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Androgen-Induced Oxidative Stress in Human LNCaP Prostate Cancer Cells Is Associated with Multiple Mitochondrial Modifications

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ABSTRACT

We investigated the role of androgen-induced oxidative stress in prostate cancer using the androgen-responsive LNCaP human prostate cancer cell line exposed to a 1-n*M* concentration of the synthetic androgen R1881 (which correlates with serum androgen levels). Such exposure, which decreases growth rate and increases oxidative stress in LNCaP cells, induced statistically significant mitochondrial changes. A 40% increase in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) reduction, indicative of mitochondrial dehydrogenase activity, occurred 24 hr after androgen treatment. This change preceded 50–110% increases, 40–96 hr after R1881 exposure, in levels of cellular peroxides and hydroxyl radicals as measured by 2′7′-dicholorofluorescin diacetate (DCF) fluorescence. On the basis of electron microscopy measurements, R1881 treatment increased the area fraction of mitochondria per cell by ~100% at 72 hr. In agreement, mitochondrial mass at 96 hr, evaluated by the fluorescent dye nonyl acridine orange (NAO), was 80% higher in treated cells. R1881 exposure for 24 hr lowered the activities of electron transport system (ETS) complexes, I, II, and IV by 17–27% and ATP levels by 50%. The ETS inhibitors, rotenone and antimycin A, lowered androgen-induced DCF fluorescence readings to control levels thereby suggesting ETS involvement in androgen-induced oxidant production. Addition of α-tocopherol succinate abrogated R1881-induced elevations in MTT reduction. In sum, androgens may, directly or indirectly, contribute to oxidative stress in LNCaP cells by regulating mitochondrial number, activity, and oxidant production by mechanisms that are, at least in part, sensitive to an antioxidant. Antiox. Redox Signal. 1, 71–81.

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INTRODUCTION

INCREASED OXIDATIVE STRESS/DAMAGE is associated with aging (Sohal and Weindruch, 1996) and many age-associated diseases, including cancer (Cerutti, 1985; Oberley and Oberley, 1993). The oxidative stress hypothesis of aging postulates that oxidative damage to critical molecules accumulates over the life span and eventually impairs function and contributes to several diseases of aging (Sohal and Weindruch, 1996). Recently, reactive oxygen species (ROS)-associated oxidative damage was documented in human prostate cancer tissue samples (Malins *et al.*, 1997). Whether or not the detected oxidative damage is a critical component of prostate carcinogenesis is unknown, as are the sources of ROS production in prostate cancer.

An accumulating body of epidemiological evidence suggests that men with higher dietary or supplemental intake of certain antioxidants, such as α-tocopherol, lycopene, and selenium, display reduced risk of developing prostate cancer (Giovannucci *et al.*, 1995; Clark *et al.*, 1998; Heinonen *et al.*, 1988). This association suggests that lowering prostatic ROS levels may delay prostate cancer development and progression whereas oxidative stress may act to favor these processes. Determining how these potentially preventative agents work would likely provide insight on how prostate cancer develops.

Increasing age is the major risk factor for developing prostate cancer (Landis *et al.*, 1998). Other etiological contributors have been suggested including androgens, such as testosterone, which are required for prostate growth and development (Meikle, 1989; Oesterling *et al.*, 1998). The main evidence for androgen involvement in prostate cancer is that it rarely occurs in men castrated before puberty, in men with low levels of the enzyme (5α-reductase) responsible for converting testosterone to its more active form, or in men who become hypopituitary before age 40 (Oesterling *et al.*, 1998). Serum testosterone levels decrease ~1% per year after age 40 and soon thereafter prostate morphology begins to change (e.g., decrease in number of epithelial foldings, increase in amyloid bodies) and the gland begins to enlarge (Gray *et al.*, 1991; Narayan, 1995). Therefore, androgen levels are highest during the reproductive years when the prostate is fully functional and not rapidly growing.

Androgen-sensitive LNCaP human prostate carcinoma cells display an increase in measures indicative of oxidative stress following exposure to concentrations of androgen that resemble those found in adult men (Ripple *et al.*, 1997). These include increased 2′,7′-dichlorofluorescin (DCF) fluorescence, catalase activity, oxygen consumption, lipid peroxidation, and decreased glutathione levels (Ripple *et al.*, 1997). The elevated oxidative stress detected in LNCaP cells exposed to 1 n*M* R1881, a synthetic form of 5α-dihydrotestosterone, is associated with decreased cellular growth rate and increased differentiated prostate function (*e.g.*, prostate specific antigen [PSA] secretion) (Schuur *et al.*, 1996; Ripple *et al.*, 1997). We propose that normal prostate functioning in adulthood is associated with a significant level of oxidative stress. This level of oxidative stress may be necessary for regulating the functional phenotype.

Recently, we demonstrated that R1881 treatment of LNCaP cells elevates the DNA binding activity of the redox-sensitive transcription factors AP-1 and NF-κB (Ripple et al., submitted). The androgen-induced binding activity of these ubiquitous transcription factors which are involved in growth, differentiation, and stress response (Angel and Karin, 1991; Sen and Packer, 1996) was lowered by treatment with α-tocopherol succinate combined with ascorbic acid in LNCaP cells. This again suggests opposing effects of high-level androgen exposure and specific antioxidants.

A source of chronic intracellular oxidative stress is the mitochondrion. Incomplete reduction of molecular oxygen by the electron transport system (ETS) has been estimated to produce 10 million superoxide radicals per mitochondrion per day (Richter, 1988). Based on our earlier findings of increased oxygen consumption and elevated 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) reduction in androgen-treated LNCaP cells (Ripple *et al.*, 1997), we hypothesized that this oxidative stress may be, in large part, of mitochondrial origin. This hypothesis is supported by the data reported herein.

MATERIALS AND METHODS

Cell culture and harvest

Androgen-responsive, androgen receptor-positive LNCaP prostate carcinoma cells were obtained from American Type Culture Collection (Rockville, MD). Cells were maintained at 37°C in an atmosphere of 5% CO_2 and 95% air in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS) (Life Technologies, Gaithersburg, MD) and 1× antibiotic/antimycotic (Sigma Chemical Co., St Louis, MO, which is the source of all chemicals, unless otherwise noted). Cells, which had previously been found to be mycoplasma free, were passaged weekly and medium was replaced every 3–4 days. Cells of passage number 40-80 were used for all experiments. The doubling time of LNCaP cells maintained under these conditions is ~36 hr.

Experiments were conducted in medium containing 1% FBS and 4% charcoal-stripped FBS (CSS) to limit the adverse growth effects noted with 5% CSS, which are unrelated to hormone depletion (Ripple *et al.*, 1997). For 96-well assays, 2,500 or 4,000 cells were plated per well in 100 μl of DMEM plus 1% FBS and 4% CSS. Four days later, cells were treated with 100 μl of DMEM plus 1% FBS and 4% CSS containing a 2× concentration of the synthetic androgen R1881 (Du Pont NEN, Boston, MA) or the vehicle control (medium containing 0.01% ethanol). The final R1881 concentration was 1 n*M* for all experiments. For the rotenone and antimycin A experiments, cells were first treated with 100 μl of a 2× concentration of R1881 or the vehicle-control. After 72 hr, the cells were treated with an additional 100 μl of media containing a 3× concentration of rotenone or antimycin A. The final concentration of rotenone or antimycin A was 1 μ*M*.

Cells harvested for electron microscopy, mitochondrial mass determination, ATP measurement, and mitochondrial isolation were plated at 10^6 cells per 100-mm tissue culture plate in medium containing 5% FBS. The following day, medium was replaced with fresh medium containing 1% FBS and 4% CSS. Cells were treated 3–4 days later with fresh medium containing R1881 or the vehicle control. After the appropriate treatment period, cells to be used for ATP determination or mitochondrial isolation were washed with phosphate-buffered saline (PBS) or cold Kreb's Ringer buffer, pipetted off the plate, and resuspended in cold buffer and centrifuged 5 min at 1000 rpm. Cell pellets were then snap frozen and stored at −80°C if they were to be used for mitochondrial isolation. For ATP samples, the cell pellet was resuspended in 100 μl of H_2O, frozen and heated for 5 min in boiling H_2O. The resulting sample was frozen at −80°C until time of assay. Cells plated for electron microscopy were treated for 3 days, washed with serum-free DMEM or Kreb's Ringer buffer, and used for transmission electron microscopy as described below.

Measurement of cellular growth (DNA fluorescence), MTT activity, and ROS levels (DCF fluorescence)

Ninety-six-well plates were harvested for estimation of DNA content, MTT reduction, and cellular ROS levels (peroxide and hydroxyl radical), after 16, 24, 40, 72, and 96 hr of treatment, as previously described (Ripple *et al.*, 1997). Androgen-treated cells were also exposed to rotenone or antimycin A, for either 0.25, 0.5, 1.5, or 22 hr.

Reduction of MTT to formazan was monitored at 515 nm (reference wavelength 630 nm) using a spectrophotometric microplate reader (Dynatech, Chantilly, VA). MTT reduction is thought to represent mainly mitochondrial dehydrogenase activity (Slater, *et al.*, 1963) but may also occur by superoxide-dependent (Madesh and Balasubramanian, 1998) or other mechanisms (York *et al.*, 1998) and may best describe cellular reductive capacity (Berridge and Tan, 1993). Peroxide and hydroxyl radical levels were evaluated using DCF (Molecular Probes, Inc., Eugene, OR) (Kane *et al.*, 1993). DCF fluorescence units were measured on a LS 50B Luminescence Spectrometer (Perkin Elmer, Norwalk, CT). Plates used to determine DCF fluorescence were subsequently frozen at −80°C, and the DNA content per well was determined using a Hoescht-based fluorometric assay (Rago *et al.*, 1990). The data are expressed as the aver-

age reading (per DNA for MTT and DCF) of six replicates ± SD.

Electron microscopy

Transmission electron microscopy was performed on cells fixed in 2% glutaraldehyde and 0.08 *M* cacodylic acid for 30 min. Cells were washed with 0.08 M cacodylic buffer and post-fixed with 0.1% OsO_4. After washing, cells were dehydrated in ethanol and embedded in Epon. Lead- and uranyl-stained sections were analyzed with a Hitachi H-600 transmission or a JEOL 1200EX scanning/transmission electron microscope.

Mitochondrial mass

Mitochondrial mass was evaluated using nonyl acridine orange (NAO) (Molecular Probes) in cells treated with or without androgen for 96 hr (Fujii *et al.*, 1997). Cells were washed with Kreb's Ringer buffer and then incubated with 50 n*M* NAO in Kreb's Ringer buffer at 37°C for 30 min. Following incubation, cells were washed with buffer and fluorescence was measured. Final results are given as the average fluorescence units per DNA ± SD ($n = 6$).

ATP assay

ATP content was assayed using a modified version of the Molecular Probes ATP Determination Kit. Each sample (10–20 μl) was mixed with 2 ml of reaction solution [25 m*M* Tricine buffer pH 7.8, 5 m*M* $MgSO_4$, 0.1 m*M* EDTA, 0.1 m*M* sodium azide, 1 m*M* dithiothreitol (DTT), 50 μ*M* luciferin (Promega, Madison, WI)] and 10 μl of luciferase (0.08 U) at 28°C. Luminescence was recorded immediately following the addition of sample on a LS 50B Luminescence Spectrometer (Perkin Elmer, Norwalk, CT). ATP levels were determined by comparing sample luminescence to an ATP standard curve. The protein content of each sample was measured with Pierce BCA reagents (Pierce, Rockford, IL) and the results are expressed as μmol/mg protein.

Isolation of mitochondria

Mitochondria were isolated from harvested cells as previously described (Rickwood *et al.*, 1987). Frozen cell pellets were resuspended in 5 ml of sucrose solution pH 7.4 (0.25 *M* sucrose, 1 m*M* EGTA, 10 m*M* HEPES) and homogenized on ice with 10 up-and-down strokes of a glass-Teflon homogenizer. The samples were then centrifuged for 10 min at 1,500 × *g*, 4°C. The supernatant was retained and the pellet was resuspended in sucrose solution and centrifuged again. The supernatants were combined and centrifuged for 30 min at 10,000 × g, 4°C. Each mitochondrial pellet was resuspended in 150 μl of sucrose solution.

ETS complex activities

Complex I, II, and IV activity of mitochondrial extracts from vehicle-control and 1 n*M* R1881-treated cells were assayed spectrophotometrically, as previously described (Birch-Machin *et al.*, 1993). For Complexes I and II, activities were calculated and expressed as nmol/min per mg protein, while Complex IV activity was calculated as the first-order rate constant (k) and expressed in min^{-1}.

Statistical analyses

Data are reported as the mean ± SD. Experiments conducted in 96-well plates had an $n = 6$. Each treatment was compared to either the vehicle-control or R1881 treatment using the unpaired, two-tailed Student's t-test and tested at the nominal 0.05 significance level. The reported p values reflect the data shown. All experiments were repeated at least two times.

RESULTS

DNA content

The growth of LNCaP cells, as determined by DNA content, was greater in vehicle-control treated cells than in those exposed to androgen (Fig. 1A). The first statistically significant androgen-induced decrease in DNA content was noted after 40 hr of treatment (30% decrease; $p < 0.005$).

MTT activity

MTT reduction, a measure of mitochondrial dehydrogenase activity and cellular reductive

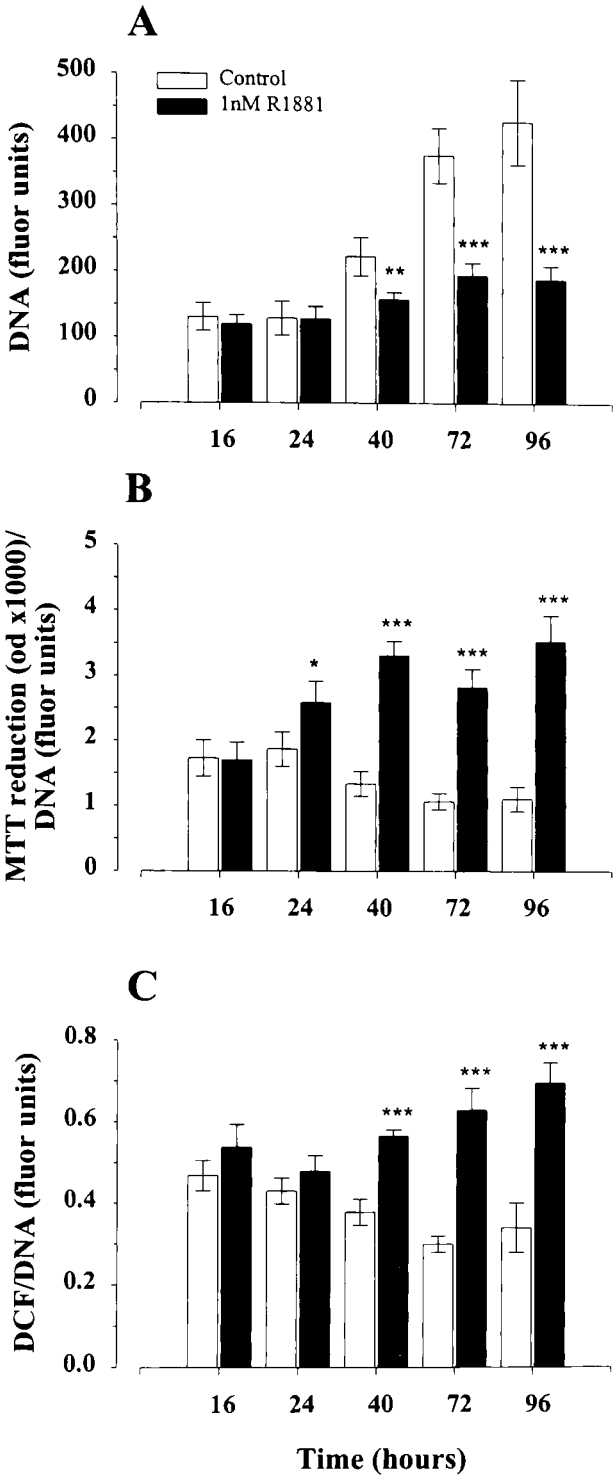

FIG. 1. LNCaP cells were treated with 1 nM R1881 (■) or a vehicle-control (□) and harvested 16, 24, 40, 72, and 96 hr later. Because the same number of cells were plated per well, DNA fluorescent units represent cellular growth (**A**). MTT reduction (MTT od × 1,000) (**B**) and DCF fluorescence (**C**) were normalized to DNA fluorescent units. Data are expressed as the average of six samples ± SD. $^{*}p < 0.05$, $^{**}p < 0.005$, and $^{***}p < 0.0001$ when compared to the respective control.

capacity, was 40% higher in LNCaP cells treated with androgen for 24 hr (Fig. 1B; $p < 0.05$). MTT activity of androgen-treated cells was 160–220% higher than that found in vehicle-control treated cells after 40–96 hr of exposure ($p < 0.001$).

ROS levels

DCF per DNA fluorescence, indicative of cellular peroxide and hydroxyl radical levels, was also greater in LNCaP cells treated with R1881 (Fig. 1C). This increase did not reach statistical significance until 40 hr and, therefore, followed the elevation in MTT per DNA which was apparent at 24 hr. R1881 treatment increased DCF per DNA fluorescence 50–100% above control levels at the 40-, 72-, and 96-hr time points ($p < 0.0001$).

Electron microscopy

Electron micrographs of LNCaP cells treated with vehicle-control or R1881 for 72 hr indicated androgen-induced morphologic alterations (Fig. 2). Androgen-exposed cells were larger and contained an increased number and complexity of mitochondria. Analysis of mitochondrial profiles from six representative micrographs provided area fractions (*i.e.*, the percent of the cytoplasmic area occupied by mitochondria) of 6 ± 4% for vehicle-control-treated cells compared to 15 ± 9% for 1 n*M* R1881-treated cells. This difference represents a statistically significant ($p \leq 0.05$) effect of androgen exposure.

Mitochondrial mass

After 96 hr of exposure to R1881, mitochondrial mass, as indicated by NAO fluorescence per DNA, was elevated by ~80% compared to levels in vehicle-control cells. The values were 0.40 ± 0.06 for R1881-treated cells and 0.22 ± 0.04 fluorescent units for control cultures ($p < 0.0001$).

ETS activities

LNCaP cells treated for 24 and 72 hr with R1881 displayed lower activities of ETS complexes I, II, and IV compared to vehicle-control treated cells (Fig. 3; $p < 0.0005$). The activities

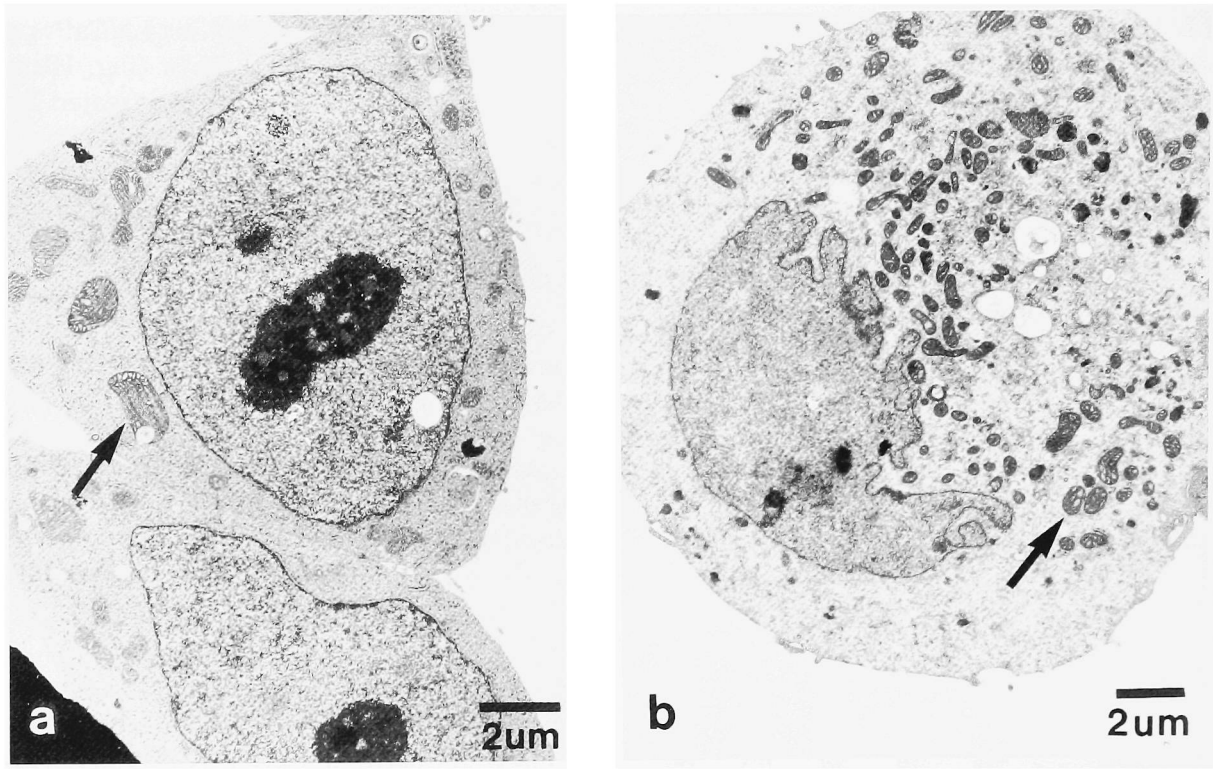

FIG. 2. Electron micrographs of LNCaP cells treated with vehicle-control (a) or 1 n*M* R1881 (b) for 72 hr. Arrows indicate mitochondria.

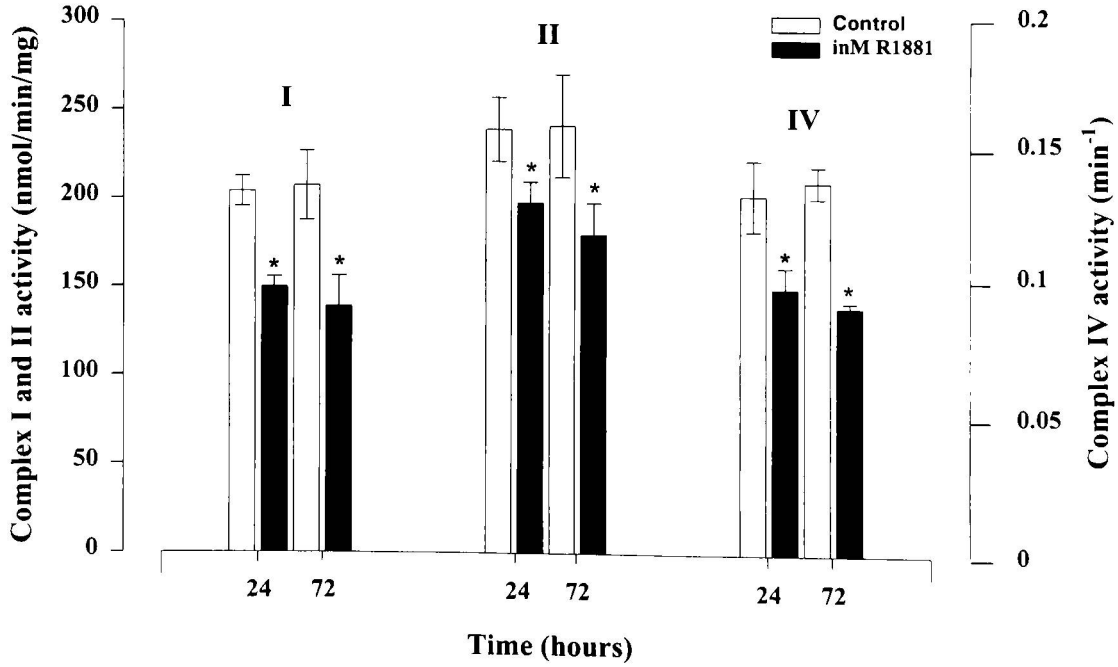

FIG. 3. ETS complex activities (I, II, and IV) of LNCaP cells treated with vehicle-control (□) or 1 n*M* R1881 (■) for 24 and 72 hr. Data are expressed as the average ± SD ($n = 7$ or 8). *$p < 0.0005$ for all R1881-treated samples when compared to the respective control.

were reduced 17–27% at 24 hr and 26–34% after 72 hr of treatment.

ATP levels

ATP content of LNCaP cells treated with 1 n*M* R1881 for 24 or 96 hr was significantly lower than that of vehicle-control treated cells ($p < 0.0005$). ATP levels expressed as μmol/mg protein were ~50% lower in androgen-treated cells compared to vehicle-control (24.2 ± 0.3 vs. 12.7 ± 0.2 at 24 hr; 57.1 ± 4.4 vs. 27.0 ± 1.2 at 96 hr).

Influence of ETS inhibitors on ROS levels

Cells treated with vehicle-control or 1 n*M* R1881 for 72 hr and followed by 15 min treatment with rotenone or antimycin A displayed a decreased ROS generation as indicated by decreased DCF per DNA fluorescence (Fig. 4). These decreases in fluorescence were statistically significant (all with $p < 0.003$). Treatment of cells with the ETS inhibitors for longer periods of time (up to 22 hr) had no further effect (data not shown).

α-Tocopherol succinate effect on MTT reduction

Both vehicle-control and androgen-treated LNCaP cells exposed to 50 or 100 μM of α-tocopherol succinate for 24 hr displayed a lower amount of MTT reduction when compared with cells not exposed to antioxidant (Fig. 5). The results showed that after 24 hr, the amount of MTT reduction was 15–30% lower in vehicle-control treated cells and 35–40% lower in cells exposed to R1881 (all $p < 0.001$). After 96 hr of treatment, the antioxidant decreased the amount of MTT reduced in LNCaP cells treated with androgen by 15–31% ($p < 0.05$ for 50 μM; $p < 0.002$ for 100 μM), but there was no statistically significant influence of α-tocopherol succinate on the vehicle-control treated cells.

DISCUSSION

Physiologic levels of androgen are capable of inducing oxidative stress in LNCaP prostate cancer cells (Ripple *et al.*, 1997). Here we observed that 1 n*M* R1881, which correlates with serum 5α-dihydrotestosterone levels found

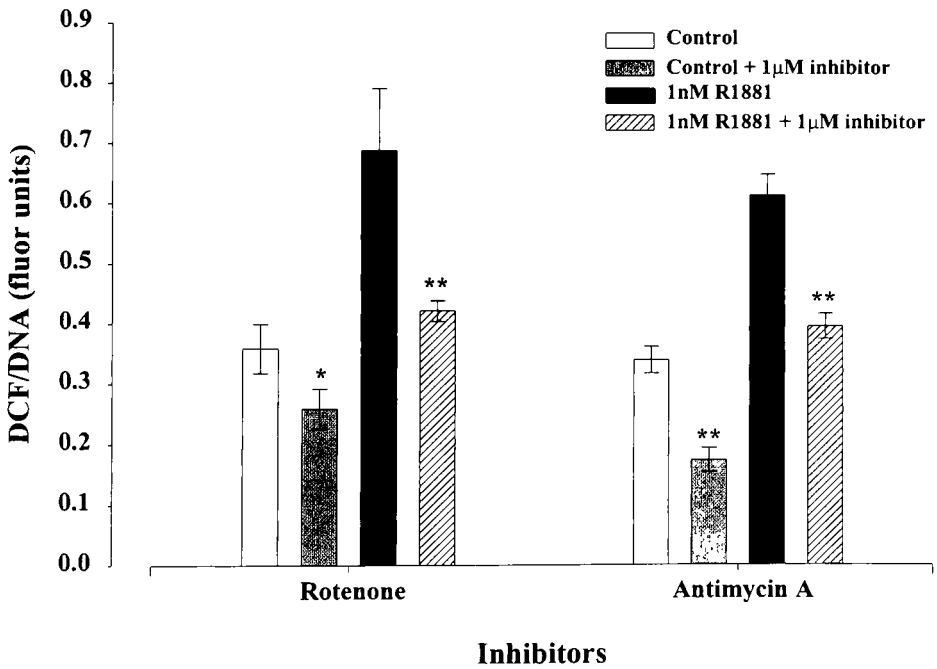

FIG. 4. LNCaP cells were treated with vehicle-control or 1 n*M* R1881 for 72 hr and then with 1 μM rotenone or antimycin A for 15 min. DCF fluorescence was normalized using DNA fluorescence. Data are expressed as the average of six samples ± SD. *$p < 0.003$ and **$p < 0.0001$ when comparing inhibitor-treated cells to the respective untreated cells.

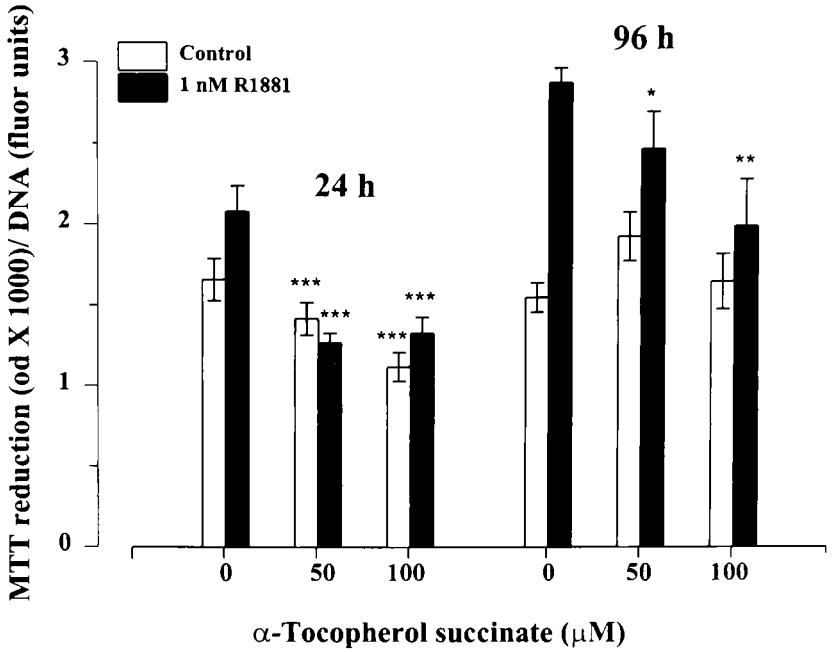

FIG. 5. LNCaP cells were treated with vehicle-control (□) or 1 n*M* R1881 (■) together with 0, 50, or 100 μM α-tocopherol succinate. MTT reduction (od × 1,000) was normalized to DNA fluorescence. Data are expressed as the average of 5–6 samples ± SD. $^{*}p < 0.05$, $^{**}p < 0.002$, and $^{***}p < 0.001$ when compared to the respective control.

normally in adult men, initially affected ETS activity, ATP levels, and MTT reduction. Within 24 hr of androgen treatment, activity of ETS complexes I, II, and IV was lowered 17–27%, MTT reduction decreased by 40%, while ATP levels dropped by ~50%. These changes were followed by a 50% elevation in DCF fluorescence and 30% decrease in growth compared to non-androgen-treated cells. α-Tocopherol succinate was most effective at lowering androgen-induced MTT reduction at 24 hr and could still do so, albeit to a lesser extent, at 96 hr.

ROS such as the superoxide radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen cause macromolecular damage, which may, in turn, affect tumor development and aging (Cerutti, 1985; Sohal and Weindruch, 1996; Oberley and Oberley, 1993). ROS are also important regulatory molecules acting as intracellular second messengers and controllers of protein function (Dargel, 1992; Ziegler, 1985; Sen and Packer, 1996; Irani *et al.*, 1997). We have previously demonstrated that androgens can increase ROS levels, as measured by DCF fluorescence, in human prostate carcinoma cells (Ripple *et al.*, 1997).

Mitochondria, by producing the superoxide radical as a normal consequence of ETS activity, are thought to be a major intracellular source of ROS (Moslen, 1994). Increased mitochondrial volume has been associated with actively secreting cells (Matthews and Martin, 1971). The concentration of androgen used in our studies correlates with the level of circulating 5α-dihydrotestosterone found in adult men and with the concentration of androgen found to induce LNCaP cells to secrete PSA and citrate (Schuur *et al.*, 1996; Costello and Franklin, 1997). The increased mitochondrial volume found in LNCaP cells treated with 1 n*M* R1881 may represent a normal response of prostate epithelial cells to androgen. Interestingly, this androgen level is associated with a higher amount of oxidative stress than occurs in cells exposed to lower androgen levels (Ripple *et al.*, 1997).

A change in mitochondrial metabolism, which could be orchestrated, in part, by hormones may be a key component in prostate carcinogenesis (Costello and Franklin, 1994). Mitochondrial function has long been known to be sensitive to testosterone (Nyden and Williams-Assman, 1953). Androgens elevate

glucose utilization and mitochondrial lipid, protein, and DNA synthesis in castrated rats (Harkonen, 1981; Doeg, 1968; Doeg *et al.*, 1971, 1972). More recently, the mechanism by which testosterone regulates mitochondrial citrate production and secretion, viewed as a major prostate function, has been described (Costello and Franklin, 1997; Costello *et al.*, 1995). The prostate is unlike most organs in that it sacrifices a substantial amount of potential biological energy by truncating the Kreb's cycle to produce citrate (Costello and Franklin, 1994). The decreased ATP levels following androgen treatment may be the result of an induction of energy-requiring processes. Alternatively, lower steady-state levels of ATP may result from an androgen-induced reduction in mitochondrial aconitase activity that would increase citrate levels while decreasing the availability of reducing equivalents to the ETS that are normally obtained through the Kreb's cycle (Costello and Franklin, 1997). The observation that the activities of ETS complexes I, II, and IV are lower in androgen-treated cells supports this latter hypothesis.

Another possibility is that steroid hormones can act as mild uncouplers of the ETS and oxidative phosphorylation; however, abnormally high hormone concentrations are required to obtain such effects (Starkov, 1997). ROS production has been shown to induce a transition in the mitochondrial membrane potential leading to a disorganization of the ETS components (Vercesi *et al.*, 1997). We utilized two separate dyes, JC-1 and DiOC6, to measure mitochondrial membrane potential and detected no significant change following androgen exposure (data not shown). Because R1881 does not elevate oxidative stress in the DU 145 prostate cancer cell line, which does not express the androgen receptor (Ripple *et al.*, 1997), it seems unlikely that R1881 is having a direct chemical effect on the inner mitochondrial membrane.

The androgen-induced mitochondrial alterations reported herein may well represent the "normal" condition ongoing in men from the end of puberty until androgen levels begin to decline, typically around age 40 (Gray *et al.*, 1991). The production of ROS may be viewed as an unwanted byproduct and/or as a means of cellular regulation. ROS are increasingly being recognized as central characters in cell signaling and apoptotic pathways (Irani *et al.*, 1997; Finkel, 1998; Mignotte and Vayssiere, 1998; Gotoh and Cooper, 1998).

Recently, we have demonstrated that androgen treatment of LNCaP cells increases the DNA binding activity at AP-1 and NF-κB sites (Ripple *et al.*, submitted). These redox-sensitive transcription factors are important regulators of growth, differentiation, and stress responses (Angel and Karin, 1991; Sen and Packer, 1996). Redox mechanisms also regulate protein state and enzyme function (Ziegler, 1985; Sen and Packer, 1996). One redox-sensitive enzyme, glyceraldehyde-3-phosphate dehydrogenase, which catalyzes the rate-limiting step in glycolysis, is known to be elevated following treatment of LNCaP cells with 1 n*M* R1881 (Ripple and Wilding, 1995).

Recent epidemiological studies of dietary and supplemental antioxidants provide encouraging data on the prevention of prostate cancer (Giovannucci *et al.*, 1995; Clark *et al.*, 1998; Heinonen *et al.*, 1998). High intakes of antioxidants such as α-tocopherol and selenium are associated with reduced risk of developing prostate cancer (Clark *et al.*, 1998; Heinonen *et al.*, 1998). These observations suggest that decreasing levels of certain ROS may delay the onset of prostate cancer and imply a causal involvement of ROS in prostate carcinogenesis.

Although androgen-induced oxidative stress may play a role in maintaining the differentiated function of the prostate, chronic exposure could lead to oxidative damage to lipids, proteins, or DNA. The protective effects of dietary antioxidants may derive from lower steady-state levels of ROS in the prostate and, consequently, less ROS-induced oxidative damage in the prostate. We provide evidence that α-tocopherol, an antioxidant already associated with reducing prostate cancer risk (Heinonen *et al.*, 1998), may act in part by lowering mitochondrial levels of ROS. Understanding the exact roles played by ROS in normal prostate function and in prostate carcinogenesis may foster the development of new preventive and treatment strategies.

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