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Androgens Regulate Expression of the Gene Coding for a Mouse Vas Deferens Protein Related to the Aldo–Keto Reductase Superfamily in Epithelial Cell Subcultures

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Mouse vas deferens protein (MVDP), a member of the aldo–keto reductase superfamily, is exclusively produced in the vas deferens. To better understand androgen-regulated MVDP gene expression we have used RNA hybridization to study the effects of androgens on the steady-state levels of MVDP mRNA in vas deferens epithelial cell subcultures. Northern blot analysis revealed that these cells only express MVDP mRNA in the presence of androgens. There was a close relationship between MVDP mRNA levels and dihydrotestosterone concentrations. MVDP mRNA is induced over a period of 24h and maximal induction is about 25-fold. Treatment of cells with cycloheximide completely abolished the observed androgen effect suggesting that the induction of the MVDP gene by androgens depends on continuous protein synthesis. Transient transfection of vas deferens epithelial cells with MMTV-CAT vector showed that these cells contained functional androgen receptors and that they are a suitable system to study androgen effect on MVDP gene regulatory elements.

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INTRODUCTION

Androgens control many aspects of cellular differentiated function by regulating the expression of a limited number of genes. The receptor for androgens has been cloned and sequenced [1, 2] and shown to belong to the steroid–thyroid–retinoid superfamily of ligand responsive transactivators [3–5]. The steroid hormone–receptor complex binds to specific recognition sequences, termed hormone responsive elements (HREs), near or within the regulated promoters [3, 6]. The existence of a unique response element for the liganted androgen receptor is still uncertain [7–12]. In the absence of normal homologous epithelial cell lines for transfection purposes, all attempts to identify functional androgen responsive elements (AREs) in androgen responsive genes have been made using heterologous cells containing androgen receptors [9, 10, 13] or in cells devoid of androgen receptors and contransfected with high levels of an androgen receptor expression vector [12, 14]. A common criticism of experiments carried out with heterologous cells is that the results obtained may not reflect the physiological cellular regulation of the androgen-responsive gene studied.

Few studies have characterized the effect of androgen on isolated epithelial cells with regard to their expression of a defined androgen-dependent specific protein or mRNA marker. Previous studies from our laboratory have demonstrated that subcultures of mouse vas deferens epithelial cells, generated from immature animals, showed differentiated cell functions and androgen responsiveness [15]. Mouse vas deferens contains a large amount of a major protein (MVDP: mouse vas deferens protein) of an apparent M_w of 34,500 [16]. On the basis of immunohistochemistry and *in situ* hybridization, MVDP has been exclusively localized in the epithelial cells of vas deferens [17, 18]. MVDP is coded by a relatively abundant mRNA of 1.4 kb in length as determined by Northern blot analysis, and the vas deferens is the only organ where MVDP mRNA has been detected [18]. Androgens have been shown to be primary regulating factors of the MVDP gene at the protein and mRNA levels [16, 18], and androgen-dependence appears in prepubertal

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males [19]. The amino acid sequence of MVDP, deduced from its cDNA nucleotide sequence, reveals a high degree of homology with members of the aldo–keto reductase superfamily, and the structure of the MVDP gene is very similar to that described for the human aldose reductase gene [20, 21]. Aldose reductase, which catalyzes the reduction of sugars to corresponding sugar alcohols, has been implicated in the development of various diabetic complications [22].

The present study represents an attempt: (a) to investigate some aspects of the androgen regulation of the MVDP gene; and (b) to determine if transiently transfected untransformed vas deferens epithelial cells are a suitable model to study the androgen effect on gene regulatory elements.

MATERIALS AND METHODS

Isolation and culture of mouse vas deferens epithelial cells

The culture of vas deferens epithelial cells was developed in this laboratory [15]. A pure epithelial cell culture was obtained from primary culture of mouse vas deferens explants. Vas deferens from sexually immature mice (CD-1 Charles River, France) were removed and placed into Costar's transwell-Col culture chambers containing serum free defined medium (SFDM). The SFDM consisted of a mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 medium (1:1, v/v) supplemented with transferrin (10 μg/ml), epidermal growth factor (EGF) (10 ng/ml), cholera toxin (10 ng/ml), selenium (17.3 ng/ml), cAMP (1.5 μg/ml), glutamine (1%), ethanolamine (0.6 μg/ml), insulin (10 μg/ml), penicillin (100 U/ml), streptomycin (100μg/ml), Hepes (4.76 mg/ml), and hydrocortisone (3 ng/ml or 10^{-8} M). Cells emerging from the explants were mainly epithelial cells.

Cellular material from primary culture was then amplified by subculturing the cells over a feeder layer of 3T3 fibroblastic cells into plastic dishes in the SFDM with 10^{-6} M hydrocortisone necessary for adhesion and proliferation. Stromal cells and 3T3 fibroblastic cells were eliminated by trypsinization at the second passage. In this system, cells reached confluency within 4 days with a cell cycle length of 29 ± 2 h. Those epithelial cells expressed cytokeratin but not the tissue-specific protein MVDP. Epithelial cell lines, maintained for 15–27 passages, were used in the present study.

For induction of differentiation and polarized secretion, epithelial cells from subculture over a 3T3 feeder layer were seeded at confluency (4×10^5 cells/cm^2) into Costar's transwell-Col culture chambers without a 3T3 feeder layer. These chambers allow polarization of the cells and improve access of the basolateral surface of the cells to the culture medium. Cells were maintained for 48 or 72 h in SFDM before androgenic induction. This period corresponded to the time required for polarization of cells. In the absence of androgens, polarized cells did not express MVDP but, in the presence of androgens, significant amounts of MVDP were produced. For all experiments, 5α-dihydrotestosterone (DHT) was added to cultures when cells were polarized. As shown previously, androgenic induction of expression of MVDP does not require hydrocortisone [15]. In all experiments reported here, MVDP expression was assayed in polarized cells cultured in SFDM depleted for hydrocortisone. The protein synthesis inhibitor cycloheximide was used at 2.8 μg/ml. Medium was changed every 2 days. Finally, cells were trypsinized and centrifuged, after washing with calcium magnesium-free phosphate buffered saline (PBS), the pellets were recovered in guanidine thiocyanate–EDTA buffer (GnSCN 5M, EDTA 0.1M, pH 7.0) and stored at -80°C.

Isolation of total RNA

Total RNA was extracted from the cultured vas deferens epithelial cells with RNAzol according to Bioprobe Systems as described previously [23]. RNA was recovered by isopropanol precipitation after removal of proteins with RNAzol–chloroform (1:0.1, v/v) mixture. The pellet was dissolved in EDTA 1 mM. The amounts of total RNAs were estimated by measuring optical density (OD) at 260 nm. The ratio OD at 260 nm/OD at 280 nm indicated the purity of extracts.

Vasa deferentia from 20-day-old male mice were removed, quickly frozen on dry ice, and stored at -80°C until needed. Total RNA was isolated from frozen tissues as described above.

Electrophoresis and blotting of RNA

For Northern blotting, 10 μg of total RNA was treated with formaldehyde–formamide (1:2, v/v) separated by electrophoresis through 1.5% denaturing formaldehyde–formamide agarose gels and transferred to a nitrocellulose filter (Hybond N, Amersham Corp.). After electrophoresis the gel was stained with ethidium bromide to ensure the integrity of each RNA sample and equal loading of RNA. 28 and 18S RNAs were used as internal size markers. For RNA slot blotting and RNA dot blotting, total RNA was denatured by heating at 65°C for 15 min in formaldehyde denaturing solution [24]. Nitrocellulose filters ruled into squares were satured with 20 × SSC (3M NaCl, 0.3 trisodium citrate, pH 7.0), air dried (Manifold Hybrid Dot, Gibco BRL) and blotted with denatured RNA. The filters were baked for 2 min in UV.

Probe labeling

The $EcoR_1$–$BamH_1$ fragment of the 1200 bp cDNA coding for MVDP was used for Northern and dot blot analysis [18]. Radioactive labeling of cDNA was done with [α^{32}P]dATP [25] using the random hexanucleotide primer labeling kit (Boerhinger Mannhein) according to the recommendation of the supplier. The specific activity of the probe was 10^8 cpm/μg DNA.

Northern and dot blot analysis

Prehybridization of RNA to radioactively labeled cDNA probes was carried out as described by Thomas

[26]. Blots were prehybridized for 6 h at 42°C in a solution containing 50% formamide, 6 × SSC (1 × SSC is 15 mM sodium citrate, 150 mM NaCl, pH 7.0), 5 × Denhart's solution (100 × is 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.02% Ficoll), 0.5% SDS and 200 μg/ml salmon sperm DNA carrier. Hybridization was performed overnight at 42°C in the same solution containing 10^6 cpm/ml MVDP cDNA probe. Membranes were washed twice in 2 × SSC, 0.1% SDS for 10 min at 42°C, followed by two washes in 1 × SSC, 0.1% SDS for 30 min at 42°C.

Autoradiography was carried out by exposing the blots to XAR-S films (Kodak) for 24 h with one intensifying screen at −70°C. The relative expression of transcript in total RNA was determined by quantitative densitometric scanning with an LKB Ultro-scan XL laser densitometer (LKB, Bromma, Sweden). All MVDP mRNA levels were corrected for differences in loading or transfer of the RNA samples to the filters by comparison to the level of poly(A)$^+$ RNA, measured by reprobing the stripped blots with oligo (dT) (Pharmacia, Uppsala) that is end-labeled with [γ^{32}P] ATP using the enzyme T4 polynucleotide kinase [27]. Relative expression was calculated using the following equation: absorbance units MVDP mRNA/absorbance units poly(A)$^+$ RNA = amount MVDP mRNA in cells.

Transient transfection and CAT assays

For each transfection experiment pure mouse vas deferens epithelial cells were seeded at a density of 1.5 × 10^6 cells per 100 mm plastic dish and transfected 24 h later with 15 μg MMTV-CAT vector using the DEAE-dextran method [28]. The MMTV-CAT vector, which contains the upstream sequence of the long terminal repeat promoter of the mouse mammary tumor virus (MMTV) driving the bacterial chloramphenicol acetyl transferase (CAT) reporter gene, was used as positive control of androgen induction. Following 1 h incubation with the DEAE-dextran/DNA precipitate and DMSO shock, the cells were washed and incubated in fresh serum-free medium. Cells were harvested 24 h later and replated into Costar's transwell-Col culture chambers which allow polarization of the cells. After polarization (3 days), the cells were incubated in fresh serum-free medium with or without 10^{-6} M DHT. Cells were harvested after 24 h hormone exposure for CAT assays. CAT activity of cell extracts was assayed according to the method of Neumann [29].

Western blotting

Samples were electrophoresed through a 15% polyacrylamide gel according to the method of Laemmli [30]. After electrophoresis, gels were electrophoretically transferred to nitrocellulose 2 h at 60 mA constant current in electrophoresis buffer. Nitrocellulose blots were stained with Ponceau S (0.5% in 1% acetic acid) in order to visualize total proteins, and then destained in rinsing buffer (0.15 M NaCl, 50 mM Tris, pH 8). Blots were preincubated for 15 min with blocking buffer (2% BSA in rinsing buffer) and then incubated for 2 h with a polyclonal anti-MVDP antibody (1:2000) according to the method described previously [31]. After a brief washing in blocking buffer, filters were incubated for 2 h in 1:500 dilution of the second antibody (anti-rabbit IgG peroxidase labeled). Blots were then washed extensively in rinsing buffer and revealed with chromogene reaction (H_2O_2 300 μl, ethanol 2.7 ml, solution chloronaphtol 300 μl in 15 ml rinsing buffer and 17.7 ml H_2O).

RESULTS

Detection of MVDP transcripts in cultured vas deferens epithelial cells

Cells were plated at confluency onto Costar's transwell-Col culture chambers with basal medium. After cells became polarized (72 h), they were cultured for 24 h in the absence or in the presence of DHT. Northern blot analysis was used to identify MVDP transcripts. As shown in Fig. 1, when 10 μg of total RNA extracted from vasa deferentia of 20-day-old mice were analyzed by Northern blot and hybridized with α^{32}P-labeled MVDP cDNA, an intense 1.4 kb band was observed indicating that the MVDP mRNA is expressed at a high level in agreement with a high abundance of the protein [16]. The overexpressed lane d is a single band as shown previously [19]. In agreement with previous observations [18], the labeled

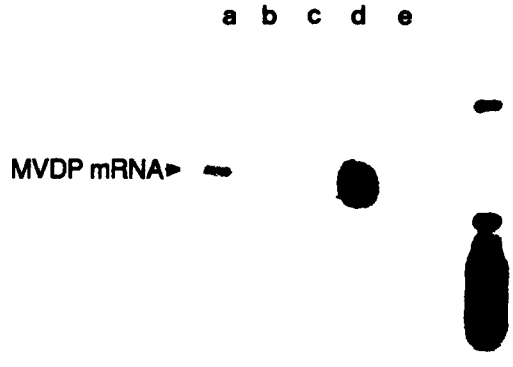

Fig. 1. Northern blot analysis of MVDP mRNA. Total RNA was extracted from epididymides (e) and vasa deferentia (d) from 20-day-old mice, and from epithelial cell cultures in Costar's transwell-Col culture chambers. Cells were cultured 24 h in basal serum free medium in the presence (a) or in absence (b) of 10^{-7} M DHT. T47D cells, that do not produce MVDP, were included as negative control (c). 10 μg total RNA per condition were subjected to Northern blot analysis. Total RNA was electrophoresed, transferred to nylon-N-membrane, probed with ^{32}P-labeled MVDP cDNA and subjected to autoradiography. Integrity and equal loading of RNA were confirmed by ethidium bromide staining of 28 and 18S ribosomal RNA bands. End-labeled λ phage DNA, which was digested by Hind III, was used as size marker (right side of the panel). MVDP was observed as single band of 1.4 kb. Arrow indicates the position of MVDP mRNA.

probe did not hybridize specifically to RNAs from mouse epididymis. The MVDP mRNA was undetectable when the cultured vas deferens epithelial cells were grown without androgens in the basal medium. Northern blot hybridization analysis of RNA extracted from vas deferens epithelial cells cultured in the presence of DHT, demonstrated MVDP transcripts similar in size to those of the whole vas deferens (Fig. 1). Greater abundance of MVDP mRNA in whole vas deferens, compared to cultured epithelial cells, explains the apparent difference of mobility between hybridization bands on lanes a and d. This indicates that cultured vas deferens epithelial cells were able to express MVDP gene over 24 h period if DHT was added to basal medium.

Dose-dependence of androgenic induction of MVDP mRNA

Since MVDP mRNA was observed as a single band, mRNA levels were evaluated by dot blot analysis for the rest of the experiments. Cells were cultured for 24 h in the presence of DHT at various concentrations. The autoradiograph is shown in Fig. 2(A) together with a graph showing the quantitation of these results by scanning densitometry. The level of poly(A)$^+$ RNAs was not significantly affected by DHT treatment indicating that oligo(dT) labeling can be used to correct for differences in loading and/or transfer of the RNAs to the nylon membrane. DHT significantly increased the levels of MVDP mRNA at concentration of 10^{-9} M and the stimulation of MVDP transcripts continued to rise with higher concentrations.

Time course of androgenic induction of MVDP mRNA

To determine the time course of MVDP steady-state RNA induction, cultured vas deferens epithelial cells were treated with 10^{-6} M DHT for increasing periods of time. Undetectable prior to 5 h of induction, MVDP mRNA was expressed at low levels at 8 and 12 h, and increased sharply between 12 and 24 h [Fig. 3(A)]. Maximal induction is about 25-fold after 24 h, compared to levels determined after 5 h induction. This induction was specific and not a result of a generalized increase in mRNAs, since hybridization of the blots with the oligo (dT) showed no significant change in the poly(A)$^+$ RNA level throughout the induction (Fig. 3). On the basis of Western blot analysis of cell extracts, the accumulation of MVDP and of its mRNA had a closely related developmental pattern [Fig. 3(B and C)]. However, whereas the significant increase in mRNA level occurred 16 h after DHT addition, the significant increase in protein level was detected only at 24 h. This lag period probably corresponds to the translation of the mRNA accumulated at 16 h.

Effect of cycloheximide on MVDP mRNA levels

To determine whether DHT exerts its action through newly synthesized protein(s), the effect of cycloheximide, a protein synthesis inhibitor (which blocks the peptidyl transferase reaction on ribosome in eucaryotes), on MVDP mRNA levels was examined. Cultured vas deferens epithelial cells were treated for 24 h with 2.8 μg/ml cycloheximide alone or using various combinations of cycloheximide and DHT as described in Fig. 4. The number of viable cells did not change during incubation with cycloheximide for up to 24 h. As shown in Fig. 4, when cycloheximide was given in concert with DHT during 24 h, the stimulatory effect of DHT on MVDP mRNA levels

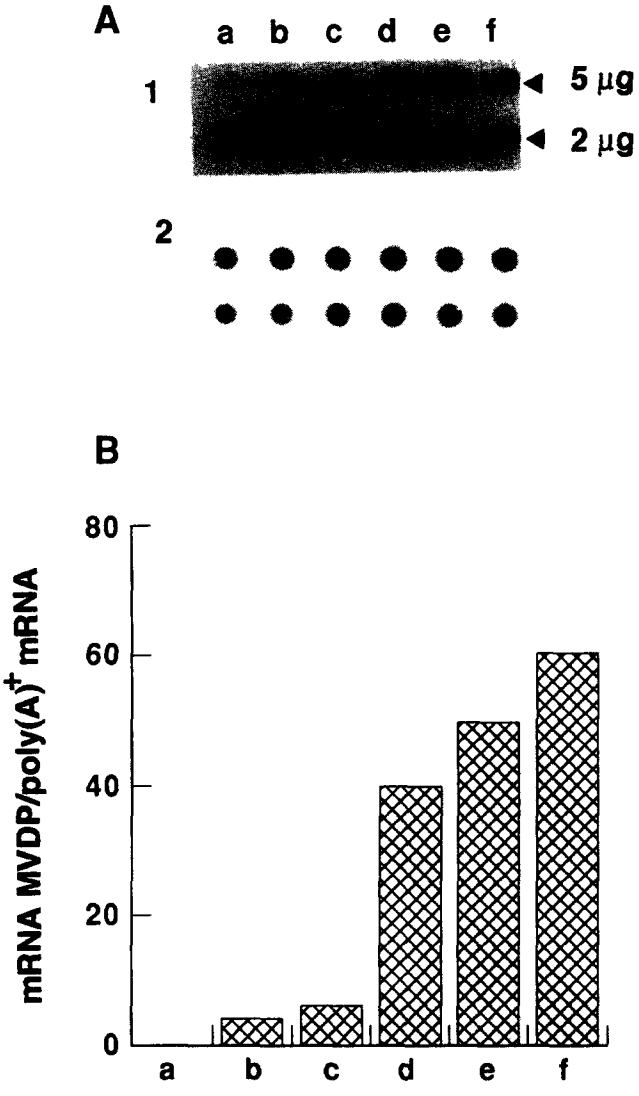

Fig. 2. Dose-dependent effect of androgen on MVDP mRNA levels in epithelial cell cultures. Cells were cultured for 24 h in basal serum free medium without (a) or with DHT at 10^{-10} M (b), 10^{-9} M (c), 10^{-8} M (d), 10^{-7} M (e) and 10^{-6} M (f). (A) Dot blot analysis of 5 and 2 μg total RNA extracted from cells in the presence of various concentrations of DHT. RNA was spotted to nylon membrane using a commercial manifold and probed with MVDP cDNA (1). Subsequently the probe was washed off and the membrane was rehybridized with an oligo (dT) probe (2). (B) Autoradiograms were scanned densitometrically and the results are shown schematically in a block diagram. To correct for small variations in the amounts of input RNA, the data were normalized with respect to poly(A)$^+$ RNA levels. The different lanes were scanned and the amount of MVDP mRNA was determined relative to poly(A)$^+$ RNA. The background observed in the absence of DHT (a) was deduced from specific hybridizing signals. The experiment was repeated three times with essentially the same results.

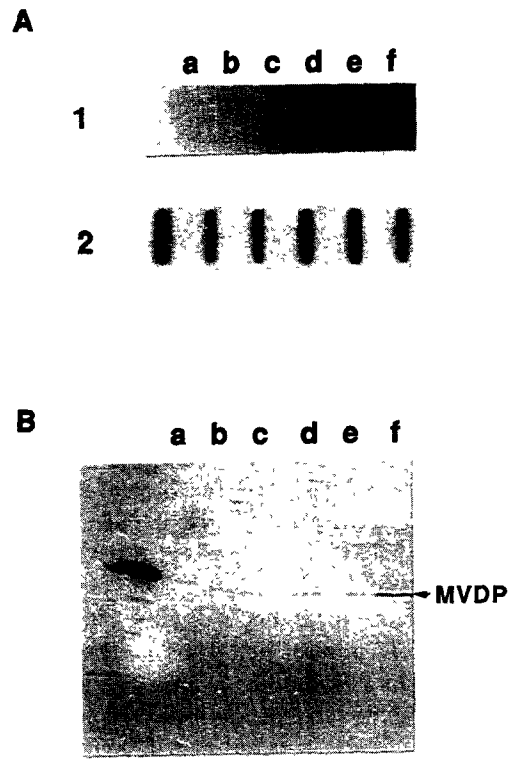

Fig. 3. Time-dependent effect of androgen on MVDP and MVDP mRNA levels in epithelial cell cultures. Cells were cultured in basal serum free medium in the presence of 10^{-6} M DHT for 0 (a), 5 (b), 8 (c), 12 (d), 16 (e) and 24 (f) h. (A) Slot blot analysis of 10 μg total RNA extracted from cells cultured in the presence of 10^{-6} M DHT for various periods. Hybridization signals were obtained with a labeled MVDP cDNA probe (1). Subsequently the probe was washed off and the membrane was rehybridized with an oligo(dT) probe (2). (B) Western blot analysis of 100 μg proteins from total vas deferens (left side of the panel) and from cells cultured in the presence of 10^{-6} M DHT for various periods. After SDS–PAGE, the proteins were transferred to nitrocellulose and incubated with polyclonal antibody specific of MVDP (1/2000). (C) Autoradiograms and Western blots were scanned densitometrically and the results are shown schematically in a block diagram. The data for MVDP mRNA were normalized with respect to poly(A)$^+$ RNA levels. The amount of MVDP mRNA was determined relative to poly(A)$^+$ RNA. For MVDP, values are expressed as percentage of the maximal induction obtained after 24 h induction. The experiment was repeated three times with essentially the same results.

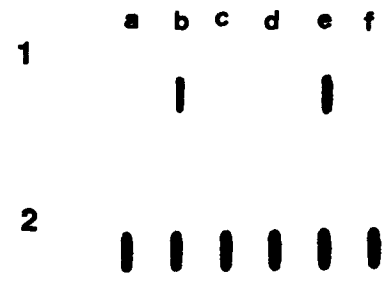

Fig. 4. Effect of inhibition of protein synthesis on MVDP transcription in epithelial cell cultures. Slot blot analysis of 5 μg total RNA obtained from cells cultured 24 h in various conditions. Hybridization signals were obtained with a labeled MVDP cDNA probe (1). Subsequently the probe was washed off and the membrane was rehybridized with an oligo(dT) probe (2). a, Basal serum free medium without DHT (24 h); b, basal serum free medium with 10^{-6} M DHT (24 h); c, cycloheximide (24 h) in combination with 10^{-6} M DHT (24 h); d, cycloheximide alone (24 h); f, 10^{-6} M DHT (24 h) + cycloheximide (16 h) added after 8 h induction by DHT. The experiments were repeated three times with essentially the same results.

was abolished. A similar observation was made when cycloheximide was added 8 h after DHT. During a 24 h period, the level of poly(A)$^+$ RNA remained essentially unchanged [Fig. 4 (lane 2)] indicating that cycloheximide abolishes the androgen-induced transcription but not transcription in general.

Effect of DHT on expression of MMTV-CAT

MMTV-CAT was used to test the presence of functional androgen receptors in polarized vas deferens epithelial cells since it is well known that the hormone response elements of MMTV are activated by a variety of steroids in different cell lines [9]. As shown in Fig. 5 significant induction of CAT activity was observed with extract from cells stimulated by DHT. In contrast, CAT activity in the absence of DHT was undetectable.

Fig. 5. Assays of transfection of subcultured vas deferens epithelial cells using MMTV-CAT plasmid. Vas deferens epithelial cells were seeded at a density of 1.5×10^6 cells per 100 mm plastic dish and transfected 24 h later with 15 μg MMTV-CAT vector using the DEAE-dextran method. Following 1 h incubation with the DEAE-dextran/DNA precipitate and DMSO shock, the cells were washed and incubated in fresh serum-free medium which allow polarization of the cells (3 days). After polarization the cells were incubated for 24 h in fresh, serum free medium without (a) or with (b) DHT at 10^{-6} M. CAT activity of cell extracts was assayed.

DISCUSSION

A method for establishing a subculture of mouse vas deferens epithelial cells as well as morphological and immunohistochemical characterization of these cells in culture has been reported previously. During the course of this characterization, the contribution of androgens to the synthesis and secretion of the major protein of the mouse vas deferens (MVDP) has been evaluated [15]. In this report, the functional status of subcultured vas deferens epithelial cells was evaluated by both Northern blot analysis and Western blotting showing that the MVDP gene is induced by androgens at mRNA and protein levels, and by transfection with a positive control of androgen induction, indicating the presence of functional androgen receptors. With respect to androgen regulation, our data confirm previous *in vivo* studies which have shown that MVDP is regulated at the protein and mRNA levels [16, 18]. The time course of the induction demonstrates that this is indeed a relatively early transcriptional response of vas deferens epithelial cells to androgens. The transcripts of the MVDP gene were first detected after 5 h of DHT treatment and were fully induced within 24 h. MVDP mRNA levels do not increase in magnitude with longer exposure to androgens, suggesting full occupancy of the HREs by the liganted androgen receptor. Scanning densitometry revealed that the fold induction of the transcript, within 24 h, is 25-fold over cells treated with DHT during 5 h (untreated cells could not be used as control since they do not express MVDP gene). Information concerning the expression of androgen regulated genes in normal epithelial cells is very limited. Tumor cells, especially the human prostate cancer cell line LNCaP, have generally been used for such experiments. In LNCaP cells it has been isolated a cDNA clone for a gene whose mRNA is fully induced (30 to 50-fold) within 6 h of treatment by DHT [32]. The expression of the prostate-specific antigen gene (PSA) is induced over a period of 24 h after addition of androgens and maximal induction (10-fold) is observed after 72 h compared to control levels measured in the absence of hormones [12, 33]. The endogenous C3 prostatic gene is not expressed in the T5 and C10 prostate cell lines suggesting the absence of specific transcription factors which may be necessary to androgenic induction [9]. The transcriptional activity of the genes expressed in the LNCaP cells is only modulated by androgens since a basal expression of the corresponding mRNAs is observed in unstimulated cells. In contrast, in the absence of androgens, vas deferens epithelial cells do not express MVDP; the MVDP gene appears to switch from an uninduced state to an induced state.

The MVDP gene showed a significant lag period between the time androgen was added to medium and when the steady-state levels of MVDP mRNA began to rise significantly. There are at least two types of mechanisms by which hormone responsive genes might be progressively activated. One is that induction of the MVDP gene is dependent on the ongoing synthesis of a labile protein factor which in turn reacts with the responsive gene. Cycloheximide experiments suggest that the action of androgens on the MVDP gene requires newly synthesized proteins which were produced by the vas deferens epithelial cells themselves. The delay observed between the addition of androgen and full MVDP gene induction might be due to a lag in the accumulation of an intermediate protein factor and/or to the attainment of a threshold concentration of this factor. An alternative explanation is that the lag period corresponds to an insufficient number of androgen receptors. It has been previously shown that DHT increased the amount of androgen receptors in a ductus deferens smooth muscle tumor cell line, DDT1MF-2 [34]. In LNCaP cells, the level of androgen receptor protein increased by 200% in a 12 h culture in the presence of DHT [35]. In vas deferens epithelial cells, MVDP mRNA levels raised sharply between 12 and 16 h suggesting that number of androgen receptors may also be a rate-limiting factor involved in the delay observed in MVDP mRNA accumulation. A similar progressive induction of mRNA synthesis for androgen-responsive genes, in mouse kidney, has been reported previously [36].

Androgen-responsive mRNA expression can be regulated by transcriptional (directly and/or indirectly) or by posttranscriptional (nuclear processing of primary transcripts and/or RNA stability) mechanisms. Whether the hormonal effect on transcription of the gene is mediated by direct activation of the androgen receptor is unknown for most of the androgen-regulated genes [12]. Of interest in this report is the observation that administration of cycloheximide, a protein synthesis inhibitor, simultaneously with DHT treatment, completely abolishes the androgenic induction of MVDP mRNA. This demonstrates that the androgen effect on MVDP mRNA requires protein synthesis. In this way, an androgen-induced increase in androgen receptor, ongoing protein synthesis, or both, may be important for MVDP and mRNA expression. In this relation, the half-life of androgen receptor, in the presence of androgen, has been reported to be relatively long in transfected Cos cells [37]. In LNCaP cells, the half-life of androgen receptor was determined to be 3.3 or 7.5 h in the absence or presence of DHT, respectively, and this effect of DHT was exerted at the level of posttranslation by reducing the turn over rate of the protein [35]. The transcripts of the MVDP gene were first detected after 5 h of DHT suggesting the presence of a significant concentration of androgen receptors. When cycloheximide was added 8 h after DHT, androgen receptors were present in cells and their half-life could be increased by the hormone [35, 37]. The abolition of MVDP mRNA, observed in these conditions, shows that short-living proteins are required for androgen induced MVDP mRNA. This suggests that androgenic induction of MVDP transcripts is not a primary response to the addition of DHT. Alternatively, liganted androgen receptors may

interact directly with the MVDP gene, but additional short-living transcription factor(s) may be required for the maximal induction of MVDP transcripts. Several lines of evidence suggest that MVDP mRNA expression is a primary response to androgen. First, *in vitro* transcription assays on isolated nuclei have shown that the hormonal induction of the MVDP gene is achieved mainly at transcriptional level [18]. Second, the present study showed that the accumulation of MVDP and of its mRNA had a closely related developmental pattern. Third, several gene fragments have been shown to act as androgen-dependent enhancers of transcription. These are the long terminal repeat of MMTV [8], an upstream element of the mouse sex limited protein gene [13], a part of the first intron of the rat prostatic binding protein gene C3 [10] and a fragment of the promoter of the PSA gene [12]. Region upstream of the MVDP gene contained several motives that closely resemble the consensus sequence of the androgen response elements of the genes described above. These sequences require functional testing (Fabre *et al.*, unpublished data) and further experiments were necessary to conclude that MVDP mRNA expression is a primary response. Among the androgen-regulated genes studied, the situation is quite variable concerning the necessity of continued protein synthesis to induce androgen response. The synthesis of short-living protein has been shown to be required for the full induction of some genes such as those encoding rat prostatic 22 kDa proteins [38]. The androgenic induction of a human prostatic gene encoding a 4.5 kb mRNA does not require protein synthesis as it is maintained in the presence of protein synthesis inhibitors [32]. The basal transcription of the PSA gene strictly depends on continuous protein synthesis, whereas transcriptional induction by androgens does not [33].

The transfection of expression vectors containing a reporter gene into cultured cells has been extensively used to characterize *cis*-regulatory elements such as promoters and enhancers. Transformed or tumor cells have been used in most of these studies, and information concerning the transfection of normal cells is very limited. Recently it has been shown that transiently transfected endometrial cells in subculture were a suitable system to study the estrogen effect on gene regulatory elements [39]. In our study, subcultured vas deferens epithelial cells were transfected with MMTV-CAT plasmids. The results show that DHT stimulated CAT activity indicating that subcultured epithelial vas deferens cells can now be used as a model system for investigating the androgen effect on MVDP gene regulatory elements.

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