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Characterization of the Promoter of the Gene for a Mouse Vas Deferens Protein Related to the Aldo-keto Reductase Superfamily: Effect of Steroid Hormones and Phorbol Esters

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Mouse vas deferens protein (MVDP) is a member of the aldo-keto reductase family regulated by androgens. The expression of a hybrid gene containing the promoter of the MVDP gene and the chloramphenicol acetyl transferase (CAT) gene coding region was analyzed in two cell lines that do not normally express the MVDP gene: T47D and CV1 cells. A small region of the promoter (−121 to +41) was able to direct significant expression of the reporter gene in both cell lines. Additional elements, between −510 and −121 modulate basal expression in a cell-dependent manner. Interestingly, the 162 bp fragment serves as an androgen-dependent enhancer, and mutation of the consensus ARE sequence located between positions −111 and −97 resulted in a loss of androgen response in both cell lines. Additional elements, upstream of the enhancer, modulate induction positively or negatively in relation to the cell line used. The expression of different MVDP-CAT constructs was more effectively induced by androgens than by glucocorticoids at physiological hormonal concentrations. In addition to the 162 bp enhancer, sequences upstream of −510 were also required for specific androgen regulation. Phorbol 12–myristate 13–acetate (TPA) had no effect on basal activity of the 1.8 kb MVDP-CAT construct but strongly enhanced the induction by androgens.

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INTRODUCTION

Aldo-keto reductases represent a multigene superfamily of monomeric proteins that are widely distributed in man and animals [1, for review]. The various aldo-keto reductases show broad overlapping substrate specificities for aldehydes and ketones including xenobiotics as well as endogenous compounds [2]. It has been recently shown that degradation of steroids by reduction of their aldehyde and ketone groups may be primarily due not to specific steroid dehydrogenases but result from the broad specificity of enzymes of the aldo-keto reductase superfamily [3–5]. Recent data also suggest that members of the aldo-keto reductase family may be used as detoxifying system [6, 7]. Among the aldo-keto reductase family, aldose reductase has received special attention because of its potential role in the development of diabetic complications [8].

Adult mouse vas deferens contains a large amount of a major protein (MVDP: mouse vas deferens protein) expressed in a tissue-specific and temporally regulated manner [9–11]. The deduced amino acid sequence shows that MVDP has a high degree of homology with members of the aldo-keto reductase family [12], and the structure of the gene is very similar to that of the human aldose reductase gene [13, 14]. A tumor-associated protein variant during rat hepatocarcinogenesis, identical to aldose reductase, exhibits a strong homology with MVDP [15]. The normal physiological role(s) of aldose reductase and MVDP are not known [16]. An osmoregulatory role has been suggested by the specific increase in aldose reductase gene expression in the renal medulla and a variety of other cells upon exposure to hyperosmotic stress [17, 18]. Expression of the aldose reductase gene in the rat lens is reportedly

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induced by excess of galactose, although the specificity of this induction has been questioned [19]. Analysis of aldose reductase gene promoters should yield important information on the role that genes from the aldo-keto reductase family may play in physiological and pathological conditions. It has been shown that testosterone is the signal that triggers MVDP gene expression at the protein and mRNA levels [9–11]. Sequence analysis of the MVDP 5′-flanking region, and transient expression assays, have demonstrated the presence of a functional androgen responsive element (ARE), mutation of which resulted in almost complete loss of androgen-dependent transcriptional activity [20].

Gene transcription is regulated by the combined action of multiple trans-acting factors on distinct enhancer regulatory elements [21]. One of the major signal transduction pathways involves steroid hormone receptors that represent an important class of trans-acting factors that are activated on binding their cognate ligand. When bound to specific consensus sequences (hormone responsive elements: HREs), they control gene transcription in either a positive or negative direction [22 for review]. A second signaling pathway results from membrane receptor-mediated extracellular stimulation of cells. Recently, evidence has been presented for the coupling of multiple signal transduction pathways with steroid response mechanisms [23]. Aldose reductases, and other members of the aldo-keto reductase family, are involved in cellular homeostasis [1]. However, little is known about the DNA sequences and nuclear factors that mediate the response of these genes to disparate signal transduction pathways. The present study represents an attempt: (a) to further characterize the elements involved in the specificity of hormonal response of MVDP gene; and (b) to determine if activation of the protein kinase C (PKC) can modulate the androgen responsiveness of the MVDP gene.

MATERIALS AND METHODS

Plasmid construction

The plasmids used in this work have been described [20] and are shown schematically in Fig. 1. They contain fragments of MVDP 5′-flanking region fused to the coding sequence of bacterial chloramphenicol acetyltransferase (MVDP-CAT). The MMTV-CAT vector contains the upstream sequence of the long terminal repeat promoter of the mouse mammary tumor virus in front of the CAT reporter gene. The pSVARo vector contains an SV40 promoter directing the transcription of the full-length human androgen receptor cDNA [24]. The HG4 vector contains the human glucocorticoid receptor and was from Stratagene.

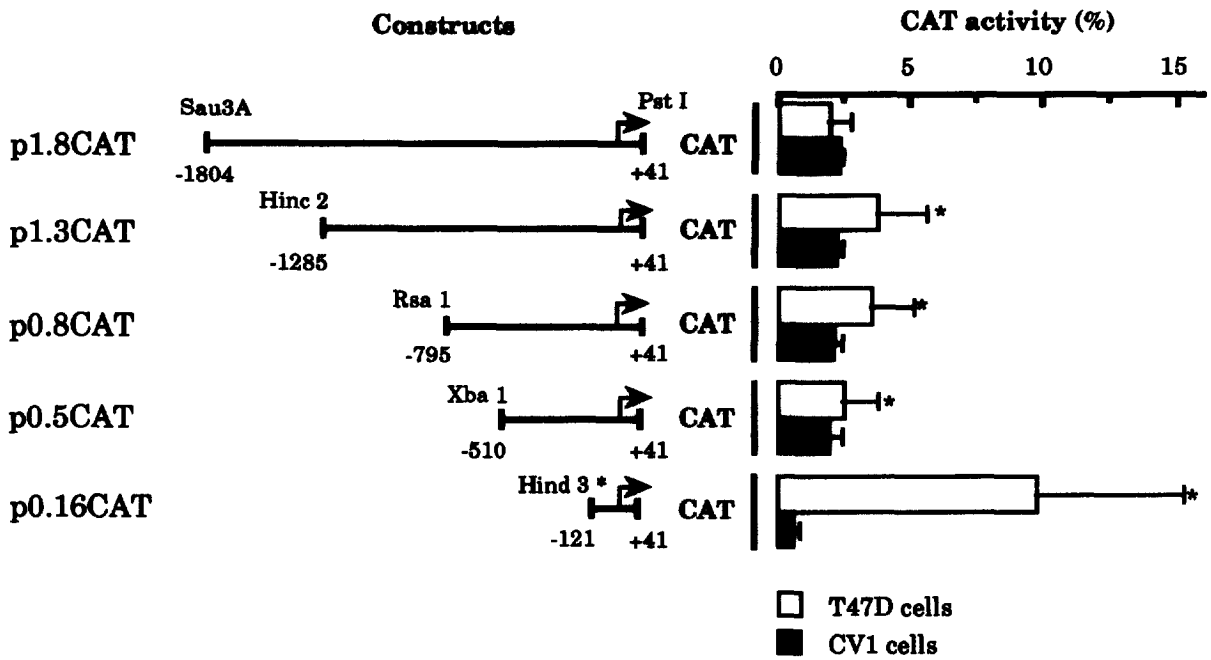

Fig. 1. Transient transfection anaylsis of MVDP promoter activity. Restriction fragments of MVDP 5′-flanking region were cloned upstream of the CAT reporter gene in pBLCAT3. Nucleotide numbering is according to the start site of transcription which is indicated by the arrow. CAT activity was determined in extracts of T47D and CV1 cells transfected 60 h earlier with 15 μg of the indicated constructs. CAT activity is shown as percent conversion from chloramphenicol to acetylated chloramphenicol. Values are means ± SEM for 4 independent experiments. *Significantly different from CV1 cells ($P < 0.05$).

Cells and transfection

Human mammary carcinoma cells, T47D, and monkey kidney cells, CV1, were used in gene transfer as they can regulate androgen-responsive genes following transfection. T47D and CV1 cells were grown in Dulbecco's modified Eagle's medium supplemented with glutamine (2 mM), insulin (4 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml) and 10% fetal calf serum. For each transfection experiment cells were seeded at a density of 1.6×10^6 cells/dish and transfected 24 h later with plasmid DNAs using the DEAE-dextran method [25]. Following a 1 h incubation with the DEAE-dextran/DNA precipitate and dimethyl sulfoxide shock, the cells were incubated in fresh Dulbecco's modified Eagle's medium supplemented with 10% steroid-free donor calf serum containing different hormones. Cells were harvested after 60 h of hormone exposure for CAT assays. Transient transfection of CV1 cells was performed as outlined for T47D cells except that the CV1 cells were transfected with the calcium phosphate/DNA precipitation method [26].

Vas deferens epithelial cells were cultured as previously described [27]. For each transfection experiment pure mouse vas deferens epithelial cells were seeded at a density of 1.8×10^6 cells per 100 mm plastic dish and transfected 24 h later with 15 μg MMTV-CAT vector by the DEAE-dextran method. An MMTV-CAT vector, which contains HRE_s activated by a variety of steroids [28], was used to detect the presence of steroid receptors in vas deferens epithelial cells. 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 allowed polarization of the cells. After polarization the cells were incubated for 72 h in fresh serum-free medium in the presence of progesterone, dexamethasone or DHT.

CAT assays

CAT activity of cell extracts was assayed according to the method of Neumann [29]. Average inductions and standard deviations were calculated from at least four independent transfections.

PCR-mediated of the androgen responsive element (ARE)

Mutation of the ARE (−111 to −97) contained in the MVDP promoter has been described previously [20].

RESULTS

Inter-species sequence homology of the 5′-flanking region

This analysis was conducted since sequences that are highly conserved are likely to have functional significance. A computer-assisted alignment of the sequences

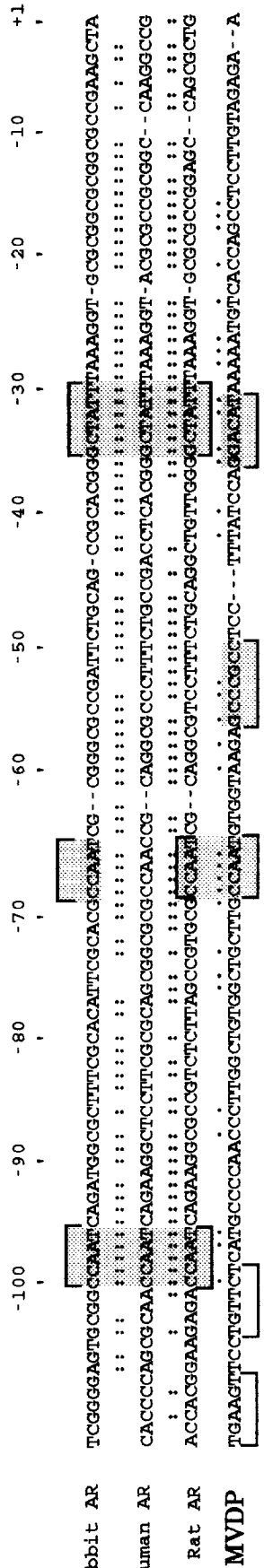

Fig. 2. Alignment of the 5′ sequences of human, rat and rabbit aldose reductase genes and MVDP gene.

of the 5′-flanking DNA of aldose reductase genes of rat [30], human [31], rabbit [32] and MVDP gene [20] has been established from the transcription start site to −609 (limits available for every species). No evident homology was seen between the 5′-flanking region of the MVDP gene and aldose reductase genes of other species. When rat sequences were compared with human and rabbit, the overall similarity was 54 and 49%, respectively; human and rabbit sequences display 69% homology. The best similarity was observed about 120 nt upstream the start site: 74 and 75% homology when rat sequences were compared to those of human and rabbit, and 80% between human and rabbit (Fig. 2). Despite the lack of recognizable sequence homology between MVDP and aldose reductase genes, computer analysis revealed many motifs reminiscent of reported consensus sequences for transcription factor binding sites. A TATA box and a CAAT box are located at about the same distance from the cap site in the four genes (−30 and −66, respectively). The TATA box sequence TATTTA is conserved in the three aldose reductase genes but not in the MVDP gene (CATAAA). The sequence CCAAT (−66 to −62), conserved in rabbit, rat, and mouse (CCAAC in human), may be part of the proximal promoter of these genes. A second CCAAT sequence is conserved at position −98 in human, rat and rabbit aldose reductase genes but not in the MVDP gene. The computer analyses also revealed potential AREs in the human aldose reductase gene (−396) [31] and in the MVDP gene (−1181; −111).

Identification of MVDP sequences responsible for androgen-stimulated expression in two different cell lines

Further evidence for a crucial role in basal and androgen-induced expression of 5′-flanking sequences of the MVDP gene was obtained by comparing MVDP-CAT construct expression in different cell types. To test the ability of MVDP sequences to direct androgen-stimulated expression, constructs containing 5′-flanking sequences from MVDP gene linked to the indicator gene CAT were transfected into two different cell lines that do not normally produce MVDP: T47D and CV1 cells. To study androgen regulation of the MVDP promoter, T47D and CV1 cells were cotransfected with each of the MVDP-CAT constructs and pSVARo in the absence and the presence of 10^{-6} M DHT. As shown in Fig. 1, in the absence of DHT all

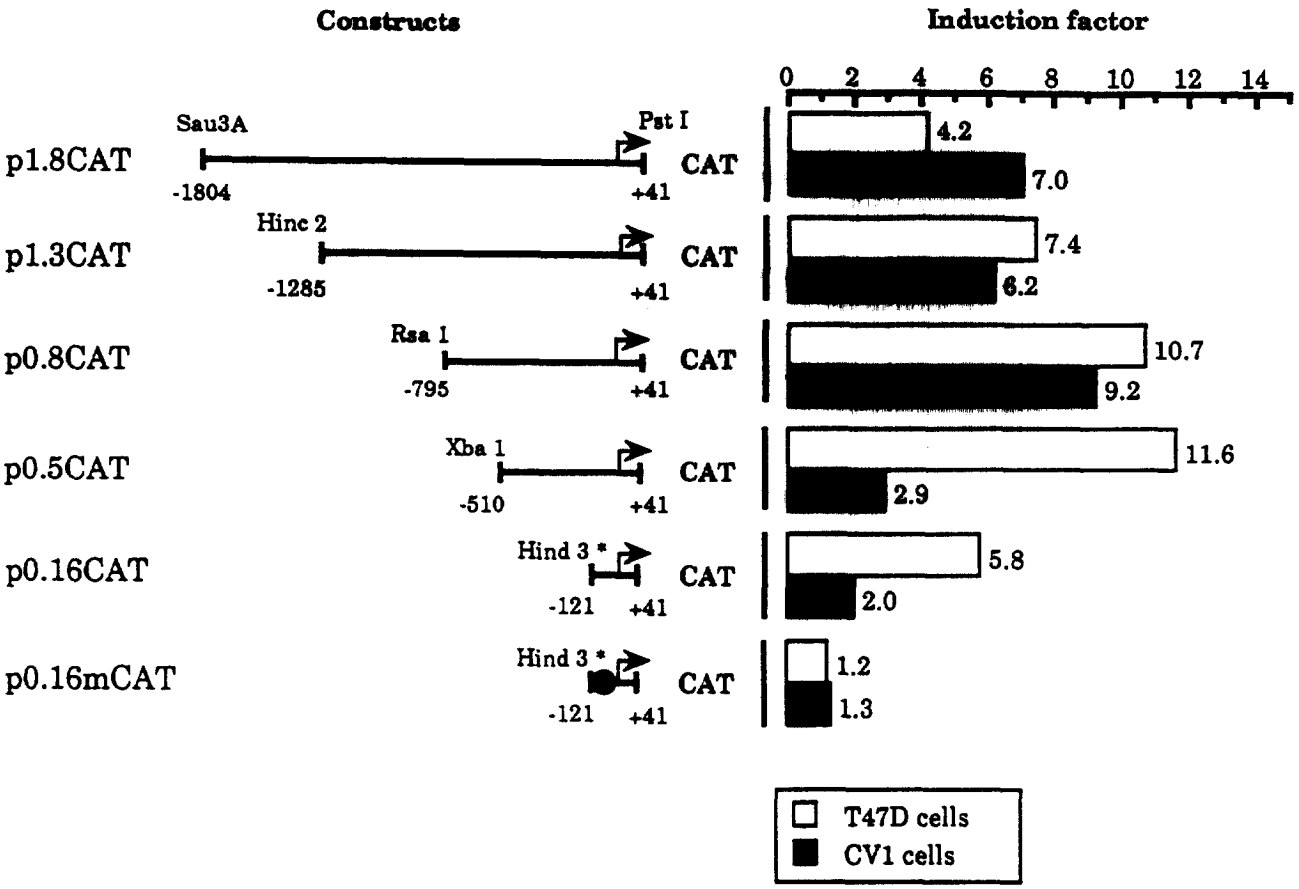

Fig. 3. Comparison of androgen-dependent enhancer activities of the 5′-flanking region in CV1 and T47D cells. CAT activity was determined in extracts of cells transfected 60 h earlier with different MVDP-CAT constructs (15 μg) and with the androgen receptor expression vector pSVARo (2.5 μg). Cells were grown in the presence of 10^{-6} M DHT. CAT activity was expressed as fold increase over basal level. In the construct p0.16mCAT, the ARE sequence (−111/−97) TGAAGTtccTGTTCT (p0.16CAT) was mutated to TGAAGTtccTTTTTT.

MVDP-CAT constructs transfected in CV1 and T47D cells supported detectable levels of expression of the CAT gene (from 1.5 to 10% acetylation), indicating that this is indeed a functional promoter. Deletions up to nt position −510 relative to the start site did not affect promoter activity in either cell line. Further deletion, to nt position −121, resulted in a loss in activity in CV1 cells, whereas basal promoter activity was enhanced in T47D cells. Apparently the region between −510 to −121 is important for basal expression of the MVDP promoter in both cell lines.

Good induction of CAT enzyme activity was seen with DHT in both cell lines that had been transfected with constructs containing 1.8 kb of the upstream region of the MVDP gene (Fig. 3). To localize the MVDP sequences required for induction by androgen, deletion mutants that removed successively larger segments of the 5′-flanking region were tested in both cell lines. As shown in Fig. 3, the ability to be induced by androgens was retained with the deletion of sequences to −121. Thus sequence elements located between nt −121 to +41 are sufficient to confer androgen-stimulated expression. Mutation of the ARE located at position −111 resulted in a loss of responsiveness of the construct in both cell lines.

Addition of DHT to the medium of cells transfected with constructs containing either 1.8, 1.3, and 0.8 kb of the upstream region of the MVDP gene resulted in similar fold increase in CAT activity in the T47D and CV1 cell homogenates. The region −1804 to −795 has a marked negative effect on the DHT-induced up-regulation of the promoter, without affecting basal activity in T47D cells. Deletion up to nt position −510 resulted in a strong decrease of androgen responsiveness in CV1 cells but not in T47D cells. The sequence spanning nt position −795 to −510 of the MVDP promoter thus appears to contain important element(s) needed for full androgen inducibility in CV1 cells. Further deletion up to nt position −121 induced an apparent decrease in androgen-induced CAT activity in T47D cells. However, the change in fold induction is artificially decreased by increased basal level expression (see above). When expressed as absolute level, CAT activity is similar for both 0.5 and 0.16 kb fragments (not shown). Finally, in cells transfected with constructs containing either 0.5 or 0.16 kb fragments of the 5′-flanking region of the MVDP gene, androgen-induced CAT activity was very much reduced in CV1 cells but not in T47D cells.

Specificity of the hormonal response

Since the proximal MVDP ARE is very similar to the glucocorticoid response element (GRE) and progesterone response element (PRE) consensus sequence [20], the effect of other hormones was tested to explore how the response of the MVDP upstream region to androgen

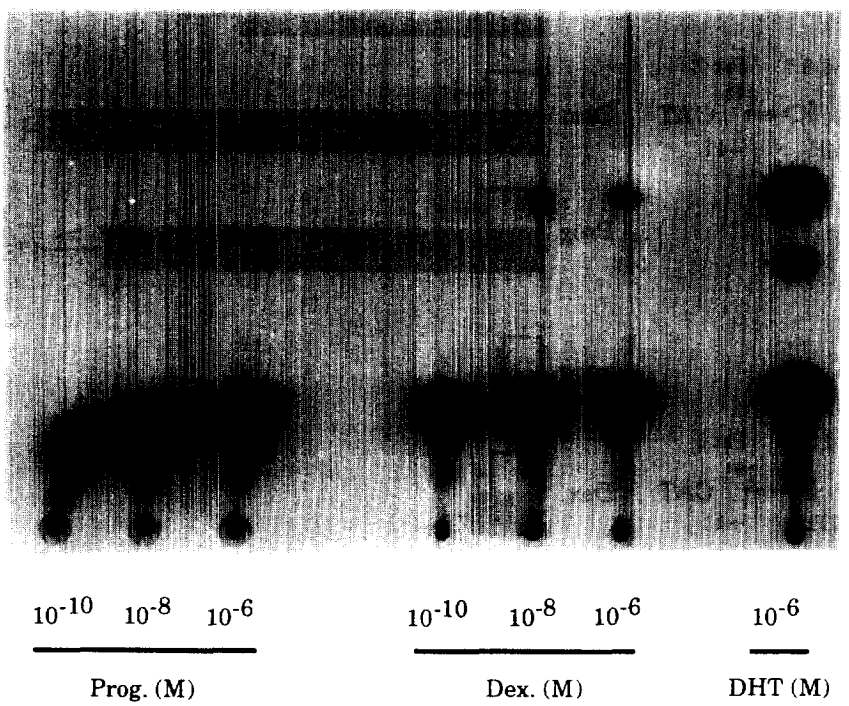

Fig. 4. Identification of functional steroid receptors in subcultured vas deferens epithelial cells. Vas deferens epithelial cells were seeded at a density of 1.8×10^6 cells per 100 mm plastic dish and transfected 24 h later with 15 μg MMTV-CAT vector by the DEAE-dextran method. Following 1 h incubation with the DEAE-dextran/DNA precipitate and DMSO shock, the cells were washed and incubated in fresh serum-free medium which allowed polarization of the cells. After polarization (72 h), the cells were incubated for 72 h in fresh serum-free medium with progesterone, dexamethasone and DHT at different concentrations. CAT activity of cells extracts was assayed.

was specific. First, the nature of steroid receptors present in vas deferens epithelial cells, which express MVDP *in vivo*, was determined. We transfected these cells with MMTV-CAT plasmid, the transcription of which can be induced by glucocorticoid, progesterone and androgen receptors [33]. As shown in Fig. 4, MMTV-CAT does not respond to progesterone concentrations up to 10^{-6} M, suggesting that vas deferens epithelial cells lack progesterone receptors. When the cells were grown in the presence of various concentrations of dexamethasone, CAT activity was induced in a dose-dependent manner, indicating the presence of functional glucocorticoid receptors in the cells. At the 10^{-6} M concentration, DHT stimulated a greater increase in CAT activity than did dexamethasone (Fig. 4). This suggests that androgen receptor concentration is higher than that of glucocorticoid receptor in vas deferens epithelial cells.

The ability of the 0.16 kb fragment of the MVDP gene to enhance androgen receptor as well as glucocorticoid and progesterone receptors transactivation, was tested in T47D cells. These cells contain low levels of androgen and glucocorticoid receptors [20, 28] and require cotransfection with expression vectors for the androgen receptor and glucocorticoid receptor before a response to their respective hormones could be elicited. On the contrary, since T47D cells have abundant progesterone receptors [28], the construct was tested for response to progesterone.

The addition of 10^{-6} M dexamethasone or progesterone to the growth medium of cells transfected with androgen responsive 0.16 kb fragment resulted in approx. 10- and 8-fold increase of CAT activity in the cell homogenates (Fig. 5). Here too, the simple mutation of the half-site of the ARE completely knocked the responsiveness of the construct to dexamethasone and progesterone. Then, MVDP ARE consensus confers glucocorticoid, progesterone and androgen induction after transfection in T47D cells. Since the ARE domain by itself did not confer androgen specificity, possible effects of additional elements were investigated. The ability of the 1.8, 0.5, and 0.16 kb fragments to enhance glucocorticoid receptor as well as androgen receptor transactivation was tested by cotransfection analysis in T47D cells cultured in the presence of physiological and pharmacological

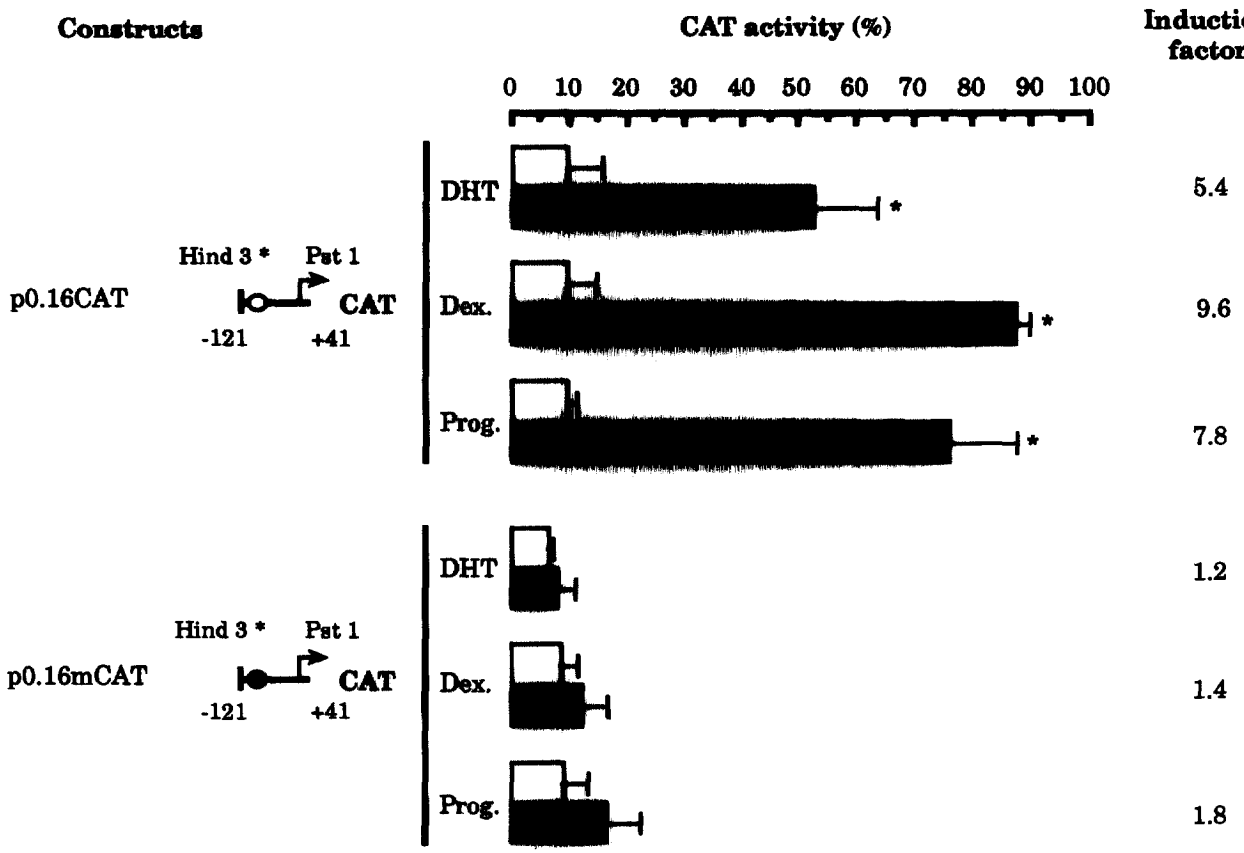

Fig. 5. Comparison of androgen-, glucocorticoid- and progesterone-dependent enhancer activities of minimal MVDP promoter. The T47D cells were cotransfected with 0.16 kb MVDP fragment (15 μg) and with the androgen receptor expression vector pSVARo (2.5 μg) or the glucocorticoid receptor HG4 (2.5 μg). Since T47D cells have abundant progesterone receptor, the construct was tested for response to progesterone without cotransfection of progesterone receptor expression vector. Cells were grown 60 h in the absence or presence of 10^{-6} M DHT, progesterone, or dexamethasone. The effects of three hormones on CAT activity were also determined for the p0.16mCAT construct (see Fig. 3). Values are means ± SEM ($n = 4$). *Significantly different from control ($P < 0.05$). Open histograms: without hormone. Solid histograms: with hormone.

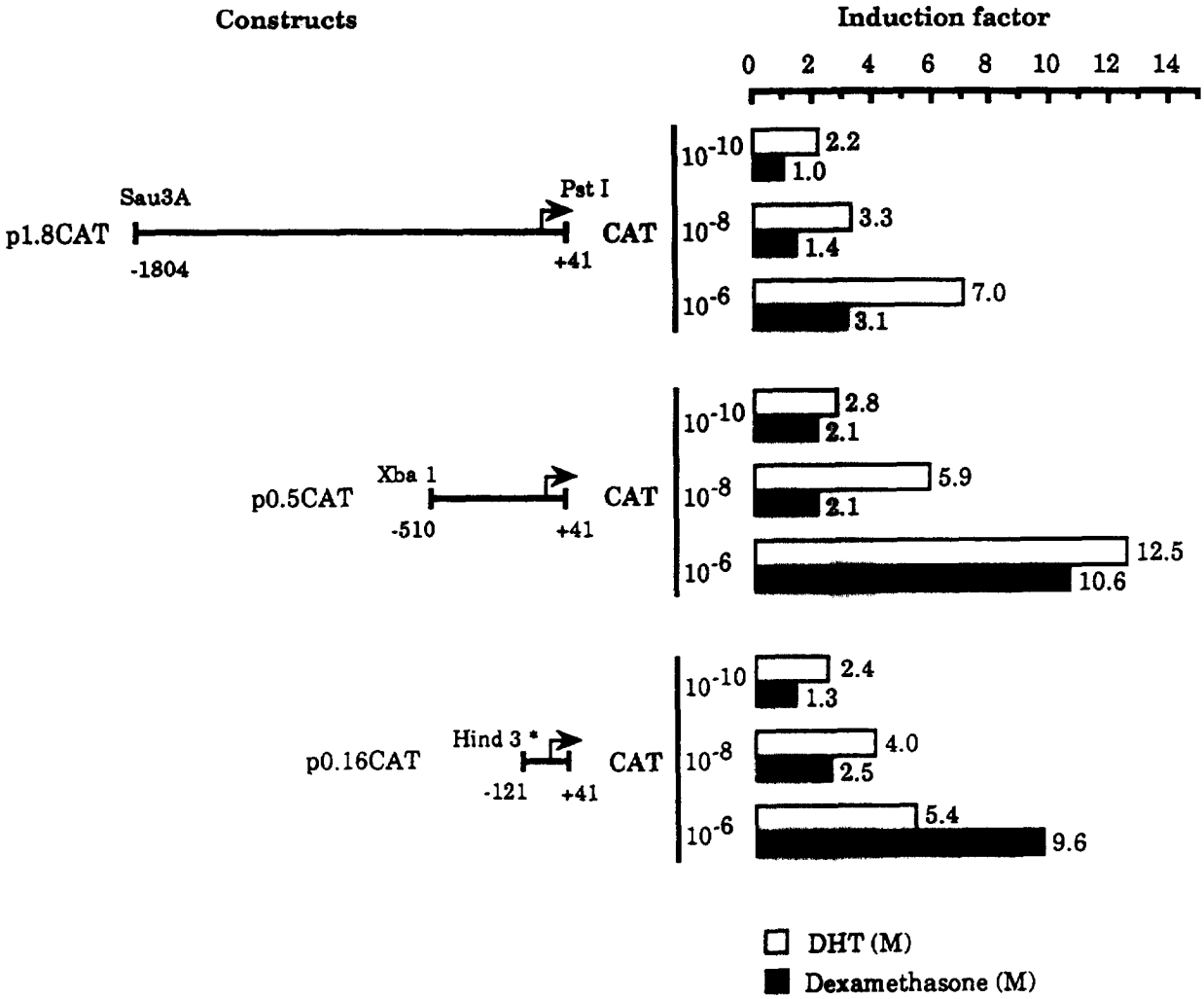

Fig. 6. Androgen and glucocorticoid dose–response induction of MVDP-CAT constructs. The T47D cells were cotransfected with different MVDP-CAT constructs (15 μg) with either androgen receptor and glucocorticoid receptor expression vectors (2.5 μg of each). Cells were grown (60 h) in the presence of increasing concentrations of DHT or dexamethasone.

concentrations of DHT and dexamethasone (Fig. 6). With the three fragments used, CAT activity increased in a dose-dependent manner with androgen receptor and DHT in concentrations up to 10^{-6} M. With glucocorticoid receptor and dexamethasone, the increases observed with physiological hormonal concentrations were not significant and always lower than those observed with DHT (Fig. 6). Only pharmacological concentrations (10^{-6} M) of dexamethasone induced CAT activity. These observations suggest that the ARE preferentially binds androgen receptors. The response to dexamethasone was maximal with 0.5 and 0.16 kb fragments indicating that other factors, interacting with sequences upstream of the 0.5 kb region, are implicated in androgen specificity.

Synergistic properties of the PKC-activating phorbol esters upon androgen-dependent transcription of the MVDP-CAT construct

In the MVDP gene, the 5′-flanking region contains putative response elements for the AP-1 family of transcription factors [20]. Accordingly, we investigated the effect of phorbol esters on androgen receptor-dependent transcription from the MVDP-CAT plasmid. T47D cells transfected with 1.8 kb MVDP-CAT construct and pSVARo, were grown in the presence of DHT and TPA alone or in combination. As shown in Fig. 7 basal CAT activity is not affected by TPA. The addition of DHT to the growth medium resulted in an approx. 4-fold increase in CAT activity. The response was increased an additional 5-fold by the presence of TPA.

DISCUSSION

The characterization of the human aldose reductase gene [14], the rat aldose reductase gene [30], the MVDP gene [13], and most recently the rabbit aldose reductase gene [32], has provided the opportunity to investigate the mechanisms involved in regulation of aldose reductase and related gene expression. These genes are homologous in structure but differ markedly

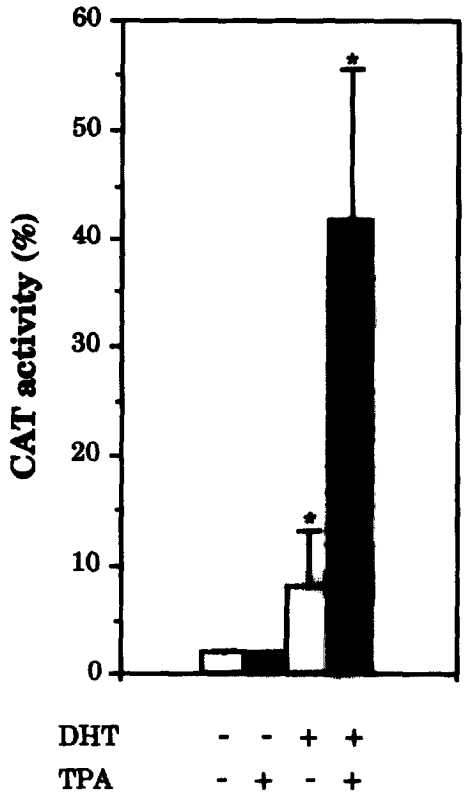

Fig. 7. Potentiation of the androgenic induction of transiently transfected MVDP-CAT construct by activation of PKC. The T47D cells were cotransfected with 1.8 kb MVDP fragment (15 μg) and androgen receptor expression vector (2.5 μg). Cells were grown in the absence or in presence of 10^{-6} M DHT alone or in combination with TPA (100 ng/ml). Values are means ± SEM ($n = 8$). *Significantly different ($P < 0.05$) from untreated cells and from cells treated with TPA alone or with DHT alone.

in their pattern of expression: whereas aldose reductases are widely distributed, MVDP is restricted to vas deferens epithelial cells. As a first step in the characterization of cis-acting elements responsible for specific expression and hormonal regulation, we compared the proximal 5′-flanking regions of the aldose reductase and MVDP genes, and searched for conserved motifs. A striking similarity occurs between human, rat and rabbit 5′-flanking sequences of aldose reductase genes extending over the entire region examined. When mouse (MVDP) sequences were compared to rat, human and rabbit aldose reductase 5′-flanking sequences, no significant homology was seen. Since tissue-specific expression of a number of genes is conferred by enhancer elements that have been localized in their vicinity [21], the differences observed between proximal 5′-flanking sequences of MVDP gene and those of other aldose reductase genes, may explain their different pattern of expression.

Promoters of the four genes contain a TATA box located at the same position. However, these four promoters differ in the sequence of their TATA boxes: for the MVDP gene the sequence is CATAAA, whereas for the three aldose reductase genes the sequence is TATTTA. It has been suggested that TATA box heterogeneity reflects differential interactions with distinctive TATA box-binding factors [34]. A putative CAAT box, which does not completely fit the consensus sequence, is present at the same position [−66] in MVDP, rat, human and rabbit aldose reductase genes. The CAAT family of transcription factors has been implicated in the expression of a number of steroid-regulated promoters [35] including the androgen-regulated gene for the C3 polypeptide of the prostatic steroid binding protein [36].

The 15 bp motif 5′-GGA/TACAnnnTGTTCT-3′ has been identified both upstream and downstream of androgen responsive genes, and has been functionally and structurally defined as an ARE [37, 20]. Two AREs have been delineated upstream of the MVDP gene at nucleotide positions −1186 and −111. Transcriptional regulation of the MVDP gene has been shown to occur via the proximal ARE [20]. Whether the expression of MVDP and aldose reductase genes from other species, which have a common evolutionary origin, is submitted to the same androgenic regulation is unknown. The presence of a putative ARE in the human aldose reductase promoter suggests the potential of androgenic control [31]. Aldose reductase is implicated in the pathogenesis of diabetic complications which do not occur until after puberty. It is also known that aldose reductases are expressed in male accessory sex organs [1]. Recently, it has been shown that the region between base pair −3429 and base pair −192, in the rabbit aldose reductase gene, contains putative osmotic response elements capable of conferring osmotic response to a heterologous promoter [32].

In both the cell lines used, promoter activity is obtained with fragments that span 1.8 kb of MVDP upstream sequence, as well as 1.3, 0.8, 0.5 and 0.16 kb fragments. Deletion between nucleotides at positions −510 and −121 resulted in a loss in basal promoter activity in CV1 cells, whereas it was enhanced in T47D cells. This suggests that the sequence between −510 and −121 can function as a negative transcription element in T47D cells, and as a positive transcription element in CV1 cells. Two potential recognition sequences showing 100% homology with the consensus for C/EBP and AP-2 regulatory factors are present in this fragment [20].

Androgen-dependent enhancer activities of the MVDP gene 5′-flanking region were compared in T47D and CV1 cells. Although some variations in the level of expression were seen with successive deletions, the ability to be induced by androgen was retained with deletion of sequences to −121. Thus, sequence elements located within 162 bp (−121 to +41) are sufficient to confer androgen-stimulated expression in both cell lines studied. Transient expression assays in CV1 cells provide confirmation of the presence of a functional cis-acting proximal ARE [20]. However,

because in CV1 cells the 0.16 kb enhancer by itself did not account for the induction attained with longer fragments, additional elements were sought. It was found that a more upstream region (−795 to −510) acts synergistically with the proximal ARE domain for maximal androgen inducibility in CV1 cells. In transfection assays in T47D cells, deletion of the MVDP 5′-flanking region resulted in a progressive increase in androgen inducibility. Thus, it appeared that some positive and negative factors differed in the two cell lines either qualitatively or quantitatively. This situation resembles that described for the sex-limited protein gene: a GRE consensus, upstream of the gene, is necessary for androgen inducibility, but additional elements modulate induction, positively or negatively and exhibit cell-specific behavior [38]. In the promoter of the prostate-specific antigen gene, in addition to the functional ARE, an upstream region, between −539 to −320, is also needed for optimal androgen responsiveness [39].

Taken together, these results suggest that the presence of other tissue-specific, as well as ubiquitous factors, which can bind to specific DNA sequences to cooperate with androgen receptor, are required for the androgen activation of gene expression. A 94 nucleotide long stretch of purines, interrupted by only 10 pyrimidine residues, is located at position −696 in the upstream region of MVDP gene [20]. A similar sequence exists in androgen-regulated genes such as those encoding the complex androgen-controled prostatic binding protein [40] and human androgen receptor [41]. This region can potentially adopt a Z-DNA conformation [42], and Z-DNA has been reported to be a preferential target for proteins acting on transcriptional events [43].

An ARE consensus element, located at position −111 in the MVDP gene, confers glucocorticoid, progesterone and androgen induction in transfection assays. Mutation of the half-site of the ARE completely abolished hormonal inducibility. However, in dose–response experiments comparing androgens to glucocorticoids, androgens preferentially induced CAT activity at concentrations between 10^{-10} and 10^{-8} M. Dexamethasone also stimulated CAT activity but only at concentration of 10^{-6} M. The transcription activation observed with dexamethasone is probably mediated by glucocorticoid receptor. However, an action via mineralocorticoid receptor, for which dexamethasone has relatively low affinity, cannot be excluded because the presence of a functional mineralocorticoid receptor has been demonstrated in the human prostate cancer cell LNCaP [44]. Thus, transcriptional activation by androgen of the MVDP-CAT construct was androgen-specific only at physiological hormonal concentrations.

The region −1.8 to −0.5 kb in the MVDP promoter has a marked inhibitory effect on the dexamethasone-induced up-regulation of the promoter, suggesting the requirement of additional DNA sequences for the specific gene expression. Glucocorticoids, progestins and androgens differ in their physiological effects. However, the varied effects of these hormones are mediated by receptor proteins that are closely related and that appear to recognize a common DNA binding sequence [45, 46]. Glucocorticoids, progestins and androgens can all induce a reporter gene via the consensus glucocorticoid response element sequence (GGTACAnnn-TGTTCT) in transfection assays [33, 46]. Thus, the specificity and complexity of hormone effects must be conferred by other means.

Several explanations have been suggested: (1) a given target cell might express only one of the closely related receptors; (2) participation of cis-acting sequences distinct from the common DNA element; (3) the receptors might cooperate differentially with other factors. In support of (1) it has been demonstrated that an endogenous glucocorticoid-responsive gene, as well as a transfected reporter, could be rendered responsive to progestin by cotransfected progesterone receptor in hepatoma cells [47]. Thus, *in vivo*, the MVDP gene may be unresponsive to progestins solely because vas deferens epithelial cells do not express the appropriate receptor. Present results (Fig. 4) showed that androgen receptor concentration is higher than that of glucocorticoid receptor in cultured vas deferens epithelial cells, suggesting that this difference can account for the androgen specificity observed in *in vivo* experiments [9]. Differences in the concentration of glucocorticoid and androgen receptors cannot account for the androgen preference observed in our experiments in T47D cells since, in each case, the appropriate expression vector was cotransfected with MVDP-CAT constructs. A similar situation has been described for the androgen-regulated probasin gene: probasin-CAT gene expression was more effectively induced by androgens than by glucocorticoids or progestins [48]. Similar observations have been reported for androgen-regulated genes such as those encoding human prostate-specific kallikrein [49] and 20 kDa protein of rat ventral prostate [50].

These results suggest that transcriptional specificity could be determined, at least in part, by subtle sequence differences in hormone responsive elements. However, a few native androgen-dependent elements have been characterized independently of other enhancer sequences to conclude that androgen specificity resides exclusively in the AREs. An enhancer that is activated by androgens but not by glucocorticoids has been characterized in the upstream region of the mouse sex-limited protein [38, 51]. This stringent hormonal control does not reside in the androgen receptor binding site itself but in adjacent sequences within a 120 bp DNA fragment [51, 52]. Thus it appears that the specific transcriptional response to androgens may result from combined actions of different elements including intrinsic properties of AREs, and cell-type dependent interactions with other factors via regions distinct from the androgen receptor binding domain. Alternatively, a given target cell might metabolically

inactivate one of the hormonal ligands. For example, in the kidney collecting tubule, which contains both glucocorticoid and mineralocorticoid receptors, glucocorticoids but not mineralocorticoids are converted to inactive metabolites by 11β-hydroxysteroid dehydrogenase [53]. It has also been suggested that this enzyme may have a role in tissue specificity of androgen action in LNCaP cells [44].

The present results also show that phorbol esters strongly enhanced androgen-dependent activity of the MVDP promoter, suggesting interaction between the PKC pathway and androgen receptor-mediated signaling. Evidence has been presented for interaction between PKC and glucocorticoid receptor signaling pathways [23, 54] but, to our knowledge, in no instance has a cellular androgen-regulated gene been demonstrated to respond to PKC activation. As the androgen receptor, like other steroid receptors, is a phosphoprotein, regulation of its phosphorylation state could be the link between androgen receptor and PKC signaling pathways as demonstrated for estrogen receptor [55]. Alternatively the AP-1 complex, the effector molecule of the PKC pathway, could enhance MVDP gene transcription by binding to specific response elements [reviewed in 56].

Whether the expression of other members of the aldose reductase superfamily is subject to the same regulation as MVDP is as yet unknown.

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