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Δ^5-Androstenediol is a natural hormone with androgenic activity in human prostate cancer cells

(ARA70/hydroxyflutamide/casodex/testosterone/7-oxo-DHEA)

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ABSTRACT **It is known that androst-5-ene-3β,17β-diol (Adiol), a precursor of testosterone (T), can activate estrogen target genes. The androgenic activity of Adiol itself, however, is poorly understood. Using a transient transfection assay, we here demonstrate in human prostate cancer cells that Adiol can activate androgen receptor (AR) target genes in the presence of AR, and that AR coactivator ARA70 can further enhance this Adiol-induced AR transcriptional activity. In contrast to this finding, an active metabolite of dehydroepiandrosterone, 7-oxo-dehydroepiandrosterone, does not activate AR target gene in the absence or presence of ARA70. Thin layer chromatography analysis reveals that T, dihydrotestosterone, and 17β-estradiol are undetectable in human prostate cancer DU145 cells after treatment with Adiol. Additionally, a proteolysis assay shows that a distinct ligand-receptor conformational difference exists between T-AR and Adiol-AR. Together, the above findings and the fact that T, but not Adiol, can induce transcriptional activity in a mutant AR (mtAR708), suggest that, without being metabolized into T, Adiol itself may represent a natural hormone with androgenic activity in human prostate cancer cells. Because two potent antiandrogens, hydroxyflutamide (Eulexin), and bicalutamide (casodex), that are widely used for the treatment of prostate cancer, fail to block Adiol-mediated induction of AR transcriptional activity in prostate cancer cells, the effectiveness of so-called "total androgen blockage," a standard treatment for prostate cancer, may need to be reevaluated.**

Androst-5-ene-3β,17β-diol (Δ^5-Androstenediol or Adiol), derived from dehydroepiandrosterone (DHEA) and convertible into testosterone (T) (for detail, see Fig. 1) (1), has been suggested to play a role in the regulation of immune responses (2), obesity (3), and the genesis of estrogen-sensitive carcinomas, such as breast cancer (4, 5). Since 1954 (6), Adiol has been known to have estrogenic activity at physiological concentrations (1, 7). Accordingly, the estrogenic effect of Adiol, which is mediated by the estrogen receptor (ER), has been proposed as an essential female hormone that can partially replace the loss of 17β-estradiol (E2) for postmenopausal women. Using transient transfection assay, Kokontis *et al.* (8) also reported that Adiol may convert to 5α-dihydrotestosterone (5α-DHT) and then induce the androgen target gene in human prostate cancer PC-3 cells. The androgenic activity of Adiol, despite its ultimate conversion to T, is poorly understood, and there is no reported evidence of androgen receptor (AR)-mediated effects of Adiol itself. Using the yeast growth assay, the mammalian two-hybrid system, and a transient transfection assay, we investigated the possible androgenic effect of Adiol in the presence of AR and ARA70. Our results suggest that Adiol itself can activate AR transcriptional activity in human prostate cancer cells, and that ARA70 can further enhance Adiol-mediated activation of the AR.

MATERIALS AND METHODS

Chemicals and Plasmids. Adiol, DHEA, androst-5-ene-7,17-dione (Adione), T, DHT, and E2 were purchased from Sigma, 7-oxo-DHEA was synthesized from DHEA as described (9), trilostane was provided by T.-M. Lin (University of Wisconsin, Madison), hydroxyflutamide (HF, Eulexin), and bicalutamide (casodex) were provided by G. Wilding (University of Wisconsin, Madison). pSG5-wild-type AR (wtAR) and pSG5-ARA70 were constructed as described (10), the two mutant ARs, mtAR877 [codon 877 mutation, threonine to serine, derived from a prostate cancer (11)] and mtAR708 [codon 708 mutation, glutamic acid to lysine, derived from a partial androgen insensitive syndrome patient (12)], were provided by S. P. Balk (Beth Israel Hospital, Boston) and H. Shima (Hyogo Medical College, Japan), respectively. pGAL0-mtAR877 and pGAL0-mtAR708 were constructed by inserting fragments—mtAR877 and mtAR708, respectively—into the pGAL0 vector that contained the GAL4 DNA binding domain, as described (13). Similarly, pGAL4-VP16 that contained the GAL4 DNA binding domain-linked to activation domain (AD) of VP16 was used to construct the ARA70 fusion.

Yeast Growth Assay. Yeast cells (gifts from A. J. Caplan, Mount Sinai Medical Center, New York, NY) (14) transformed with the reporter and expression plasmids were grown at 30°C overnight in -3SD medium (-histidine, -leucine, and -tryptophan) with 25 mM 3-aminotriazole (Sigma) and hormones. Yeast transformations were performed by using the modified lithium acetate transformation procedure (15). The cell density was determined from the OD_{660} value.

Cell Culture, Transfections, and Reporter Gene Expression Assays. Human prostate cancer cell lines, DU145, PC-3, and LNCaP, were maintained in DMEM containing 5% fetal calf serum. Transfections and chloramphenicol acetyltransferase (CAT) assays were performed as described (13). Briefly, 4×10^5 cells were plated on 60-mm dishes 24 h before transfection, and the medium was changed to phenol red free DMEM with 5% charcoal-stripped fetal calf serum 1 h before transfection. The cells were transfected by using the calcium phosphate precipitation method. The total amount of DNA was adjusted

Abbreviations: Adiol or Δ^5-androstenediol, androst-5-ene-3β,17β-diol; DHEA, dehydroepiandrosterone; T, testosterone; ER, estrogen receptor; AR, androgen receptor; E2, 17β-estradiol; Adione, androst-5-ene-7,17-dione; DHT, dihydrotestosterone; HF, hydroxyflutamide; wtAR, wild-type AR; CAT, chloramphenicol acetyltransferase; TLC, thin layer chromatography, MMTV, mouse mammalian tumor virus.

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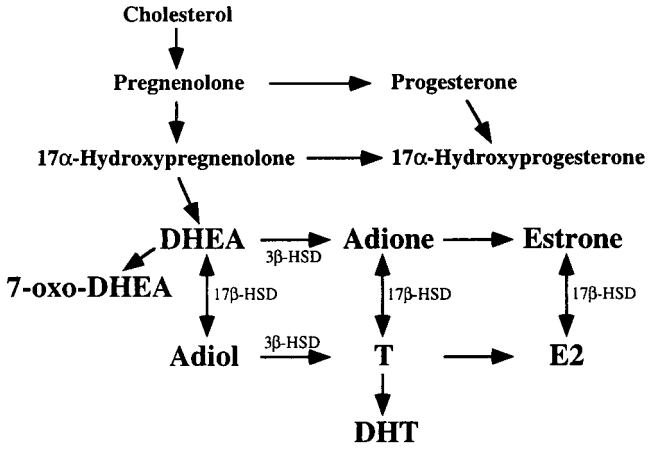

FIG. 1. The steroid biosynthesis pathway involved Adiol and T. 3β-HSD, 3β-hydroxysteroid dehydrogenase.

to 10.5 μg with pSG5 or pVP16 in each transfection assay. Twenty-four hours after transfection, the medium was changed again and the cells were treated with hormones for another 24 h. The cells were then harvested and whole cell extracts were used for CAT assay. Transfection efficiency was normalized by β-galactosidase activity. The CAT activity was quantitated by PhosphorImager (Molecular Dynamics).

Mammalian Two-Hybrid Assay. DU145 cells were transiently cotransfected with a GAL4-hybrid expression plasmid, a VP16-hybrid expression plasmid, and a reporter plasmid pG5CAT. Transfections and CAT assays were performed as described above.

Thin Layer Chromatography (TLC) Analysis. DU145 cells at 4×10^5 cells/dish were cultured for 48 h, and the medium was changed to DMEM with 5% charcoal-stripped fetal calf serum and [^{3}H]Adiol (1 μCi, 23 nM; 1 Ci = 37 GBq) (DuPont/NEN) or [^{3}H]DHEA (1 μCi, 16 nM) (DuPont/NEN). After 24 h, the products were extracted from the medium and cells with diethyl ether and then methanol, as described (16). Aliquots and non-radioactive standard steroids were applied on the same TLC plate. The plate was then exposed twice to toluene: 95% ethanol (9:1). Spots by standard steroids were visualized with specific spray [90% methanol, 5% H_2SO_4, and 5% acetic acid containing p-anisaldehyde (Sigma)], and the running distances were measured. Then, from each lane of the plate 5-mm sections were scraped and radioactivity was determined on the scintillation counter.

Limited Proteolysis Assay. *In vitro* transcriptions/translations were performed in TNT-coupled reticulocyte lysate systems (Promega) in the presence of [^{35}S]methionine. Three-microliter aliquots of labeled translation mixture were incubated for 60 min at room temperature with 3 μl of hormones diluted in water. Then, 1 μl of 25 μg/ml trypsin solution was added, followed by incubation for 15 min at room temperature. The samples were then loaded on 0.1% SDS/12.5% polyacrylamide gel, and autoradiography was carried out as described (13, 17, 18).

RESULTS AND DISCUSSION

Adiol Induces AR Transcriptional Activity. To explore Adiol-mediated AR transcriptional activity, we first investigated the growth of yeast cells containing the human AR and androgen response element (ARE), in the presence of Adiol (Fig. 2A). When treated with 1 nM DHT, the growth of yeast cells that contained wtAR was 3.8× faster than with mock treatment. The presence of 10 nM Adiol stimulated cell growth (2.3-fold increase compared with mock), but 10 nM DHEA did not. We then tested yeast cells that contained mutated AR (mtAR708), which was derived from a partial androgen insensitive syndrome patient, and responds to T and DHT, but not E2, or antiandrogens such as HF (12, 13). When treated with 1 nM DHT, the cells that contained mtAR708 grew as well as those containing wtAR. However, in the presence of Adiol or DHEA, the growth was similar to mock treatment.

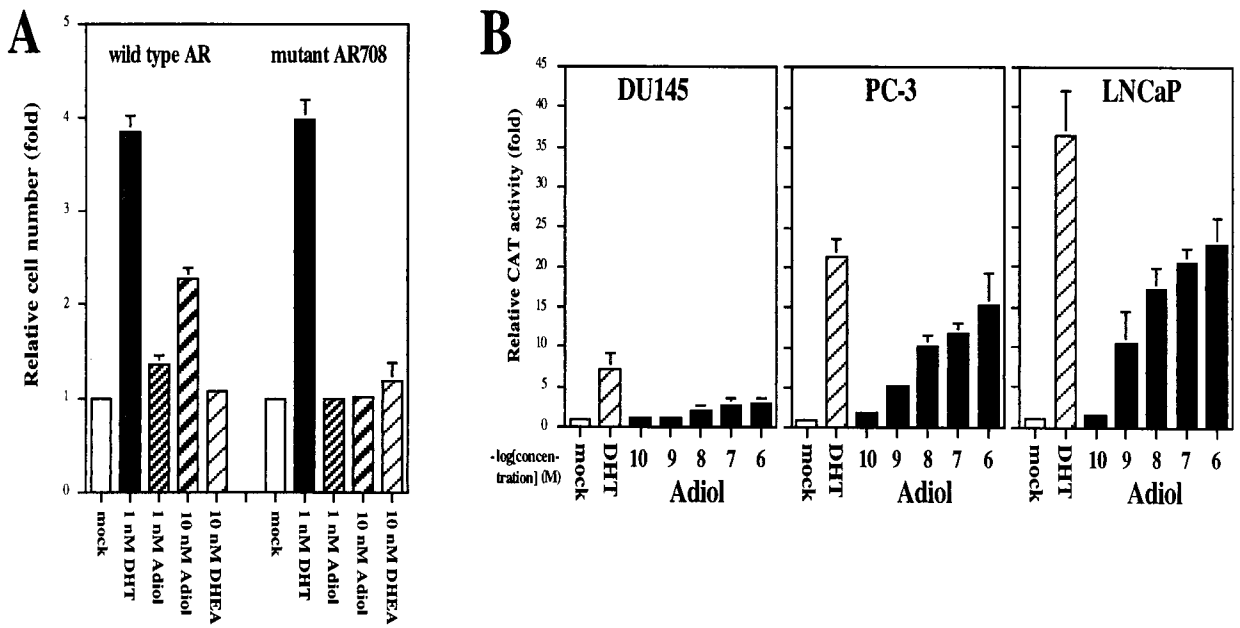

FIG. 2. The effects of Adiol on the growth of yeast cells and on the AR transcriptional activity in human prostate cancer cells. (*A*) Yeast strain, w3031b, transformed with either pG1-wtAR or pG1-mtAR708 and reporter plasmid (pARE-his3) were cultured in -3SD medium (-histidine, -leucine, and -tryptophan) with 25 mM 3-aminotriazole in the absence or presence of hormones as indicated. When the OD_{660} value (X) was between 0.6–1.2, the cell density ($Y \times 10^7$ cells/ml) was calculated by the formula: $Y = 0.276 \times 10^{0.826x}$. Relative cell number represents the means ± SD of three independent experiments. (*B*) DU145, PC-3, or LNCaP cell line was transfected with MMTV-CAT (3.5 μg) and with (for DU145 and PC-3) or without (for LNCaP) wtAR expression plasmid (1.5 μg). Twenty-four hours after transfection, cells were cultured an additional 24 h in the absence or presence of 1 nM DHT or increasing concentrations of Adiol. The CAT activity was determined and the mock treatment was set as 1-fold. Values are the means ± SD of at least three determinations.

The Adiol-induced AR transcriptional activity was further investigated in the prostate cancer cell lines, AR-negative DU145 and PC-3 with cotransfection of an AR plasmid or AR-positive LNCaP carrying endogenous mutated AR (codon 877 mutation, threonine to alanine) (19) without AR plasmid cotransfection. The results were obtained by transient transfection of the ARE-reporter plasmid, mouse mammalian tumor virus (MMTV)-CAT into these cell lines. As shown in Fig. 2*B*, when AR and/or its ARE-reporter were expressed in these 3 cell lines without adding ligands, there was no CAT activity. When treated with 1 nM DHT, CAT activity was induced 7–36-fold over the mock treatment. In the presence of Adiol, CAT activity in DU145 showed only marginal inductions (within 3-fold), whereas the CAT activity in PC-3 or LNCaP was increased up to 15–23-fold in a dose-dependent manner. Interestingly, the induction was detected at the physiological concentration (nM range) of Adiol. Moreover, when MMTV-CAT was replaced with prostate specific antigen-CAT, another AR target gene, similar results were obtained (data not shown). Together, these results indicate that Adiol can activate AR target genes via AR.

ARA70 Enhances Adiol-Induced AR Transcriptional Activity. We have previously reported that ARA70, an AR relatively specific coactivator, can enhance AR transcriptional activity in DU145 cells (10); we thus wanted to know whether ARA70 could enhance Adiol-induced AR transcriptional activity also. As shown in Fig. 3, without cotransfection of ARA70, Adiol can induce the wtAR or mtAR877 transcriptional activity only <3-fold in DU145 cells. However, when ARA70 was cotransfected with wtAR or mtAR877, CAT activities were significantly further induced by Adiol. The level of CAT activity induced was similar to that seen in PC-3 or LNCaP without cotransfection of exogenous ARA70. Similarly, when cotransfected with ARA70 and wtAR or mtAR877, in the presence of DHEA, the inductions can go up to 12-fold in a dose-dependent manner. We also tested the effect of 7-oxo-DHEA, an active metabolite of DHEA that is not metabolically converted to T (9), and could not detect any CAT activity in the presence or absence of ARA70 with AR. These functional differences between two distinct DHEA metabolites (Adiol vs. 7-oxo-DHEA) supplement the previous report that 7-oxo-DHEA is an active metabolite of DHEA (9). When mtAR708 was cotransfected with or without ARA70, at most an only marginal induction was observed in the presence of Adiol, DHEA, or 7-oxo-DHEA. In contrast, when treated with T, transcriptional activity of wtAR or mutants (mtAR877 or mtAR708) was induced 4–8-fold, and ARA70 could enhance the induction by 30–54-fold. This T-mediated induction of mtAR708 transcriptional activity was similar to the effects of DHT-mediated mtAR708 induction as reported (12, 13). The relatively small induction of wtAR or mtAR877 transcriptional activity by DHEA, compared with none by 7-oxo-DHEA, may be due to the metabolic conversion of DHEA to Adiol. (See following section and Fig. 4.)

The Possibility of Conversion of Adiol to T during Transfection. To rule out the possibility that Adiol-induced AR transcriptional activity resulted from the conversion of Adiol to T, four different approaches were applied. (*i*) Trilostane (10 μM; IC_{50} is 1 μM), an inhibitor of 3β-hydroxysteroid dehydrogenase that blocks the conversion of Adiol to T and DHEA to Adione (20, 21), was added in the transfection assay and the results (data not shown) indicated no difference in the presence or absence of this inhibitor. The lack of 3β-hydroxysteroid dehydrogenase in yeast and our demonstration that Adiol-induced AR transcriptional activity in yeast also supports our above finding. (*ii*) TLC analysis (Fig. 4) showed that in a transient transfection assay with or without transfection of AR and ARA70 in DU145 cells, only 7% of [^{3}H]Adiol was

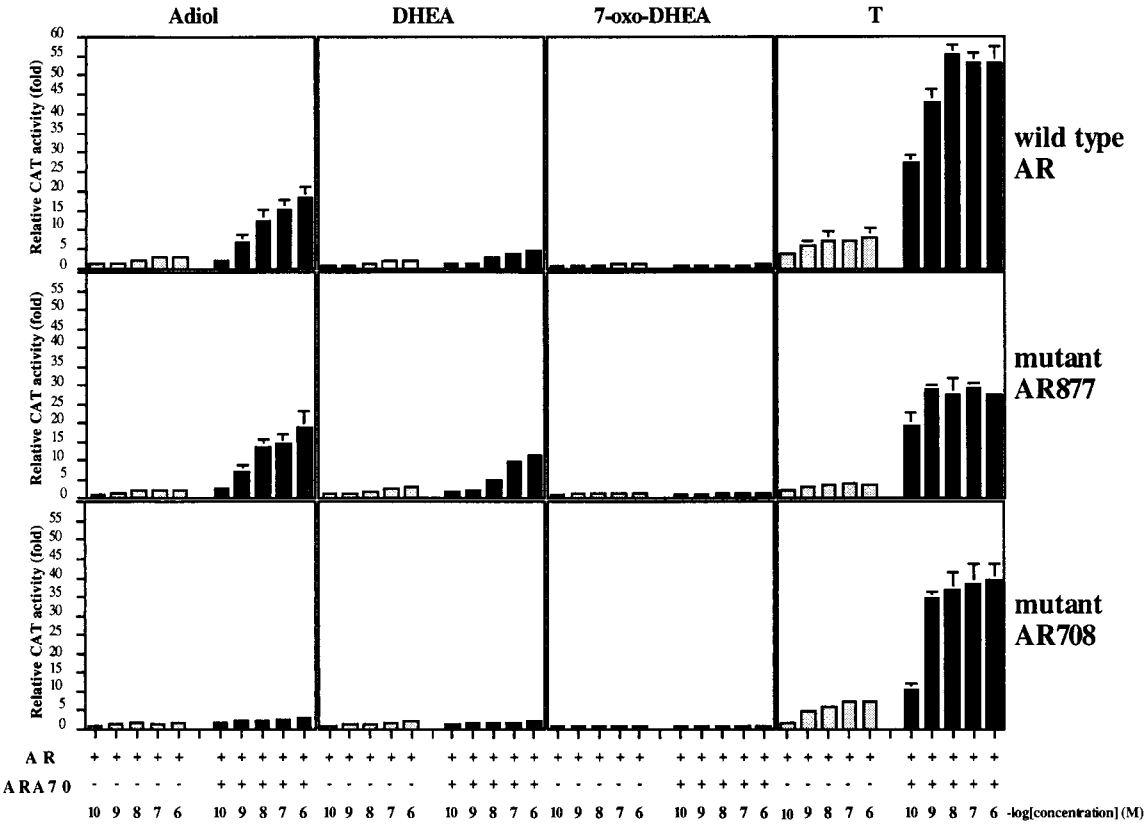

FIG. 3. Activation of AR by Adiol in the presence of an AR coactivator, ARA70, in DU145 cells. DU145 cells first cotransfected with 3.5 μg reporter gene and 1.5 μg AR expression plasmid (wtAR, mtAR877, or mtAR708) with or without 4.5 μg ARA70, were treated with increasing concentrations of Adiol, DHEA, 7-oxo-DHEA, or T. The induction ratios relative to control (mock) are the means ± SD of at least three determinations.

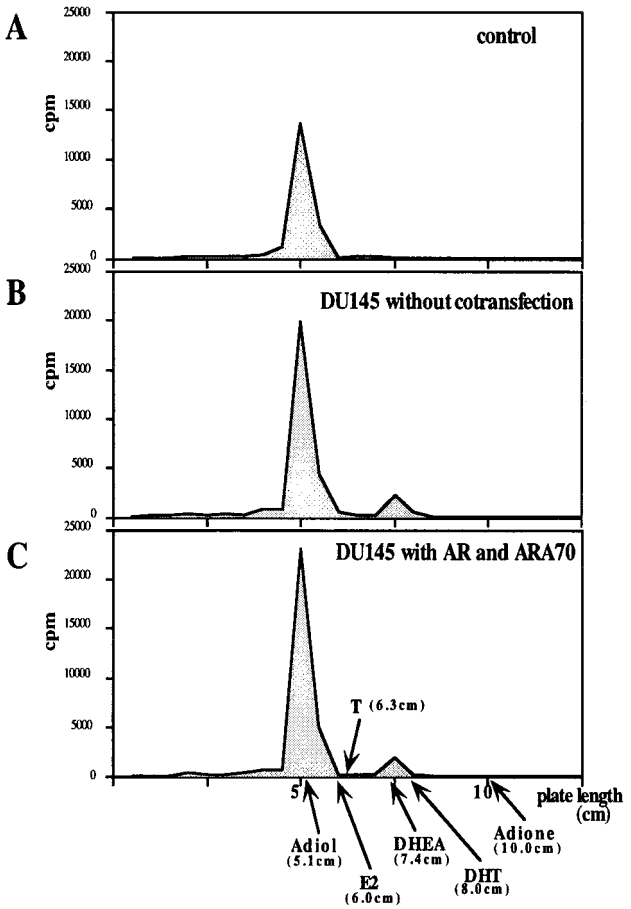

FIG. 4. Metabolite pattern of Adiol in DU145 cells. DU145 cells with (*C*) or without (*B*) cotransfection of AR and ARA70 were incubated with 1 nM [^{3}H]Adiol for 24 h. Steroid metabolites were extracted, dissolved, spotted onto TLC plate, and chromatographed. The plate was then scraped and radioactivity was determined. For the standards, running distances of nonradioactive Adiol, DHEA, Adione, T, DHT, and E2, applied on the same TLC plates, were measured. Control (*A*) was examined without DU145 cells (media only).

converted back to its precursor DHEA. Other Adiol potential metabolites, such as T, E2, DHT, or Adione, were all undetectable. In the same system, 5% of [^{3}H]DHEA could be converted to Adiol, but not other metabolites (data not shown). (*iii*) The results of a limited protease digestion assay (Fig. 5) revealed that, in the presence of T or DHT, only a 29-kDa proteolysis resisting fragment was apparent. In contrast, a specific 35-kDa fragment, as well as the original 29-kDa fragment, was observed in the presence of Adiol. The distinctions between the conformations of the T-AR and Adiol-AR complexes strongly suggest that Adiol-induced AR transactivations are at least structurally different from T-induced AR transactivations. (*iv*) Using wtAR and mtAR708 as comparison, both our yeast growth assay (Fig. 2) and CAT reporter assay (Fig. 3) showed that although T and Adiol can induce wtAR transcriptional activity in the presence of AR and ARA70, only T, but not Adiol, could induce mtAR708 transcriptional activity. This obviously different ability to induce mtAR708 transcriptional activity by T vs. Adiol further strengthens our hypothesis that Adiol has intrinsic androgenic activity, and does not need to be converted to T for this function. With the above four different approaches resulting in similar conclusions, our data therefore strongly support the hypothesis that Adiol-induced AR transcriptional activity may not be due to the conversion of Adiol to T and suggest Adiol itself is a natural hormone with androgenic activity in prostate cancer cells.

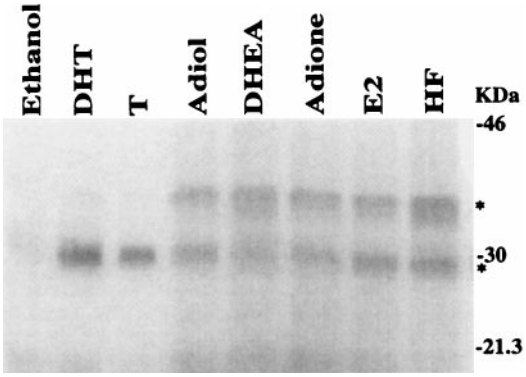

FIG. 5. Conformational changes of the T- or DHT-bound AR vs. Adiol-bound AR. *In vitro* translated AR was incubated with 10 nM DHT, 100 nM T, 10 nM Adiol, 100 nM DHEA, 10 nM Adione, 100 nM E2, 10 μM HF, or 0.01% (vol/vol) ethanol for 60 min at room temperature before limited proteolytic digestion with trypsin. Digestion products were analyzed by electrophoresis on SDS/12.5% polyacrylamide gels and visualized by autoradiography. Trypsin resistant bands are indicated by asterisks. Molecular mass markers are indicated at right.

Adiol-Dependent Interaction between AR and ARA70. To further study the mechanism through which ARA70 enhances Adiol-induced AR transcriptional activity, a mammalian two-hybrid assay was utilized to determine if Adiol can promote the interaction between AR and ARA70. Two fusion plasmids, one of three AR fragments (wtAR, mtAR877, or mtAR708) fused to the GAL4 DNA binding domain, and an ARA70

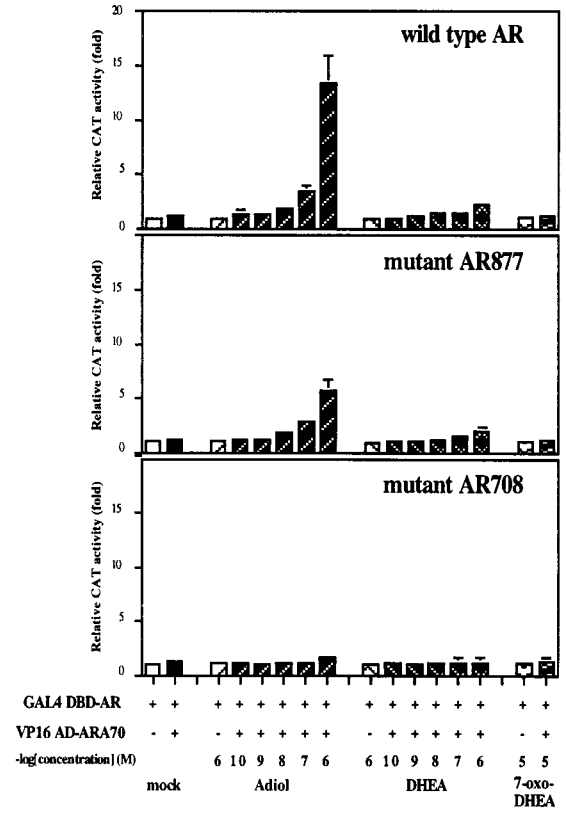

FIG. 6. Dose-dependent physical interaction between AR and ARA70 in the presence of Adiol by mammalian two-hybrid assay. DU145 cells were cotransfected with 3.5 μg pGAL0-wtAR (*A*), 3.5 μg pGAL0-mtAR877 (*B*), or 3.5 μg pGAL0-mtAR708 (*C*), 3.5 μg pVP16-ARA70, and 2.5 μg CAT reporter gene, and treated 24 h with Adiol, DHEA, or 7-oxo-DHEA. Data represent the means ± SD of at least three determinations.

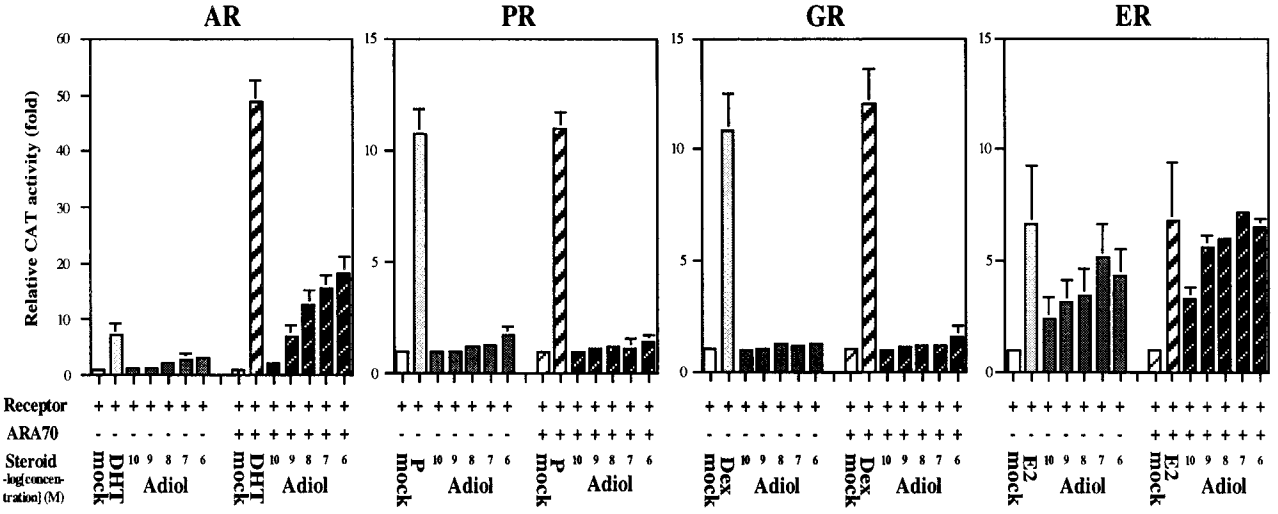

FIG. 7. The effects of Adiol on the transcriptional activity of different steroid receptors. DU145 cells were cotransfected with 1.5 μg receptor and 3.5 μg of its reporter (AR/MMTV-CAT, progesterone receptor (PR)/MMTV-CAT, glucocorticoid receptor (GR)/MMTV-CAT, ER/ERE-CAT) with or without 4.5 μg ARA70, and treated with ligand [1 nM DHT, 10 nM progesterone (P), 10 nM dexamethasone (Dex), or 10 nM E2] or increasing concentrations of Adiol. Values represent the means ± SD of at least three determinations.

fragment fused to the VP16 AD, were coexpressed in DU145 cells with a CAT reporter plasmid. Cells were then treated with Adiol. Thus, the interaction between various AR species and ARA70 in the presence of Adiol could be examined with induced CAT activity. Transient transfection of AR and ARA70 with mock treatment, or transient transfection of either AR or ARA70 with T or Adiol, showed no effect on induction of CAT activity. However, cotransfection of wtAR or mtAR877 with ARA70 increased the CAT activity 3–14-fold in the presence of Adiol (10^{-7}–10^{-6} M) and 2–3-fold in the presence of DHEA (10^{-7}–10^{-6} M) (Fig. 6). In contrast, the addition of a higher concentration (10^{-5} M) of 7-oxo-DHEA showed no activation. As expected, there were no inductions by cotransfection with mtAR708 and ARA70 in the presence of Adiol or DHEA. These results suggest that Adiol itself can promote the interaction between ARA70 and either the wtAR or mtAR877.

The Specificity of Adiol-Induced AR Transcriptional Activity. To evaluate the possibility that Adiol-induced MMTV-CAT activity was mediated by other steroid receptors, such as the progesterone receptor or glucocorticoid receptor, we replaced AR with these receptors in our CAT assay. Because it is known that Adiol can bind directly to the ER and functions as an estrogen (1, 4–7), we also tested the effect of Adiol on ER transcriptional activity using a reporter, ERE-CAT. As shown in Fig. 7, MMTV-CAT activity was induced significantly only via AR, but not progesterone receptor or glucocorticoid receptor, in the presence of ARA70 and Adiol. As expected, ERE-CAT activity could also be induced by Adiol, with the induction fold similar to that achieved by 10^{-8} M E2. These results suggest that Adiol possesses AR-mediated androgenic activity as well as ER-mediated estrogenic activity and can activate the estrogen target gene via ER.

HF and Casodex Fail to Block Adiol-Induced AR Transcriptional Activity. The treatment of advanced stages of prostate cancer, the most commonly diagnosed noncutaneous malignancy in the United States, and the second leading cause of cancer death in North American men, with so-called "total androgen blockage," with a combination of surgical or medical castration with antiandrogen, such as casodex or HF, still has very limited effectiveness (22). Most of the patients will no longer respond to this therapy within 18–24 months of treat-

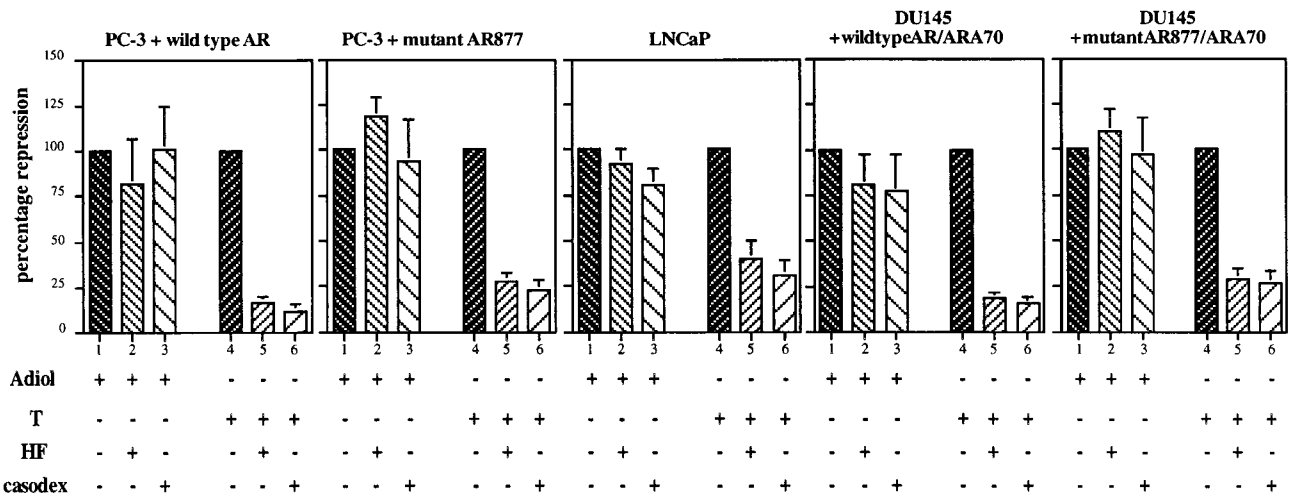

FIG. 8. The effects of antiandrogens on the Adiol-induced transcriptional activity of AR. CAT activity was determined in PC-3 cells transiently cotransfected with wtAR or mtAR877, in DU145 cells with ARA70 and wtAR or mtAR877, or in LNCaP cells without cotransfection of AR expression vector. After transfection, 1 μM HF or 1 μM casodex was added simultaneously with 2 nM Adiol or 1 nM T. The first bars show the activity of Adiol alone or T alone, respectively (set as 100%). Values represent the means ± SD of at least three determinations.

ment. Little or no benefit for the combined approach over castration therapy alone, has been found in patients with regional or metastatic prostate cancer. Labrie *et al.* (23) demonstrated that total androgen blockage caused more than a 90% reduction in serum T level, but only a 26–49% reduction in the serum adrenal androgen level. Blocking the activity of the remaining Adiol (1–2 nM in a patient's serum) may, therefore, be worthy of consideration. To investigate the effects of antiandrogens in conditions mimicking those of patients with advanced prostate cancer, we set up a transient transfection system by using human prostate cancer cell lines. PC-3 and DU145 cotransfected with wtAR or mtAR877, and LNCaP without transfection of the AR, were cultured in media with charcoal-stripped serum, in the presence of 2 nM Adiol plus 1 μM HF or 1 μM casodex. As shown in Fig. 8, these antiandrogens only marginally reduced CAT activities mediated by Adiol, although T-mediated AR transcriptional activity could be reduced significantly. These only marginal reductions were observed irrespective of whether wtAR or mutant AR species were present. This observation suggests that treatment with HF or casodex, in combination with castration may be insufficient to block Adiol's action in AR-positive prostate cancer and may provide a possible explanation for the well documented disappointing clinical findings. This becomes even more of an issue as antiandrogenic therapies, either alone or in combination with castration, have been advocated for earlier stages, and even prevention of prostate cancer (24, 25). The development of new therapeutic approaches that block Adiol's androgenic action, therefore, are worth investigating.

CONCLUSION

In summary, the discovery that Adiol, without conversion to T, has androgenic activity plus the fact that HF and casodex fail to block this Adiol-induced AR transcriptional activity, may not only help us to better understand the molecular mechanisms of Adiol, but may raise critical questions and also open the discussion about the possible role of Adiol in overcoming the effects of androgen ablation therapy for prostate cancer. Because virtually all of the over 39,000 American men who will die of this cancer in 1998 will succumb to disease that is refractory to antiandrogenic therapy, the potential clinical importance of the observations we report here may be considerable.

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1. Adams, J. B. (1985) *Mol. Cell. Endocrinol.* **41,** 1–17.
2. Padgett, D. A. & Loria, R. M. (1994) *J. Immunol.* **153,** 1544–1552.
3. Azziz, R., Potter, H. D., Bradley, E. L., Jr., & Boots, L. R. (1994) *Fertil. Steril.* **62,** 321–326.
4. Adams, J., Garcia, M. & Rochefort, H. (1981) *Cancer Res.* **41,** 4720–4726.
5. Poulin, R. & Labrie, F. (1986) *Cancer Res.* **46,** 4933–4937.
6. Huggins, C., Jensen, E. V. & Cleveland, A. S. (1954) *J. Exp. Med.* **100,** 225–240.
7. Poortman, J., Prenen, J. A. C., Schwarz, F. & Thijssen, J. H. H. (1975) *J. Clin. Endocrinol. Metab.* **40,** 373–379.
8. Kokontis, J., Ito, K., Hiipakka, R. A. & Laio, S. (1991) *Receptor* **1,** 271–279.
9. Lardy, H., Partridge, B., Kneer, N. & Wei, Y. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 6617–6619.
10. Yeh, S. & Chang, C. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5517–5521.
11. Taplin, M.-E., Bubley, G. J., Shuster, T. D., Frantz, M. E., Spooner, A. E., Ogata, G. K., Keer, H. N. & Balk, S. P. (1995) *N. Engl. J. Med.* **332,** 1393–1398.
12. Yeh, S., Miyamoto, H., Shima, H. & Chang, C. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 5527–5532.
13. Miyamoto, H., Yeh, S., Wilding, G. & Chang, C. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 7379–7384.
14. Caplan, A. J., Langley, E., Wilson, E. M. & Vidal, J. (1995) *J. Biol. Chem.* **270,** 5251–5257.
15. Gietz, D., Jean, A. S., Woods, R. A. & Schiestl, R. H. (1992) *Nucleic Acids Res.* **20,** 1425.
16. Lin, T.-M. & Chang, C. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 4988–4993.
17. Allan, G. F., Leng, X., Tsai, S. Y., Weigel, N. L., Edwards, D. P., Tsai, M.-J. & O'Malley, B. W. (1992) *J. Biol. Chem.* **267,** 19513–19520.
18. Kuil, C. W., Berrevoets, C. A. & Mulder, E. (1995) *J. Biol. Chem.* **270,** 27569–27576.
19. Veldacholte, J., Ris-Staplers, C., Kuiper, G. G. J. M., Jenster, G., Berrevoets, C., Claassen, E., van Rooij, H. C. J., Trapman, J., Brinkmann, A. O. & Mulder, E. (1990) *Biochem. Biophys. Res. Commun.* **173,** 534–540.
20. Potts, G. O., Creange, J. E., Harding, H. R. & Schane, H. P. (1978) *Steroids* **32,** 257–267.
21. Honma, S., Takezawa, Y. & Yamanaka, H. (1995) *Nippon Naibunpi Gakkai Zasshi-Folia Endocrinol. Jap.* **71,** 679–694.
22. Prostate Cancer Trialists' Collaborative Group. (1995) *Lancet* **346,** 265–269.
23. Labrie, F., Dupont, A., Giguere, M., Borsanyi, J.-P., Lacourciere, Y., Monfette, G., Emond, J. & Bergeron, N. (1988) *Br. J. Urol.* **61,** 341–346.
24. Corn, B., Winter, K. & Pilepich, M. (1998) *J. Urol.* **159,** 337A.
25. Pilepich, M. V., Caplan, R., Byhardt, R. W., Lawton, C. A., Gallaher, M. J., Mesic, J. B., Hanks, G. E., Coughlin, C. T. & Porter, A. (1995) *Int. J. Radiat. Oncol. Biol. Phys.* **32** (Suppl.), 188.