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Differential expression of determinants of glucocorticoid sensitivity in androgen-dependent and androgen-independent human prostate cancer cell lines

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ABSTRACT

Glucocorticoids (GCs) are widely used for the treatment of hormone refractory prostate cancer. However, few data are available on the expression and regulation of glucocorticoid and mineralocorticoid receptors (GR and MR) and 11β-hydroxysteroid dehydrogenase (11β-HSD) 1 and -2 activities in prostate cancer cells. Here we show that GR is expressed in both the androgen-independent PC-3 cell line and, at very low levels, in the androgen-dependent LNCaP cells, and MR is expressed in both cell lines. IL-1β increased GR expression in both cell lines. In LNCaP cells IL-1β also increased MR expression. Significant 11β-HSD oxidase activity and 11β-HSD2 protein were found in LNCaP cells, but not in PC3 cells, and no ketoreductase activity was detected in either cell lines. GR function was assessed by measuring the inhibitory effect of dexamethasone on constitutive and IL-1β-inducible IL-6 and osteoprotegerin (OPG) production. In PC-3 cells, IL-1β stimulated IL-6 and OPG release, and dexamethasone dose-dependently inhibited IL-1β-inducible IL-6 release, and constitutive and IL-1β-inducible OPG release. In LNCaP cells, IL-1β stimulated only OPG release. While dexamethasone was ineffective, cortisol dose-dependently inhibited IL-1β-inducible OPG release. Eplerenone (Epl), a selective mineralocorticoid antagonist, reverted this effect. We conclude that different patterns of expression of receptors and 11β-HSD activity were associated with different responsiveness to GCs in terms of regulated gene expression. GR and MR expression may vary as a function not only of the malignant phenotype, but also of local conditions such as the degree of inflammation. Inhibition of IL-6 and OPG release by GCs may contribute to the antitumor efficacy in prostate cancer.

1. Introduction

For years, glucocorticoids (GCs) have been extensively used for the treatment of hormone refractory prostate cancer (HRPC). The combination of paclitaxel and dexamethasone is nowadays a standard treatment for HRPC patients and GCs (mostly prednisone and dexamethasone) have been used in the comparative arm of several phase II–III trials for novel strategies of treatment in such patients [1]. However, the underlying mechanisms by which GCs affect HRPC progression are still elusive.

First, GCs may exert an antitumor effect by suppression of adrenal androgens [1]. Moreover, they may inhibit androgen signalling, since GC receptor (GR) and androgen receptor (AR) can associate through their DNA-binding domain to form a heterodimer which inhibits androgen-specific transcription *in vitro* [2]. Second, GCs can inhibit prostate cancer cell growth by modulating local secretion of cytokines and growth factors and by interfering with their signalling. Dexamethasone increases IκBα protein levels and the cytosolic accumulation of NF-κB in DU145 cells, and inhibits release of IL-6 [3]. Moreover, it induces the expression of TGF-β1 and TGF-β receptor type II, and enhances TGF-β1 signalling in prostate cancer cells [4]. Dexamethasone also suppresses PTHrP expression [5] and inhibits tumor-associated angiogenesis by decreasing VEGF and IL-8 [6], and lymphangiogenesis by downregulating VEGF-C [7]. As a third mechanism, GCs may regulate cell responses to estrogen, by increasing tumor aromatase activity [8].

Although the overall beneficial effect of GCs in HRCP patients is well known, objective responses are found only in 20–25% of patients [1], suggesting a variable GC sensitivity. It is held that the magnitude of genomic effects induced by GCs within a given target cell results from a number of cellular and molecular determinants [9]: intracellular steroid metabolism, GC receptors isoforms and availability of binding sites, nuclear translocation and interactions of activated GR with other transcription factors/cofactors

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upon specific DNA sequences, recycling/degradation of receptor. GC receptors are distinguished in type I (mineralocorticoid receptors, MR) and type II (glucocorticoid receptors *tout court*, GR), which are encoded by different genes and expressed as different variants as a function of alternative transcription, splicing, and translation [9]. Different GC molecules show different affinities for the two receptor types. Intriguingly, receptor expression has been shown to be regulated by cytokines and growth factors in a number of tissues [10,11]. Yet, the cortisol–cortisone interconversion that occurs at a pre-receptorial level *via* activities of 11β-hydroxysteroid dehydrogenase (HSD) type 1 and 2 has received increasing attention as an important mechanism accounting for GC sensitivity [12].

Few data are available on the expression and regulation of GR, MR and 11β-HSD1 and -2 activities in prostate cancer cells [13–15]. The aim of the present study was to assess GR and MR number and affinity, and 11β-HSD1 and -2 activities, under basal conditions and after exposure to the inflammatory cytokine IL-1β, in two human prostate cancer cell lines, the androgen-dependent LNCaP and the androgen-independent PC-3 cells.

2. Materials and methods

2.1. Materials

Dexamethasone, cortisol, aldosterone, mifepristone (RU486), 11β,17β-dihydroxy-6-methyl-17α-(1-propynyl)-androsta-1,4,6-trien-3-one (RU28362), triamcinolone and methyltrienolone (R1881) (Sigma–Aldrich, Milan, Italy) were initially dissolved in 95% ethanol and then diluted in phosphate buffered saline (PBS) to give a 1 mM stock solution, which was stored at −20 °C. They were promptly diluted to final concentrations before each experiment and binding assay. Eplerenone (Epl) (Tocris, Bristol, UK) was dissolved in DMSO to obtain a 1 mM stock solution which was stored at −20 °C. The final dilution of DMSO (01%) did not by itself have any effect on cell vitality.

Recombinant human IL-1β was purchased from R&D Systems (Abingdon, UK); it was reconstituted using PBS containing 1% bovine serum albumin (BSA), stored at −20 °C, and diluted to the appropriate concentrations immediately before experiments.

[1,2,4,6,7-^{3}H]Dexamethasone and [1,2,6,7-^{3}H]aldosterone were purchased from Amersham Biosciences (Milan, Italy); [17α-methyl-^{3}H]methyltrienolone was purchased from NEN (PerkinElmer, Milan, Italy); before each binding assay, solvent was removed under a nitrogen stream and labelled steroid was then dissolved to final concentrations in appropriate medium. Scintillation fluid and scintillation vials were purchased from Beckman Coulter (Milan, Italy).

Anti-GRα polyclonal antibody [GR(P-20) sc-1002] and anti-MR polyclonal antibody [MCR(C-19) sc-6861] were purchased from Santa Cruz Biotechnology Inc. (Tebu-Bio, Milan, Italy). Anti-human 11β-HSD type 1 and 2 antibodies (PC544 and PC545) were purchased from The Binding Site (Birmingham, United Kingdom). Bradford protein assay kit and horseradish peroxidase-labelled protein A were purchased from Biorad Laboratories (Hercules, CA, USA); all other reagents for Western blotting were purchased from Sigma–Aldrich.

2.2. Cells and culture conditions

The established human prostate cancer cell lines PC-3 and LNCaP were purchased from Interlab Cell Line Collection (Banca di Materiale Biologico Servizio Biotecnologie IST c/o CBA, Genova, Italy). PC-3 and LNCaP cells were cultured in Ham's F12 and RPMI 1640 medium, respectively, enriched with heat-inactivated fetal bovine serum (FBS—10% and 20%, respectively), 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma–Aldrich), at 37 °C in a 95% air/5% CO_2 humidified atmosphere.

For experiments, cells were plated into 6-well plates and treated when subconfluent. When the effects of IL-1β on GR, MR and 11β-HSD activities were assessed, both cell lines were incubated with IL-1β (0.1–10 ng/mL) for 24 h. When the effects of dexamethasone on constitutive and IL-1β-inducible IL-6 and osteoprotegerin (OPG) release were assessed, both cell lines were treated with dexamethasone (0.01–1 μM) or vehicle, in the presence or absence of IL-1β (10 ng/mL) for 24 h. When the effects of cortisol on IL-1β-inducible OPG release in LNCaP cell line were assessed, cells were incubated with IL-1β (10 ng/mL) in the presence or in absence of cortisol (*F*, 0.01–1 μM) or eplerenone (Epl, 1 μM) or *F* (0.01–1 μM) + Epl (1 μM) for 24 h.

2.3. Radioligand binding assay for GR, MR, and AR

After treatment, cells were washed twice with PBS and incubated with fresh phenol red free media containing 10% charcoal-stripped FBS for 1 h; subsequently, media were removed and cells were incubated for 1 h with binding solutions containing high specific activity ligand: for GR, [1,2,4,6,7-^{3}H]dexamethasone (82 Ci/mmol) at six progressively decreasing concentrations (46-2.25 nM), in the presence or absence of 200-fold molar excess of unlabelled dexamethasone; for MR, [1,2,6,7-^{3}H]aldosterone (76 Ci/mmol) at six progressively decreasing concentrations (46–2.25 nM), in the presence of 200-fold molar excess of a specific GR antagonist (RU486 or RU28362), and in the presence or absence of 200-fold molar excess of unlabelled aldosterone; for AR, [17α-methyl-^{3}H]-R1881 (82 Ci/mmol) at six progressively decreasing concentrations (5–0.15 nM), in the presence of 500-fold molar excess of triamcinolone acetonide and in the presence or absence of 200-fold excess of unlabelled R1881.

After incubation, cells were washed five times with ice-cold PBS buffer, scraped by 1 M NaOH and transferred to scintillation vials with scintillation fluid. Activity was counted by a scintillation β-counter (Packard Instr., Canberra, Australia). Six tubes with labelled dexamethasone/aldosterone/R1881 alone were assessed for total activity. At each dexamethasone/aldosterone/R1881 concentration, specific binding was calculated as the difference between the totally bound radioactivity and the non-specifically bound radioactivity. Non-specific binding was calculated from the aliquots containing the 200-fold molar excess of non-radioactive dexamethasone/aldosterone/R1881, assuming that non-specific binding was non-saturable and linearly related to the concentration of free ligand. GR, MR and AR numbers and equilibrium dissociation constants (K_d) were determined by Scatchard method.

2.4. Western blot

LNCaP and PC-3 cells were harvested and resuspended in lysis RIPA buffer (10% glycerol, 1% Triton X-100, 150 mM NaCl, 20 mM Hepes, pH 7.2, 5 mM EDTA) supplemented with protease inhibitor cocktail. Cell lysates were then sonicated, centrifuged for 20 min at 16,000 × *g* at 4 °C and immediately frozen at −20 °C. A sample was taken for protein estimation by Bradford protein assay.

When appropriate, cell lysates (0.5–1.0 mg of total proteins) were incubated with the specific primary antibodies (6–10 μg) overnight at 4 °C. Agarose-conjugated protein A/G beads were then added to the supernatants, and the mixtures were incubated for 2 h at 4 °C. The immunoprecipitates were washed five times in lysis buffer and proteins were recovered by boiling the beads in SDS sample buffer.

The immunoprecipitates or cell lysates (30–100 μg of total proteins) were resolved by electrophoresis through 8% or 12% SDS-polyacrylamide gels and electrophoretically transferred to

nitrocellulose in Tris–glycine buffer (25 mM Tris–HCl, pH 8.3, 250 mM glycine, 0.05% SDS, 20% methanol). Membranes were stained with Ponceau S (0.2% in 5% trichloroacetic acid) to evaluate loading equivalency and transfer efficiency, and blocked with PBS containing 5% nonfat milk and 0.1% Tween 20. The blot was then washed with PBS and incubated overnight at 4 °C with the appropriate antibodies: the anti-GRα polyclonal antibody (1:500), the anti-MR polyclonal antibody (1:500), the anti-human 11β-HSD type 1 and 2 antibodies (1:4000). Subsequently, membranes were washed with PBS containing 0.5% Tween 20, reacted for 1 h at room temperature with the appropriate secondary antibodies, washed with PBS containing 0.5% Tween 20, reacted with chemiluminescent reagents (Amersham Biosciences) and then processed for autoradiography. Autoradiographic films were analyzed by densitometric scanning using the Kodak Image Station 440 System, supported by Kodak 1D Image Analysis Software. Results were expressed as arbitrary densitometric units.

2.5. 11β-HSD activity

Both cell lines were incubated with either cortisone (0.1 and 1 μM) or cortisol (0.1 and 1 μM), with or without 5 μM 18β-glycyrrhetinic acid (Sigma–Aldrich) for 6, 12 and 24 h to assess 11β-HSD ketoreductase and dehydrogenase activity, respectively. Thereafter, culture medium was collected and concentrations of cortisol and cortisone were measured by high pressure liquid chromatography with ultraviolet detection (HPLC-UV). HPLC analysis was performed by a Merck-Hitachi System (LaChrom instrument); separation was achieved with a C18 reversed-phase column (Lichrocart 250-4, Lichrospher 100 RP-18, 5 μm, VWR, Germany). The mobile phase, in isocratic condition, consisted of methanol/water 63:37 (v/v) at the constant flow rate of 0.8 mL/min, 35 °C. The eluate was monitored at 254 nm UV wavelength.

2.6. ELISAs

After treatment, cell culture media were collected, spun for 10 min at 200 g at 4 °C, and the supernatants were frozen at −20 °C. Supernatants were diluted when necessary before assay. IL-6 and OPG were measured in duplicate by sensitive ELISAs using commercially available kits (IL-6 and OPG DuoSet, R&D Systems). Detection limits were less than 0.8 pg/mL and 26 pg/mL for IL-6 and OPG, respectively; intra- and inter-assay CV values were <10%; samples with CV > 10% were re-assayed.

2.7. Statistics

Statistical analysis was performed with Statistica 6.0 (Statsoft Inc., Tulsa, OK, USA). When the effects of IL-1β and GC were studied, multiple measures ANOVA followed by LSD post hoc comparison test were used. $P < 0.05$ was considered to represent statistical significance.

3. Results

3.1. AR, GR and MR

AR was detected in LNCaP but not PC-3 cells (Fig. 1). GR was detected by both radioligand binding assay and Western blot in PC-3; in LNCaP cells very low levels of protein were found by Western blot (Fig. 2), together with minimal binding of the radioligand (not shown). GR affinity for dexamethasone in PC-3 cells was 2.4 ± 0.3 (mean ± SE) nM. The MR was detected by Western blot and radioligand binding assay in both cell lines (Fig. 3). MR number was distinctly higher in LNCaP cells (9500 ± 136, mean ± SE, sites/cell)

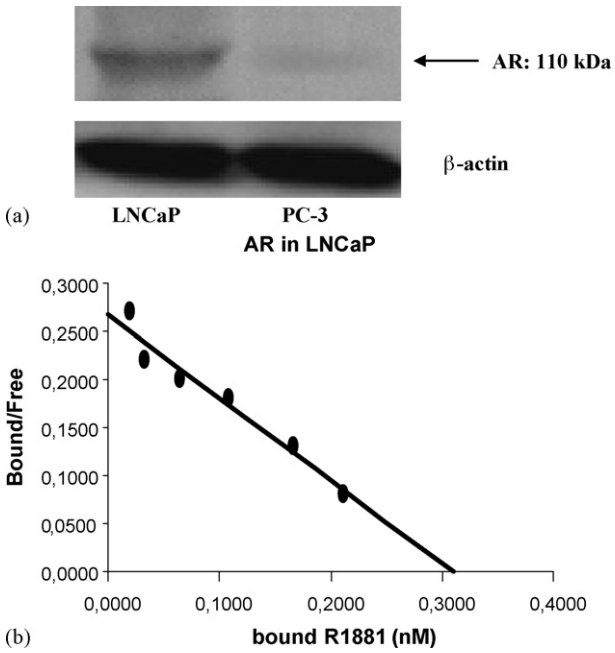

Fig. 1. AR in LNCaP and PC-3 cells. (a) Representative Western blot for AR. (b) Representative Scatchard plot of radioligand binding assay for AR in LNCaP cells. The slope gives the negative reciprocal of the binding affinity, the intercept on the *x*-axis the number of specific binding sites. Similar results were obtained in other two experiments.

with respect to PC-3 (3877 ± 77 sites/cell); MR affinities for aldosterone resulted 1.6 ± 0.1 nM and 0.30 ± 0.03 nM, respectively. In PC-3 cells the Western blot analysis showed a double band (Fig. 3).

GR and MR modulation was assessed by measuring the effect of IL-1β. In both cell lines IL-1β clearly increased GR protein expression, as assessed by both Western blot and radioligand binding assay (Figs. 4 and 5). In PC-3 cells maximal effect was observed with IL-1β 1 ng/mL, while in LNCaP cells an inverse dose–response relationship was found. IL-1β also increased MR protein (Fig. 6);

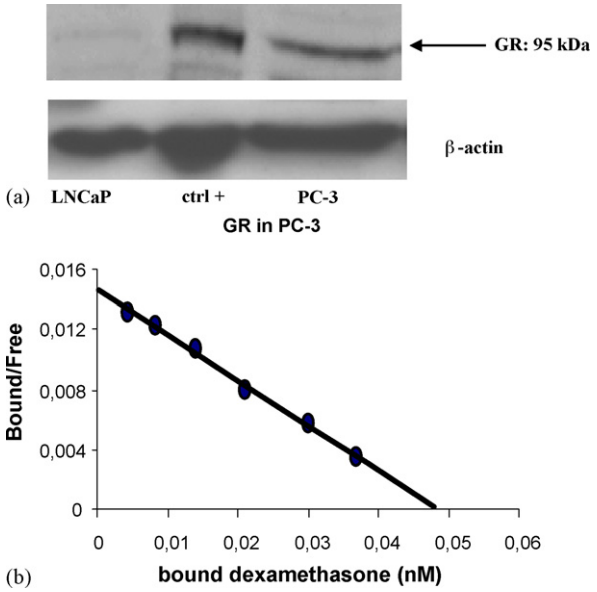

Fig. 2. GR in LNCaP and PC-3 cells. (a) Representative Western blot for GR. ctrl+, positive control (rat hippocampus). (b) Representative Scatchard plot of radioligand binding assay for GR in PC-3 cells. The slope gives the negative reciprocal of the binding affinity, the intercept on the *x*-axis the number of specific binding sites. Similar results were obtained in other two experiments.

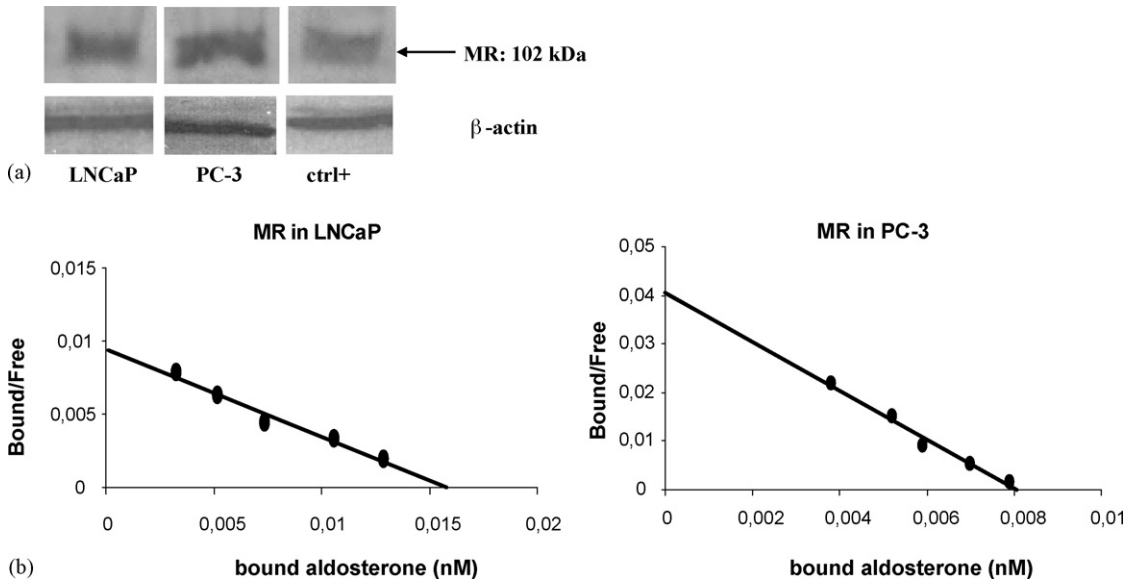

Fig. 3. MR in LNCaP and PC-3 cells. (a) Representative Western blot for MR. ctrl+, positive control (K562 cells). (b) Representative Scatchard plots of radioligand binding assay for MR. The slope gives the negative reciprocal of the binding affinity, the intercept on the *x*-axis the number of specific binding sites. Similar results were obtained in another experiment.

in PC-3, with limitations due to the presence of a double band, no clear modulator effect on MR was apparent (Fig. 6).

3.2. 11β-HSD activity

When LNCaP cells were incubated with cortisol (0.1–1 μM), dose- and time-dependent generation of cortisone and consumption of cortisol were observed (Fig. 7), consistently with 11β-HSD oxidase activity. For 0.1 μM cortisol, complete conversion to cortisone was observed. Oxidase activity was partially reduced and slowed by co-incubation with the 11β-HSD inhibitor 18β-glycyrrhetinic acid. Western blot showed expression of 11β-HSD type 2, but not type 1, isozyme in LNCaP cells (Fig. 8). No conversion of cortisol to cortisone was observed in PC-3 cells (not shown), and no consistent ketoreductase activity (i.e. conversion of cortisone to cortisol) was detected in either cell lines; finally, no effect of IL-1β on either oxidase or reductase 11β-HSD activity was found (not shown).

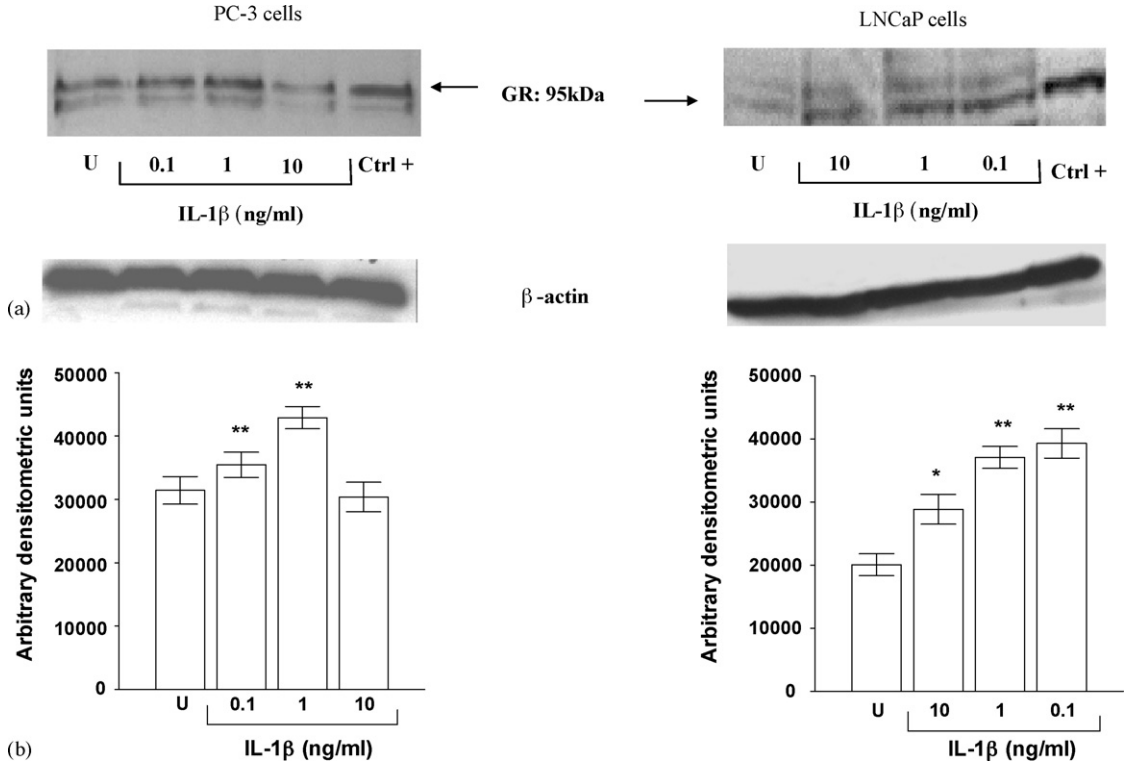

Fig. 4. IL-1β up-regulates GR in LNCaP and PC-3 cells. (a) Representative Western blots. (b) Densitometric analysis. Bars are means and whiskers are ±SE of three experiments. U, untreated; ctrl+, positive control (rat hippocampus). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. untreated by multiple measures ANOVA and LSD post hoc comparison test.

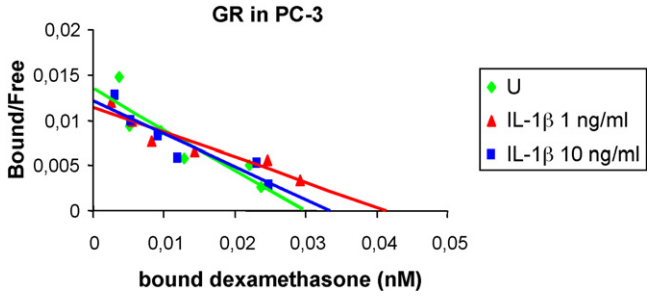

Fig. 5. IL-1β up-regulates GR in PC-3 cells. Representative Scatchard plot. Cells were incubated with IL-1β for 24 h; GR was subsequently measured on intact cells by radioligand binding assay. The slope gives the negative reciprocal of the binding affinity, the intercept on the *x*-axis the number of specific binding sites. Similar results were obtained in other two experiments.

3.3. GC responsiveness

GR function was assessed by measuring the inhibitory effect of dexamethasone on constitutive and IL-1β-inducible IL-6 and OPG production. In PC-3 cells, IL-1β stimulated IL-6 and OPG release in the medium, with a more apparent effect for the latter ($P = 0.08$ and $P < 0.05$ by ANOVA, respectively). Dexamethasone dose-dependently inhibited IL-1β-inducible IL-6 release ($P = 0.03$ by ANOVA—Fig. 9), and constitutive and IL-1β-inducible OPG release ($P < 0.001$ by ANOVA 1 μM—Fig. 10). In LNCaP cells IL-6 was undetectable in cell medium either under basal conditions or after exposure to IL-1β. OPG was undetectable under basal conditions, but reaches very high levels after IL-1β treatment ($P < 0.001$ by ANOVA—Fig. 11); no effect of dexamethasone in LNCaP cells was observed, but a clear-cut, dose-dependent inhibition of IL-1β-inducible OPG release was apparent when cortisol was added (>50% inhibition at 1 μM concentration; $P < 0.01$—Fig. 11). Eplerenone, a mineralocorticoid antagonist with enhanced selectivity for MR compared to spironolactone [16] did not affect IL-1β-inducible OPG release, but completely reverted the effect of cortisol (Fig. 11).

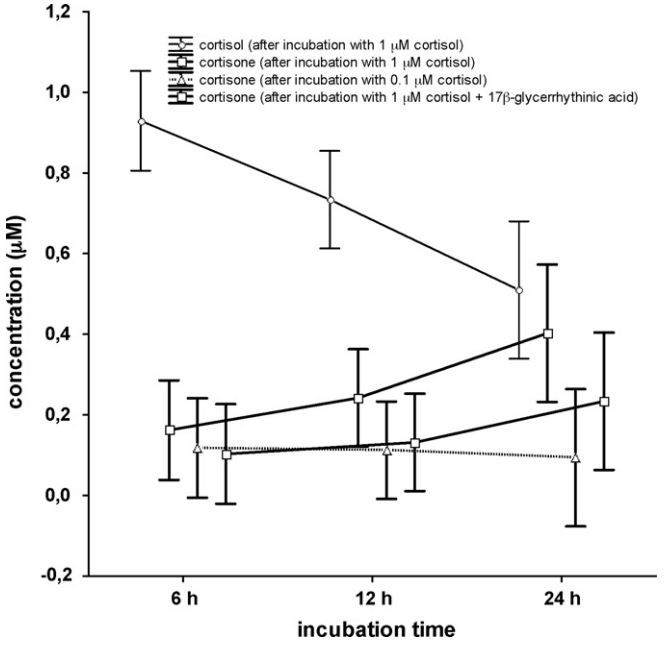

Fig. 7. 11β-HSD activity. LNCaP cells were incubated with cortisol 0.1 and 1 μM, with or without 18β-glycyrrhetinic acid, for 6, 12, and 24 h. Cortisol and cortisone concentrations were measured by HPCL-UV method.

4. Discussion

Despite their widespread use in patients with HRPC, mechanisms of action of GCs and determinants of sensitivity in relation

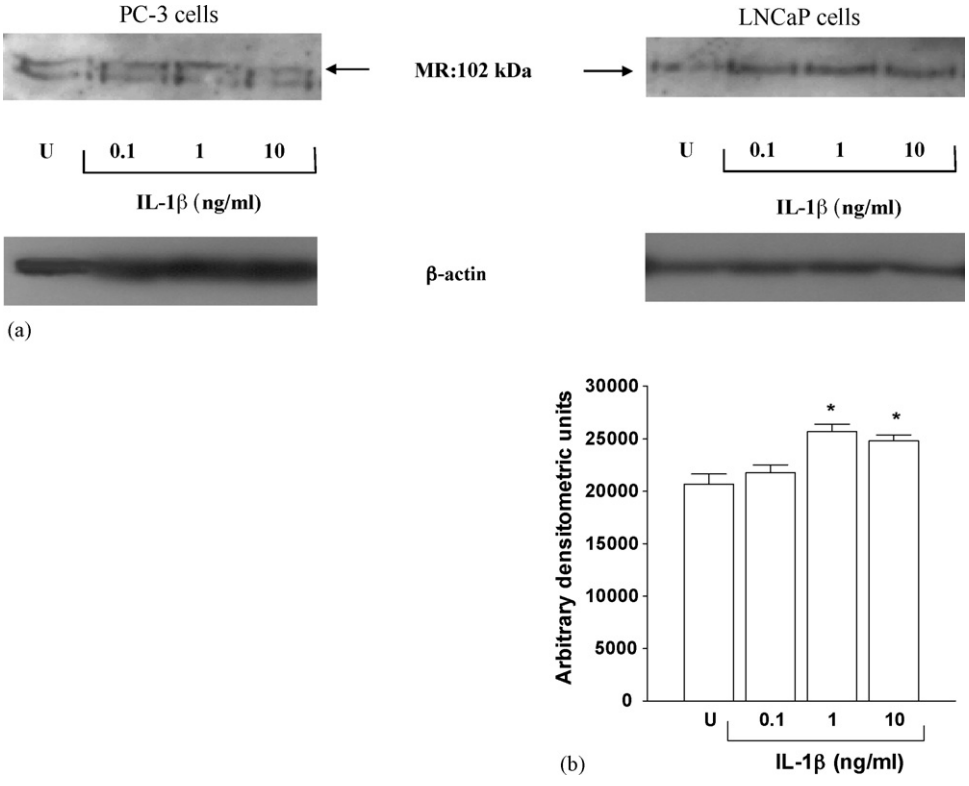

Fig. 6. IL-1β up-regulates MR in LNCaP cells. The effect of IL-1β in PC-3 cells is difficult to resolve. (a) Representative Western blots. (b) Densitometric analysis. Bars are means and whiskers are ±SE of three experiments. U, untreated; *$P < 0.01$ vs. untreated by multiple measures ANOVA and LSD post hoc comparison test.

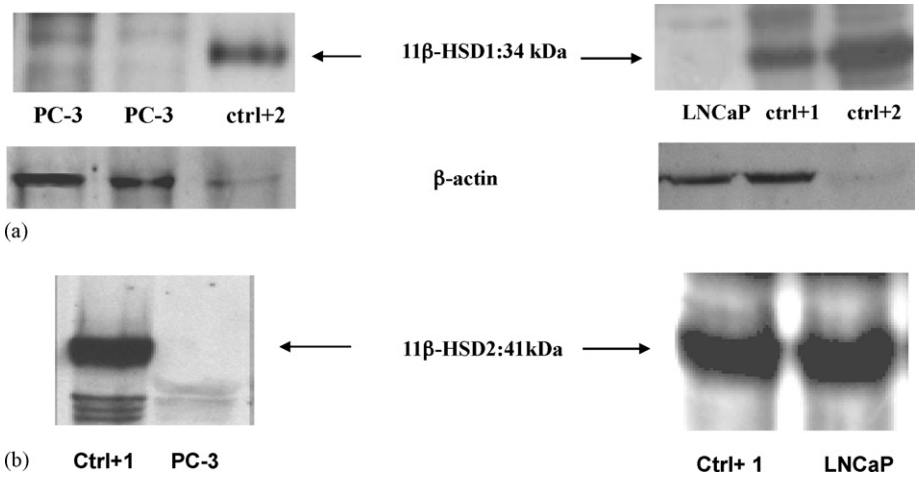

Fig. 8. 11β-HSD in PC-3 and LNCap cells. (a) Representative Western blots for 11β-HSD1. (b) Representative Western blots for 11β-HSD2 (immunoprecipitates). ctrl+1, positive control (rat kidney); ctrl+2, positive control (rat liver).

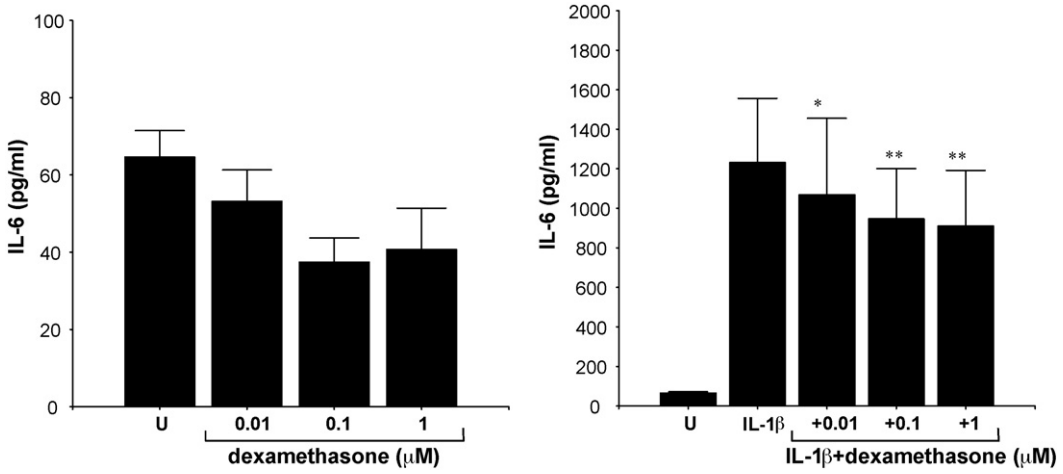

Fig. 9. Inhibitory effects of dexamethasone on constituive and IL-1β-inducible release of IL-6 in PC-3 cells. Cells were incubated for 24 h with increasing concentrations of dexamethasone (0.01–1 μM), in absence or presence of IL-1β (10 ng/mL); after treatment, cell culture media were assayed for IL-6 by ELISA. Bars represent the mean ± SE of three independent experiments.*$P < 0.05$, **$P < 0.01$ vs. IL-1β by multiple measures ANOVA and LSD post hoc comparison test.

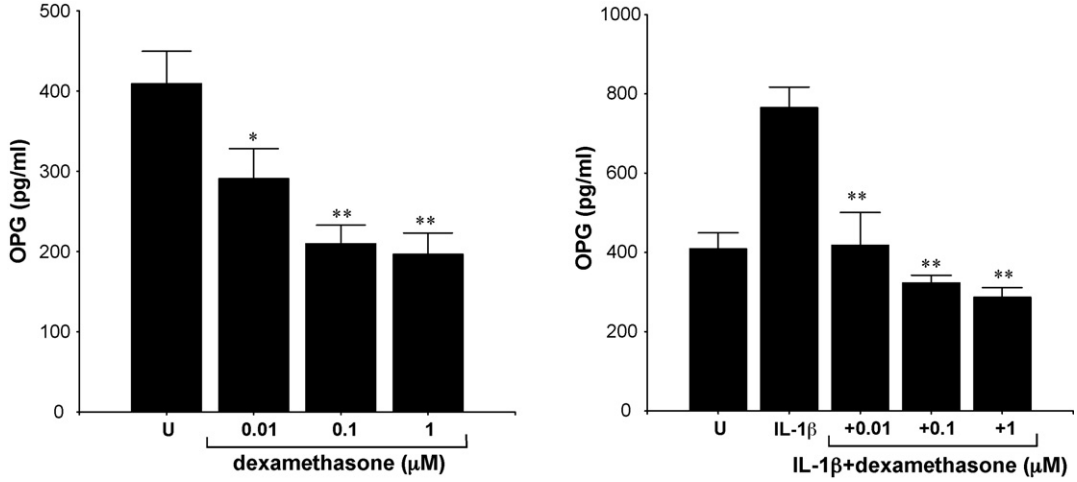

Fig. 10. Inhibitory effects of dexamethasone on constituive and IL-1β-inducible release of OPG in PC-3 cells. Cells were incubated for 24 h with increasing concentrations of dexamethasone (0.01–1 μM), in absence or presence of IL-1β (10 ng/mL); after treatment, cell culture media were assayed for OPG by ELISA. Bars represent the mean ± SE of three independent experiments.*$P < 0.01$, **$P < 0.001$ vs. untreated (left panel) or IL-1β (right panel) by multiple measures ANOVA and LSD post hoc comparison test.

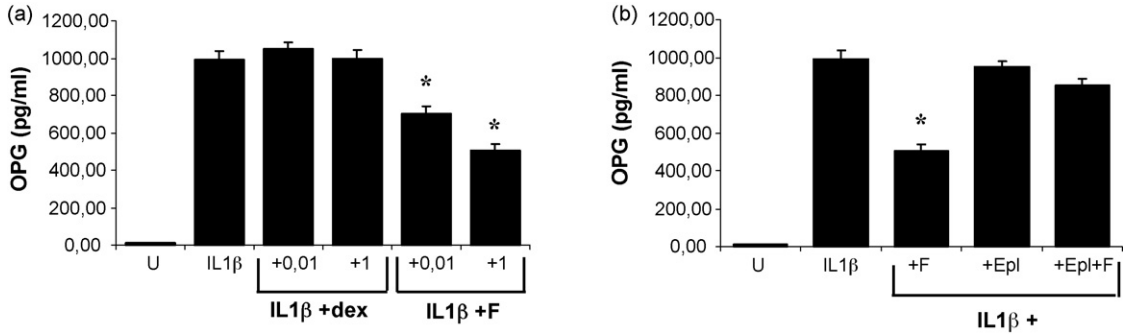

Fig. 11. Effects of dexamethasone and cortisol on IL-1β-inducible release of OPG in LNCap cells. (a) Cells were incubated for 24 h with IL-1β (10 ng/mL) in the presence or in absence of dexamethasone (0.01 and 1 μM) or *F* (0.01 and 1 μM). (b) Cells were incubated for 24 h with IL-1β (10 ng/mL) in the presence or in absence of *F* (1 μM) or Epl (1 μM) or *F* (1 μM) + Epl (1 μM). After treatment, cell culture media were assayed for OPG by ELISA. Bars represent the mean ± SE of 5 independent experiments.*$P < 0.01$ vs. IL-1β by multiple measures ANOVA and LSD post hoc comparison test.

to cancer cell phenotype have been scarcely investigated. To our knowledge, MR expression in androgen-dependent LNCaP cells has been reported in only two previous papers [13,14], and the present work is the first to document MR in androgen-independent PC-3 cells. In these cells Western blot analysis with a polyclonal antibody raised against the N-terminus revealed two bands that are compatible with different isoforms [17]. Given the great similarity in molecular weight, the two bands could reflect the A and B isoforms of MR reportedly resulting from alternative translation [18]. With regard to LNCaP cells, they have been repeatedly found to be GC-responsive [8,19], though most authors did report little, if any, GR expression [3,20,21]. The most credited interpretation is that GCs act through mutated AR [2,21]. The role of MR deserves consideration, given their high expression. Data obtained in the present study by exposing LNCaP cells to dexamethasone, cortisol and eplerenone, a selective mineralocortocoid antagonist, lend support to the view that MR mediates important glucocorticoid effects in these cells. Since MR binds with high affinity cortisol and only a few 11-hydroxy-derivatives, it is reasonable to think at differential effects of the various GCs on PC cells as a function of two variables: molecular individuality and receptor expression. Unfortunately, no data are available on MR expression in tissue specimens coming from human primary and/or metastatic prostate cancer.

As far as GR is concerned, our results confirm previous data and definitely indicate very low or even minimal expression in LNCaP and remarkable expression in PC-3 cells [3,20,21]. Only few and contradictory data are available on GR expression in specimens coming from human prostate cancer vs. benign hyperplasia and normal tissue [3,22,23]. A progressive decrease of GR expression in PC cells while progressing tumor growth has been hypothesized, yet the intensity of GR staining by immunohistochemistry did not correlate with Gleason grade, circulating prostate specific antigen or pathological stage [22,23].

We provide here the first report on the modulation of GR and MR by IL-1β in PC cells, thus extending our previous observations in osteoblasts [10]. It is conceivable that GR and MR expression may vary as a function not only of the malignant phenotype, but also of local, possibly transient conditions such as the degree of inflammation. Importantly enough, reduced levels or even absence of GR and/or MR in cancer cells do not necessarily translate into clinical inefficacy of GCs given to patients. Specific receptors are expressed in stromal cells [22,23], which are involved in many aspects of malignant progression [24].

As far as 11β-HSD activities are concerned, our data are consistent with previous reports on the predominant dehydrogenase activity and expression of type 2 isoenzyme in LNCaP cells [13–15]. 11β-HSD2 enzyme is generally co-expressed with MR, and thought to "protect" MR from cortisol, hence preserving MR specificity for aldosterone [12]. Surprisingly, 11β-HSD2 activity was absent in PC-3 cells despite appreciable MR expression. No change in 11β-HSD2 activity was observed when cells were incubated with IL-1β, which has been reported to increase type 1 reductase activity in a number of cell types [12]. In both our cell lines, 11β-HSD1 activity was not apparent even after exposure to the cytokine.

Interestingly, different patterns of expression of GR and MR, and 11β-HSD activity were associated with different responsiveness to GCs in terms of regulated gene expression. In PC-3 cells, inhibition of both constitutive and/or IL-1β-inducible release of IL-6 and OPG by dexamethasone suggests GR-mediated trans-repression of the two genes. IL-6 is an established growth factor for PC-3 cells [3]; pertinently, it has been shown that high serum IL-6 concentrations were predictive of shorter survival and lower probability of response to chemotherapy in patients with HRPC [25,26]. OPG has been shown to protect PC-3 cells from TRAIL-induced apoptosis [27]. Inhibition of the release of these two key molecules of autocrine/paracrine modulation conceivably contributes to the antitumor efficacy of GCs after escape from androgen dependency. At variance with PC-3 cells, LNCaP could be viewed as able to "protect" themselves from GC action through both low constitutive expression of GR and intracellular conversion of GCs to their inactive 11-keto-derivatives. Accordingly, in our experimental conditions dexamethasone had no appreciable effect in terms of inhibition of cytokine release by these cells. However, in these cells cortisol was capable to inhibit IL-1β-inducible OPG release; eplerenone, a mineralocorticoid antagonist with enhanced selectivity for MR and remarkably lower activity at other steroid receptors as compared to spironolactone [16], completely blocked this effect. Pertinently, IL-1β increased MR expression in LNCaP cells.

In conclusion, we have assessed expression and modulation by the inflammatory cytokine IL-1β of MR and GR and 11β-HSD activities in two human PC cell lines characterized by the presence and absence of AR, respectively. We have found a clear diversity in GC sensitivity and provided further evidence of the complex modulation of major determinants for such sensitivity. Studies are needed to better ascertain the role of MR expression in mediating GC effects in LNCaP cells and to assess whether expression of GR, MR and 11β-HSD enzymes in prostate cancer is associated with clinical responses to GCs as a class or as individual molecules.

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