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Specificity of Glucuronosyltransferase Activity in the Human Cancer Cell Line LNCaP, Evidence for the Presence of at Least Two Glucuronosyltransferase Enzymes

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Recent findings obtained by our group showed that incubation of LNCaP cells with labeled steroids leads to the formation of 3- and 17-hydroxysteroid glucuronides. In this study, the specificity and the kinetic properties of 3-hydroxy-C_{19}steroid uridine diphospho-glucuronosyltransferase (3-OH-UGT) and 17-hydroxy-C_{19}steroid UGT (17-OH-UGT) activities in LNCaP cells were investigated. Results indicate that the UGT has a high affinity for testosterone, dihydrotestosterone (DHT), androsterone (ADT) and androstane-3α,17β-diol (3α-DIOL), with K_m values ranging from 0.25 to 0.68 μM. The K_m values are approx. 10-fold higher for androst-5-ene-3β,17β-diol (5-ene-DIOL) and androstane-3β,17β-diol (3β-DIOL). The relative specificities (V_{max}/K_m) also showed higher turnover rates for testosterone, DHT, ADT and 3α-DIOL with values ranging from 2.93 to 5.71, than for 3β-DIOL and 5-ene-DIOL with ratios of 0.41 and 1.10, respectively. Dixon plot and Cornish–Bowden analysis demonstrate that testosterone, DHT, ADT, and 3α-DIOL inhibit the glucuronidation of DHT and ADT in a competitive fashion. In contrast, when the studies are performed with 3β-diol and 5-ene-DIOL the inhibition of ADT glucuronidation is uncompetitive while the glucuronidation of DHT is inhibited competitively, suggesting the presence of two UGT enzymes, one for glucuronidation of the 17β-OH group and a second for the 3α-OH group. Further evidence for the presence of two UGTs in LNCaP cells was obtained by incubation with a variety of 3β-OH-C_{19} steroids which caused a marked inhibition of DHT-G formation but had no effect on the glucuronidation of ADT. In summary, our data demonstrate the presence of at least two UGTs in the human prostate cancer cell line LNCaP. The relative specificity of the 17-OH-UGT in LNCaP cells is 3α-DIOL > DHT > testosterone, while ADT is glucuronidated by the 3-OH-UGT.

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Abbreviations: 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; FBS, fetal calf serum; UGT, uridine diphospho-glucuronosyltransferase; 17-OH-UGT, 17-hydroxy-C_{19} steroid UGT; 3-OH-UGT, 3-hydroxy-C_{19} steroid UGT; ADT, 5α-androstane-3α-ol-17-one; 5-ene-DIOL, androst-5-ene-3β,17β-diol; 3α-DIOL, 5α-androstane-3α,17β-diol; DHT, 5α-androstane-17β-ol-3-one; 3β-DIOL, 5α-androstane-3β,17β-diol; testosterone, 4-androstene-17β-ol-3-one; testosterone-G, testosterone-glucuronide; DHT-G, DHT-glucuronide; 3α-DIOL-G, 3α-DIOL-glucuronide, 3β-DIOL-G, 3β-DIOL-glucuronide, ADT-G, ADT-glucuronide; 5-ene-DIOL-G, 5-ene-DIOL-glucuronide; EPI-ADT, 5α-androstane-3β-ol-17-one; 6α-OH-EPI-ADT, 5α-androstane-3β,6α-diol-17-one; 7α-OH-EPI-ADT, 5α-androstane-3β,7α-diol-17-one; 16α-OH-EPI-ADT, 5α-androstane-3β,16α-diol-17-one; 3β, 6α,17β-TRIOL,5β-androstane-3β,6α,17β-triol; 3β,7β,17β-TRIOL, 5β-androstane-3β,7β,17β-triol; 3β,16α,17β-TRIOL, 5β-androstane-3β,16α,17β-triol; etiocholanolone, 5β-androstane-3α-ol-17-one; 7β,17β, 3-ONE, 5α-androstane-7β,17β-diol-3-one; 7α-OH-testosterone, 4-androstene-7α,17β-diol-3-one; 19-nor-testosterone, 19-nor-4-androstene-17β-ol-3-one; 1-dehydrotesto, 1,4-androstadiene-17β-ol-3-one; 6-dehydrotesto, 4,6-androstadiene-17β-ol-3-one; estradiol, 1,3,5(10)-estratriene-3,17β-diol; estrone, 1,3,5(10)-estratriene-3-ol-17-one; estriol, 1,3,5(10)-estratriene-3,16α,17β-triol; 2-OH-estrone, 1,3,5(10)-estratriene-2,3-diol-17-one; 4-OH-estradiol, 1,3,5(10)-estratriene-3,4,17β-triol; 11β-OH-ADT, 5α-androstane-3α,11β-diol-17-one; 3β,11α,17-ONE, 5α-androstane-3β,11α-diol-17-one; 3β,11α,17β-TRIOL, 5α-androstane-3β,11α,17β-triol; 11β-OH-testosterone, 4-androstene-11β,17β-diol-3-one; LCMS; liquid chromatography ion spray mass spectrometry.

INTRODUCTION

Despite the essential role of 5α-reductase in the conversion of testosterone to DHT, there is now good evidence that several steroid-transforming enzymes are also involved in the regulation of prostatic DHT levels. In fact, enzymes such as 3β-HSD, 3α-HSD and 17β-HSD can convert DHT into a series of 5α-reduced-C_{19} steroid metabolites including, androstanedione, 3α-DIOL, ADT, 3β-DIOL and EPI-ADT [1, 2]. Recent work has also shown that, in the prostate, these C_{19} steroids can also be hydroxylated by cytochrome *P*450 enzymes [3–5] or converted to more polar derivatives by UGT [6, 7].

UGT is particular by the fact that once the glucuronide derivative is formed the conjugated product becomes highly soluble in serum. UGTs are grouped into several families of similar enzymes [8, 9] catalysing the transfer of glucuronic acid to the hydroxyl position of a variety of metabolites, including, 5α-reduced C_{19} steroids. In the rat, four steroid UGTs have been isolated from liver and encode isoenzymes that are responsible for glucuronidation of 3-hydroxy-C_{19} steroids and 17-hydroxy-C_{19} steroids [10–14]. More recently, a cDNA from human liver was isolated and the expressed protein showed specific activity towards 17-hydroxy-C_{19} steroids such as DHT [15, 16]. Interestingly, the presence of mRNA encoding this isoenzyme has been detected in the human prostate [15].

Recent findings suggest that the prostate tumor cell line LNCaP contains most of the steroidogenic enzymes present in the human prostate [17–19]. Previous studies by our group indicate that incubation of LNCaP cells with labeled steroids leads to the formation of polar metabolites. Complete characterization of these metabolites was performed using LCMS and showed the formation of 3-hydroxysteroid and 17-hydroxysteroid glucuronides [19]. In the present study the specificity and the kinetic properties of 3-hydroxy-C_{19} steroid UGT (3-OH-UGT) and 17-hydroxy-C_{19} steroid UGT (17-OH-UGT) activities in LNCaP cells were investigated. Our data suggest the presence of at least two UGT enzymes in this prostatic human cancer cell line, one responsible for the glucuronidation of steroids at position 3 such as ADT and the other responsible for the glucuronidation of the 17 hydroxyl group of steroids such as testosterone, DHT and 3α-DIOL.

MATERIALS AND METHODS

Chemicals

RPMI-1640 medium, was obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). Fetal bovine serum was obtained from Immunocorp (Montreal, Québec, Canada) and 24-well culture plates from Flow Laboratories (McLean, VA, U.S.A.). [9,11-^{3}H]ADT (59 Ci/mmol), [9,11-^{3}H]3α-DIOL (56 Ci/mmol) and [1,2-^{3}H]5-ene-DIOL (56 Ci/mmol) were obtained from NEN Dupont (Boston, MA, U.S.A.). [1,2-^{3}H]DHT (47 Ci/mmol), [1,2-^{3}H]3β-DIOL (48.6 Ci/mmol) and [1,2,6,7-^{3}H]testosterone (90 Ci/mmol) were obtained from Amersham (Oakville, Ontario, Canada). Purity of labeled substrates was measured by HPLC as previously described [19] and was higher than 96%. Unlabeled steroids were purchased from Steraloids Inc. (Wilton, NH, U.S.A.) and from Research Plus (Bayonne, NJ, U.S.A.).

Maintenance of stock cultures

The LNCaP cell line was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) [20]. Cells were routinely cultured in RPMI-1640 medium phenol red-free supplemented with 10% (v/v) FBS, 2 mM glutamine and antibiotics (100 IU of penicillin/ml and 100 μg of streptomycin/ml). The cultures were kept in a humidified atmosphere of 5% CO_2 in air, at a temperature of 37°C. The cells were subcultivated at weekly intervals by digestion in a solution of 0.05% trypsin and 0.01% EDTA.

Cell experiments

Cell cultures obtained between passages 20–28 were plated at a density of 0.25×10^5 cells per well in 24-well plastic plates and were allowed 48 h for adhesion. The experiments were performed in RPMI-1640 medium containing the above-mentioned antibiotics and 2% (v/v) FBS treated twice with dextran-coated charcoal to remove endogenous steroids. In order to determine enzymatic activity, cells were incubated with 10 nM of labeled steroids in the presence or absence of unlabeled steroids namely, testosterone, DHT, 3α-DIOL, 3β-DIOL, 5-ene-DIOL or ADT in fresh medium. Results were normalized according to DNA content measured at the end of the incubation period, as previously described [19]. For K_m and V_{max} determination, cells were incubated for a period which allowed conversion of approx. 20% of the appropriate substrate. At the end of the incubation, the medium was collected, centrifuged to remove cellular debris and stored until required for analysis. In each experiment, triplicate dishes were used for each treatment. The cell medium samples were extracted twice with diethyl ether and an aliquot of the aqueous phase was counted by liquid scintillation counting, and the percentage of glucuronidation was expressed by dividing the radioactivity in the aqueous phase by the total radioactivity present in both phases multiplied by 100. The amount of radioactivity in the aqueous phase corresponds to the amount of steroid glucuronides formed since we did not detect any sulfate derivatives in LNCaP cells. In each experiment, metabolites were analyzed by HPLC showing that steroid glucuronides were the unique polar product formed. Apparent kinetic parameters K_m and V_{max} from the primary data were estimated by Lineweaver–Burk plots using Enzfitter Software

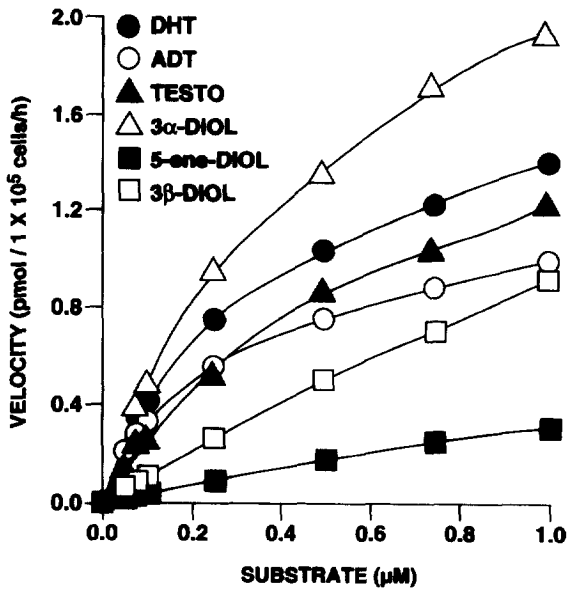

Fig. 1. Relative efficiency of conversion of testosterone (TESTO), dihydrotestosterone (DHT), androsterone (ADT), androstane-3α,17β-diol (3α-DIOL), androstane-3β,17β-diol (3β-DIOL), and androst-5-ene-3β,17β-diol (5-ene-DIOL) by UGT enzymes present in LNCaP cells in culture for 4 h of incubation. Values represent the means of triplicate incubations.

(Biosoft, Cambridge, U.K.). For experiments used to calculate Dixon plots, Cornish–Bowden analysis [21] and competition data using hydroxy-C_{19} steroids, cells were incubated with two fixed concentrations of labeled substrates, in the same conditions as mentioned above and for an identical period of time.

Calculations

Experimental data are presented as mean ± SEM and each experiment was performed at least three times. Where appropriate, statistical significance was determined according to the multiple range test of Duncan–Kramer [22].

RESULTS

Kinetic properties of UGT activity present in the LNCaP cell line

Kinetic analysis of UGT activities in LNCaP cells was performed by using substrates such as testosterone, DHT, ADT, 3α-DIOL, 5-ene-DIOL and 3β-DIOL; knowing that these C_{19} steroids are present in the human prostate and that previous studies obtained with LNCaP cells show that testosterone, DHT and 3α-DIOL are glucuronidated at position 17-hydroxy and ADT at position 3-hydroxy. It should be noted that the position of the monoglucuronide of 5-ene-DIOL-G and 3β-DIOL-G has not yet been determined. Figure 1 illustrates the velocity of formation of glucuronide conjugates by LNCaP cells as a function of the respective substrate concentration. The double reciprocal plot of velocity and substrate concentration was linear for all five substrates as shown in Table 1. The apparent maximal velocity (V_{max}) and the relative specificity (V_{max}/K_m) are also summarized in Table 1. These results demonstrate that the relative specificity of the substrates can be classified in two groups with values for testosterone, DHT, ADT and 3α-DIOL ranging from 2.93 to 5.71 whereas those for 5-ene-DIOL and 3β-DIOL were 0.41 and 1.10, respectively.

Specificity of UGT activity in LNCaP cells

The substrate specificity and inhibitory properties of various steroids for UGT activity in LNCaP cells are shown in Table 2 and Figs 2 and 3. Our data demonstrate that conversion of both ADT and DHT into glucuronide conjugates was markedly inhibited by the addition of testosterone, DHT, 3α-DIOL or ADT while 3β-DIOL and 5-ene-DIOL decreased the formation of polar metabolites to a lesser extent. Dixon plot analyses shown in Fig. 2(A and B) indicate that ADT and 3α-DIOL are competitive inhibitors of DHT glucuronidation with K_i values of 0.40 and 0.28 μM, respectively. Using labeled ADT as substrate [Fig. 2(C and D)], the K_i values of DHT and 3α-DIOL were 0.12 and 0.37 μM, respectively. It must be noted that 3β-DIOL showed a competitive inhibition when labeled DHT was used as substrate [Fig. 3(A)] but uncompetitive in the presence of labeled ADT [Fig. 3(C)]. In agreement with these results, Cornish–Bowden analysis [Fig. 3(B and D)] also demonstrated competitive and uncompetitive inhibitions of DHT-G and ADT-G formation, respectively, in the presence of 3β-DIOL, suggesting the presence of at least two UGTs, namely 3-OH-UGT and 17-OH-UGT, in LNCaP cells.

Table 1. Kinetic properties of UGT enzymes present in LNCaP cells

Substrate	K_m (μM)	Relative V_{max} (pmol/1 × 10^5 cells/h)	Relative specificity (relative V_{max}/K_m)
TESTO	0.68	1.99	2.93
DHT	0.37	1.85	5.00
ADT	0.25	1.16	4.64
3α-DIOL	0.49	2.80	5.71
3β-DIOL	4.09	4.50	1.10
5-ene-DIOL	2.44	0.99	0.41

Kinetic parameters were determined using Lineweaver–Burk plot (1/v vs 1/[S]).

Table 2. Competition studies using substrates of UGT activities in LNCaP cell line

	K_i (μM)					
	Substrates					
Competitor	TESTO	DHT	ADT	3α-DIOL	3β-DIOL	5-ene-DIOL
TESTO	—	0.44*	0.52*	0.56*	3.06*	0.32*
DHT	0.48*	—	0.12*	0.40*	2.62*	0.55*
ADT	0.64*	0.40*	—	0.60*	10.45†	3.77†
3α-DIOL	0.29*	0.28*	0.38*	—	2.34*	0.61*
3β-DIOL	1.50*	2.25*	8.50†	2.50*	—	1.40*
5-ene-DIOL	2.35*	2.52*	3.80†	3.70*	1.32*	—

Competition experiments were performed in duplicate, three times, at two fixed concentration of labeled substrate, as described in Materials and Methods. K_i values were calculated by Dixon plot analysis.

*Competitive inhibition.

†Uncompetitive inhibition.

Further evidence for the presence of two distinct UGTs in LNCaP cells was obtained by incubation with various 3β-hydroxy-17-keto-C_{19} steroids at a concentration of 1 μM in the presence of fixed concentrations of labeled DHT or ADT (Table 3). The steroids EPI-ADT, 6α-OH-EPI-ADT, 7α-OH-EPI-ADT, or 16α-OH-EPI-ADT inhibited the formation of DHT-G by 47, 37, 37 and 27%, respectively, whereas the formation of ADT-G was not affected. Moreover, addition of 3β-hydroxy-C_{19} steroids, namely, 3β-DIOL, 3β,6α,17β-TRIOL, 3β,7β,17β-TRIOL, or 3β,16α,17β-TRIOL had a small inhibitory effect on

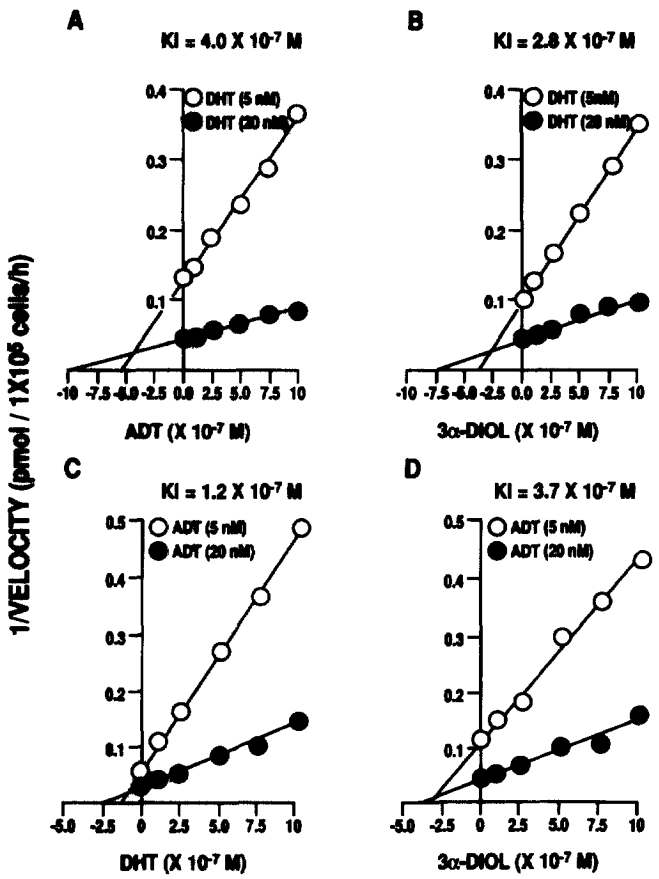

Fig. 2. Dixon plot analysis of the effect of androsterone (ADT) (A), dihydrotestosterone (DHT) (C), and androstane-3α,17β-diol (3α-DIOL) (B) and (D), on the 17-OH-UGT and on the 3-OH-UGT activities using two concentrations of DHT (A, B) and ADT (C, D), respectively, as labeled substrate with increasing concentrations of unlabeled steroids during 4 h incubation with cells. Results are expressed in pmol of metabolites formed per 1 x 10^5 cells per hour.

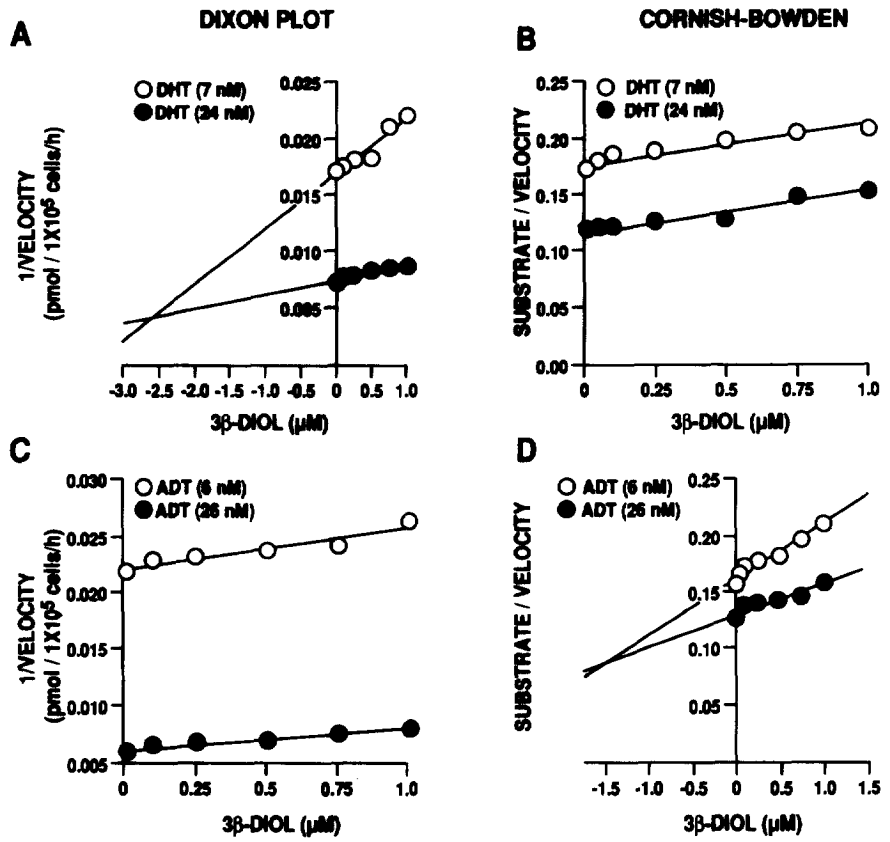

Fig. 3. Dixon plot (A, C) and Cornish–Bowden (B, D) analysis of the effect of androstane-3β,17β-diol (3β-DIOL) on the 17-OH-UGT and 3-OH-UGT activities using two concentrations of DHT (A, B), and ADT (C, D), respectively, as labeled substrates with increasing concentrations of unlabeled steroids during 4 h incubation with LNCaP. Results are expressed in pmol of metabolites formed per 1×10^5 cells per hour.

ADT-G formation whereas the glucuronidation of DHT was diminished substantially. Table 3 also illustrates a differential inhibition in the glucuronidation of DHT and ADT in the presence of etiocholanolone. Taken together, these results further support the hypothesis that 3-OH-UGT present in LNCaP cells is specific for steroids possessing an hydroxyl at position 3α, while 17β-hydroxysteroids may be glucuronidated by 17-OH-UGT.

Studies with testosterone derivatives indicate that the inhibition on ADT-G and DHT-G production may be altered when the testosterone molecule is slightly modified. Incubation with 7α-OH-testosterone, 19-nor-testosterone, 1-dehydrotestosterone and 6-dehydrotestosterone had a small inhibitory effect on both ADT-G and DHT-G formation as compared to that observed using testosterone. There was a small or no effect on the glucuronidation of DHT and ADT by estradiol, estrone and estriol whereas 2-hydroxy- or 4-hydroxy-estradiol altered the glucuronidation of both C_{19} steroids. It must however be noted that for all estrogens studied, there is a tendency to find a higher inhibition on DHT-G than on ADT-G production. Finally it is of interest to observe that a small or no effect was observed on DHT and ADT glucuronidation when 11-hydroxy-C_{19} steroids were used, indicating that steroids hydroxylated at position 11 are not suitable substrates for the UGTs specific for DHT and ADT.

DISCUSSION

LNCaP is an androgen-dependent cell line derived from a fast growing colony of a lymph node carcinoma of a prostate cancer patient [20, 23] and due to a mutation in the androgen receptor, these cells also respond to estrogens, glucocorticoids and antiandrogens such as hydroxyflutamide [24–26]. In response to androgens, prostate-specific antigen is also secreted by LNCaP cells and its production appears to be dose-dependent [27–30]. Despite the inconvenience of having a mutated androgen receptor for the study of steroid action, there is now good evidence that LNCaP cells possess all the enzymes necessary to transform C_{19} steroids by a metabolic pathway identical to that occurring in the human prostate. In fact, in addition to 5α-reductase, LNCaP cells contain 3β-HSD, 3α-HSD and 17β-HSD and their reductase counterparts. Characterization of polar C_{19} steroid metabolites formed by LNCaP cells was recently performed and

showed that glucuronide derivatives are the major polar steroids whereas sulfate derivatives could not be detected [17–19]. Taken together, these observations indicate that LNCaP cells represent a very good model for the study of steroid-transforming enzymes.

In this study we have examined the kinetic properties and specificity of UGTs present in LNCaP cells. As an initial study we incubated the LNCaP cells with potential substrates and demonstrated that several steroids are glucuronidated. Kinetic studies revealed that the glucuronosyltransferase activity has a high specificity for steroids. Moreover, the ratio between V_{max} and K_m revealed higher relative specificities for DHT, testosterone, ADT and 3α-DIOL than for 3β-DIOL and 5-ene-DIOL. These results demonstrate that substrates which have an hydroxyl group at position 17β and/or 3α have a higher affinity for the UGTs and are glucuronidated with a higher turnover rate than steroids hydroxylated at position 3β. It must however be mentioned that the enzyme assays were performed with intact cells and that the uptake, transport and release of steroids may interfere with the measurement of enzymatic activity. Nevertheless, in the present study, the range of polarity between the steroid substrates and between the glucuronidated products is approximately the same and should therefore minimize these interferences. Moreover, in a previous study, it was shown that homogenization of LNCaP cells caused a major reduction in UGT activities thus suggesting that the assay of glucuronidation in intact cells is a reflection of UGT activities in intact tissue.

Table 3

Steroids Abbreviations	Inhibition of glucuronidation (%) DHT 17-OH-UGT	ADT 3-OH-UGT
5α-reduced, 3α-OH-steroid		
ADT	78 ± 2	80 ± 2
3α-DIOL	77 ± 2	77 ± 1
5α-reduced, 17β-OH-steroid		
DHT	82 ± 1	77 ± 2
5α-reduced, 3β-OH-steroid		
EPI-ADT	47 ± 2	8 ± 1*
6α-OH-EPI-ADT	37 ± 1	3 ± 0*
7α-OH-EPI-ADT	37 ± 1	13 ± 0*
16α-OH-EPI-ADT	27 ± 1	9 ± 0*
5α-reduced, 3β, 17β-OH-steroid		
3β-DIOL	31 ± 0	3 ± 0*
3β, 6α, 17β-TRIOL	36 ± 1	7 ± 0*
3β, 7β, 17β-TRIOL	37 ± 1	3 ± 0*
3β, 16α, 17β-TRIOL	50 ± 2	14 ± 0*
5β-reduced, 3α-OH-steroid		
Etiocholanolone	23 ± 0	35 ± 1**
Testosterone derivatives		
TESTO	65 ± 1	58 ± 2
7α-OH-TESTO	12 ± 1	3 ± 0*
19-nor-TESTO	15 ± 0	7 ± 1*
1 Dehydrotesto	15 ± 1	17 ± 0
6 Dehydrotesto	10 ± 0	10 ± 0
Estrogen derivatives		
Estradiol	12 ± 0	6 ± 0*
Estrone	9 ± 0	7 ± 0*
Estriol	10 ± 0	3 ± 0*
2-OH-Estrone	31 ± 3	20 ± 1*
4-OH-Estradiol	24 ± 2	20 ± 2
11-OH-steroid derivatives		
11β-OH-ADT	8 ± 0	6 ± 0**
3β, 11α, 17-ONE	3 ± 0	3 ± 0
3β, 11α, 17β-TRIOL	1 ± 0	1 ± 0
11β-OH-TESTO	12 ± 0	7 ± 0*

*$P < 0.001$; **$P < 0.01$.

Inhibition experiments were performed using 10 nM of labeled DHT and ADT and 1 μM of unlabeled steroids as competitors. Glucuronide formation was determined by extraction using ether as described in Materials and Methods. Values represent the mean ± SEM of triplicate incubations.

To determine if different UGT enzymes in LNCaP cells are used to glucuronidate steroids hydroxylated at different positions we conducted competition studies. Dixon plot and Cornish–Bowden analysis demonstrate that testosterone, DHT or ADT, and 3α-DIOL inhibit the glucuronidation of DHT and ADT in a competitive fashion. In contrast, when the studies are performed with 3β-DIOL and 5-ene-DIOL the inhibition of ADT glucuronidation is uncompetitive while the glucuronidation of DHT is inhibited competitively. These differential types of inhibition suggest the presence of two UGT enzymes, one for glucuronidation of the 17β-hydroxyl group and a second for the 3α-hydroxyl group. This is not surprising since several UGT cDNAs have been cloned from the rat liver where the expressed proteins specifically catalyze the glucuronidation of 3-hydroxysteroids and 17-hydroxysteroids [10–14]. As well, two groups have reported the isolation of a cDNA encoding a protein which have high activity for 17-hydroxysteroids [15, 16]. In addition, Chen *et al.* have reported the expression of a 17-OH-UGT mRNA in the human prostate [15]. Further evidence for the presence of two UGTs in LNCaP cells was obtained by incubation with a variety of 3β-hydroxy-C_{19} steroids and 3β-hydroxy-17-keto-C_{19} steroids which caused a marked inhibition of DHT-G formation but had no effect on the glucuronidation of ADT.

Based on the position of their hydroxyl groups, testosterone and DHT are specific substrates for a putative 17-OH-UGT whereas ADT would be glucuronidated by a 3-OH-UGT. Results obtained by LCMS analysis have clearly demonstrated that 3α-DIOL, which has a 3α- and a 17β-hydroxyl group, is glucuronidated at position 17 by a putative 17-OH-UGT [19]. Due to the low levels of 3β-DIOL-G formed by LNCaP cells we were unable to determine the position of the glucuronide group as 3β or 17β. However, the inhibition of DHT-G formation by both 3β-hydroxy-C_{19} steroids and 3β-hydroxy-17-keto-C_{19} steroids suggest that 3β-DIOL may be glucuronidated at position 3β by the 17-OH-UGT or by an additional uncharacterized UGT. Further evidence for the glu-

curonidation of steroid substrates at position 3β was obtained when LNCaP cells were incubated with labeled EPI-ADT to yield a polar metabolite as identified by HPLC (data not shown). It is reasonable to speculate that the polar metabolite is a glucuronide derivative, however, due to the low levels of labeled EPI-ADT conjugated we could not confirm the product as a 3β-OH-glucuronide by LCMS analysis.

The presence of at least two distinct UGTs in LNCaP cells is also supported by the specificity of the UGT activities observed in this cell line. Among the steroids used in the present study it is shown that 3α-DIOL is the best substrate for a putative 17-OH-UGT. Similar results showing the high specificity for 3α-DIOL as substrate were also obtained with purified 17-OH-UGT as well as with protein expressed from a rat 17-OH-UGT cDNA transfected in COS cells [12, 14]. In addition, our studies show that DHT has a higher relative specificity than testosterone which is in accordance with previous observations showing that DHT is a significantly better substrate than testosterone for the human 17-OH-UGT encoded by UGT2B15 [16].

The specificity of the putative 3-OH-UGT in LNCaP cells for 3α-hydroxysteroids was elucidated by competition studies where incubation with several 3β-hydroxy-17-keto-C_{19} steroids including EPI-ADT and its hydroxylated derivatives inhibited the glucuronidation of DHT but not ADT. These results which indicate the presence of a 3-OH-UGT in LNCaP cells agree with studies performed with purified enzymes showing that the rat 3-OH-UGT reacts with ADT, etiocholanolone, and 3α-hydroxy derivatives of bile acids whereas EPI-ADT, which is a 3β-hydroxysteroid, was not a good substrate for 3-OH-UGT [31]. In addition, etiocholanolone, which has a 3α-hydroxyl group, showed higher competition for ADT than for DHT glucuronidation in LNCaP cells suggesting that the 3-OH-UGT present in these cells is specific for the alpha orientation of the 3-hydroxyl group.

The specificity of the UGTs expressed in LNCaP cells was further demonstrated by incubation with testosterone derivatives where alteration of the testosterone molecule was shown to markedly decrease the affinity of the substrate for the enzyme. As well, in contrast to the inhibition of ADT-G and DHT-G formation observed by testosterone, ADT and 3α-DIOL, the use of 11-hydroxyl derivatives demonstrated very little inhibitory effect. Thus suggesting the importance of this position since the addition of a hydroxyl group at position 11 alleviates this compound as a substrate for the UGT enzymes. Subsequent studies with LNCaP cells show that estrogens and catechol-estrogens are not glucuronidated and do not inhibit the formation of ADT-G and DHT-G, which is in agreement with previous observations using purified UGT enzymes where it was shown that estrogens and catechol-estrogens are poor substrates of 3-OH-UGT and 17-OH-UGT enzymes [8, 32, 33].

In summary, our data demonstrate the presence of at least two UDP-glucuronosyltransferases in the human prostate cancer cell line LNCaP, a 3-OH-UGT and a 17-OH-UGT activity responsible for the conjugation of C_{19} steroids. The presence of steroid specific UGTs in the prostate reinforce a possible role of this catalytic pathway in regulating the level of steroids in peripheral tissues.

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