Comparative effects of DHEA vs. testosterone, dihydrotestosterone, and estradiol on proliferation and gene expression in human LNCaP prostate cancer cells

Julia T. Arnold,†§ Hanh Le,†§ Kimberly K. McFann,‡ and Marc R. Blackman§

1Endocrine Section, Laboratory of Clinical Investigation, Division of Intramural Research, and 2Office of Clinical and Regulatory Affairs, National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, Maryland

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Arnold, Julia T., Hanh Le, Kimberly K. McFann, and Marc R. Blackman. Comparative effects of DHEA vs. testosterone, dihydrotestosterone, and estradiol on proliferation and gene expression in human LNCaP prostate cancer cells. Am J Physiol Endocrinol Metab 288: E573–E584, 2005. First published November 9, 2004; doi: 10.1152/ajpendo.00454.2004.—Serum levels of the adrenal androgen dehydroepiandrosterone (DHEA) peak in men and women in the third decade of life and decrease progressively with age. Increasing numbers of middle-aged and older individuals consume over-the-counter preparations of DHEA, hoping it will retard aging by increasing muscle and bone mass and strength, decreasing fat, and improving immunologic and neurobehavioral functions. Because DHEA can serve as a precursor to more potent androgens and estrogens, like testosterone (T), dihydrotestosterone (DHT), and 17β-estradiol (E2), supplemental DHEA use may pose a cancer risk in patients with nascent or occult prostate cancer. The steroid-responsive human LNCaP prostate cancer cells, containing a functional but mutated androgen receptor (AR), were used to compare effects of DHEA with those of testosterone, dihydrotestosterone, and 17β-estradiol (E2). Cell proliferation assays revealed significant stimulation by all four steroids. DHEA- and E2-induced responses were similar but delayed and reduced compared with that of T and DHT. All four hormones increased gene and/or protein expression of PSA, IGF-I, IGF-I receptor (IGF-IR), IGF-II, IGF-binding proteins-2, -3, and -5, (IGFBPs-2, -3, and -5), and estrogen receptor-β (ERβ). Cell proliferation assays revealed significant stimulation by all four steroids. DHEA- and E2-induced responses were similar but delayed and reduced compared with that of T and DHT. All four hormones increased gene and/or protein expression of PSA, IGF-I, IGF-I, and IGFBP-2 and decreased that of AR, ERβ, IGF-II, and IGFBP-3. There were no significant effects of hormone treatment on IGFBP-5 mRNA. DHEA and E2 responses were similar, and distinct from those of DHT and T, in time- and dose-dependent studies. Further studies of the mechanisms of DHEA effects on prostate cancer epithelial cells of varying AR status, as well as on prostate stromal cells, will be required to discern the implications of DHEA supplementation on prostatic health.

dehydroepiandrosterone; androgen receptor; prostate-specific antigen; insulin-like growth factor axis; estrogen receptor-β; lymph node-derived cancer of prostate

DEHYDROEPIANDROSTERONE (DHEA) is produced by the adrenal cortex and is the most abundant steroid in humans. DHEA exerts its actions via androgen receptor (AR)- and estrogen receptor (ER)-mediated effects directly or after enzymatic conversion to androgen or estrogen (34), as well as by sex steroid receptor-independent mechanisms (58). Serum DHEA levels in aged men and women decrease by 75–80% from peak levels (44). DHEA is available as a dietary supplement and is increasingly self-prescribed for its alleged antiaging effects, with reported beneficial effects on body composition and cardiometabolic, immune, and neurobehavioral functions (3). Humans and other primates are unique among animal species in having adrenals that secrete large amounts of the inactive steroid precursors DHEA and its sulfate DHEA-S (33, 38).

In aged adults, the use of DHEA as a supplement is of potential concern in that DHEA can be metabolized to androgens or estrogens and may stimulate proliferation of cancer cells within the prostate or breast. In that context, controversy exists as to whether DHEA enhances or reduces the risk of prostate and breast cancer (1). Although DHEA has been reported to exert potent cancer-protective effects in preclinical rodent models of prostate cancer (48, 50), its effects on human prostate cancer cells remain to be elucidated. Epidemiological data are confusing, in that the age-related decline in DHEA, testosterone, and estrogens may protect against increasing occurrence of hormone-sensitive cancers (40), yet elevated serum levels of DHEA and DHEA-S have been associated with decreased cancer rates (16).

In this study, the well-characterized, AR-positive human lymph node-derived cancer of prostate (LNCaP) cell line was used to compare the effects of DHEA with those of testosterone (T), dihydrotestosterone (DHT), and 17β-estradiol (E2) on cell proliferation and gene and/or protein expression of AR, PSA, ERβ, IGF-I and IGF-II, IGF type 1 receptor (IGF-IR), and IGF-binding proteins (IGFBPs)-2, -3, and -5.

MATERIALS AND METHODS

Cell Culture

LNCaP-FGC cells [androgen-dependent, prostate adenocarcinoma cells derived from lymph node metastasis (CRL1435); American Type Culture Collection, Manassas, VA] were grown in DMEM-F12 (1:1) medium (Invitrogen, Gaithersburg, MD) with penicillin (100 U/ml), streptomycin (100 µg/ml), l-glutamine (292 µg/ml, Invitrogen), and 5% fetal bovine serum (FBS; HyClone Laboratories, Logan, UT) at 37°C in 5% CO2 and propagated at 1:5 dilutions.

Cell Proliferation Assays

Cell proliferation was estimated using the CellTiter 96 Assay (MTT assay; Promega, Madison, WI). The assay measures the cellular

*J. T. Arnold and H. Le were equal contributors to this paper.

Address for reprint requests and other correspondence: J. T. Arnold, Endocrine Section, LCI-NCCAM, NIH, 9 Memorial Dr., Rm 1N105, Bethesda, MD 20892-0933 (E-mail: jarnold@mail.nih.gov).

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Hormone Treatments for Gene and Protein Expression

LNCaP cells were seeded into 96-well plates precoated with Matrigel matrix film (growth factor reduced, phenol red free; Becton-Dickinson Biosciences, Bedford, MA; 1:10 in sterile H2O and allowed to dry overnight) at a density of 20,000 cells/well in medium 199-F12 (1:1) phenol red-reduced media (Invitrogen) supplemented with 2% charcoal-dextran-treated FBS (CDS, Hyclone Laboratories) for 2–3 days. Hormones were dissolved in ethanol (ethanol concentrations not exceeding 0.02%) and added at various doses (DHEA, DHT, T, and E2; Sigma, St. Louis, MO) with a final CDS concentration of 1%. In the time course experiments, cell growth was assessed at hormone concentrations ranging from 0.1 to 10,000 nM and compared with untreated controls at each time point. Three replicates per point were assayed, and data from three separate experiments are presented.

RNA Extraction and Real-Time Quantitative RT-PCR

RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer’s manual. The resulting RNA pellet was extracted a second time with TRIzol reagent to increase the RNA purity. RNA was quantified spectrophotometrically at a 260-nm wavelength. cDNA synthesis and real-time RT-PCR were performed as described in Latil et al. (35), with some modifications. Briefly, RNA was reverse transcribed with Superscript III RNase H- reverse transcriptase from Invitrogen, according to the manufacturer’s manual, with 10 units of RNasin RNase inhibitor (Promega), 50 units of Superscript III, 150 ng of random hexamer (Amersham Biosciences), and 1.5 μg of total RNA. PCR was performed using a SYBR Green PCR Core Reagents kit (Applied Biosystems, Foster City, CA) and quantitated by an ABI Prism 7900 Sequence Detection System (Applied Biosystems) according to the manufacturer’s manual. Relative mRNA expression level was calculated using the ΔΔCt method, where the calibrator is the RPLPO gene. RPLPO encodes human acidic ribosomal phosphoprotein P0 and was used as the endogenous RNA control to correct for the amount of total RNA used in each PCR reaction. Primers for RPLPO, AR, ERβ, and PSA were described by Latil et al. (35);

Table 1. Sequences of primers used for quantitative RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Oligonucleotide Sequence</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPLPO (BC003655)</td>
<td>5'-GGCCAGCTGGAGTCTCAACT-3'</td>
<td>149</td>
</tr>
<tr>
<td>AR (M23263)</td>
<td>5'-CCATCAGGACACCCGCGCTC-3'</td>
<td>168</td>
</tr>
<tr>
<td>ERβ (AF051427)</td>
<td>5'-GGACTTGCGATCGGCATCTCA-3'</td>
<td>143</td>
</tr>
<tr>
<td>PSA (X05332)</td>
<td>5'-CCCCGAATCCCGGACAGA-3'</td>
<td>161</td>
</tr>
<tr>
<td>IGF-IR (X04434)</td>
<td>5'-GGGATGCGGGTGTGTTTG-3'</td>
<td>107</td>
</tr>
<tr>
<td>IGF-I (M27544)</td>
<td>5'-TTTGGCTGATCGCTCTGTCG-3'</td>
<td>135</td>
</tr>
<tr>
<td>IGF-II (J03242)</td>
<td>5'-AGAGGAGTCATAGCAAGA-3'</td>
<td>125</td>
</tr>
<tr>
<td>IGFBP-2 (X16302)</td>
<td>5'-GCATGCCCTTCTCAGATT-3'</td>
<td>147</td>
</tr>
<tr>
<td>IGFBP-3 (X64875)</td>
<td>5'-AAAAGCTACCGCGGAAATG-3'</td>
<td>126</td>
</tr>
<tr>
<td>IGFBP-5 (M62782)</td>
<td>5'-GGAGATGCGGTGTG-3'</td>
<td>107</td>
</tr>
</tbody>
</table>

primes for IGF-I, IGF-II, IGF-IR, IGFBP-2, IGFBP-3, and IGFBP-5 were described by Nickerson et al. (42). The sequences for the primers used are summarized in Table 1.

**PSA ELISA**

LNCaP cells were seeded in triplicate into 24-well tissue culture plates precoated with Matrigel matrix film (1:10) at a density of 5 × 10^5 cells/well with medium and hormone treatments similar to those in the proliferation assay. The cells were grown in medium containing 2% CDS for 2 or 3 days. Hormones were added as above for 3 days. Hormone-containing medium was replaced and allowed to condition for 48 h. Medium was collected and assayed directly or frozen at −80°C. Time course experiments were set up in a similar manner, and cells were plated for 3 days and then treated with 100 nM hormone for multiple time points (day 1 to day 5). Fresh hormone-containing medium was added 48 h before harvesting conditioned medium for each time point.

Total PSA ELISA kits (DSLabs, Webster, TX) were used to determine PSA concentrations. In the assay, standards, controls, and medium samples are incubated in 96 wells following the manufacturer's instructions. Each original triplicate experimental sample was assayed in duplicate. PSA values (ng/ml) were determined using log-log curve fit to standard curve. PSA values were then normalized to cell numbers as determined by the modified MTT assay. Cell number was estimated by comparing the absorbance of the sample with a cell number standard curve. A cell number standard curve was created by plating known cell numbers, from 10^5 to 2 × 10^6, in 1 ml of medium onto a 24-well plate. After 24 h, 75 μl of MTT dye were added per well to standards or sample. Stop solution was then added after 4 h at 400 μl/well. After solubilization of the formazan product, 250 μl of each sample were transferred to a 96-well plate and read on a spectrophotometer at 570 nm. Three replicates per condition were assayed, and data from three separate experiments are presented.

**Statistical Analysis**

Data are expressed as the mean values ± SD derived from three to eight replicates within each of three separate experiments. Analysis of covariance (ANCOVA) was performed to test for the homogeneity of regression slopes among hormones when the dependent variable was regressed over time or dose. Where appropriate, a quadratic term was included in the model. The 95% confidence intervals (CIs) for parameter estimates were calculated for each hormone and compared to identify which regression slopes differed. To further delineate effects of hormones across time or dose, fixed effects ANOVA was performed using the Tukey-Kramer adjustment for multiple comparisons to compare least square means. An adjusted P value of 0.05 was considered significant. Effects of three separate experiments were included in each of the analyses.

**RESULTS**

**Effects of DHT, T, DHEA, and E2 on LNCaP Cell Proliferation**

LNCaP epithelial cell cultures were treated with 100 nM each of DHT, T, DHEA, or E2 for 1–9 days. Cell proliferation increased significantly by day 2 in DHT- and T-treated vs. control cells (P < 0.0001; Fig. 1A). In contrast, addition of DHEA and E2 did not increase cell growth significantly compared with control until day 3 (P < 0.0001). Cell growth induced by DHEA, DHT, T, and E2 peaked on day 8 with 296, 252, 273, and 247% of control, respectively (P < 0.0001 for all points after day 2 vs. control). From days 1 to 5, DHEA-induced proliferation was less than that induced by all other hormones (P < 0.0001, except at day 5 DHEA vs. E2, P = 0.04). At day 8, DHEA-stimulated proliferation exceeded those induced by DHT and E2 (P < 0.0001). The morphology of the LNCaP cells was not altered by any of the hormonal treatments.

Further analysis of results using ANCOVA revealed a significant difference in the regression slopes between the control and DHEA, DHT, T, and E2 (P < 0.0001). In addition, comparison of the 95% CIs for the slope of each treatment indicated that cell growth induced by DHEA was significantly lower than that induced by DHT or T but similar to that induced by E2.

Cells were also treated with 0.1 to 10,000 nM DHT, T, DHEA, or E2 for 5 days to determine comparative dose response among the hormones. Increased dose-dependent cell proliferation occurred with all hormone treatments (Fig. 1B). Cell growth induced by DHT and T was significantly different from that of control at all concentrations tested, from 0.1 to 10000 nM (P < 0.0001). In contrast, cell growth induced by DHEA or E2 differed from that of control at concentrations of 10 nM or higher for DHEA (P < 0.0001) and 1.0 nM or higher for E2 (P < 0.016, 1 nM; P < 0.001, 10–10,000 nM).
Expression by LNCaP Cells of control values by DHT, T, and E2 (with no significant differences found among the hormone mRNA levels during a 5-day period (P < 0.0001; Fig. 2A), with no significant differences found among the hormone treatments. At 24 h, AR expression was decreased to 48–55% of control values by DHT, T, and E2 (P < 0.01), whereas DHEA decreased AR mRNA to 47% of control after 48 h of treatment (P < 0.0003).

In dose-response experiments, AR mRNA fold change was reduced to 43 and 52% of control values by DHEA and E2, respectively, in a dose-dependent manner (all P < 0.01, except E2 at 100 nM at P = 0.0366; Fig. 2B). There was a nonsignificant 30% downward trend of AR mRNA in DHT- and T-treated cells. The responses induced by DHEA did not differ significantly from those of the other hormones. However, E2 demonstrated greater downregulation of AR expression than did DHT at concentrations of 100, 1,000, and 10,000 nM.

AR protein. Compared with the changes seen in the AR mRNA, the AR protein expression did not change significantly in time- or dose-dependent manner in steroid- vs. control-treated LNCaP cells (data not shown).

PSA mRNA. DHT and T increased PSA mRNA expression (Fig. 3A) twofold within 6 h (P < 0.01), proceeding in a linear fashion to peak at six- and sevenfold over control, respectively, at 48 h (P < 0.0001) and at 72 h (P < 0.0001). In contrast, there was a 24-h delay in PSA gene induction by DHEA and E2. DHEA-induced expression peaked at fivefold increase vs. control (P < 0.0001) and remained unchanged, whereas the E2 response peaked at fourfold and decreased at 95 and 120 h. The patterns of DHEA and E2 were similar to each other and differed from the patterns of DHT and T. Comparison of least square means revealed that DHT and T had significantly higher fold changes in PSA mRNA expression than did DHEA at earlier time points, but by 72 h, expression was not different among DHEA, DHT, and T. E2 responsivity did not differ from that of DHEA at 6 and 24 h but was significantly lower than that for DHEA at 72 h and beyond.

In dose-response experiments, DHT, T, and E2 stimulated PSA mRNA significantly from 0.1 to 10,000 nM (P < 0.0001; Fig. 3B), with DHT and T levels peaking at a 10-fold increase over control and E2 peaking at a 4.6-fold increase over control. DHEA-induced PSA mRNA expression was significantly increased over control after 1 nM and peaked at fivefold increase. Comparison of least square mean values revealed significantly greater increments in PSA mRNA (nearly 2-fold) at almost all hormone doses of DHT or T vs. DHEA or E2. The PSA responses to DHEA and E2 did not differ significantly.

PSA protein. DHT and T increased PSA protein secretion to a maximum of 342% (DHT) and 325% (T) of control values at 96 and 120 h, respectively (P < 0.0001; Fig. 3C). DHEA- and E2-stimulated PSA levels were delayed and significantly increased compared with control values after 72 h for DHEA (P < 0.0001) and 48 h for E2 (P = 0.003), with peak values of 222 and 257%, respectively, at 120 h (P < 0.0001). DHEA-stimulated PSA secretion was less than the levels induced by DHT and T after 48 h (P < 0.0006). ANCOVA revealed a significant difference in the regression slopes when PSA secretion was regressed against time for all treatments (P < 0.0001). The 95% CI for the B1 (slope) value for DHEA overlapped with that for E2 but not with those for DHT and T.

PSA protein levels increased significantly after DHT, T, and E2 treatments (Fig. 3D), starting at the lowest concentration of 0.01 nM (DHT and E2 vs. control, P < 0.0001; T vs. control, P = 0.04) and reaching a plateau at 450–500% (DHT and T) and 400% (E2) of control values (all P < 0.0001). In contrast, DHEA-induced increases in PSA became significant at 1 nM (310%, P = 0.0007 vs. control) but were similar to those of other hormones by 10 nM and higher. ANCOVA revealed no significant differences in the regression slopes for DHEA, DHT, T, and E2 when PSA was regressed against dose; however, comparison of least square means revealed lower PSA responses to DHEA than to DHT at concentrations of 0.01, 0.1, and 1 nM (P < 0.05) but similar DHEA and DHT responses at the higher concentrations. DHEA responses did not significantly differ from those induced by T or E2.

IGF-IR mRNA. IGF-IR mRNA expression increased three- to fourfold (Fig. 4A) within 6 h (P < 0.0001) of 100 nM treatment with DHT, T, or E2, decreasing to 1.4- to 2.5-fold stimulation (P ≤ 0.0015) by 5 days. ANCOVA revealed significant differences in regression slopes among DHEA, DHT, E2, and T when fold change was regressed against time (P = 0.0007). Whereas the slopes of DHT, E2, and T were similar to one another, a delay was observed in DHEA-induced IGF-IR expression, which increased to threefold vs. control after 24 h (P < 0.0001) and remained elevated during the next 4 days. Comparison of least squares means revealed that the
The response to DHEA treatment was significantly lower than those to DHT, T, or E2 treatments at 6 h. By 48 h, the response to DHEA treatment was similar to those of DHT and E2 but lower than that of T. By 120 h, DHEA-induced IGR-IR mRNA expression was not significantly different from that induced by DHT, E2, or T.

IGF-IR mRNA was increased in response to escalating doses of all four hormones (Fig. 4B). Augmented expression was evident in DHT- and T-treated cells even at the lowest concentration of 0.1 nM (P < 0.0005) and increased further with concentrations ≤100 nM, wherein the response leveled at 5.8- to 6.0-fold (P < 0.0001). ANCOVA revealed significant dose-related differences in regression slopes for DHEA, DHT, T, and E2 (P < 0.0001). The 95% CI for the β1 (slope) value for DHEA did not overlap those of DHT or T but did overlap that of E2. The peak responses to DHT and T were greater (5- to 6-fold) than those of DHEA or E2 (3- to 4-fold). E2 exerted the weakest effect on IGF-IR induction, with a 2- to 3.5-fold increase between 10 and 10,000 nM. The response of DHEA was similar to those of DHT and T up to 100 nM and resembled that of E2 at higher doses.

IGF-IR protein. DHT, T, DHEA, and E2 stimulated time-dependent increases in IGF-IR protein expression up to 96 h, reaching ~170~200% of control values, followed by decreases at 120 h to ~150% of control (Fig. 4C). Significant responses to DHT and T were first evident at 24 h, whereas DHEA- and E2-induced IGF-IR expressions were not significant until 72 h. There were no significant differences among hormone responses at any time point.

Each of the four hormones also augmented IGF-IR protein expression in a dose-dependent manner (Fig. 4D), reaching plateau stimulation of ~200% at 100 nM. Neither ANCOVA nor comparison of least square means revealed any significant differences among the steroids in hormone-modulated IGF-IR protein expression, except for a marginally significant difference in the effects of DHEA and T at a concentration of 1 nM (P = 0.0474).

IGF-I mRNA. IGF-I mRNA increased twofold by 6 h after treatment with 100 nM DHT, T, and E2 (P ≤ 0.03; Fig. 5A), whereas DHEA stimulated a twofold increase after 24 h (P = 0.01). IGF-I mRNA expression increased to threefold (DHT and E2) and fourfold (T and DHEA) by 48 h (P < 0.0001) and then stabilized in response to DHEA, DHT, and T but diminished in response to E2 at 96 and 120 h.

IGF-I mRNA levels increased in a dose-responsive manner after administration of each of the four hormones (Fig. 5B). DHT and T induced twofold increases in IGF-I mRNA at 0.1 nM, which peaked at threefold at 1,000 nM (DHT) and at 2.5-fold at 100 nM (T). DHEA- and E2-induced increases were significant only at 10 nM or higher for DHEA (P < 0.0001) and at 1 nM or higher for E2 (P = 0.0025). DHT- and T-induced mRNA expression exceeded those of DHEA and E2.
ANCOVA demonstrated no significant differences in regression slopes among the four hormones. Comparison of least square means revealed that DHEA elicited significantly lower responses than did DHT at concentrations of 0.1, 1, 10, and 1,000 nM but not at concentrations of 100 or 10,000 nM. DHEA responses did not differ from those of T or E2, except for a slightly lower response of DHEA vs. T at the lowest hormone dose (0.1 nM).

**IGF-II mRNA.** IGF-II mRNA expression decreased insignificantly in response to all four hormones up to 48 h (Fig. 5C) and appeared to increase slightly after 48 h. T and E2 responses differed ($P = 0.0357$) at 120 h. ANCOVA demonstrated no significant difference in regression slopes when fold change was regressed on time for DHT, T, DHEA, or E2.

All four hormones led to dose-dependent decreases in IGF-II mRNA expression (Fig. 5D). Significant reductions were found with DHEA at 10 and 100 nM (44 and 46% vs. control, $P = 0.0004$ and $P = 0.0001$, respectively); with E2 at 10, 100, and 1,000 nM to 45, 46, and 43%, respectively, vs. control ($P < 0.001$); and with T at 1 and 1,000 nM (36 and 44% vs. control; $P = 0.002$, $P = 0.01$). Neither ANCOVA nor comparison of least square means revealed any significant differences in dose responsivity among the four steroid hormones.

**IGFBP-2, -3, and -5 mRNA.** DHEA and E2 increased IGFBP-2 mRNA expression at 48 h by 32% ($P = 0.0068$) and 46% ($P < 0.0001$), respectively (Fig. 6A), with no significant changes at any other time point. In contrast, neither DHT nor T elicited significant effects at any time point. There were no significant differences between the response to DHEA and those of the other hormones at any time point. DHT and T increased IGFBP-2 mRNA expression 40 and 60%, respectively, above control values ($P < 0.0001$) at 0.1 nM, followed by decreases at concentrations of 100 nM and higher (Fig. 6B). IGFBP-2 expression increased by 30–47% at DHEA concentrations of 1, 1,000, and 10,000 nM ($P < 0.001$) and increased 41–56% with E2 treatment at doses of 10–1,000 nM ($P < 0.001$), respectively. DHEA-induced increases were greater than those of DHT and T at doses of 10,000 and 1,000 nM, respectively.

DHT, T, DHEA, and E2 each decreased IGFBP-3 mRNA message levels by a maximum of 30% at 48 h ($P < 0.0001$; Fig. 6C). All hormones showed similar patterns of IGFBP-3 suppression except for E2, which lost its inhibitory effect after 72 h.

All four hormones reduced IGFBP-3 expression with increasing doses (Fig. 6D). The inhibitory effects of DHT and T were greater than that of DHEA at 0.1 and 1 nM ($P < 0.0001$) but at 10 nM or higher there were no significant differences found among the four hormones.

There were no significant time- or dose-dependent changes in IGFBP-5 mRNA expression in response to varying times and differing hormone concentrations of DHT, T, DHEA, or E2
Moreover, there were no differences in IGFBP-5 responses among the four steroids.

**ER** mRNA. DHT, T, DHEA, and E2 each decreased **ER** mRNA with maximum suppression of 61–74% at 72 h (P < 0.0001; Fig. 7A). There were no significant differences among the hormone responses at any time point.

**ER** mRNA was decreased 48–64% by DHT at concentrations of 1 nM and higher (P < 0.02; Fig. 7B) and 50–60% by DHEA and T at doses 10 nM and higher (P < 0.02). There was no significant effect of E2 treatment at any dose. DHEA responses did not differ from those of DHT, T, or E2 at any dose.

**DISCUSSION**

In the United States, DHEA is widely used as an over-the-counter dietary supplement on the basis of its purported, yet controversial, antiaging benefits to improve body composition, endocrine-metabolic balance, immune and psychological functions, and quality of life (3, 54). Because DHEA is a precursor for more potent androgens and estrogens, there is some concern that DHEA supplementation may promote prostate cancer growth or deleterious functions in men with preexisting prostate cancer (1).

This study aimed to evaluate the effects of DHEA on human prostate cancer cells. Well-characterized, hormonally responsive LNCaP human prostate cancer cells (29) were used to compare the effects of administration of DHEA with the effects of T, DHT, and E2 on cell proliferation and gene and protein expression of AR, ER, and PSA and members of the IGF axis. These are important biomarkers of prostate cancer progression and were chosen so as to characterize steroid regulation of prostate cancer cells. We found that DHEA, DHT, T, and E2 each increased cell proliferation and mRNA and/or protein expression of PSA, IGF-IR, IGF-I, and IGFBP-2, whereas they each decreased mRNA levels of AR, ER, IGF-II, and IGFBP-3. IGFBP-5 mRNA expression was unaffected by any of the hormone treatments.

DHEA administration stimulated LNCaP cell proliferation in a time- and dose-responsive manner, but when compared with DHT and T treatments the responses were delayed (by 24–48 h) and reduced, with DHEA concentrations nearly 1,000 times those of DHT and T being needed to effect similar responses. E2 administration exerted up to 50% greater cell growth, occurring 24 h earlier, compared with the responses to DHEA. Prior studies (60) have demonstrated DHEA- and E2-induced proliferation of LNCaP cells of 220 and 200%,
respectively, over 6 days. Our results confirm and extend these
prior observations by comparing DHEA- and E2-induced in-
creases in cell growth (200 and 225% increases, respectively,
after 5 days) with the greater cell proliferation induced by DHT
and T (250%). Taken together, the data highlight the distinctly
reduced and delayed cell-proliferative responses induced by
DHEA and E2 compared with those modulated by DHT and T.

The dynamics of steroid-induced cell proliferation observed
in the present study differ from the biphasic response of
LNCaP to DHT previously reported (20, 53). This apparent
discrepancy may have resulted from differences in the culture
conditions employed. The protocol used in the present study
included plating of cells on a film of basement membrane
(Matrigel) and using slightly different basal medium and lesser
amounts of serum than reported previously. The conditions
reported herein were optimized to provide a more physiologi-
cal microenvironment for epithelial cells (4).

Hormonal effects on steroid receptor levels included de-
creases in AR mRNA of 80% with DHT and T treatments and
of 70–75% with DHEA and E2 administration, with no signif-
icant effects on AR protein expression. These results confirm
and extend prior studies showing a reduction of AR mRNA by
DHT with a stabilization of the AR protein (59). It is possible
that E2 and DHEA interactions with ERβ could have contrib-
uted to the decrease in AR mRNA levels, as has been
reported in LNCaP cells treated with genistein (7).

LNCaP cell expression of ERβ mRNA also decreased after
administration of DHT, T, DHEA, and E2. This effect in
human prostate cancer cells contrasts with previous observa-
tions in the rat prostate, in which the ERβ hybridization signal
was eliminated following orchidectomy (51). To our knowl-
dge, this is the first report of reduction of ERβ mRNA levels
in LNCaP cells by these sex steroid hormones. Given that ERβ
may play an inhibitory role in the prostate (57), these findings
suggest another mechanism whereby sex hormones increase
proliferation of prostate cells by decreasing the “brakes” to cell
proliferation.

DHEA increased PSA mRNA and protein expression in a
pattern similar to that induced by E2 but different from those
stimulated by DHT and T. PSA mRNA expression was delayed
by 24 h in DHEA-treated cultures and reached levels 75% of
that induced by T in the time response experiments and 50% of
those of DHT and T in the dose response studies. Prior studies
reported a 10-fold increase in PSA expression after 6 days of
exposure to 100 nM DHEA and 18-fold increase with T and E2
treatment (60). In the present experiments, 5 days of treatment
revealed a repeated distinction between the greater DHT- and
T-induced PSA protein expression (320 and 325%, respec-
tively) and the lesser DHEA- and E2-induced PSA release (222
and 257%, respectively). Importantly, the steroid-induced PSA
expression, as determined by ELISA, was standardized by
comparing PSA concentrations with total cell numbers to adjust for the effects of the hormones on cell proliferation.

Steroid-induced responses in gene expression of members of the IGF axis were compared among DHEA, DHT, T, and E2 treatments. The prostatic IGF axis includes IGF-I and IGF-II, the IGF-IR, and IGFBPs-2–5, which have been well characterized in normal and diseased prostate (14, 45). Increased circulating levels of IGF-I (27, 37), IGFBP-2 (15), and IGF-IR (2) have been associated with increased risk of prostate cancer (27, 37), accounting for up to one-sixth of total prostatic DHT (22). Additionally, steroid-metabolizing enzymes and steroid precursors are present in primary prostatic tumors and lymph node metastases (31). LNCaP cells possess the major enzymes involved in androgen metabolism, including the hydroxy-steroid dehydrogenases (HSD), 3α-HSD, 3β-HSD (23), and 17β-HSD4 (10), involved in conversion of DHEA to androstenedione and T, as well as 5α-reductase for conversion of T to DHT (21, 32). Aromatase activity of LNCaP cells may also contribute to the production of E2 from DHEA via T or androstenedione. However, the existence of aromatase activity in LNCaP is controversial, having been reported to be absent (41), weak (9), or present and modulatable (19).

To our knowledge, this study represents the first demonstration that DHEA, DHT, T, and E2 can increase mRNA and/or protein levels of both IGF-I and IGF-IR in LNCaP cells. The increases in IGF-I and IGF-IR were accompanied by a decrease in IGFBP-3; this latter finding has been reported previously (25). Here again, the effects of DHT and T were greater than those of DHEA and E2. There were no significant effects on IGFBP-5 levels by any of the four steroid hormones, in contrast to previous reports of stimulation by androgens (26). DHEA, DHT, T, and E2 all decreased IGF-II expression. Whereas IGF-II treatment has been shown to decrease AR expression (24), whether hormonal suppression of IGF-II affects AR activity in prostate cancer cells remains to be determined. In conjunction with the increased secretion of PSA, these data suggest a mechanism whereby sex steroids may modulate proliferation of prostate cancer epithelial cells in part by modulating an ensemble of effects on members of the IGF axis.

Taken together, the effects of DHEA that we observed on the various biomarkers of steroid regulation of prostate cancer cells indicated responses similar to those elicited by E2 but less than those induced by DHT or T.

There are several possible mechanisms that, singly or in combination, may explain these findings. First, DHEA (and E2) may act as weak androgens in these cells. LNCaP cells are known to have a mutation in the ligand-binding region of the AR (T877A), which can be transactivated by multiple steroid hormones, including DHEA and E2 (18, 60); therefore, DHEA and E2 may exert their effects by binding directly to the mutant AR (56). Whether the AR mutation characterized in the LNCaP cell line is present in a substantial number of patients with androgen-independent prostate cancer (55), and whether DHEA could induce similar proliferative and gene or protein expression effects in these patients as are found in our in vitro study, remain to be clarified.

Second, DHEA may exert its effects in LNCaP cells by undergoing enzymatic conversion to more potent androgens or estrogens, with consequent binding to AR or ERβ. DHEA circulates in the plasma in a sulfated form, DHEA-S. The DHEA-S levels are 100–500 times higher than those of T and 1,000–10,000 times higher than those of E2. Both DHEA-S and its sulfatase are present at high levels in the prostate (31). Nonhyperplastic prostate tissue specimens exhibit concentrations of 300 nM DHEA-S and 90 nM DHEA (5). These tissues maintain the capacity to metabolize adrenal androgens to DHT (33), accounting for up to one-sixth of total prostatic DHT (22). Additionally, steroid-metabolizing enzymes and steroid precursors are present in primary prostatic tumors and lymph node metastases (31). LNCaP cells possess the major enzymes involved in androgen metabolism, including the hydroxy-
response element (ERE) (8), an effect that could be blocked with an ER antagonist, suggesting that DHEA was not metabolized to E2 but directly interacted with ER. However, this is unlikely to be the explanation, because DHEA at 100 nM was unable to stimulate ERE previously, whereas DHEA significantly induced cell proliferation at 10 nM in the present studies.

If DHEA were to mimic the effects of estrogen in the prostate, then DHEA would present the same paradoxical inhibitory and proliferative influence on the prostate that estrogens do. Excessive estrogen induces squamous metaplasia and can act synergistically with androgens to induce glandular hyperplasia (30). Estrogens have long been used in prostate cancer therapy, and their effects on the prostate, as mediated through ERα and ERβ, have been reviewed (12, 52). Estrogens can inhibit prostate cancer xenograft growth in female intact and ovariectomized mice in the absence of androgens (17). These inhibitory effects were postulated to occur by direct actions via the ER or by E2 effects on other cells secreting secondary factors that influence cancer cell growth. Additionally, ERβ knockout mice exhibit increased epithelial proliferation compared with that observed in wild-type mice (57), suggesting that ERβ may inhibit prostate growth.

Finally, a recent study defines another important metabolite of DHEA resulting from 7α-hydroxylation of DHEA to 7α-hydroxy-DHEA (7HD) by CYP7B, an epithelial-specific P450 enzyme (39). The authors identified both CYP7B and the ERβ in human prostate samples and postulated that CYP7B metabolizes prostatic DHEA to 7HD, which directly activates ERβ. This pathway may be important in regulating the balance of androgens and estrogens in the prostate. Of additional note was the finding that coculture with stromal cells increases epithelial CYP7B mRNA.

Whether the aforementioned possible mechanisms to explain the effects of DHEA on prostate cancer cells with mutated AR pertain to prostate cancer cells with wild-type AR or to in vivo effects of DHEA on the prostate remains to be clarified. Further studies using ER and AR antagonists, and assessing aromatase and other endogenous steroid metabolizing enzyme levels, will be required to understand the pathophysiological mechanisms underlying DHEA effects in prostate cancer cells.

Controversy exists concerning the safety of hormonal supplements like DHEA including their use in performance sports (1). It is important to resolve whether DHEA is cancer promoting or preventing in prostate health in young and elderly men. Adrenal androgens alone do not promote prostatic growth, as was recognized in early retrospective clinical studies (43). Those studies in normal prostate do not address the consequence of DHEA supplementation on preneoplastic or neoplastic prostate cells, which may be more complex. DHEA and DHEA-S were stimulatory to proliferation in an androgen-sensitive mammary cancer cell line through the AR (6). Conversely, many in vivo studies demonstrate potent cancer-preventive activity of DHEA in rodent prostate, breast, lung, skin, liver, colon, and lymphatic tissues (for review see Ref. 50). However, it is unclear whether the data generated in rodent studies can be extrapolated to humans, as the amounts of DHEA and DHEA-S in rodents are lower than those in humans and these steroids are not physiologically important in rodents.

Until more safety information is available, it would appear prudent for men with known or suspected prostate cancer to avoid use of exogenous DHEA.

It is noteworthy that there are few suitable prostate in vitro models to evaluate hormonal responsiveness. Of the small number of human prostatic carcinoma cell lines available, LNCaP is valuable in that it maintains a high level of responsivity to hormone administration. Ideally, primary prostate cell cultures would be used, but these cells have limited growth potential and lose the AR very quickly once placed in culture (49). Further research is warranted to compare DHEA effects on cells containing mutant vs. wild-type AR, prostatic intraepithelial neoplasia (PIN) cells, basal cells, and primary epithelial and stromal cells.

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REFERENCES


24. Gnanapragasam VJ, McCahy PJ, Neal DE, and Robson CN.


18. Klein H, Bressel M, Kastendieck H, and Voigt KD.


14. Gingras S and Simard J.


