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11β-Hydroxysteroid Dehydrogenase and Tissue Specificity of Androgen Action in Human Prostate Cancer Cell LNCaP

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Incubation of whole LNCaP cells in suspension with tritium labeled cortisol revealed two major and one minor radioactive product. Of the major products, one migrated with an R_f value identical to cortisol (Kendall's compound "F"), and the second migrated with an R_f value similar to nonradioactive cortisone (Kendall's compound "E"); the third minor product comigrated with 21-acetylated cortisol. The conversion of cortisol to cortisone was linear with respect to cell number, and conversion reached a plateau after 120 min of incubation at 37°C. One half of the cortisol was converted to cortisone within 2 h of incubation at 37°C. This conversion was nicotine amide dinucleotide (NAD) dependent. Low levels of transcription activation by cortisol were documented in LNCaP cells transfected with glucocorticoid and androgen responsive mouse mammary tumor virus-bacterial chloramphenicol acetyltransferase chimeric gene (MMTV-CAT). Hormone binding assay and transactivation analysis revealed the presence of a functional mineralocorticoid receptor in LNCaP cells. Treatment of transfectants with F in the presence of carbenoxolone, a potent inhibitor of 11β-hydroxysteroid dehydrogenase (11β-HSD), resulted in a two orders of magnitude increase in measurable CAT activity. The addition of the reduced form of nicotine amide dinucleotide (NADH) in the presence of 10^{-7} M E stimulated measurable CAT activity in LNCaP cells. In conferring aldosterone specificity in mineralocorticoid target tissues, 11β-HSD may have an important role as "gate keeper" in allowing a specific androgen response in hormone responsive LNCaP prostate cancer cells.

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INTRODUCTION

Differentiation and proliferation of human prostate cells, as well as the evolution of prostate cancer, depend upon androgens. These hormonal effects are mediated by intracellular receptors which, after binding specific steroids, recognize high affinity *cis*-acting regulatory sites on the genome [1, 2]. LNCaP human prostate cancer cells contain a mutant androgen receptor (AR^T868_A) with a single base mutation at codon 868, and functional glucocorticoid receptor [3–9]. Prostate-specific antigen (PSA) is a 240-amino acid glycoprotein, a serine protease with high substrate specificity [3] that is upregulated in LNCaP cells by androgens, progesterone (R5020), estradiol and epiandrosterone but not by glucocorticoids or diethylstilbestrol [4]. Thus PSA expression in LNCaP cells is influenced predominantly by androgens via its receptor, and the mutation of AR affects the expression of this cellular gene by steroids other than androgens [4–9].

The DNA binding domains of glucocorticoid receptor (GR), progesterone receptor (PR), mineralocorticoid receptor (MR) and AR share sequence similarities up to 94%, consistent with their recognizing similar response elements [1, 2]. Although glucocorticoids are known to affect the growth of a number of cells in culture, their precise mechanism of action is as yet unknown [10]. Glucocorticoids cause a decrease in the level of AR in prostate cancer cells [11–14] and the AR mutant in LNCaP has been shown not to bind glucocorticoids [4]. The demonstration of the presence of both GR and AR in a number of cells in culture, such as LNCaP, DDT, MF-2 and R3327H-G8-A, suggests that a cell specific mechanism may exist to confer tissue specificity of androgen action [11–14].

Most tissues contain 11β-hydroxysteroid dehydrogenase (11β-HSD) and 11–oxo reductase activity, with the ratio varying within tissues and cell types [15–19].

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In liver, glucocorticoid action is enhanced by the reduction of cortisone to cortisol [15–17], and the conversion of cortisol to an inactive metabolite has been documented in kidney cells [15, 16, 18]. The physiological role of these enzymes appears to be developmental in some tissues. For example in the placenta and fetus, substantial dehydrogenase activity prevents high tissue levels of cortisol in the early stages of pregnancy [18, 20, 21]. As in the tissues involved in salt and water balance such as the kidney [15], the consequences of the conversion of 11-ketosteroids in prostate may be pivotal. First, the conversion of glucocorticoids to receptor-inactive 11-ketosteroids may enable androgen bound AR in prostate to function unhindered despite the much higher circulating concentrations of cortisol. Secondly, this metabolic activity lowers cortisol occupancy of GR in the same tissue. In this report we have documented the presence of the enzyme 11β-HSD in LNCaP cells and its ability to inactivate the potent glucocorticoid cortisol. In addition we studied the specificity of 11β-HSD to oxidize cortisol to cortisone and vice versa in LNCaP cells in suspension. We have investigated the level of MR expression in these cells by a classical hormone binding assay and by immunodetection with MR specific polyclonal antibodies. We also analyzed the effect of 11β-HSD dependent inactivation of cortisol and vice versa by transactivation analysis of mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) transfected into LNCaP cells with or without carbenoxolone (CBX) treatment, which inhibits 11β-HSD. Our results are compatible with a "gatekeeper" function of the enzyme 11β-HSD enabling selective androgen action in LNCaP human prostate cancer cells.

EXPERIMENTAL

Cell culture and transfection

LNCaP cells were obtained from American Type Culture Collection at passage 19 (Buffalo, NY). Cells were grown at 37°C in a humidified atmosphere of 5% carbon dioxide and air, and maintained in RPMI 1640 supplemented with 2% glutamine, 10% heat inactivated fetal bovine serum, 100 U/ml gentamycin and 5 μg/ml fungizone. The cells, grown to 70–80% confluency in 250 ml culture flasks and placed in 5% fetal bovine serum containing media prior to beginning transfection, were collected by mild trypsinization and 1.5 million cells/100 mm Petri dishes were plated. A transfection experiment consisted of 19 Petri dishes plated with cells derived from a single pool of cells. Two hours prior to transfection the medium from the plates was aspirated and 4 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum were added. 960 μl of calcium phosphate precipitated DNA (5 μg MMTV-CAT + 5 μg CH110, β-galactosidase expression plasmid used for standardization of transfection from Pharmacia, Canada) was added dropwise to the medium [22]. The calcium phosphate precipitate was left in contact with the cells for 4 h. Then, 2 ml of 15% glycerol in Hepes buffered saline (HBS) were added to the medium and gently mixed. This step was found to be crucial for an efficient transfection and for removal of calcium phosphate precipitate quantitatively. After 3–5 min, the medium was removed by aspiration and the cells were washed once with 5 ml of 25 mM Tris–HCl, 137 mM NaCl, 5 mM KCl, 0.7 mM $MgCl_2$ and 0.6 mM Na_2HPO_4, pH 7.4 (TBS) and 4 ml of RPMI 1640 supplemented with 5% dextran coated charcoal treated fetal bovine serum (DDC-FBS) were added to the transfectants. Hormones were added as indicated [a (10^{-11} M), b (10^{-10} M), c (10^{-9} M), d (10^{-8} M), e (10^{-7} M), f (10^{-6} M) and g (10^{-5} M) final concentrations] from a 1000 × concentrated stock solution in ethanol and the incubation was continued for additional 24 h.

The following day the medium from the Petri dishes was collected in 15 ml plastic centrifugation tubes (Falcon) and the cells were washed with 5 ml of phosphate buffered saline (PBS). The cells were briefly trypsinized and the enzyme was blocked by the addition of the medium collected from each Petri dish. The cells were collected by centrifugation at 2000 rpm for 10 min at room temperature, suspended in 500 μl of PBS, transferred into 1.5 ml reaction tubes and centrifuged at 2500 rpm in a microcentrifuge for 5 min to collect the cells. The PBS was removed by aspiration and the cells were resuspended in 100 μl of 0.25 M Tris–HCl, pH 7.8 containing 0.05 mM phenyl methyl sulfonyl fluoride (PMSF). The cells were lysed by three cycles of freeze–thaw and the cell extracts were collected by centrifugation at 12,000 rpm for 5 min at room temperature. The transfection efficiency was measured by determining the activity of β-galactosidase in 10 μl of the cell extract [23]. Extracts containing 10 U of β-galactosidase activity were used in assaying the CAT activity (usually between 30 and 50 μl of the extract).

Assay of 11β-HSD activity

The LNCaP cells were grown to confluence in RPMI 1640 as described above, scraped with a rubber policeman, centrifuged at 2500 rpm for 10 min to collect the cells, washed with PBS and resuspended at 10^6 cells/50 μl in DMEM–HEPES (DMEM with 15 mM HEPES pH 7.4). The 11β-HSD assay was essentially as described by Quirk *et al.* [24] with minor modifications. Briefly, the assay was carried out in glass vials in a total volume of 100 μl, comprising of cells in 85 μl of assay buffer (DMEM–HEPES), 10 μl 5 mM NAD, NADP or NADH and 5 μl 50 μM [^{3}H]cortisol (25 μM unlabeled cortisol and 25 μM [^{3}H]cortisol, NET-396 hydrocortisone, [1,2,6,7-^{3}HN)]cortisol, (sp. act. 2590 GBq/mmol). Triplicate incubations were conducted for various times at 37°C in a shaking water

bath. With appropriate incubation times, the conversion of cortisol to cortisone was linear with respect to cell number and time of incubation up to ~50% conversion. At the end of the incubation period, 1 ml of ethyl acetate (extraction efficiency >85%) was added and the steroids were extracted by vortexing twice for 1 min. The phases were separated by centrifugation at 3000 rpm at room temperature and the organic phase was transferred into 1.5 ml reaction tubes. Ethyl acetate was removed by speed vac, and the steroids were redissolved in 10 μl of ethyl acetate and spotted onto fluorescein coated Merck silica gel aluminium plates with 5 μl of 10 mM nonradioactive cortisol and 5 μl of nonradioactive cortisone to visualize the products. The thin layer was developed in chloroform–methanol (9:1, v/v), dried and autoradiographed on Kodak X'O MAT film for 4 days. The areas corresponding to authentic cortisol and cortisone were located, recovered, and the radioactivity in each area determined for calculation of the conversion of cortisol to cortisone. No significant activity was localized in areas other than cortisol or cortisone. This was established by subdividing the TLC into zones and counting the activity in all zones after cutting. The percentage of the conversion of cortisol to cortisone was calculated by adding the radioactive counts identified as cortisol and cortisone and expressing the radioactive cortisone as a percentage thereof.

Synthesis of [^{3}H]cortisone

Synthesis of [^{3}H]cortisone was performed by incubation at 37°C for 3 h with 5×10^6 cells in a final volume of 1 ml with 50 μl radioactive cortisol (sp. act. 250 GBq/mmol). The radioactive steroids were extracted with 3 × 1 ml ethyl acetate and the products formed were separated on a preparative thin layer (Fig. 3) as described above. The radioactive band from the preparative thin layer was cut out and the steroids were extracted from the silica gel by extraction with ethyl acetate. The final products were collected by evaporating the solvent, the cortisol and cortisone redissolved in 200 μl of ethanol for use in conversion assay.

Assay of 11-ketoreductase activity

The assay was carried out in glass vials in a total volume of 100 μl, comprising of cells in 85 μl of assay buffer (DMEM–HEPES), 5 mM NADH (final) and 1 μM [^{3}H]cortisone (250 GBq/mmol). Incubations were in triplicate for various times at 37°C in a shaking water bath. At the end of the incubation period, 1 ml of ethyl acetate (extraction efficiency >85%) was added and the steroids were extracted by vortexing twice for 1 min. The phases were separated by centrifugation at 3000 rpm at room temperature and the organic phase was transferred into 1.5 ml reaction tubes. Ethyl acetate was removed by speed vac, and the steroids were redissolved in 10 μl of ethyl acetate and spotted onto fluorescein coated Merck silica gel aluminium plates with 5 μl of 10 mM nonradioactive cortisol and 5 μl of nonradioactive cortisone to visualize the products. The thin layer was developed in chloroform–methanol (9:1, v/v), dried and autoradiographed on Kodak X'O MAT film for 4 days. The areas corresponding to authentic cortisol and cortisone were located, recovered, and the radioactivity in each area determined for calculation of the conversion of cortisone to cortisol. The percentage of the conversion of cortisol to cortisone was calculated by adding the radioactive counts identified as cortisone and cortisol and expressing the radioactive cortisol as a percentage thereof.

Hormone binding assay

LNCaP cells were grown in RPMI medium supplemented with DCC-FBS and harvested in HBS containing 1.5 mM EDTA by scraping with a rubber policeman. The cells were placed on ice, collected by centrifugation and washed with ice-cold PBS or TKEG buffer (20 mM Tris–HCl pH 7.4, 50 mM KCl, 1 mM EDTA 0.1 mM PMSF and 10% glycerol) and resuspended in 250 μl/plate homogenization buffer (50 mM Tris–HCl pH 7.4, 1.5 mM EDTA, 10 mM sodium molybdate, 2.5 mM β-mercaptoethanol, 50 mM KCl, 0.1 mM PMSF and 10% glycerol). Cells were homogenized by 25–30 strokes in a glass Dounce homogenizer (B pestle). The homogenates were centrifuged at 65,000 rpm at 4°C for 60 min. The protein concentration was determined with the Biorad reagent.

The protein concentration of the high speed supernatant (cytosol) was adjusted to 10–20 mg/ml. Duplicate aliquots of 50 μl were incubated with 10 nM [1,2,6,7-^{3}H]aldosterone (sp. act. 2.6–3.9 TBq/mmol; Amersham, U.S.A.) in the presence and absence of varying concentrations of the competitor steroids. After over night incubation at 4°C, an equal volume of 0.5% Norit A charcoal, 0.05% dextran T-70 in TKEG was added. The samples were incubated for 10 min on ice with occasional mixing centrifuged at 4000 *g* for 10 min at 4°C and 125 μl was transferred into scintillation vials. The radioactivity was determined by adding 4 ml of scintillation cocktail and counting in a scintillation counter.

RESULTS

11β-HSD activity in LNCaP cells in suspension

The activity of 11β-HSD in LNCaP cells to convert **F** to **E** was analyzed by incubating 50,000–250,000 cells in suspension with labeled **F** in the presence of NAD or NADP as coenzymes. The conversion of **F** to **E** was not observed with incubations in the absence of either NAD or NADP. The conversion of **F** to **E** in LNCaP cells was linear with respect to cell number. This reaction reached a plateau after 120 min of incubation at 37°C. Addition of 0.25 mM NAD or 0.25 mM

NADP during the incubation revealed that the metabolic activity of converting **F** to **E** was 50% higher in the presence of NAD. The 11β-HSD activity in the incubates was blocked by adding 1 μM CBX, a potent inhibitor of the enzyme (data not shown). The conversion of **F** to **E** was drastically decreased by freezing the cells. The addition of neither NAD nor NADP to the frozen cell suspension resulted in an activation of the enzyme.

Synthesis of [^{3}H]cortisone and assay of reductase activity

Tritium labeled cortisone was synthesized with [^{3}H]cortisol as a substrate, to assay the reverse reaction in LNCaP cell suspension. The overall yield of [^{3}H]cortisone was 40% (Fig. 1). With this radiolabeled cortisone the 11-ketoreductase activity was assayed in LNCaP cell suspension and found to be absent. Addition of 5 mM NADH (final concentration) did not influence the 11-ketoreductase activity (during shorter incubation periods). However, 12–24 h incubations in the presence of NADH resulted in a 9% conversion of E to F (Fig. 2). Assay of 11β-HSD with the nonconverted cortisol purified from the preparative thin layer was identical to the radioactive cortisol conversion observed previously. In the preparative conversion of cortisol to cortisone in LNCaP cells, an additional minor band was observed which could be the 21-acetylated derivative of cortisol.

Specificity of aldosterone (ALDO) binding in LNCaP cells

The binding of tritium labeled ALDO provided evidence for the presence of MR in LNCaP cells. Dihydrotestosterone (DHT), R5020 and the antiglucocorticoid RU486 did not compete with radioactive ALDO binding. Dexamethasone (DEX), at higher concentrations, displaced ALDO binding by 50% (Fig. 3).

11β-HSD dependent inactivation of cortisol assessed by transcription activation

We have analyzed the ability of endogenous GR and MR in LNCaP cells to induce the transcription activation of GR, MR, PR and AR responsive MMTV-CAT by transient transfection. Following transfection, the LNCaP cells were treated with DEX, ALDO, DHT, **E** and **F**. As shown in Fig. 4, the MMTV-CAT was induced by DEX (Fig. 4, lanes 2–5) and ALDO (Fig. 4, lanes 7–10) in a dose dependent manner. Similarly, LNCaP cells transfected with MMTV-CAT following treatment with DHT show the dose dependent hormonal response mediated by AR (Fig. 5, lanes 2–10). LNCaP cells transfected with MMTV-CAT

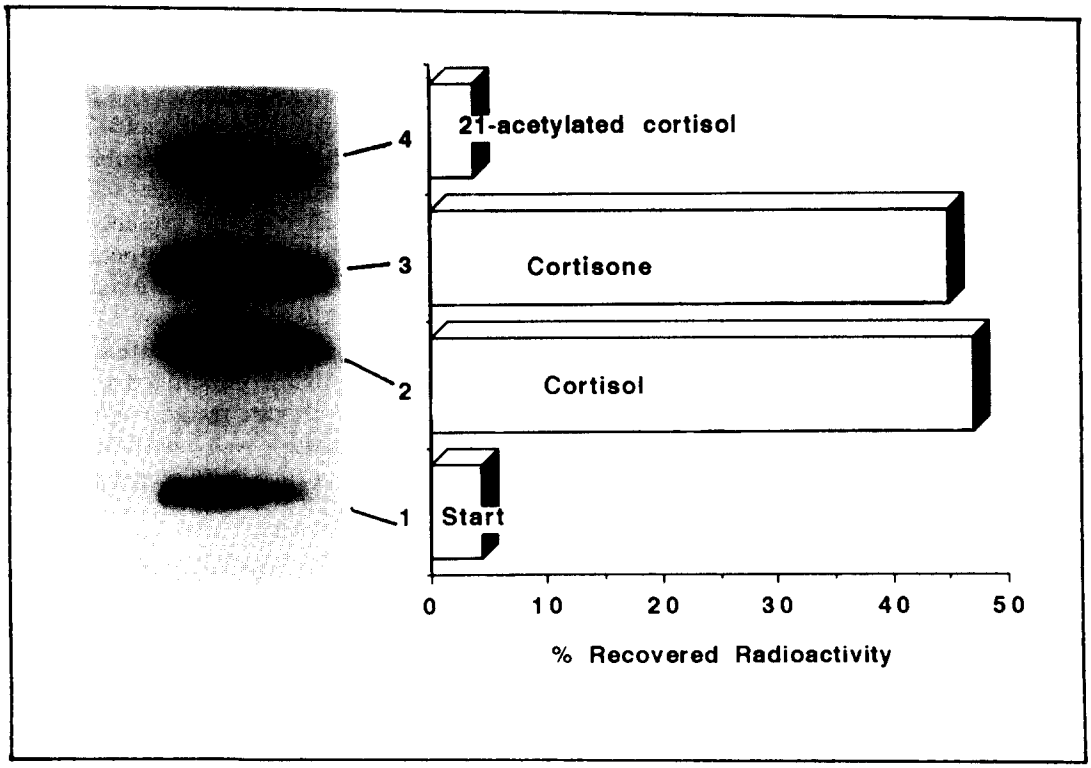

Fig. 1. Enzymatic synthesis of radioactive cortisone from tritium labeled cortisol. The synthesis performed in a final volume of 1 ml with 5×10^6 cells. The products were purified as described in Experimental. The autoradiogram of the preparative thin layer is also shown in Fig. 1. Exposure was for 2 days at room temperature. The percentage represents the actual radioactivity recovered from the starting 100% used in the synthesis. This radioactive tracer was used in determining the 11-ketoreductase activity in LNCaP cell suspensions.

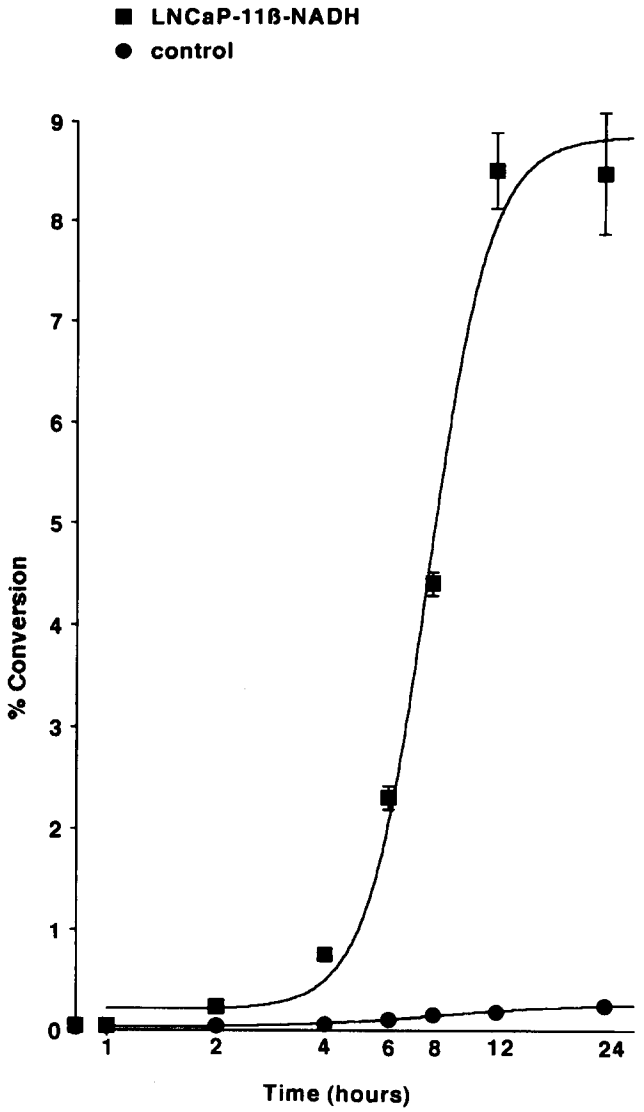

Fig. 2. 11-Ketoreductase activity in LNCaP cells in the presence and absence of exogenous NADH. Cells suspended in DMEM–HEPES were incubated in a final volume of 100 μl in the presence and absence of 5 mM NADH with 1 μM radioactive cortisone. Following various incubation periods the steroids were extracted with ethyl acetate and the percentage of conversion determined by counting the radioactivity in the respective spots visualized by UV. The bars represent standard deviation.

show a limited response to F (Fig. 6, lanes 2–7). The induction did not reach the maximum level even at 10 μM F (Fig. 6, lanes 2–7). However, the concomitant treatment of the transfectants with 10^{-10}–10^{-5} M F in the presence of 10^{-7} M of the 11β-HSD inhibitor CBX produced an elevation of CAT activity (Fig. 6, lanes 8–13). The difference in the levels of activation in the presence and absence of 100 nM CBX in the presence of 0.1–10 μM F was ~2 orders of magnitude (Fig. 6, lanes 11, 12 and 13, respectively). The inability of E to activate transcription was not influenced by the addition of increasing concentrations of CBX (data not shown). These data indicate that CBX in the presence of E does not elicit mineralocorticoid like activity. However, transcription activation of MMTV-CAT was observed by treating the LNCaP transfectants for 24 h with 10^{-6}–2×10^{-3} M NADH in the presence of 100 nM E (Fig. 6, lanes 15–19). Thus, the conversion of E to F was activated in the transcription activation assays of LNCaP cells transfected with MMTV-CAT and treated with E and NADH (Fig. 6, lanes 14–19).

DISCUSSION

The human prostate epithelial cell line LNCaP is responsive to several steroid hormones as described by Horoszewicz *et al.* [25]. The presence of GR mRNA and GR protein has been established previously [4, 26]. The AR in LNCaP has an altered affinity for a number of steroids and their analogs such as progesterone (R5020), antiprogesterone (RU 486), the antiandrogens

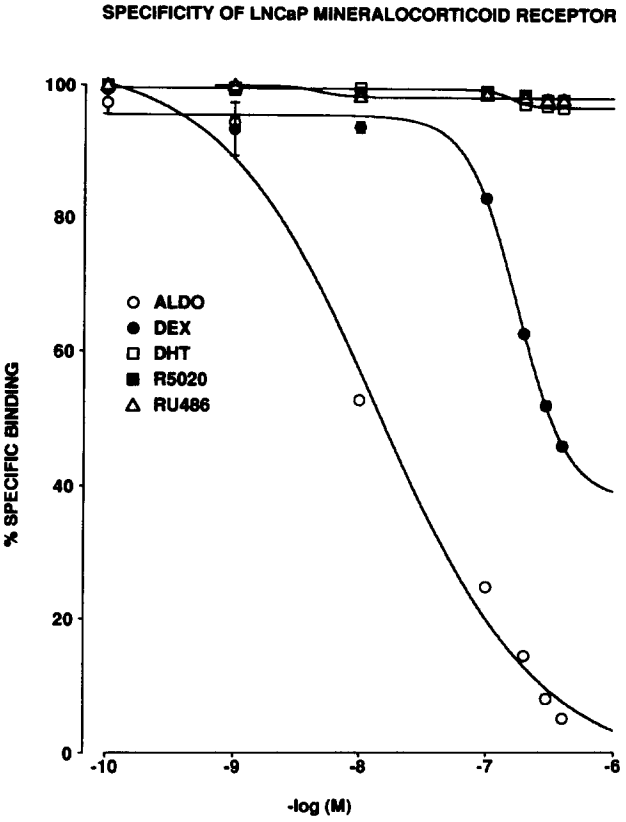

Fig. 3. Determination of relative affinities of various steroids to the MR by competition assay. Cytosol was prepared from LNCaP cells. Aliquots of cytosol were incubated in duplicate with 10 nM [^{3}H]ALDO in the absence and in the presence of various concentrations (0–5 μM) of nonradioactive competitors over night at 4°C. Unbound steroids were removed by treating with an equal volume of charcoal suspension (0.5% Norit A, 0.05% dextran T-70 in TKEG buffer for 10 min at 0°C). Charcoal was removed by centrifugation at 4000 g for 10 min at 4°C. Receptor bound radioactivity was measured from the charcoal treated cytosol. Each point represents an average of duplicate samples and is expressed as a percentage of the control incubates in the absence of the nonradioactive competitor.

	1	2	3	4	5	6	7	8	9	10
MMTV CAT 5μg	+	+	+	+	+	+	+	+	+	+
CH 110 5μg	+	+	+	+	+	+	+	+	+	+
LNCaP	+	+	+	+	+	+	+	+	+	+
DEX	-	b	c	d	e	-	-	-	-	-
ALDO	-	-	-	-	-	-	b	c	d	e

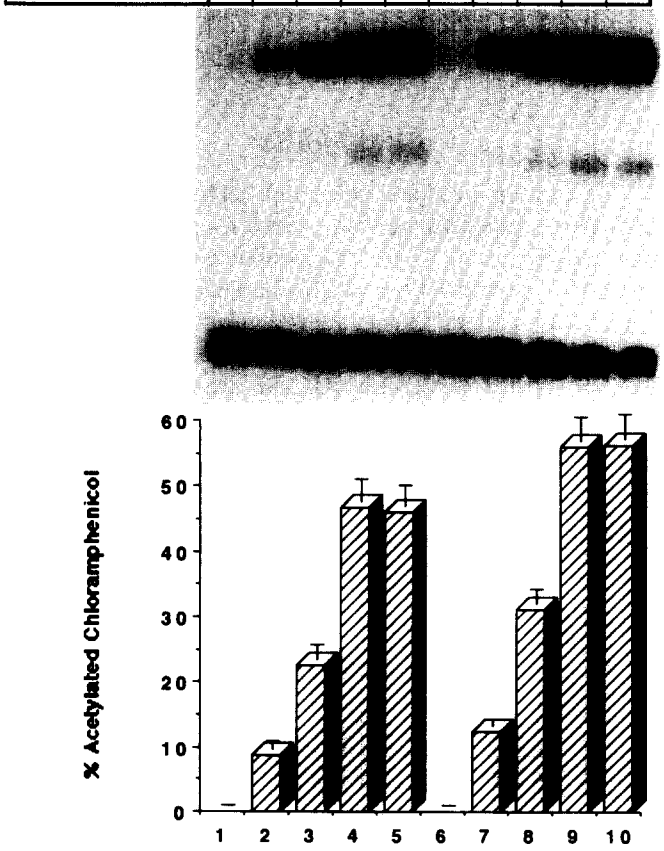

Fig. 4. Transcription activation of MMTV-CAT transfected into LNCaP cells. MMTV-CAT in the presence of CH110 (β-galactosidase expression plasmid) was transiently transfected into LNCaP cells as described in Experimental. The transfections were with a single pool of cells. Transfection efficiency was measured by determining the β-galactoside activity and CAT activity was subsequently measured with extracts containing 10 U of β-galactosidase. The hormone concentrations were as follows: b, 10^{-10}; c, 10^{-9}; d, 10^{-8} and e, 10^{-7} M. Numbers 2–5 represent treatments with DEX and 7–10 show treatments of the transfectants with ALDO. The histogram shows the percentage of acetylated chloramphenicol determined by scratching and counting the radioactive spots in the thinlayer plates histogram represents the results of three independent experiments. The bars represent standard deviation.

(cyproterone acetate and hydroxyflutamide), and androgen metabolite epiandrosterone [4–9]. However, the mutant AR in LNCaP does not bind potent glucocorticoids [4]. Cortisol is the principal glucocorticoid secreted in man (~15–20 mg/day) and its main site of action is thought to be the liver [27]. Conversion of F to the inactive steroid E occurs early in the metabolic transformation of F and is catalyzed by the microsomal enzyme complex 11β-HSD [15, 24]. Many tissues such as liver, kidney, adipose tissue, hippocampus, testis, uterus, lung, parotid and mammary gland show 11β-HSD activity [15, 16, 28–30]. Our understanding of the physiological significance of 11β-HSD was underlined from the investigation of the enzyme-deficient state [31].

LNCaP cells produce the prostate acid phosphatase [32], prostate specific antigen [33] and contain AR and GR [4–9]. However, the affinity of AR for other androgens such as DHT and testosterone is unchanged. We have combined experimental procedures reported by Quirk *et al.* [24] and Blankenstein *et al.* [34] to assay 11β-HSD activity in whole cells in suspension. Blankenstein *et al.* described the free entry of exogenous NADPH across the Leydig cell plasma membrane. Similarly, Stein and Tesone studied the effects of the NADPH generating system on steroidogenic response

	1	2	3	4	5	6	7	8	9	10
MMTV CAT 5μg	+	+	+	+	+	+	+	+	+	+
CH 110 5μg	+	+	+	+	+	+	+	+	+	+
LNCaP	+	+	+	+	+	+	+	+	+	+
DHT	-	A	B	C	D	E	F	G	H	I

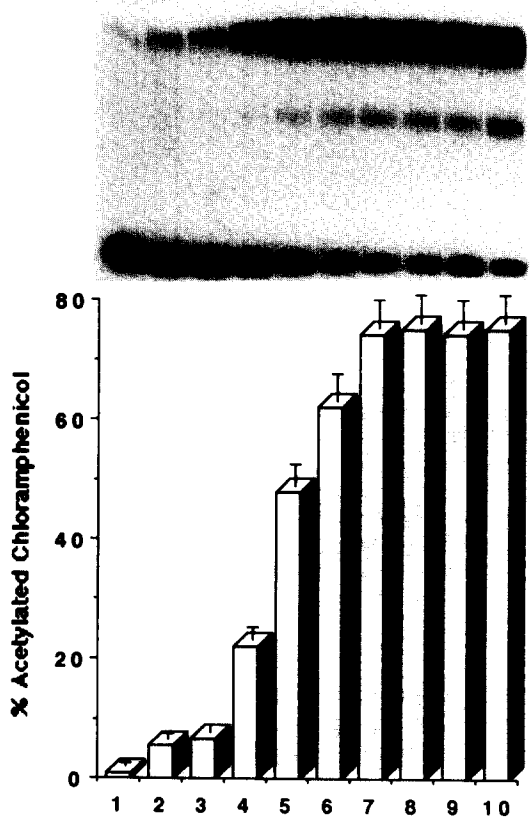

Fig. 5. Transcription activation of MMTV-CAT by DHT. The transfectants were treated with increasing concentrations of DHT following transient transfection. These cells contained approx. 31,000 high affinity ($K_d = 9 \times 10^{-10}$ M) androgen binding sites per cell (5). The concentrations of DHT used in treating the transfectants were: A, 10^{-12}; B, 10^{-11}; C, 10^{-10}; D, 10^{-9}; E, 3×10^{-9}; F, 10^{-8}; G, 3×10^{-8}; H, 10^{-7} and I, 10^{-6} M, respectively. The histogram represents the results of three independent experiments. The bars represent standard deviation.

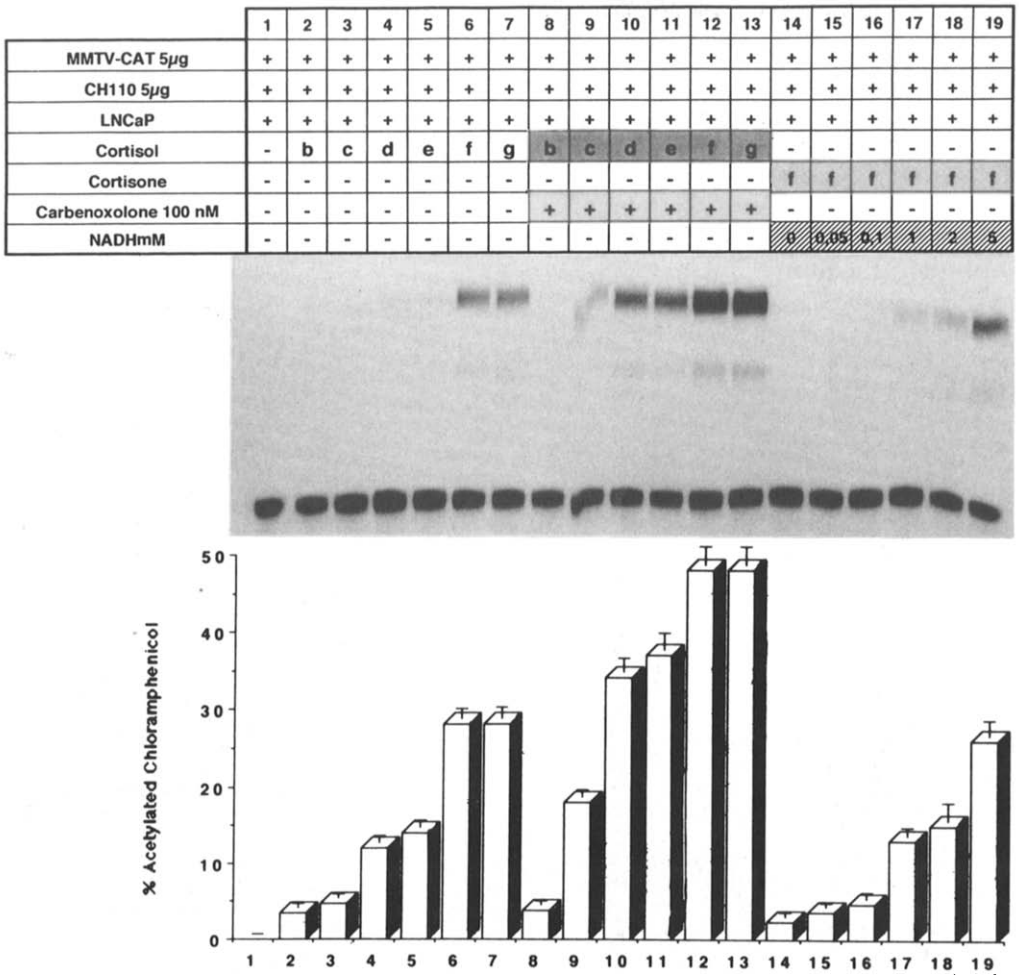

Fig. 6. Cotransfection of LNCaP cells with MMTV-CAT and treatment with cortisol and cortisone. LNCaP cells cotransfected with MMTV-CAT and CH110 were treated with cortisol (lanes 2–13) and cortisone (lanes 14–19). The hormone concentrations were as follows b, 10^{-10}; c, 10^{-9}; d, 10^{-8}; e, 10^{-7}; f, 10^{-6} and g, 10^{-5} M. The treatments in lanes 8–13 were in the presence of 100 nM CBX and the treatments in lanes 14–19 were in the presence of increasing concentrations of NADH in the medium. The histogram represents the results of three independent experiments. The bars represent standard deviation.

in rat luteal cells [35, 36]. To test the effects of coenzymes on 11β-HSD activation, LNCaP cells were incubated in suspension. Our data document a considerable level of 11β-HSD activity. We have established that LNCaP cells possess an extensive metabolism of **F** to **E**. The metabolism experiments were conducted in suspension to eliminate the influence of serum factors to the tissue culture medium. In addition to **F** and **E**, we have detected the presence of a third metabolite as previously reported by Hampel *et al.* [37] and Quirk *et al.* [24]. According to Hampel *et al.* this third metabolite was the 21-acetylated derivative of corticosterone.

Montgomery *et al.* [4] have demonstrated the inability of the mutant AR in LNCaP cells to bind dexamethasone. This leads us to conclude that the transcription activation observed with dexamethasone is mediated by GR. However, we have established the presence of a functional MR in LNCaP cells by a competitive hormone binding assay. The localization of 11β-HSD activity in LNCaP cells suggests a general mechanism in the prostate tissue to lower **F** concentration levels, a mechanism not confined to a particular cell type. In LNCaP cells treated with **F**, 11β-HSD activity lowers transcription activation of MMTV-CAT. This activity appears to assign the enzyme with yet another important function namely "conferring androgen specificity" by preventing **F** occupied hGR and hMR to compete with AR for binding to androgen responsive element (ARE) since ARE is similar if not identical to glucocorticoid responsive element (GRE). The **F** dependent transcription activation is enhanced $\sim$2 orders of magnitude by inhibiting the 11β-HSD with CBX [38]. The NAD dependent specificity of conversion may be an indication of the presence of a family of 11β-HSD enzymes [39], a member of which has been purified from rat liver [28]. Northern blot analysis has detected 11β-HSD in liver, lung, kidney,

and testis [40] but not in the parotid [41]. The enzyme was demonstrated to be present by immunofluorescence in proximal convoluted tubes of the kidney and absent in the corticol collecting tubules [19]. High levels of this species were also present, as demonstrated by immunofluorescence in the testis, where a developmental role involving modulation of GR occupancy has been suggested [42]. Even though, we have shown that the enzyme in LNCaP cells modulates GR induced transcription activation, its physiological role affecting AR specificity in the prostate remains to be established.

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