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The Human Progesterone Receptor A-Form Functions as a Transcriptional Modulator of Mineralocorticoid Receptor Transcriptional Activity

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The human progesterone receptor (hPR) exists as two distinct molecular forms in most cells, hPR-A and -B. These receptor isoforms display distinct biological functions and demonstrate a cell and promoter specific ability to regulate gene transcription. In cellular contexts where hPR-A is transcriptionally inactive it can function as a ligand dependent inhibitor of mineralocorticoid receptor (MR) transcriptional activity. Inhibition occurs by a non-competitive mechanism as direct binding to MR is not required. Interestingly, PR agonists differ in their ability to facilitate the inhibitory function of hPR-A, suggesting that a specific receptor conformation may be preferred for this activity. Those compounds derived from 19-nor-testosterone are the most effective. The antiprogestins RU486, ZK98299 and ZK112993 are effective MR antagonists in the presence of coexpressed hPR-A. The mechanism of hPR-A mediated inhibition of MR transcriptional activity is unknown. We propose that inhibition results from a competition of hPR-A with MR for a common transcription factor and that the association of hPR-A with this factor is not transcriptionally productive.

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INTRODUCTION

Progesterone is a key hormone central to the regulation of reproductive function in the body [1]. Its mechanism of action is similar to other nuclear hormones in that it utilizes a specific intracellular receptor which transduces its chemical message to the nuclei of target cells. The interaction of progesterone with its specific receptor induces allosteric changes in receptor structure, promotes phosphorylation and displaces tightly bound heat-shock proteins which act to repress receptor function. These events ultimately lead to an association of the progesterone receptor (PR) with specific DNA elements within the regulatory sequences of progesterone responsive target genes [2–4]. The resulting changes in cellular phenotype are a consequence of the combinatorial effects of the positive or negative regulation of multiple genes in target tissues.

The human progesterone receptor (hPR) is unique among the classic steroid hormone receptors as it is present in the cell as two distinct molecular forms, hPR-A and -B [5]. Controversy exists as to the occurrence of multiple forms of PR in all species, but to date two PR isoforms have been identified in the reproductive organs of human [5], rat [6], mouse [7] and chicken [8]. In humans, both PR isoforms are encoded by the same gene and differ only in that the amino terminus of hPR-B extends 164 amino acids longer than hPR-A [9]. Recent evidence has suggested that both of these receptors arise from alternate initiation of transcription from two promoters within the same gene [9]. Interestingly, in chicken, both forms of PR are also derived from a single gene. However, unlike hPR, both chicken PRs (cPR) arise by alternate initiation of translation from a single mRNA [10]. The existence of complex systems regulating production of these proteins in species as diverse as chickens and humans suggests that both protein forms are critically important for manifestation of progesterone activity.

A genetic analysis of the functional domains of hPR has revealed that the structural elements required for DNA binding, hormone binding, dimerization, nuclear

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localization, and transactivation (transactivation activation function 1 and 2; TAFs) are wholly contained within the region common to hPR-A and -B. Recently, however, it has been shown that a region in the hPR-B-specific 164 amino acids is required for maximal activity of the amino-terminal TAF1 function in some promoter contexts [9, 11]. This information suggested that TAF1 in PR-A and -B are functionally different. In addition, it is suggested that genes which were predominantly under TAF2 control could be regulated by both PR-A and -B, whereas those genes that were predominantly regulated by TAF1 would demonstrate a differential responsiveness to hPR-A and -B. In support of this we and others have shown that progesterone receptor A and B isoforms do in fact display a promoter- and cell-specific ability to function as transcriptional regulators ([12, 13, 18] and Wen, D. X. and McDonnell, D. P., unpublished data). In particular, hPR-B was transcriptionally active in most cellular contexts. However, hPR-A transcriptional activity was more restricted.

We were intrigued by the fact that even though hPR-A transcriptional activity was context restricted its production was tightly regulated in most cells. It was possible that in cells where PR-A was transcriptionally inactive that it had additional functional roles that previously were unexplored. Indeed, production of two transcriptional regulators from a common gene is not unique. Recent studies have indicated that the transcription factors LAP [14], mTFE3 [15], and thyroid hormone receptor-α [16], all encoded by single genes, give rise to two distinct proteins of different molecular weight. In all these cases it was observed that the smaller isoform functioned as a transdominant inhibitor of the transcriptional activity of the larger protein [17]. We have shown previously that in cells where hPR-A is transcriptionally inactive it functions as a hormone-dependent inhibitor of glucocorticoid (GR), progesterone-B and androgen receptor (AR) transcriptional activity, whereas it had no effect on vitamin D receptor mediated gene transcription [18]. In order to determine if all members of the GR subfamily of receptors were modulated in a similar manner we examined the role of hPR-A as a modulator of mineralocorticoid receptor (MR) function.

MATERIALS AND METHODS

Chemicals

Restriction and modification enzymes were obtained from Promega Biotec (Madison, WI), Boehringer Mannheim (Indianapolis, IN), or New England Biolabs (Bethesda, MD). PCR reagents were obtained from Perkin Elmer Cetus (Norwalk, CT). Chemicals were purchased from Sigma (St Louis, MO). The antiprogestins ZK112993 and ZK98299 were a generous gift from Dr David Henderson (Schering-AG, Berlin).

Cell culture

Monkey kidney CV-1 fibroblasts were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) (Biowittaker, MD) supplemented with 10% fetal bovine serum (FBS, obtained from Hyclone Laboratories, UT).

Transient transfection assays

Cells were seeded in 12-well, 96-well or 10 cm tissue culture plates. DNA was introduced into cells using calcium phosphate coprecipitation [19]. 20 μg of DNA/ml of transfection buffer were used in each transfection reaction. In this mix, the concentration of the luciferase plasmids and that of the internal control plasmid (pCH110, which contains the gene for the β-galactosidase enzyme) remained constant (5 μg of each plasmid DNA), while the receptor plasmid concentration varied as indicated for each experiment. Different amounts of receptor parental plasmid, pSV2-neo was included to keep constant the total amount of the SV40 enhancer containing vectors. pGEM4 plasmid DNA was added to balance the total DNA concentration to 20 μg/reaction. For the 96-well plate experiments, transfections were performed on a Biomek 1000 Automated Laboratory Workstation (Beckman, Fullerton, CA) and cells were incubated with the precipitate for 6 h. Cells were washed with PBS and incubated for 40 h with or without hormones as indicated in the text. Cell extracts were prepared as described previously [22] and assayed for luciferase and β-galactosidase activities.

Plasmid constructions

The construction of all the plasmids used in this paper with the exception of pRST7hPR-A and -B have been described previously [18–22]. These plasmids were constructed as follows. The plasmids YepPR-B and -A891, containing the full length hPR-B and a truncated hPR-A were cleaved with BamHI. This released the PR-A and -A891 DNA's, respectively. These fragments were cloned into the cognate site of the pRST7 expression vector [21], giving rise to pRST7hPR-A and -A891.

The construct pRST7hPR-B891 was derived as follows; YephPR-B891 was digested with AflII and KpnI. The 3 kb fragment arising from this digestion was purified and modified with T4 DNA polymerase and digested with BamHI. The resulting fragments (0.2 and 2.8 kb) were cloned into an EcoRV/BamHI prepared pRST7 vector. The plasmid pRST7hPR-B was constructed by replacing the BstEII/KpnI fragment of pRST7hPR-B891 with the analogous fragment from pRST7hPR-A. All the constructions were sequenced for validation.

RESULTS

hPR-A is a transdominant repressor of hPR-B function

We have shown previously that the transcriptional activity of the hPR-A and -B was dependent on cell

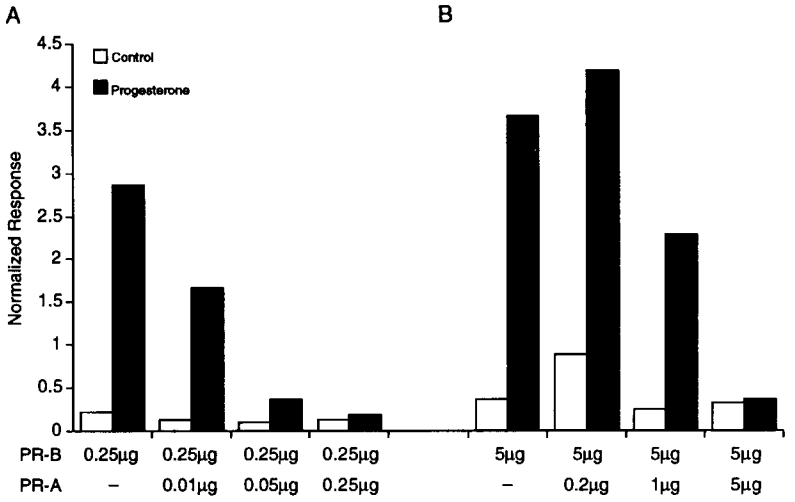

Fig. 1. The hPR-A functions as a transdominant repressor of hPR-B function. CV-1 monkey kidney cells were transiently transfected with (A) 0.25 μg phPR-B alone or in the presence of increasing concentrations of phPR-A (as indicated) together with 5 μg of an MMTV-LUC reporter plasmid or (B) 5 μg of pRST7hPR-B alone or in the presence of increasing concentrations of pRST7hPR-A together with 5 μg of the MMTV reporter. Cells were treated with 10^{-7} M progesterone as indicated and assayed for β-galactosidase and luciferase (LUC) activity (LUC activity was normalized for β-galactosidase activity). The normalized LUC activity was calculated by dividing the raw luciferase activity ($\times 10^4$ units) for each point by the β-galactosidase activity [($A_{415\,nm} \times 10^5$]/time in minutes) at that point. A representative experiment is detailed above. Each data point shown represents the average of triplicate determinations of the transcriptional activity under a given experimental condition.

type and promoter context [18]. Additionally, it was shown that in cellular contexts where hPR-A was transcriptionally inactive it functioned as a transdominant inhibitor of hPR-B, GR and AR function [18]. In order to extend and confirm these results we established two independent assays in the PR negative CV-1 cell line. One assay used the SV40 promoter to direct the synthesis of hPR-A and -B [Fig. 1(A)]*, whereas the other used the Rous Sarcoma Virus promoter (RSV) [Fig. 1(B)]. In both of these cases it is clear that ligand activated hPR-B was an effective regulator of the mouse mammary tumor virus (MMTV) promoter. In the presence of increasing concentrations of hPR-A the transcriptional activity of hPR-B in both assays was attenuated. Hormone binding and Western immunoblot analysis confirmed that half maximal inhibition occurred when hPR-A expression was 20–25% that of hPR-B. These data suggest that this inhibitory event is occurring sub-stoichiometrically and that hPR-A's biologic activity is "catalytic" in nature. It is clear from these results that the modulation of hPR-B by -A occurs independently of the vector systems used to produce the effector molecules. These results confirm that in a cellular context where hPR-A is transcriptionally inactive it can function as a transdominant inhibitor of hPR-B mediated gene transcription.

Reconstitution of a mineralocorticoid responsive transcription system in mammalian cells

We wished to determine if the transdominant role of hPR-A as a modulator of AR, GR, and hPR-B extended to MR. To accomplish this we reconstituted a mineralocorticoid responsive transcription unit in MR negative CV-1 cells. A vector (pRShMR) directing the synthesis of authentic MR was transfected into CV-1 cells and its ability to regulate the MMTV promoter in a ligand-dependent manner was measured. The results are shown in Fig. 2(A). In this assay MR functioned as an effective regulator of MMTV gene transcription in the presence of aldosterone, demonstrating an EC_{50} of 1×10^{-10} M. In the presence of cotransfected hPR-A there was a minor attenuation of MR activity at the highest concentrations of aldosterone. However, at concentrations above 10^{-8} M aldosterone it is clear that aldosterone interacts directly with hPR-A leading to a stimulation of the inhibitory activity of hPR-A. The fidelity of the reconstituted assay was confirmed by demonstrating that the MR antagonist, spironolactone, was capable of inhibiting aldosterone activation of MR. The antimineralocorticoid activity of spironolactone was unaffected by coexpression of hPR-A. These results suggest that MR functions as a ligand-dependent activator of MMTV gene transcription and that coexpresssion of hPR-A in the absence of a PR ligand has minimal effects on MR activity.

hPR-A functions as a hormone-dependent inhibitor of MR transcriptional activity

Previously, we have shown that PR ligands derived from 19-nortestosterone are effective in inducing the inhibitory activity of hPR-A [23]. For this reason we examined the ability of norethynodrel and norethindrone to modulate MR in the presence and absence of transfected hPR-A. The results are shown in Fig. 3. In

*The data presented in Fig. 1(A) have been presented before in another format [18]. It is included in this manuscript as a reference to quantitate inhibition.

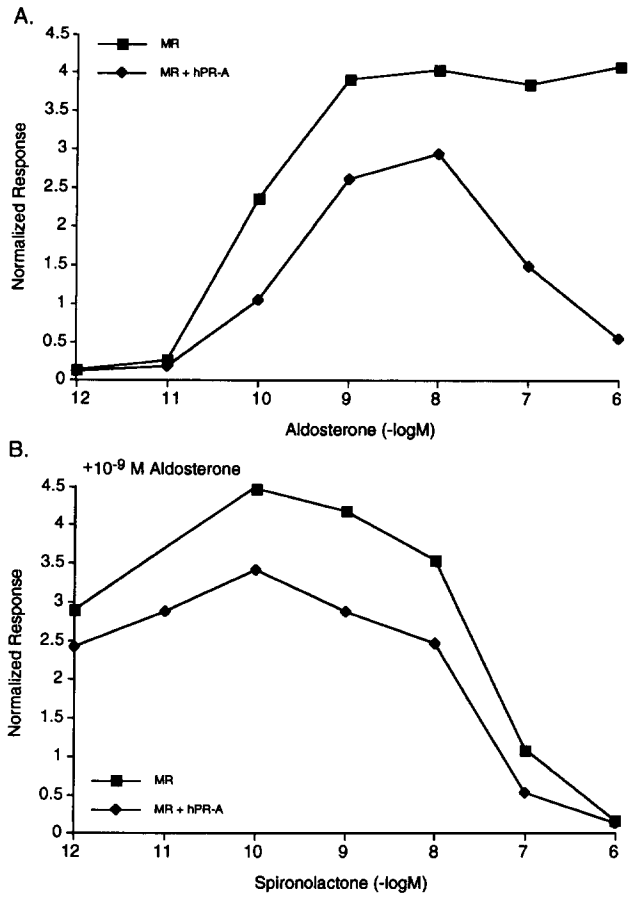

Fig. 2. The MR functons as a hormone-dependent transcription factor in transfected mammalian cells. Monkey kidney cells (CV-1) were transiently transfected with vectors expressing human MR alone (pRShMR) or in combination with vectors expressing phPR-A as indicated. The transcriptional activity in these setups was measured following the addition of (A) increasing concentrations of aldosterone or (B) increasing concentrations of the mineralocorticoid antagonist spironolactone in the presence of a saturating concentration of aldosterone (10^{-9} M). Treated cells were harvested and assayed for β-galactosidase and luciferase (LUC) activity (LUC activity was normalized for β-galactosidase activity). The normalized LUC activity was calculated as in Fig. 1. A representative experiment is detailed above. Each data point shown represents the average of triplicate determinations of the transcriptional activity under a given experimental condition.

this assay MR transcriptional activity was induced by 10^{-9} M aldosterone. We then added increasing concentrations of the test compounds in the presence or absence of transfected hPR-A and measured their ability to interfere with MR activity. Interestingly, these 19-nor-testosterone derived derivatives had minimal effects on MR directly, however, they were extremely active as MR antagonists in the presence of hPR-A. It is clear that cotransfection of hPR-A increases the inhibitory activity of these compounds by at least 3 orders of magnitude. In this assay norethindrone and norethynodrel are equally effective as spironolactone as antimineralocorticoids. These data in combination with our other published results indicate that hPR-A functions as a ligand-dependent inhibitor of all members of the GR subfamily of intracellular receptors. In addition, it suggests that it is possible to modulate the biological activity of GR, PR, AR, MR with ligands that do not competitively interact with these receptors.

Antiprogestins exhibit antimineralocorticoid receptor activity in cells containing hPR-A

Clearly, progestin agonists can inhibit MR transcriptional activity through their interaction with hPR-A. It was of interest therefore to determine whether progestin antagonists could function analogously. Specifically, the modulatory activity of RU486, ZK98299 and ZK112993 was examined in the presence or absence of transfected hPR-A. The results of this analysis are shown in Fig. 4. Notably, none of these antiprogestins had any significant direct effects on MR (<20% inhibition at 10^{-6} M). However, in the presence of transfected hPR-A all three compounds

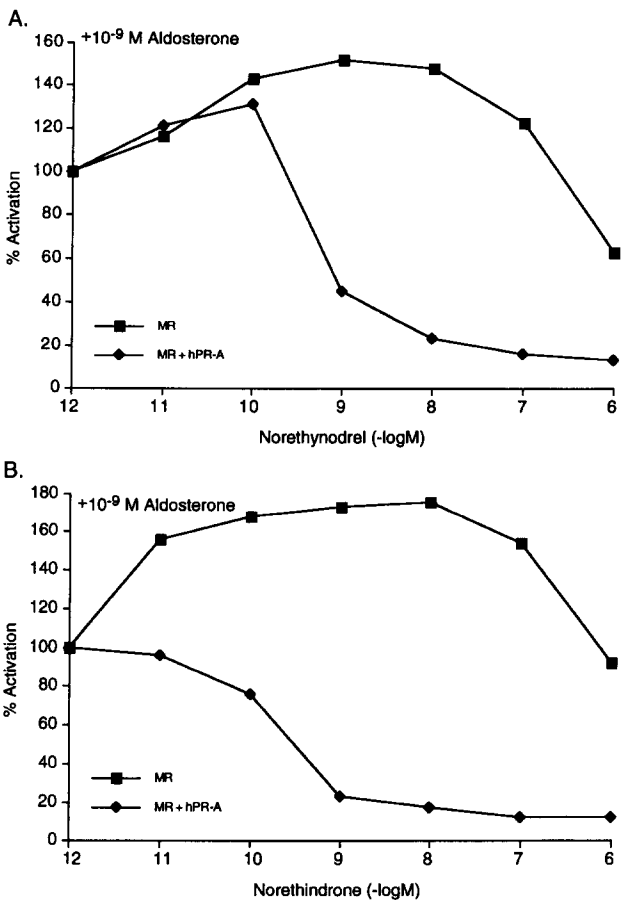

Fig. 3. hPR-A functions as a hormone-dependent inhibitor of MR transcriptional activity. Monkey kidney cells (CV-1) were transiently transfected alone with a vector expressing human MR (pRShMR) or in combination with a vector expressing phPR-A. The transcriptional activity of MR in this experiment was measured following the addition of 10^{-9} M aldosterone alone or in combination with increasing concentrations of (A) norethynodrel or (B) norethindrone. The data are presented as % activation where the 100% value represents maximally activated MR in the presence of 10^{-9} M aldosterone. A representative experiment is detailed above. Each data point shown represents the average of triplicate determinations of the transcriptional activity under a given experimental condition.

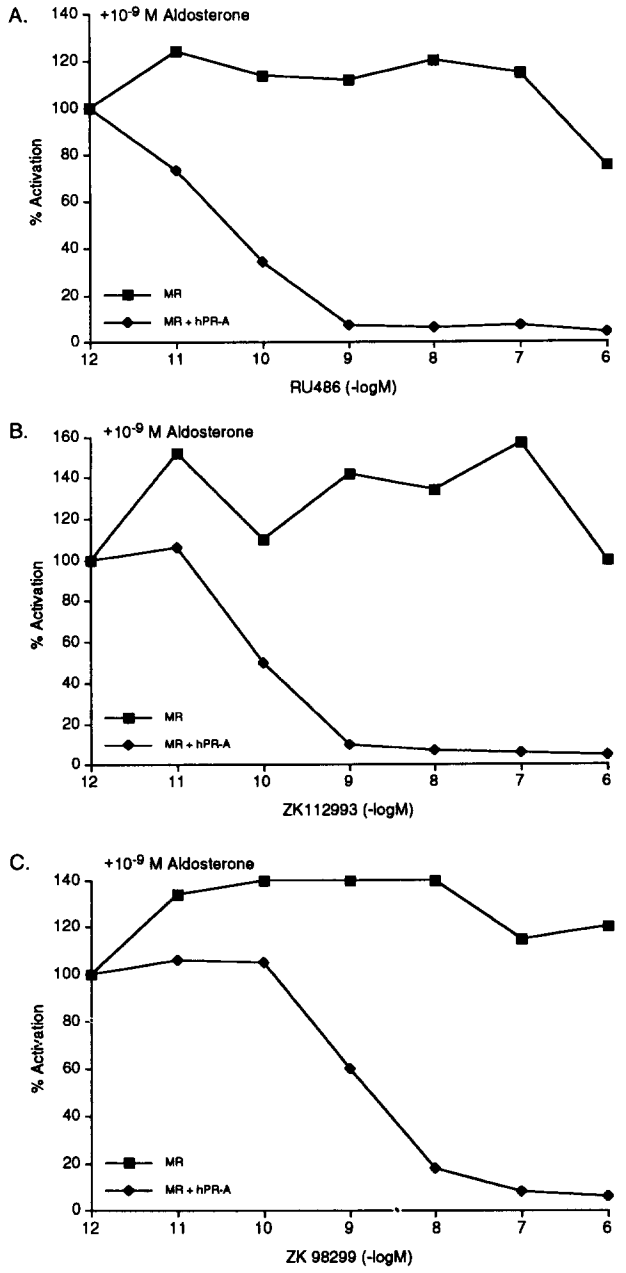

Fig. 4. Non-competitive inhibition of ER transcriptional activity by PR antagonists. Monkey kidney cells (CV-1) were transiently transfected alone with a vector expressing human MR (pRShMR) or in combination with a vector expressing phPR-A. The transcriptional activity of MR in this experiment was measured following the addition of 10^{-9} M aldosterone alone or in combination with increasing concentrations of the antiprogestins (A) RU486 (mifepristone) (B) ZK112993 or (C) ZK98299 (onapristone). The data are presented as % activation where the 100% value represents maximally activated MR in the presence of 10^{-9} M aldosterone. A representative experiment is detailed above. Each data point shown represents the average of triplicate determinations of the transcriptional activity under a given experimental condition.

Functioned as potent MR antagonists. Maximal inhibition (90%) occurred at 10^{-9} M RU486 and ZK112993. The less potent activity of ZK98299 (10^{-8} M) is most probably related to its lowered affinity for PR. These data suggest that in cellular contexts where hPR-A and MR are coexpressed that PR antagonists have the potential to function as potent antimineralocorticoids.

Not all PR agonists function identically as inhibitors of MR

It is interesting that all the compounds tested thus far (both PR agonists and antagonists) are derived from 19-nor-testosterone. It was of interest therefore, to examine the inhibitory activity of additional PR agonists. The results obtained using progesterone, 17α-hydroxyprogesterone and medroxyprogesterone acetate (Provera™) are shown in Fig. 5. As before, the ability of these compounds to inhibit aldosterone activated MR was examined in the presence or absence of transfected hPR-A. The results indicate that all three of these progesterone ligands function as direct inhibitors of MR function. The IC_{50} for progesterone in this system is 10^{-8} M. The antagonist properties of progesterone on MR have been noted previously. The addition of hPR-A to this system increases the potency of progesterone by one order of magnitude. Because of the direct effect of this class of PR ligands on MR transcriptional activity it is difficult to determine whether their ability to inhibit MR through hPR-A is as dramatic as that observed with the 19-nor-testosterone derived compounds. However, it appears that the efficacy of 17α-hydroxyprogesterone and medroxyprogesterone acetate as MR inhibitors in the presence or absence of hPR-A is only marginally significant. A further difficulty encountered is determining whether the lowered biological activity of 17α-hydroxyprogesterone and medroxyprogesterone acetate is merely a reflection of their lowered affinity for PR. Nonetheless, these data suggest that PR ligands differ in their ability to inhibit MR through a hPR-A mediated mechanism.

DISCUSSION

Very little is known about the function of the individual PR isoforms. In most tissues examine[d], both hPR-A and -B appear to exist in approximately stoichiometric concentrations. However, in certain tissues and under specific endocrine circumstances the ratio of these two isoforms changes considerably. In humans, the hPR-A isoform is expressed at a constant level in the endometrium throughout the menstrual cycle whereas hPR-B levels vary [24]. In chicken, the PR isoforms display seasonal variations in their relative expression levels [25]. In the mouse the ratios of PR-A and -B change during development [26]. Clearly therefore, delineation of the precise molecular function of these two receptors is central to our understanding of the hormonal actions of progesterone. The importance of this scientific question was highlighted by a recent Institute of Medicine Report on the clinical application of antiprogestins where it was recommended that considerable research effort should be expended to examine the significance of hPR-A and -B expression levels in pathological conditions such as endometriosis and breast cancer [27].

We have examined the effect of hPR-A coexpression on the biological activity of hPR-B, GR, and AR and have now extended these studies to include MR. The results from these experiments suggest that in cellular contexts where hPR-A in itself is transcriptionally inactive it can act as a cell and promoter specific inhibitor of the entire GR sub-family of nuclear receptors. One of the most important findings from these studies is that the synthetic progestins and antiprogestins can exhibit antiMR, GR, AR and PR activities through an indirect mechanism. This data will impact on the design of approaches to develop a "clean" antiprogestin, one with absolute PR binding specificity as it is clear that PR ligands can regulate the biological activity of other receptors through this "cross-talk" mechanism. However, since all antiprogestins currently available are derived from the same chemical class it is difficult to predict whether novel classes of antiprogestins will function similarly.

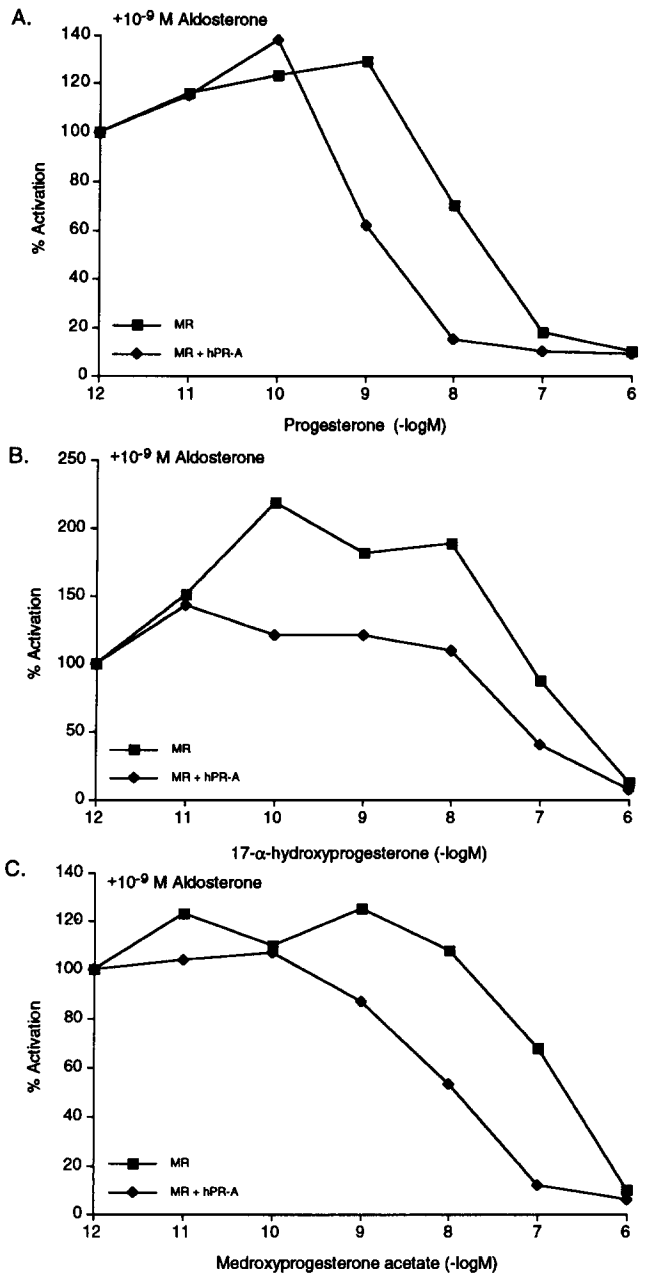

Fig. 5. PR agonists differ in their ability to inhibit MR transcriptional activity. Monkey kidney cells (CV-1) were transiently transfected alone with a vector expressing human MR (pRShMR) or in combination with a vector expressing phPR-A. The transcriptional activity of MR in this experiment was measured following the addition of 10^{-9} M aldosterone alone or in combination with increasing concentrations of the PR agonists (A) progesterone (B) 17-α-hydroxyprogesterone or (C) medroxyprogesterone acetate (Provera™). The data are presented as % activation where the 100% value represents maximally activated MR in the presence of 10^{-9} M aldosterone. A representative experiment is detailed above. Each data point shown represents the average of triplicate determinations of the transcriptional activity under a given experimental condition.

It is difficult to extrapolate from the *in vitro* results in this study showing hPR-As effect on MR, to an *in vivo* situation as it is not known if there are any cells in which hPR-A and MR are coexpressed. This remains to be determined. In the case of hPR's effect on GR function however, the ubiquitous nature of GR expression make it likely that this inhibitory process is physiologically relevant. Minimally, these results extend our understanding of how hPR-A functions as a transcriptional inhibitor. Taking what we know of the specificity, stoichiometry and the ligand dependence of this process we propose a model in which hPR-A in cellular contexts where it is transcriptionally inactive can function as a potent transdominant inhibitor of MR, GR, AR and PR-B by competing for a common transcription factor that is required by this entire class of receptors (Fig. 6). Specifically, in the case of MR, we propose that in the absence of hPR-A, aldosterone activated MR will bind to this common transcription factor or "adapter" and upon subsequent or simultaneous binding to DNA will productively interact with the general transcription apparatus (GTA) [Fig. 6(A)]. However, in the presence of ligand activated hPR-A, a competition for this adapter exists [Fig. 6(B)]. In the absence of the "adapter" MR cannot interact with the general transcription apparatus. In this cell context hPR-A binds to the adapter but fails to alter its structure in such a way as to allow a productive association of the PR-A/adapter complex with the transcription machinery. Our data indicates that inhibition by hPR-A of -B occurs sub-stoichiometrically. This observation would be consistent with our model if hPR-A displays a higher affinity for the adapter protein than for other members of the GR sub-family of receptors.

These experiments and those already reported indicate that there are distinct cellular roles for the two isoforms of PR. It remains to be seen if the molecular events that we have observed *in vitro* occur also *in vivo*. This critical question is the focus of our current research and will provide the necessary information to determine if the cross-reactivity observed *in vitro* is sufficiently predictable to permit its use in the development of new antiprogestins.

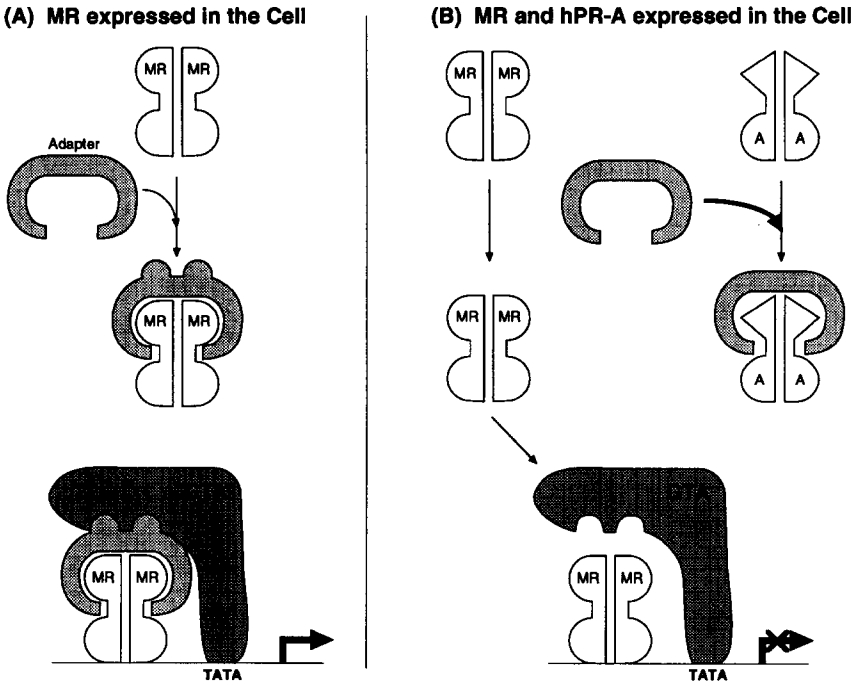

Fig. 6. PR agonists and antagonists exert antiMR activities through a novel PR-A mediated mechanism. (A) In cells where MR alone is expressed in the absence of hPR-A, hormone activation of MR facilitates an association of MR with a required "adapter" protein. This interaction induces a conformational change in the adapter allowing the complex to productively associate with general transcription apparatus (GTA). When hPR-A is coexpressed with MR in the presence of a PR ligand (B) a competition for the common adapter exists. MR in the absence of bound adapter is transcriptionally inactive. In addition, the complex of hPR-A and the adapter protein is transcriptionally inactive as it does not induce the conformational changes in the adapter required to fit the transcription apparatus.

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REFERENCES

1. Clark C. L. and Sutherland R. L.: Progestin regulation of cellular proliferation. *Endocrine Rev.* 11 (1990) 266–301.
2. O'Malley B. W.: The steroid receptor superfamily: more excitement predicted for the future. *Molec. Endocr.* 4 (1990) 363–369.
3. Beato M.: Gene regulation by steroid hormones. *Cell* 56 (1989) 335–344.
4. Evans R. M.: The steroid and thyroid receptor superfamily. *Science* 240 (1988) 889–895.
5. Horwitz K. B. and Alexander P. S.: *In situ* photolinked nuclear progesterone receptors of human breast cancer cells: subunit molecular weights after transformation and translocation. *Endocrinology* 113 (1983) 2195–2201.
6. Henchuk T. T. and Walters M. R.: Rat uterine progesterone receptor analyzed by [^{3}H]R5020 photoaffinity labeling: evidence that A and B subunits are not equimolar. *Endocrinology* 120 (1987) 1449–1456.
7. Schneider W., Ramachandran C., Satyaswaroop P. G. and Shyamala G.: Murine progesterone receptor exists predominantly as the 83-kilodalton "A" form. *J. Steroid Biochem. Molec. Biol.* 38 (1991) 285–291.
8. Schrader W. T. and O'Malley B. W.: Progesterone-binding components of chick oviduct: Characterization of purified subunits. *J. Biol. Chem.* 247 (1972) 51–59.
9. Kastner P., Krust A., Turcotte B., Stropp U., Tora L., Gronemeyer H. and Chambon P.: Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO Jl.* 9 (1990) 1603–1614.
10. Conneely O. M., Maxwell B. L., Toft D. O., Schrader W. T. and O'Malley B. W.: The A and B forms of the chicken progesterone receptor arise by alternate initiation of translation of a unique mRNA. *Biochem. Biophys. Res. Commun.* 149 (1987) 493–501.
11. Meyer M. E., Pornon A., Ji J., Bocquel M. T., Chambon P. and Gronemeyer H.: Agonist and antagonist activities of RU486 on the functions of the human progesterone receptor. *EMBO Jl.* 9 (1990) 3923–3932.
12. Tora L., Gronemeyer H., Turcotte B., Gaub M. P. and Chambon P.: The N-terminal region of the chicken progesterone receptor specifies target gene activation. *Nature* 333 (1988) 185–188.
13. Bocquel M. T., Kumar V., Stricker C., Chambon P. and Gronemeyer H.: The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. *Nucleic Acids Res.* 17 (1989) 2581–2595.
14. Desombres P. and Schibler U.: A liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP are translated from the same mRNA. *Cell* 67 (1991) 569–579.
15. Roman C., Cohn L. and Calame K.: A dominant negative form of transcription activator mTFE3 created by differential splicing. *Science* 254 (1991) 94–97.
16. Koenig R. J., Lazar M. A., Hodin R. A., Brent G. A., Larsen R., Chin W. and Moore D. D.: Inhibition of thyroid hormone action by a non-hormone binding c-erbA protein generated by alternate mRNA splicing. *Nature* 337 (1989) 659–661.
17. Foulkes N. S. and Sassone-Corsi P.: More is better: activators and repressors from the same gene. *Cell* 68 (1992) 411–414.
18. Vegeto E., Shahbaz M. M., Wen D. X., Goldman M., O'Malley B W. and McDonnell D. P.: Human progesterone receptor A form is a cell-and promoter specific repressor of human progesterone receptor B function. *Molec. Endocr.* 6 (1993). 124–1253.
19. Berger T. S., Parandoosh Z., Perry B. W. and Stein R. B.: Interaction of glucocorticoid analogues with the human glucocorticoid receptor. *J. Steroid Biochem. Molec. Biol.* 41 (1992) 733–738.

20. Vegeto E., Allan G. F., Schrader W. T., Tsai M. J., McDonnell D. P. and O'Malley B. W.: The mechanism of RU486 antagonism is dependent on the conformation of the carboxy-terminal tail of the human progesterone receptor. *Cell* **69** (1992) 703–713.
21. Tzukerman M. T., Esty A., Santiso-Mere D., Danielian P., Parker M. G., Stein R. B., Pike J. W. and McDonnell D. P.: Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. *Molec. Endocr.* **8** (1993) 21–30.
22. Arriza J. L., Weinberger C., Cerelli G., Glaser T. M., Handelin B. L., Housman D. E. and Evans R. M.: Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with glucocorticoid receptor. *Science* **237** (1987) 268–275.
23. McDonnell D. P. and Goldman M. E.: RU486 exerts antiestrogenic activities through a novel progesterone receptor A-form mediated mechanism. *J. Biol. Chem.* (1994) In press.
24. Feil P. D., Clarke C. L. and Satayaswaroop P. G.: Progestin-mediated changes in progesterone receptor forms in the normal human endometrium. *Endocrinology* **123** (1988) 2506–2513.
25. Boyd P. A. and Spelsberg T. C.: Seasonal changes in the molecular species and nuclear binding of the chicken oviduct progesterone receptor. *Biochemistry* **18** (1979) 3685–3690.
26. Shyamala G., Schneider W. and Schott D.: Development regulation of murine mammary progesterone receptor gene expression. *Endocrinology* **126** (1990) 2882–2889.
27. Donaldson M. S., Dorflinger L., Brown S. S. and Benet L. Z.: *Clinical Applications of Mifepristone (RU486) and Other Antiprogestins.* National Academy Press, Washington (1993) pp. 36–51.