

# A Glucocorticoid-Responsive Mutant Androgen Receptor Exhibits Unique Ligand Specificity: Therapeutic Implications for Androgen-Independent Prostate Cancer

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The cortisol/cortisone-responsive AR (AR<sup>CCR</sup>) has two mutations (L701H and T877A) that were found in the MDA PCa human prostate cancer cell lines established from a castrated patient whose metastatic tumor exhibited androgen-independent growth. Cortisol and cortisone bind to the AR<sup>CCR</sup> with high affinity. In the present study, we characterized the structural determinants for ligand binding to the AR<sup>CCR</sup>. Our data revealed that many of the C17, C19, and C21 circulating steroids, at concentrations that are found *in vivo*, functioned as effective activators of the AR<sup>CCR</sup> but had little or no activity via the wild-type AR or GR $\alpha$ . Among the synthetic glucocorticoids tested, dexamethasone activated both GR $\alpha$  and AR<sup>CCR</sup>, whereas triamcinolone was selective for GR $\alpha$ . In MDA PCa 2b cells, growth and prostate-specific antigen production were stimulated by potent AR<sup>CCR</sup> agonists such as cortisol or 9 $\alpha$ -fluorocortisol but not by triamcinolone (which did not bind to

or activate the AR<sup>CCR</sup>). Of the potential antagonists tested, bicalutamide (casodex) and GR antagonist RU38486 showed inhibitory activity. We postulate that corticosteroids provide a growth advantage to prostate cancer cells harboring the promiscuous AR<sup>CCR</sup> in androgen-ablated patients and contribute to their transition to androgen-independence. We predict that triamcinolone, a commonly prescribed glucocorticoid, would be a successful therapeutic agent for men with this form of cancer, perhaps in conjunction with the antagonist casodex. We hypothesize that triamcinolone administration would inhibit the hypothalamic-pituitary-adrenal axis, thus suppressing endogenous corticosteroids, which stimulate tumor growth. Triamcinolone, by itself, would not activate the AR<sup>CCR</sup> or promote tumor growth but would provide glucocorticoid activity essential for survival. (*Endocrinology* 143: 1889–1900, 2002)

THE BIOLOGICAL ACTIONS of androgens are mediated by the AR, a member of the nuclear hormone receptor superfamily (1). The AR has been implicated in the development, growth, and progression of prostate cancer (2–7). In some prostate cancers, AR levels are elevated because of gene amplification and/or overexpression (8, 9), whereas in others, the AR is mutated (10–12). A number of mutations in the AR have been identified in metastatic prostate cancers, and these mutations are most frequently located in the ligand-binding domain (LBD) of the receptor (4, 5, 11–14). ARs with LBD mutations, such as the T877A found in the LNCaP human prostate cancer cell line (15) and in many prostatic cancers (11, 12), exhibit broadened ligand specificity (14–16). For example, the T877A mutant AR is capable of responding to hydroxyflutamide, progesterone, and estrogens (15), although the circulating levels of both progesterone and estrogens in men are low and may not be clinically significant (17). The role of AR mutations in the transition of prostate cancers to androgen-independent growth and in the subsequent failure of endocrine therapy is the focus of recent studies (2–7).

We recently identified an AR with a double mutation (L701H and T877A) in its LBD in the human prostate cancer

cell lines MDA PCa 2a and MDA PCa 2b, established from a bone metastasis of a castrated patient whose prostate cancer exhibited androgen-independent growth (18, 19). This double-mutant AR binds the prostatic androgen, dihydrotestosterone (DHT), with reduced affinity, compared with the wild-type AR or AR with the T877A mutation (20). We have also shown that the double-mutant AR responds to corticosteroids such as cortisol and cortisone (20). We designated this mutant AR as the cortisol/cortisone-responsive AR (AR<sup>CCR</sup>). The AR<sup>CCR</sup> is a promiscuous receptor exhibiting relaxed ligand specificity, responding to glucocorticoids, androgens, progesterone, and E2, but not aldosterone (20, 21).

In the present study, we investigated the structural requirements of ligands for the AR<sup>CCR</sup>, in comparison with ligands for the human GR $\alpha$ . We tested natural steroids in the steroidogenic pathway, as well as synthetic corticosteroids, for their potential to act as AR<sup>CCR</sup> ligands. The steroids were evaluated in functional assays, which included binding to AR<sup>CCR</sup> and activation of AR<sup>CCR</sup>-mediated transcription. Selected corticosteroids were also tested for their ability to cause transactivation through the single-mutant L701H AR. The abilities of key steroids to regulate the growth of MDA PCa 2b cells, which harbor the AR<sup>CCR</sup>, were evaluated; and their effects on the androgen-responsive target gene prostate-specific antigen (PSA) were determined. Structure-activity relationships were addressed by studying a series of structurally related steroids.

Our studies reveal that the AR<sup>CCR</sup> can be activated by a

Abbreviations: AR<sup>CCR</sup>, Cortisol/cortisone-responsive AR; BRFF, Biological Research Faculty and Facility; DHT, dihydrotestosterone; FluF, 9 $\alpha$ -fluorocortisol; K<sub>d</sub>, dissociation constant; LBD, ligand-binding domain; MMTV, mouse mammary tumor virus; PSA, prostate-specific antigen; RBA, relative binding affinity; RU486, GR antagonist RU38486.

number of circulating corticosteroids and their precursors. Cortisol and 9 $\alpha$ -fluorocortisol (FluF), the most potent agonists for AR<sup>CCR</sup>, stimulate the growth of MDA PCa 2b cells and PSA secretion. The presence of AR<sup>CCR</sup> would therefore provide a growth advantage to prostate cancer cells harboring these mutations by responding to cortisol and other steroids in the steroidogenic pathway and thus contribute to androgen-independent growth and the progression of prostate cancer seen in androgen-ablated patients. The antiandrogen bicalutamide (casodex), as well as the GR antagonist RU38486 (RU486), acted as antagonists through the AR<sup>CCR</sup> and inhibited growth and PSA stimulation in MDA PCa 2b cells. Interestingly, the synthetic glucocorticoid triamcinolone was selective for GR $\alpha$  and did not bind to or activate the AR<sup>CCR</sup>. Because triamcinolone did not stimulate the growth of MDA PCa 2b cells or increase PSA secretion by these cells, it might be useful as a novel therapeutic agent to suppress endogenous corticosteroids in patients whose cancers express the AR<sup>CCR</sup> mutant receptors.

## Materials and Methods

### Materials

All steroids were purchased from either Sigma (St. Louis, MO) or Steraloids, Inc. (Newport, RI). Tritiated DHT, cortisol, and dexamethasone were obtained from Amersham Pharmacia Biotech (Piscataway, NJ). The mouse mammary tumor virus (MMTV) reporter plasmid pMMTV-luc and expression vectors (pSG5-AR and pSG5-GR $\alpha$ ) were gifts from Dr. Ron Evans (Salk Research Institute, San Diego, CA), Dr. Zoran Culig (University of Innsbruck, Innsbruck, Austria), and Dr. Peter Kushner (University of California, San Francisco, CA), respectively. Biological Research Faculty and Facility (BRFF)-HPC1 medium was obtained from Biological Research Faculty and Facility (Ijamsville, MD), and DMEM:F12 and LipofectAMINE were from Life Technologies, Inc. (Rockville, MD). RU486 was a kind gift from Roussel-Uclaf (Romainville, France).

### Radioligand-binding assay, Scatchard analysis, and competition-binding analysis

COS-7 cells were transfected with pSG5-AR, pSG5-GR $\alpha$ , or pSG5-AR<sup>CCR</sup> expression vectors using LipofectAMINE (Life Technologies, Inc.) (20). After 48 h, cell monolayers were harvested, and high-salt nuclear extracts were made as previously described (22, 23). Protein concentration of the extract was determined by the method of Bradford (24). Binding assays were done as described (22, 23). In a typical binding assay, 200  $\mu$ l soluble extract (0.5–1 mg protein/ml) were incubated with 0–100 nM of [<sup>3</sup>H]hormone, for 16–20 h at 0 C. Bound and free hormones were separated by hydroxylapatite. Specific binding was calculated by subtracting nonspecific binding obtained in the presence of a 250-fold excess of radioinert ligand from the total binding measured in the absence of radioinert steroid. Data were expressed as femtomoles of bound hormone per milligram of protein.

Competition-binding assays were performed with extracts of COS-7 cells expressing AR<sup>CCR</sup>, in the presence of 20 nM [<sup>3</sup>H]cortisol as the ligand and various nonradioactive molecules as competitors at 1-, 10-, and 100-fold excess.

### Reporter assay

CV-1 monkey kidney cells (ATCC, Manassas, VA) were transfected with the expression vectors pSG5-AR<sup>CCR</sup>, pSG5-GR $\alpha$ , or pSG5-L701H AR, as well as the reporter MMTV-luc, as previously described (20). Five nanograms of pRL-SV40 (Promega Corp., Madison, WI) renilla luciferase were cotransfected in each sample as an internal control for transfection efficiency. The cells were treated with various steroids alone or in the presence of antagonists, for 16–30 h, and luciferase activity was determined using the dual-luciferase assay system (Promega Corp.).

### Cell growth and PSA assays

MDA PCa 2b cells were routinely cultured in BRFF-HPC1 medium supplemented with 20% FBS as previously described (18, 19). The BRFF-HPC1 medium contains a high concentration of cortisol (hydrocortisone, 280 nM) as well as DHT at 0.1 nM. To test the effects of AR<sup>CCR</sup> agonists, such as cortisol and other steroids, on cell growth and PSA secretion, we developed a test medium whose composition was comparable to BRFF-HPC1 except for the lack of cortisol and DHT. For these assays, cells were seeded in 6-well plates ( $2 \times 10^5$  cells/well) in BRFF-HPC1 medium. After 48 h, the BRFF-HPC1 medium was replaced with DMEM:F12 medium supplemented with epidermal growth factor (10 ng/ml), insulin (1  $\mu$ M), bovine pituitary extract (40  $\mu$ g/ml), cholera toxin (25 ng/ml), phosphoethanolamine (5  $\mu$ M), selenic acid (30 nM), BSA (250  $\mu$ g/ml), and trypsin inhibitor (10  $\mu$ g/ml), along with 20% FBS. We refer to this medium as test medium. Various steroids were added at the indicated concentrations in test medium. Fresh test medium and compounds were replenished every 3 d. The conditioned media were collected, and the PSA levels were measured as described (22). DNA content and [<sup>3</sup>H]thymidine incorporation were assayed as measures of cell proliferation (25). The effects of casodex and RU486 on cell growth and PSA were assessed in BRFF-HPC1 medium, and their abilities to antagonize the stimulatory effects of endogenous cortisol and DHT present in the BRFF-HPC1 medium were evaluated.

### Structural models of the LBDs

Molecular models were based on an AR homology model produced in an earlier study (16) using the crystal structure of PR LBD as template (Protein Data Base accession code 1A28) (26). After this study was initiated, the crystal structure of the human AR LBD was solved (27, 28). Because there is a strong structural homology between the template structure of PR LBD and AR LBD (the root mean square deviation between  $\alpha$ -carbons is 0.84 Å), predictions about the structural effects of mutations can be made from the homology model. Based on the x-ray crystallographic findings on the T877A mutant AR, Sack and co-workers (28) modeled the double mutant AR (AR<sup>CCR</sup>) bound to DHT (described in Ref. 6) and obtained results similar to our modeling data reported in this paper.

The sequences of the PR and AR LBD are 52% identical. A molecular model of the mutant AR<sup>CCR</sup> LBD was produced from this model by substitution of histidine for leucine at residue 701 and alanine for threonine at residue 877. The histidine side chain was oriented using a rotamer library derived from crystallographically determined protein structures (29). For comparison, a homology model of GR $\alpha$  LBD (54% identical with PR) was constructed, in the present study, using essentially the same protocol described by McDonald *et al.* (16). Briefly, residues of PR LBD were changed to sequences of GR $\alpha$  at homologous sites with the program MODELLER (30), and the initial homology model was generated automatically. A molecule of cortisol and water molecules were added based on corresponding positions of steroid rings or bound waters in the template. The model was adjusted manually to optimize side chain rotamer positions (29) guided by the progesterone structure. A few local corrections employed molecular mechanics energy minimization using CHARMm (31) within QUANTA 97.0 (Molecular Simulations, Inc., San Diego, CA).

### Ligand-receptor docking analyses

Molecular coordinates for steroids with crystallographically determined structures were retrieved from the Cambridge Crystallographic Database (32) for docking analyses to AR, AR<sup>CCR</sup>, or GR $\alpha$  LBD pockets. Ligands were manually docked into the binding pocket, orienting each molecule by superimposition of steroid rings onto the position of progesterone in the PR crystal structure (26). Molecular mechanics energy minimization calculations using CHARMm were implemented, imposing harmonic restraints on all nonligand atoms. A number of starting positions/configurations were manually generated for each ligand in the binding pocket, and the structure with the lowest energy was selected for further analysis.

### Statistical analysis

Data were evaluated by ANOVA using the StatView 4.5 software (Abacus Concepts, Inc., Berkeley, CA), and  $P < 0.05$  was considered significant.

### Results

#### *DHT and glucocorticoids bind to the AR<sup>CCR</sup>*

Because AR<sup>CCR</sup> is a mutant AR that responds to cortisol, we first determined the binding affinity of androgens and glucocorticoids for AR<sup>CCR</sup>, and compared the results to the wild-type AR and GR $\alpha$ . Dissociation constant ( $K_d$ ) values of the AR<sup>CCR</sup>, wild-type AR, and GR $\alpha$  in COS-7 cells were measured for the binding of DHT, the major prostatic androgen, cortisol, the major circulating glucocorticoid, and dexamethasone (a potent synthetic glucocorticoid). Scatchard analyses (Fig. 1A) revealed that [<sup>3</sup>H]DHT, [<sup>3</sup>H]cortisol, and [<sup>3</sup>H]dexamethasone bound specifically to AR<sup>CCR</sup>, with  $K_d$  values of 10, 5, and 50 nM, respectively. Compared with the wild-type AR (Fig. 1B), the AR<sup>CCR</sup> had a 50-fold reduced affinity for DHT binding ( $K_d = 0.2$  nM for wild-type AR *vs.* 10 nM for the AR<sup>CCR</sup>). When compared with the GR $\alpha$  (Fig. 1C), the AR<sup>CCR</sup> had a 10-fold higher affinity for cortisol and a 25-fold lower affinity for dexamethasone. These results indicate that the AR<sup>CCR</sup> has a unique ligand specificity distinct from either wild-type AR or GR $\alpha$ .

#### *Natural corticosteroids are AR<sup>CCR</sup> agonists*

Because the AR<sup>CCR</sup> exhibits a high affinity for cortisol, we evaluated a series of cortisol-related steroids for their ability to bind to and activate the AR<sup>CCR</sup> (Fig. 2). The structures of these steroids are depicted in Fig. 2C. They included natural corticosteroids like cortisol (11 $\beta$ -hydroxycortisol), corticosterone, and their corresponding precursors (11-deoxycortisol and 11-deoxycorticosterone, respectively) as well as 18-hydroxycorticosterone and the mineralocorticoid hormone, aldosterone. We also tested 11 $\alpha$ -cortisol (the biologically inactive synthetic stereoisomer of cortisol) and cortisone, which has a keto group at the 11 position (the natural metabolite of cortisol that does not bind to or activate GR $\alpha$ ). Competition-binding analyses were performed using [<sup>3</sup>H]cortisol as the ligand and unlabeled steroids at 1, 10, and 100 molar excess as competitors. The relative binding affinity (RBA) values of these steroids for AR<sup>CCR</sup> ranked as follows (Table 1): cortisol 100% = cortisone 100% > DHT 41% > 11 $\alpha$ -cortisol 16% = 11-deoxycortisol 16% > corticosterone 11% > 11-deoxycorticosterone 9%  $\gg$  aldosterone <1%  $\gg$  18-hydroxycorticosterone (< 0.01%).

These steroids were also tested for their transactivation potential, using a cotransfection assay in CV-1 cells. These cells were selected because they are devoid of the steroid receptors under investigation and lack 11 $\beta$ -hydroxysteroid dehydrogenase (33), the enzyme that catalyzes the reversible conversion of cortisol to cortisone. The expression plasmids for the wild-type AR, AR<sup>CCR</sup>, or GR $\alpha$  were cotransfected into CV-1 cells with the MMTV-luc reporter. The cells were treated with 10 nM of each steroid for 30 h. We observed a significant difference in the extent of activation of the luciferase reporter between AR<sup>CCR</sup> and GR $\alpha$ . This observation can be explained by the fact that wild-type AR has approximately

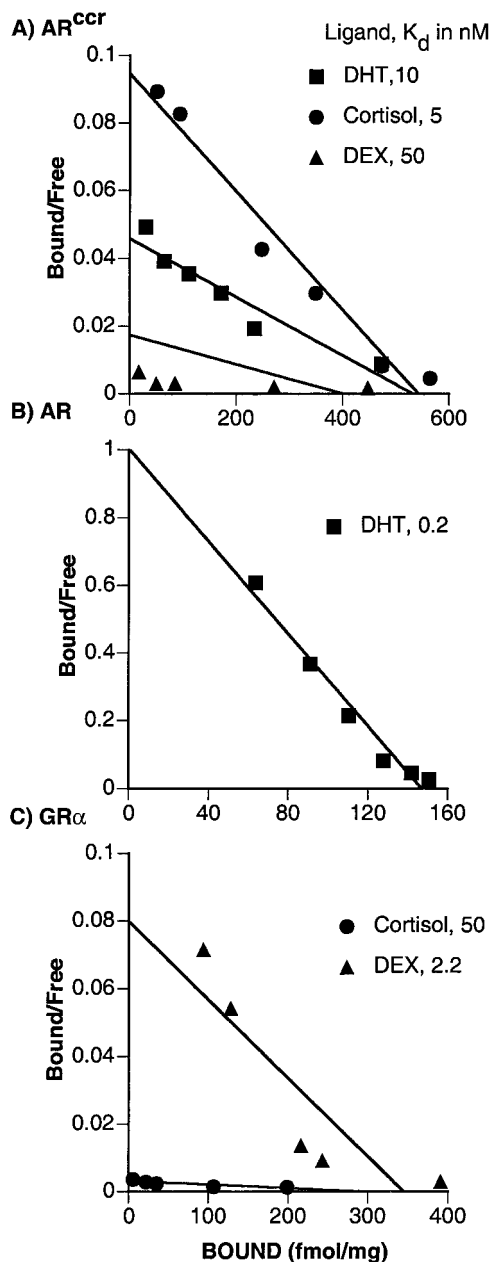


FIG. 1. The AR<sup>CCR</sup> binds both androgen and glucocorticoids. COS-7 cells were transfected with expression vectors for AR<sup>CCR</sup>, wild-type AR, or GR $\alpha$ . High-salt extracts from transfected cells were incubated with various doses of radioligand, at 0 C, in equilibrium-binding assays, as described in *Materials and Methods*. DEX, Dexamethasone. A, Scatchard plots of [<sup>3</sup>H]DHT, [<sup>3</sup>H]cortisol, and [<sup>3</sup>H]DEX binding to AR<sup>CCR</sup>; B, Scatchard plot of [<sup>3</sup>H]DHT binding to AR; C, Scatchard plots of [<sup>3</sup>H]cortisol and [<sup>3</sup>H]DEX binding to GR $\alpha$ .

20% of the maximal transcriptional activity of GR $\alpha$  on the MMTV-promoter (34). The wild-type AR could be activated only by androgens such as DHT and R1881. We tested a selected panel of corticosteroids for their ability to cause transactivation through the wild-type AR, and none of them activated the wild-type AR (data not shown).

AR<sup>CCR</sup> and GR $\alpha$  displayed distinct activation profiles in response to the various steroids (Fig. 2, A and B). In these transactivation assays, DHT and most of the cortisol-related



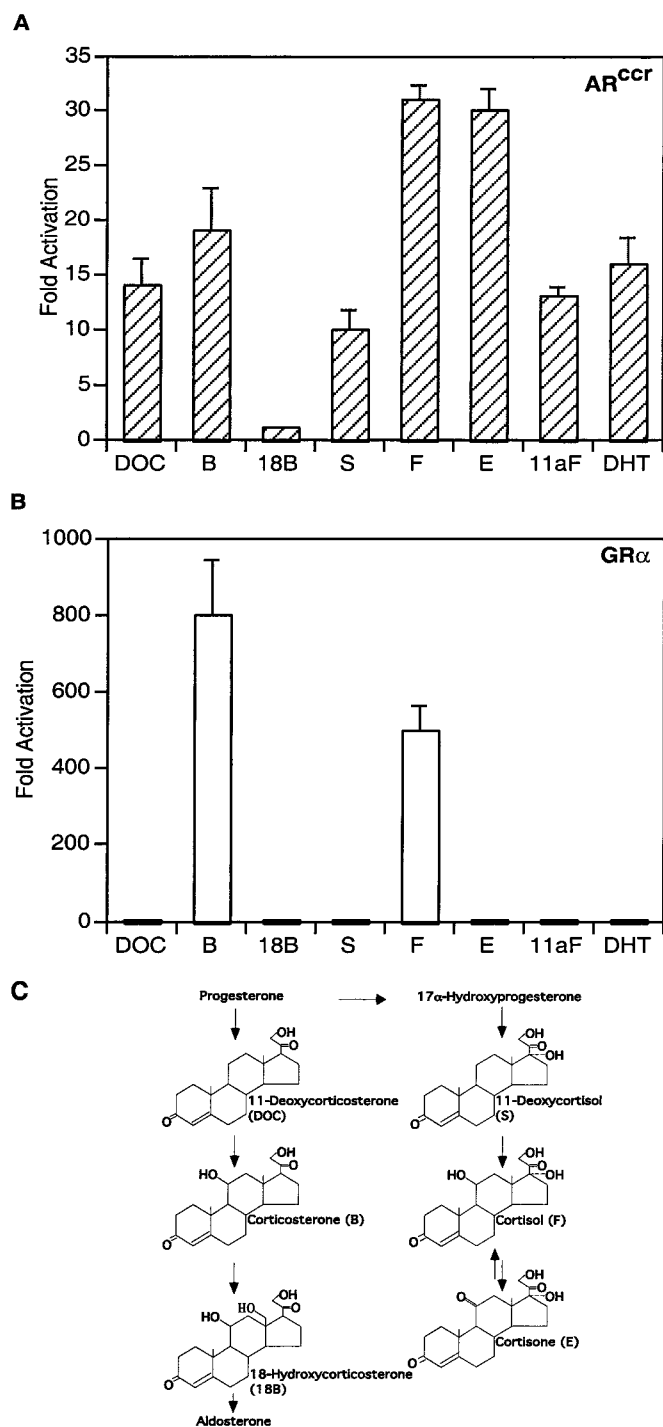


FIG. 2. The AR<sup>CCR</sup> differs from the GR $\alpha$  in structural requirements of ligands at the C11 position. CV-1 cells were transfected with expression vectors for AR<sup>CCR</sup> (panel A) or GR $\alpha$  (panel B) and the reporter MMTV-luc as well as renilla luciferase plasmids. Cells were treated with the indicated steroids at 10 nM in steroid-depleted medium for 30 h. Cell extracts were subsequently assayed for luciferase activity by the dual-luciferase system (Promega Corp.). Values are given as fold activation over activity found in control cells treated with ethanol. Data represent the mean of assays performed in triplicate  $\pm$  SEM. Panel C, Chemical structures of the naturally occurring corticosteroids tested in panels A and B. DOC, 11-Deoxycorticosterone; B, corticosterone; 18B, 18-hydroxycorticosterone; S, 11-deoxycortisol; F, cortisol; E, cortisone; 11aF, 11 $\alpha$ -cortisol.

TABLE 1. RBA of different molecules for AR<sup>CCR</sup>

Competitor	RBA	Competitor	RBA
9 $\alpha$ -Fluorocortisol	300	17 $\beta$ -E2	11
Cortisol	100	Corticosterone	10.6
Cortisone	100	T	9
R1881	65	DOC	9
17-Hydroxyprogesterone	60	Progesterone	8
DHT	41	Aldosterone	<1%
Spirolactone	30	Casodex (bicalutamide)	0.05
Prednisolone	26	Pregnenolone	<0.01
Prednisone	23	DHEA	<0.01
Dexamethasone	18	Androstenedione	<0.01
RU486	16.4	4-Hydroxytamoxifen	<0.01
11-Deoxycortisol	16.4	ICI 182780	<0.01
11 $\alpha$ -Cortisol	16.4	Triamcinolone	<0.01
Hydroxyflutamide	16	18-Hydroxycorticosterone	<0.01

RBA is expressed as the ratio of concentration of cortisol over concentration of competitor, each of which produces a 50% decrease in specific [<sup>3</sup>H]cortisol binding  $\times$  100 (mean, n > 3). In competition binding assays, the dose response curves have been done twice for each competing molecule, and the RBA values represent means from two experiments.

DOC, 11-Deoxycorticosterone; DHEA, dehydroepiandrosterone.

steroids, except for 18-hydroxycorticosterone (18B), activated the AR<sup>CCR</sup> and induced luciferase activity (Fig. 2A). Cortisol and cortisone were the most effective activators of the AR<sup>CCR</sup>, inducing reporter levels over 30-fold above the basal level. Corticosterone increased reporter levels 20-fold. The precursor molecules of cortisol and corticosterone (11-deoxycortisol and 11-deoxycortisol, respectively) were also potent AR<sup>CCR</sup> activators. Remarkably, the C11 isomer of cortisol, 11 $\alpha$ -cortisol, which is inactive through GR $\alpha$ , also increased AR<sup>CCR</sup>-mediated gene transactivation by 13-fold. Thus, the AR<sup>CCR</sup> exhibited only limited stereoisomer specificity for the C11 position of the corticosteroids. In contrast, only cortisol and corticosterone, both harboring the 11 $\beta$ -hydroxyl group, functioned as GR $\alpha$  agonists (Fig. 2B). Importantly, changing the stereochemistry at C11 of cortisol from the naturally occurring ( $\beta$ ) to the synthetic ( $\alpha$ ) configuration resulted in a complete loss of GR $\alpha$ -mediated transactivation, in contrast to the AR<sup>CCR</sup>. Cortisone, which has a keto group at the C11 position, had no agonist activity for GR $\alpha$ , as expected. In contrast, it was as effective as cortisol (which has a hydroxyl group at C11) in activating the AR<sup>CCR</sup>. Overall, these data suggest that the AR<sup>CCR</sup> has an activation profile distinct from those of wild-type AR and GR $\alpha$  and that both active glucocorticoids (cortisol and corticosterone) and inactive corticosteroids (cortisone and 11 $\alpha$ -cortisol) are potent activators of the AR<sup>CCR</sup>.

#### Synthetic glucocorticoids exhibit differential agonist activity for the AR<sup>CCR</sup>

We next tested several commonly prescribed synthetic glucocorticoids, which are potent GR $\alpha$  agonists, for their possible agonist activity via the AR<sup>CCR</sup>. These steroids each contain the 11 $\beta$ -hydroxyl group except prednisone, which has a keto group in that position (Fig. 3C). They also contain modified A rings that are unsaturated at C1–C2 except the mineralocorticoid/glucocorticoid FluF. In competitive binding assays (Table 1), FluF exhibited a 3-fold increase in binding affinity for the AR<sup>CCR</sup>, compared with cortisol. The potent

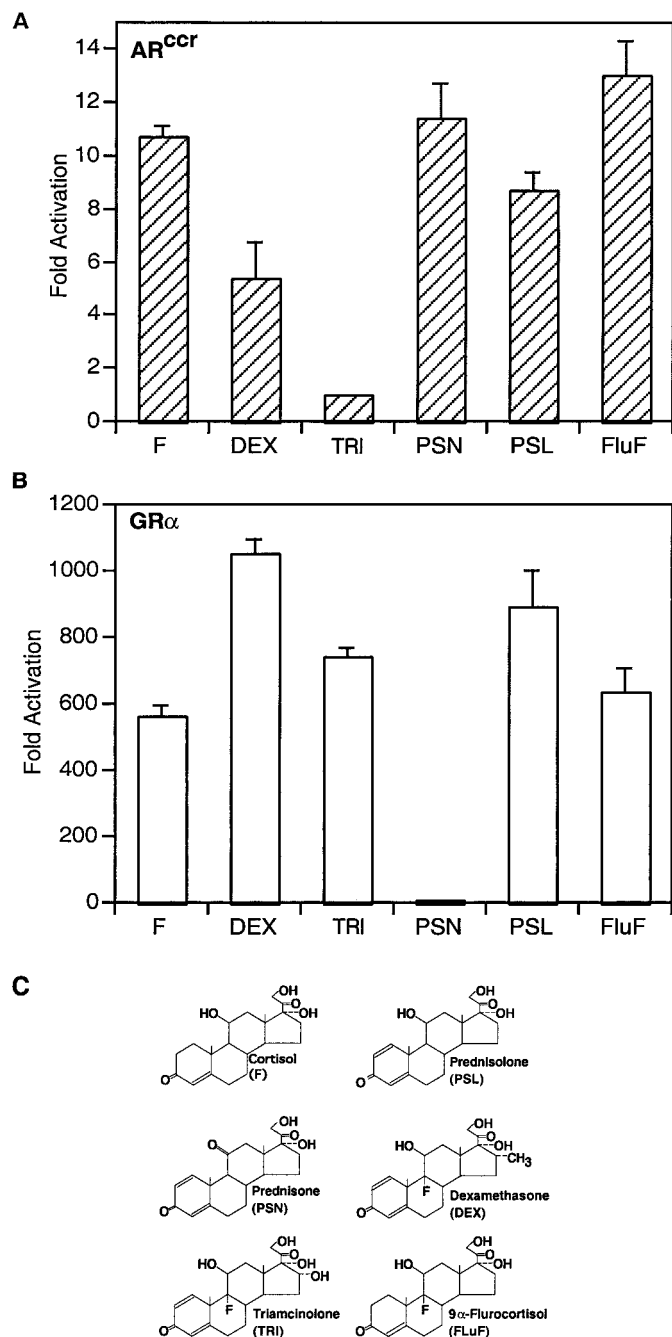


FIG. 3. Synthetic glucocorticoids exhibit differential agonist activity for the AR<sup>CCR</sup>. CV-1 cells were transfected with expression vectors for AR<sup>CCR</sup> (A) or GR $\alpha$  (B) and the reporter MMTV-luc as well as renilla luciferase plasmids. Cells were treated with the indicated steroids at 5 nM in steroid-depleted medium for 30 h. Cell extracts were subsequently assayed for luciferase activity by dual-luciferase system (Promega Corp.). Values are given as fold activation over activity found in control cells treated with ethanol. Data represent the mean of assays performed in triplicate  $\pm$  SEM. C, Structures of the synthetic corticosteroids tested in A and B. TRI, Triamcinolone; PSN, prednisone; PSL, prednisolone.

glucocorticoids, prednisone ( $\Delta^1$ -dehydrocortisone), prednisolone, ( $\Delta^1$ -dehydrocortisol), and dexamethasone (9 $\alpha$ -fluoro-16 $\alpha$ -methylprednisolone), bound to AR<sup>CCR</sup> with binding affinities approximately 5-fold lower than cortisol and

cortisone (Table 1). Thus, the double bond at C1–C2 in the A ring decreased the binding affinity of the steroids for AR<sup>CCR</sup>. Interestingly, triamcinolone (9 $\alpha$ -fluoro-16 $\alpha$ -hydroxyprednisolone), a potent synthetic glucocorticoid, which has a hydroxyl group in the D ring of the sterol structure replacing the C16 methyl group of dexamethasone, did not bind to AR<sup>CCR</sup>.

In transactivation assays, the MMTV-reporter-transfected CV-1 cells were treated with each compound at a suboptimal concentration (5 nM) to detect differences in agonist activity between cortisol and other drugs. All of these synthetic compounds are known agonists for the GR $\alpha$ - and activated GR $\alpha$ -mediated transactivation (Fig. 3B). Prednisone, with a keto group at C11 position, was inactive through GR $\alpha$  as expected, because CV-1 cells are deficient in 11 $\beta$ -hydroxysteroid dehydrogenase, the enzyme that catalyzes the *in vivo* conversion of prednisone to the active molecule prednisolone with a hydroxyl group at the C11 position. As shown in Fig. 3A, FluF had a somewhat greater activity than cortisol, consistent with its increased affinity for AR<sup>CCR</sup> (Table 1). The AR<sup>CCR</sup>, which did not distinguish between a keto or hydroxyl group at C11 position, was activated by both prednisone and prednisolone. Both prednisone and prednisolone were comparable with cortisol in AR<sup>CCR</sup>-mediated transcription, although they exhibited lower affinities for binding to AR<sup>CCR</sup>. Dexamethasone, which contains a 16 $\alpha$ -methyl group and a 9 $\alpha$ -fluoro group in addition to the A-ring double bond, showed reduced activity via the AR<sup>CCR</sup>. Interestingly, triamcinolone containing a C16 hydroxyl group did not promote AR<sup>CCR</sup>-mediated transactivation. Thus, the C16 hydroxyl group seems to abolish AR<sup>CCR</sup> binding and gene activation through this receptor.

In summary, our transactivation studies revealed that the following hormones were AR<sup>CCR</sup> agonists: androgens [DHT, T, androstenedione, and R1881 (data not shown)]; corticosteroids (cortisol, cortisone 11-deoxycorticosterone, corticosterone, 11-deoxycortisol); synthetic glucocorticoids (dexamethasone, prednisone, prednisolone); and the mineralocorticoid/glucocorticoid (FluF). The synthetic glucocorticoid triamcinolone did not bind to or activate the AR<sup>CCR</sup>.

#### Casodex and RU486 antagonize AR<sup>CCR</sup>-mediated transactivation

In search of AR<sup>CCR</sup> antagonists that may have therapeutic utility in the treatment of prostate cancers harboring this type of mutated receptor, we evaluated several known receptor antagonists. These included the AR antagonists hydroxyflutamide and casodex, the GR/PR antagonist RU486, and the MR/AR antagonist spironolactone. In competition-binding assays (Table 1), these antagonists exhibited significant binding to the AR<sup>CCR</sup>. Their RBA values ranked as follows: cortisol 100% > spironolactone 30% > RU486 16.4% > hydroxyflutamide 11.3%  $\gg$  casodex 0.05% (Table 1). Transactivation assays demonstrated that both hydroxyflutamide (20) and spironolactone (data not shown) functioned as AR<sup>CCR</sup> agonists in CV-1 cells, whereas casodex and RU486 acted as antagonists through the AR<sup>CCR</sup>. As shown in Fig. 4, both of these antagonists caused significant inhibition of R1881, cortisol, FluF, or corticosterone-induced activation of the MMTV-luc

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