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Advances in Brief

Alteration of Glyceraldehyde-3-phosphate Dehydrogenase Activity and Messenger RNA Content by Androgen in Human Prostate Carcinoma Cells[1]

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Abstract

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) activity and mRNA content is altered in the androgen-responsive human prostate carcinoma cell line LNCaP after exposure to the synthetic androgen R1881. Elevation in GAPDH activity is noted as early as 24 h after treatment with 1 nM R1881 and lasts at least 96 h. R1881 has no effect on the activity of GAPDH in androgen-independent DU145 cells. LNCaP GAPDH mRNA content is lowered by treatment with 1 nM R1881; the magnitude of reduction appears to depend on the length of exposure. The results present at least one means by which androgen-responsive tissues may develop alterations in GAPDH mRNA or activity, as is found in certain tumor tissues.

Introduction

GAPDH[3] is primarily recognized for its role as a key regulatory enzyme of glycolysis. However, recent studies suggest that the M_r 37,000 protein may have alternate biological activities and that its function may depend on its oligomeric state (1). The tetramer produces 1,3-diphosphoglycerate, a high energy compound of glycolysis (2), but the monomeric form has been shown to possess uracil DNA glycosylase activity and is involved in DNA repair (1). GAPDH, which has been localized immunohistochemically to both the cytoplasm and nucleus (3, 4), has the ability to bind single-stranded DNA and has been shown to accumulate at the time of cell cycle arrest and of terminal differentiation. It has also been proposed to be involved in transcription (3). These data suggest that alterations in GAPDH may not only effect glycolysis but may also have impact on other critical cellular processes.

Recently Epner *et al.* (5) have provided evidence for an association of cell motility and metastatic potential with GAPDH mRNA expression in rat prostatic adenocarcinoma tissue. Other groups have previously reported that GAPDH mRNA is elevated in tumor tissues (6, 7). Although the reason for this accumulation is not clear, it has been suggested to be related to the increased rate of aerobic glycolysis long known to occur in tumor cells (reviewed in Ref. 8). This report provides evidence that both GAPDH mRNA and activity are altered after exposure to the synthetic androgen R1881 in the androgen-responsive human prostate carcinoma cell line LNCaP. The results present at least one means by which androgen-responsive tissues may develop alterations in their GAPDH mRNA or activity.

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[3] The abbreviations used are: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FBS, fetal bovine serum; CSS, charcoal-stripped FBS.

Materials and Methods

Cell Culture and GAPDH Activity. LNCaP and DU145 cells obtained from American Type Culture Collection (Rockville, MD) were maintained at 37°C in 5%CO_2/95% air atmosphere in DMEM supplemented with 5% heat-inactivated FBS (GIBCO, Grand Island, NY) plus antibiotics/antimycotics (Sigma, St. Louis, MO). For the following experiments, 10^6 cells were plated in triplicate in 100-mm tissue culture plates containing DMEM plus 5% FBS. Twenty-four h after plating, medium was replaced with DMEM containing 1% FBS and 4% CSS. Three days later medium was again removed and replaced with DMEM plus 1% FBS/4% CSS containing the synthetic androgen R1881 (New England Nuclear, Boston, MA). Control media contained the ethanol vehicle. Plates were harvested by trypsinization at various times after treatment, suspended in 5 ml DMEM plus 1% FBS/4% CSS and spun at 1000 rpm at 4°C. The pellets were resuspended in cold PBS, centrifuged, and resuspended again. Cell counts were performed on a hemacytometer. Cells were spun once again and resuspended in 1 ml cold PBS. This suspension was then split, and 500 μl were combined with 500 μl 0.5 M sucrose and stored at −80°C. At the time of assay cells were thawed at 4°C and sonicated. GAPDH activity was determined by measuring the oxidation of NADH spectrophotometrically at 340 nm for 4 min at 37°C (9). Protein content was determined using the Bradford assay. GAPDH activity is expressed as IU/mg protein or per cell (IU = Δ absorbance/min/6.22).

Northern Blotting and Hybridization. Total RNA was isolated from control and R1881-treated cultures 72 h after treatment and extracted using the guanidine-isothiocyanate method described by Davis *et al.* (10). Electrophoresed RNA (15 μg) was blotted and fixed onto GeneScreen Plus membranes (New England Nuclear). Membranes were prehybridized at 42°C and then hybridized with a 1.3 kb-^{32}P-labeled *Pst*I insert of rat GAPDH cDNA (11) overnight. The next day filters were washed first with 2× SSC for 10 min at room temperature followed by 0.1% SDS/2× SSC for 60 min at 55°C. Membranes were also probed with a 2.8-kb ^{32}P-labeled pT7RNA 18s linearized plasmid (Ambion, Austin, TX) to normalize any loading differences. The labeled bands were imaged using a Molecular Dynamics PhosphorImager and quantitated using ImageQuant software (Version 3.3).

Results and Discussion

The data presented show that androgens can effect both GAPDH activity and mRNA content in androgen-responsive human prostate carcinoma cells. The synthetic androgen R1881 was chosen for these studies because, unlike 5α-dihydrotestosterone, it is metabolically stable. R1881 treatment had no effect on the androgen-independent cell line DU145. This, along with the finding that R1881 alone had no effect on the activity of the purified enzyme, suggests that the results seen were dependent on the presence of an operable androgen receptor and were not due to metabolism of the added androgen.

GAPDH Activity Is Altered in Androgen-responsive LNCaP, but not in Androgen-independent DU145 Prostate Carcinoma Cells after Exposure to R1881. GAPDH activity measured after a 72-h exposure to R1881 is altered in LNCaP cells depending on the dose. Androgens elicit a bell-shaped growth response in LNCaP cells. In this experiment 0.01 and 0.05 nM R1881 increased growth to approximately 130 and 145% above control, while 0.1 and 1 nM R1881 inhibited growth to 85 and 91% of control. At R1881 doses

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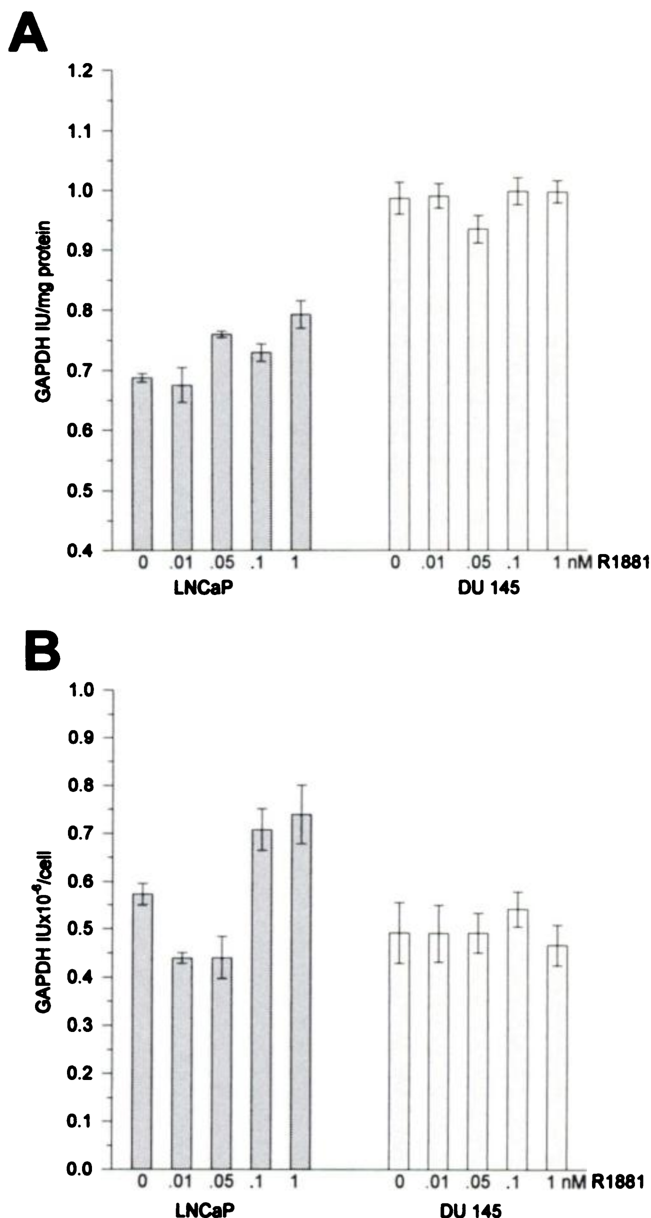

Fig. 1. LNCaP and DU145 cells were plated in triplicate at a density of 10^6 cells/100-mm plate and were treated with 0.01, 0.05, 0.1, and 1 nM R1881 or vehicle control for 72 h. GAPDH activity was determined spectrophotometrically by measuring the rate of NADH oxidation at 340 nm. Results are presented per mg protein (*A*) and per cell (*B*). R1881 treatment at 0.05, 0.1, and 1 nM significantly ($P < 0.001$, 0.02, and 0.002) increases GAPDH activity/mg protein above control. R1881 at 0.1 and 1 nM also increases GAPDH activity on a per cell basis ($P \leq 0.01$), while lower doses, 0.01 and 0.05, result in significantly lower GAPDH activity/cell ($P < 0.001$ and 0.01). R1881 had no effect on the GAPDH activity of DU145 cells.

that stimulate growth above control level, GAPDH activity per cell is significantly reduced compared to control. In contrast, doses which inhibit growth increase GAPDH activity significantly. R1881 has no effect on the GAPDH activity of DU145 cells (Fig. 1*B*). When expressed per mg total protein, GAPDH activity is elevated in LNCaP exposed to 0.05, 0.1 ,and 1 nM R1881. Again, the treatment has no effect on DU145 cells (Fig. 1*A*).

The increase in GAPDH activity at R1881 doses of 0.1 and 1 nM implies an increase in glycolysis is occurring at these doses. Since these doses happen to inhibit cell growth, an increase in glycolytic activity may seem counterintuitive; however, the evolving role of GAPDH in cellular functions other than glycolysis (1, 3, 12) provides an interesting alternative explanation. The findings of Epner *et al.* (4) show that GAPDH distribution within the cell and between individual cells of human prostate tumors is heterogeneous as compared to normal and benign prostatic hypertrophy tissues. Androgens may play a role in the disregulation of GAPDH noted in these tumors.

Time Course of GAPDH Activity in LNCaP Exposed to 1 nM R1881. LNCaP cells treated with 1 nM R1881 display a significant elevation in GAPDH activity per cell as early as 24 h after treatment, and this elevation above control is maintained for at least 96 h (Fig. 2). The GAPDH activity per cell declines over time in untreated

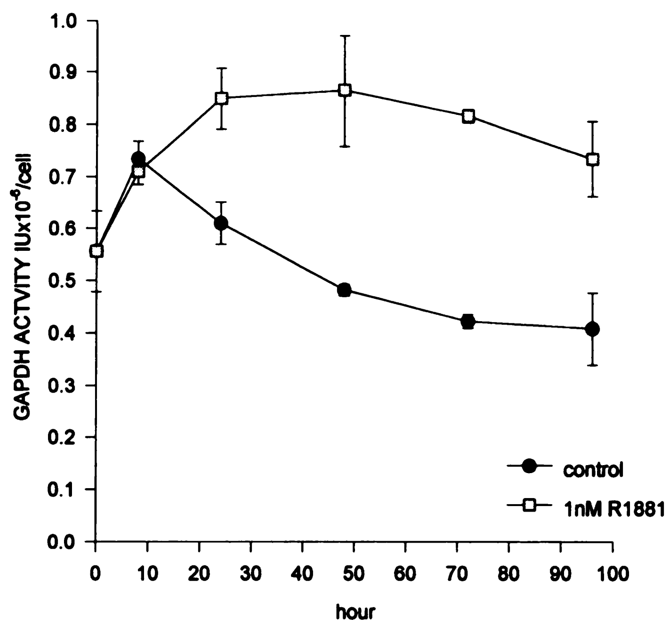

Fig. 2. Time course of GAPDH activity of LNCaP cells treated with 1 nM R1881. Cells were plated in triplicate and treated with 1 nM R1881 or vehicle control 4 days after plating. GAPDH activity was determined as described in the legend to Fig. 1 and is expressed per cell. GAPDH activity is significantly ($P < 0.005$) elevated above control as early as 24 h after treatment and remained elevated for 96 h.

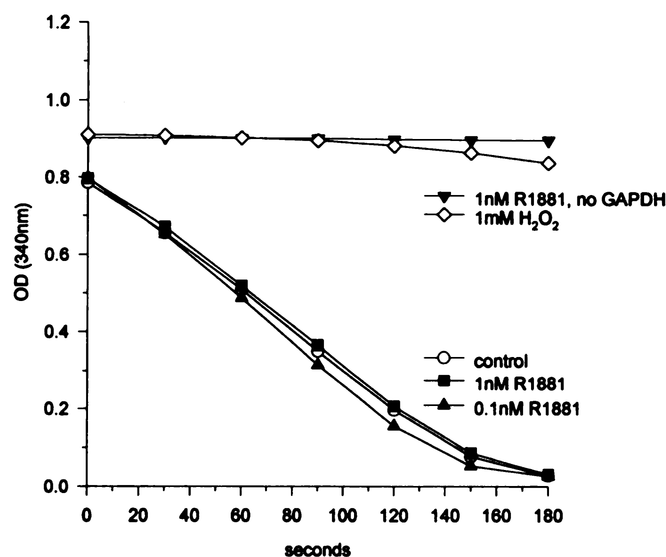

Fig. 3. Effect of R1881 on purified GAPDH. Purified GAPDH was incubated with 0.1 and 1 nM R1881 for 30 min at 37°C. GAPDH activity was determined spectrophotometrically, and the actual absorbance for each sample is shown above. R1881-treated GAPDH gave readings nearly identical to those of vehicle-treated GAPDH, suggesting that R1881 had no effect on the activity of purified enzyme. R1881, in the absence of enzyme, had no GAPDH activity. Treatment of the purified enzyme with 1 mM H_2O_2 for 30 min inhibited GAPDH activity as expected.

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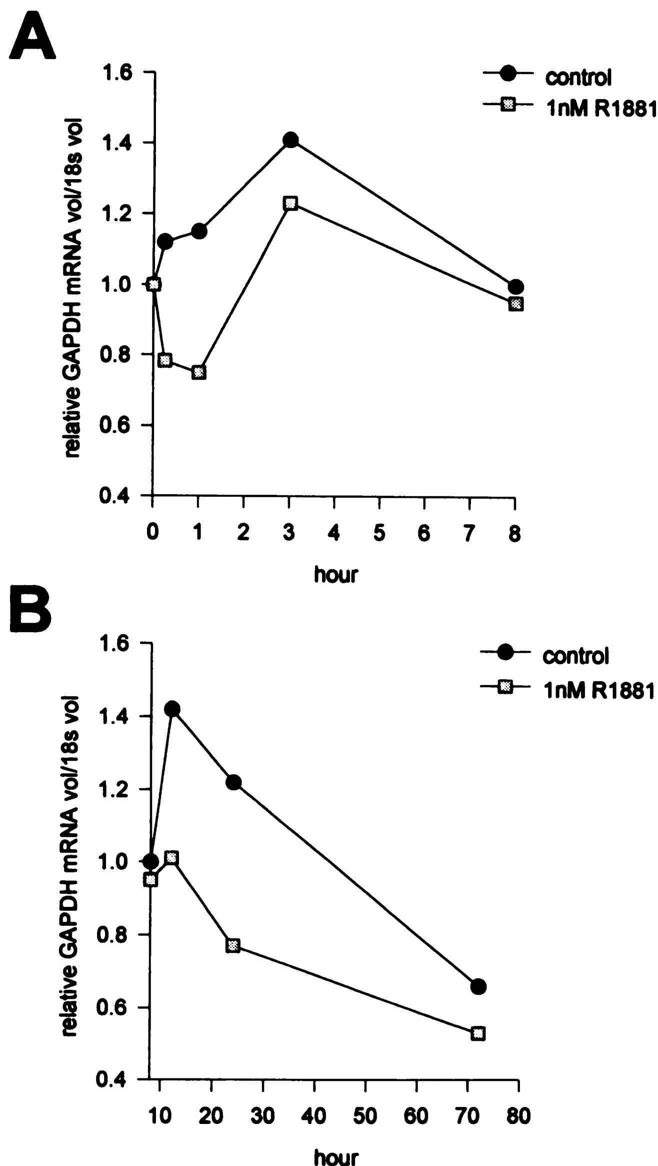

Fig. 4. LNCaP cells were exposed to media containing 1 nM R1881 or vehicle control and harvested for RNA at various time points. RNA was electrophoresed, Northern blotted, and probed with a labeled 1.3-kb insert of rat GAPDH cDNA. GAPDH mRNA expression is normalized to ribosomal 18s expression. *A*, R1881-treated cells display a rapid drop in GAPDH expression within the first 2 h, while expression in the untreated cells rises steadily for 4 h. Eight h after treatment, both control and R1881 GAPDH mRNA expression is back to the initial starting level. *B*, GAPDH mRNA expression is elevated approximately 40% between 8 and 12 h in control cells, but it changes little in the treated cells. Both control and treated GAPDH mRNA expression decreases steadily between 24 and 72 h.

control cells, possibly in response to decreased glycolysis induced by increased cell density and decreased nutrients. Although 1 nM R1881 inhibits cell growth, the density of treated cells at 48–96 h is comparable to the density of the control cells at 24 h (data not shown). As shown in Fig. 2, GAPDH activity of the treated cells at 48–96 h is significantly greater than control cells at 24 h, suggesting that GAPDH activity is not merely a reflection of cell density.

R1881 Has No Effect on Purified GAPDH. When purified GAPDH was incubated with 0.1 and 1 nM R1881 for 30 min at 37°C, there was no detectable change in GAPDH enzymatic activity (Fig. 3). When incubated with 1 mM H_2O_2 for the same period, GAPDH activity was nearly eliminated, which is consistent with previous reports showing inhibition of GAPDH by H_2O_2 (13).

Effect of R1881 on GAPDH mRNA Steady-State Level Normalized to 18s. LNCaP cells exposed to 1 nM R1881 for 72 h reproducibly display a lower GAPDH mRNA content compared to untreated cells. Data from three separate RNA preparations, run on six separate gels, show that the GAPDH mRNA content of R1881-treated cells is reduced to 67 ± 14% of control. Fig. 4 shows the results of a time course treatment of LNCaP with 1 nM R1881 (*A*, 0–8 h and *B*, 8–72 h). Within the first hour after treatment, the GAPDH mRNA content drops in R1881-treated cells. At the same time control GAPDH mRNA begins to rise. Between 1 and 3 h, control and treated cells show an increase in GAPDH mRNA, but by 8 h expression in both returns to the level found at time 0. GAPDH mRNA content is elevated approximately 40% between 8 and 12 h in control cells, while it changes little in the treated cells. There is a steady decline noted in both treated and untreated cells from 12 to 72 h, but GAPDH mRNA expression remains reduced compared to control over this period.

The fact that we see a reduction in GAPDH mRNA at the same time GAPDH activity is elevated suggests that the increase in activity is due to some posttranscriptional modifications in GAPDH following androgen treatment. In contrast, the GAPDH mRNA expression of the vehicle-treated control cells corresponds well with GAPDH activity. The highest level of GAPDH mRNA expression occurred at 12 h, which is the time of greatest GAPDH activity in the control cells. Alterations in GAPDH mRNA levels by certain doses of androgen suggest that this would be a poor marker for Northern blot normalization, but are especially intriguing in light of the evidence that GAPDH mRNA content is elevated in certain tumors (6, 7). Also, since recent reports link GAPDH mRNA content to the metastatic ability of rat prostate cancer cell lines (5), a better understanding of the possible role of androgens in regulation of both GAPDH activity and mRNA expression seems warranted, especially in the case of prostate cancer.

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