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Hormonal Regulation of the Androgen Receptor Expression in Human Prostatic Cells in Culture

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The regulation of the androgen receptor (AR) expression was studied using immunocytochemical and Western blot techniques on separate cultures of epithelial cells (PNT2) and fibroblasts of human prostate. In both cell types, immunocytochemistry revealed both nuclear and cytoplasmic staining. Treatment with DHT (5×10^{-9} M) increased both the intensity of nuclear staining and the number of cells stained. The increase, observed after DHT treatment was markedly decreased by cyproterone acetate (5×10^{-7} M), confirming a direct action of DHT via the AR. This autoregulation of AR was confirmed by Western blot, and seems to involve transcription and protein synthesis, since it was suppressed by actinomycin D and cycloheximide. In fibroblasts, known to contain an estrogen receptor, estradiol treatment (5×10^{-7} M) also increases the AR immunostaining. In addition, coculture studies show that epithelial cells require the presence of fibroblasts for optimal expression of the AR. These results demonstrate that prostate epithelial cells and fibroblasts have retained in culture, an hormonal sensitivity correlated with the presence of specific receptors and can serve as a model for the study of hormone action in this tissue in normal or pathological conditions. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

The prostate gland depends on androgens for its development and the maintenance of its differentiated structures and secretory functions. Little is known, however, about the precise mechanisms by which androgens exert these actions, except for the fact that they are mediated via the androgen receptor (AR). The AR belongs to the steroid/thyroid receptor superfamily [1] and as such acts as a ligand-activated transcription factor in the nuclei of androgen-sensitive cells. Any factor regulating the AR expression may therefore modify the action of androgens on prostate tissue. Other steroid receptors have been shown to be regulated by their cognate ligand, a process referred to as "autoregulation". As concerns the AR, although there is ample evidence of androgens controlling the expression of their own receptor, contradictory results have been reported: down-regulation of the mRNA and up-regulation of the protein [2–5], up-regulation of both mRNA and protein [6], or no effect on the protein [7]. Prins *et al.* have shown that in the rat prostate, AR regulation was dependent on the prostate lobe and/or the age of the animals [8, 9]. In addition, other factors may contribute to AR regulation. A better knowledge of AR regulation in target tissues should lead to a better understanding of androgen action in these tissues.

To gain some insights in this question, we have studied the AR regulation in separate cultures of epithelial and stromal cells from human prostate. We show, in this study, that androgens stimulate the expression of the AR in both epithelial and stromal cells. In addition, our data suggest a paracrine regulation of AR expression in epithelial cells by a diffusible factor secreted by the stroma. These regulations may play a significant role in modulating the prostate tissue sensitivity to androgens. Any alterations in this process may result in the imbalance in hormone sensitivity which in aging men leads to prostate pathology.

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MATERIALS AND METHODS

Materials

Tissue culture media and reagents were purchased from Life Technologies (France). Fetal calf serum (FCS) was from Dutscher (France) and penicillin and streptomycin were from Assistance Publique-Paris and Sigma respectively. 5α-dihydrotestosterone (DHT), and estradiol (E_2), were purchased from Sigma. Cyproterone acetate (CPA) was kindly provided by Schering AG (France), 4-hydroxytamoxifen (4-OHTam) was obtained from ICI.

Antibodies

The first antibody was the rabbit polyclonal anti-rAR N27 antibody [10] and was a generous gift of Dr Chang (Madison, WI). We have previously shown that this antibody specifically stains the AR in human prostatic epithelial and stromal cells [11]. The second antibody, peroxydase-labelled goat anti-rabbit IgG was from Diagnostics Pasteur, France.

Cell cultures

The epithelial cells used in this study (PNT2) are derived from normal human prostatic epithelium and have been immortalized by transfection of the large SV40 T antigen [12].

Hyperplastic human prostate tissue was obtained, with informed consent, from patients undergoing transurethral resection of the prostate for benign prostatic hyperplasia (BPH). Fibroblasts (FPH) were derived from explants, as previously described [13]. Fibroblasts and epithelial cells have been characterized by the expression of vimentin and cytokeratin KL1 respectively and testosterone metabolism has been characterized in each cell type [14].

The cells were maintained in medium RPMI 1640 supplemented with 8% FCS, glutamine (4 mM), penicillin (100 IU/ml), streptomycin (100 μg/ml). For epithelial cells, this basal medium was supplemented with hydrocortisone 2.3×10^{-7} g/l, bovine insulin 10^{-2} g/l, spermine 1.7×10^{-4} g/l, cholera toxin 10^{-4} g/l, *O*-phosphoethanolamine-$2H^+$ 1.4×10^{-4} g/l, bovine albumin 10^{-2} g/l (Sigma), EGF 2×10^{-6} g/l (Boehringer). Cells were maintained at 37°C in a humid atmosphere of 95% air/5% CO2 and were routinely subcultured by trypsinization every week (1/10 for PNT2; 1/6 for FPH).

Hormonal treatments

All studies were carried out on subconfluent cells, 6 days after subculture. Before all experiments, cells were placed in serum free medium for 24 h, to achieve deprivation of steroid hormones and growth factors. Hormonal treatments were then applied for various amounts of time and cells were collected by trypsinization. Nuclear proteins were extracted for Western blot analysis. For immunocytochemical analysis, cells were cytocentrifuged and fixed on slides as described below.

Immunocytochemical studies of hAR

Treated or control untreated cells were harvested with trypsin–EDTA (Life Technologies) and resuspended at the final concentration of 250 000 cells/ml in PBS (154 mM NaCl, 3.2 mM Na_2HPO_4, 2.7 mM KCl, 1.2 mM KH_2PO_4). 200 μl aliquots of the cell suspension were spun in a cytospin 2 Device (Shandon, Eragny) for 8 min at 400 rpm. The slides were immediately fixed in 3.7% formaldehyde for 20 min, and permeabilized in 0.2% Triton X100 (Prolabo) for 4 min. These slides can thereafter be stored at −20°C in a preservative solution (42.3 g saccharose (Prolabo), 0.33 g anhydrous $MgCl_2$, 250 ml glycerol (Sigma), PBS qsp 500 ml) until use and for no more than one month.

For immunocytochemical analysis of the AR, the slides were first incubated with 3% H_2O_2 (Merck) for 10 min to eliminate endogenous peroxidase, and blocked with horse serum (1/20^e in PBS) for 30 min. The slides were then incubated with the polyclonal N27 antibody (21 μg/ml horse serum 1/50^e in PBS) overnight at room temperature in a humidified chamber. The next day, the slides were incubated with the peroxidase-labelled bridging goat anti-rabbit IgG (20 μg/ml horse serum 1/50^e in PBS) for 30 min at room temperature. The cell areas were covered with 0.7 mg/ml diaminobenzidine-2 mg/ml H_2O_2 (Fast DAB tablets, Sigma) as a chromogen for 10 min in the dark. The staining was amplified by a bath in 5% cupric sulfate (Sigma) for 10 min and cells were counterstained with Harris hematoxylin (Reactifs RAL, Paris). In the negative controls, the primary antibody was omitted and replaced by horse serum 1/50^e in PBS.

After sequential dehydration with ethanol (Carlo Erba) and cleaning with toluene (Prolabo), the slides were mounted with Entellan (Merck) and representative sections were photographed at a 200-fold magnification.

Androgen receptor staining was appreciated as follow: slides were put on a grid and a total of 500 cells were counted on 3 different area. Cells were divided into negative, weakly stained, strongly stained. The number of strongly stained cells was counted and expressed as % of total.

Effect of coculture on AR immunocytochemical staining

For each experiment, fibroblasts, originating from one dish, were plated at D_0 in the appropriate number of 100 mm diameter culture dishes (3×10^4 cells per dish; Falcon). From D_5 to D_6, fibroblasts were cultured in the epithelial cell culture medium. At D_6, epithelial cells, originating from one 75 cm^3 flask, were plated in the appropriate number of tissue culture inserts (3×10^5 cells per insert; two inserts for

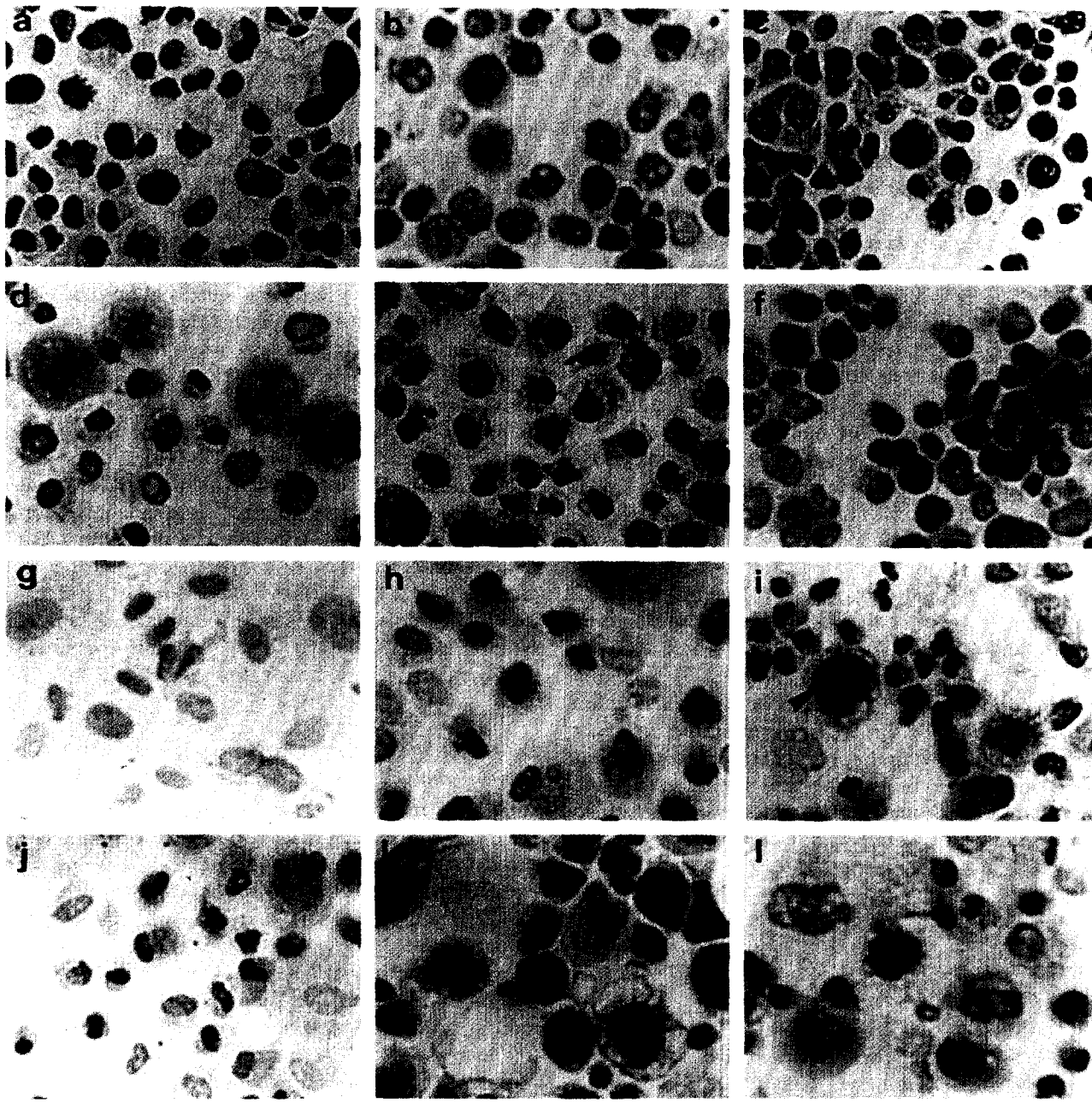

Fig. 1. Effects of hormonal treatments or coculture on AR levels in human prostatic cells in culture. After 24 h in serum-free medium, cells were treated as indicated, before harvesting, cytocentrifugation and fixation as described in Section 2. This figure illustrates typical results obtained reproducibly in repeated (5–10) experiments. (a–f) epithelial cells (PNT2). (a) non immune serum. (b) untreated cells. (c) epithelial cells cultured in presence of fibroblasts. (d) DHT: 5×10^{-9} M, 2 h. (e) DHT: 5×10^{-9} M, 6 h. (f) DHT: 5×10^{-9} M + CPA: 5×10^{-7} M, 6 h. (g–l) fibroblasts (FPH). (g) non immune serum. (h) untreated cells. (i) DHT: 5×10^{-9} M, 4 h, the arrow points to a perinuclear cistern. (j) DHT: 5×10^{-9} M + CPA: 5×10^{-7} M, 4 h. (k) E_2: 5×10^{-7} M, 24 h. (l) E_2: 5×10^{-7} M + 4 – OHTam: 5×10^{-7} M, 24 h.

one fibroblast dish; NUNC). The controls (fibroblasts or epithelial cells cultured alone) were also plated at D_6 and cultured in the same conditions than cocultured cells. D6 represents the time t_0 of coculture. After 48 h of coculture, epithelial cells were harvested with trypsin–EDTA, washed twice with PBS, cytocentrifuged and stained as described above.

Western blot analysis

Western blots were performed on nuclear proteins. Cells were harvested with trypsin–EDTA and lysed in NP-40 lysis buffer (10 mM Tris–HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$ and 0,5% NP-40) at 0°C for 5 min. After centrifugation (5 min, 2500 rpm,

4°C) nuclear proteins were extracted from nuclei in warm (85°C) SDS loading buffer (50 mM Tris, 2% SDS, 10% glycerol, 100 mM dithiothreitol pH 6.8, Sigma) [15], immediately boiled for 10 min and sonicated (4 × 30 s). Tubes were then centrifuged 10 min at 12 000 rpm at room temperature. The supernatant, containing the nuclear proteins was stored at −80°C until use.

Nuclear proteins were assayed in the supernatant by the Lowry method [16] using Sigma procedure No. P5656. Samples (200 μg nuclear protein per lane) were loaded on an SDS–polyacrylamide gel (4% stacking gel, 8% resolving gel) and run for 1 h at 25 mA, then for 3 h at 50 mA. After electrophoresis, gels were blotted onto Immobilon-P membranes (Millipore) for immunoblot analysis.

Non specific binding sites were blocked with 10% skimmed milk (Gloria, France), plus 1% goat serum in TBS buffer (50 mM Tris pH 7.5, 150 mM NaCl). Membranes were incubated first with the N27-antibody (0.8 μg/ml) for 18 h at 4°C, then with the peroxydase-labeled 2nd antibody, (0.3 μg/ml) in TBS supplemented with 1% goat serum, 1% skimmed milk and 0.1% Tween 20 (Sigma), for 1 h at 4°C.

Each of these two steps was followed by a 30 min wash in TBS containing 0.2% Tween 20. Detection was performed with the ECL chemiluminescence kit (Amersham) following the manufacturer's instructions and exposed to a Fuji X-ray film for times ranging from 10 min to 4 hours. Immunoblots were quantified by the Shimadzu CS-930 scanner.

RESULTS

Immunocytochemical study of AR expression in cultured human epithelial and stromal prostatic cells

In untreated cells, both epithelial and stromal, about 75% of the cells were marked and staining was cytoplasmic and nuclear (Fig. 1(b) and (h)). Treatment of the cells with DHT (5×10^{-9} M) increased both the number of cells stained and the intensity of staining. In addition, staining became predominantly nuclear with the appearance of perinuclear cisterns indicative of protein synthesis (Fig. 1 (i), arrow). When the first antibody was replaced by non immune serum, only the blue countercoloration was visible confirming the specificity of the reaction (Fig. 1(a) and (g)).

Positive autoregulation of hAR expression by DHT: Immunocytochemical studies

The increase in hAR staining observed in the presence of DHT is both time- and dose-dependent in both cell types.

The dose-dependence was studied in both cell types with similar results, with DHT concentrations ranging from 2×10^{-9} M to 10^{-7} M: staining was already increased in the presence of 2×10^{-9} M of DHT and was maximal with 5×10^{-9} M. It remained stable with the higher doses tested.

PNT2 were treated with DHT (5×10^{-9} M) for periods of time ranging from 2 to 48 h. After 2 h of treatment, the increase in staining intensity was mainly seen in the cytoplasm (Fig. 1(d)); the staining increased thereafter and became predominantly nuclear, with a maximum being observed after 6 h of treatment (Fig. 1(e)), and remained stable up to 48 h. A similar pattern was observed in fibroblasts (increase first in the cytoplasm, then in the nucleus) but the maximal staining was reached in 4 h (Fig. 1(i)) and began to decrease after 15 h. After 48 h, the staining was similar to that of untreated plates (not shown), probably due to the high level of androgen metabolism in these cells [14].

To confirm the involvement of the AR in its own regulation, cells were treated simultaneously for 4 (fibroblasts) or 6 (PNT2) hours with DHT (5×10^{-9} M) and the antiandrogen cyproterone acetate (CPA, 5×10^{-7} M). As expected, CPA inhibited the DHT induced increase in staining in both cell types (Fig. 1(f) and (j)). The quantitative evaluation of these data is shown on Table 1.

Positive autoregulation of hAR expression by DHT: Western blot analysis

To confirm that the increased staining observed in the presence of DHT was indeed due to increased protein content of the cells, nuclear proteins were extracted from PNT2 and fibroblasts before or after DHT treatment (15 h) and analyzed on Western blots. Figure 2 shows on a typical experiment, the increased intensity of the 110 kDa band representative of the AR. On five separate western blots, with nuclear proteins originating from different extractions, this increase varied from 6 to 12 fold, but was always observed. The DHT induced increase of AR was suppressed by actinomycin D (1 μg/ml, 6 h) or cycloheximide (15 μg/ml, 6 h) (Fig. 3) confirming that it

Table 1. Positive autoregulation of hAR expression in human prostatic cells in culture. Cells were treated for the indicated time with DHT (5×10^{-9} M) alone or together with CPA (5×10^{-7} M). Each value represents the % of strongly stained cells (mean ± SEM of 5–10 separate experiments)

PNT2	NS	48 ± 1
	DHT 6 h	61 ± 1.5*
	DHT 24 h	58 ± 2.5*
	DHT + CPA 6 h	34 ± 2.3**
FPH	NS	39 ± 1.4
	DHT 4 h	56 ± 2.8*
	DHT 24 h	45 ± 3.6
	DHT + CPA 4 h	39 ± 1.7**

*significantly different from control $p < 0.05$ (Student's t test).
**significantly different from DHT treated cells $p < 0.05$.

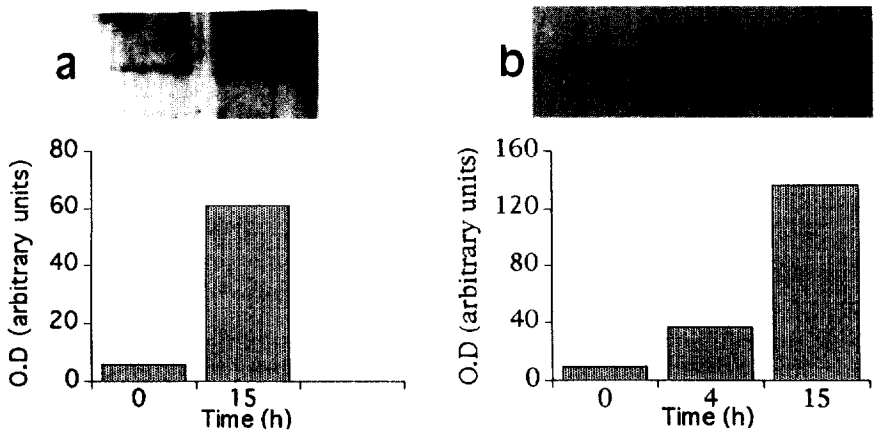

Fig. 2. Western blot analysis of AR in epithelial cells (a) and fibroblasts (b) from human prostate. Cells were treated for the indicated times with DHT (5×10^{-9} M) and nuclear proteins were extracted before SDS-PAGE electrophoresis. Exposition was for 15 min for epithelial cells and 3.5 h for fibroblasts. The lower part of the figure represents the scan of the bands shown above.

implies both transcriptional activation and protein synthesis.

It thus appears that androgens are capable of positively regulating their own receptor concentration in both cultured human prostatic epithelial cells and fibroblasts, by a process mediated by the AR and involving transcriptional activation and protein synthesis.

Estradiol regulation of AR

Because estrogens are suspected to play a role in the development of benign prostatic hyperplasia, we have studied the effect of estradiol on the AR expression in cultured human prostatic cells. When PNT2 were treated with estradiol (5×10^{-7} M) for 6 or 24 h, no effect on AR expression was observed (not shown). In contrast, in the same conditions, estradiol induced a strong increase in AR staining in fibroblasts (Fig. 1(k)): the % of strongly stained cells increased from 39 ± 1.4 to 54 ± 2.5 ($n = 4$). This effect was greatly decreased in the presence of the antiestrogen 4-hydroxytamoxifen (4-OH Tam) confirming that it was specific and involved the presence of a functional estrogen receptor.

Effect of coculture of fibroblasts with epithelial cells: Evidence for stromal/epithelial interactions

We have previously shown that the androgen binding capacity of prostatic epithelial cells was greatly increased when these cells were cocultured with fibroblasts [11]. We therefore tested whether a similar increase in immunostaining could be observed in the same coculture conditions. As shown in Fig. 1(c), the AR nuclear staining was greatly increased in epithelial cells grown in the presence of fibroblasts, as compared to control non stimulated cells (Fig. 1(b)).

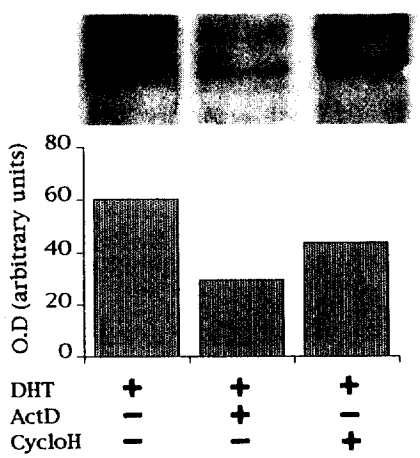

Fig. 3. Effect of actinomycin D (ActD) and cycloheximide (CycloH) on DHT induced AR expression. Epithelial cells were treated for 6 h with DHT (5×10^{-9} M) ± ActD (1 μg/ml) or CycloH (15 μg/ml). Nuclear proteins were extracted for Western blot analysis.

DISCUSSION

In the prostate gland, as in all androgen target tissues, the first step in androgen action is the binding to the AR, a member of the steroid/thyroid nuclear receptor superfamily [1], which thereafter activates the transcription of specific genes. Mechanisms regulating the AR concentration in target tissues are therefore essential to maintain and control the sensitivity of these tissues to androgens. However these mechanisms are, as yet, but poorly understood and contradictory data have been reported, in particular, concerning the effect of androgens themselves on their cognate receptor. We have studied the effect of androgens on the AR expression in human prostatic cells in culture. We show that androgen treatment of these cells result in an increase in AR protein concentration, both in immunocytochemical and Western blot studies, and that this effect is suppressed by actinomycin D and cycloheximide indicating a mechanism involving both transcription and protein

synthesis. In addition, we show, using coculture studies, that epithelial cells require the presence of fibroblasts for optimal expression of the AR.

Cell culture provides a useful tool for the study of hormone action since defined conditions can be used. This is especially pertinent to the study of the prostate, a complex organ composed of two compartments, glandular epithelium and stroma, both androgen target tissues; the interactions between these two compartments are known to be essential for the development and the maintenance of the prostate [17]. We have used epithelial cells obtained from normal prostate and immortalized by transfection of the large T SV40 antigen. These cells are indeed not normal and if they have retained most of the characteristics of prostatic epithelial cells [12], they also present some of the genetic alterations described in prostatic tumors. However, they do not induce the formation of tumors when injected into nude mice [18] and, in contrast to normal epithelial cells, they can be easily subcultured, allowing the production of sufficient material for reproducible studies. Fibroblasts have been derived from the periurethral zone of hyperplastic prostates, known to be involved in the development of BPH [19]. We have previously characterized the cell strains used as concerns their expression of vimentin and keratin to establish their stromal or epithelial nature and have shown that human epithelial and stromal cells in culture maintain their main metabolic characteristics [14].

For immunological characterization of the AR, we have used a polyclonal antibody made against the rat AR, which cross-reacts with the human AR [10]. We have previously shown that the 110 kDa band, characteristic of the AR protein disappears when the antibody is replaced by non-immune serum and that the intensity of the band obtained is proportional to the amount of protein present, confirming the specificity of this antibody [11].

Surprisingly, a commercially available monoclonal antihuman AR antibody (clone F39.4.1), recognizing an epitope localized on the N-terminal end of the AR (AA 301-320) did not stain the AR in PNT2 cells, while it stains efficiently frozen tissue sections of prostate [20] and human genital skin fibroblasts (our unpublished results). We wondered whether the immortalization process or the prolonged time in culture could have produced a mutation of the AR gene, either as has been described in the LNCaP androgen-dependent cell line [21], or resulting in a conformational change of the protein. However, using SSCP analysis, we were unable to detect any mutation in the exons 2 to 8 of the AR gene in these cells (unpublished data). Another possibility could be that interaction with specific protein(s) of the prostate cells could conceal the epitope recognized by this antibody. At any rate, the polyclonal antibody has been used throughout these studies.

We have studied both subcellular and tissular localization of AR expression. As concerns the cellular localization of the AR, we show that in the absence of hormone it is both cytoplasmic and nuclear in both cell types. The nuclear staining increases in the presence of DHT. Whereas immunocytochemical studies have clearly shown that the estradiol and the progesterone receptors were nuclear, even in the absence of hormones [22, 23], discussions are still ongoing concerning the glucocorticoid receptor (GR) and the AR. Concerning the GR, some studies have shown that it was cytoplasmic in the absence of hormone [24] but Brink *et al.* have suggested that the cytoplasmic localization was an artefact due to fixation techniques [25]. As concerns the AR, Jenster et al have shown that its cellular localization in the absence of hormone was cell specific [26]. At any rate, the AR, like all nuclear receptors, contains two nuclear localization signals [26, 27] and is probably subject to cell-trafficking as has been shown for the progesterone receptor [28].

As concerns the tissue localization of the AR in the prostate, our data suggest that it is expressed both in the stroma and in the epithelium, and that the optimal expression in the epithelium requires the presence of stromal cells. In most developing tissues of the urogenital tract, including the prostate, the AR is first expressed in the mesenchyme and its expression seems to be induced by androgens [17, 29]. The expression of the AR in the epithelium appears only after birth [30] and is optimal in mature epithelium while it decreases in the mesenchyme derived tissue. This is particularly true in the rat prostate and Prins *et al.* [9] have proposed that in adult rat prostate, the AR, in the epithelium, exerted a modulatory effect on stromal growth thus explaining why the rat never develops prostatic hyperplasia. To support this hypothesis, these authors have shown that in the dog, where prostatic hyperplasia is a constant feature of aging, the AR remained confined to the stroma throughout life [9]. The human prostate could follow an intermediary pattern: immunostaining of fixed frozen sections of adult prostate tissue revealed a predominant staining in the nuclei of epithelial cells with a very weak staining in the stroma [31, 32]. However, our previous study in separate cultures of fibroblasts and epithelial cells, have shown that the AR binding capacity was higher in fibroblasts than in epithelial cells [11] and the present study shows a similar immunostaining in fibroblasts and epithelial cells. This discrepancy between cultured cells and tissue frozen sections can perhaps be explained by the disruption, in cell cultures, of the normal architecture of prostate. This would suppress the interactions between the stromal and epithelial compartments which are necessary to maintain the normal function of the prostate tissue. Isolated fibroblasts, not submitted to an inhibitory effect from the epithelium

resume an "immature-like" expression of the AR. A similar effect has been shown in adult rat penis smooth-muscle cells which in culture have a high level of AR expression in contrast to a low level *in vivo* [33]. Similarly a growth inhibiting effect of prostate epithelial cells on stromal cells has been reported [34]. Our coculture data show that the expression of the AR in epithelial cells is optimal only in the presence of stromal cells, studied as binding capacity [11] or immunostaining (present study). They suggest the existence of diffusible factor(s), secreted by fibroblasts and acting on epithelial cells, to modulate AR expression, and emphasize the importance of stromal-epithelial interactions in androgen mechanism of action in the prostate.

The regulation of the AR gene expression by its cognate ligand has been the subject of numerous studies with contradictory results probably due to different techniques and models used. Negative regulation of the mRNA has been demonstrated using Northern blot analysis of whole tissue [2, 3], LNCap cells [4], or COS cells transfected with AR [5]. However, using *in situ* hybridization techniques Takeda *et al.* [6] have shown that the AR mRNA in the rat prostate disappears following castration and is restored by androgen treatment, suggesting a positive control of androgens on the AR. As concerns the AR protein, the literature seems more consistent and would conclude to a positive regulation but with cell-type, tissue, and age specificity [4, 6, 8, 35]. The data reported here, showing an increased expression of the AR protein after androgen treatment, are consistent with these latter studies. Similar results have been recently reported by Webber *et al.* [36] using an immortalized adult human prostatic epithelial cell line. These data demonstrate that human prostatic cells in culture have retained some degree of androgen responsiveness characteristic of the prostate. They can therefore be used as tools in the study on androgen action in this tissue. The mechanism involved in the positive autoregulation of the AR by androgens in the human prostate however, is not clear. It seems to imply directly the AR as it is, at least partially, suppressed by the antiandrogen cyproterone acetate and to involve both transcriptional and translational mechanisms as shown by the inhibition observed in the presence of actinomycin D and/or cycloheximide ([35], and this study). However, androgens have been shown to increase the AR half-life from 1 to 6 hours in transfected COS cells [37] and this stabilization could result in a more intense staining if it was effective in cultured human prostate cells.

Estrogens have been thought to share in the responsibility of the development of BPH [38] and the presence of an estrogen receptor in the prostate stroma, but not the epithelium, has been reported [39, 40]. Consistent with this notion, we show that estrogens increase AR staining in fibroblasts but not in epithelial cells. This effect is a true estrogenic effect suppressed by the antiestrogen 4-OH Tamoxifen. Should this action of estradiol apply *in vivo*, it could contribute to an increased sensitivity to androgens of the prostate stroma at an age of life when the androgen/estrogen ratio tends to be modified in favor of the latter. The prostate stroma however is not a homogeneous tissue and in addition to fibroblasts, it contains an important proportion of smooth muscle cells (SMC). A recent study [41] have shown that these cells are especially sensitive to estradiol for growth and expression of cell specific SMC markers. As both SMC and fibroblasts express vimentin [41], the possibility exists that in the culture system used in our laboratory, SMC participate in the observed response to estradiol.

In conclusion, androgens stimulate the expression of the AR in both epithelial and stromal cells. This positive autoregulation is inhibited by antiandrogens and may play a significant role in modulating the prostate tissue sensitivity to androgens. In addition, our data suggest a paracrine regulation of AR expression in epithelial cells by a diffusible factor secreted by the stroma. Alterations of these regulations may result in the imbalance in hormone sensitivity which in aging men leads to prostate pathology. The culture of androgen sensitive epithelial cells and fibroblasts either isolated or in coculture represents a good model for the study of this process.

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