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Regulation of Steroid Glucuronosyltransferase Activities and Transcripts by Androgen in the Human Prostatic Cancer LNCaP Cell Line*

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ABSTRACT

Although much attention has been focused on the synthesis of dihydrotestosterone (DHT), the inactivation and elimination of active androgens can also be key points in regulating androgen levels in tissues such as the prostate. Recent data suggest that 5α-reduced C_{19} steroids can be converted to glucuronide derivatives in the human prostate, leading to complete inactivation of these steroids. These results are supported by the recent finding of at least two steroid uridine diphosphoglucuronosyltransferase (UGT) enzymes in the prostate as well as in the human prostatic cancer LNCaP cell line.

To ascertain the role of UGTs in regulating active steroid levels, we investigated the modulation of UGT levels in response to steroid treatments in LNCaP cells. Results demonstrate the down-regulation of UGT activities specific for 3-hydroxysteroids and 17-hydroxysteroids after treatment with androgens and estrogens. Treating the cells with DHT or R1881 for 7 days inhibited UGT activity by 60%; however, 80% of the total activity was recovered after 5 days in the absence of the androgens. The inhibition of UGT activities by DHT and R1881 increases with the time of incubation and with increasing concentrations of the androgens used. The decrease in UGT enzyme activity occurred in parallel with a diminution in UGT transcript levels, as observed in Northern blot analyses. A correlation between the effect of steroids on the androgen-dependent growth response of LNCaP cells, the secretion of prostate-specific antigen, and the inhibition of UGT activities was clearly demonstrated, which implicates the androgen signaling pathway. Treating cells with Casodex, an androgen antagonist that binds the mutated androgen receptor expressed in LNCaP cells, partially blocked the androgen- and estrogen-induced decrease in UGT activity, suggesting that the regulation of UGT levels involves the androgen receptor.

In addition to the formation of DHT, the inactivation of steroids by glucuronidation, which is regulated by steroids themselves, is an important mechanism controling the level of androgens in the prostate. (*Endocrinology* **137:** 2872–2879, 1996)

DIHYDROTESTOSTERONE (DHT), the major androgen in the human prostate, plays an important role as a modulator of several parameters of this tissue, including its growth and secretion of a variety of proteins, such as prostate-specific antigen (PSA) (1–3). Moreover, DHT is a determining factor in benign prostatic hyperplasia and prostate cancer (4–8). The action of DHT in the prostate depends upon several factors, including the androgen receptor machinery, the amount of steroid precursors in the circulation, and the production and catabolism of DHT in the prostate.

In human adult males, the adrenals and testicles contribute to the production of DHT in steroid target tissues (9–11); however, these tissues secrete very low amounts of DHT into the circulation, and the plasma DHT levels are extremely low (12–14). In addition to 5α-reductase, which converts testosterone (TESTO) to DHT, it is now well accepted that the human prostate contains 3β-hydroxysteroid dehydrogenase 4-ene-5-ene isomerase, which is responsible for the transformation of dehydroepiandrosterone (DHEA) to TESTO (15–17). Despite a marked decrease in the secretion of adrenal DHEA during aging in the human, approximately 40–50% of DHT in the prostate of men between 60–70 yr of age is produced from adrenal C_{19} steroids (18).

The human prostate also contains several DHT-transforming enzymes, such as 3β-hydroxysteroid dehydrogenase (3βHSD), 3αHSD, and 17βHSD, which induce the formation of several 5α-reduced C_{19} steroid metabolites, such as androsterone (ADT) and androstane-3α,17β-diol (3α-DIOL) (3, 16, 19–21). However, DHT is the predominant unconjugated 5α-reduced C_{19} steroid found in the human prostate, and the levels of ADT or 3α-DIOL reach only 10–20% those of DHT (22). Although the metabolism of DHT into other 5α-reduced C_{19} steroids markedly reduces the androgenic activity due to their low affinity for the androgen receptor, it must be taken into account that 17βHSD, 3βHSD, and 3αHSD do not irreversibly metabolize DHT (Fig. 1). Recently, the presence of a steroid uridine diphosphoglucuronosyltransferase (UGT) that converts C_{19} steroids into polar derivatives has been reported in the human prostate (23, 24). Therefore, the glucuronidation of DHT or its unconjugated metabolites, such as ADT and 3α-DIOL, may markedly alter the levels of active androgens in the prostate because this enzymatic reaction is irreversible.

To demonstrate the role of steroid UGT enzymes in the prostate, we recently studied these conjugating enzymes in LNCaP cells, a human prostatic adenocarcinoma cell line that expresses the androgen receptor and exhibits androgen-dependent proliferation (25–29). We have shown that LNCaP cells, similar to the human prostate, are capable of converting

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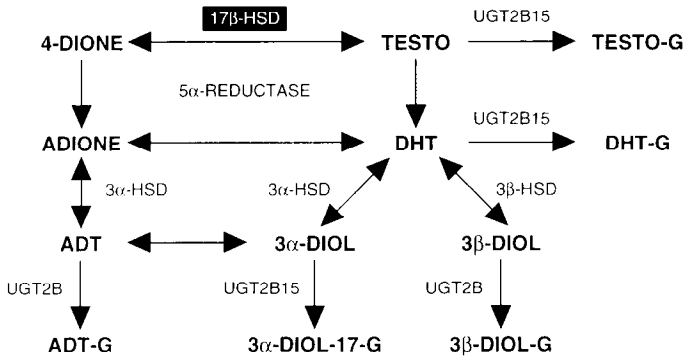

FIG. 1. Metabolism of C_{19} steroids in LNCaP cells.

5α-reduced C_{19} steroids into glucuronide conjugates and of expressing UGT transcripts such as UGT2B15 (20, 24, 30–32), which was isolated from human liver and is responsible for the glucuronidation of 17-hydroxysteroids such as TESTO, DHT, and 3α-DIOL (23). Moreover, we recently demonstrated that the glucuronidation of DHT is elevated in LNCaP cells with a low androgen response, whereas LNCaP cells with a higher androgen response display a lower level of DHT glucuronidation (31).

In the present study, we investigated the effects of androgens on the modulation of UGT activity in LNCaP cells. Although evidence exists that UGT activity present in liver may be regulated by several exogenous compounds (33), no study has been performed to address the effect of steroids on steroid UGT activity in extrahepatic tissues. This study shows, for the first time, that the UGT activities specific for 3-hydroxysteroids and 17-hydroxysteroids and UGT transcript levels are regulated by androgens.

Materials and Methods

Materials

[9,11-^{3}H]Androsterone (59 Ci/mmol) and R1881 (methyltrienolone) were obtained from New England Nuclear-DuPont (Boston, MA), and [1,2-^{3}H]DHT (47 Ci/mmol) was purchased from Amersham (Oakville, Canada). All nonradioactive steroids used for cell treatments were purchased from Steraloids (Wilton, NH). ICI 176 334 (Casodex) was obtained from ICI Pharmaceuticals (Macclesfield, UK). Cycloheximide was purchased from Boehringer Mannheim (Laval, Canada). RPMI 1640 medium was obtained from Sigma Chemical Co. (St. Louis, MO), and FBS was purchased from Immunocorp (Montreal, Canada).

LNCaP cell line

The LNCaP cell line from American Type Culture Collection (Rockville, MD) was obtained at passage 20 (34). The cell line was routinely maintained as monolayer cultures in RPMI 1640 phenol red-free medium supplemented with 10% (vol/vol) FBS, 2 mM glutamine, and antibiotics (100 IU penicillin/ml and 100 μg streptomycin/ml) and kept in a humidified atmosphere of 5% CO_2 in air at 37 C. The cells were subcultivated at weekly intervals using a mixture of 0.05% trypsin and 0.01% EDTA.

Treatments of cells

Cell cultures between passages 21–28 were plated at the indicated density in 24-well plastic plates previously treated for 24 h with poly-L-lysine. Cells were grown for 48 h to allow for adhesion. For steroid treatments, RPMI-1640 medium containing the added exogenous steroid and 2% (vol/vol) FBS, which had been treated twice with dextran-coated charcoal to remove endogenous steroids, was used in each experiment. Exogenous steroids were added to the medium as an ethanolic stock solution to obtain a final ethanol concentration of 0.1%. Medium was changed every 2 days, and at the end of treatments, cells were washed with fresh medium, and labeled substrate diluted in medium was added for a period of 3 h. The medium was then carefully removed and immediately stored in a freezer (−20 C) until measurement of glucuronide formation. For studies of cell proliferation, 150 μl methanol were added to the cells, and plates were left to dry at room temperature in the absence of light. The DNA content, which represents the number of cells, was determined as previously described (21) and used to normalize glucuronide formation and PSA secretion. The amount of PSA secreted in the medium during the last 48 h of treatment was measured as previously described (35).

Steroid glucuronide analysis

The formation of steroid glucuronides was determined as previously described (21), using HPLC, which allows for the separation of unconjugated from conjugated steroids. The identification of steroid glucuronides formed was performed by liquid chromatography ion spray mass spectrometry (21).

Northern blot

Total RNA was isolated by the trireagent acid-phenol method and quantified by optical density. Twenty micrograms of total RNA were separated on a 1% agarose gel and transferred to a Hybond-N membrane (Amersham, Oakville, Canada). A solution containing 40% formamide, 5 × Denhardt's solution, 5 × SSPE, 0.1% SDS, and 100 μg salmon sperm DNA was used for prehybridization and hybridization. Prehybridization was performed for 12 h at 42 C. Hybridization was performed in the presence of a radiolabeled full-length UGT2B15 complementary DNA (cDNA) probe (10^6 cpm/ml) for 24 h at 42 C. The probe was radiolabeled by the random hexamer primer technique with [α-^{32}P]deoxy-CTP as previously described (24). The blot was then washed twice in a solution of 0.1 × SSC-0.1% SDS at room temperature for 15 min followed by another two washes of 15 min each at 55 C. The blot was exposed for 10 days at −80 C on XAR film with an intensifying screen (Eastman Kodak, Rochester, NY). The resulting autoradiograph was analyzed by densitometry using the Amersham RAS image analyzer system. Results of the hybridization with a glyceraldehyde-3-phosphate dehydrogenase cDNA probe were used for normalization.

Statistics

Dose-response curves and IC_{50} values were calculated using a weighted iterative nonlinear least squares regression. Statistical significance was determined according to the multiple range test of Kramer (36) and Student's *t* test.

Results

Treatments of LNCaP cells with androgens and estrogens at concentrations of 1 and 1000 nM were performed for 10 days, after which the glucuronidation of DHT and ADT were determined. The glucuronidation of DHT and ADT corresponds to the conjugation of the hydroxyl group at positions 17 and 3, respectively, and previous results have shown that for the period of time studied, glucuronidation was the unique transformation of the steroid observed. The basal glucuronidation of DHT was 1.45 ± 0.02 pmol glucuronide/μg DNA·3 h, whereas the basal glucuronidation of ADT was 1.29 ± 0.02 pmol of glucuronide/μg DNA·3 h. Our results show that several steroids are able to cause an inhibition of UGT activity for both substrates, and the degree of

decrease is related to the relative binding affinity of the inhibitor for the androgen receptor (Table 1).

The modulation of cell growth and PSA secretion in response to the steroid treatments was also determined to ascertain the androgenicity of the various steroids used in our studies (Table 1). Cell proliferation was stimulated at a physiological concentration (1 nM) of androst-5-ene-3β,17β-diol (5-DIOL), androstane-3β,17β-diol (3β-DIOL), TESTO, DHT, and R1881; however, a supraphysiological concentration (1 μM) of DHEA, ADT, androstenedione (DIONE), and 3α-DIOL was required for stimulation of cell growth. In contrast, treating cells with the supraphysiological concentration of 3β-DIOL, TESTO, DHT, and R1881 had no effect and in some cases decreased cell proliferation. This inhibitory effect on cell proliferation observed at high concentrations of steroid treatment corresponds to the well described characteristic biphasic growth response of LNCaP cells (26, 27, 31, 32). A positive correlation can be observed between the stimulation of LNCaP cell proliferation after steroid treatment (1 nM) and the inhibitory effect on UGT activities for DHT ($r^2 = 0.86$; $P < 0.001$) and ADT ($r^2 = 0.83$; $P < 0.001$; Table 1). A significant correlation can also be demonstrated between the affinity of steroids for the androgen receptor and inhibition of the UGT activity for DHT and ADT after steroid treatments, with r^2 values of 0.75 ($P < 0.001$) and 0.77 ($P < 0.001$), respectively. PSA secretion was also increased by treating the cells with 5-DIOL, 3β-DIOL, TESTO, or DHT (Table 1).

Knowing that estrogens can also stimulate proliferation of LNCaP cells via the mutated androgen receptor (27), we also treated LNCaP cells with estrone, estradiol, or estriol. As illustrated in Table 1, treating the cells with 1 nM estradiol inhibited the glucuronidation of DHT and ADT by 18.7% and 19.9%, respectively, whereas estrone only decreased glucuronidation at 1000 nM. In contrast, estriol had no effect on glucuronidation, cell proliferation, or PSA secretion at the two concentrations used.

To further investigate the inhibitory effect of androgens on steroid glucuronidation, we treated LNCaP cells with increasing concentrations of DHT or R1881 in a dose-response study. As illustrated in Fig. 2A, DNA content was stimulated by increasing concentrations of DHT or R1881 ranging from 0.01–0.5 nM. With all treatments, the cells responded in the characteristic biphasic pattern, as shown in Fig. 2A. When the effect on the glucuronidation of DHT and ADT was determined after these treatments, it was clearly demonstrated that increasing concentrations of DHT or R1881 led to a decreased formation of DHT glucuronide (DHT-G) and ADT glucuronide (ADT-G; Fig. 2B). The effect of the androgens on the conjugation of DHT was observed with an IC_{50} of 0.5 nM; no significant difference was observed between 3-hydroxysteroid-UGT (3-OH-UGT) and 17-hydroxysteroid-UGT (17-OH-UGT) activities. It is apparent that the stimulation of proliferation of LNCaP cells by DHT and R1881 (≤0.5 nM) is correlated to their inhibitory effect on glucuronidation, where the r^2 values were 0.68 ($P < 0.05$) and 0.70 ($P < 0.05$) for DHT and R1881, respectively. The effects of the androgens on the secretion of PSA were also determined; they increased secretion in a dose-dependent manner (data not shown).

Treating LNCaP cells with 1 nM DHT or R1881 for increasing periods of time demonstrated an increased inhibition of steroid glucuronidation. The results show no significant inhibitory effect on DHT-G formation before 48 h of treatment (Fig. 3); however, 2 days of treatment with DHT or R1881 led to an inhibition of DHT-G formation ranging from

TABLE 1. Correlation among stimulation of LNCaP cell proliferation, secretion of PSA, and inhibition of dihydrotestosterone glucuronide (17-OH-UGT) and androsterone glucuronide (3-OH-UGT) formation

Steroid treatments (10 days)	Proliferation of DNA (μg/well);[a] *control = 1.46 ± 0.09*		PSA secretion (ng/ml · 48 h);[b] *control = 0.10 ± 0.05*		17-OH-UGT inhibition of control (%)[a,c]		3-OH-UGT inhibition of control (%)[a,c]		Relative binding affinity for androgen receptor; DHT = 100%[d]
	1 nM	1 μM	1 nM	1 μM	1 nM	1 μM	1 nM	1 μM	
Androgens									
DHEA	1.80 ± 0.15[e]	2.64 ± 0.12[f]	0.70 ± 0.11[f]	3.07 ± 0.42[f]	13.0 ± 0.1[f]	18.2 ± 5.8[f]	9.7 ± 0.9[f]	23.5 ± 2.2[f]	ND
ADT	1.98 ± 0.09[f]	2.98 ± 0.12[f]	0.13 ± 0.04	13.63 ± 1.24[f]	24.3 ± 2.1[f]	38.8 ± 4.1[f]	25.7 ± 0.9[f]	47.8 ± 4.4[f]	0.6
DIONE	1.52 ± 0.03	2.63 ± 0.17[f]	0.13 ± 0.03	10.80 ± 1.28[f]	4.4 ± 1.3	44.2 ± 2.8[f]	ND	43.4 ± 4.1[f]	0.7
3α-DIOL	1.81 ± 0.08[e]	2.37 ± 0.10[f]	0.50 ± 0.40	9.20 ± 0.38[f]	8.2 ± 0.6[e]	60.3 ± 6.8[f]	5.2 ± 3.6	67.1 ± 5.2[f]	1.5
5-DIOL	2.92 ± 0.11[f]	2.25 ± 0.17[f]	8.87 ± 1.19[f]	10.83 ± 0.52[f]	33.8 ± 0.9[f]	46.1 ± 2.4[f]	31.2 ± 0.6[f]	43.9 ± 4.6[f]	6.6
3β-DIOL	2.64 ± 0.14[f]	1.88 ± 0.07[f]	8.20 ± 0.37[f]	10.05 ± 0.72[f]	54.6 ± 4.8[f]	65.7 ± 4.9[f]	52.6 ± 4.5[f]	68.8 ± 7.5[f]	41
TESTO	2.72 ± 0.08[f]	1.58 ± 0.12	9.47 ± 0.61[f]	11.08 ± 0.98[f]	63.7 ± 6.4[f]	68.1 ± 6.2[f]	59.5 ± 2.8[f]	76.8 ± 3.6[f]	43
DHT	3.08 ± 0.11[f]	1.38 ± 0.16	10.03 ± 1.16[f]	11.53 ± 1.19[f]	58.7 ± 5.2[f]	83.5 ± 3.9[f]	59.9 ± 7.4[f]	83.3 ± 3.8[f]	100
R1881	3.13 ± 0.09[f]	1.43 ± 0.09	10.53 ± 1.42[f]	11.67 ± 0.68[f]	66.6 ± 10.4[f]	88.4 ± 9.2[f]	63.1 ± 1.8[f]	86.4 ± 5.2[f]	100
Estrogens									
Estrone	1.45 ± 0.07	2.41 ± 0.03[f]	0.10 ± 0.20	9.85 ± 0.95[f]	8.4 ± 1.2[e]	19.1 ± 1.4[f]	7.4 ± 1.1[e]	14.2 ± 1.0[f]	ND
Estradiol	1.96 ± 0.05[f]	2.47 ± 0.08[f]	8.43 ± 0.19[f]	12.80 ± 2.54[f]	18.7 ± 8.2[f]	42.7 ± 2.7[f]	19.9 ± 2.2[f]	32.7 ± 1.1[f]	24
Estriol	1.46 ± 0.07	1.62 ± 0.14	0.10 ± 0.22	1.76 ± 0.12[f]	ND	ND	ND	7.4 ± 4.4	ND

DHEA, Dehydroepiandrosterone; ADT, androsterone; DIONE, androstenedione; 3α-DIOL, androstane-3α,17β-diol; 5-DIOL, androst-5-ene-3β,17β-diol; 3α-DIOL, androstane-3β,17β-diol; TESTO, testosterone; DHT, dihydrotestosterone; R1881, methyltrienolone. ND, Not detectable.

[a] Proliferation and determination of steroid glucuronidation are expressed as the mean ± SEM of three experiments for DHT and R1881 and two experiments for other steroids; each experiment was performed in triplicate.

[b] Duplicate determinations of PSA secretion were made for each of three samples in one experiment.

[c] Not detectable (<3%).

[d] See Ref. 28.

[e] $P < 0.05$ *vs.* control.

[f] $P < 0.01$ *vs.* control.

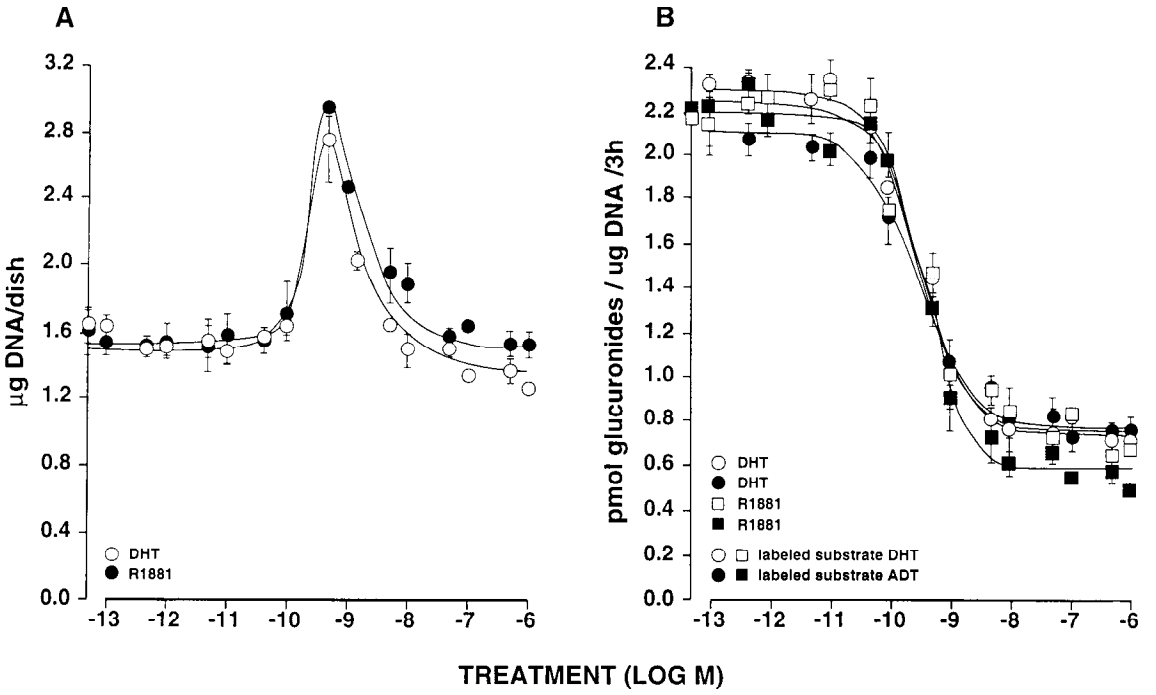

FIG. 2. Effects of increasing androgen concentration on LNCaP cell proliferation (A) and the formation of androgen glucuronides (B). Cells were cultured at a density of 12,500 cells/well for 2 days before treatment with DHT or R1881 in increasing concentrations from 10^{-13}-10^{-6} M for 10 days. After treatment, cells were washed with fresh medium and then incubated in the presence of 10 nM [^{3}H]DHT and [^{3}H]ADT for 3 h. DNA content and glucuronide formation were measured as described in *Materials and Methods*. Values represent the mean ± SEM of triplicate incubations in one experiment. The experiment was performed three times, and similar results were obtained.

10–22%. This inhibitory effect was shown to be time dependent, reaching an inhibition of 50–60% in the presence of either androgen for 12 days. Similar results were observed when ADT was used as substrate, demonstrating an inhibition of ADT-G formation (Fig. 3). To determine the half-life of the UGT enzyme activity, cells were treated with a noncytotoxic concentration of a protein synthesis inhibitor, cycloheximide (25 µg/ml), for 30 min to 24 h. The results illustrated in Fig. 4 show a gradual decrease in UGT activity; however, the inhibition obtained after 24 h was only 30%, suggesting the high stability of the UGT protein.

To determine whether the inhibitory effect on steroid glucuronidation was reversible in LNCaP cells, we studied the recovery of the UGT activities after androgen treatment. Cells were treated for 8 days with 1 nM DHT, after which the androgen was removed, and UGT activities were measured daily for 5 days (Fig. 5). As shown previously, long term treatment with the androgen led to an inhibition of DHT and ADT glucuronidation by 60%. Recovery of UGT activities was observed after 48 h, where 50% of the lost UGT activities were recovered after 72 h. Incubation of the cells in steroid-free medium for 5 days after treatment led to a near recovery of UGT activities. No significant difference was observed between the recoveries of 3-OH-UGT and 17-OH-UGT activities.

As mentioned above, the inhibitory effect of androgens on UGT activities is correlated to their affinity for the androgen receptor. To further demonstrate the involvement of the androgen receptor, cells were treated with Casodex, which was previously shown to be an androgen antagonist that binds the mutated androgen receptor expressed in LNCaP cells (37–42). The results indicate that glucuronidation of DHT was stimulated when cells were treated with 5 µM Casodex for 6 days; DHT-G formation was increased by 11.4% (Fig. 6). The results of treating cells with DHT (0.1, 0.5, and 1 nM) and Casodex (5 µM) showed that the antiandrogen can partially block the inhibitory effect of DHT on UGT activity (Fig. 6). It was previously shown that 5-DIOL and E_2 can stimulate cell proliferation via their binding to the mutated androgen receptor expressed in LNCaP cells. In the present study, the combined treatment of Casodex with 5-DIOL and E_2 also indicated that the antiandrogen can partially reverse the inhibition of DHT-G formation induced by these steroids alone.

To determine whether the decrease in 3-OH-UGT and 17-OH-UGT activities caused by DHT or R1881 treatment is correlated with UGT transcript levels, a Northern blot analysis was performed. After treatment of LNCaP cells with 1 nM DHT or R1881 (8 days), the level of UGT2B15 transcript, which encodes a 17-hydroxysteroid UGT enzyme, was decreased (Fig. 7).

Discussion

The present study describes the effect of steroids on UGT activities in an *in vitro* model of human prostate cancer, the LNCaP cell line. Our data indicate that androgens induce, in LNCaP cells, a marked diminution in the glucuronidation of 5α-reduced C_{19} steroids, namely DHT and ADT, and this effect appears to occur through the androgen receptor.

Characterization of the effects of steroids on UGT activity

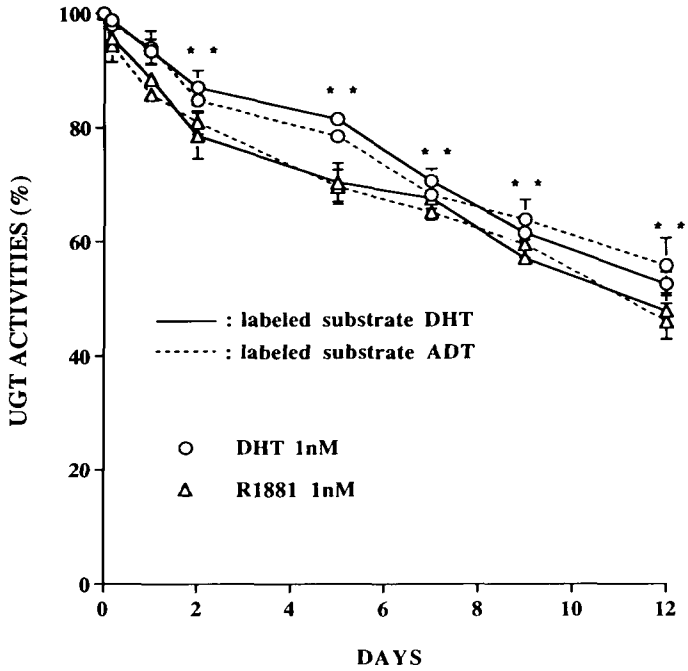

FIG. 3. Time course of the effects of androgens on the formation of androgen glucuronides. Cells were cultured at a density of 10,000 cells/well for 2 days before steroid treatment with 1 nM DHT or R1881 for 4 h, 1 day, 2 days, 5 days, 7 days, 9 days, and 12 days. After treatment, cells were washed with fresh medium and then incubated in the presence of 10 nM [^{3}H]DHT or [^{3}H]ADT for 3 h. Glucuronide formation was measured as described in *Materials and Methods* and is expressed as a percentage of UGT activities. Values represent the mean ± SEM of three experiments, each performed in triplicate. **, $P < 0.005$ *vs.* control.

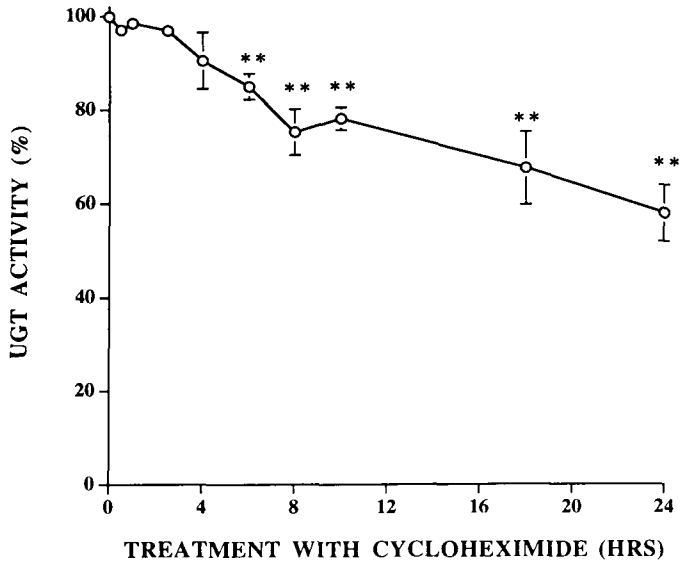

FIG. 4. Effect of cycloheximide on DHT glucurosyltransferase activity. Cells were cultured at a density of 30,000 cells/well for 2 days before treatment with 25 μg/ml cycloheximide for 30 min to 48 h. After treatment with cycloheximide, cells were washed with fresh medium and then incubated in the presence of 10 nM [^{3}H]DHT for 3 h. Glucuronide formation was measured as described in *Materials and Methods* and is expressed as a percentage of UGT activity. Values represent the mean ± SEM of three experiments, each performed in triplicate. **, $P < 0.005$ *vs.* control.

showed that exposure of LNCaP cells to androgens decreased the 3-OH-UGT and 17-OH-UGT activities in a dose- and time-dependent manner, with a significant effect detectable after 48 h. In addition, the inhibition of UGT was shown to be reversible, as recovery of the UGT activities was observed in the absence of steroid hormone and reached 100% of the basal activity after 5 days. When LNCaP cells were treated with cycloheximide to arrest protein synthesis, UGT activities persisted at basal levels for an extended period of time; 70% of the activity was retained after 24 h of treatment. These results demonstrate the high stability of the steroid UGT enzymes and may explain the relatively slow inhibitory response to androgen treatment. Northern blot analysis with LNCaP cell RNA showed that the steady state level of UGT2B15 transcript is negatively regulated by androgen treatment.

To determine the role of the androgen receptor in the steroid regulation of UGTs, cells were treated with Casodex, which is an androgen antagonist for the mutated androgen receptor in LNCaP cells. It has been previously shown that Casodex is able to reverse the stimulatory effect of DHT on cellular proliferation and compete for the binding of R1881 to the androgen receptor (37–42). At a low concentration of steroid, including estradiol, which also binds to the mutated androgen receptor of LNCaP cells (28), the inhibition of glucuronidation was partially blocked by a high concentration of Casodex. This result clearly indicates that the regulation of steroid UGT activities is mediated through the androgen receptor. The mechanism by which steroids can inhibit the glucuronidation of androgens is still unknown, but binding of the steroid to the androgen receptor is suggested to be an important step in the regulation of UGTs.

In the human prostate, DHT is found in the range of 10–20 nM, a level capable of inducing a maximal inhibitory effect on UGT activity in LNCaP cells. The human prostate also contains several 5α-reduced C_{19} steroids, namely ADT and 3α-DIOL, but at lower concentrations than DHT (9, 22). It is, however, interesting to note that ADT-G and 3α-DIOL-G levels in the prostate exceed the levels of their nonconjugated counterparts by almost 10- to 20-fold, thus indicating a high steroid UGT activity for ADT and 3α-DIOL in the prostate despite the presence of high concentrations of DHT (22). It is generally believed that DHT is converted to ADT and 3α-DIOL, which are conjugated by two different UGTs, 3-OH-UGT and 17-OH-UGT, respectively. The recent characterization of UGT2B15 has shown that 3α-DIOL is more easily conjugated than DHT by this enzyme (23, 43). Nevertheless, these observations suggest that the basal level of glucuronidation by both 3-OH-UGT and 17-OH-UGT in the prostate ensures the elimination of androgens in this tissue. In addition, there have been several observations made in humans suggesting an increase in glucuronide conjugate production when the secretion of adrenal and testicular precursors is diminished (18).

A large body of evidence indicates that the level of circulating 5α-reduced C_{19} steroid glucuronides, namely ADT-G and 3α-DIOL-G, reflects the production of DHT from the adrenal and gonadal C_{19} steroids by steroid target tissues (18, 21, 44–50). By increasing the levels of available precursors,

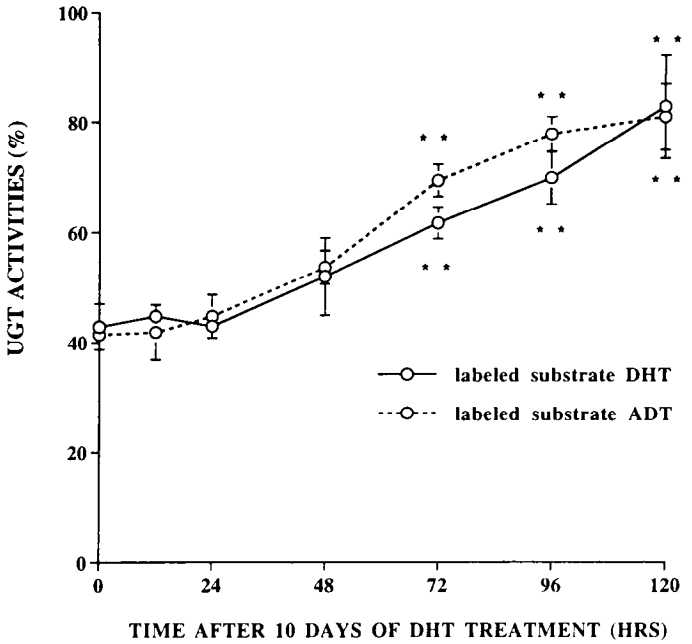

FIG. 5. Recovery of UGT activities after treatment with androgen. Cells were cultured at a density of 10,000 cells/well for 2 days before treatment with 1 nM DHT or R1881 for 7 days. After treatment, cells were washed with fresh medium and then incubated in the presence of 10 nM [^{3}H]DHT or [^{3}H]ADT for 3 h each day for 5 days. Glucuronide formation was measured as described in *Materials and Methods* and is expressed as a percentage of UGT activities. Values represent the mean ± SEM of three experiments, each performed in triplicate. **, $P < 0.005$ *vs.* control.

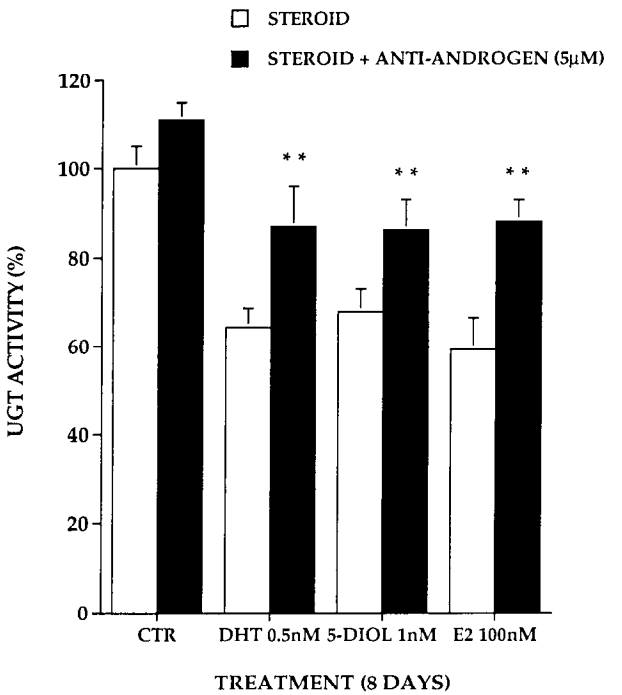

FIG. 6. Effect of Casodex on DHT-G formation. Cells were cultured at a density of 10,000 cells/well for 2 days before treatment with steroids. Cells were treated with DHT (0.5 nM), 5-DIOL (1 nM), or E_2 (100 nM) with or without Casodex (5 μM) for 6 days. After steroid treatment, cells were washed with fresh medium and then incubated in the presence of 10 nM [^{3}H]DHT for 3 h. DNA content and DHT-G formation were measured as described in *Materials and Methods*. Values represent the mean ± SEM of three experiments, each performed in triplicate. **, $P < 0.005$ *vs.* control.

the elevation of androgen production also induces higher secretion of 5α-reduced C_{19} steroid glucuronides in the circulation. It is, however, important to note that any alteration in the level of circulating ADT-G and 3α-DIOL-G will be the result of changes in the activity of one or many steroid-transforming enzymes, namely 3βHSD, 5α-reductase, 3αHSD, 17βHSD, and UGTs, in steroid target tissues. In the case of increased plasma levels of adrenal and gonadal C_{19} steroid precursors, it may be possible that the basal activity of steroid UGT enzymes in the target tissues is high enough to induce the glucuronidation of 5α-reduced C_{19} steroids despite the presence of a high level of DHT. However, in previous studies, we reported that UGT activities, reflected by the circulating levels of ADT-G and 3α-DIOL-G, may be altered by lowering DHT production. As an example, in castrated prostate cancer patients treated with a combination of flutamide and aminoglutethimide, an inhibitor of DHEA and cortisol production by the adrenals, we observed that the ratio between plasma levels of DHEA and steroid glucuronides was decreased (51). Moreover, during aging in the human, we observed a dramatic decrease in adrenal and gonadal C_{19} steroid precursors, whereas the concentrations of 5α-reduced C_{19} steroid glucuronides were less affected (18). Taken together, these data suggest that the negative modulation of UGT activity observed in the present study using LNCaP cells may also occur in steroid target tissues in the human. In the case of decreased levels of DHT in the tissue, the UGT activity may be increased to further enhance the elimination of DHT.

Although regulation of steroid UGT activity has received little attention until now, it is well established that administration of synthetic drugs to rats is capable of inducing increased liver UGT activities (33, 52). In fact, until recently, the classification of rat liver UGTs was carried out by determination of the sensitivities of different drugs to specific UGTs. It was also shown that the increase in UGT activity caused by inducers is found in parallel with increased gene expression and protein synthesis. In the rat, Munzel *et al.* (53) demonstrated that UGT1A1, a phenol UGT, is expressed at low levels in the liver but at high levels in the kidney, testis, epididymis, and ovary. After administration of tetrachlorodibenzodioxin, a polycyclic aromatic hydrocarbon, the researchers observed an increase of approximately 9-fold in liver UGT1A1 expression, but only a 2-fold increase in other tissues, thus suggesting complex tissue-specific regulation of UGTs. Our data clearly show a negative modulation of steroid UGT messenger RNA levels and enzyme activity in response to DHT in LNCaP cells. To our knowledge, this is the first observation that the expression of steroid UGTs in extrahepatic tissue is regulated by androgens.

In addition to other conversions of DHT (3, 16, 21, 22–24, 30–31, 48), our data suggest that glucuronidation plays an important role in regulation of the androgen level in the prostate. It is possible that the inactivation by glucuronidation of DHT in androgen-dependent prostate cancer cells is inhibited in the presence of high DHT levels to help maintain the high level of DHT required to stimulate the growth and

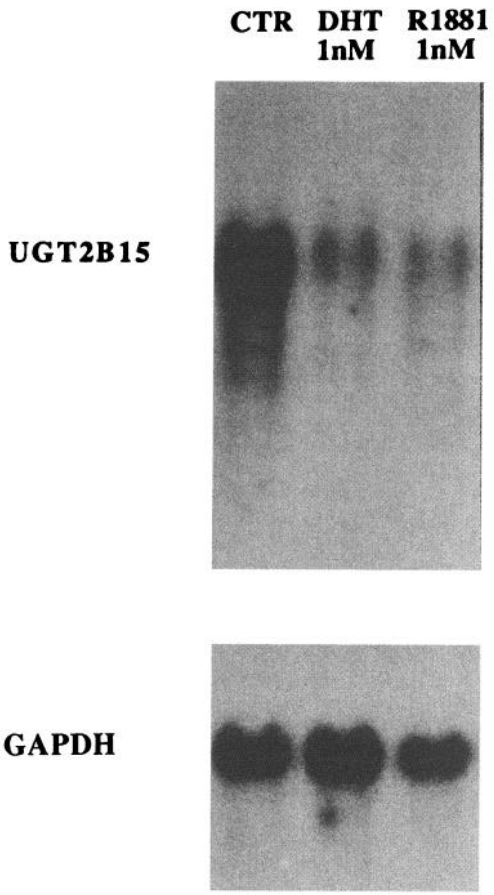

FIG. 7. Inhibitory effect of androgen on UGT transcript levels in LNCaP cells. Cells were cultured in the presence of 1 nM DHT or R1881 for 8 days. Twenty micrograms of total cellular RNA were subjected to Northern blot analysis using the full-length UGT2B15 cDNA (A) and a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (B) as probes. Relative UGT mRNA levels were determined by densitometric quantification and normalized relative to GAPDH levels; relative values were 1 × for the control, 0.22 × for DHT, and 0.18 × for R1881.

progression of the tumor, thus suggesting that androgens protect themselves from their own degradation by inhibition of steroid UGT enzymes.

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