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Both Androgen Receptor and Glucocorticoid Receptor Are Able to Induce Prostate-Specific Antigen Expression, but Differ in Their Growth-Stimulating Properties of LNCaP Cells*

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ABSTRACT

Androgen receptor-positive LNCaP cells were stably transfected with a rat glucocorticoid receptor (GR) expression plasmid. Ligand-binding studies in the generated cell lines revealed high-affinity binding of the cognate ligands to their receptors. Transfection experiments with the newly derived cell lines showed that, like androgen receptor, GR can induce activity of a prostate-specific antigen promoter fragment linked to the luciferase gene. Similarly, dexamethasone can stimulate expression of endogenous prostate-specific antigen messenger RNA. Cell proliferation could be induced by R1881. In contrast, dexamethasone treatment of the GR-positive sublines had no stimulatory effect on cell growth. Using the differential display technique, a so far unknown complementary DNA fragment, designated 21.1, specifically induced by androgens and not by glucocorticoids, has been identified. In conclusion, the newly generated cell lines, together with the parental LNCaP cell line, form an attractive system with which to study the mechanism of specificity of steroid hormone regulation of gene expression. (*Endocrinology* **138:** 5293–5300, 1997)

STEROID HORMONES affect many biological activities of the cell by modulating gene activity via interaction with specific nuclear receptors (1–3). Upon ligand binding, steroid receptors interact with specific DNA sequences and regulate the transcriptional activity of target genes (1–4). The glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), and androgen receptor (AR) bind with high affinity to a DNA element composed of an inverted repeat, separated by a 3-bp spacer sequence. The consensus high- affinity binding site for GR, MR, PR, and AR (HRE: hormone response element) is identical (5–8). Although there are genes that can be regulated by more than one specific steroid hormone receptor, the biological function of the different receptors is quite distinct. This presents the problem of specific gene activation in the event that multiple receptors, which recognize the same DNA-binding site, are present in one and the same cell. Several mutually nonexclusive mechanisms to explain specificity, including subtle differences in receptor-DNA interaction, specificity of the interaction of the receptor with other proteins, receptor levels, and ligand availability, have been proposed (1–3).

LNCaP is an androgen-sensitive human prostatic carcinoma cell line (9) that expresses AR, but lacks GR and PR (10, 11). It was shown previously that growth of LNCaP cells, maintained in steroid-depleted culture medium, is stimulated by the addition of androgens (11). The synthetic androgen R1881, at a concentration of 10^{-10} M, increases the growth rate; a higher R1881 concentration is suboptimal to cell proliferation and might even have no stimulatory effect. Androgen treatment of LNCaP cells increases the messenger RNA (mRNA) level and production and secretion of prostate-specific antigen (PSA) (12–14).

In the present study we describe the generation and initial characterization of LNCaP sublines, in which the GR was stably expressed. In this way a system was established for comparison of AR and GR molecular and biological functions in a well defined cell line expressing comparable amounts of both receptors.

Materials and Methods

Cell culture

LNCaP prostate cells were cultured in RPMI 1640 supplemented with 5% FCS (Boehringer, Mannheim, Germany) and antibiotics. For transfection, cells were grown in DMEM supplemented with 5% FCS.

Plasmids and probes

The rat GR expression plasmid PSTC-GR(3–795) and the selection plasmid pSV_2Neo were described previously (15, 16). PSA-61-LUC was generated by integration of a 6-kb *Hin*dIII-*Hin*dIII (−6000/+12) fragment of the PSA promoter in the multiple cloning site of pLUC (17). Southern and Northern blots were hybridized with a rat (r) GR complementary DNA (cDNA) fragment (nucleotide 2256–2543) obtained by PCR amplification with primers: 5′-GAGTCTCACAAGACACTTCG-3′ and 5′-GAAACATCCATGAGTACTG-3′ and plasmid PSTC-GR(3–795) as template using standard methods. Northern blots were hybridized with the 320-bp *Eco*RI-*Cla*I fragment of PSA75 cDNA (18), and a 1.2-kb *Pst*I-*Pst*I hamster actin cDNA fragment as a control.

Transfections

Stable transfection. LNCaP cells were transfected according to the calcium phosphate precipitation method essentially as described (19), using 3 ×

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10^6 cells per 10-cm dish, 10 μg PSTC-GR(3–795) and 2 μg pSV_2Neo. After overnight incubation with the precipitate, the culture medium was replaced by PBS, containing 15% glycerol (incubation for 90 sec at room temperature). Subsequently, transfected cells were incubated in culture medium for 24 h. Next, culture medium was replaced by maintenance medium supplemented with G418 (GIBCO BRL, Grand Island, NY) at a concentration of 1.2 mg/ml. The resulting G418-resistant clones were seeded into 96-well plates and selected for GR expression by immunohistochemistry (see below).

Transient transfection. The GR-positive clones LNCaP-1B7 and LNCaP-1F5 and the parental LNCaP cells were transiently transfected according to the calcium phosphate method using 1×10^6 cells per 25-cm^2 flask and 5 μg PSA-61-LUC. After overnight incubation with the precipitate, the culture medium was removed and cells were shocked in PBS, containing 15% glycerol (90 sec at room temperature). Subsequently, transfected cells were incubated in culture medium in the absence or presence of 10 nM of the synthetic glucocorticoid dexamethasone (Dex) (Sigma, St. Louis, MO) or 1 nM R1881 (DuPont NEN, Boston, MA) for 24 h. Transfections were performed three times in duplicate, using two independent plasmid isolates. Luciferase activities were corrected for variations in protein concentrations in 100-μl cell extract samples.

Luciferase assay

Cells were washed once in PBS and subsequently lysed in 300 μl lysis buffer (25 mM Tris-phosphate, pH 7.8, 8 mM $MgCl_2$, 1 mM dithiothreitol, 1% Triton X-100, 15% glycerol). Next, 100 μl 0.1 μM luciferin (Sigma)/0.25 μM ATP were added to 100 μl extract, and luciferase activity was measured in a LUMAC 2500 M Biocounter (LUMAC, Landgraaf, The Netherlands). After a delay of 2 sec (according to the supplier), the light emission during 5 sec was recorded.

Immunohistochemistry

Immunostaining for GR was performed with the monoclonal anti-GR (rat) antibody Mab 7 (20). Cells were seeded at a density of 3×10^5 cells per well on sterile micro slides in four-well tissue culture plates (Heraeus Instruments, Hanau, Germany) in maintenance medium supplemented with G418 and cultured until 50–60% confluence. Next, 10 nM Dex was added, and the incubation was continued for 24 h. Cells were washed in PBS and fixed for 10 min in 10% phosphate-buffered formalin (pH 7.4). Subsequently, the slides were rinsed in PBS (pH 7.4), and attached cells were made permeable in methanol (−20 C, 5 min) and acetone (−20 C, 2 min). After rehydration in PBS, the slides were incubated in 5% nonimmune rabbit serum in PBS followed by overnight incubation in 1:1000 diluted monoclonal antibody Mab7 at 4 C. Excess antibody was removed, and rGR immunoreactivity was visualized using rabbit anti-mouse Ig (DAKO, Glostrup, Denmark) as secondary antibody and mouse monoclonal peroxidase antiperoxidase complexes (DAKO) as third-step reagent. After three PBS washes, the slides were incubated with diaminobenzidine. The reaction was stopped in water. Cells were counterstained with Mayers hematoxylin.

Southern and Northern blot analysis

Total cellular DNA of LNCaP- and GR-positive sublines LNCaP-1F5 and LNCaP-1B7 was isolated using standard procedures (21). Ten micrograms of DNA were digested with *Eco*RI for 16 h, electrophoresed on 0.8% agarose gel, and transferred to a Hybond N^+ membrane (Amersham, Cardiff, UK). Filters were hybridized at high stringency with random primed ^{32}P-labeled probes. Both DNA transfer and filter hybridization were carried out according to the protocol of the manufacturer.

Isolation of total cellular RNA from the different cell lines was carried out by the guanidinium thiocyanate method (22). Glyoxal denatured RNA (10 μg/lane) was separated by electrophoresis on a 1% agarose gel and transferred to a nylon membrane (Gene Screen, DuPont NEN, Boston, MA). The blot was hybridized with random primed ^{32}P-labeled rGR, PSA, or actin cDNA probes in 50% formamide at 42 C, using standard conditions.

Radioligand binding assay

Cells were cultured in maintenance medium until 50–60% confluence. To deplete for steroids, cell culturing was continued in RPMI 1640 supplemented with dextran-charcoal-stripped (dcc) serum for 48 h.

[^{3}H]-R1881 (87 Ci/mmol) and unlabeled R1881 (methyl-trienolone) were purchased from DuPont NEN. [^{3}H]-Dex (94 Ci/mmol) was obtained from Amersham. For radioligand-binding analysis, cells were rinsed in PBS and harvested as a single-cell suspension by trypsinization. Cells were washed four times, counted, and resuspended in ice-cold PBS. To measure the cellular AR and GR content and ligand affinity of both receptors, cells were incubated with serial [^{3}H]R1881 dilutions (0.125–16 nM, in the absence and presence of a 100-fold molar excess of unlabeled R1881) or [^{3}H]Dex dilutions (0.5–32 nM, in the absence and presence of a 100-fold molar excess of unlabeled Dex) for 90 min at room temperature. Free steroid was removed by extensive washing of the cells in ice-cold PBS. Radioactivity was measured in a scintillation counter. Specific binding of [^{3}H]R1881 and [^{3}H]Dex was calculated by subtraction of nonspecifically bound radioactivity from total bound radioactivity and used for Scatchard analysis with the radioligand-binding analysis program Ebda/Ligand by G. A. Pherson from Elsevier-BIOSOFT (Cambridge, UK). All assays were performed in triplicate.

Cell growth studies

Cells were trypsinized, seeded in RPMI medium containing 5% FCS in 25-cm^2 tissue culture flasks at a density of 5×10^4 cells, and cultured for 3 days. Subsequently, medium was replaced by medium containing 5% dcc serum, and the incubation was continued for an additional 3 days. At this time point (t = 0), medium was replaced by RPMI medium containing 5% dcc serum and indicated hormones at different concentrations. R1881 was added to final concentrations of 10^{-11}, 10^{-10}, and 10^{-9} M; Dex was added to final concentrations of 10^{-10}, 10^{-9}, and 10^{-8} M, respectively. Control cultures without steroids were supplemented with 0.1% (vol/vol) ethanol. At day 4, medium was renewed. At day 8, cells were washed in PBS and trypsinized, and the cell number in each tissue culture flask was determined using a Bürker's cell-counting chamber. Experiments were performed in triplicate.

Identification of differentially expressed genes

The mRNA Differentially Display PCR (DD-PCR) procedure used was essentially identical to van Belzen *et al.* (23). In short, LNCaP-1F5 cells were cultured in maintenance medium until 50–60% confluence and for an additional 72 h in RPMI 1640 supplemented with 5% dcc serum. After this period, the medium was replaced by RPMI medium containing 5% dcc serum supplemented with 1 nM R1881, or 10 nM Dex, or without hormone, and cells were incubated for an additional 24 h. Isolation of total cellular RNA from the different cell lines was carried out according to Chirgwin *et al.* (22). First-strand cDNA was synthesized from 1 μg total RNA with M-MuLV RT and a mix of three anchored oligo-T primers: 5′-TTTTTTTTTTTG-3′, 5′-TTTTTTTTTTTC-3′, and 5′-TTTTTTTTTTTA-3′. The resulting cDNAs from RNA of LNCaP cells grown under the three different growth conditions were amplified for two cycles at low stringency annealing conditions (1 min at 94 C, 5 min at 35 C, 5 min of gradual heating from 35 C to 72 C, and 5 min at 72 C), followed by 30 cycles at high stringency annealing (1 min at 94 C, 2 min at 50 C, and 2 min at 72 C) using a 15- to 25-nucleotide arbitrary primer. All reactions were performed in triplicate. The resulting PCR products were separated on a 7.5% polyacrylamide gel containing 7 M urea. The dried gel was exposed to x-ray film. Bands of interest were eluted from gel by incubation for 1 h at 65 C in TE (10 mM Tris-HCl, pH 7.4; 1 mM EDTA). Subsequently, the eluted fragments were amplified by an additional PCR step (30 cycles: 1 min at 94 C, 2 min at 50 C, and 2 min at 72 C). The amplified cDNAs were cloned in pGEM-T (Promega, Madison, WI), and sequenced.

Results

Generation of GR-expressing LNCaP transfectants

LNCaP cells were transfected with the available rat GR expression vector PSTC-GR and the pSV_2Neo selection plas-

mid. Because of the high structural homology between the rat and human GR, human GR is expected to give identical results. Cells were grown in medium supplemented with G418 to select for transfected cells. After 3 weeks, G418-resistant clones were selected. Clones were immunohistochemically stained with the GR antibody MAb 7. Of 60 G418-resistant clones, five showed strong reactivity with the antibody, indicating high GR expression. Thirty-two clones showed low or heterogeneous GR expression, whereas in the remaining clones, no GR immunoreactivity was observed. Staining of two clones with high levels of GR expression (LNCaP-1B7 and LNCaP-1F5) is shown in Fig. 1, A and B; staining of the parental LNCaP cells for GR was negative (Fig. 1C). Note that the morphology of GR+ sublines is different from that of the parental cells, indicating a thus far unexplained specific effect of the activated GR on cell physiology. LNCaP-1B7 and LNCaP-1F5 were selected for more detailed studies.

Characterization of LNCaP-1B7 and LNCaP-1F5

Southern blot analysis of *Eco*RI-digested genomic DNA isolated from the LNCaP-1B7 and LNCaP-1F5 transfectants demonstrated the presence of one or more copies of the complete CMV-GR cDNA fragment in both clones (lanes 2 and 3, Fig. 2A; indicated by an *arrow*). The rat GR probe showed cross-hybridization to two fragments corresponding to the endogenous human GR gene (bands a and b; see also lane 1, containing parental LNCaP DNA). Both LNCaP-1B7 and LNCaP-1F5 DNA contained also at least one incomplete PSTC-GR fragment (bands c and d). Densitometric scanning of the blot revealed the presence of 6 to 7, and two complete copies of CMV-GR cDNA in LNCaP-1B7 and LNCaP-1F5, respectively.

Northern blot analysis of LNCaP-1B7 and LNCaP-1F5 RNA showed expression of GR mRNA of the expected size (2.7 kb) in both clones; no hybridization signal was observed in the parental LNCaP cell line (Fig. 2B). The presence of more GR mRNA in LNCaP-1F5 as compared with LNCaP-1B7 cells, despite the higher number of integrated GR cDNA copies in LNCaP-1B7 cells, can be due to different genomic integration sites. Radioligand-binding assays on LNCaP, LNCaP-1B7, and LNCaP-1F5 cells were performed to establish the number of GR and AR molecules per cell and the respective dissociation constants for both receptors (Fig. 3).

The parental LNCaP cell line showed for R1881 a B_{max} of 75 pM, which is equivalent to approximately 15,000 AR molecules per cell (see legend to Fig. 3). LNCaP-1B7 and LNCaP-1F5 contained approximately 30,000 and 32,000 AR molecules per cell, respectively. This 2-fold higher level of AR in the newly generated cell lines in comparison to the parental LNCaP is probably due to a small variation in AR content in the parental LNCaP cells. All three cell lines showed an identical binding affinity for R1881 (K_d 1.1 nM).

As expected, in the parental LNCaP cell line, binding of Dex could not be detected. From the B_{max} values it could be deduced that LNCaP-1B7 contains approximately 31,000 GR copies per cell, and LNCaP-1F5 contains 115,000 copies. The K_d for Dex binding was 3.1 nM in both LNCaP sublines.

In the four *lower panels* of Fig. 3, the values obtained at the two highest ligand concentrations deviate from the calculated straight line. The apparent curvilinearity of the Scatchard data are most likely due to the low percentage of specific binding at high ligand concentrations, which makes these data less reliable. The program used for Scatchard and linear regression analyses took the differences in specific binding into account. An alternative explanation, interaction between GR and AR, is less likely, because experiments were done in the presence of one hormone at a time (or R1881 or Dex).

GR activity in GR-positive LNCaP sublines

To investigate whether the GR present in LNCaP-1B7 and LNCaP-1F5 cells was functionally active, the cells were transiently transfected with PSA-61-LUC, which contains a 6-kb PSA promoter fragment. This promoter contains a strong androgen-dependent enhancer region, approximately 4.2 kb upstream of the transcription start site of the PSA gene (17, 24). As expected, PSA-61-LUC showed clear, R1881-induced

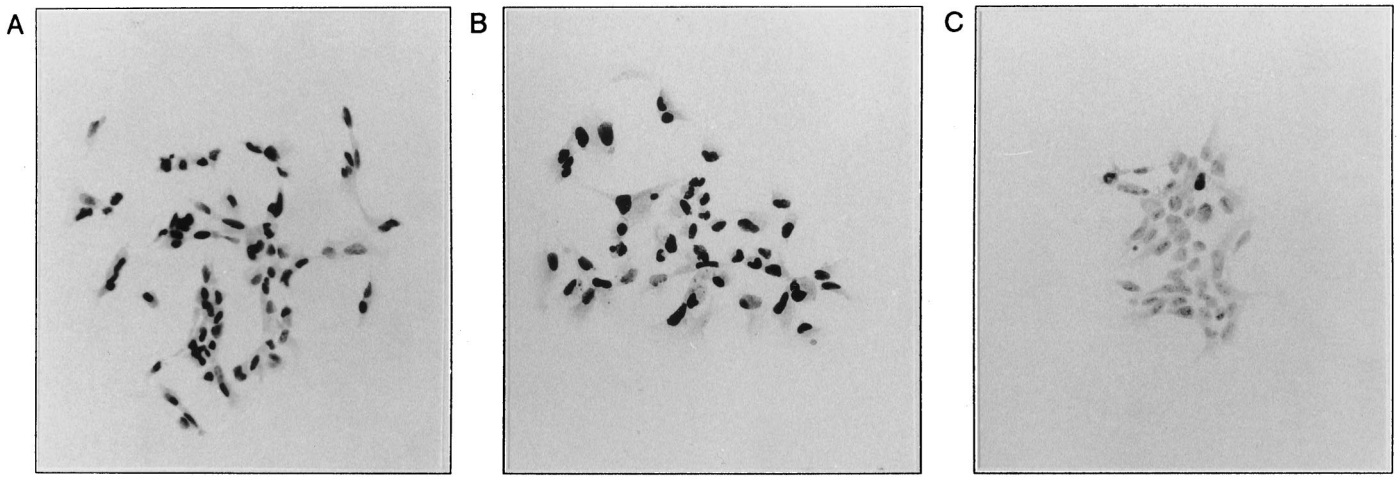

FIG. 1. Immunohistochemical staining of GR-positive LNCaP sublines LNCaP-1B7 (A), LNCaP-1F5 (B), and the parental LNCaP cells (C) with the anti-rat-GR monoclonal antibody Mab7, using the indirect PAP technique (magnification 200 ×). Cells were counterstained with Mayer's hematoxylin. Before immunohistochemical analysis, cells were cultured for 24 h in the presence of Dex.

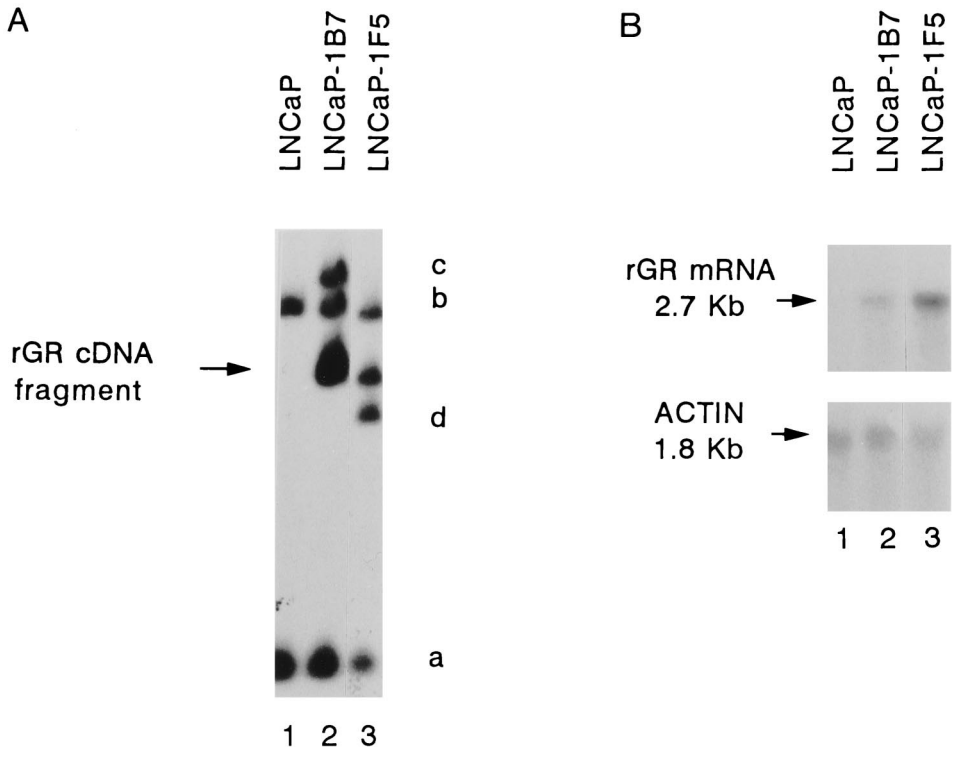

FIG. 2. Southern and Northern blot analysis of the integrated GR cDNA (A), and GR mRNA expression (B) in GR+ LNCaP sublines and parental LNCaP cells. A, Southern blot analysis of *Eco*RI-digested genomic DNA from LNCaP (lane 1), LNCaP-1B7 (lane 2), and LNCaP-1F5 (lane 3). DNA (10 μg/lane) was hybridized with a rat GR cDNA probe, homologous to human GR cDNA. Bands a and b, Endogenous human GR gene; bands c and d, integrated, partial rGR cDNA fragments. The *arrow* indicates the position of integrated, complete CMV-GR cDNA copies. B, Northern blot analysis of 10 μg total RNA from LNCaP (lane 1), LNCaP-1B7 (lane 2), and LNCaP-1F5 (lane 3) hybridized with a rat GR cDNA probe. The *lower panel* shows β-actin mRNA expression.

luciferase activity in transfected parental LNCaP cells. No luciferase activity was detected upon incubation of the PSA-61-LUC-transfected parental LNCaP cells with Dex (Fig. 4). Transient transfection of LNCaP-1B7 and LNCaP-1F5 cells with the PSA-61-LUC construct resulted in a comparable R1881-induced luciferase activity, both in absolute luciferase activity and in induction level (1310- and 1940-fold, respectively). Dex induced a slightly higher PSA-61-LUC activity, clearly indicating the transactivating capacity of the GR encoded by the stably integrated rGR expression vector (Fig. 4).

Regulation of endogenous PSA mRNA expression

In previous studies we and others (12–14) have shown that PSA mRNA expression is induced upon androgen incubation of LNCaP cells. To investigate whether GR can replace AR in induction of the endogenous PSA gene, we performed Northern blot analysis with RNAs isolated from the parental LNCaP cell line, and from LNCaP-1B7 and LNCaP-1F5 cells, grown in the absence and in the presence of hormone (10 nM Dex or 1 nM R1881). Hybridization with a PSA-specific cDNA probe demonstrated that GR can replace AR in high, steroid hormone-induced expression of the PSA gene (Fig. 5). In the parental LNCaP cell line, PSA mRNA expression was induced by R1881 but not by Dex. In contrast, in the two GR-positive clones, Dex treatment clearly resulted in stimulation of PSA mRNA expression, although to a slightly lower level (approximately 3-fold) than R1881 up-regulated PSA mRNA.

Regulation of cell proliferation

Growth of LNCaP cells depends on androgens in a concentration-dependent fashion. Previous studies indicated a bell-shaped dose-response curve for the stimulatory effect of androgens (11). Maximal growth stimulation of R1881 was observed at a concentration of 0.1 nM. To find out whether GR was able to replace AR in growth regulation of LNCaP cells, we compared the effects of different R1881 and Dex concentrations on growth of LNCaP cells and of the two GR-positive LNCaP sublines. At day 8 after addition of hormone, all three cell lines showed a clear growth stimulation upon treatment with 0.1 nM R1881 and to a somewhat lower extent at 1 nM R1881. However, at none of the tested Dex concentrations was a growth-stimulatory effect observed (Fig. 6). Similar negative results were obtained with hydrocortisone (data not shown). These results clearly indicated that GR was unable to replace AR in growth stimulation of LNCaP cells.

Detection of androgen-specific gene expression in LNCaP-1F5 cells

Using the DD-PCR method, a search for novel, differentially expressed genes in the LNCaP-1F5 subline, grown in the presence of R1881, or Dex, or in the absence of hormone, was initiated. Using 35 different arbitrary PCR primers, one fragment that was differentially expressed was detected. The fragment, designated 21.1, was amplified from RNA of LNCaP-1F5 cells, cultured in the presence of R1881 (*arrow* in Fig. 7A), and not from RNA of Dex- incubated LNCaP-1F5 cells, or cells incubated in the absence of hormone. Using fragment 21.1 as a hybridization probe, on a Northern blot of LNCaP-1F5 RNA, a mRNA of approximately 5 kb was detected, and the differential expression pattern was confirmed (Fig. 7B). The sequence of the 504-bp 21.1 fragment is available in GenBank under accession number AF007835.

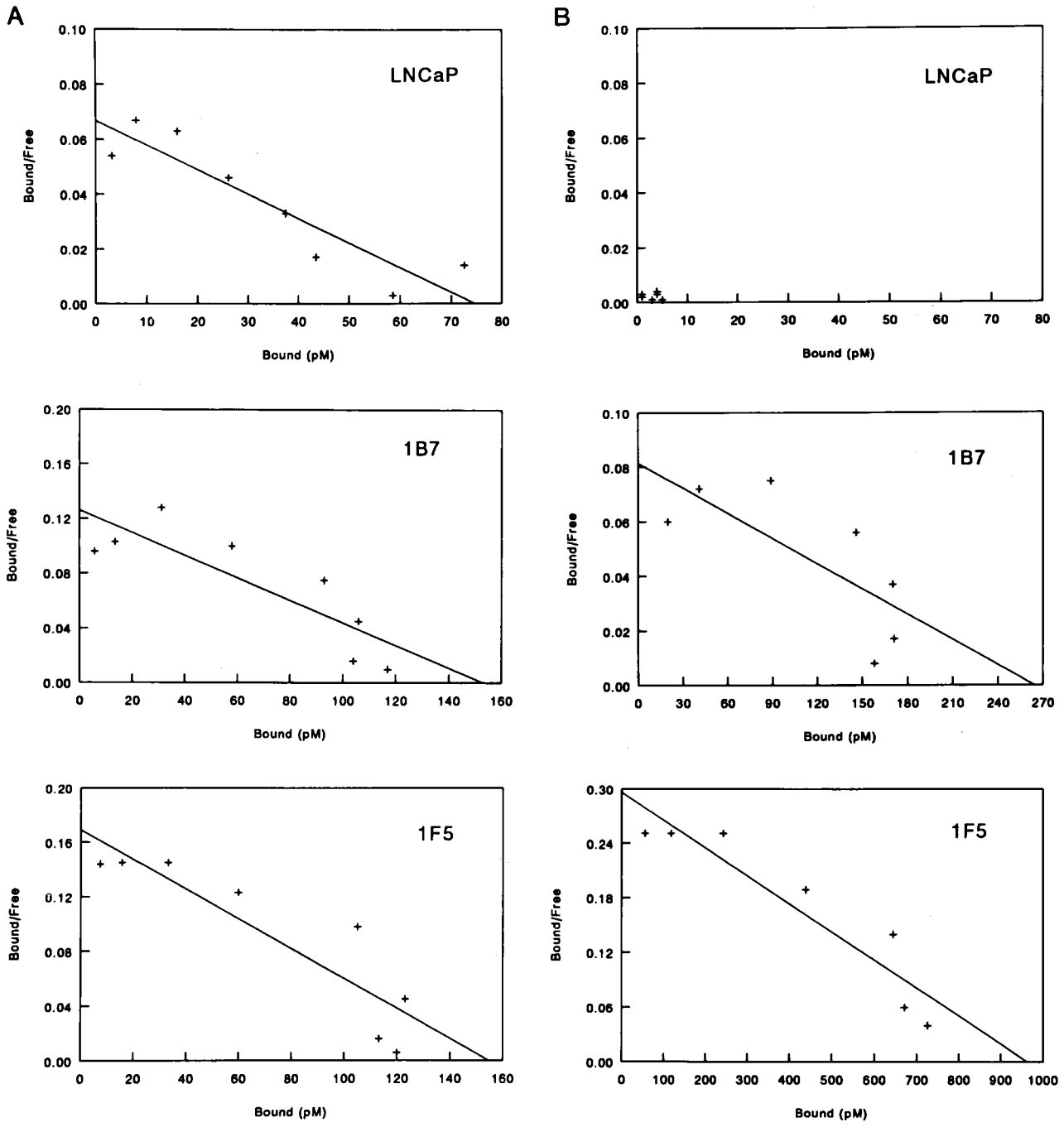

FIG. 3. Analysis of glucocorticoid- and androgen-binding activity in parental LNCaP and the GR transfectant 1B7 and 1F5 cells. Scatchard representation of R1881-binding activity (panel A) and Dex-binding activity (panel B) of LNCaP, LNCaP-1B7, and LNCaP-1F5 cells. Cellular AR and GR concentrations were deduced from (B_{max} (M) $\times$ N_{av})/number of cells per liter (3×10^9 in panel A and 5×10^9 in panel B). Receptor-bound ^{3}H-labeled steroid was measured by a whole cell assay in the presence and absence of a 100-fold molar excess of unlabeled hormone. The values shown are the mean of a triplicate experiment and represent specific binding after subtraction of nonspecific binding.

Discussion

In this paper we describe the generation and properties of LNCaP sublines that express not only AR but also GR. These novel cell lines facilitate the direct comparison of GR and AR effects on cellular functioning. Furthermore, they can be employed for investigation of interference between GR- and AR-activated molecular and biological processes. We demonstrated that AR- and GR-positive cells behave identically in up-regulation of the expression of a transfected PSA promoter-driven reporter gene, and the expression of the endogenous PSA gene. Interestingly, they were found to differ in hormone- induced effects on cell proliferation. Additionally, one novel, differentially expressed gene was identified.

At least part of the effects of steroid hormone receptors on

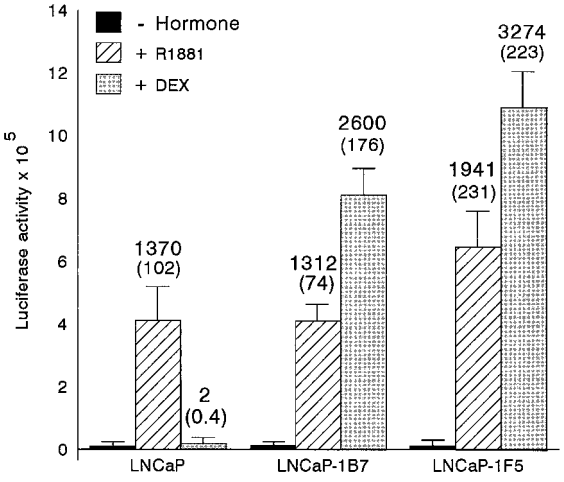

FIG. 4. R1881 and Dex regulation of the PSA promoter activity in LNCaP and GR- positive LNCaP sublines. LNCaP, LNCaP-1B7, and LNCaP-1F5 cells were transfected with the PSA-61-LUC reporter gene construct as described in *Materials and Methods*. After overnight incubation with the precipitate, cells were incubated for 24 h either in the presence or absence of 1 nM R1881 or 10 nM Dex. Activity in the absence of hormone is indicated by a *solid bar*; activity in the presence of R1881 is shown by a *hatched bar* and in the presence of Dex by a *gray bar*. Fold-induction is displayed on *top* of the bars. The absolute activity and relative induction factor were calculated as the mean of three independent transfection experiments, which were all done in duplicate. The SE of the mean (SEM) of the absolute activity is represented by a *vertical stripe*; the SEM of induction is given in *parentheses*.

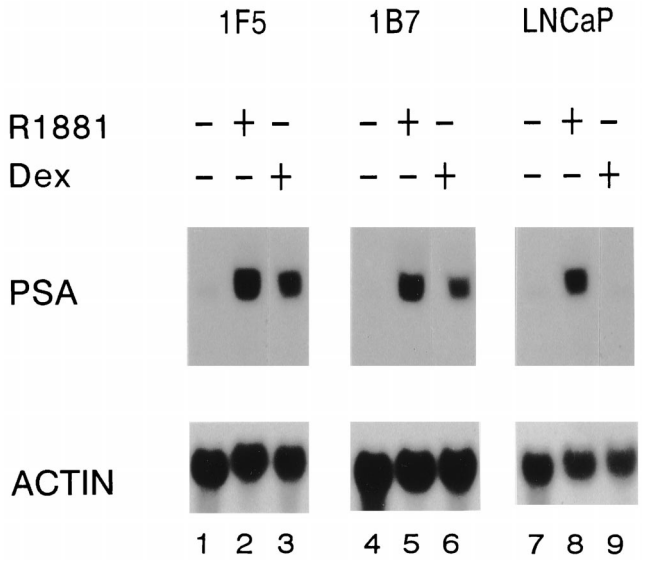

FIG. 5. Expression of the rat GR renders the endogenous androgen-regulated PSA gene inducible by Dex in the LNCaP sublines 1F5 and 1B7. Northern blot analysis of 10 μg total RNA of LNCaP-1F5 (lanes 1–3), LNCaP-1B7 (lanes 4–6), and LNCaP (lanes 7–9) cells hybridized with a PSA cDNA probe. Cells were treated for 24 h with 10 nM Dex (lanes 3, 6, and 9), 1 nM R1881 (lanes 2, 5, and 8), or were grown in the absence of hormone (lanes 1, 4, and 7). The *lower panel* shows hybridization of the β-actin cDNA probe as a control.

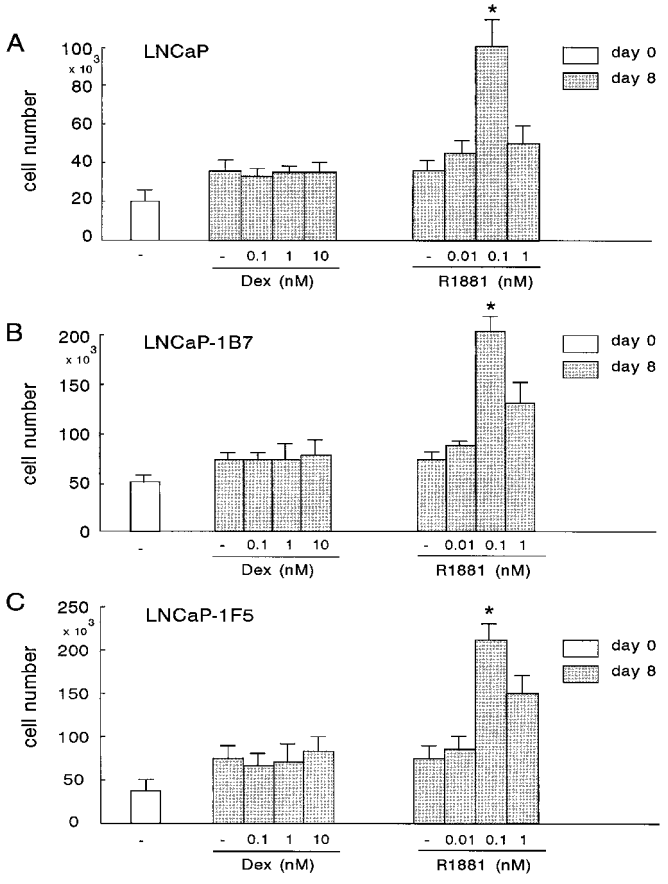

FIG. 6. Effects of Dex and R1881 on cell growth of parental LNCaP, and the GR+ 1B7 and 1F5 cells. At day 0, and at day 8 of incubation in the absence or presence of hormone, cells were washed, trypsinized, and counted. The values shown are the mean of experiments in triplicate; the SEM is represented by a *vertical stripe*. *, $P < 0.05$ compared with cells grown in the absence of hormone, or in the presence of different Dex concentrations.

gene expression is on transcription initiation. Upon ligand binding, steroid receptors interact with specific DNA sequences (HREs) and regulate the transcription of target genes. The GGT/AACAnnnTGTTCT consensus sequence for high-affinity DNA binding of GR, MR, PR, and AR is identical (5–8). Despite this common DNA-binding site, the different receptors mediate distinct cellular responses. Many independent mechanisms to achieve specificity of the steroid hormone response have been proposed (see for reviews Refs. 3 and 25). These include differential affinities to natural receptor-binding sites, or to binding sites in their natural DNA context (26–30), differential affinity to general and specific transcription factors (31–34), differences in interaction with receptor-specific accessory proteins or coactivators (35–37), differential modification of specific chromatin structures (38, 39), differences in cellular concentration of the specific receptors (40), and variations in ligand availability (41, 42).

The observation that PSA mRNA was Dex inducible in the GR-expressing LNCaP sublines showed that the steroid receptor content determines, at least in part, the specific activation of the PSA gene in wild-type LNCaP cells. Transient transfection of the 6-kb PSA promoter to the LNCaP sublines resulted also in activation by both AR and GR. In contrast, in the case of the MMTV promoter, differences have been reported on the effect of GR and PR on transiently transfected reporter gene constructs and stably integrated plasmids (38,

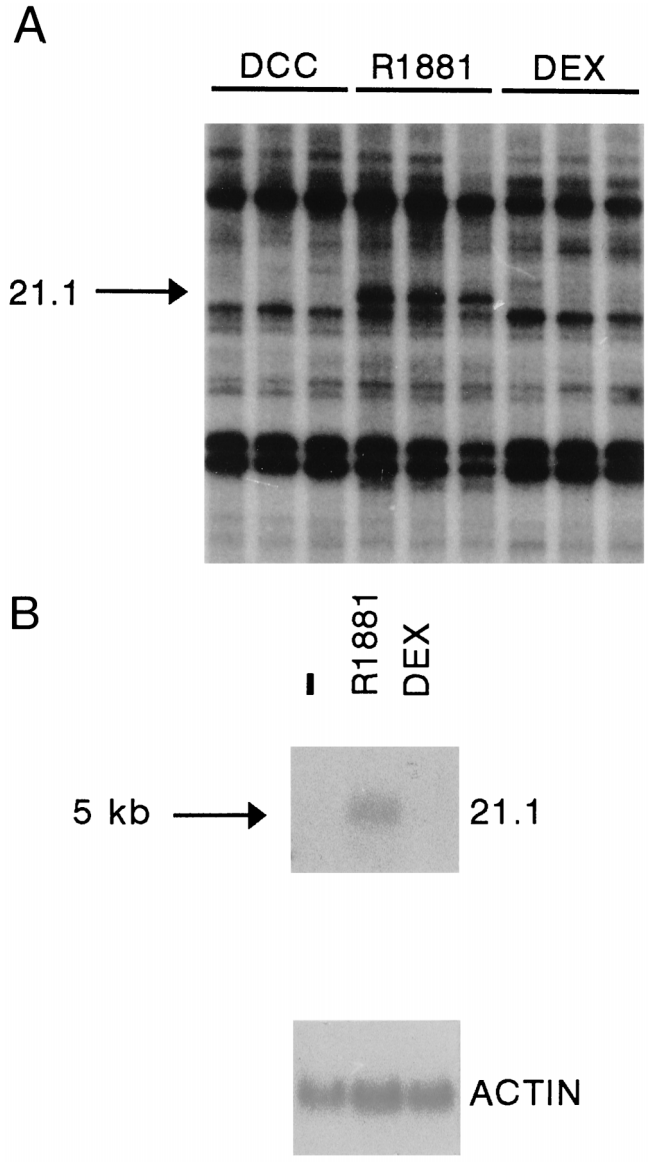

FIG. 7. Identification and regulation of expression of 21.1 mRNA. A, DD-PCR was performed on LNCaP-1F5 RNA as described in *Materials and Methods*, using primer 5′-ATGACAGCCTGGTTG-3′. Experiments were done in triplicate. The *arrow* indicates the position of band 21.1, which is present in the R1881 lanes and absent in the Dex and minus hormone lanes. B, Northern blot hybridization of 10 μg RNA from LNCaP-1F5 cells grown in the presence of 1 nM R1881, or 10 nM Dex, or in the absence of hormone, using purified fragment 21.1 as a probe. The *lower panel* shows the control hybridization, using the β-actin probe.

39). It appears that the native chromatin structure can prevent PR activation and permits GR stimulation of the stably integrated target gene.

AR activation of the PSA promoter involves at least three androgen response elements, two in the 600-bp proximal promoter region and one in a far upstream enhancer region (17, 43). Although ultimate PSA promoter induction is comparable for AR and GR, it cannot be excluded as yet, that AR and GR affect the individual regulatory regions differentially. Such an observation has been made for GR and PR activation of the MMTV promoter, which contains four HREs. Differences might be accounted for by distinct chromatin structures over the individual HREs, and the ability of the different receptors to affect these structures, and/or the interaction with other specific transcription factors (34).

As shown above, in contrast to normal human prostate tissue, GR expression cannot be detected in wild type LNCaP cells (44). However, in normal prostate highest GR expression is in the stromal compartment. In epithelial cells expression of AR appears much higher than GR expression. In prostate cancer, GR expression seems even to decrease. Therefore, GR might not be a major factor in PSA expression. No data are available relating to PR expression in prostate tissue, another candidate for regulation of PSA expression (17).

The stimulatory effect of androgens on LNCaP cell proliferation shows a bell-shaped dose-response curve (11, 45). At low androgen concentrations (up to 10^{-10} M R1881), LNCaP cells proliferate in a dose-dependent manner; at higher hormone concentration, the proliferation rate is less. The molecular mechanism of growth stimulation by androgens, including the remarkable dose response, is not fully understood, although it has been proposed that TGFβ1 mediates at least part of the growth arrest observed at high androgen concentration (46). The cell growth studies clearly demonstrated that Dex was unable to induce proliferation of LNCaP-1B7 and LNCaP-1F5 cells. The molecular mechanism responsible for the differential effects of glucocorticoids and androgens on growth remains to be investigated. In a probably oversimplified view it can be hypothesized that a limited number of differentially expressed genes are involved. Differential AR and GR regulated TGFβ and other growth factors or inhibitors, and their corresponding receptors should be considered in this regard.

The novel clone 21.1 described here might be another candidate involved in a specific AR function in LNCaP. Isolation and characterization of full-length 21.1 cDNA and the accompanying gene, which might provide information on 21.1 function and regulation of expression, are in progress.

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