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Human Progesterone Receptor A and B Isoforms in CHO Cells. I. Stable Transfection of Receptor and Receptor-responsive Reporter Genes: Transcription Modulation by (Anti)progestagens

R. Dijkema,[1]* W. G. E. J. Schoonen,[2] R. Teuwen,[1] E. van der Struik,[1] R. J. H. de Ries,[2] B. A. T. van der Kar[2] and W. Olijve[1]

[1]Department of Biotechnology and Biochemistry, Scientific Development Group, N.V. Organon, P.O. Box 20, 5340 BH Oss, The Netherlands and [2]Department of Endocrinology, Scientific Development Group, N.V. Organon, P.O. Box 20, 5340 BH Oss, The Netherlands

A hormone-dependent transcription modulation system was established on the basis of a two-step transfection procedure of the human progesterone receptor isoforms (hPR-A and hPR-B, respectively) and a progesterone receptor-responsive reporter (MMTV-Luc). In the first step, stable transfection of the hPR-A and hPR-B isoform-encoding cDNAs was performed in the steroid receptor-negative CHO K1 cell line. Individual clones were characterized for hPR-isoform expression with respect to Western immuno-blotting, transcriptional activation and hormone binding. With respect to the latter characteristic, individual hPR-isoforms demonstrated similar dissociation constants (K_d for hPR-A: 0.5 ± 0.3 and hPR-B: 0.8 ± 0.3 nM, respectively) irrespective of the amount of receptor isoform expressed (B_{max} varying from 4.1 to 33.2 nM). The K_d values observed for individual hPR-isoforms were comparable to those found for human breast tumor MCF-7 cells (K_d for hPR-A + hPR-B: 0.6 ± 0.3 nM). In the second step, hPR-isoform expressing CHO clones were supertransfected with a MMTV-Luc reporter construct resulting in permanent cell lines useful for testing the activity of natural and synthetic steroids in their ability to modulate gene transcription. Both isoform-specific reporter cell lines responded in a similar ranking order towards different progesterone reference compounds such as Org 2058, progesterone (Prog), R5020, norethisterone (NE), and medroxy progesterone acetate (MPA). Moreover, a good correlation was observed between the relative binding affinity (RBA) and the transcriptional activation potency of these compounds towards the individual hPR-isoforms. The latter correlation could not only be demonstrated for the progestagenic agonist reference compounds but was also observed for the progestagenic antagonist reference compounds like Org 33628, Org 31710, RU 38486 and ZK 98299. The major difference observed between the individual PR-isoforms was related to the degree of stimulation of the reporter gene (MMTV-based) within the cellular CHO context. Therefore, these cell lines can be used for the determination and quantitation of the activity of (anti)progestagenic compounds *in vitro* but may also be useful to predict the activity of compounds *in vivo* (see also II Comparison of binding, transactivation and ED_{50} values of several synthetic (anti) progestagens *in vitro* in CHO and MCF-7 cells and *in vivo* in rabbits and rats).

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INTRODUCTION

The human progesterone receptor (hPR), initially cloned in 1987 [1], belongs to the steroid–thyroid–retinoic acid family of nuclear receptor proteins. These

*Correspondence to R. Dijkema. Fax: 31 412 662617.
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receptor proteins act as transcriptional factors able to induce complex gene networks after interaction with their cognate ligands [2, 3]. The association of progesterone to its specific receptor initiates a number of events including the allosteric change in receptor structure, the increase in receptor phosphorylation, and the displacement of a repertoire of heat shock proteins acting to repress receptor function. Altogether, these events lead to the interaction of the steroid receptor complex with specific hormone response elements (HREs) within the regulatory regions of progesterone-responsive target genes [4, 5].

The hPR is unique within the steroid hormone receptor family, since it exists in two distinct molecular isoforms in cells; the hPR-B isoform containing 933 amino acids and the (truncated) hPR-A isoform lacking the N-terminal 164 amino acids present in the hPR-B isoform [6]. At present, it is a matter of debate whether both PR-isoforms do occur in all species or whether rabbit PR represents an exception [7, 8]. In addition, within the different species analyzed so far, both PR-isoforms may originate from either two alternate promoters within the single copy PR-gene [9] or alternate initiation from a single PR-mRNA [10]. In this respect, it is likely that the PR-isoform ratio can differ depending on the origin and nature of the target tissue under investigation [11, 12].

Although a specific role for the individual hPR-isoforms is unclear, however, important differences have been shown with respect to the extent of transcriptional regulation of progesterone responsive target genes [13–16]. The latter phenomenon can be explained on the basis of the different N-termini as present in both hPR-isoforms and the role of this region in constituting one of the transcription activation functions (TAF-1) of the receptor [15, 17]. In this way, the differential modulation of target genes by the PR-isoforms has been demonstrated to depend largely on the cellular context in combination with the promoter (DNA) context. This finding implicates a possible role of additional transcription factors in the modulation of the overall transcriptional efficiency [13, 15, 16].

Since a detailed characterization of (anti)progestagenic hormones is of interest for their potential therapeutic use, a study was initiated towards hormone action mediated by the individual PR-isoforms *in vitro*. To this end, the commonly used "cotransfection assay" was performed using a combination of receptor and receptor-responsive reporter plasmids in a steroid receptor-negative cell background. However, data obtained this way demonstrated a considerable inter/intra assay variation not allowing the proper quantitative estimation of the hormonal activity. In order to minimize this variation, permanent cell lines were made using the sequential stable transfection of the individual hPR-isoforms and the PR-responsive MMTV-Luc reporter plasmid. These isoform-specific cell lines can provide an accurate tool for the quantitation of (anti)progestagenic hormone activity at both the level of receptor binding and receptor-mediated transcription modulation.

MATERIALS AND METHODS

Hormones and anti-hormones

The non-radioactive hormones Org 2058, progesterone (Prog), R 5020 (promegestone), norethisterone (NE), medroxyprogesterone acetate (MPA), 17β-estradiol (E2), 5α-dihydrotestosterone (DHT), dexamethasone (Dex), aldosterone (Ald) and the anti-hormones Org 33628, Org 31710, RU 38486 (mifepristone) and ZK 98299 (onapristone) were synthesized by N.V. Organon. Radioactive Org 2058 (1.7 Tbq/mmol), dexamethasone (3.3 Tbq/mmol) and aldosterone (2.7 Tbq/mmol) were obtained from Amersham, and estradiol (4.7 Tbq/mmol) and 5α-dihydrotestosterone (4.1 Tbq/mmol) from New England Nuclear.

Plasmids

The full-length hPR cDNA [1] was kindly provided by Dr E. Milgrom (INSERM U135, Paris, France) and used for the construction of hPR-A and hPR-B expression plasmids in the following way. The hPR-B encoding cDNA was isolated as a 2847 bp *Eco*RI/*Dra*I fragment (the *endo*R *Eco*RI site being artificially introduced at position −13 upstream of the first AUG codon), whereas the hPR-A encoding cDNA was isolated as a 2357 bp *Eco*RI/*Dra*I fragment (the *endo*R *Eco*RI site being artificially introduced at position +477 upstream of the second AUG codon). In addition, a modified extended version of the hPR-B isoform was constructed in which the TGA stopcodon at position +2800 was replaced for a 20 amino acid encoding peptide(-Tag), thereby allowing immunodetection by Tag-specific monoclonal antibodies. The hPR-A and hPR-B encoding cDNAs were inserted in the mammalian expression vectors pKCR [18] and pSG5 [19], that are based on the SV40 early promoter and rabbit β-globin/SV40 splice and polyadenylation signals. In the case of hPR-B Tag, the cDNA was inserted into a pKCR-based vector, modified in a way that the insert is now under the control of the metallothionein-II gene promoter [20]. The pMMTV-Luc reporter plasmid was composed of the mouse mammary long terminal repeat promoter linked to the luciferase cDNA as present in pMAM and pMAMneoLUC, respectively (Clontech). The dominant selection markers for neomycin and hygromycin were present on the plasmids pAG60 [21] and pDR2 (Clontech), respectively; the latter selection plasmid was modified by digestion with *endo*Rs *Cla*I and *Pvu*II, blunted by Klenow DNA polymerase, and reli-

gation. In addition, plasmid pAG60/MTII was constructed by the insertion of the metallothionein-II gene (as a 3 kb *end*oR *Hin*dIII fragment, [20]) in the *end*oR *Hin*dIII site of pAG60.

Cell culture

Chinese Hamster Ovary cells (CHO K1) were obtained from ATCC (CCL61). They were cultured in M505 medium consisting of a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM, Gibco) and Nutrient Medium F12 (Ham's F12, Gibco) supplemented with 5% charcoal-treated defined bovine calf serum (Hyclone).

The human breast tumor cells T47D and MCF-7 were kindly provided by Dr R. Sutherland (Garvan Institute, Sydney, Australia) and Dr M. McGrath (Michigan Cancer Foundation, U.S.A.), respectively; they were cultured as described [22].

DNA transfections

CHO cells were transfected with the cDNAs encoding hPR-A, hPR-B and/or hPR-B Tag together with the selection plasmid pAG60 (in the case of hPR-A and hPR-B) or pAG60/MTII (in the case of hPR-B Tag). Transfections were performed by either the calcium phosphate coprecipitation method [23], followed by a glycerol shock or the lipofection method (Gibco) using a 10:1 molar ratio of receptor plasmid and selection plasmid. Stable transfected cells were selected in culture medium supplemented with neomycin (800 μg/ml, Gibco) and subsequent increments in $CdCl_2$ (in the case of hPR-B Tag). Single cell clones were obtained by dilution titration of positive selected cell pools. Receptor-positive single cell clones containing hPR-A or hPR-B were super-transfected with the reporter plasmid pMMTV-Luc together with the selection plasmid pDR2A using similar methods and plasmid ratios. Stable super-transfected cells were selected in culture medium supplemented with hygromycin (400 μg/ml, Boehringer) and single cell clones were obtained as described.

Characterization of CHO transfectants

Western immunoanalysis. CHO transfectants were tested for receptor expression by analyzing cell extracts by Western immunoblotting [24], using monoclonal antibodies specific for the NH-terminal A/B-domain (Novocastra, NCL-PGR 1A6) or Tag-peptide (Tag-MoAb 39C, gift of Dr H. de Haard, Organon Teknika, Boxtel, The Netherlands).

Hormone binding analysis. CHO transfectants were tested for receptor content by saturation binding analysis using labelled Org 2058, E2, DHT, Dex, and Ald as described [25]. Shortly, transfected cells were harvested, homogenized and cytosolic preparations obtained by centrifugation at 100 000 *g* for 45 min at 4°C. Cytosol was diluted into 96-well plates and incubated overnight at 4°C with labelled ligand (0.1–10 nM) in the presence or absence of 1 μM unlabelled ligand. Bound and unbound hormone were separated by the addition of dextran-coated charcoal. After centrifugation, radioactivity in the supernatant was counted in a Topcount microplate scintillation counter (Packard). Specific binding was determined by subtracting non-specific (sample with excess unlabelled ligand) from total binding (sample without excess unlabelled ligand). Binding data were transformed and displayed in a Scatchard plot as bound over free vs bound. For the calculation of the dissociation constant (K_d) and the maximal number of binding sites (B_{max}) the non-linear least square curve-fitting program LIGAND was used as described [26].

Luciferase reporter assay. Functional receptor in stable CHO transfectants obtained after the primary transfection/neomycin selection, was demonstrated using transient transfection of pMMTV-Luc reporter plasmid and testing for single point hormone-dependent transcription activation (10 nM Org 2058). In addition, functional receptor/reporter in stable CHO transfectants obtained after the secondary transfection/hygromycin selection, was tested by dose-dependent transcription activation. Cells at 24–48 h after hormone addition were washed twice with phosphate buffered saline and incubated with a minimal volume of lysis buffer for 5–10 min at room temperature. Cell debris was removed by centrifugation and a sample corresponding to 5–10 μg protein was added to 100 μl luciferase reagent (Promega). The light emission was measured in a luminometer (Berthold Biolumat) for 10 s at 562 nm. Alternatively, LucLite (Packard) was used for combined lysis of cells and luminescence measurements in a 96 well-plate format (TopCount, Packard).

RESULTS

Stable transfection of hPR-isoforms in CHO cells

In order to obtain an isoform-specific *in vitro* transcription modulation system, the hPR-A and hPR-B encoding cDNAs were introduced in the receptor-negative CHO cell line. The expression of the individual PR-isoforms is regulated by the SV40 promoter present in pKCR and pSG5-based expression plasmids, known to result in the efficient synthesis of steroid hormone receptors [19]. In addition, a hPR-B variant containing an additional peptide-Tag at the COOH-terminus, was constructed and inserted in a modified pKCR in that the insert is now regulated by the metallothionein-II gene promoter. The latter expression plasmid in combination with the selection plasmid pAG60/MTII offers the possibility of additional gene amplification by the addition of $CdCl_2$, thereby resulting in an increase in the receptor expression level.

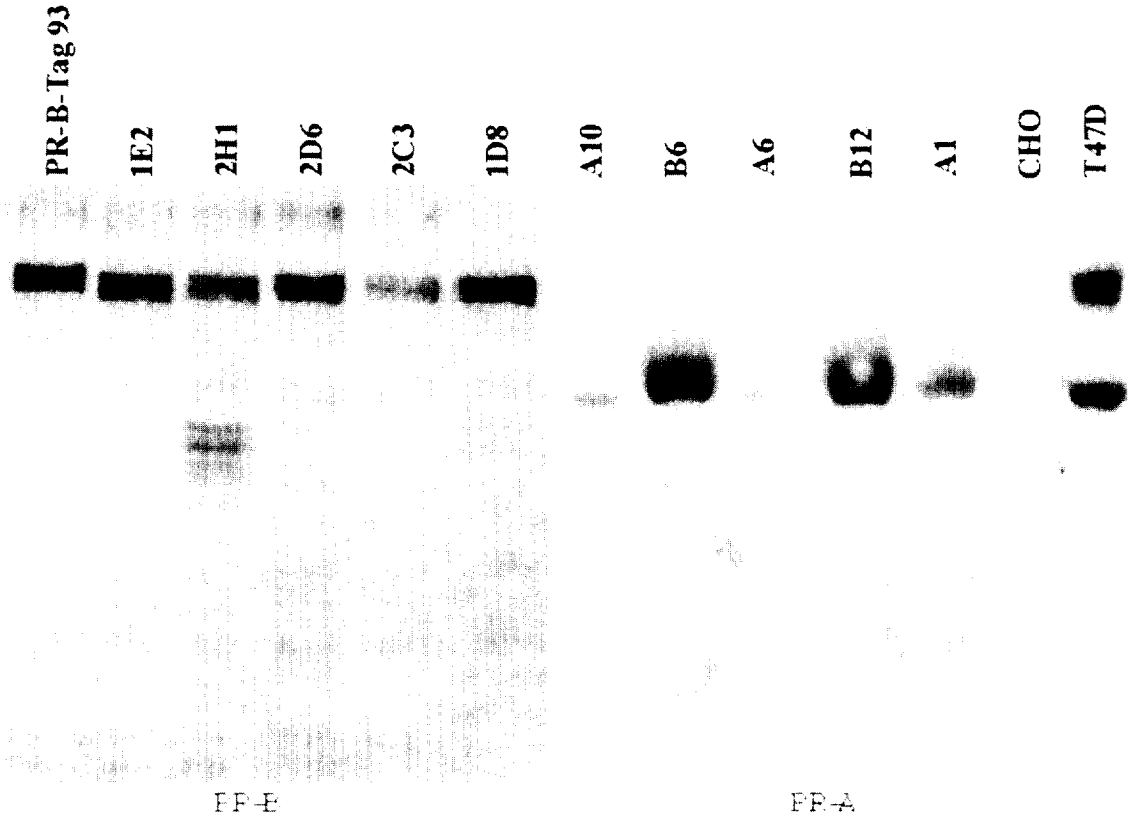

Fig. 1. Western blot analysis for identification of PR-isoform expression within CHO cell clones. Neomycin-resistent CHO cell clones were made as described in Section 2. Extracts were prepared from cells grown in selective medium and protein (50–100 μg) was loaded and processed for 7.5% SDS-PAAGE. Subsequently, immunodetection was performed by a PR-specific monoclonal antibody recognizing both receptor isoforms (Novocastra, NCL-PGR 1A6).

After the initial selection by neomycin (and in the case of hPR-B Tag, the subsequent selection/amplification by 2.5 μM $CdCl_2$), individual cell pools of each transfection were subjected to a single cell cloning procedure. A representation of individual cell clones was analyzed by Western immunoblotting using a PR-specific antibody and is shown in Fig. 1. Both recombinant expressed hPR-B (and its enlarged variant hPR-B Tag) as well as hPR-A comigrated with the isoforms as naturally expressed in the T47D human breast tumor cells [27]. Variation was observed in the PR-isoform expression levels in between individual clones as well as the integrity of the immuno-pattern observed.

Apart from the demonstration of the hPR-isoform expression at the protein level, an impression of functional hPR-isoform activity in cell clones was obtained by analyzing the effect of 10 nM Org 2058 on transiently transfected pMMTV-Luc reporter (Fig. 2). In this way, a clear distinction between hPR-B and hPR-A CHO transfectants became visible in that hormone-dependent activation mediated by hPR-B was more efficient as compared to the modulation mediated through hPR-A for the standard progestagen Org 2058 (stimulation factor 10 to 29 for hPR-B and stimulation factor 1.5 to 4 for hPR-A, respectively). The hPR-B-Tag clone 93, when analyzed by the same method, showed a hormone-dependent stimulation factor of 24, indicating the lack of interference of the additional COOH-terminal peptide sequence in reporter gene activation by Org 2058.

To quantitate the different expression levels of a number of individual CHO transfectants, saturation binding assays were performed with [^{3}H] Org 2058 and cellular extracts of a subset of hPR-isoform expressing clones (Table 1). Data presented in this table substantiated the presence of the different number of hPR binding sites within individual cell clones (indicated as B_{max}) as well as the high affinity nature of the hPR binding sites in these cell clones (indicated as K_d). In addition, the K_d values observed for the individual hPR-isoforms was in the same order of what has been found for the hPR-A/hPR-B combination as present in the MCF-7 human breast tumor cells. Moreover, the use of other radioactive steroid ligands (e.g. E2, DHT, Dex, and Ald) did not demonstrate any detectable binding towards the individual hPR-isoform transfected CHO cell clones (data not shown).

Because of the level of PR-isoform expression, clones hPR-B Tag 93 and hPR-A B6 were chosen for future competition binding analysis. In addition,

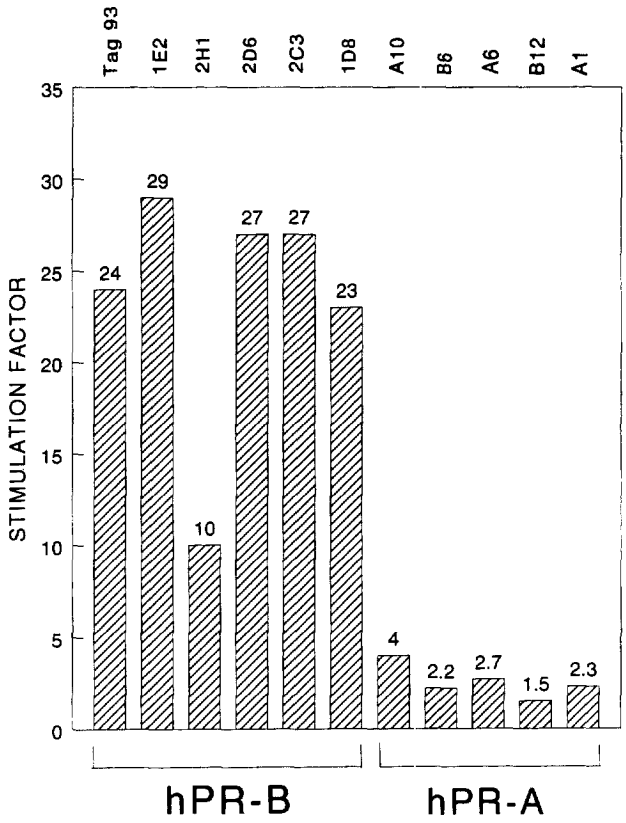

Fig. 2. Induction of luciferase activity of PR-isoform expressing CHO cell clones. Neomycin-resistant CHO cell clones were transiently transfected with pMMTV-Luc by lipofection (Gibco). 5 h posttransfection cells were cultured in selective medium in the presence or absence of 10 nM Org 2058 for 48 h. Luciferase activity was determined in 10 μg protein extract as described under Section 2 (Promega) and is illustrated as stimulation factor; the latter representing the ratio of luciferase activity in the presence and absence of hormone.

clones hPR-B 1E2 and hPR-A B6 and A10 were selected for stable supertransfection with the MMTV-Luc reporter on the basis of the functional characteristics described above.

Table 1. Quantitative determination of the hormone binding characteristics (K_d and B_{max} values) for a number of PR-isoform expressing CHO cell clones

Cell line	K_d (nM)	B_{max} (nM)
CHO hPR-B 1E2	0.2 ± 0.1	9.5 ± 0.5
CHO hPR-B-Tag 93	0.8 ± 0.3	33.2 ± 14.9
CHO hPR-A B6	0.5 ± 0.3	19.3 ± 3.7
CHO hPR-A A10	0.3 ± 0.1	4.1 ± 0.7
MCF-7 (hPR-B/hPR-A)	0.6 ± 0.3	5.0 ± 0.9

Binding experiments were performed on the basis of equivalent protein concentrations within cytosolic extracts and calculations were made using the non-linear least square program as described in Section 2.

Stable supertransfection of pMMTV-Luc in PR-isoform-transfected CHO cells

In the subsequent secondary transfection, the pMMTV-Luc reporter plasmid was transfected in hPR-B and hPR-A-containing CHO transfectants using the hygromycin resistance gene as a dominant marker. In this way, neomycin and hygromycin resistant cell pools as well as single cell clones derived from them were obtained. All cell clones were selected on the basis of their hormone-dependent luciferase activation. Figure 3 illustrates the concentration-dependent progestagen (Org 2058) activation of individual supertransfected single cell clones possessing either hPR-B [based on 1E2, Fig. 3(A)] or hPR-A [based on B6 and A10, Fig. 3(B) and (C), respectively].

As already demonstrated above using transient transfection of the MMTV-Luc reporter into hPR-B and hPR-A transfectants (see Fig. 2), also stable hPR-B/MMTV-Luc and hPR-A/MMTV-Luc supertransfectants responded in a quantitative different way with respect to the stimulation factor upon hormone addition (stimulation factor for the hPR-B/1E2 series of 25 to 30 and stimulation factor for the hPR-A/A10 series of 5 to 10, respectively). In addition, the hormone concentration range to achieve maximal

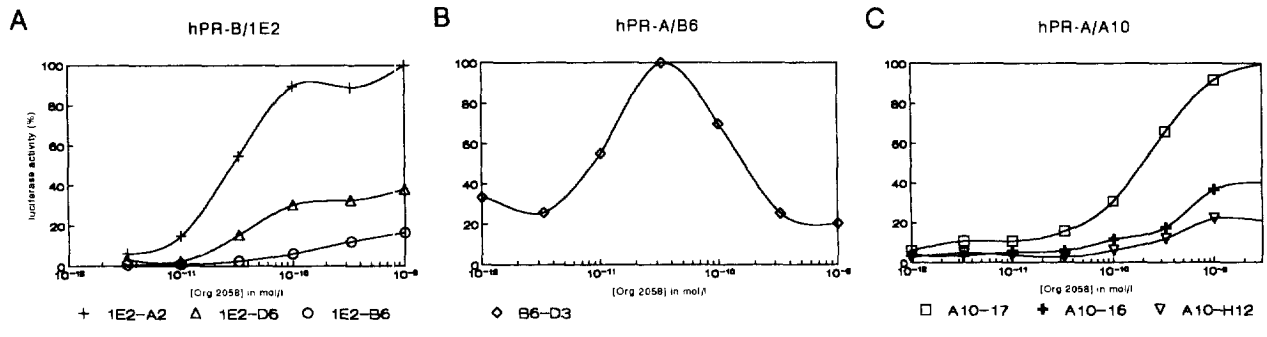

Fig. 3. Hormone-dependent stimulation of luciferase activity of PR-isoform and reporter expressing CHO cell clones. Individual supertransfectants containing PR-B (A) and PR-A (B and C) in combination with the pMMTV-Luc reporter plasmid were cultured in selective medium in the presence of increasing concentrations of Org 2058. 48 h after the addition of hormone cell extracts were prepared and assayed for luciferase activity (Promega).

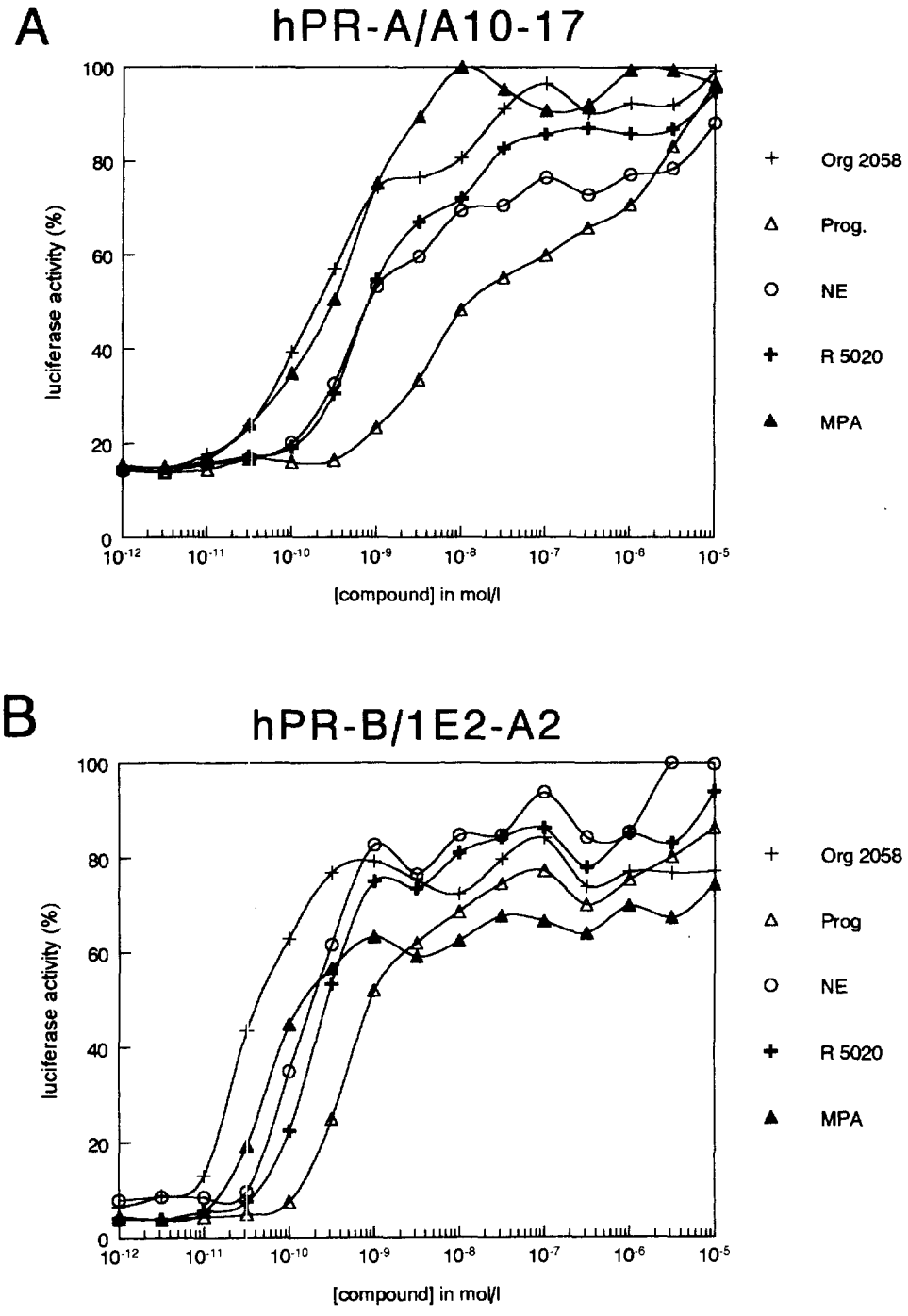

Fig. 4. Dose-dependent stimulation of luciferase activity by different progestagen agonists of CHO supertransfectants containing PR-isoforms and reporter. Individual supertransfectants specific for PR-A (A) and PR-B (B) were cultured in non-selective medium in the presence of increasing concentrations of hormones as indicated. 16 h after the addition of hormones cell extracts were prepared and assayed for luciferase activity (Packard). Data points reflect the mean of duplicate measurements varying less than 5%.

stimulation was more or less comparable for all single cell clones within a hPR isoform-specific series. Interestingly, the supertransfectant obtained from the hPR-A/B6 parental clone (e.g. hPR-A/B6 D3) showed an optimum in hormone-dependent activation. The major difference between the hPR-A/B6 and hPR-A/A10 parental clones was the amount of the hPR-A protein as determined by the number of binding sites in the saturation binding analysis (see Table 1).

The isoform-specific CHO supertransfectants hPR-B/1E2-A2 and hPR-A/A10-17 were selected for further study with respect to the effects of different progestagenic agonists and antagonists.

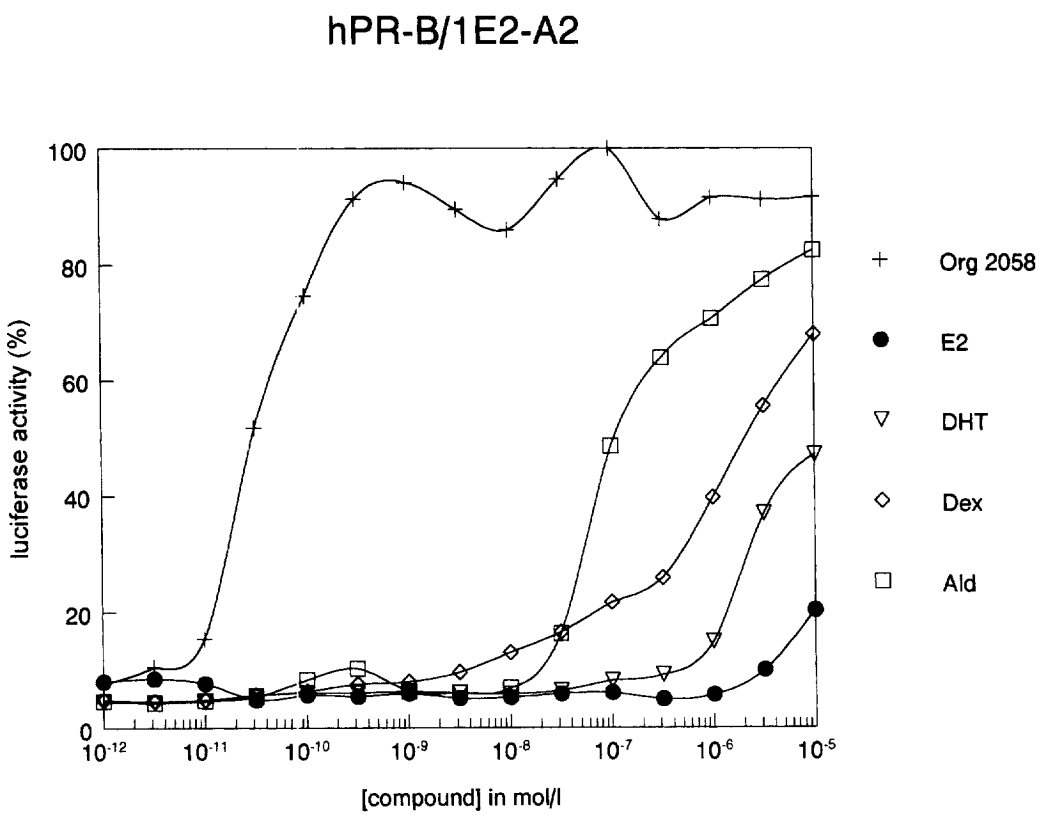

Fig. 5. Dose-dependent stimulation of luciferase activity by different steroid hormones of the CHO supertransfectant containing PR-isoform B. The procedures for cell culturing, hormone addition and luciferase activity determination were as described in legend to Fig. 4.

hPR-isoform mediated transcription modulation by (anti)-progestagens

The effect of a number of compounds possessing different affinities towards hPR were evaluated for gene transactivation potency towards the CHO supertransfectants containing individual hPR-isoforms and functional reporter (Fig. 4). As illustrated in this figure, both hPR-isoforms mediated transcription activation in a similar dose-dependent way as evidenced by the high sensitivity towards the progestagenic reference compounds Org 2058, Prog, R5020, NE and MPA. Moreover, both hPR-isoforms responded in a similar ranking order with respect to the binding affinities of these compounds. In addition to the sensitivity and ranking order of both hPR-isoforms as mentioned above, the selectivity in steroid transactivation was also demonstrated with E2, DHT, Dex and Ald for the hPR-B isoform transfected CHO cell clone 1E2/A2 (Fig. 5). In this respect, the latter compounds were all able to induce gene transcription modulation, albeit at a 1000-fold higher concentration as compared with the progestagen reference compound Org 2058.

In order to test the effect of different progestagen antagonists mediated by the individual hPR-isoforms, stable transfected cells were treated with 1 nM Org 2058 and increasing amounts of test compound (Fig. 6). All compounds tested were able to suppress the agonist-mediated (Org 2058) transcriptional activation of both hPR-isoforms. Moreover, and similar to what has been demonstrated above for the progestagenic agonists, the progestagenic antagonists Org 33628, RU 38486, Org 31710 and ZK 98299 all responded in an identical ranking order and dose-dependent way. All progestagen antagonists if tested for their intrinsic agonist properties towards both hPR-isoforms did not result in any stimulation of reporter gene activity up to concentrations of 0.1 μM (data not shown).

DISCUSSION

A two-step transfection procedure was used to obtain CHO cell-based transfectants containing functional hPR-isoforms and a hPR-responsive reporter. In the primary transfection, cDNA's encoding individual hPR-isoforms were introduced and single cell clones analyzed for hPR-isoform characteristics at both the protein and functional level. As suggested by the immuno-analysis and accurately quantitated by the hormone binding studies, the expression level of hPR-isoforms in between individual cell clones showed great variation. In this respect, the analysis of receptor content over a prolonged period of cell culturing (with and without selective agents) did not

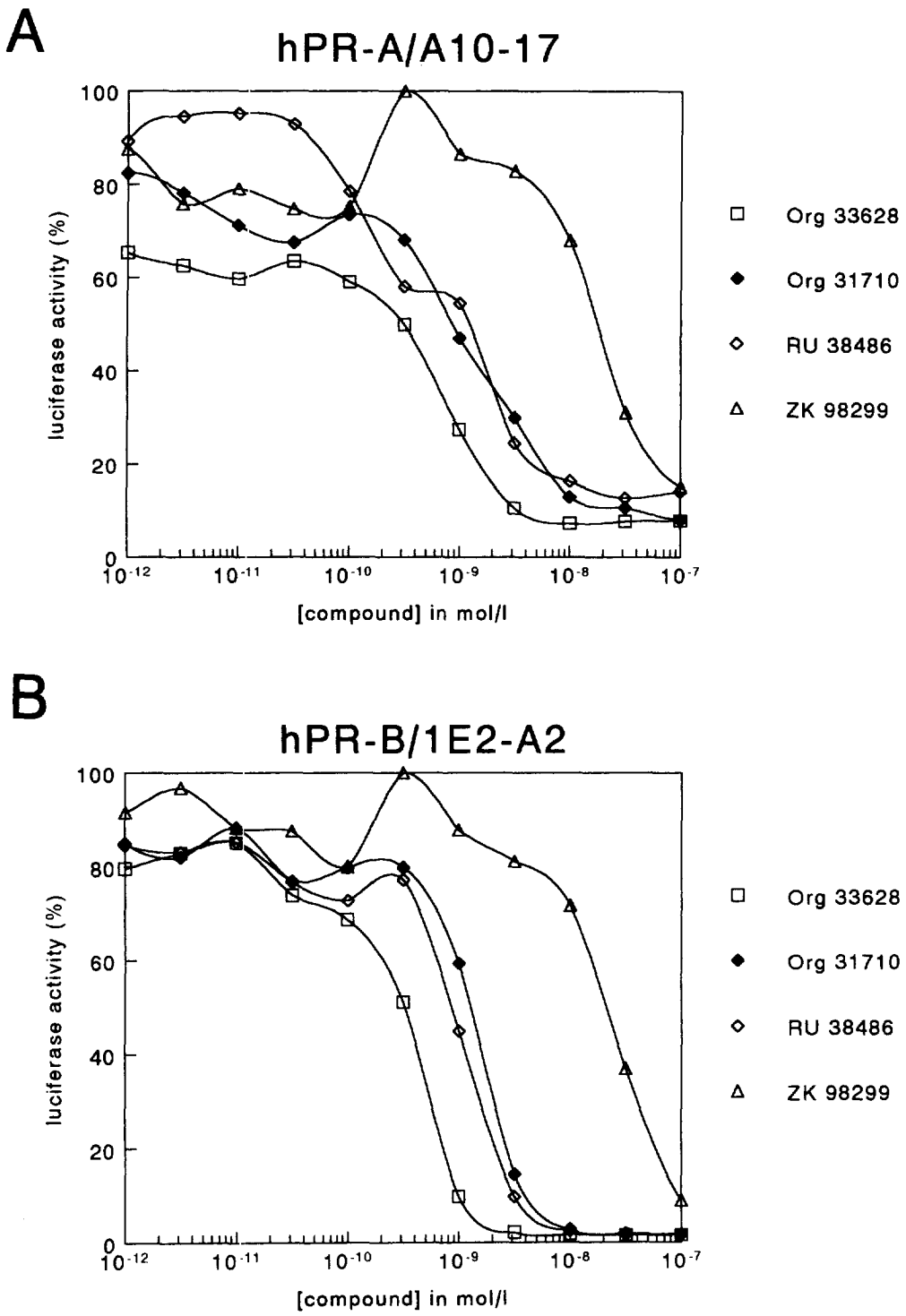

Fig. 6. Dose-dependent inhibition of luciferase activity by different progestagen antagonists of CHO supertransfectants containing PR-isoforms and reporter. Individual supertransfectants specific for PR-A (A) and PR-B (B) were cultured and tested as described in legend to Fig. 4, except that the progestagen agonist Org 2058 was included in all test samples.

result in any significant change in the expression level of the receptor protein (data not shown). Therefore, CHO cells possessing high levels of hPR-isoforms were not deleterious whatsoever with respect to cell growth and survival as has been documented for the human estrogen receptor [28]. PR-isoforms expressed by the CHO transfectants showed the typical molecular weight heterogeneity caused by basal receptor phosphorylation [29]; the latter post-translational modification has been demonstrated in multiple human breast tumor cell lines expressing both hPR-isoforms [27, 30].

Functional properties of the individual hPR-isoforms in CHO transfectants were tested by hormone-dependent activation of transiently transfected reporter. All clones showed a clear hPR-isoform related difference with respect to the magnitude of gene transcription activation. Thus, hPR-A being active towards the MMTV promoter with a 2 to 5-fold stimulation factor, whereas hPR-B could activate with a 10 to 35-fold stimulation factor. The lower (10-fold) stimulation factor of hPR-B was primarily based on hPR-B clone 2H1. This clone contained, in addition to the full-length hPR-B receptor protein, a number of incomplete NH-terminal receptor protein fragments (see Fig. 1). The latter characteristic might well be responsible for the relative high hormone-independent basal luciferase level, thereby lowering the overall stimulation factor. In addition, the hPR-isoform related transcriptional efficiency was independent of the receptor content within the cells, since both high and low isoform-expressing transfectants indicated comparable stimulation factors. The observed differential modulation of the MMTV promoter using different hPR-isoforms in transient transfection have been documented before for human [14–16] and chicken [13, 31] PR.

In the secondary transfection, the MMTV-Luc reporter was transfected in isoform-containing primary CHO transfectants. Resulting supertransfectants were directly screened for dose-dependent hormone activation. Multiple isoform-specific supertransfectants were obtained, that all possessed similar dose-dependent hormone activation albeit with different (absolute) activation plateau levels. As an exception to this, the supertransfectant derived from the hPR-A B6 parental clone (e.g. hPR-A/B6-D3), demonstrated an optimum in hormone-dependent activation; the latter optimum was evidenced for all progestagenic compounds tested (data not shown), indicating a "squelching" effect as described before [16, 17, 32]. This squelching effect appeared to be specific for hPR-A, since hPR-B transfectants expressing comparable (and higher) amounts of receptor protein did not show any indication of an optimum in dose-dependent hormone activation [16, 17]. Moreover, this effect might explain the relative low stimulation factor within the relative high expressing hPR-A transfectants B6 and B12 as determined by single point hormone-dependent transient transfection of the MMTV-Luc reporter (see Fig. 2).

Testing of (anti)progestagenic compounds for isoform-mediated transcription modulation was obtained by the dose-dependent titration of compounds in the culture medium of isoform-specific CHO supertransfectants. The (anti)progestagenic compounds tested all differ in their affinity towards hPR as determined by competitive hormone binding studies using cell extracts of the hPR-isoform containing breast tumor cell line MCF-7 ([25], see accompanying article II). If analyzed for their transcription modulation potencies mediated by the individual hPR-isoforms, all progestagenic agonists and antagonists tested, were active towards the individual PR-isoforms in a similar if not identical way. This was illustrated not only by a comparable ranking order of the activity of compounds with respect to their relative binding values (RBA's), but was also evidenced by a good correlation that existed for the half maximal stimulation and/or inhibition values.

Therefore, these CHO permanent cell lines expressing hPR-isoforms and PR-responsive reporter can provide an efficient and accurate tool for the quantitative determination of (anti)progestagens *in vitro*. The latter characteristic and the linkage of *in vitro* transcription modulation data towards the *in vivo* situation will be the subject of the follow-up article II.

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