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Two-Stage Glucocorticoid Induction of *CYP3A23* through Both the Glucocorticoid and Pregnane X Receptors

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ABSTRACT

Glucocorticoid inducibility of the *CYP3A23* gene is conferred by a multisite unit comprising binding sites for several members of the nuclear receptor superfamily of transcription factors, including the chicken ovalbumin upstream promoter-transcription factor COUP-TF, pregnane X receptor (PXR), and hepatocyte nuclear factor 4 (HNF-4). The presence of three binding sites, each of which interacts with more than one factor, contributes to the complexity of the *CYP3A23* glucocorticoid-responsive region. Despite the glucocorticoid sensitivity of this gene, direct binding of ligand-activated glucocorticoid receptor (GR) to the *CYP3A23* dexamethasone-responsive region (DexRE) is not required for induction. This study demonstrates that DexRE-2 is the key element within the *CYP3A23* proximal promoter, conferring ligand sensitivity via its interaction with the PXR/RXRα heterodimer. The DexRE-1 and HNF-4 sites are not ligand-responsive, but are essential accessory elements required for full promoter inducibility. In addition to ligand-mediated activation of PXR, the overall induction response involves a GR-mediated stimulation of PXR and RXRα expression. Hence, the induction pathway can be divided into two stages. In stage one, maximal induction requires a GR-dependent increase in PXR and RXRα expression, and stage two is characterized by direct transcriptional activation of *CYP3A23*, which is dependent on ligand-activated PXR as well as accessory factors bound at the DexRE-1 and HNF-4 sites. Because multiple proteins bind at each element within the glucocorticoid-responsive region, factors not contributing to ligand responsiveness, such as chicken ovalbumin upstream promoter-transcription factor, may modulate the response through competitive interactions.

The cytochrome P450 (CYP) superfamily of heme-thiolate mono-oxygenases is involved in biosynthetic pathways (steroids, prostaglandins) as well as in the metabolism of both endogenous and foreign compounds (Nelson et al., 1996). Approximately 6 isoforms of the 40 found in humans metabolize most clinically administered drugs; therefore, direct substrate competition for a given isozyme presents a potential mechanism for drug interactions (Rendic and DiCarlo, 1997; Michalets, 1998). Expression of the xenobiotic metabolizing enzymes is regulated transcriptionally by several classes of compounds, including hormones, peroxisome proliferators, anticonvulsants, and polycyclic aromatic hydrocarbons; therefore, both substrates and compounds not metabolized by CYP can influence the cellular levels of these enzymes (Dogra et al., 1998). Glucocorticoid activation of the CYP3A subfamily of enzymes has long been a topic of clinical interest, because CYP3A4, the major human isoform in adults, metabolizes 50 to 60% of drugs (Wilkinson, 1996).

Studies aimed at elucidating the precise mechanism of *CYP3A* activation by glucocorticoids have predominantly focused on the rat isoforms, particularly the major glucocorticoid-inducible form, *CYP3A23* (Schuetz and Guzelian, 1984; Schuetz et al., 1984; Gonzalez et al., 1985; Simmons et al., 1987; Ribeiro and Lechner, 1992; Cooper et al., 1993; Komori and Oda, 1994; Sidhu and Omiencinski, 1995). Insights gained from studying rat genes have led directly to a greater understanding of the regulation of *CYP3A* isoforms from various species (Wrighton et al., 1985; Barwick et al., 1996; Lehmann et al., 1998). Within the *CYP3A23* proximal 5′-flanking region, three elements, dexamethasone response element 1 (DexRE-1), DexRE 2 (DexRE-2), and Site A, were shown to be essential for the full induction response (Huss et al., 1996). All of the *CYP3A23* elements contain imperfect

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ABBREVIATIONS: CYP, cytochrome P450; CMV, cytomegalovirus; COUP-TF, chicken ovalbumin upstream promoter-transcription factor; CYP3A, cytochrome P450 3A subfamily; ds, double-stranded; DexRE-1, dexamethasone response element 1; DexRE-2, dexamethasone response element 2; FCS, fetal calf serum; GR, glucocorticoid receptor; HNF-4, hepatocyte nuclear factor 4; Me_2SO, dimethyl sulfoxide; MMTV, mouse mammary tumor virus; PCN, pregnenolone 16α-carbonitrile; PXR, pregnane X receptor; RLU, relative light units; SV, simian virus; TK, thymidine kinase; RXR, retinoid X receptor.

AGGTCA direct repeats and bind members of the nuclear receptor superfamily of ligand-activated transcription factors (Huss and Kasper, 1998; Quattrochi et al., 1998). Chicken ovalbumin upstream promoter-transcription factors (COUP-TFs) bind the two distal elements, DexRE-1 (−144 to −169) and DexRE-2 (−118 to −136). Hepatocyte nuclear factor 4 (HNF-4) binds to the proximal site, Site A (−85 to −110), and directly activates the *CYP3A23* promoter in HeLa cells, which lack endogenous HNF-4 (Huss and Kasper, 1998). Interactions at each of the sites were demonstrated to correlate with functional activity in mutagenesis experiments; therefore, it is likely that these factors play a direct role in the response of *CYP3A23* to glucocorticoids (Huss and Kasper, 1998). The glucocorticoid-responsive *CYP3A* genes from all other species examined contain homologous DexRE-1 elements. In fact, the DexRE-1 of the rabbit and human *CYP3A6* and *CYP3A4*, respectively, confers dexamethasone responsiveness on a heterologous promoter (Barwick et al., 1996; Lehmann et al., 1998). The DexRE-2 and Site A elements, however, are present only in mouse and rat isoforms (Burger et al., 1992; Telhada et al., 1992; Hashimoto et al., 1993; Jounaïdi et al., 1994; Barwick et al., 1996; Huss et al., 1996; Toide et al., 1997).

Interestingly, gene regulatory studies have ruled out the likelihood of direct glucocorticoid receptor (GR) involvement via binding to the *CYP3A23* promoter (Quattrochi et al., 1995; Huss et al., 1996). However, whether GR plays an indirect role in the overall induction pathway is still a focus of debate (Schuetz and Guzelian, 1984; Schuetz et al., 1984; Burger et al., 1992; Huss et al., 1996). Early pharmacologic studies revealed an atypical profile for *CYP3A23* induction compared with other glucocorticoid-responsive genes whose mechanisms are GR-dependent (Schuetz and Guzelian, 1984; Schuetz et al., 1984; Burger et al., 1992). These studies demonstrated that the response was specific for glucocorticoids, with the exception of pregnenolone 16-α carbonitrile (PCN); the *CYP3A23* dexamethasone induction response was partially inhibited by RU486; a 100-fold higher glucocorticoid concentration was required to induce *CYP3A23* than was necessary for maximal GR activation; and the potencies of various glucocorticoids for inducing *CYP3A23* did not parallel their potencies for activating GR. This work provides a feasible model for GR involvement that is consistent with the pharmacologic characteristics of the response.

Recently, a novel orphan receptor isolated from mouse, the pregnane X receptor (PXR), was shown be activated by the *CYP3A* inducers PCN and dexamethasone (Kliewer et al., 1998). The PXR/RXRα heterodimer binds the dexamethasone-responsive region at DexRE-2 and mediates ligand-dependent activation of a thymidine kinase reporter construct containing multiple upstream copies of this element (Kliewer et al., 1998). This study investigates the role of PXR in activating *CYP3A23* promoter constructs in both CV-1 and H4IIE cells and examines the role of these response elements within the context of the wild-type promoter, rather than a heterologous system. These findings demonstrate, that although PXR plays a key role in mediating the *CYP3A23* response to glucocorticoids, DexRE-1 and Site A, which bind other nuclear receptors, including COUP-TF and HNF-4, respectively, are also required for full induction. Although GR does not directly transactivate the *CYP3A23* promoter (Quattrochi et al., 1995; Huss et al., 1996), GR is involved in the regulation of *PXR* and *RXR*α gene expression. Finally, the capacity of the DexRE-1 and -2 elements from various species to bind PXR/RXRα is examined, and the nucleotide requirements for strong PXR/RXRα binding are precisely defined.

Experimental Procedures

Materials. Oligonucleotides were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). Polymerase chain reactions were performed with a GeneAmp kit and *Taq* polymerase (PE Applied Biosystems, Foster City, CA). Restriction enzymes, T4 ligase, and T4 polynucleotide kinase were from New England Biolabs (Beverly, MA). Radioactive isotopes, [α-^{32}P]UTP (800 Ci/mmol) and [γ-^{32}P]dATP (3000 Ci/mmol), were from NEN Life Science Products (Boston, MA). Luciferase reagents were from Promega (Madison, WI). Dexamethasone was purchased from Sigma (St. Louis, MO), and dexamethasone *t*-butylacetate from Research Plus, Inc. (Bayonne, NJ). RU486 and pregnenolone 16α-carbonitrile were from Biomol (Plymouth Meeting, PA).

Construction of Reporter Constructs. Construction of *CYP3A23* deletion constructs has been described previously (Huss et al., 1996). The nucleotide numbering for *CYP3A23* constructs is based on a submitted *CYP3A23* promoter sequence (Genbank accession number S82239). The mutant construct P3–175/TTG was generated by using the polymerase chain reaction overlap extension technique to make substitutions at positions −167 and −165 of the P3–175 deletion construct (Huss et al., 1996; Huss and Kasper, 1998). Construction of the heterologous promoter constructs has been described previously (Huss et al., 1996). To define the region of the *CYP3A23* promoter cloned upstream of the thymidine kinase (TK) promoter (−110 to +50), the −170TK construct is identified by its 5′ base and has nucleotide −60 as its 3′ terminus. The other constructs are identified by their 5′/3′ termini. Constructs with multiple element copies were made using oligonucleotides designed such that the annealed oligonucleotides had overhanging *Bam*HI and *Kpn*I sites; these were ligated with the *Bam*HI/*Kpn*I-digested TK-Luc vector. All constructs were sequenced by the dideoxy chain termination method with a T7 Sequenase V2.0 DNA-sequencing kit or with BigDye sequencing reagents according to the manufacturer's protocol (PE Applied Biosystems).

Cell Culture, Transfection, and Luciferase Assays. H4IIE cells were maintained, transfected, and treated as described previously (Huss et al., 1996; Huss and Kasper, 1998). In cotransfection experiments, 2 μg/ml of expression vector was used. CV-1 cells were generously provided by S. A. Kliewer (Glaxo-Wellcome); overnight (14 h) transfections of 2 μg/ml reporter and 0.1 μg/ml expression vector were performed using Lipofectin (Life Technologies, Grand Island, NY). Transfected CV-1 cells were treated for 24 h with inducers. Luciferase assays were carried out as described previously (Huss and Kasper, 1998). Activities from CV-1 experiments were normalized to β-galactosidase activity (0.4 μg/ml transfected plasmid).

Electrophoretic Mobility Shift Assays. Probes were generated by annealing complementary oligonucleotides. Sequences for *CYP3A23* DexRE-1 and DexRE-2 have been described (Huss and Kasper, 1998). Other probes included 3A2DexRE-1:5′AGAATGTTAGCTCAAGAAGGTCAAAGAAGCTGT3′; 3A2DexRE-2:5′TGTAGATGAACTTTATGAACTGTTTAGG3′, 5′TAAACAGTTCATAAAGTTCATCTACAGC3′; 3A6DexRE-1:5′CAGCACATGAACTCAGAGGAGGTCACCACGGATT3′; 3A4DexRE-1:5′GAATATGAACTCAAAGGAGGTCAGTGAGT3′; 3a11DexRE-1:5′CAGAATGTTAGCTCAAAGTAGGTCAAGTTGGGCT3′; 3a11DexRE-2:5′CTGTGGATGAACTATACGAACTGCCTAG3′; and TTGDexRE-1:5′CCCAGAATTTGAACTCAAAGGAGGTCAAAATAG3′ (exact complementary oligonucleotides were used for opposite strands unless otherwise indicated). Nuclear extracts from H4IIE cells were prepared using a protocol described by Dignam, with modifica-

tions (Dignam et al., 1983; Ausubel et al., 1987). Briefly, cells are incubated in hypotonic buffer (10 mM HEPES, pH 7.6, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) and disrupted with a glass homogenizer. The nuclei are collected by centrifugation (3300*g*, 15 min), and proteins are extracted stepwise using low-salt buffer (20 mM HEPES, pH 7.6, 25% glycerol, 1.5 mM $MgCl_2$, 20 mM KCl, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol) and high-salt buffer (substitute 1.2 M KCl in low-salt buffer). Recombinant PXR was synthesized from the pSG5-PXR cDNA clone (kindly provided by S. A. Kliewer) in rabbit reticulocyte lysate, using TNT in vitro coupled transcription/translation system (Promega). The GST-RXRα fusion protein (provided by J. E. Mertz, McArdle Laboratory, Madison, Wisconsin) was purified from 1 liter JM109 bacterial cultures, using glutathione-Sepharose 4B (Amersham Pharmacia Biotech), according to the manufacturer's protocol.

RNase Protection Assays. Antisense probes were synthesized using T7 RNA polymerase and corresponded to the 151 to 1101 region of mouse PXR, the 850 to 1740 region of human COUP-TFII (cDNA clone provided by M. J. Tsai, Baylor College of Medicine, Houston, TX), the 1340 to 1764 region of rat HNF-4 (cDNA clone provided by F. M. Sladek, University of California-Riverside), and a 334-nucleotide mouse β-actin probe (provided in a MAXIscript kit; Ambion, Austin, TX) in the presence of [^{32}P]UTP (β-actin probe was labeled at 50-fold lower specific activity). Full-length probes were purified by denaturing polyacrylamide gel electrophoresis. Isolated probes were incubated with 10 μg total RNA from 5×10^7 H4IIE cells isolated with a Qiagen RNeasy Midi kit. Overnight hybridization of probe and RNA at 42°C and RNase (A/T1) digestion (30 min) were performed according to the manufacturer's protocol (RPAII kit; Ambion). RNA digestion products were resolved on 5% denaturing polyacrylamide electrophoresis gels and visualized by phosphorimage analysis. Band intensities were measured by Imagequant software and normalized to the β-actin reaction included in each analysis.

Results

PXR/RXRα Binds to Distinct Elements in *CYP3A* Genes from Various Species. Previous work has established that the dexamethasone response elements DexRE-1 and DexRE-2 are required for glucocorticoid activation of *CYP3A23* (Huss and Kasper, 1998). DexRE-2 binds several nuclear receptors, including COUP-TFI and II, and the recently described pregnane X receptor (PXR) (Huss and Kasper, 1998; Kliewer et al., 1998; Quattrochi et al., 1998). PXR (also known as steroid and xenobiotic receptor (SXR) in humans) binds as a PXR/RXRα heterodimer to AGTTCA hexamers arranged as direct, inverted, or everted repeats (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998). Homologous elements are present in rodent, human, and rabbit *CYP3A* genes (Fig. 1A) (Hashimoto et al., 1993; Tukey, 1995; Toide et al., 1997). A direct repeat of AGTTCA hexamers (DR3) comprises the DexRE-2 of the rodent isoforms, although the mouse *Cyp3a11* element differs by one mismatch. In contrast, the human *CYP3A4* and rabbit *CYP3A6* lack a DexRE-2 homology region. Their DexRE-1 elements, however, contain an AGTTCA everted repeat separated by six nucleotides (IR6) and have been shown to confer activation by glucocorticoids on a heterologous promoter (Barwick et al., 1996). The IR6 components of the rodent DexRE-1 elements are disrupted within their upstream hexamers, but their ability to support glucocorticoid activation or interact with PXR/RXRα has not been tested.

To further investigate the role of PXR in glucocorticoid regulation of these *CYP3A* isoforms, binding of PXR/RXRα to these elements was analyzed by gel shifts (Fig. 1B). The DexRE-2 elements of rat *CYP3A2* and *CYP3A23* and mouse *Cyp3a11* bind the PXR/RXRα heterodimer, whereas *CYP3A4* and *CYP3A6* bind PXR/RXRα at DexRE-1. The mouse DexRE-2 (Fig. 1B, lane 12) displayed a much weaker binding for PXR/RXRα compared with the rat DexRE-2 probes under identical conditions (Fig. 1B, lanes 4 and 8), presumably because of the single DR3 mismatch. The functional effects of this relatively weak PXR/RXRα binding on the *Cyp3a11* induction response are not known. Although the upstream hexamer of the IR6 within *CYP3A23* DexRE-1 (AGTTAA) differs by only one mismatch from the *CYP3A4* and *CYP3A6* elements, PXR/RXRα binds less strongly to the *CYP3A23* DexRE-1 (Fig. 1B, lane 2) than to the *CYP3A4* and *CYP3A6* DexRE-1 elements (Fig. 1B, lanes 14 and 16). The *CYP3A23* DexRE-1 element also binds PXR/RXRα less strongly than *CYP3A23* DexRE-2 (Fig. 1B, lane 4). As might be expected, the *CYP3A2* DexRE-1, with two mismatches in the IR-6 upstream hexamer, has virtually no affinity for PXR/RXRα (Fig. 1B, lane 6).

A Single Nucleotide Mismatch Confers Differential Binding and Function on Human and Rat DexRE-1 Elements. Mutagenesis experiments were performed to determine whether the single nucleotide mismatch within DexRE-1 that gave rise to the differences in PXR/RXRα bind-

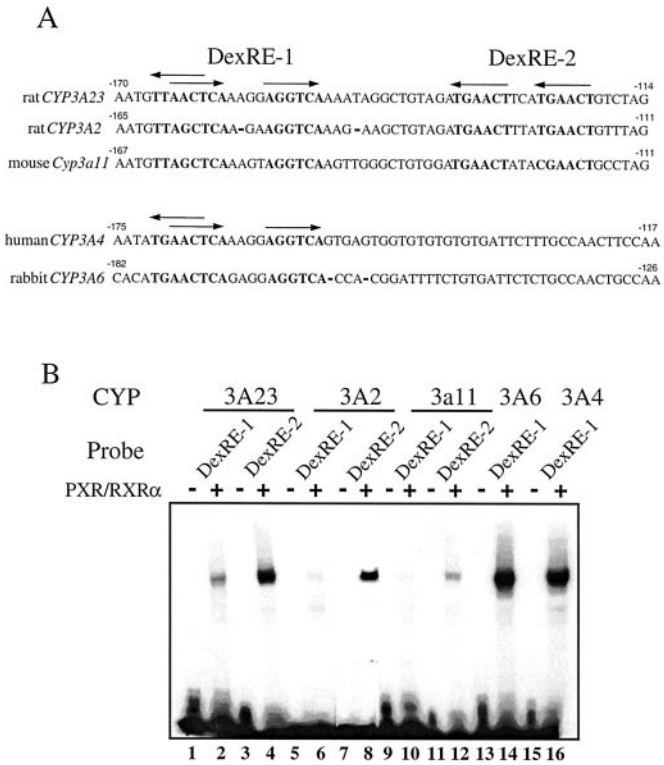

Fig. 1. Comparison of PXR/RXRα binding within *CYP3A* genes. A, the 5′-flanking regions of several *CYP3A* genes, homologous to the *CYP3A23* glucocorticoid-responsive region, are aligned. The DexRE-1 and DexRE-2 elements, containing nuclear receptor consensus binding sites, are in bold type. Arrows indicate the relative arrangements of the identified hexameric half-sites. B, PXR/RXRα binding to homologous elements of the *CYP3A* isoforms identified in A. In gel-shift reactions probes corresponding to either the DexRE-1 or DexRE-2 of the various *CYP3A* genes were incubated in the absence (−) or presence (+) of recombinant PXR and GST-RXRα as indicated.

ing between *CYP3A4* and *CYP3A23* also affected the glucocorticoid response. A mutant, designated mP3–175/TTG, was made that substituted a G for T at position −165 of the P3–175 construct, making the *CYP3A23* IR6 sequence identical with that of *CYP3A4* (Fig. 2). In the cloning process, the G at position −167 of *CYP3A23*, which is outside the IR6 consensus, was changed to T and therefore does not correspond to either *CYP3A4* or *CYP3A23* in that position. The mP3–175/TTG mutant displayed an enhanced dexamethasone induction response 2.5 times that of the 15-fold induction exhibited by wild-type constructs P3–175 and P3–210 and 15 times that of P3–144, which lacks the DexRE-1 (Fig. 2A). When binding was assessed, the mutant DexRE-1 oligonucleotide displayed a strong affinity for PXR/RXRα compared with the wild-type *CYP3A23* element (Fig. 2B, lanes 2 and 6). These results suggest that the single nucleotide difference between the DexRE-1 IR6 of *CYP3A23* and that of *CYP3A4* does account for the observed difference in PXR/RXRα binding affinity. Hence, in the case of wild-type *CYP3A23* DexRE-1, the imperfect IR6 renders the element unable to interact strongly with the ligand-receptor complex and, therefore, to directly mediate ligand activation (see below).

Multimerized CYP3A23 DexRE-2 Can Support Ligand Activation through PXR. To determine whether PXR/RXRα binding to DexRE-2 correlated with PXR-dependent transcriptional activation, the *CYP3A23* elements were examined individually for their ability to confer glucocorticoid responsiveness on the thymidine kinase promoter. Figure 3A shows the results of PXR cotransfection experiments in CV-1 monkey kidney cells, using constructs in which each of the three elements within the *CYP3A23* dexamethasone-responsive region was multimerized. Consistent with previous observations (Kliewer et al., 1998), a 6-fold, PXR-dependent induction by 10 μM dexamethasone *t*-butylacetate (DtBu) was shown for the DexRE-2-containing construct. In contrast, neither the *CYP3A23* DexRE-1 nor Site A constructs displayed responsiveness to DtBu in the presence of PXR. Site A supports basal and dexamethasone-induced activity through the binding of HNF-4 and has been shown to also bind the upstream stimulatory factor, a bHLH/leucine zipper transcription factor (Huss et al., 2000). Notably, the DexRE-1, despite the fact that it shows measurable binding affinity for PXR/RXRα, did not support PXR-dependent induction by DtBu in CV-1 cells.

The DexRE-1 and DexRE-2 multimer constructs were further characterized in H4IIE cells, which support the *CYP3A* glucocorticoid response, for their ability to respond to dexamethasone independent of PXR cotransfection (Fig. 3B). As shown previously, single copies of the DexRE-1 or DexRE-2 were unable to confer inducibility on the TK promoter (Huss et al., 1996). However, a construct containing multiple copies

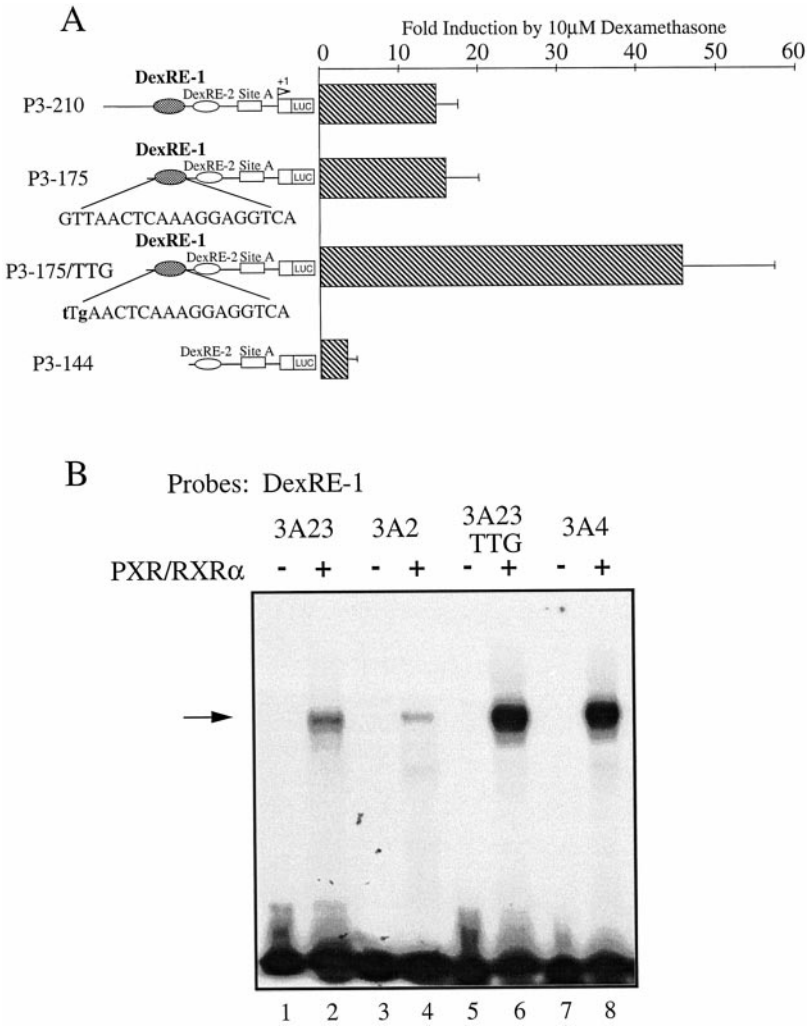

Fig. 2. Conversion of *CYP3A23* DexRE-1 to an AGTTCA indirect repeat enhances dexamethasone responsiveness and PXR/RXRα binding. A, deletion constructs P3–210, P3–175, and P3–144 are truncations of the wild-type *CYP3A23* 5′-flanking region. The DexRE-1 mutant was constructed using the P3–175 construct for which the wild-type DexRE-1 sequence is shown. Substitutions made in the mutant are indicated by bold lowercase letters. Transient transfection experiments were performed in H4IIE cells. Activities were measured after 60 h of 10 μM dexamethasone or vehicle (Me_2SO) treatment and are reported as mean fold dexamethasone induction ± S.D. (dexamethasone-treated/control activity) for a minimum of five experiments. B, gel-shift reactions comparing wild-type DexRE-1 sites from *CYP3A23*, *CYP3A2*, *CYP3A4*, and the mutated 3A23DexRE-1 (3A23TTG) were performed. Probes were incubated in the absence (−) or presence (+) of recombinant PXR and GST-RXRα. The arrow indicates the PXR/RXRα complex.

of DexRE-2 displayed more than an 11-fold induction response by dexamethasone, whereas no response was observed with a multimerized DexRE-1 construct. This further supports our hypothesis that DexRE-2, but not DexRE-1, directly mediates ligand-dependent activation of *CYP3A23* through an interaction with PXR/RXRα. The DexRE-1, which is necessary for full glucocorticoid induction of *CYP3A23*, may contribute to the response as an accessory factor binding site.

Factors in Addition to PXR Are Required for Full *CYP3A23* Induction in CV-1 Cells. Thus far, the ability of PXR to directly mediate ligand-dependent activation has been studied using multimerized constructs (Kliewer et al., 1998) (Fig. 3A). These conditions do not reflect the natural organization of elements in the wild-type *CYP3A* genes, because *CYP3A23* as well as the human (*CYP3A4*), rabbit (*CYP3A6*), and mouse (*Cyp3a11*) isoforms contain only single copies of their PXR/RXRα binding elements. Indeed, the high-magnitude induction response observed in hepatic cells was not obtained in CV-1 cells cotransfected with PXR and constructs containing single copies of the essential elements (Fig. 4). Compared with the multimerized DexRE-2 construct that is activated 6-fold by DtBu, a single-copy construct, 144/110TK, is activated by only 2-fold under the same conditions (Fig. 4). Similarly, −170TK, which contains the entire *CYP3A23* glucocorticoid-responsive region (−60 to −170), mediates only a 2- to 3-fold PXR-dependent induction by DtBu. Hence, PXR/RXRα cannot mediate a full glucocorticoid induction response in the absence of additional transcription factors that bind at DexRE-1 and Site A, factors that are presumably absent from CV-1 cells. We predict that a higher magnitude response would be observed if the additional *trans*-acting proteins binding at Site A and DexRE-1 were present in the system.

PXR/RXRα Do Not Enhance the *CYP3A23* Induction Response to Dexamethasone in H4IIE Cells. To determine whether increased expression of PXR could enhance the level of glucocorticoid activation observed in a cell line replete with all necessary *trans*-acting factors, cotransfection experiments were performed in H4IIE cells (Fig. 5). Cotransfection of PXR/RXRα activated *CYP3A23* promoter constructs in the absence of added ligand. This so-called ligand-independent increase in activity required the presence of both the DexRE-1 and DexRE-2 elements (Fig. 5A) and, furthermore, required cotransfection of both PXR and RXRα (data not shown). The addition of 10 μM dexamethasone elicited a much lower fold induction over untreated PXR/RXRα cotransfected controls (3-fold) than the 15-fold induction typically observed in the absence of cotransfected PXR/RXRα (Fig. 5B). This graph, in which data are reported relative to P3–210 activity without PXR/RXRα or dexamethasone treatment, clearly shows a biphasic response, in which P3–210 is activated 6-fold by PXR/RXRα, then further induced 2.5-fold by the addition of 10 μM dexamethasone. The resulting activity level is equivalent to that observed on treatment of non-cotransfected cells with 10 μM dexamethasone. No activation by PXR/RXRα or dexamethasone is observed with the P3–110 construct, which lacks DexRE-1 and DexRE-2.

Expression of PXR and RXRα Is Increased by Dexamethasone Treatment in H4IIE Cells. Because an increase in PXR/RXRα alone can mediate *CYP3A23* activation, presumably by mediating the response to an endogenous ligand, the effect of glucocorticoids on PXR expression was evaluated. RNase protection assays were performed to measure PXR message levels in untreated and dexamethasone-treated H4IIE cells. For comparison, the effect of dexamethasone treatment on the expression of COUP-TFII, which binds to DexRE-1 and DexRE-2, and on HNF-4, which binds to Site A, was determined as well. Increased PXR expression was observed with 10 μM dexamethasone, which was attenuated by cotreatment with RU486, a GR antagonist (Fig. 6A). Dose-response experiments revealed that peak PXR mRNA induction was achieved at 0.1 to 1.0 μM concentrations of dexamethasone (Fig. 6B). In addition, a time course experiment revealed that PXR message reached maximum levels after 7 h and remained high through 24-h dexamethasone treatment (Fig. 6B). None of the treatment levels caused a large change in COUP-TFII or HNF-4 message levels, although at the highest dexamethasone concentration, a 25% drop in COUP-TFII and HNF-4 message was observed (data not shown). Finally, PXR expression was unaffected by 10

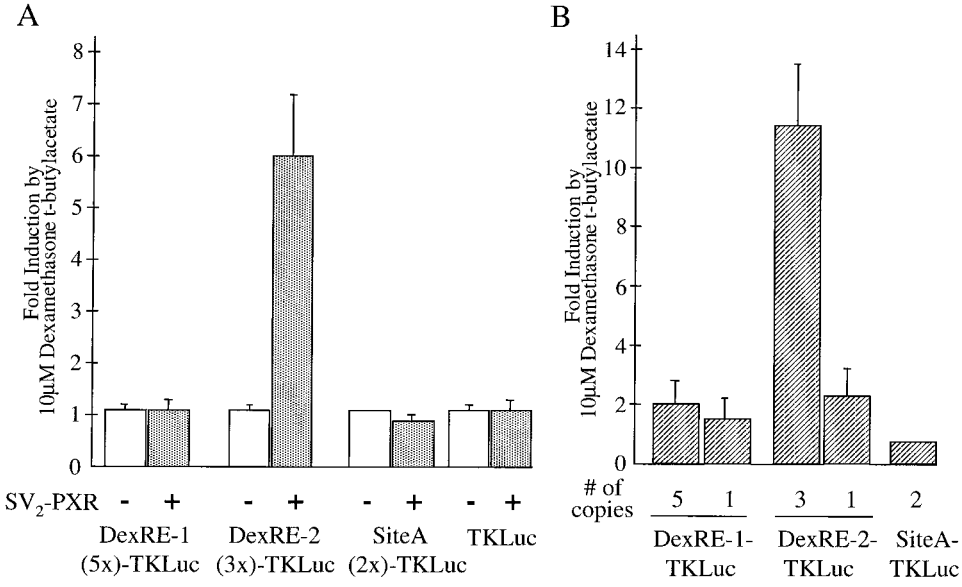

Fig. 3. Ability of multimerized elements of the *CYP3A23* dexamethasone-responsive region to confer activation by inducers. A, reporter constructs containing multiple copies of DexRE-1, DexRE-2, or Site A upstream of the thymidine kinase (TK) promoter were transiently cotransfected with empty expression vector (−) or the SV_2-PXR vector (+) into CV-1 cells. Activities are reported as fold induction by 24 h of treatment with 10 μM dexamethasone *t*-butylacetate. Data represent mean ± S.D. for at least three experiments. B, reporter constructs containing either multimerized elements as described in A or single copies of DexRE-1 or DexRE-2 upstream of the TK promoter were analyzed in H4IIE cells. Constructs were assayed as described in Fig. 2. Data represent mean fold induction ± S.D. by dexamethasone for three experiments.

μM pregnenolone16α-carbonitrile (PCN), a PXR agonist and *CYP3A23* inducer, excluding the possibility of autoregulation through the activation of PXR (data not shown).

The effects of dexamethasone treatment on RXRα expression were also determined. Immunoblotting was performed with antibody specific for RXRα, using nuclear extracts from either control or dexamethasone-treated H4IIE cells. An obvious increase in RXRα protein was observed in cells treated with 10 μM dexamethasone (data not shown). These results are in agreement with the reported induction of RXRα (but not RXRβ or RXRγ) message levels in H4IIE cells treated with 0.5 μM dexamethasone (Wan et al., 1994; Steineger et al., 1997).

Differential Dose Response of CYP3A23 and Mouse Mammary Tumor Virus-LTR to Dexamethasone. A well-characterized feature of the *CYP3A23* response to glucocorticoids that distinguishes it from typical GR-mediated responses is the high concentration of dexamethasone required for maximal induction (Schuetz et al., 1984; Schuetz and Guzelian, 1984). The dose curves shown in Fig. 7 demonstrate this point by displaying the *CYP3A23* dose response relative to that of mouse mammary tumor virus-LTR (MMTV-LTR). At submicromolar dexamethasone concentrations, *CYP3A23* was induced by less than 50% of the maximum, which is achieved at 10 μM dexamethasone. The *CYP3A23* dose-response curve is clearly shifted to higher ligand concentrations when compared not only to that of MMTV-LTR (Fig. 7), which is a GR-regulated promoter, but also to the dose curve reported for PXR message induction (Fig. 6B). At submicromolar dexamethasone concentrations, PXR message and MMTV-LTR transcriptional activity are maximally induced, suggesting a common mode of regulation. Hence these data support GR involvement in PXR induction and, therefore, an indirect role for GR in the *CYP3A23* transcriptional response to glucocorticoids.

Submicromolar Dexamethasone Concentrations Are Synergistic with 10 μM PCN. The results implicating GR in the *CYP3A23* response directly address a key observation concerning the synergistic *CYP3A23* response to combined PCN and dexamethasone treatment (Burger et al., 1992; Quattrochi et al., 1995). Cotreatment with 10 μM PCN and submicromolar dexamethasone (0.1 μM) resulted in transcriptional activity exceeding that reached with either individual treatment. This effect was demonstrated for the P3–210 construct in H4IIE cells (Fig. 8). When administered individually, 10 μM PCN and 0.1 μM dexamethasone each elicited an approximate 3-fold induction response, whereas when cells were cotreated with both inducers a synergistic activation (18-fold) was observed. The resulting level of activation by cotreatment is equivalent to that normally seen with 10 μM dexamethasone alone. One possible explanation for these results is that low concentrations of dexamethasone induce expression of PXR and RXRα, and, in turn, PXR is activated directly by PCN, at a high concentration. In CV-1 cotransfection experiments, DexRE-2 multimer constructs were strongly activated by PCN in the presence of PXR, verifying that PCN is a potent PXR ligand (Kliewer et al., 1998, and data not shown).

Discussion

This study advances our understanding of the multisite regulatory unit that mediates *CYP3A23* glucocorticoid inducibility (Huss and Kasper, 1998) by defining precisely the roles of individual elements within the glucocorticoid-responsive region. DexRE-2, through its ability to bind PXR/RXRα, imparts ligand sensitivity to the *CYP3A23* glucocorticoid-responsive region. This is supported by CV-1 experiments in which PXR-dependent activation by glucocorticoids was mediated solely through the DexRE-2. Neither DexRE-1 nor Site A supported or enhanced the response in this experimental system, presumably because CV-1 cells do not express the factors binding at these sites. We propose that DexRE-1 and Site A act with DexRE-2 as accessory factors to maximize the induction response. DexRE-1 and Site A bind, respectively, the nuclear receptors COUP-TF and HNF-4 but do not bind PXR/RXRα and cannot directly support the induction response. However, they are essential for the full response observed in H4IIE cells (Huss et al., 1996; Huss and Kasper, 1998). By acting together, the elements optimize interactions with general transcription factors, thereby conferring full activation in response to glucocorticoids. Additional support for these conclusions would be gained by showing that the full response can be reconstituted in CV-1 cells on the expression of transcription factors that bind at DexRE-1 and Site A (i.e., COUP-TF, complex B protein, HNF-4, etc.).

These findings, when considered with the GR-dependent regulation of PXR and RXRα, form the basis for the mecha-

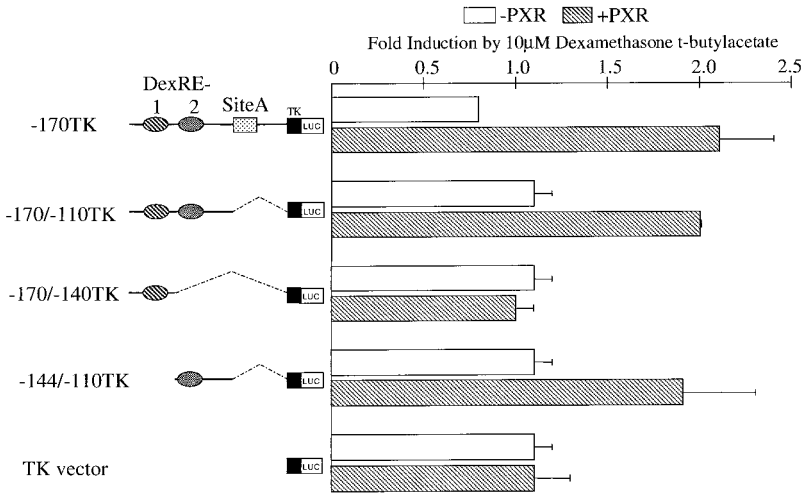

Fig. 4. Ability of segments within the *CYP3A23* dexamethasone-responsive region to confer responsiveness. Reporter constructs with either the *CYP3A23* DexRE-1 or DexRE-2 alone or the −110 to −170 region cloned upstream of the TK promoter were cotransfected with empty vector or with SV_2-PXR vector into CV-1 cells as described in Fig. 3A. Data are reported as mean fold induction by 10 μM dexamethasone *t*-butylacetate ± S.D. for three experiments.

nistic model presented in Fig. 9. According to this hypothesis, the response to glucocorticoids is divided into two steps: one mediated by submicromolar glucocorticoid concentrations and the other triggered at high concentrations of PXR ligands. At low dexamethasone levels, activation of GR causes an increase in cellular PXR and RXRα levels. This is predicted to increase the occupancy of DexRE-2 by the PXR/RXRα heterodimer to the exclusion of COUP-TF binding. The increased occupancy of DexRE-2 by the PXR/RXRα complex is predicted to elicit a moderate transcriptional activation, based on experimental results showing that PXR/RXRα cotransfection directly activated *CYP3A23* promoter constructs in H4IIE cells. This activation in the absence of added ligand may, in fact, be due to the presence of endogenous PXR ligand in H4IIE cells. Previous work has established that the most likely endogenous candidates are pregnanes (Kliewer et al., 1998).

Based on several observations, we propose that the first stage is GR-dependent. First, the dose response for PXR message induction by dexamethasone paralleled that of an MMTV-LTR-driven reporter but was distinct from the *CYP3A23* dose response. Second, cotreatment with 10 μM dexamethasone and 10 μM RU486 blocked the expected increase in PXR expression observed with dexamethasone alone. Finally, cellular levels of RXRα protein increased in response to dexamethasone.

The second stage of the mechanism involves direct activation of PXR by high concentrations (10 μM) of PXR-specific ligands, such as dexamethasone or PCN. The activated re-

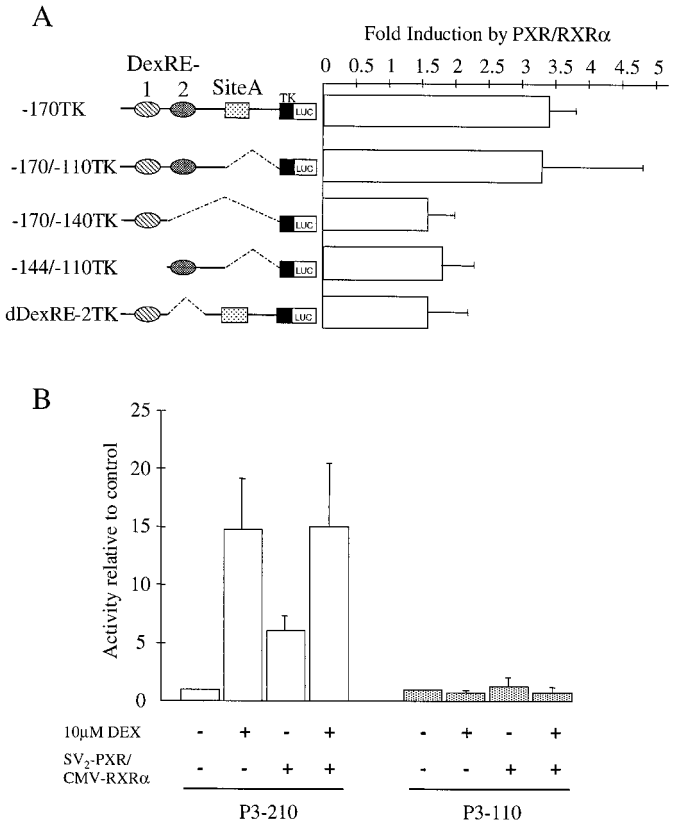

Fig. 5. Activation of *CYP3A23* by PXR/RXRα in H4IIE cells in the absence of added ligand. A, reporter constructs containing regions of the *CYP3A23* 5′-flanking region cloned upstream of the TK promoter were cotransfected into H4IIE cells with either SV2-PXR and CMV-RXRα or the corresponding empty expression vectors. Fold activation by PXR/RXRα 48 h after transfection is reported (activity with PXR/RXRα/activity with empty expression vectors). Data represent mean ± S.D. for three experiments. B, *CYP3A23* deletion constructs P3–210 and P3–110 were used in cotransfection experiments with PXR/RXRα as described in A. The effects of PXR/RXRα expression and of 10 μM dexamethasone treatment in the absence (−) or presence (+) of exogenous receptors were measured. Data are reported for activity relative to that of each construct in the absence of PXR/RXRα or dexamethasone. Data represent the mean relative activity ± S.D. for at least three experiments.

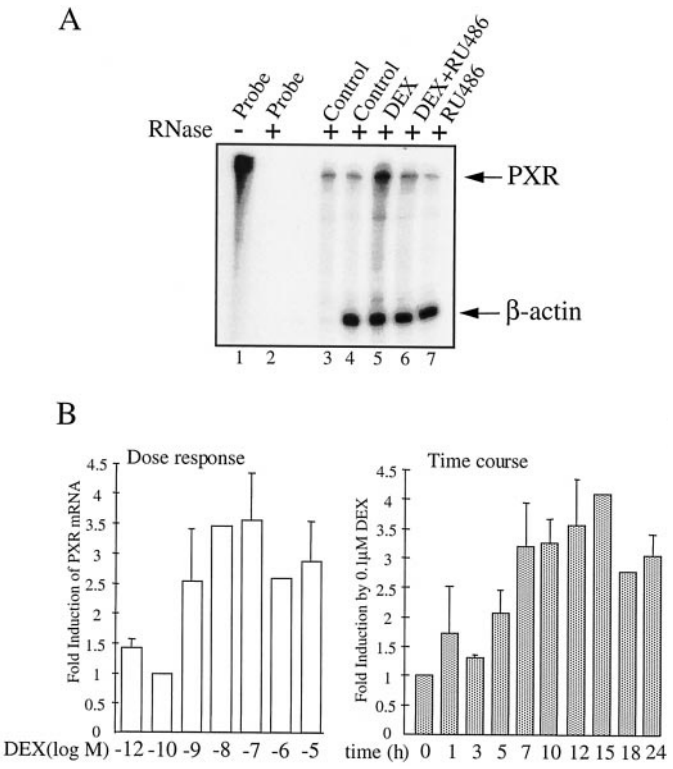

Fig. 6. Analysis of PXR, COUP-TFII, and HNF-4 mRNA levels by RNase protection. A, representative RNase protection experiment using PXR and β-actin probes. Lanes 1 and 2 contain probe with yeast RNA in the absence (−) or presence (+) of RNase, respectively; lane 3 contains PXR probe only; and lanes 4–7 contain PXR and β-actin probes with 10 μg of total RNA from Me_2SO-treated H4IIE cells or cells treated for 12 h with 10 μM dexamethasone, 0.1 μM RU486, or both. B, dose response and time course for dexamethasone effects on PXR expression. Fold inductions relative to Me_2SO-treated controls are reported for each dose (M) or time point (h). The data with error bars represent the mean ± S.D. for three RNA samples, whereas the columns without bars represent one trial.

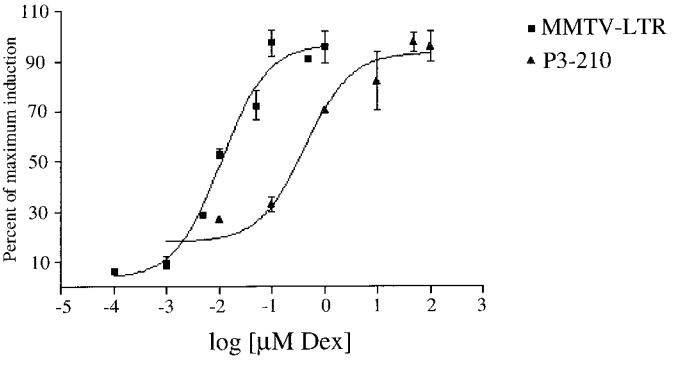

Fig. 7. Differential dose-response curves for MMTV-LTR- and *CYP3A23* promoter-driven reporter constructs. Dose response curves were generated for transiently transfected constructs of either the TK promoter controlled by a region of the MMTV-LTR (squares) or the P3–210 *CYP3A23* deletion construct (triangles).

ceptor, in cooperation with DexRE-1 and Site A bound factors, transactivates the downstream promoter. Previous characterization of the induction response in H4IIE cells, utilizing 10 μM dexamethasone, simultaneously activated both pathways and did not permit differentiation of the two stages (Huss and Kasper, 1998). To separate the GR-mediated stage of the pathway from the PXR-mediated stage, PCN, which is a ligand for PXR but not for GR, was used. Cotreatment of H4IIE cells with 0.1 μM dexamethasone activated GR, whereas 10 μM PCN directly activated PXR. Activation of both stages of the pathway resulted in a synergistic transcriptional response for *CYP3A23* constructs containing the glucocorticoid-responsive region. Consistent with our hypothesis, the response to cotreatment was of the same magnitude as observed with high concentrations of dexamethasone alone. Interestingly, this synergistic response to low-dose glucocorticoids and high-dose PCN is a well-known characteristic of *CYP3A23* regulation, and, until now, it was thought to serve as evidence that the two inducers acted through different mechanisms (Heuman et al., 1982; Schuetz and Guzelian, 1984; Quattrochi et al., 1995). Our model provides a feasible and testable hypothesis for how this synergy occurs.

Because multiple proteins bind to DexRE-1 and DexRE-2, competition for binding among *trans*-acting factors will likely occur, creating a potential for functional antagonism. This proposal is particularly relevant with respect to COUP-TF, because it binds at DexRE-1 along with another protein(s) in complex B, and at DexRE-2, which is also the PXR/RXRα binding site (Huss and Kasper, 1998). COUP-TF frequently represses transcription via several mechanisms, including direct competition with other nuclear receptors for their binding sites (Liu et al., 1993; Miyata et al., 1993; Galson et al., 1995; Leng et al., 1996; Tsai and Tsai, 1997). Because PXR/RXRα and COUP-TF bind at DexRE-2, the ability of PXR/RXRα to occupy the site and to activate *CYP3A23* will be influenced by cellular levels of COUP-TF, PXR, and RXRα and the relative binding affinities of the complexes. COUP-TF binding would also be predicted to influence DexRE-1 function, because competition gel-shift experiments using recombinant COUP-TF showed that COUP-TF binding and complex B binding are mutually exclusive (data not shown). Although the relative importance of COUP-TF binding versus complex B binding at DexRE-1 has yet to be determined, binding of both complexes correlates with function (Huss and Kasper, 1998).

Considering that GR is indirectly involved in the *CYP3A23* response through PXR/RXRα induction and that PXR directly mediates ligand-dependent transcriptional responsiveness, results from early pharmacologic studies on the *CYP3A* induction response might be understood in light of this new model (Schuetz et al., 1984; Schuetz and Guzelian, 1984; Burger et al., 1992). For instance, the high concentration of dexamethasone or PCN typically required for transcriptional activation of *CYP3A23* reflects the relatively low potency of these ligands for activating PXR (Kliewer et al., 1998). The rank order potencies of various glucocorticoids for inducing *CYP3A23* do not parallel their potency for activating GR, but instead may parallel their relative abilities to activate PXR. With respect to the observation that RU486 partially inhibits

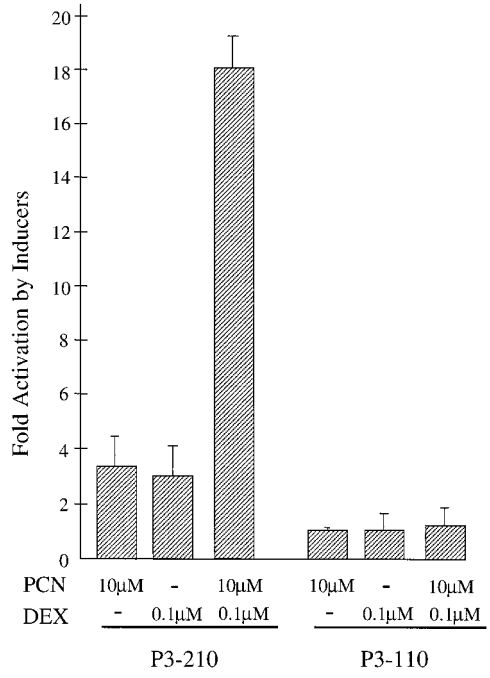

Fig. 8. Synergistic activation of the *CYP3A23* promoter by PCN and dexamethasone. Induction of *CYP3A23* deletion constructs P3–210 and P3–110 by the indicated treatments were analyzed in transiently transfected H4IIE cells as described in Fig. 2 legend. Data are reported as mean fold induction ± S.D. for at least three experiments.

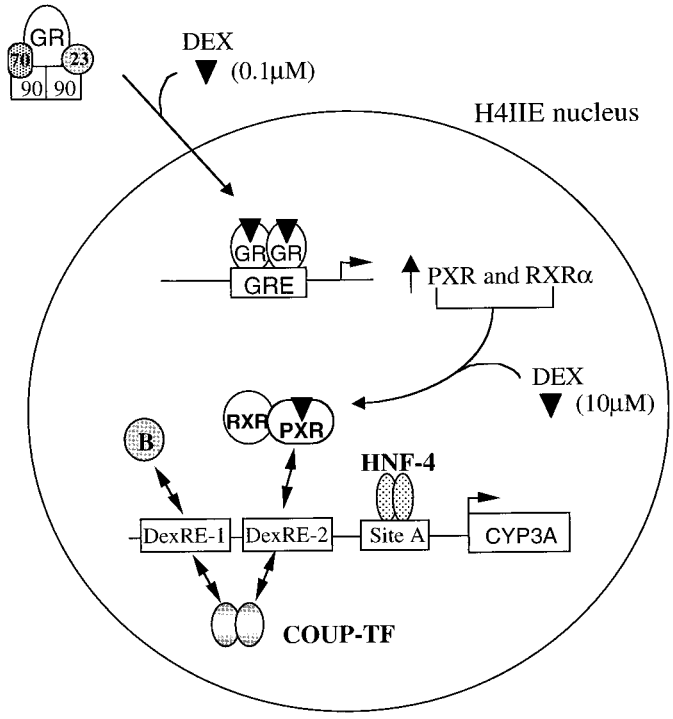

Fig. 9. Model summarizing the proposed roles of nuclear receptors in the transcriptional activation of *CYP3A23*. The pathway can be divided into two steps. First, submicromolar concentrations of glucocorticoids increase cellular PXR and RXRα levels via a GR-mediated mechanism. This leads to increased occupancy of DexRE-2 by PXR/RXRα and a low level transcriptional activation. Second, PXR is activated by PCN or dexamethasone at high concentrations resulting in further induction. The DexRE-1 and HNF-4 sites are required for maximal dexamethasone activation. DexRE-1 does not strongly bind PXR and may contribute to the full induction response by binding accessory factors such as the proteins associated with complex B.

dexamethasone induction of *CYP3A23* (Burger et al., 1992), a GR antagonist would block the GR-dependent increase in PXR and RXRα expression; however, because RU486 does not antagonize stage two, a total inhibition would not be expected. Hence moderate activation would still occur through PXR/RXRα. Finally, the mechanism predicts that GR activation should cause moderate *CYP3A23* induction of the same magnitude as that caused by PXR and RXRα cotransfection in H4IIE cells. To bypass the use of glucocorticoids that might also act as PXR ligands, a GR mutant, rendered constitutively active by truncation at the ligand binding domain, was able to specifically activate a glucocorticoid-responsive *CYP3A23* reporter construct but not a noninducible construct in H4IIE cells (data not shown).

With respect to PXR regulation of other *CYP3A* family members, there are interesting species differences among genetic elements through which PXR acts. The abilities of homologous DexRE-1 or DexRE-2 elements from rabbit, human, mouse, and rat *CYP3A* genes to interact with the PXR/RXRα complex were compared. A dichotomy exists between the rabbit (*CYP3A6*) and human (*CYP3A4*) isoforms, which bind PXR/RXRα at DexRE-1, and the rodent isoforms, *CYP3A23*, *CYP3A2,* and *Cyp3a11*, which bind PXR/RXRα at their DexRE-2 elements. This differential binding reflects functional differences between the elements. The *CYP3A23* DexRE-2 was required for ligand-dependent activation in PXR-expressing CV-1 cells, whereas the DexRE-1 could not support an induction response. In contrast, DexRE-1 of *CYP3A4* and *CYP3A6* directly mediates ligand-dependent induction (Barwick et al., 1996). In the human and rabbit genes, DexRE-1 contains the same imperfect DR4 as *CYP3A23* (AACTCA(n)$_4$AGGTCA), but more important is the IR6 (TGAACT(n)$_6$AGGTCA), because the *CYP3A23* DexRE-1 could be converted to a PXR/RXRα binding site by changing the upstream hexamer of the IR6 to match that of *CYP3A4*. The *CYP3A23* reporter construct containing this mutation displayed enhanced responsiveness to glucocorticoids because of the creation of a PXR/RXRα binding site in addition to the native DexRE-2 site. Our results are consistent with the findings that PXR/RXRα binds to hexamers of AGTTCA rather than to typical AGGTCA elements but displays no strong preference for a specific hexamer arrangement (Blumberg et al., 1998; Lehmann et al., 1998). Therefore, evidence supports the hypothesis that PXR/RXRα is mediating ligand-dependent activation through DexRE-2 in the rodent *CYP3A* genes, but through DexRE-1 in the human and rabbit genes, but that in the rat *CYP3A23* gene additional accessory sites are essential for full induction.

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References

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K (1987) *Current Protocols in Molecular Biology,* John Wiley and Sons, New York.

Barwick JL, Quattrochi LC, Mills AS, Potenza C, Tukey RH and Guzelian PS (1996) Trans-species gene transfer for analysis of glucocorticoid-inducible transcriptional activation of transiently expressed human CYP3A4 and rabbit CYP3A6 in primary cultures of adult rat and rabbit hepatocytes. *Mol Pharmacol* **50:**10–16.

Bertilsson G, Heidrich J, Svensson K, Åsman M, Jendeberg L, Sydow-Bäckman M, Ohlsson R, Postlind H, Plomquist P and Berkenstam A (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci USA* **95:**12208–12213.

Blumberg B, Sabbagh W, Juguilon H, Bolado J, van Meter CM, Ong ES and Evans RM (1998) SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev* **12:**3195–3205.

Burger HJ, Schuetz JD, Schuetz EG and Guzelian PS (1992) Paradoxical transcriptional activation of rat liver cytochrome P-450 3A1 by dexamethasone and the antiglucocorticoid pregnenolone 16 *alpha*-carbonitrile: Analysis by transient transfection into primary monolayer cultures of adult rat hepatocytes. *Proc Natl Acad Sci USA* **89:**2145–2149.

Cooper KO, Reik LM, Jayyosi Z, Bandiera S, Kelley M, Ryan DE, Daniel R, McClusky SA, Levin W and Thomas PE (1993) Regulation of two members of the steroid-inducible cytochrome P450 subfamily (3A) in rats. *Arch Biochem Biophys* **301:**345–354.

Dignam JD, Lebovitz RM and Roeder RG (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* **11:**1475–1489.

Dogra SC, Whitelaw ML and May BK (1998) Transcriptional activation of cytochrome P450 genes by different classes of chemical inducers. *Clin Exp Pharmacol Physiol* **25:**1–9.

Galson DL, Tsuchiya T, Tendler DS, Huang E, Ren Y, Ogura T and Bunn HF (1995) The orphan receptor hepatic nuclear factor 4 functions as a transcriptional activator for tissue-specific and hypoxia-specific erythropoietin gene expression and is antagonized by EAR3/COUP-TFI. *Mol Cell Biol* **15:**2135–2144.

Gonzalez FJ, Nebert DW, Hardwick JP and Kasper CB (1985) Complete cDNA and protein sequence of a pregnenolone 16 alpha-carbonitrile-induced cytochrome P-450. A representative of a new gene family. *J Biol Chem* **260:**7435–7441.

Hashimoto H, Toide K, Kitamura R, Fujita M, Tagawa S, Itoh S and Kamataki T (1993) Gene structure of CYP3A4, an adult-specific form of cytochrome P450 in human livers, and its transcriptional control. *Eur J Biochem* **218:**585–595.

Heuman DM, Gallagher EJ, Barwick JL, Elshourbagy NA and Guzelian PS (1982) Immunochemical evidence for induction of a common form of hepatic cytochrome P-450 in rats treated with pregnenolone 16 alpha-carbonitrile or other steroidal or non-steroidal agents. *Mol Pharmacol* **21:**753–760.

Huss JM and Kasper CB (1998) Nuclear receptor involvement in the regulation of rat cytochrome P450 3A23 expression. *J Biol Chem* **273:**16155–16162.

Huss JM, Wang SI, Astrom A, McQuiddy P and Kasper CB (1996) Dexamethasone responsiveness of a major glucocorticoid-inducible CYP3A gene is mediated by elements unrelated to a glucocorticoid receptor binding motif. *Proc Natl Acad Sci USA* **93:**4666–4670.

Huss JM, Wang S-I and Kasper CB (2000) Differential glucocorticoid responses of CYP3A23 and CYP3A2 are mediated by nucleotide mismatches between homologous regulatory regions. *Arch Biochem Biophys* **372:**321–332.

Jounaïdi Y, Guzelian PS, Maurel P and Vilarem MJ (1994) Sequence of the 5′ region of human CYP3A5, a gene related to the glucocorticoid inducible liver cytochrome P450. *Biochem Biophys Res Commun* **205:**1741–1747.

Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterström RH, Perlmann T and Lehmann JM (1998) An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **92:**73–82.

Komori M and Oda Y (1994) A major glucocorticoid-inducible P450 in rat liver is not P450 3A1. *J Biochem* **116:**114–120.

Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT and Kliewer SA (1998) The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* **102:**1016–1023.

Leng X, Cooney AJ, Tsai SY and Tsai M-J (1996) Molecular mechanisms of COUP-TF-mediated transcriptional repression: Evidence for transrepression and active repression. *Mol Cell Biol* **16:**2332–2340.

Liu Y, Yang N and Teng CT (1993) COUP-TF acts as a competitive repressor for estrogen receptor-mediated activation of the mouse lactoferrin gene. *Mol Cell Biol* **13:**1836–1846.

Michalets EL (1998) Update: Clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy* **18:**84–112.

Miyata KS, Zhang B, Marcus SL, Capone JP and Rachubinski RA (1993) Chicken ovalbumin upstream promoter transcription factor (COUP-TF) binds to a peroxisome proliferator-responsive element and antagonizes peroxisome proliferator-mediated signaling. *J Biol Chem* **268:**19169–19172.

Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC and Nebert DW (1996) P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6:**1–42.

Quattrochi LC, Mills AS, Barwick JL, Yockey CB and Guzelian PS (1995) A novel *cis*-acting element in a liver cytochrome P450 3A gene confers synergistic induction by glucocorticoids plus antiglucocorticoids. *J Biol Chem* **270:**28917–28923.

Quattrochi LC, Yockey CB, Barwick JL and Guzelian PS (1998) Characterization of DNA-binding proteins required for glucocorticoid induction of CYP3A23. *Arch Biochem Biophys* **349:**251–260.

Rendic S and Di Carlo FJ (1997) Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metab Rev* **29:**413–580.

Ribeiro V and Lechner MC (1992) Cloning and characterization of a novel CYP3A1 allelic variant: Analysis of CYP3A1 and CYP3A2 sex-hormone-dependent expression reveals that the CYP3A2 gene is regulated by testosterone. *Arch Biochem Biophys* **293:**147–152.
Schuetz EG and Guzelian PS (1984) Induction of cytochrome P-450 by glucocorticoids in rat liver. II. Evidence that glucocorticoids regulate induction of cytochrome P-450 by a nonclassical receptor mechanism. *J Biol Chem* **259:**2007–2012.
Schuetz EG, Wrighton SA, Barwick JL and Guzelian PS (1984) Induction of cytochrome P-450 by glucocorticoid in rat liver. I. Evidence that glucocorticoids and pregnenolone 16α-carbonitrile regulate *de novo* a common form of cytochrome P-450 in cultures of adult rat hepatocytes and in the liver *in vivo*. *J Biol Chem* **259:**1999–2006.
Sidhu JS and Omiencinski CJ (1995) Modulation of xenobiotic-inducible cytochrome P450 gene expression by dexamethasone in primary rat hepatocytes. *Pharmacogenetics* **5:**24–36.
Simmons DL, McQuiddy P and Kasper CB (1987) Induction of the hepatic mixed-function oxidase system by synthetic glucocorticoids. Transcriptional and post-transcriptional regulation. *J Biol Chem* **262:**326–332.
Steineger HH, Arntsen BM, Spydevold Ø and Sørensen HN (1997) Retinoid x receptor (RXRα) gene expression is regulated by fatty acids and dexamethasone in hepatic cells. *Biochimie* **79:**107–110.
Telhada MB, Pereira TM and Lechner MC (1992) Effect of dexamethasone and phenobarbital on run-on transcription rate and CYP3A mRNA concentration in rat liver: Changes during development. *Arch Biochem Biophys* **298:**715–725.
Toide K, Itoh S, Nagasaka Y, Yanagimoto T and Kamataki T (1997) Gene structure of mouse Cyp3a11: Evidence for an enhancer element within its 5′ flanking sequences. *Arch Biochem Biophys* **338:**43–49.
Tsai SY and Tsai M-J (1997) Chicken ovalbumin upstream promoter-transcription factors (COUP-TFs): Coming of age. *Endocr Rev* **18:**229–240.
Tukey R (1995) EMBL/GenBank Data Libraries, Accession no. U40569.
Wan YJ, Wang L and Wu TC (1994) Dexamethasone increases the expression of retinoid x receptor genes in rat hepatoma cell lines. *Lab Invest* **70:**547–552.
Wilkinson GR (1996) Metabolism: Prediction of *in vivo* activity in humans. *J Pharmacokinet Biopharm* **24:**475–490.
Wrighton SA, Schuetz EG, Watkins PB, Maurel P, Barwick J, Bailey BS, Hartle HT, Young B and Guzelian PS (1985) Demonstration in multiple species of inducible hepatic cytochromes P-450 and their mRNAs related to the glucocorticoid-inducible cytochrome P-450 of the rat. *Mol Pharmacol* **28:**312–321.

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