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Induction of NAD$^+$-Linked 15-Hydroxyprostaglandin Dehydrogenase Expression by Androgens in Human Prostate Cancer Cells[1]

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Prostate cancer cells are known to express cyclooxygenases (COXs) and synthesize prostaglandins. Catabolism of prostaglandins in these cells remains to be determined. Induction of NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a key metabolic inactivation enzyme, was investigated in androgen-sensitive LNCaP cells and in hormone-independent PC3 cells. 15-PGDH was found to be induced by dihydrotestosterone or testosterone in a time- and dose-dependent manner in LNCaP but not in PC3 cells as shown by activity assay and immunoblot analysis. However, prostaglandin synthetic enzymes, COX-1 and COX-2, were not found to be induced by androgens. Induction was also achieved by 17β-estradiol and progesterone, although to a lesser extent. Induction of 15-PGDH was not blocked by steroid receptor antagonist, RU 486, nor by antiandrogen, flutamide. However, induction was inhibited by tyrosine kinase inhibitor, genistein, and by ERK kinase inhibitor, PD 98059, but not by protein kinase C inhibitor, GF109203X. These results suggest that androgens induce 15-PGDH gene expression through an unconventional nongenomic pathway.

Prostaglandins are a family of biologically potent fatty acids derived from arachidonic acid. Transformation of arachidonic acid to prostaglandins is initiated by oxidative cyclization of the fatty acid catalyzed by cyclooxygenase (COX) (1). Two isoforms of COX have been recognized. COX-1 is believed to be a constitutively expressed enzyme, whereas COX-2 is generally found to be induced by growth factors, tumor promoters and pro-inflammatory cytokines although constitutive expression also occurs in some tissues and cells (2). These prostaglandins are rapidly metabolized and inactivated. The key enzyme responsible for metabolic inactivation of prostaglandins is NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) which catalyzes the oxidation of 15(S)-hydroxyl group of prostaglandins to a keto function (3). The 15-keto derivatives exhibited greatly reduced biological activities.

Biosynthesis of prostaglandins has been demonstrated in most mammalian tissues and cells (4). Prostate tissue is well known to actively synthesize prostaglandins. Prostate cancer cells such as androgen sensitive LNCaP cells and androgen independent PC3 cells have also been shown to express high levels of either forms of cyclooxygenase (5, 6). However, whether prostate and its cancer cells possess the capacity to catabolize prostaglandins has not been reported. We have found that resting prostate cancer cells exhibit little prostaglandin catabolic activity. However, androgens and to a lesser extent estrogens are able to induce the expression of 15-PGDH in hormone responsive LNCaP cells but not in nonresponsive PC3 cells. This study represents the first report that androgens may stimulate the catabolism of prostaglandins in prostate cancer cells which constitutively generate high levels of prostaglandins.

EXPERIMENTAL PROCEDURES

Materials. Testosterone, dihydrotestosterone, dithiothreitol (DTT), bovine liver glutamate dehydrogenase, dexamethasone, mifepristone (RU486), flutamide, hydrocortisone, corticosterone, 17β-estradiol, progesterone, genistein, phenylmethylsulfonyl fluoride (PMSF), NAD$^+$ and RPMI 1640 were obtained from Sigma Chemical Co. GF 109203X, PD 98059 and wortmannin were purchased from Alexis Biochemical. PGE_2 was supplied by Cayman Chemical Co. ECL$^+$ plus Western Blotting Detection System RPN 2132 was obtained from Amersham Pharmacia Biotech. Rabbit antiserum against human placental 15-PGDH was generated as described previously (7). Rabbit antisera against human COX-1 (LLPPLPVLLADPGAPTPV) and COX-2 (NASSSRSGLDDINPTVLLK) specific sequences were generated using glutathione-S-transferase fusion protein as antigens (8). 15(S)-[15-^{3}H]PGE_2 was prepared according to a previously

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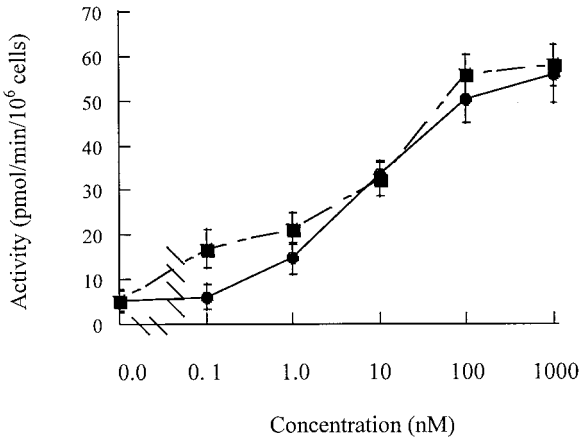

FIG. 1. Effect of dihydrotestosterone (DHT) and testosterone (TS) on the induction of 15-PGDH activity. LNCaP cells were treated with the indicated concentrations of DHT (■) or TS (●) for 24 h. Cells were washed and sonicated for the determination of 15-PGDH activity as described under Experimental Procedures.

published procedure (9). LNCaP and PC3 cells were obtained from the American Type Culture Collection. Other reagents were obtained from the best commercial sources.

Cell culture. LNCaP and PC3 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum, 100 units of penicillin per ml and 100 μg of streptomycin per ml at 37°C in a humidified atmosphere of 5% CO_2. The cells were plated in 6-well plate (2 ml per well) at about 5×10^5 cells per ml in duplicate and grown for 36 h before treatment.

Treatment of prostate cancer cells. After LNCaP or PC3 cells were grown for 36 h, cells in each well received treatment with dihydrotestosterone, testosterone or other steroids either alone or in different combinations with other reagents as described in the figure legends.

Preparation of cell homogenate. Prostate cancer cells from the above culture were scraped and spun down at maximal speed in a microfuge for 2 min and washed once with saline. Approximately 1×10^6 cells were suspended in 1 ml of 0.05 M Tris-HCl buffer, pH 7.5 containing 1 mM DTT and sonicated in an ice bath for 3×10 s by an ultrasonic sonicator set at 4. The crude homogenate was used as an enzyme preparation.

Enzyme assay. 15-PGDH was routinely assayed by measuring the transfer of tritium from 15(S)-[15-^{3}H]PGE_2 to glutamate by coupling 15-PGDH with glutamate dehydrogenase as described previously (7). Briefly, the reaction mixture contained NH_4Cl (5 μmol), α-oxo-glutarate (1 μmol), NAD^+ (1 μmol), 15(S)-[15-^{3}H]PGE_2 (1 nmol, 20,000 cpm), glutamate dehydrogenase (100 μg), DTT (1 μmol) and 15-PGDH enzyme preparation in a final volume of 1 ml of 0.05 M Tris-HCl, pH 7.5. The reaction was allowed to continue for 10 min at 37°C, and was terminated by the addition of 0.3 ml of 10% aqueous charcoal suspension. After incubation for 5 min the mixture was centrifuged at 2000*g* for 5 min. The radioactivity in the supernatant was determined by liquid scintillation counting. Calculation of the amount of PGE_2 oxidized was based on the assumption that no kinetic isotope effect was involved in the oxidation of 15(S)-hydroxyl group of 15(S)-[15-^{3}H]PGE_2 as a substrate. Enzyme activity of each sample was always assayed in duplicate.

SDS–PAGE and immunoblot analysis. LNCaP cells either treated or incubated with different stimuli were homogenized in phosphate-buffered saline containing 1 mM PMSF, 1 mM DTT, 50 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor and 1 mM benzamidine by sonication in an ice bath for 3×10 s. Approximately 50–150 μg of cellular extract was resolved by SDS–PAGE (10%) gel according to the method of Laemmli (10). Electrophoretic transfer of proteins from the gel to PVDF membrane was performed according to the method of Towbin *et al.* (11). The membrane was blocked with 5% non-fat dry milk in 0.02 M Tris-HCl, pH 7.6 containing 0.8% NaCl and 0.1% Tween 20 (TBST) followed by incubation with a rabbit antiserum against human placental 15-PGDH (1 to 2000 dilution in TBST with 5% non-fat milk) at room temperature for 1 h. After washing with TBST three times, the membrane was incubated with goat anti-rabbit IgG-horse radish peroxidase conjugate (1 to 5000 dilution in TBST with 5% nonfat milk) at room temperature for 1 h. The immunoreactive bands were detected with ECL$^+$ Plus Western Blotting Detection System.

Statistical analysis. Each enzyme sample was performed in duplicate. The data were expressed as the mean ± SE. Statistical significance was assessed by Student's *t* test using a *P* value of <0.05. Each figure is a representative of 2–4 replications.

RESULTS

Human prostate cancer cells, either hormone-responsive LNCaP cells or hormone-independent PC3 cells, are known to express both isoforms of cyclooxygenase (5, 6). Accordingly, biosynthesis of prostaglandins is evident in these cell lines. Whether these cell lines also possess catabolic activity of prostaglandins is

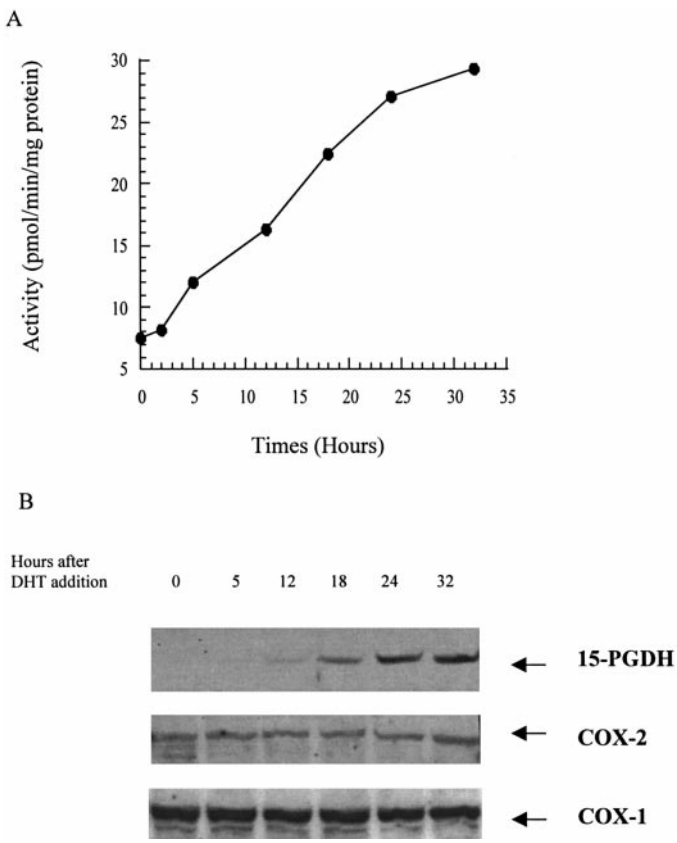

FIG. 2. The time course of 15-PGDH induction by dihydrotestosterone (DHT). LNCaP cells were treated with 1 μM DHT for the indicated amount of time. Cells were then washed and sonicated for the determination of (A) 15-PGDH activity and (B) 15-PGDH immunoreactivity as described under Experimental Procedures.

TABLE 1

Effect of Several Steroids on the Induction of 15-PGDH Activity

Steroids (1 μM)	Activity (pmol/min per 10^6 cells)
Control	6.1 ± 1.2
Dihydrotestosterone	58.5 ± 7.0
Testosterone	56.5 ± 6.3
17β-Estradiol	28.3 ± 2.7
Progesterone	40.4 ± 4.2
Hydrocortisone	6.5 ± 0.7
Corticosterone	16.1 ± 2.8
Dexamathasone	6.5 ± 0.8
Mifepristone (RU 486)	5.9 ± 3.1

Note. LNCaP cells were treated with 1 μM of each steroid for 24 h. Cells were washed and sonicated for the determination of 15-PGDH activity as described under Experimental Procedures.

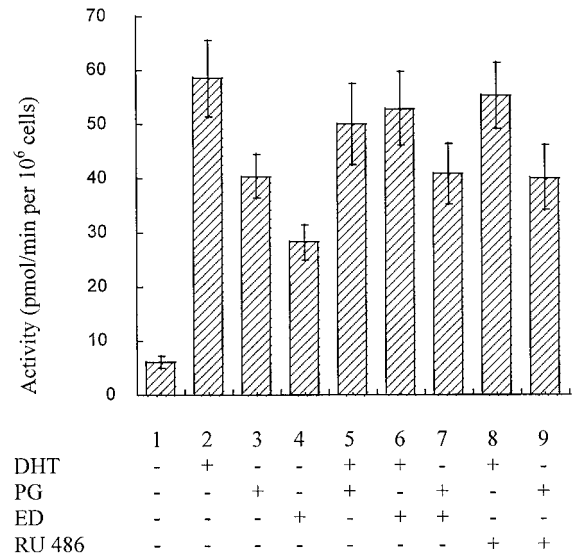

FIG. 3. Effect of steroid receptor antagonist, RU 486 on the induction of 15-PGDH by different combinations of steroids. LNCaP cells were treated with 1 μM each of various steroids or in different combinations for 24 h before washing and determination of 15-PGDH activity as described under Experimental Procedures. Each column represents: vehicle (1), dihydrotestosterone (DHT) (2), progesterone (PG) (3), 17β-estradiol (ED) (4), dihydrotestosterone plus progesterone (5), dihydrotestosterone plus 17β-estradiol (6), progesterone plus 17β-estradiol (7), dihydrotestosterone plus RU-486 (8), progesterone plus RU 486 (9).

not clear. When the 15-PGDH activity was assayed in either cell lines, LNCaP exhibited low basal activity, whereas PC3 cells showed no detectable activity. When an androgen, testosterone or dihydrotestosterone, was incubated with these prostate cancer cells for 24 h, significant increase in the 15-PGDH activity was observed in LNCaP cells but not in PC3 cells. A concentration-dependent increase in the 15-PGDH activity in LNCaP cells is shown in Fig. 1. Testosterone or dihydrotestosterone at 1 nM increased significantly the 15-PGDH activity. Maximal stimulation was nearly observed at 1 μM of androgens. Dihydrotestosterone appeared to be more effective than testosterone at lower concentrations. The time course of induction of the 15-PGDH activity is shown in Fig. 2A. A steady increase in the 15-PGDH activity was observed over a period of 34 h. The increase in the 15-PGDH activity appeared to correlate well with the increase in the expression of enzyme protein detected by immunoblot as shown in Fig. 2B. Interestingly, both COX-1 and COX-2 expression remained unchanged during the course of androgen stimulation. Aside from the androgens, other sex hormones and glucocorticoids were also examined for their ability to stimulate the 15-PGDH induction. Progesterone, 17β-estradiol and corticosterone at 1 μM were found to increase the 15-PGDH activity to a different degree although the increase was not as dramatic as those of the androgens as shown in Table 1. Either male or female sex hormones were able to stimulate the 15-PGDH activity. Whether these hormones act on different pathways or converge to the same pathway remains to be determined. Combination of any of the two hormones did not show any additive effect as shown in Fig. 3. These results indicate that these sex steroid hormones may act through the same pathway or receptor. However, steroid receptor antagonist, mifepriston (RU 486) (12) at 1 μM did not antagonize the effect of either dihydrotestosterone or progesterone indicating that these hormones did not act through the traditional steroid receptors. Similar findings were observed with anti-androgen, flutamide (13) at 1 μM (data not shown). To study other possible modes of action of the androgens, various protein kinase inhibitors were examined if they affected dihydrotestosterone induced 15-PGDH expression. Table 2 shows that protein kinase C inhibitor, GF 109203 X (14) at 1 μM and PI 3-kinase inhibitor, wortmannin (15) at 0.1 μM did not inhibit dihydrotestosterone induced 15-PGDH expression. However, MAPK/ERK ki-

TABLE 2

Effect of Various Kinase Inhibitors on Dihydrotestosterone-Induced 15-PGDH Activity

Kinase	Concentration (μM)	Activity (pmol/min/10^6 cells)	Inhibition (%)
Control		56.2 ± 6.5	0
PD 98059	50.0	20.9 ± 5.3	62.8
Genistein	50.0	11.9 ± 4.2	78.8
Wortmannin	0.1	55.4 ± 3.8	1.4
GF109203X	1.0	55.6 ± 4.5	1.1

Note. LNCaP cells were incubated with 1 μM dihydrotestosterone in the presence or absence (control) of each protein kinase inhibitor for 24 h. Cells were washed and sonicated for the determination of 15-PGDH activity as described under Experimental Procedures.

nase inhibitor, PD 98059 (16) at 50 μM and tyrosine kinase inhibitor, genistein (17) at 50 μM, attenuated significantly dihydrotestosterone induced 15-PGDH expression.

DISCUSSION

We have found that androgens and to a lesser extent estrogens are able to induce 15-PGDH expression in LNCaP cells but not in PC3 cells in a dose and time dependent manner. The unresponsiveness of PC3 cells may be related to their lack of the androgen receptor in this cell line (5). As low as subnanomolar concentrations of testosterone or dihydrotestosterone were effective in LNCaP cells. This is in contrast to our previous finding that submicromolar concentrations of anti-inflammatory steroids are needed to induce 15-PGDH expression in HEL cells (18). Dexamethasone and other glucocorticoids but not sex steroids were effective in this cell line. Induction of 15-PGDH expression by androgens and estrogens was found to be unrelated to classical androgen receptor as anti-androgens such as flutamide and mifepristone failed to antagonize the action of sex steroids. Induction of 15-PGDH expression was also found to be insensitive to GF 109203X, a specific inhibitor of protein kinase C indicating the non-involvement of protein kinase C in androgen induced 15-PGDH expression. This appears to be in direct contrast to an earlier report in which androgens were found to induce elevation of intracellular calcium concentration and inositol 1,4,5-triphosphate and diacyl glycerol production in rat osteoblasts (19). However, induction appeared to be inhibited by MAPK/ERK kinase inhibitor, PD 98059 and by tyrosine kinase inhibitor, genistein, at concentrations commonly used in assessing the role of ERK kinase and tyrosine kinase in receptor signalling. Apparently, induction is mediated by a non-classical androgen receptor which can be also activated by estrogens and which may lead to the activation of ERK prior to stimulation of gene expression.

The mechanism of action of androgens is not fully understood. Androgens classically exert their effects by interacting with the androgen receptor, a latent transcriptional factor found both in the nucleus and in the cytoplasm as a large complex containing heat shock proteins and other associated proteins (20). Androgen receptor undergoes conformational changes with dissociation of associated proteins and phosphorylation of the receptor following androgen binding (13). Activated receptor will then enter into the nucleus and bind to the androgen response elements in target genes. After a complex interaction with the transcriptional apparatus, activation or repression of gene expression is then elicited (21). Such a genomic responses to androgen is known to be inhibited by androgen antagonists including flutamide which binds to the androgen receptor and stabilizes the receptor with associated proteins (13). In addition to the genomic responses, non-genomic actions of androgens have been described. Steinsapir *et al.* (22) have reported that dihydrotestosterone triggers a rapid increase of intracellular calcium concentration in LNCaP prostate cancer cells. Zhu *et al.* (23) have described that androgens activate ERK through tyrosine phosphorylation in a rapid and transient manner in human breast cancer cells. This protein kinase has been shown to have an important role in the regulation of gene expression and cell growth (24). Our finding that ERK kinase inhibitor, PD 98089, and tyrosine kinase inhibitor, genistein, attenuated androgen induced 15-PGDH expression is consistent with such a non-genomic mode of action of androgens.

The significance of androgen induced 15-PGDH expression in LNCaP prostate cancer cells remains to be determined. Elevated synthesis of prostaglandins has been found in many cancerous tissues including prostate. Chaudry *et al.* (25) first showed that human malignant prostate tissue had significantly reduced arachidonic acid concentration compared with benign tissue. When these investigators examined the metabolism of arachidonic acid, significant synthesis of PGE_2 was found in both benign and malignant prostatic tissues, with the malignant tissues having 10 fold higher rate of synthesis than benign tissues (26). The data suggest a specific role for PGE_2 in maintaining the growth of malignant prostate tissue. Prostate cancer cells such as LNCaP and PC-3 cells have also been shown to express constitutively COX-2 (5, 6), whereas human fetal prostate fibroblasts was found to have barely detectable basal COX-2 expression (27). This finding is consistent with the observations made in other cancer cell lines such as colon cancer cells (28) and breast cancer cells (29) in which COX-2 is constitutively expressed. Enhanced expression of COX-2 may result in elevated synthesis of PGE_2 which may lead to growth stimulation. In fact, a non-metabolizable PGE_2 analog, 16,16-dimethyl PGE_2 has been found to stimulate PC-3 cell growth (5). We have found that androgens did not increase the expression of COX-1 and COX-2 in androgen sensitive LNCaP cells. However, androgens did increase the expression of 15-PGDH in a dose and time dependent manner in hormone sensitive LNCaP cells but not in hormone independent PC-3 cells. This finding suggests that androgens may control the levels of prostaglandins by facilitating their catabolism and curtail the growth induced by PGE_2 as a growth regulatory mechanism.

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