

Effect of Culture Conditions on Androgen Sensitivity of the Human Prostatic Cancer Cell Line LNCaP

Erna G. Langeler, Connie J.C. van Uffelen, Marinus A. Blankenstein, Gert Jan van Steenbrugge, and Eppo Mulder

Departments of Endocrinology and Reproduction (E.G.L., E.M.) and Urology (C.J.C.v.U., G.J.v.S.), Erasmus University, Rotterdam; Department of Endocrinology, University of Utrecht, Utrecht (M.A.B.), The Netherlands

Several effects of androgens on LNCaP-FGC prostate tumor cells showed a biphasic pattern. Stimulation of growth and inhibition of secretion of prostatic acid phosphatase (PAP) was observed at low androgen concentrations (below 1 nM of the synthetic androgen R1881), and inhibition of growth and stimulation of PAP secretion was observed at higher concentrations. In contrast, prostate specific antigen (PSA) secretion did not show this biphasic response pattern. Comparable effects were found for two sublines of the LNCaP-FGC cells: an early (passage 20, androgen-dependent) and relatively late (passage 70, androgen-sensitive) passage of the cells. Culturing of both sublines in the presence of a high concentration of androgens (10 nM R1881) resulted initially in a decrease in growth rate, but the cells started to proliferate within 3 weeks. These cells became less sensitive to androgens, lost their biphasic response pattern, and showed reduced androgen receptor levels. Three weeks after removal of the excess of androgens, the passage 70 cells regained a biphasic growth response to androgens. Culture in medium without steroids but with EGF resulted in a decrease of both androgen sensitivity and androgen receptor level.

In conclusion, rapid changes of the androgen sensitivity and receptor level of the LNCaP cells occurred under the influence of culture conditions. These changes were partly reversible and, therefore, were most likely due to adaptation of the cells.

© 1993 Wiley-Liss, Inc.

Key words: LNCaP cells, androgen sensitivity, androgen receptor, prostatic acid phosphatase, prostate specific antigen

INTRODUCTION

The prostate gland is dependent on androgens for the maintenance of its normal growth and functional integrity. In addition, prostatic tumors are initially androgen-responsive, and decrease in size in response to androgen ablation or anti-androgen therapy. The extent and duration of the response is variable, and ultimately, prostate cancer relapses to an androgen-independent state. The molecular mechanism of this loss of growth control by androgens is poorly understood [1].

Only a few permanent growing human prostate cell lines are available for study

Received for publication December 7, 1992; accepted February 19, 1993.

Address reprint requests to Dr. E. Mulder, Department of Endocrinology and Reproduction, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

of prostatic tumors in vitro [2]. Among them, only the LNCaP (lymph node carcinoma of the prostate) cell line [3] shows androgen dependent growth. LNCaP cells contain considerable amounts of androgen receptors, which are involved in growth regulation [3,4] and in secretion of prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) [5,6]. The androgen receptor of the LNCaP cells has an unusual broad specificity for other steroids such as progestins and estradiol [7–9], and some anti-androgens do not inhibit, but stimulate cell growth [10–13]. It has been shown that a point mutation in the steroid binding domain of the androgen receptor in LNCaP cells is responsible for this phenomenon [14,15]. No other sex-steroid receptors could be detected in LNCaP cells [4,7].

For the growth response of LNCaP cells to androgens, a characteristic biphasic effect has been observed: increased growth rate at low androgen concentrations, and no effect or even cell loss at higher concentrations. The higher concentrations used in these studies were in the range of 1–10 nM of the natural ligand dihydrotestosterone or the synthetic androgen R1881 [7,16]. Effects of steroids at these concentrations are mediated by androgen receptors, and non-specific, toxic effects of steroids are generally minimal. A biphasic pattern with opposite direction was shown for apolipoprotein-D secretion (i.e., higher secretion correlates with lower growth rate) [17], and for the secretion of PAP [18]. The growth response to $1\alpha,25$ -dihydroxyvitamin D_3 also showed biphasic characteristics [19].

In this report, we describe the effects of culturing LNCaP cells under different conditions for several weeks, on growth, PAP/PSA secretion, and androgen receptor levels. Two sublines of the LNCaP-FGC cell line with different levels of androgen receptors and degrees of androgen dependency have been used. We show that rapid changes occur with respect to androgen sensitivity and receptor concentration. In addition, we show that biphasic response to increasing steroid concentrations is a reversible phenomenon that appears and disappears within a few weeks, depending on culture conditions.

MATERIALS AND METHODS

Materials

The synthetic androgen R1881 (methyltrienolone, 17β -Hydroxy- 17α -methyl-estra-4,9,11-trien-3-one) and labeled R1881 ($[17\alpha$ - Me - 3H]-methyltrienolone; 87 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Epidermal growth factor (EGF) from mouse submaxillary gland was obtained from Sigma (St. Louis, MO), and fetal calf serum (FCS) from Hyclone (Logan, UT).

Cell Culture

The LNCaP-FGC cell line used in the present study was a gift of Dr. Horoszewicz (Buffalo, NY), and was identical with the cell line present at the American Type Culture Collection. Two different sublines were used: the parental LNCaP-FGC line, cultured for 2–3 passages (subline L20; passage number 20 from the original beginning cultures of Dr. Horoszewicz), and cells which were maintained in our laboratory (subline L70). The L70 cells were obtained from the L20 cells after 1 year culture (replating once weekly, in total 50 times) in complete medium containing 7.5% fetal calf serum [8].

Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY) supple-

TABLE I. Effects of Different Culture Conditions on the Concentration of Androgen Receptors

Cell type	Culture condition	Receptors (fmol/mg protein)
L20-FCS	5 weeks FCS medium	441 ± 32
L20-Andr	5 weeks 10 nM R1881	318 ± 35
L20-EGF	5 weeks 1 nM EGF	83 ± 20
L70-FCS	5 weeks FCS medium	1619 ± 22
L70-Andr	5 weeks 10 nM R1881	612 ± 4
L70-EGF	5 weeks 1 nM EGF	328 ± 8
L70-Andr-FCS	5 weeks 10 nM R1881 and 3 weeks FCS medium	862 ± 28
L70-EGF-FCS	5 weeks 1 nM EGF and 3 weeks FCS medium	1513 ± 3

Androgen receptor levels in femtomoles of R1881 bound per milligram of protein in nuclear extracts were determined as described in Materials and Methods. Data are expressed as means ± SD of three determinations. Culture media contain either 5% FCS-medium or 5% ccFCS with R1881 or EGF.

mented with penicillin and streptomycin. Further additions were made as indicated in the legends of Table I and Figure 1. Either 5% FCS or 5% steroid-depleted fetal calf serum (ccFCS) (obtained by treatment twice with dextran-coated charcoal; charcoal 1%, dextran 0.1%) was added. During the culture period of several weeks, cells were only trypsinized when cultures were (nearly) confluent. Medium was changed twice a week. Cells were maintained in a humidified atmosphere of 5% CO₂ in air.

Androgen Sensitivity

The LNCaP cells were trypsinized and plated out in 24 multi-well dishes (Falcon, Oxnard, CA) at a density of 2×10^4 cells/cm² in RPMI-1640 medium with 5% ccFCS medium. Cells were allowed to attach for 2–3 days, after which medium with 5% ccFCS and a variable concentration of R1881 were added. After 3 days, the media were refreshed and, after an additional 3 days, cells were carefully washed with phosphate-buffered saline (pH 7.5) and dissolved in 1 M NaOH for determination of DNA content [20].

PAP and PSA Assays

The LNCaP cells were cultured as described above for study of androgen sensitivity. When the cells were harvested, the conditioned media of the last 3 days of culture were sampled, centrifuged, and stored at –20°C.

The PAP concentration in the media was assayed immunologically with a commercially available test kit according to the instructions of the supplier (Enzygnost PAP; Behring Diagnostica, Frankfurt am Main, Germany). The limit of detection was 0.2 µg enzyme/L. The antibodies of the kit did not cross-react with other acid phosphatase isoenzymes. PAP secretion was expressed as nanograms of PAP per 100 micrograms of DNA (estimated at the end of the culture period).

The PSA concentration in the media was measured with an automated micro-particle-based enzyme immunoassay (IMx-MEIA; Abbott Diagnostics, Wiesbaden-Delkenheim, Germany). Sensitivity of this assay was 0.03 ng/mL, and the inter-assay coefficients of variation at 4, 15, and 45 ng/mL were 3.6, 5.8, and 4.6, respectively (n = 10). PSA secretion was expressed as nanogram of PSA per microgram of DNA (estimated at the end of the culture period).

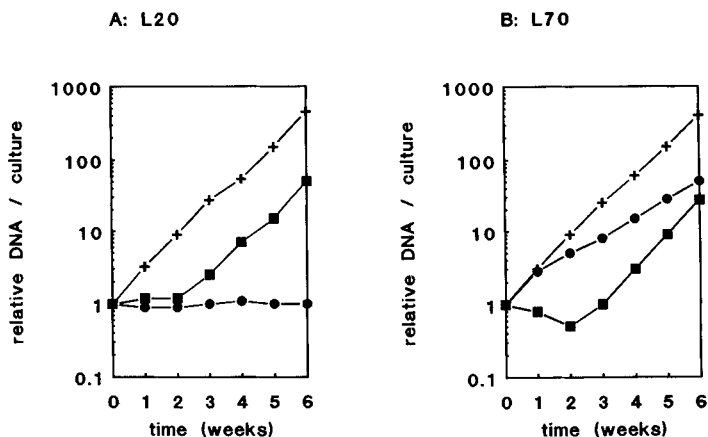


Fig. 1. Effects of R1881 on the growth of LNCaP cells. LNCaP cells (A: cells of subline L20; B: cells of subline L70) were cultured in the steroid depleted medium (●), in the presence of 0.1 nM R1881 (+), or 10 nM R1881 (■). The relative increase in cell mass was calculated from DNA measurements in two culture flasks at weekly intervals.

Nuclear Androgen Receptor Assay

Androgen receptor concentrations were estimated in LNCaP cells, which were cultured for several weeks in different experimental media, as indicated in Table I. For the binding assay, the cultures were incubated additionally for 1 hour at 37°C in charcoal-treated medium supplemented with 10 nM ^3H -R1881. Cells cultured in the presence of 10 nM non-radioactive R1881 were maintained for 1 week in the presence of 10 nM ^3H -R1881 (decreased specific activity; 4.3 Ci/mmol) to obtain a complete exchange with the radioactive tracer. Thereafter, cells were collected by scraping and homogenized by freeze-thawing (three times in liquid nitrogen, followed by thawing at 10°C.). Nuclear extracts were prepared and specific binding of ^3H -R1881 was measured by separation of bound and unbound steroids by protamine precipitation, as described previously [21,22]. Non-specific binding of ^3H -R1881 in the nuclear extracts was less than 5% of the total binding.

RESULTS

Growth Studies and Androgen Receptor Levels

In previous studies, we showed that LNCaP cells proliferated well in medium with FCS containing small amounts of endogenous steroids (e.g., 0.1 nM testosterone). When transferred to steroid-depleted medium, maximal growth was obtained after the addition of 0.1 nM of the synthetic androgen R1881. It is advantageous to use this steroid because the cells rapidly convert dihydrotestosterone, the natural ligand of the androgen receptor in prostatic cells [4]. To study possible adaptation processes to culture conditions, two androgen sensitive sublines of the LNCaP cell line were followed during several weeks in culture, in medium with different androgen concentrations. Cells of passage 20 (subline L20) and cells of passage 70 (subline L70) were compared. In the presence of 10 nM R1881, growth of both cell types was initially inhibited, but cells started to proliferate after a lag period of approximately

2–3 weeks (Fig. 1). During the lag period, morphological changes were observed: Cells lost their epithelial-like structure and rounded up. These changes disappeared when cells started to grow. The effect of the increased androgen concentration was more pronounced for the L70 cells. For these cultures the addition of 10 nM R1881 resulted initially in a prominent reduction of total cell number (Fig. 1). In contrast, cells cultured in steroid-depleted medium with 0.1 nM R1881 continued to grow at a constant rate. In steroid-depleted medium without supplements (or supplemented with 1 nM EGF), the L20 cells did not grow, but remained viable, whereas the L70 cells continued to grow at a decreased rate.

After adaptation of the cells to medium with 10 nM R1881 (at 5 weeks of culture), the androgen receptor level in L20 cells showed a limited decrease, while in L70 cells the receptor level substantially decreased to approximately 40% of the initial value (Table I). A decrease in the amount of androgen receptors to approximately 20% was noted in the presence of steroid-depleted medium with EGF. In steroid-depleted medium without EGF, receptor levels decreased to a lesser extent (250 and 527 fmole/mg protein for L20 and L70 cells, respectively); therefore, the addition of EGF contributes to the loss in steroid binding. The effects of different culture media on androgen receptor levels were partially reversible: after medium change to the original medium with FCS, the concentration of androgen receptors increased (Table I: compare line 5 with 7, and 6 with 8; without medium change, receptor values remained constant during an additional culture period of 3 weeks).

Androgen Sensitivity

Subsequently, the subcultures obtained after 5 weeks of culture under various conditions (Table I) were tested for their androgen responsiveness. Three types of responses were found: 1) The characteristic biphasic effect of androgens on the growth rate was retained by cells cultured in medium with FCS (Fig. 2, L20-FCS and L70-FCS) and by cells of the L70 subline cultured in steroid-depleted medium with EGF (Fig. 2, L70-EGF). 2) The cells cultured in medium with increased androgen concentration did not show the biphasic effect and were less sensitive to androgens (Fig. 2, L20-Andr and L70-Andr). 3) Complete loss of response was observed for cells of subline L20 after culture in steroid-depleted medium supplemented with EGF (Fig. 2, L20-EGF). The reversibility of the effects induced by the different media were studied for the L70 subline. Three weeks after change to the original medium with FCS, the biphasic dose-response to R1881 was restored (Fig. 3), although sensitivity to androgens remained less pronounced (compare Figs. 2 and 3).

Secretion of PAP and PSA

Secretion of PAP and PSA by the LNCaP cells was studied for the L20 subline. An inverse relationship between stimulation of LNCaP cells by R1881 (Fig. 2) and secretion of PAP was observed (Fig. 4A: L20-FCS cells). This biphasic effect disappeared after culture in the presence of 10 nM R1881 for 5 weeks (Fig. 4A: L20-Andr cells). These results showed that adaptation of the L20 cells to medium with increased androgen concentrations affected not only growth rate, but also PAP secretion.

In contrast to the effects of R1881 on cell growth and PAP secretion, no biphasic pattern was observed for PSA secretion (Fig. 4B). PSA secretion of all tested sublines could be stimulated by concentrations of R1881 up to 10 nM. The absolute

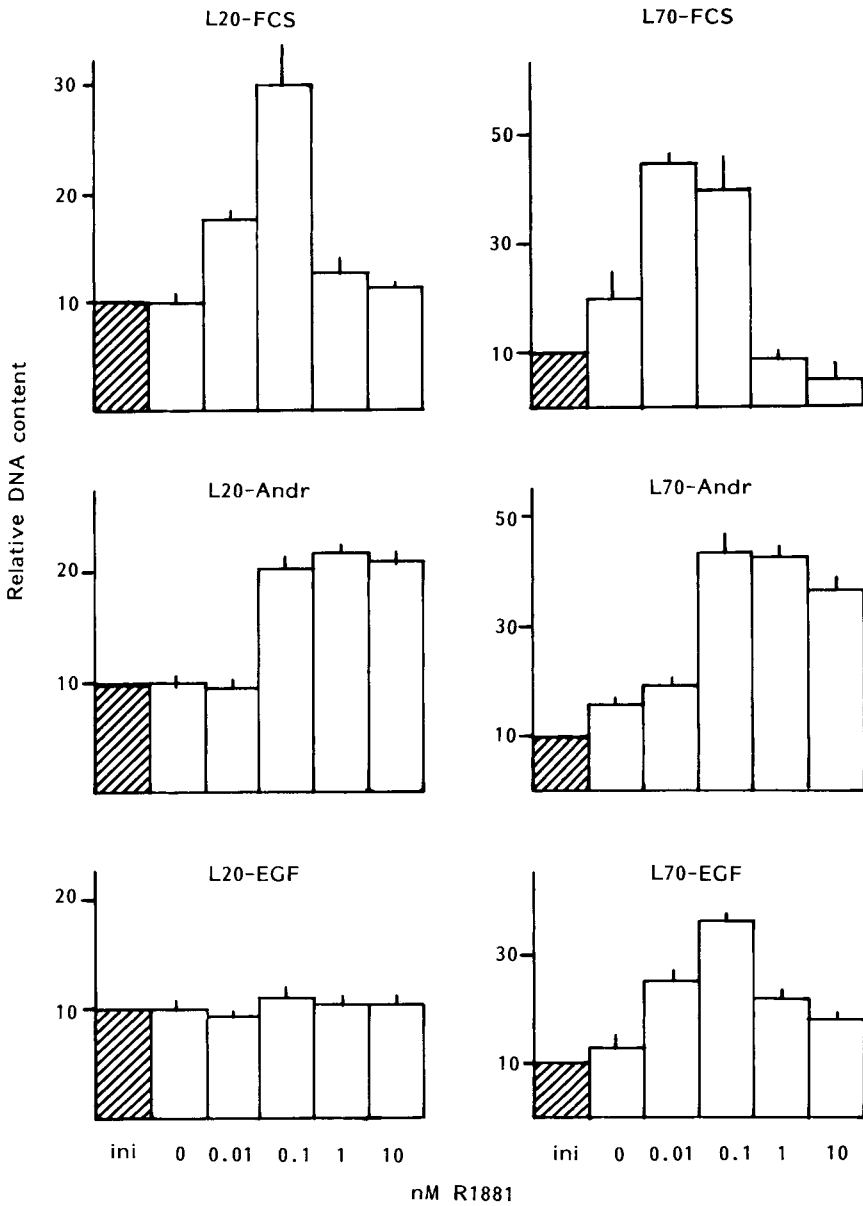


Fig. 2. Androgen sensitivity of LNCaP cells adapted to different culture conditions. Cells of subline L20 and subline L70 were cultured as indicated in Table I for 5 weeks in medium with FCS (subcultures L20-FCS and L70-FCS), in medium with an elevated androgen concentration (subcultures L20-Andr and L70-Andr), or in steroid-depleted medium supplemented with EGF (subcultures L20-EGF and L70-EGF). Growth responses of these subcultures to different concentrations of R1881 were estimated as described in Materials and Methods in a 6-day test period and were expressed as the relative amount of DNA per culture (micrograms of DNA per culture divided by initial value at day zero). Initial values for DNA at the start of the 6-day test period were arbitrarily set at 10 (striped bar, abbreviation "ini"). Data are expressed as means and SD of four determinations.

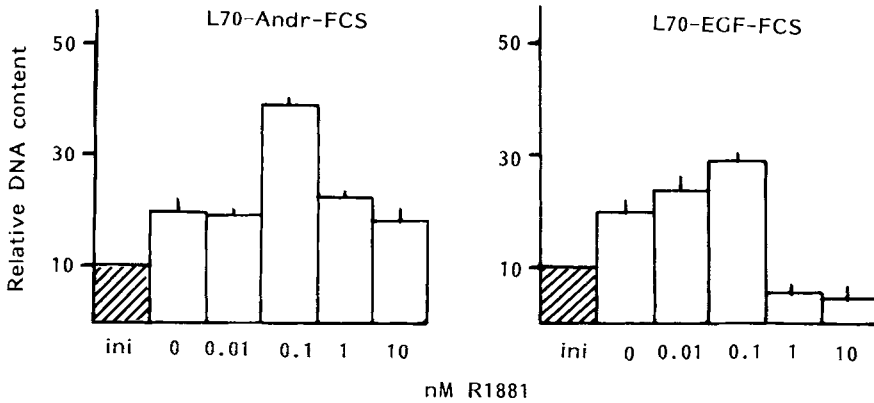


Fig. 3. Androgen sensitivity after medium change. Cells of subline L70 were adapted either to 10 nM R1881 or to steroid-depleted medium with EGF in a 5-week culture period. Subsequently, the cells were cultured for 3 weeks in medium with FCS. Abbreviations: L70-Andr-FCS, cells originally adapted to 10 nM R1881; L70-EGF-FCS, cells originally adapted to 1 nM EGF. Growth response of these subcultures to different concentrations of R1881 was estimated as described in Figure 2.

amount of PSA secretion was lower for the cells adapted to increased androgen concentrations (Fig. 4B: L20-Andr cells). We also noted that PSA secretion by LNCaP cells was strictly androgen-dependent. EGF-stimulated cell growth was not accompanied by a significant secretion of PSA (results not shown).

DISCUSSION

In the present study, we showed that large changes in sensitivity to androgens of two different sublines (L20 and L70) of the prostate cancer cell line LNCaP could be induced within a few weeks under the influence of changes in androgen concentrations in culture media. The L20 subline was derived from a fast-growing colony of the original LNCaP cells (LNCaP-FGC) [3]. The L20 subline was androgen-dependent and did not grow in the absence of steroids. Also, the addition of EGF to the steroid-depleted medium did not induce growth within a few weeks. The L70 subline was derived from the original L20 line after continuous (routine) culture in medium with FCS for more than 1 year. L70 cells lost strict androgen dependence, grew in the absence of steroids, and were stimulated by EGF in agreement with previous studies [23]. Increase in basal growth rate and loss of steroid sensitivity has been found for several mammary carcinoma cell lines [24], e.g., for MCF-7 cells [25], and for ZR-75 and T47-D cells [26]. In contrast to these observation for mammary tumor cells, the increased basal growth rate of the L70 cells was not accompanied by loss of steroid sensitivity. This subline showed increased sensitivity to androgens and increased androgen receptor levels. This is not a general phenomenon for LNCaP cells, as other sublines have been described with different characteristics and degrees of androgen sensitivity [27]. It is not known which factors are involved in changes of the different characteristics of these various sublines.

Both sublines L20 and L70 showed the characteristic biphasic growth response to androgens that has also been observed in previous studies with LNCaP cells [7,16].

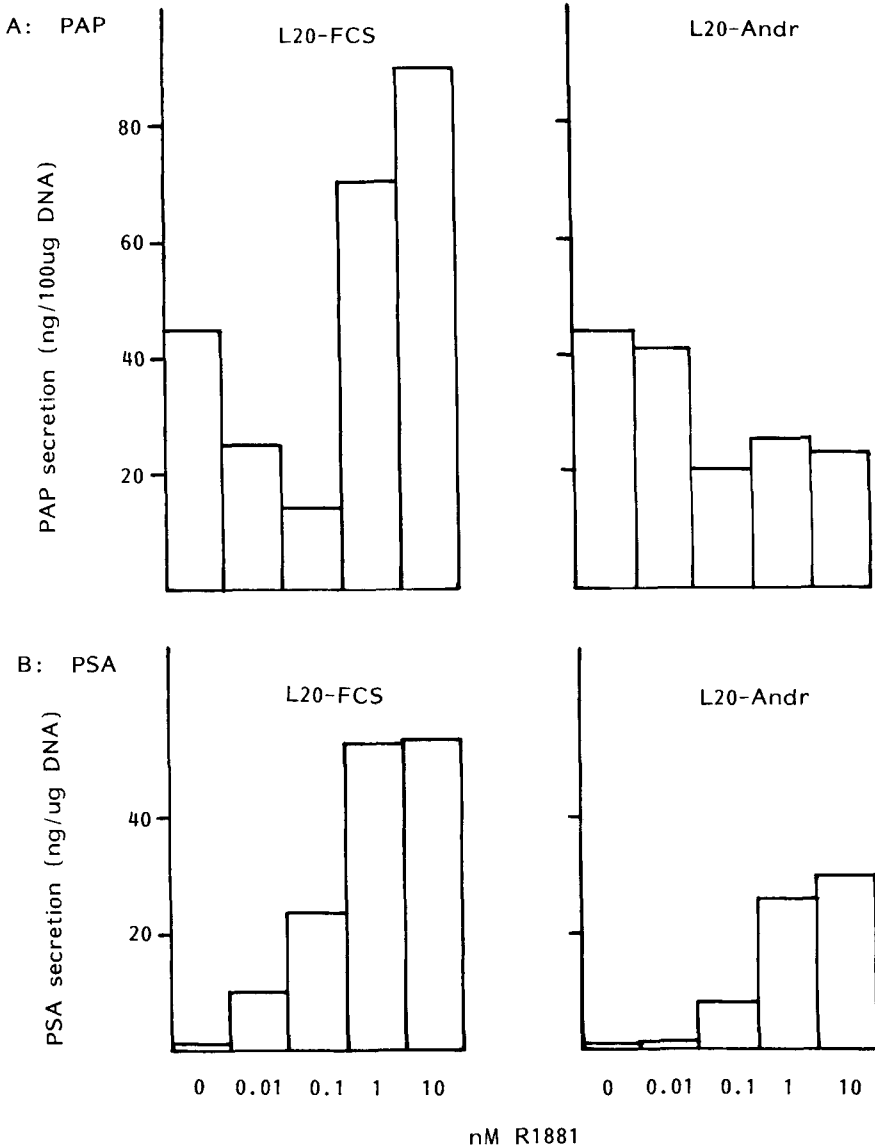


Fig. 4. PAP and PSA secretion in response to androgens. Cells adapted to medium with FCS or to medium with an elevated androgen concentration (subcultures L20-FCS and L20-Andr, see Table I) were plated out in dishes with steroid-depleted medium without further supplements. R1881 was added to the indicated concentration after 2 days of culture. After an additional 6 days, PAP and PSA concentrations were measured in conditioned media of the last 3 days of culture. Values represent the mean of two to four determinations. Standard deviation is less than 5%.

After culture for 5 weeks in the presence of 10 nM of the synthetic androgen R1881 (a steroid concentration too high to stimulate the L20 and L70 cells at the beginning of the culture period), the cells adapted to the increased androgen concentration in the medium, and started to proliferate. Androgens no longer exerted a biphasic effect on cell proliferation. This effect was partially reversible, as after withdrawal of hormone

during 3 weeks, L70 cells regained a biphasic growth response. An inhibitory effect of high doses of steroids on growth has been observed for tumor cells transfected with an artificially large number of estrogen receptors [28]. These cells are inhibited by high concentrations of estrogens, probably due to removal ("squelching") of an essential co-activator during transcription activation [29]. In our studies with LNCaP cells, release from the inhibitory effect of high doses of steroids was accompanied by a decrease in androgen receptor levels. On the other hand, the changes in steroid sensitivity were more pronounced than the changes in receptor level (e.g., for the L20 cells only a 30% decrease in receptor concentration is seen after adaptation to androgen-rich medium). In addition, the absolute level of androgen receptors did not predict the type of response observed, as L70 cells cultured in steroid-depleted medium enriched with EGF still showed a biphasic growth response, although androgen receptor levels had decreased to 20% of the original value (L70-EGF: Table I and Fig. 2).

The relative large amount of androgen receptors in the original LNCaP cells alone is, therefore, not sufficient to explain the biphasic growth curve. One could speculate that in cells showing a biphasic growth response, high levels of steroids induce a differentiated state of the cells and decrease growth rate. The mechanistic basis of differentiation and proliferation is found in the activation of specific sets of transcription factors, which regulate the specific genetic programs. Mutual interference between these pathways occurs, as has been shown for several genes regulated by both differentiation-inducing members of the steroid hormone receptor family (e.g., glucocorticoids) and proliferation-inducing protein products of the *fos* and *jun* genes [30]. In line with the concept that the phenotype of a cell is determined by the action of either differentiation or proliferation factors, we observed for a characteristic of differentiated cells the secretion of PAP [31], with opposite effects of different steroid concentrations as for growth rate. The biphasic dose-response curve for PAP secretion showed an inverted shape as compared with the growth curve, with minimal PAP secretion at the concentration of steroids, inducing the highest growth rate. Down-regulation of both PAP production and PAP messenger level by androgens in LNCaP cells has also recently been shown in other studies [18,31]. Cells that were adapted to an increased concentration of androgens after a culture period of a few weeks lost the biphasic response to androgens also with respect to PAP secretion.

The secretion of PSA, a member of the kallikrein family of serine proteases, is another differentiated function of prostatic epithelial cells. PSA secretion could be induced by androgens in LNCaP cells, in agreement with other studies [9,18]. However, none of the different cell cultures showed an androgen-dependent biphasic effect on PSA secretion. Culture in medium with the growth factor EGF stimulated cell growth of the L70 subline, but did not induce PSA secretion. A recent study [32] showed that prostatic stroma-derived factors exhibited the opposite effect on LNCaP cells: decrease in (androgen-induced) growth rate and considerable stimulation of both PSA and PAP secretion.

In conclusion, we have shown that rapid changes of the androgen sensitivity of LNCaP cells occur under the influence of increased concentrations of androgens. This phenomenon of adaptation to culture conditions is largely reversible, and, therefore, is likely due to phenotypic changes of the cells between differentiated and proliferative states. The concomitant changes in androgen receptor levels play a limited role in this process.

It is tempting to extrapolate the results of this study to the situation in the patient, and predict that also in vivo androgen-sensitive prostate tumor cells with respect to their growth characteristics rapidly and reversibly adapt to changes in androgen levels. This effect should be distinguished from a gradual loss of androgen sensitivity that occurs during tumor progression. However, additional complexity could be expected for a heterogeneous tumor mass growing in close contact with several other cells of various tissues.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of Mrs. M. van Loon, Department of Clinical Chemistry, Academic Hospital, Utrecht, The Netherlands, in performing the PSA assays, and Dr. A. Vockel, Abbott Diagnostics Division Scientific Affairs, Wiesbaden-Delkenheim, Germany, for providing the PSA reagents. This study was supported by the Dutch Cancer Society (KWF) through Grant IKR 90-13.

REFERENCES

1. Davies P, Eaton CL: Regulation of prostate growth. *J Endocrinol* 131:5-17, 1991.
2. Isaacs JT: Development and characteristics of the available model systems for the study of prostatic cancer. In Coffey DS, et al. (eds): "Current Concepts and Approaches to the Study of Prostate Cancer." *Prog Clin Biol Res* 239:513-576, 1987.
3. Horoszewicz JS, Leong SS, Kawinsky E, Karr JP, Rosenthal H, Ming Chu T, Mirand EA, Murphy GP: LNCaP model of human prostatic carcinoma. *Cancer Res* 43:1809-1818, 1983.
4. Berns EMJJ, de Boer W, Mulder E: Androgen-dependent growth regulation of and release of specific protein(s) by the androgen receptor containing human prostate tumor cell line LNCaP. *Prostate* 9:247-259, 1986.
5. Schulz P, Bauer HW, Fittler F: Steroid hormone regulation of prostatic acid phosphatase expression in cultured human prostatic carcinoma cells. *Biol Chem Hoppe Seyler* 366:1033-1039, 1985.
6. Hasenson M, Lundh B, Stege R, Carlström K, Pousette A: PAP and PSA in prostatic carcinoma cell lines and aspiration biopsies: relation to hormone sensitivity and to cytological grading. *Prostate* 14:83-90, 1989.
7. Schuurmans ALG, Bolt J, Voorhorst MM, Blankenstein MA, Mulder E: Regulation of growth and epidermal growth factor receptor levels of LNCaP prostate tumor cells by different steroids. *Int J Cancer* 42:917-922, 1988.
8. Veldscholte J, Voorhorst-Ogink MM, Bolt de Vries J, van Rooij HCJ, Trapman J, Mulder E: Unusual specificity of the androgen receptor in the human prostate tumor cell line LNCaP: high affinity for progestagenic and estrogenic steroids. *Biochem Biophys Acta* 1052:187-194, 1990.
9. Montgomery BT, Young CY-F, Bilhartz DL, Andrews PE, Prescott JL, Thompson NF, Tindall DJ: Hormonal regulation of prostate-specific antigen (PSA) glycoprotein in the human prostatic adenocarcinoma cell line, LNCaP. *Prostate* 21:63-73, 1992.
10. Wilding G, Chen M, Gelmann EP: Aberrant response in vitro of hormone-responsive prostate cancer cells to anti-androgens. *Prostate* 14:103-115, 1989.
11. Olea N, Sakabe K, Soto AM, Sonnenschein C: The proliferative effect of "anti-androgens" on the androgen-sensitive human prostate tumor cell line LNCaP. *Endocrinology* 126:1457-1463, 1990.
12. Schuurmans ALG, Bolt J, Veldscholte J, Mulder E: Stimulatory effects of antiandrogens on LNCaP human prostate tumor cell growth, EGF-receptor level and acid phosphatase secretion. *J Steroid Biochem Molec Biol* 37:849-853, 1990.
13. Veldscholte J, Berrevoets CA, Brinkmann AO, Grootegoed A, Mulder E: Anti-androgens and the mutated androgen receptor of LNCaP cells: differential effects on binding affinity, heat-shock protein interaction, and transcription activation. *Biochemistry* 31:2393-2399, 1992.
14. Veldscholte J, Ris-Stalpers C, Kuiper GGJM, Jenster G, Berrevoets C, Claassen E, van Rooij HCJ, Trapman J, Brinkmann AO, Mulder E: A mutation in the ligand binding domain of the androgen

- receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. *Biochem Biophys Res Commun* 173:534–540, 1990.
15. Harris SE, Harris MA, Rong Z, Hall J, Judge S, French FS, Joseph DR, Lubahn DB, Simental JA, Wilson EM: Androgen regulation of HBGF-I (aFGF) mRNA and characterization of the androgen-receptor mRNA in the human prostate carcinoma cell line –LNCAP/A-dep. In Karr JP, Coffey DS, Smith RG, and Tisdell DJ (eds) "Molecular and Cellular Biology of Prostate Cancer." New York: Plenum Press, 315–322, 1991.
 16. De Launoit Y, Veilleux R, Dufour M, Simard J, Labrie F: Characteristics of the biphasic action of androgens and of the potent antiproliferative effects of the new pure antiestrogen EM-139 on cell cycle kinetic parameters in LNCaP human prostatic cancer cells. *Cancer Res* 51:5165–5170, 1991.
 17. Simard J, Veilleux R, de Launoit Y, Haagensen DE, Labrie F: Stimulation of apolipoprotein D secretion by steroids coincides with inhibition of cell proliferation in human LNCaP prostate cancer cells. *Cancer Res* 51:4336–4341, 1991.
 18. Henttu P, Vihko P: Steroids inversely affect the biosynthesis and secretion of human prostatic acid phosphatase and prostate-specific antigen in the LNCaP cell line. *J Steroid Biochem Molec Biol* 41:349–360, 1992.
 19. Miller GJ, Stapleton GE, Ferrara JA, Lucia MS, Pfister S, Hedlund TE, Upadhyay P: The human prostatic carcinoma cell line LNCaP expresses biologically active specific receptors for $1\alpha,25$ -Dihydroxyvitamin D_3 . *Cancer Res* 52:515–520, 1992.
 20. Hinegardner RT: An improved fluorometric assay for DNA. *Anal Biochem* 39:197–201, 1971.
 21. Kuiper GGJM, De Ruiter PE, Grootegoed JA, Brinkmann AO: Synthesis and post-translational modification of the androgen receptor in LNCaP cells. *Mol Cell Endocrinol* 80:65–73, 1991.
 22. Mulder E, Vrij L, Foekens JA: Extraction of nuclear androgen receptors from rat prostate with different reagents. *Mol Cell Endocrinol* 23:283–296, 1981.
 23. Schuurmans ALG, Bolt J, Mulder E: Androgens stimulate both growth-rate and epidermal growth factor receptor activity of the human prostate tumor cell LNCaP. *Prostate* 12:55–63, 1988.
 24. King RJB: Progression from steroid sensitive to insensitive state in breast tumours. *Cancer Surveys* 14:131–146, 1992.
 25. Katzenellenbogen BS, Kendra KL, Norman MJ, Berthois Y: Proliferation, hormonal responsiveness and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogens. *Cancer Res* 47:4355–4360, 1987.
 26. Daly RJ, Darbre, PD: Cellular and molecular events in loss of estrogen sensitivity in ZR-75-1 and T-47-D human breast cancer cells. *Cancer Res* 50:5868–5875, 1990.
 27. Van Steenbrugge GJ, Van Uffelen CJC, Bolt J, Schröder FH: The human prostatic cancer cell line LNCaP and its derived sublines—an in vitro model for the study of androgen sensitivity. *J Steroid Biochem Mol Biol* 40:207–214, 1991.
 28. Kushner PJ, Hort E, Shine J, Baxter JD, Greene GL: Construction of cell lines that express high levels of the human estrogen receptor and are killed by estrogens. *Mol Endocrinol* 4:1465–1473, 1990.
 29. Gronemeyer H: Transcription activation by estrogen and progesterone receptors. *Ann Rev Genetics* 25:89–123, 1991.
 30. Ponta H, Cato ACB, Herrlich P: Interference of pathway specific transcription factors. *Biochem Biophys Acta* 1129:255–261, 1992.
 31. Lin M-F, DaVolio J, Garcia-Arenas R: Expression of human prostatic acid phosphatase activity and the growth of prostate carcinoma cells. *Cancer Res* 52:4600–4607, 1992.
 32. Fong C-J, Sherwood ER, Braun EJ, Berg LA, Lee C, Kozlowski JM: Regulation of prostatic carcinoma cell proliferation and secretory activity by extracellular matrix and stromal secretions. *Prostate* 21:121–131, 1992.