dependent growth of some prostate cancers can occur in the absence of detectable androgens.

MATERIALS AND METHODS

Reagents and Plasmids. All of the chemicals used in this study were obtained from Sigma Chemical Co. (St. Louis, MO), except for the bicalutamide, which was a generous gift from Nobex Corp. (Research Triangle Park, NC). The RS-AR and VP16-AR were gifts from K. Marschke (Ligand Pharmaceuticals, San Diego, CA). The RS-AR/T877A and VP16-AR/T877A containing the T877A AR mutant were generated using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The 5xGal4Luc3, MMTV-Luc, and pM-D30 plasmids were described previously (22). All of the cell culture media and supplements were purchased from Life Technologies, Inc. (Grand Island, NY). LNCaP and CV-1 cells were obtained from American Type Culture Collection (Manassas, VA), and the PSA ELISA assay kit was purchased from ICN Pharmaceuticals (Orangeburg, NY).

Cell Culture and Transfection. Human prostate cancer LNCaP cells were maintained in RPMI medium supplemented with 8% fetal bovine serum, essential amino acids, and sodium pyruvate. Monkey Kidney CV-1 cells were grown in MEM plus 8% fetal bovine serum, essential amino acids, and sodium pyruvate. Cells were seeded on 25-cm² tissue culture flasks a day before transfection. Lipofectin-mediated transfection was performed essentially as described (22). Transfection was stopped after 5 h by replacing the DNA/lipofectin mixture with fresh medium containing charcoal-stripped serum and incubated overnight, allowing cells to recover. Cells were then trypsinized and seeded on 96-well plates the next morning. After cells were attached, hormones were added, and the cells were incubated for 16 h before assaying. Luciferase and β -galactosidase activities were determined as described (22).

Cell Proliferation Assays. LNCaP cells were seeded in 24-well plates with $\sim 4 \times 10^4$ cells/well and maintained in phenol red-free RPMI plus 8% charcoal-stripped serum for 3 days. On day 3, cells were treated with fresh medium containing different concentrations of compounds, and the medium was replaced every other day for 6 days. Cell numbers were determined at the end of 6 days by DPA DNA assays (23, 24).

DPA DNA Assays. The DPA DNA assay was performed as described with minor modification (23, 24). Medium was removed by suction from attached LNCaP cells. Prechilled 20% hyperchloric acid and 0.16% acetaldehyde were mixed at a 5:1 ratio and added to each well at 120 μ l/well. Subsequently, 200 μ l of 4% DPA/acetic acid were added. The plates were sealed with parafilm and incubated at room temperature overnight. Color development was measured with a microtiterplate reader with absorption wavelength 595 nm and reference wavelength 750 nm.

PSA ELISA. LNCaP cells were seeded at a density of 1×10^5 cells/well in phenol red-free RPMI plus 8% charcoal-stripped serum and incubated for 3 days. Fresh medium (500 μ l) containing the hormones to be tested were added on day 3 and incubated with the cells for 20 h. For the PSA ELISA, 50 μ l of medium from each well were used, and total PSA secreted in the medium was determined using a PSA enzyme immunoassay test kit (ICN Pharmaceuticals) following the manufacturer's protocol. Total PSA secreted in the medium was normalized to total DNA content (measured by DPA DNA assay) in the wells.

RT-PCR. Monolayer LNCaP cells were maintained in phenol red-free RPMI plus 8% charcoal-stripped serum for 3 days and then induced with different concentrations of DHT, DOC, or Dex for 24 h. Total RNA was collected using Ultraspec RNA isolation system (Biotech, Houston, TX), and RT-PCR was carried out with Qiagen One-Step RT-PCR kit (Qiagen, Valencia, CA). Primer sets used in RT-PCR were: β -actin-5': 5'-TGTGATGGTGGGAATGGGTCAG-3', β -actin-3': 5'-TTTGATGTCACGCACGAT-TTC-3', PSA-617: 5'-CCTCCTGAAGAATCGATTCC-3', and PSA-814: 5'-GAGGTCCACACACTGAAGTT-3' (25).

RESULTS

Earlier studies have identified progesterone, estradiol, and certain weak adrenal androgens as potent activators of the AR T877A mutant. In this study, we tested a broader spectrum of compounds for their ability to activate either the wild-type or the T877A mutant AR in transient transfection assays in CV-1 cells using the MMTV-Luc reporter gene. Compounds tested include several adrenal androgens, their precursors and metabolites, and various clinically relevant glucocorticoids. In a pilot screen, we found that many compounds, at a concentration of 1 μ M, could activate the reporter gene in cells transfected with the mutant AR (Fig. 1A) while exhibiting little or no activity in cells transfected with the wild-type AR or with a control plasmid (data not shown). It was surprising to us that the mutant AR could be activated by such a structurally diverse group of ligands. This suggested that resistance to antihormonal treatment may reflect a gain-of-function property of the AR mutant, wherein ligands which would not normally activate AR can do so on the mutant. Of particular importance was the observation that the mutant AR could be activated by a series of naturally occurring and synthetic glucocorticoids. Because both AR and the GR can activate the MMTV-Luc reporter gene, it was important to confirm that the reporter gene was activated by the transfected AR and not by the low level of endogenous GR present in this cell system. This was addressed by assaying the ability of the selected compounds to induce an activating conformational change in the structure of AR. In a previous study, we reported the identification of several short peptides which interact with AR when it is in a transcriptionally active conformation (22).⁴ One of these peptides, D30, contains a leucine-rich LxxLL motif that is often present in coactivators, facilitating their interaction with the receptor ligand-binding domain (26). To evaluate the interaction between the D30 peptide and AR, we made use of a mammalian two-hybrid system. The D30 peptide was fused to the DNA-binding domain of the yeast protein Gal4 (Gal4-DBD), and the AR and its mutant were modified by insertion of a viral acidic activation domain VP16 at their NH₂ terminus. When VP16-AR is recruited to the DNA-bound Gal4DBD-D30 in the cells, it reconstitutes the transcriptional activity of the Gal4-VP16 and drives the expression of a cotransfected reporter gene containing five copies of the Gal4-response elements. Using this assay, we found that the ability of compounds to activate MMTV-Luc, through either AR or AR/T877A, correlates with their ability to effect an activating conformational change within these proteins. With this assay, we confirmed that compounds unexpectedly found to activate MMTV-Luc in our assay system were acting through AR (Fig. 1B).

The potentially important finding that compounds other than androgens could function as activating ligands for the mutant AR prompted us to perform dose-response curves to determine whether the concentrations required for AR activation are within the known physiological/pharmacological range of these compounds. For these studies, we limited analysis to those compounds which were likely to have the most clinical relevance in prostate cancer patients (Table 1). The results of this transcriptional assay indicate that the EC₅₀ for

⁴ Chang, C.-Y. and McDonnell, D. P., unpublished data.

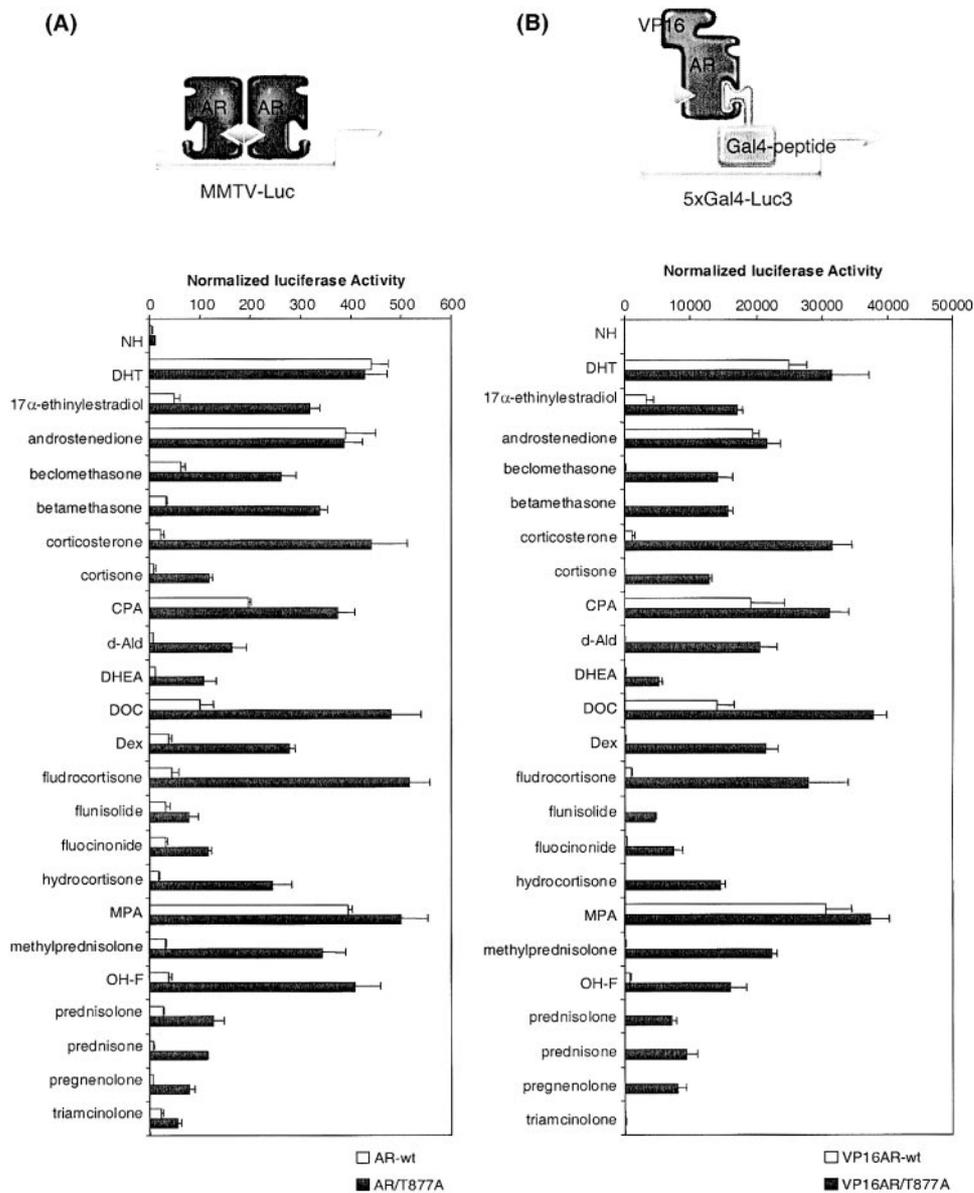


Fig. 1. A large number of structurally diverse compounds can activate the AR/T877A mutant. In A, CV-1 cells were transfected with either the wild-type AR or the AR/T877A mutant expression plasmids and the reporter gene MMTV-Luc together with a normalization vector pCMV- β gal. Transfected cells were induced with $1 \mu\text{M}$ each compound for 16 h, then the luciferase and β -galactosidase activities were measured. In B, CV-1 cells were transfected with the pM-D30 plasmid expressing the D30 peptide fused to the Gal4DBD and either VP16AR-wild type or VP16AR/T877A together with the reporter gene *5xGal4Luc3* and pCMV- β gal. Transfected cells were treated and assayed as in A. NH, no hormone; CPA, cyproterone acetate; *d-Ald*, d-aldosterone; DHEA, dehydroepiandrosterone; MPA, medroxyprogesterone. Normalized luciferase activity was obtained by dividing the luciferase activity by the β -galactosidase activity. The values shown are the mean \pm SD of three determinations. The results shown are representative of multiple experiments performed under the same conditions.

DOC and Dex are 2.8×10^{-10} and 6.4×10^{-8} M, respectively, both being within achievable serum concentrations (Fig. 2). A number of other compounds, including corticosterone and fludrocortisone, are also potent agonists of the mutant AR, exhibiting EC_{50} values of 7.7×10^{-9} and 2.6×10^{-8} M, respectively. Corticosterone and DOC are produced mainly in the zona glomerulosa of the adrenal cortex as intermediary products in the biosynthesis of aldosterone (27). The production of DOC is up-regulated in a number of disease states and is not affected by orchietomy or by the treatment with GnRH agonists or antagonists (28–30). Dex is often used in patients with advanced disease who have failed standard hormonal therapies.

The compounds which showed potential clinical relevance were further analyzed for their ability to up-regulate PSA production in prostate cancer cells. PSA, an AR-regulated, secreted glycoprotein produced mainly in the prostate, is a validated marker used to track the progression of prostate cancer (31). We chose LNCaP cells, a cell line derived from the bone metastasis of a patient with advanced prostate cancer, which carries the T877A mutation, for these studies. Semi-quantitative RT-PCR of mRNA prepared from LNCaP cells treated with DOC and Dex indicated that these two compounds can induce

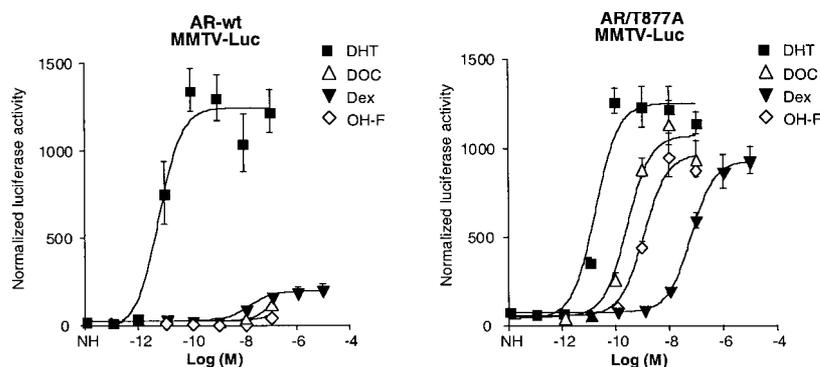
the accumulation of PSA mRNA at concentrations similar to what we determined to be optimal in the reporter gene assays in CV-1 cells (Fig. 3). Furthermore, using an ELISA assay, it was observed that cells treated with these compounds also secreted increased amounts of

Table 1 EC_{50} of different compounds on activation of wild-type and T877A mutant AR^a

Compounds	AR/wild type	AR/T877A
DHT	2.1×10^{-11} M	1.2×10^{-11} M
OHF	$>10^{-7}$ M	1.0×10^{-9} M
Corticosterone	$>10^{-7}$ M	7.7×10^{-9} M
DOC	$>10^{-7}$ M	2.8×10^{-10} M
Aldosterone	$>10^{-7}$ M	$>10^{-7}$ M
Fludrocortisone	$>10^{-7}$ M	2.6×10^{-8} M
Hydrocortisone (cortisol)	$>10^{-7}$ M	$>10^{-7}$ M
Cortisone	$>10^{-7}$ M	$>10^{-7}$ M
Beclomethasone	$>10^{-7}$ M	$>10^{-7}$ M
Betamethasone	$>10^{-7}$ M	$>10^{-7}$ M
Dexamethasone	$>10^{-7}$ M	6.4×10^{-8} M

^a CV-1 cells were transfected with either wild-type or T877A mutant AR expression plasmid together with MMTV-Luc and pCMV- β gal. Different concentrations of hormones (10^{-15} – 10^{-7} M) were added 16 h before assaying. The data were plotted, and EC_{50} was calculated using the software Prism 3 (Graphpad, San Diego, CA).

Fig. 2. CV-1 cells were transfected with expression plasmids for either the wild-type AR or AR/T877A mutant and the reporter gene *MMTV-Luc*, along with a normalization vector pCMV- β gal. Transfected cells were induced with different concentrations of compounds for 16 h, and the luciferase and β -galactosidase activities were measured. Normalized luciferase activity was obtained by dividing the luciferase activity by the β -galactosidase activity. The values shown are the mean \pm SD of three determinations. The results shown are representative of multiple experiments performed under the same conditions.



PSA into the medium when compared with untreated cells (Fig. 4A). Although it has been shown that the LNCaP cells do not express estrogen receptor, progesterone receptor, and GR (32, 33), we included the antiandrogen bicalutamide in the assay to ensure that the response observed was indeed mediated by the AR but not other receptors. We found that cotreatment with 10 μ M bicalutamide was able to significantly suppress the PSA secretion induced by all of the compounds tested, confirming the involvement of AR in this pathway (Fig. 4B). The same concentration of bicalutamide has no inhibitory effect on Dex-induced GR transcriptional activity (Fig. 4C).

Androgens are mitogenic in LNCaP cells (33, 34). We decided, therefore, to determine whether DOC and Dex, two compounds that are potent activators of the AR/T877A mutant, can induce LNCaP cell proliferation. We used a modified DPA assay to measure the total DNA content as an index of cell number. In 6-day proliferation assays, DHT and OH-F induced a \sim 2.5-fold increase in cell number, in agreement with previously published reports (Refs. 32 and 33; Fig. 5A). DOC at concentrations of <1 nM can induce significant cell proliferation with maximum induction being reached at \sim 10 nM. Interestingly, the biphasic dose-response curve seen with DHT and other steroids (32, 33) was not observed when cells were treated with DOC and OH-F. Our studies, thus far, have not produced an explanation for this phenomenon. Dex, although a potent AR agonist in AR/T877A-dependent gene transcription and in induction of PSA secretion, failed to stimulate significant cell proliferation under the conditions of our assay. When we treated the cells with increasing concentrations of Dex in the presence of 0.1 nM DHT, we found that high concentrations of Dex can actually inhibit DHT-induced cell proliferation (Fig. 5B). However, Dex did not inhibit cell proliferation down to the same level as the no hormone-treated control but rather to a level similar to what Dex alone would achieve at that concentration.

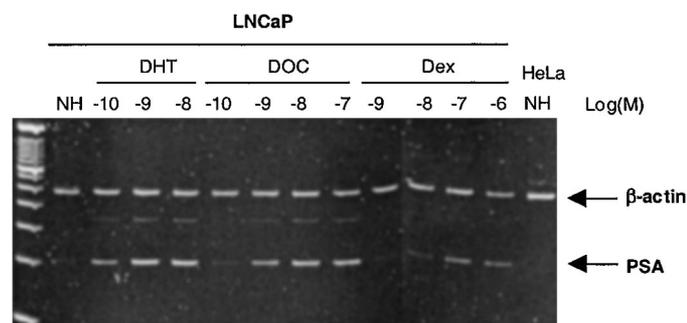


Fig. 3. Both DOC and Dex induce PSA mRNA expression in LNCaP cells. LNCaP cells were maintained in RPMI media containing charcoal-stripped serum for 3 days. Fresh media containing different concentrations of DHT, DOC, and Dex were added to the cells on day 4 and incubated for 24 h. Total RNA was isolated from cells and used in RT-PCR. Primer sets specific for PSA and β -actin produced 214- and 515-bp PCR products, respectively. Total RNA from HeLa cells was used as a negative control.

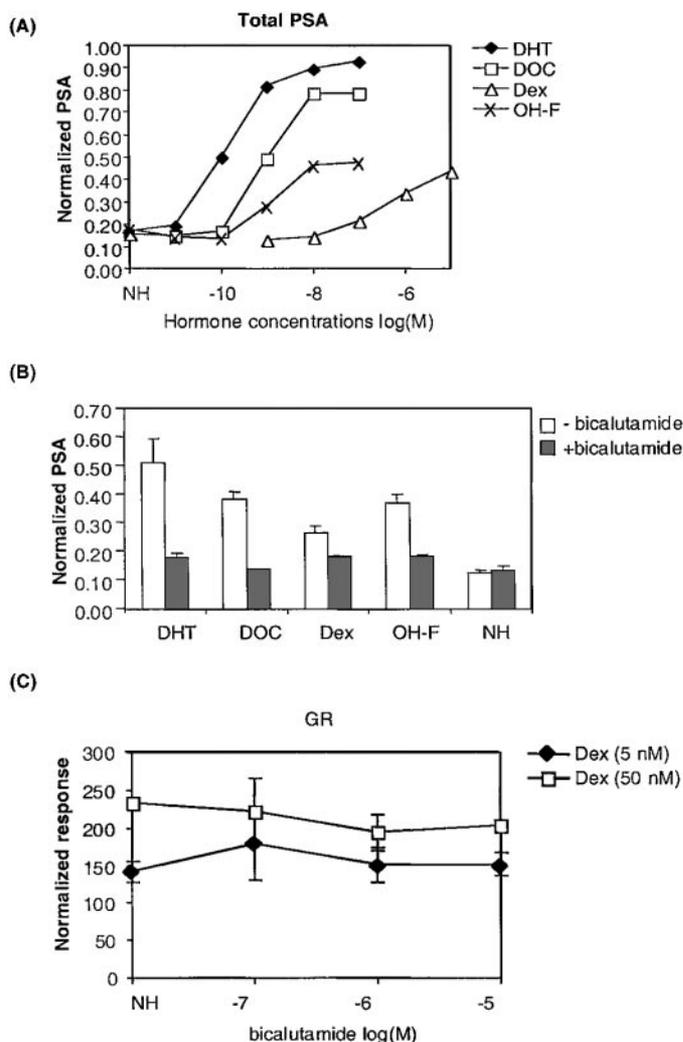


Fig. 4. Both DOC and Dex induced PSA secretion in LNCaP cells. LNCaP cells were maintained in RPMI media containing charcoal-stripped serum for 3 days. In A, fresh media containing different concentrations of DHT, DOC, and Dex were added to the cells on day 4 and incubated for 24 h. Media (50 μ l) was removed from each treatment group and analyzed using a PSA-ELISA assay kit. In B, media containing 1 nM DHT, 10 nM DOC, 5 μ M Dex, or 50 nM OH-F in the presence or absence of 10 μ M bicalutamide were added to the cells and incubated for 24 h. Secreted PSA was measured as in A. Normalized PSA value was determined by dividing the total PSA secreted in the medium by the total DNA content in the well. The values shown are the mean \pm SD of three determinations. In C, CV-1 cells were transfected with GR expression plasmid together with MMTV-Luc and pCMV- β gal. After transfection, cells were treated with various concentrations of bicalutamide in the presence of either 5 or 50 nM Dex. Luciferase and β gal activities were analyzed 16 h after hormone addition.

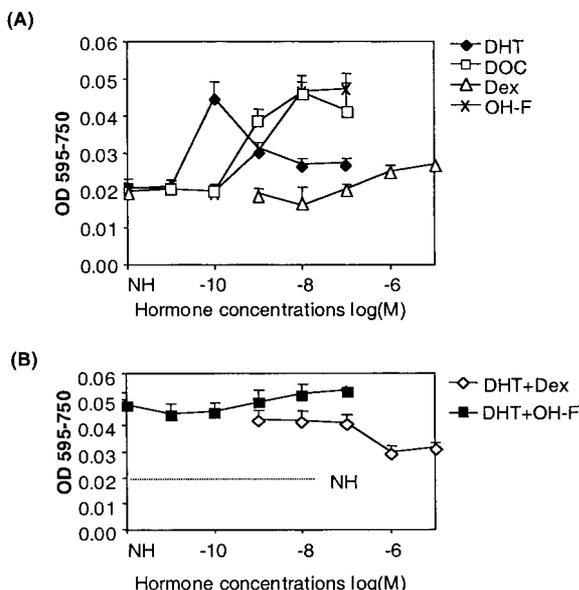


Fig. 5. DOC induces LNCaP cell proliferation. LNCaP cells were maintained in RPMI media containing charcoal-stripped serum for 3 days. In A, cells were treated with media containing different concentrations of DHT, DOC, Dex, and OH-F on day 4, and the media were changed every other day for 6 days. In B, cells were treated with media containing 0.1 nM DHT in the presence of different concentrations of Dex or OH-F on day 4, and the media were changed every other day for 6 days. Cell numbers at the end of the 6-day treatment were determined using a DPA assay. The values shown are the mean \pm SD of three determinations. The results shown are representative of multiple experiments performed under the same conditions.

This indicated to us that with respect to proliferation, Dex functions more like a weak partial agonist.

DISCUSSION

In this study, we have identified a panel of nonandrogenic activators of the AR/T877A mutant, an AR mutant frequently found to occur in late stage prostate cancers. These compounds can activate AR-mediated gene transcription at concentrations that are achievable either in normal physiological states or after administration as a drug. DOC is an endogenous hormone that is normally present in relatively low levels in the circulation, though high enough to activate AR/T877A. DOC is also elevated in certain disease states and after administration of some pharmaceuticals. Of note in this regard is ketoconazole, a drug which was used initially to treat hormone-refractory prostate cancer based on its ability to inhibit C17,20-desmolase and steroid 17 α -hydroxylase, enzymes involved in the biosynthesis of adrenal androgens (35, 36). In addition, ketoconazole inhibits 11- β hydroxylase (37), leading to increased plasma levels of DOC (14-fold) and corticosterone (3-fold) in prostate cancer patients treated with high-dose ketoconazole (38). Therefore, ketoconazole or other mechanistically related pharmaceuticals may have the potential for adverse effects in patients whose tumor characteristics are consistent with those having an AR/T877A mutation. Interestingly, when aminoglutethimide, an agent which inhibits adrenal steroidogenesis, is administered to patients with documented flutamide withdrawal, a response is seen in 48% of patients, whereas <5% respond in the absence of the withdrawal phenomenon (39). This suggests that, in patients who have this mutant receptor, adrenal glucocorticoid intermediates might exert a stimulatory effect.

Dex is used therapeutically in many prostate cancer contexts; it can relieve bone pain, diminish ureteric obstruction, and provide temporary decompression of metastasis to the epidural space compressing the spinal cord in advanced prostate cancer patients. It is also fre-

quently prescribed as a pituitary suppressant to reduce the production of adrenal androgens in patients who have failed hormonal therapy and as an antiemetic in patients undergoing chemotherapy and/or radiation therapy. Our results show that Dex activates the AR/T877A-mediated gene transcription at a dose that is achievable in the clinical setting; however, a much higher concentration of Dex is required to induce PSA secretion and cell proliferation in LNCaP cells. We believe that this may be attributable to the fact that higher levels of AR are produced in the transient transfection system, which allows the dose-response curve to be shifted to the left. Indeed, in the LNCaP cells, Dex does not activate the endogenous AR/T877A mutant-mediated reporter gene expression as efficiently as in the CV-1 system (data not shown). However, the responsiveness to Dex can be restored by increasing expression of an exogenous AR/T877A in LNCaP cells (data not shown). As noted earlier, amplification of AR has been observed in a substantial number of specimens (30%) from patients who have failed AA (9–11). These observations, coupled with our data which shows that the Dex-bound AR/T877A can acquire a transcriptionally active conformation, suggest that Dex may be able to function as an androgen in some prostate cancer cells. Therefore, we speculate that some tumors that have become refractory to prior flutamide treatment may have a less favorable response to subsequent Dex treatment.

Zhao *et al.* (19) have also recently described the loss of androgen-dependence in tumors containing the L701H mutation, which allows the AR to be activated by cortisol and cortisone. Combining our data, one might speculate that in so-called androgen-independent prostate cancer, glucocorticoids may stimulate tumor cell growth by an AR-mediated mechanism.

Our data demonstrate that the T877A mutation allows the AR to be activated by multiple endogenous hormones and pharmaceuticals, therefore circumventing the need for androgens to support tumor cell growth. Certain clinical strategies for treatment of advanced prostate cancer bear reassessment based on this work: (a) the use of TAA therapy; (b) the use of high-dose ketoconazole as second-line hormonal therapy; and (c) the indiscriminate use of Dex in a variety of contexts. The results of this study highlight the complexity of AR pharmacology and reinforce the need to use mechanism-based approaches in the search for new hormonal therapies for the treatment of prostate cancer.

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