Report

Induction of prostate specific antigen production by steroids and tamoxifen in breast cancer cell lines

He Yu, Eleftherios P. Diamandis, Nosratollah Zarghami and Linda Grass
Department of Clinical Biochemistry, Toronto Western Division, The Toronto Hospital, 399 Bathurst Street, Toronto, Ontario M5T 2S8, Canada; and Department of Clinical Biochemistry, University of Toronto, 100 College Street, Toronto, Ontario, M5G 1L5, Canada

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Summary

We demonstrate that the steroid hormone receptor-positive breast carcinoma cell lines T-47D and MCF-7 can be induced by androgens, progestins, mineralocorticosteroids, glucocorticosteroids, and antiestrogens, to produce prostate specific antigen (PSA). Estrogens failed to induce such stimulation in both cell lines and, in addition, were able to block the induction by androgens in the cell line T-47D. These data support and extend our previous report on PSA production by breast tumors and describe an *in vitro* system which can be used to study the phenomenon for possible application in prognosis and design of new therapy.

Introduction

Prostate specific antigen (PSA) is a glycoprotein produced almost exclusively by the epithelial cells of the prostate and is currently used as a marker for diagnosis and monitoring of prostate cancer [1–3]. Prostate specific antigen detection in tumors of non-prostatic origin has been reported as a rare event in a few instances, using immunohistochemical techniques [4, 5]. Recently, using a highly sensitive immunofluorometric procedure, we have reported presence of PSA in about 30% of breast tumors [6]. PSA production in breast tumors is associated with the presence of steroid hormone receptors. Preliminary data suggest that PSA may be a favorable prognostic indicator in breast cancer.

Production of PSA by cell lines other than those of prostatic origin has not, to our knowledge, as yet been reported. We here demonstrate that the steroid hormone receptor-positive breast cancer cell lines T-47D and MCF-7 can be induced by steroid hormones and tamoxifen to produce PSA. This work was undertaken in an effort to identify candidate ligands which stimulate expression of the PSA gene in breast cancer *in vivo*, and to devise methods for assessing the biological activity of candidate antitumor agents *in vitro*. We reason that if PSA production is a marker of a patent pathway starting from a steroid hormone ligand and ending at the PSA gene expression, with intermediary steps involving the receptors, then candidate antitumor agents interacting with the receptors could be evaluated *in vitro* by monitoring PSA production under conditions of competition between the putative drugs and the pathway-stimulating ligands. Moreover, the availability of a tissue culture system

would facilitate the study of PSA gene regulation in cell lines and cancer of non-prostatic origin.

Materials and methods

The steroid hormone receptor-positive breast carcinoma cell lines T-47D and MCF-7 [7–9] and the steroid hormone receptor-negative breast carcinoma cell line BT-20 [9,10] were obtained from the American Type Culture Collection, Rockville, MD 20852. All three cell lines were cultured according to the instructions of the ATCC.

All steroids used were from Sigma Chemical Co., St. Louis, MO 63178. Stock 10^{-3} mol/L solutions of steroids were prepared in absolute ethanol. The 16 steroids and tamoxifen tested were given a number and are shown in Table 1. We have subsequently studied triamcinolone acetonide and R1881. These two steroids were not numbered.

The prostate specific antigen (PSA) assay used is a highly sensitive immunofluorometric procedure described in detail elsewhere [11]. Total protein de-

terminations were carried out with the bicinchoninic acid method (BCA) commercially available from Pierce Chemical Co., Rockford, IL.

The cell lysis buffer was a 50 mmol/L Tris buffer, pH 7.80, containing 2 g/L of Nonidet NP-40 (from Boehringer Mannheim Canada, Laval, PQ).

Procedures

Cell culture and stimulation

The T-47D and MCF-7 cell lines were initially grown in flasks at 37° C and 5% CO_2 in RPMI 1640 medium supplemented with 0.2 I.U. of bovine insulin per mL, 29 g/L glutamine, and 10% fetal calf serum. The cells were then detached by trypsin-EDTA treatment and subcultured as above in 24-well microtiter plates, until confluency. Each well contained 2 mL medium. Initial stimulation experiments were performed by adding 2 µL of a 10^{-3} mol/L steroid solution in the wells. At specific time intervals, ranging from 1 h to 24 h, supernatant

Table 1. Potency of various steroids in inducing expression of the PSA gene in T-47D and MCF-7 cells

Steroid	Code Number	Biological Activity	PSA Induction	
			T-47D	MCF-7
Testosterone	1	Androgen	Strong	Weak
Estrone	2	Estrogen	None	None
11β-Hydroxyprogesterone	3	Progestin	Moderate	Strong
Dehydroisoandrosterone	4	Androgen metabolite	None	Weak
Dihydroandrosterone	5	Androgen	Strong	Weak
Estriol	6	Estrogen	None	None
17α-Hydroxyprogesterone	7	Progestin	Weak	Weak
Androsterone	8	Androgen	Strong	Weak
Hydrocortisone	9	Glucocorticoid	Strong	Strong
17α-Ethinylestradiol	10	Estrogen derivative	Weak	Weak
Norethynodrel	11	Progestin	Strong	Moderate
Tamoxifen	12	Antiestrogen	Moderate	Moderate
β-Estradiol	13	Estrogen	None	None
Betamethazone 17-valerate	14	Glucocorticoid	Strong	Strong
Norethyndrone	15	Progestin	Strong	Strong
Norgestrel	16	Progestin	Strong	Strong
Aldosterone	17	Mineralocorticoid	Strong	Strong
Triamcinolone acetonide		Progestin/Glucocorticoid	Strong	Strong
R1881		Androgen/Progestin/Glucocorticoid	Strong	Strong
Cholesterol		—	None	None
Alcohol		—	None	None

was removed (200 µL) for PSA quantification by the immunofluorometric procedure. At 24 h, a second 2 µL stimulation was initiated followed by supernatant collection. For certain experiments, the first and second stimulation was done in culture medium containing 10% fetal calf serum (FCS); 24 hours after the second stimulation the medium was aspirated, fresh medium was added without FCS, and the stimulation was continued as above for 48 h. The experiments without FCS were undertaken in order to avoid the presence of steroid-binding proteins in the FCS. Other stimulation experiments were conducted in FCS-free culture media only. At the end of each stimulation experiment, the culture media were removed and the attached cells lysed with 0.5 mL lysis buffer, for 10 min with shaking at room temperature. After spinning, PSA was assayed in the supernatant.

Blocking experiments with steroids were performed by first incubating the cells with 2 mL medium in which we added 2 µL of a 10^{-3} mol/L blocking steroid solution for 15 min followed by stimulation as described above. Blocking was repeated before each addition of the stimulating steroid.

For the purpose of producing relatively high amounts of PSA, the T-47D cells were grown to confluency in 175 cm² culture flasks in RPMI medium supplemented with insulin, glutamine, and fetal calf serum. The media were then removed, and the cells were rinsed with RPMI medium and further maintained in 200 mL RPMI without fetal calf serum. Four identical flasks, A, B, C, D, were then processed for stimulation as follows: Flask A. The cells were stimulated every 24 h with 5 µL absolute alcohol for a total of nine days. Just before each stimulation, 1 mL supernatant was removed for testing. The culture medium was not changed during the 9-day period. Flasks B, C, and D were stimulated with 5 µL of steroid # 5 (dihydroandrosterone, stock solution was 10^{-3} mol/L in absolute ethanol) every 24 h for a total of 4 days (Flask B), 7 days (Flask C), or 9 days (Flask C). Just before each stimulation, 1 mL supernatant was removed for testing. Variant stimulation procedures are described in the text.

The prostate carcinoma cell line LNCaP (obtained from ATCC) was grown in RPMI medium supplemented with 5% fetal calf serum without any stimulation. Supernatants were collected for PSA analysis. This cell line is known to produce PSA and secrete it into the culture medium, and was used as a positive control. LNCaP cells were grown in a separate CO_2 incubator to avoid the possibility of cross-contamination.

High performance liquid chromatography (HPLC)

Characterization of the PSA immunoreactivity in the culture media was performed by HPLC analysis. We used a Shimadzu system (Shimadzu Corp., Kyoto, Japan), run isocratically with a mobile phase of 0.1 mol/L Na_2SO_4 – 0.1 mol/L NaH_2PO_4, pH 6.80. Flow-rate was 0.5/min. The column used was a Bio-Sil SEC-250, 600 mm × 7.5 mm (Bio-Rad Labs, Richmond, CA). The column was calibrated with a molecular weight standard solution from Bio-Rad. Fractions of 0.5 ml were collected and analyzed for PSA by the immunofluorometric procedure.

Tissue culture supernatants were concentrated ~ 50–100-fold by using Centriprep 10 preconcentration modules from Amicon (Amicon Division, W.R. Grace and Co., Beverly, MA 01915). These disposable devices were used as recommended by the manufacturer.

Western blot analysis

All necessary reagents and equipment for Western blot analysis were purchased from Novex, San Diego, CA 92121. The manufacturer's protocols were followed throughout. Samples for Western blot analysis were electrophoresed under reducing conditions on 8–16% gradient polyacrylamide mini gels and proteins were transferred to Hybond-ECL nitrocellulose membranes (Amersham International, Arlington Heights, IL 60005). Membranes were blocked for 1 h and further processed as recommended by the manufacturer of the ECL-Western blot detection kit (Amersham). Briefly, the membrane was probed with a polyclonal anti PSA antibody (from Medix Biotech, Foster City, CA, 1 mg/
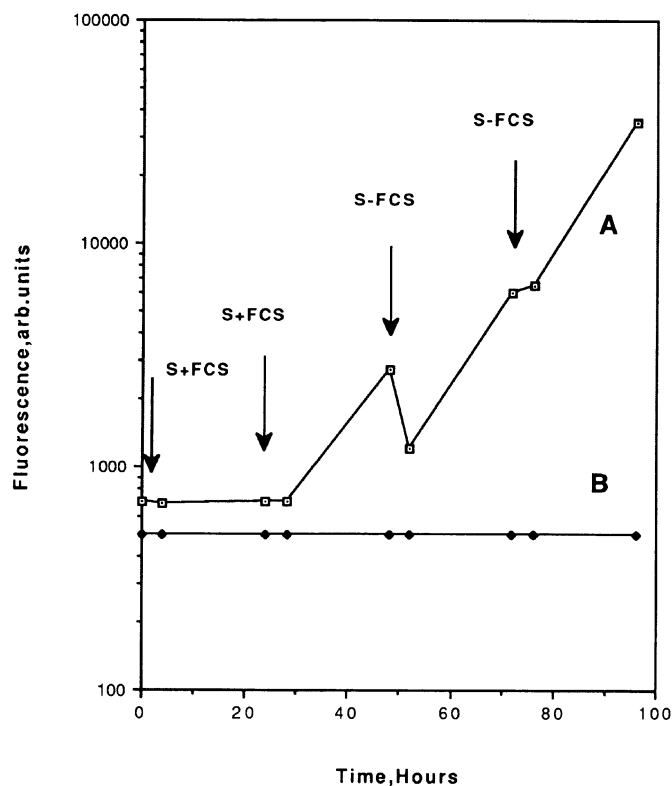
Fig. 1. Stimulation of PSA production by the breast carcinoma cell line T-47D. Curve A, stimulation with testosterone; curve B, stimulation with β-estradiol. Each stimulation consisted of 2 μL of a 10^{-3} mol/L steroid solution in ethanol added to 2 mL culture medium. S = stimulation at the time indicated; FCS, fetal calf serum, 10%. At 48 h, the culture medium was removed and replaced by culture medium without FCS. Medium obtained for PSA analysis was withdrawn just before stimulation at the times indicated by the arrows and at 96 h. One thousand arbitrary fluorescence units are equivalent to a PSA concentration of approximately 0.03 μg/L.

mL, diluted 2000-fold in the blocking buffer), washed, and reacted with a horseradish peroxidase-conjugated goat anti-rabbit antibody. The enzyme activity on the membrane was revealed by chemiluminescence, captured on X-ray film. Biotinylated molecular weight markers were visualized by reacting with a streptavidin-horseradish peroxidase conjugate added simultaneously with the goat anti-rabbit antibody.

A highly purified seminal plasma PSA preparation used as standard was a gift from Dr. T. Stamey, Department of Urology, Stanford University.

Results

Figure 1 shows a time course of PSA production by

the T-47D cells after stimulation with the androgenic steroid testosterone (Curve A) or the estrogenic steroid β-estradiol (Curve B). Similar time courses for PSA production were observed with use of the other stimulating steroids. Clearly, testosterone induces PSA production approximately 30–48 h after the initiation of the stimulation. The effect occurs in the presence or absence of fetal calf serum (FCS). After removal of the culture medium containing FCS at 48 h and replacement with a culture medium without FCS, stimulation continued to induce PSA production during the subsequent 48 h. No PSA production was observed by stimulating with β-estradiol (Fig. 1, curve B) or alcohol (the steroid solvent, data not shown).

A number of steroids were tested as possible stimulators of PSA production by the breast carcinoma cell lines T-47D, MCF-7, or BT-20 under identical stimulation conditions. The BT-20 cell line failed to produce detectable immunoreactive PSA in all cases. The T-47D cell line was successfully induced by a number of steroids as shown in Fig. 2. All data in Fig. 2 were plotted by analyzing supernatants at 48 h after stimulating twice, at time zero and 24 h. Similar experiments were performed with the cell line MCF-7. We have classified steroids into four catagories based on their ability to induce PSA production. The results are summarized in Table 1. Androgens, progestins, glucocorticoids, and mineralocorticoids were able to induce PSA gene expression in the cell line T-47D. The same classes of steroids except androgens were also strong stimulators of PSA gene expression in MCF-7 cells. Estrogens were not able to induce PSA production in either T-47D or MCF-7 cells even when tested in the concentration range between 10^{-6}–10^{-9} mol/L. We have further studied the induction of PSA production by four stimulating steroids at concentrations of 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} mol/L. The steroids tested were testosterone, dihydroandrosterone, aldosterone, and R1881. The results are shown in Fig. 3. Maximal stimulation was achieved at concentrations around 10^{-6}–10^{-7} mol/L. At lower steroid concentrations the stimulation is significantly reduced. No PSA production was observed in parallel control experiments using ethanol as stimulant (solvent) or no stimulant at all.
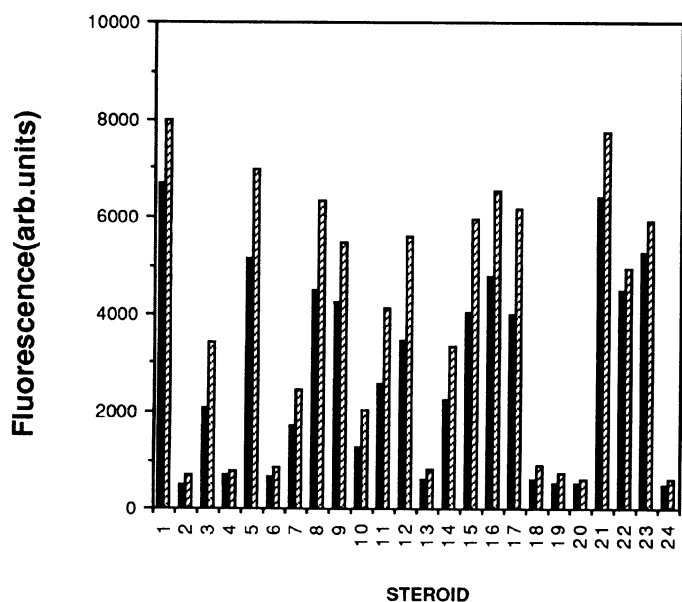
Fig. 2. Production of PSA by T-47D cells after stimulation by various steroids. In all cases, we analyzed supernatants after two stimulations at 0 and 24 h in a fetal calf serum-free culture medium, collecting supernatants at 48 h (solid bars). The cell lysates were also analyzed for PSA (hatched bars). Total protein in cell lysates was similar in all wells. The steroids tested in each well are shown in Table 1 as numbers between 1–17. Wells 18 and 24 were stimulated with absolute ethanol (the solvent), wells 19 and 20 contained cells that were not stimulated at all, and wells 21, 22, and 23 are repeats of wells 1, 5 and 8. One thousand arbitrary fluorescence units are equivalent to a PSA concentration of approximately 0.03 μg/L. In general there is concordance of PSA analysis in the supernatants and the corresponding cell lysates.

We have further examined if the estrogenic molecules estrone (steroid # 2) or estradiol (steroid # 13) were able to block the effect of stimulating molecules, if added before stimulation. We have also examined if a moderately inducing molecule, tamoxifen, which is also known to bind to the estrogen receptor, can modify the effects of more potent stimulating steroids. Some results are shown in Fig. 4. Preincubation of cells with estrone or estradiol severely impairs the stimulating effect of the two androgens. The costimulation of the cells with tamoxifen plus testosterone or dihydroandrosterone gives results which are between the stimulation of tamoxifen and either testosterone or dihydrotestosterone alone.

Stimulation experiments with the androgenic steroid dihydroandrosterone (steroid # 5) over extended periods of time were also performed in large flasks. In Fig. 5, we show that PSA production by the cell line T-47D is reproducible between flasks B,
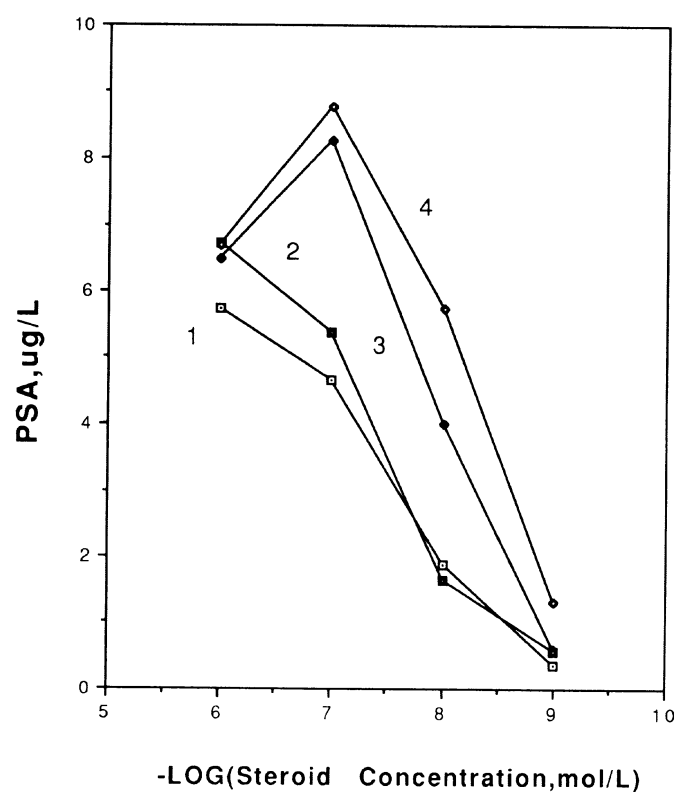

-LOG(Steroid Concentration,mol/L)

Fig. 3. Dose-response experiments using four stimulating steroids at concentrations between 10^{-9}–10^{-6} mol/L. The steroids are R1881 (1), aldosterone (2), dihydroandrosterone (3), and testosterone (4). The T-47D cell supernatants were collected 11 days post-stimulation.

C, and D, and continues for extended periods of time (up to 9 days; this experiment was not continued further). No PSA production was detected in the control flask (flask A), which was stimulated identically with absolute ethanol (the solvent of the steroid solutions) over 9 days. PSA continues to accumulate in the tissue culture supernatant. Supernatants from flasks B, C, and D were concentrated by ultrafiltration and used for characterization by HPLC and Western blot analysis.

The results of the HPLC studies are shown in Fig. 6. Highly purified PSA, immunoreactive PSA produced by T-47D cells after induction by dihydroandrosterone, PSA produced by the prostatic carcinoma cell line LNCaP, and free PSA in serum all elute at the same HPLC fraction (fraction 38) which corresponds to the molecular weight of free PSA (~ 33 kDa). We have previously shown that immunoreactive PSA produced by breast tumors also co-elutes with free PSA in serum or purified seminal plasma PSA [6]. In serum, the major immunoreactive peak elutes at fraction 30 and corresponds to a molecular
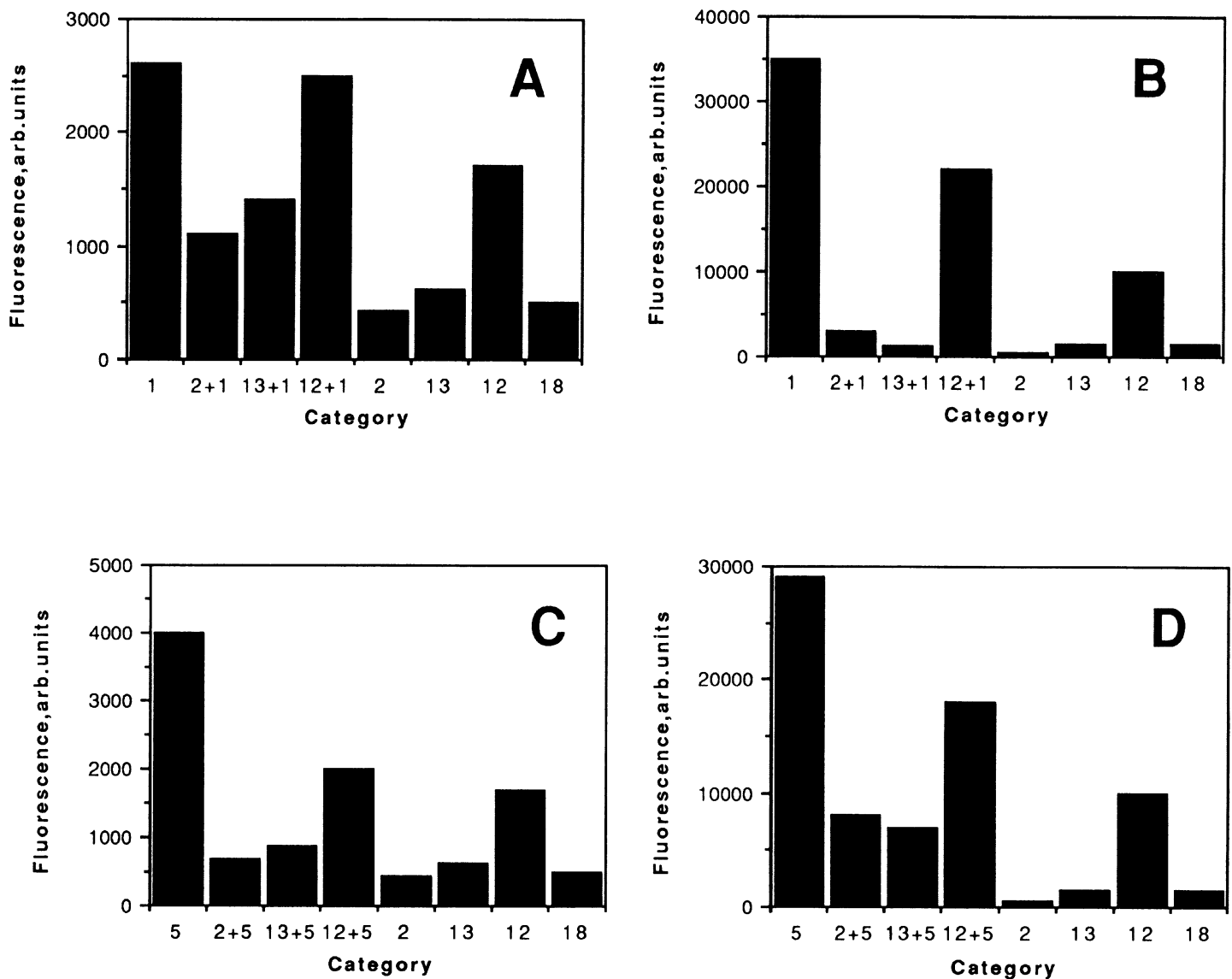
Fig. 4. Blocking of the stimulatory effects of androgens by estrogens. Molecules are coded by numbers as shown in Table 1. Single numbers indicate experiment with the steroid alone. Two numbers, e.g. 2 + 1, indicate incubation with molecule 2 (blocker) for 15 min, followed by the addition of molecule 1 (stimulant). A and C show results of analysis of supernatants at 48 h in the presence of fetal calf serum (FCS) in the culture medium. B and D show results of analysis of supernatants at 96 h without FCS in the culture medium. Analysis of cell lysates at 96 h gave results comparable to those of the supernatants and are not shown. For discussion see text.

weight of ~ 100 kDa. This immunoreactive peak corresponds to PSA bound a_1-antichymotrypsin. Another peak eluting at fraction 26 was also identified, and was described by us elsewhere [11]. In order to avoid any possibility of contamination, a new HPLC column was used and the injector was meticulously cleaned after every injection.

Western blot analysis of highly purified PSA, LNCaP cell supernatants, T-47D cell supernatants (concentrated by ultrafiltration), and breast tumor extracts positive and negative for PSA by the immunofluorometric procedure are shown in Fig. 7. Distinct bands corresponding to the molecular weight

of seminal plasma PSA are seen in the LNCaP cell supernatants, the T-47D cell supernatants, and the PSA-positive breast tumor extracts. No bands corresponding to the molecular weight of free PSA are seen in the PSA-negative breast tumor extracts. Bands corresponding to the molecular weight of seminal plasma PSA were also seen in concentrated MCF-7 cell supernatants stimulated with various inducing steroids (data not shown).
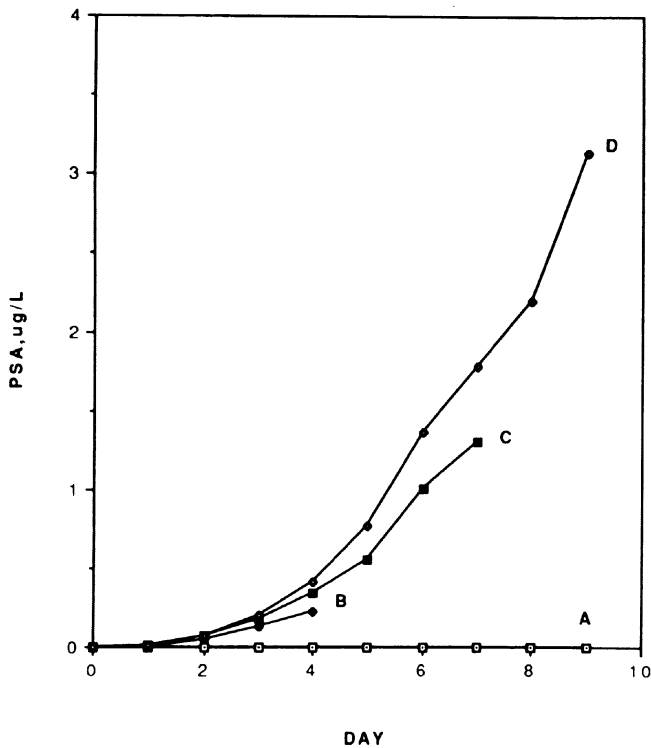
Fig. 5. Production of immunoreactive PSA by T-47D cells with the androgenic steroid dihydroandrosterone in fetal calf serum-free media for up to 9 days. Stimulation was repeated every 24 h and supernatant was removed for PSA analysis just before the stimulation. Flasks B, C, and D were stimulated identically. Flask A was stimulated only with the steroid solvent (absolute ethanol). For more details see text.

Discussion

Recently, we have demonstrated that about 30% of breast tumors contain immunoreactive prostate specific antigen (PSA), a glycoprotein that was thought to be specifically produced only by the epithelial cells of the prostate [6]. PSA presence in breast tumors is associated with the presence of estrogen and progesterone receptors, younger patient age, and early disease stage. PSA is now being evaluated as a new favorable prognostic indicator in breast cancer. In this study we demonstrate that the steroid hormone receptor-positive breast carcinoma cell lines T-47D and MCF-7 can produce PSA *in vitro*. T-47D or MCF-7 cells growing in culture do not produce measurable amounts of PSA. However, upon stimulation with a variety of steroids, PSA production is evident after 48 h. PSA continues to accumulate in the culture supernatant and can be measured either in the supernatant or in the cell lysate. In a few instances, supernatants contain-
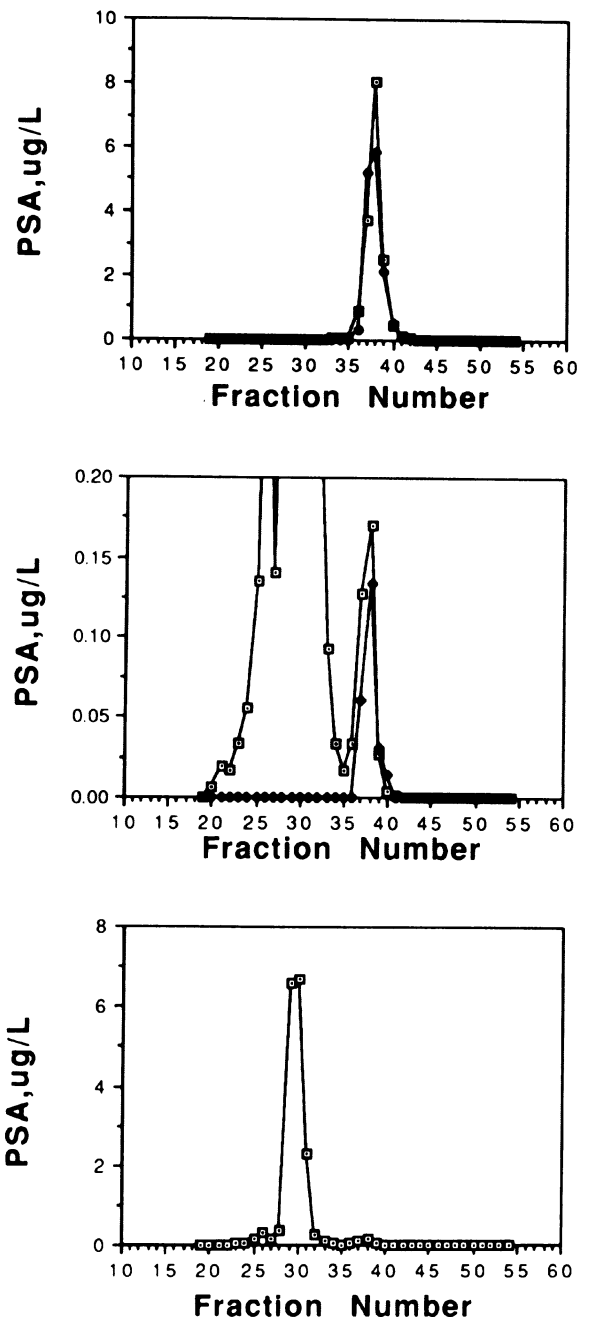

Fig. 6. Separation of PSA by high performance liquid chromatography and assay of the fractions by the time-resolved immunofluorometric procedure. Upper panel: Highly purified PSA from seminal plasma (□) and concentrated tissue culture supernatant from T-47D cells (flask C, at 7 days, see Fig. 5, preconcentrated 50-fold) (◆). Middle panel: Human serum PSA (□) and supernatant from the LNCaP prostatic carcinoma cell line (◆). The scale on the y-axis was adjusted to clearly reveal the free fraction of serum PSA. Lower panel: Human serum PSA shown with an extended y-scale. Highly purified seminal PSA, PSA from the T-47D cell supernatants, LNCaP cell supernatants, and free PSA in serum all elute at fraction 38, corresponding to a molecular weight of ~ 33 kDa. Serum PSA elutes predominantly at fraction 30 corresponding to a molecular weight of ~ 100 kDa (PSA-α_1-antichymotrypsin complex). The column was calibrated with molecular weight standards eluting at fraction 21 (thyroglobulin, 670 kDa), 28 (IgG, 158 kDa), 35 (ovalbumin, 44 kDa), 40 (myoglobin, 17 kDa), and 46 (cyanocobalamin, 1.4 kDa). For more details and discussion see text.
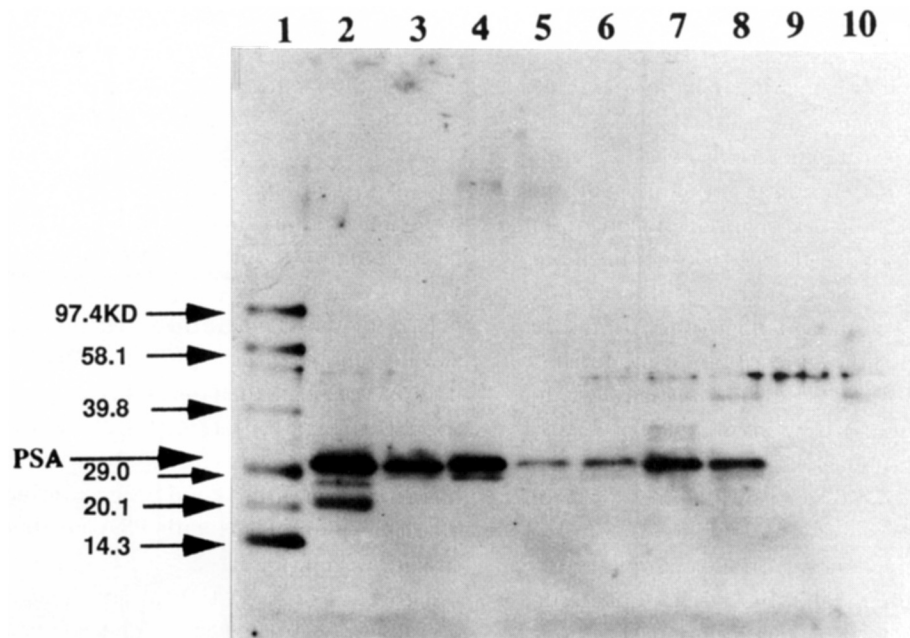
Fig. 7. Western blot analysis using a polyclonal anti-PSA antibody and chemiluminescence detection based on horseradish peroxidase. Lane 1: Molecular weight markers: Phosphorylase B (97.4 kDa); catalase (58.1 kDa); alcohol dehydrogenase (39.8 kDa); carbonic anhydrase (29.0 kDa); trypsin inhibitor (20.1 kDa); lysozyme (14.3 kDa). In brackets is the amount of PSA loaded per lane. Lane 2: Highly purified seminal plasma PSA (7 ng). Lane 3: LNCaP cell line supernatant (1 ng). Lane 4: T-47D tissue culture supernatant collected after 9 days of stimulation with dihydroandrosterone and concentrated 100-fold (3 ng). Lane 5: T-47D tissue culture supernatant collected after 7 days stimulation with the androgenic steroid R1881 and concentrated 50-fold (0.2 ng). Lane 6: As lane 5 but stimulation was with triamcinolone acetonide (0.3 ng). Lane 7: Breast tumor extract positive for PSA (700 pg). Lane 8: Another breast tumor extract positive for PSA (600 ng). Lanes 9 and 10: Two different breast tumor extracts negative for PSA. Note the visualization of PSA in identical positions in lanes 2, 3, 4, 5, 6, 7, and 8, and the complete absence of PSA in lanes 9 and 10. The molecular weight markers were biotinylated and visualized with streptavidin-horseradish peroxidase.

ing PSA levels > 0.2 µg/L were also analyzed by the IMx PSA assay (Abbott Laboratories, Chicago, IL), which confirmed the PSA results of our method [11].

Working with relatively high concentrations of steroids in the culture medium ($\sim 10^{-6}$ mol/L) we have shown that a variety of steroids including androgens, glucocorticosteroids, mineralocorticosteroids, progestins, as well as the antiestrogen tamoxifen, can induce expression of the PSA gene. Some stimulating steroids were tested at concentrations as low as 10^{-9} mol/L and were able to induce significant expression of the PSA gene even at these concentrations (Fig. 3). Notably, three estrogens failed to induce any PSA production in either of the two cell lines (Table 1). Blocking experiments have clearly shown that brief, (15 min) incubation of the cells with the estrogens estrone or β-estradiol, was

enough to severely impair the PSA production induced by androgens in T47-D cells (Fig. 4). The MCF-7 cells failed to produce high levels of PSA after stimulation by androgens. This effect may be due to either androgen receptor absence, malfunction, or to post receptor defects.

Although the PSA presence in breast tumor tissue and in the cell lines T-47D and MCF-8 is associated with steroid hormone receptors, the exact nature of the operating receptor(s) has not as yet been clarified. It is now known that the induction of gene regulation by glucocorticosteroids, progestins, androgens, and mineralocorticosteroids is mediated by a common hormone response element (HRE) in the target DNA [12]. This HRE is quite different from the HRE of the estrogen receptors. Our data are compatible with the proposal that PSA gene expression in T-47D and MCF-7 cells is mediated by

either the glucocorticosteroid, mineralocorticosteroid, progesterone, or androgen receptor bound to its cognate hormone. The receptor-steroid complex then interacts with the HRE which is associated with the PSA gene. The estrogen receptor-estrogen complex does not bind to this HRE, thus rendering estrogens inactive at the level of PSA gene regulation. Our finding that estrogens may actually block the effects of androgens is in accord with the reported ability of estrogens to bind to the androgen receptor at high concentrations [13, 14].

All measurements of PSA in this study and the previous report [6] were carried out with a highly sensitive immunofluorometric procedure which was published recently [11]. This method incorporates a monoclonal capture anti-PSA antibody and a polyclonal detection anti-PSA antibody. The method has been thoroughly evaluated and has been shown to be highly specific for PSA and to correlate well with other FDA-approved PSA methods. On many occasions, the analysis of breast tumor extracts and tissue culture supernatants from T-47D and MCF-7 cells was confirmed by the well-established and FDA-approved PSA assays including the Hybritech Tandem-E and -R assays, the IMx PSA assay, and the DPC IRMA-count PSA assay (data presented elsewhere) [6]. Further evidence for the immunoreactive compound in breast tumors and the tissue culture supernatants of stimulated T-47D and MCF-7 cells to be authenic PSA comes from our HPLC studies, which have clearly shown that highly purified PSA, immunoreactive PSA from breast tumors and stimulated T-47D cells, and PSA from the prostatic carcinoma cell line LNCaP all elute as single sharp peaks corresponding to the molecular weight of seminal PSA. The free serum PSA also elutes in the same fraction. Further evidence comes from Western blot analysis using SDS-PAGE. In this experiment, it was also found that highly purified PSA, PSA produced by LNCaP, T-47D, and MCF-7 cells, and breast tumor extracts positive for PSA all contain a sharp band corresponding to the molecular weight of seminal PSA ($\sim$ 33 kDa). This band was absent from breast tumor extracts which were found negative for PSA by the immunofluorometric procedure. Notably, three different steroids (dihydroandrosterone, R1881,

and triamcinolone acetonide) produce the same immunoreactive band in Western blots (Fig. 7).

Based on the data presented here and elsewhere [6] we believe that it is unlikely that the immunoreactive PSA identified in breast tumor extracts and T-47D and MCF-7 cells, reactive with many monoclonal and polyclonal antibodies incorporated in FDA-approved PSA assays, and exhibiting the same molecular weight as seminal PSA, is a molecule that is homologous but different from PSA of prostatic origin. However, final positive identification of the immunoreactive species by protein sequencing after purification of sufficient quantities must await further experimentation.

PSA appears to be a good prognostic indicator in breast cancer. Patients with PSA-positive tumors are a subset ($\sim$ 30% of patients) among the patients with steroid hormone receptor-positive tumors ($\sim$ 50–80% of all patients). This subset is able to complete a series of events starting from the formation of a steroid-receptor complex and ending at the level of PSA gene expression. The reason why many steroid hormone receptor-positive tumors do not produce PSA is unknown, but the following possibilities exist: (a) the inducing ligands are missing, (b) the receptors are defective, (c) competing steroids, e.g. estrogens, counterbalance the effects of the stimulating steroids. Although speculative, there is a possibility that the tumors producing PSA are the ones likely to respond to endocrine treatment. Preliminary clinical data have recently been reported by us [15]. Tamoxifen, a widely used antitumor drug, was found to induce PSA production, but the mechanism by which the effect is mediated is unknown. It will be of interest to test the effects of other antiestrogens and to establish if their pharmacological anticancer potency correlates with their ability to induce PSA production *in vitro*, using T- 47D or MCF-7 cells. Although tamoxifen is known to block the estrogen receptor, rendering estrogens inactive, we here show that a tamoxifen-receptor complex may act at the level of DNA, regulating the PSA and possibly other genes.

Our tissue culture findings using T-47D and MCF-7 cells further support and extend our previous report on PSA production by breast tumors and offer an *in vitro* system which can be used to study

the phenomenon for possible application in prognosis and design of new therapy.

Acknowledgement

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