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Identification of a human nuclear receptor defines a new signaling pathway for *CYP3A* induction

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ABSTRACT **Nuclear receptors regulate metabolic pathways in response to changes in the environment by appropriate alterations in gene expression of key metabolic enzymes. Here, a computational search approach based on iteratively built hidden Markov models of nuclear receptors was used to identify a human nuclear receptor, termed hPAR, that is expressed in liver and intestines. hPAR was found to be efficiently activated by pregnanes and by clinically used drugs including rifampicin, an antibiotic known to selectively induce human but not murine *CYP3A* expression. The *CYP3A* drug-metabolizing enzymes are expressed in gut and liver in response to environmental chemicals and clinically used drugs. Interestingly, hPAR is not activated by pregnenolone 16α-carbonitrile, which is a potent inducer of murine *CYP3A* genes and an activator of the mouse receptor PXR.1. Furthermore, hPAR was found to bind to and trans-activate through a conserved regulatory sequence present in human but not murine *CYP3A* genes. These results provide evidence that hPAR and PXR.1 may represent orthologous genes from different species that have evolved to regulate overlapping target genes in response to pharmacologically distinct *CYP3A* activators, and have potential implications for the *in vitro* identification of drug interactions important to humans.**

The recent progress made in determining the full genomic sequences of model organisms as well as the rapid accumulation of sequence data from the human genome has opened up new possibilities to determine the functional organization of genomes by computational approaches (1). Multiple alignments of members of different protein families followed by homology searching are powerful methods to infer gene function from sequence data as well as to identify novel genes within a given gene family. Hidden Markov models (HMMs) are a general statistical modeling technique that can be used as formal, fully probabilistic forms of sequence profiles (2, 3), describing the consensus of a set of sequences. This approach toward the identification and functional characterization of novel genes is particularly amenable to evolutionary conserved gene families consisting of a large number of orthologs and paralogs. The nuclear receptors constitute one such large gene family that is structurally and functionally conserved and represented within different metazoan phylae from cnidarians to vertebrates (4). These receptors are conditionally regulated transcription factors that exert their effects by interacting with small lipophilic ligands followed by sequence-specific binding of the receptor to DNA sequences called hormone response elements (HREs). Binding of the receptor to DNA results in changes in gene expression of specific target genes (5).

Steroid hormones were the first group of small, lipophilic molecules identified as nuclear receptor ligands. Today, the number of substances known to regulate the activity of this group of receptors is represented by a large and chemically diverse group of molecules including retinoids, vitamin D, thyroid hormones, eicosanoids such as prostanoids and leukotrienes, fatty acids, and oxysterols (5–11). Steroid hormones have been shown to be high-affinity ligands, interacting with their cognate receptors in the nanomolar range. In addition, a growing number of receptors termed orphan nuclear receptors that lack identified high-affinity ligands have been identified. Several of the vertebrate orphan nuclear receptors are true orphans in the sense that they act as constitutive activators or repressors of transcription (12). Other orphan nuclear receptors do not yet have any identified high-affinity ligands but have been shown to be conditionally activated by a defined group of small lipophilic molecules in the micromolar range (6, 8, 11–14).

New members of the nuclear receptor gene family traditionally have been identified by low-stringency hybridization, PCR, or yeast two-hybrid interactions. Here we describe a HMM profile-based search strategy to identify novel orphan nuclear receptors from expressed sequence tag (EST) databases. By using this approach we have identified a previously unrecognized human nuclear receptor, termed hPAR, that is activated efficiently by a group of pregnanes and by a group of clinically used drugs known to selectively induce the expression of human *CYP3A* drug-metabolizing enzymes. We suggest that hPAR is likely to mediate a novel signaling pathway that is important for regulation of *CYP3A* gene expression and have potential implications for pharmacological evaluation of drug interactions that are important to humans.

MATERIALS AND METHODS

Chemicals. 3α-Hydroxy-5β-pregnane-11,20-dione, methanesulfonate, and 5α-pregnane-3,20-dione were synthesized by Pharmacia and Upjohn. All other steroids were purchased from Sigma.

Sequence Analysis and Construction of HMMs. HMMs were built by using the HMMER 1.8.3 software (15) from amino acid sequences corresponding to the DNA- and ligand-binding domains (DBDs and LBDs, respectively) of known nuclear receptor genes. The models were improved iteratively by searching the Swiss-Prot and TREMBL databases and adding

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Abbreviations: PCN, pregnenolone 16α-carbonitrile; HMM, hidden Markov models; DBD and LBD, DNA- and ligand-binding domain, respectively; EST, expressed sequence tags; 5′-RACE, 5′-rapid amplification of cDNA ends; VDR, 1,25-dihydroxyvitamin D_3 receptor.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF084645 and AF084644).

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sequences matching the model. Multiple alignment of the DBD of selected nuclear receptor sequences was constructed manually (16). This alignment was used for constructing a phylogenetic tree by the Fitch least-square method (17).

Plasmids and Molecular Cloning of hPAR cDNAs. DNA sequence analysis of a cDNA clone corresponding to a human EST from the Incyte LifeSeq database (Incyte Pharmaceuticals, Palo Alto, CA) revealed that this clone was partially similar to the LBD of the 1,25-dihydroxyvitamin D_3 receptor (VDR). Based on this sequence information, full-length hPAR-1 and -2 cDNAs were generated by 5′-rapid amplification of cDNA ends (RACE)–PCR as described (18) using cDNA synthesized from human liver poly(A)$^+$ RNA together with hPAR gene-specific primers (5′-CAT GGC CCT CCT GAA AAA G-3′ and 5′-ATG ACA TTG AAG TGA TAG CCA GTG-3′) corresponding to positions +486 to +468 and +446 to +423 in relation to the transcriptional start site in hPAR-1. The amplified PCR products where cloned, sequenced, and verified by PCR amplification of the entire hPAR-1 and -2 ORFs with Pfu polymerase (Promega) followed by DNA sequencing. For *in vitro* translation of hPAR, an *Nco*I site was introduced at the predicted start initiation codons of hPAR-1 and -2. After PCR amplification the fragments were subcloned into the pcDNA3 vector (Invitrogen) generating pCMV-hPAR-1 and -2, respectively. The GAL4-hPAR-LBD plasmid used for transient transfections was generated by PCR amplification of the hPAR cDNA corresponding to amino acid residues 107–434 followed by subcloning of this fragment in frame after the sequence encoding the DBD of yeast GAL4 (19). A double-stranded, 68-bp oligonucleotide, corresponding to two times the human CYP3A4 IR-6 enhancer promoter element plus flanking regions (−176 to −146) 5′-GGG AGA ATA <u>TGA ACT</u> CAA AGG <u>AGG TCA</u> GTG AGT AGA ATA <u>TGA ACT</u> CAA AGG <u>AGG TCA</u> GTG AGT GAG CT-3′, was inserted upstream of the simian virus 40 promoter in pGL2-promoter (Promega) generating the *CYP3A4* IR-6 luciferase reporter plasmid.

Transient Transfection Assays. The TC7 subclone of Caco-2 cells (20) were maintained in DMEM without phenol red and supplemented with nonessential amino acids, L-glutamine, and 10% fetal bovine serum. For transient transfections, 200,000 cells were seeded in 6-well plates and transfected with Dosper (Boehringer Mannheim) according to the recommendations of the manufacturer. Each well was transfected with 0.1 μg of pRSV-AF (CLONTECH), 0.2 μg of the GAL4-hPAR-LBD plasmid or 0.6 μg of either the pCMV-hPAR-1 or pCMV-hPAR-2, and 2 μg of the 4xUAS-Luciferase reporter plasmid (19) or 1.8 μg of the *CYP3A4* IR-6 luciferase reporter plasmid. After treating the cells with the DNA–Dosper mix for 6 hr, the medium was replaced and the cells were incubated for 12 hr followed by the addition of activators to a final concentration of 10 μM for 24 hr in medium without fetal bovine serum. The medium was removed and assayed for alkaline phosphate activity (CLONTECH). Cells were lysed in 0.1 M Tris/2 mM EDTA/0.25% Triton X-100, and luciferase activity was determined and normalized for alkaline phosphate activity. Data represent the mean ± SD of two or more experiments performed in duplicate or triplicate.

Northern Blot and *in Situ* Analysis. A 700-bp *Nhe*I/*Kpn*I fragment from the 3′ untranslated region of the hPAR cDNA was ^{32}P-labeled by random priming, purified on a Sephadex G50 Nick column (Amersham, Pharmacia), and hybridized to human multiple tissue Northern blots (CLONTECH) at 68°C in ExpressHyb Solution (CLONTECH) for 30 min followed by washing at a final stringency of 2xSSC and 0.1% SDS at 55°C for 1 hr. The human embryo specimens were sectioned, mounted, pretreated, and subjected to *in situ* hybridization analysis as described (21). Sense-probe controls were performed to adjacent tissue sections on the same glass slide as the antisense probe. The hPAR-1 and -2 sense and antisense probes were generated by *in vitro* transcription of the pCMV-hPAR-1 and -2 plasmids with T7 or T3 polymerase in the presence of [^{35}S]UTP.

DNA–Protein Interaction Assay. Gel mobility-shift assays were performed as previously described (21) with hPAR-1 and -2 translated *in vitro* from pCMV-hPAR-1 and -2 plasmids by using coupled TNT rabbit reticulocyte lysate (Promega). The direct-repeat probe used (DR-3) was derived from the RAR-β2 promoter and has been described (21). The IR-6 oligonucleotide (5′-AGAATATGAACTCAAAGGAGGT-CAGTGAGT-3′) was derived from the human consensus II sequence in the *CYP3A4* and *CYP3A5* promoter (22).

RESULTS

Isolation of hPAR cDNAs. HMM profiles can be used in addition to classical FASTA and BLAST database searches in attempts to identify sequences that belong to a given gene family (2). To identify novel class II (12) orphan nuclear receptors we generated HMM profiles from a selected number of receptors and used these profiles for homology searching of EST databases. In this screen, ESTs representing known class II nuclear receptors were identified. In addition, a number of ESTs were identified that were shown to encode putative novel members of the nuclear receptor gene family. The deduced amino acid sequence of one of these ESTs showed a very high degree of conservation in a small region of the LBD to the VDR. Next, 5′-RACE–PCR was used to obtain full-length cDNAs from human liver mRNA. Two cDNAs, termed hPAR-1 and -2, were amplified that differed only in their 5′ end, probably as a result of alternative splicing (Fig. 1*A*). The hPAR-1 cDNA does not contain a classical ATG initiation codon but instead may initiate at an alternative CTG codon located at position +280 relative to the transcription-initiation site. This putative non-ATG start site is located in a favorable sequence context for efficient initiation from alternative start sites (23) and is in frame with the entire ORF and preceded by a stop codon.

Alignment and evolutionary tree analysis (16, 17) of the DBD of selected nuclear receptor sequences indicated that hPAR was grouped together with the recently cloned mouse receptor PXR (24), xONR1 (25), MB67 (26), and VDR (data not shown). Sequence comparisons of the DBD and LBD of hPAR with these receptors demonstrated that hPAR is most closely related to PXR.1 and PXR.2. In the DBD the amino acid identity between hPAR and PXR is 96% (Fig. 1*C*) as compared with 78% and 74% amino acid identity between the LBDs of hPAR and PXR.1 and PXR.2, respectively. The PXR.2 splice variant contains a deletion in the LBD as compared with PXR.1 and hPAR. The high degree of overall amino acid similarity in the DBDs of PXR and PAR indicates that these receptors may be orthologous genes. Characteristically, both PXR and hPAR have six amino acid residues between the first two cysteines in the second zinc finger as compared with five for a majority of known receptors, including xONR1.

hPAR Is Expressed in a Restricted Number of Tissues. hPAR expression was analyzed in normal adult human tissues by Northern blot analysis. Expression of hPAR mRNA could be detected only in a restricted number of adult human tissues, including liver, colon, and small intestine, but not in any other tissue examined (Fig. 2*A*). Three hPAR mRNAs of different sizes were detected both in intestines and in liver, where the approximately 3.4-kb mRNA was the most abundant as compared with the less abundant transcripts of 4.9 and 6.6 kb. *In situ* hybridization was used to determine whether hPAR was expressed also in the human embryo. As shown in Fig. 2*B*, hPAR expression was limited to cells of the intestinal mucosal layer. The finding that hPAR expression is confined only to a

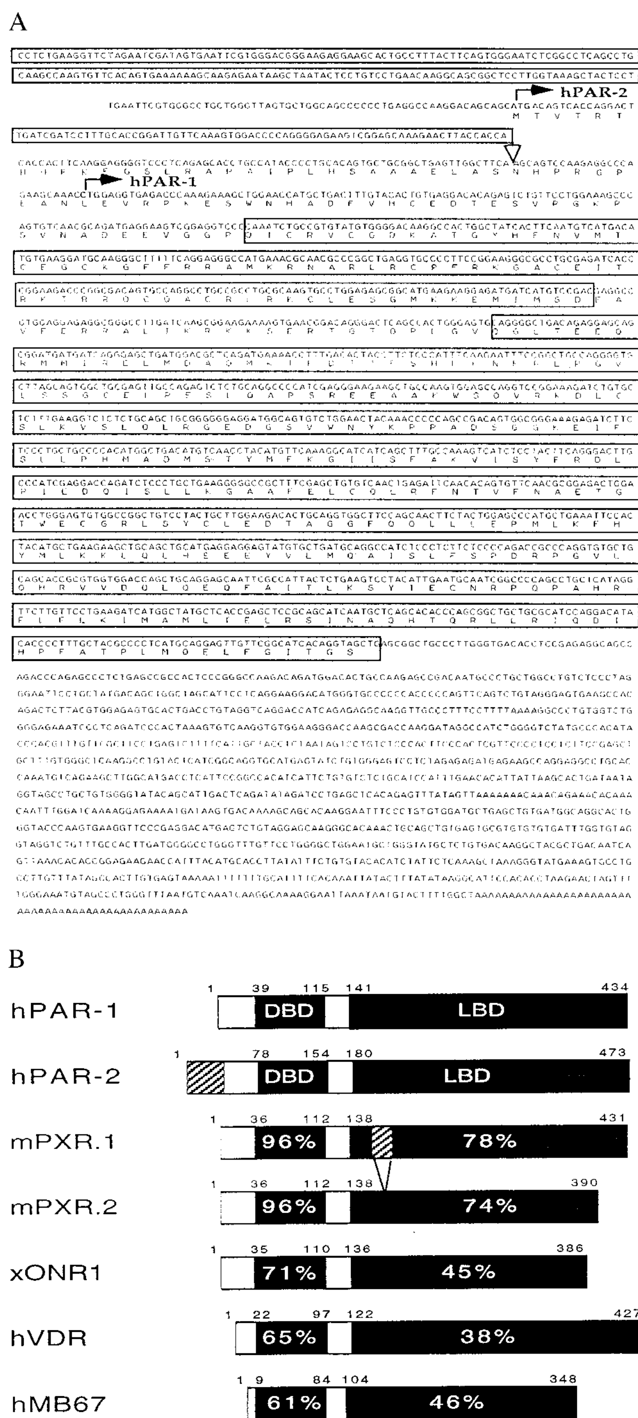

FIG. 1. hPAR is a novel member of the nuclear receptor family. (*A*) Nucleotide and predicted amino acid sequence of hPAR-1 and -2. The putative initiation codons for hPAR-1 and -2 are indicated by solid arrows. The 5′ untranslated region of hPAR-1 is boxed until the splice site indicated by an open arrow. The predicted LBD and DBD are boxed. (*B*) Amino acid sequence comparison between hPAR and related nuclear receptors. The similarity in the DBD and LBD between hPAR and related nuclear receptors is indicated as percentage amino acid identity. The N-terminal region in hPAR-2 that is different from hPAR-1 is indicated by the cross-hatched box in hPAR-2.

restricted number of tissues in the adult is corroborated by the *in situ* hybridization analysis, as we could not detect expression in a number of embryonal tissues including adrenals, skin, skeletal muscle, adipose tissue, and connective tissue (data not shown).

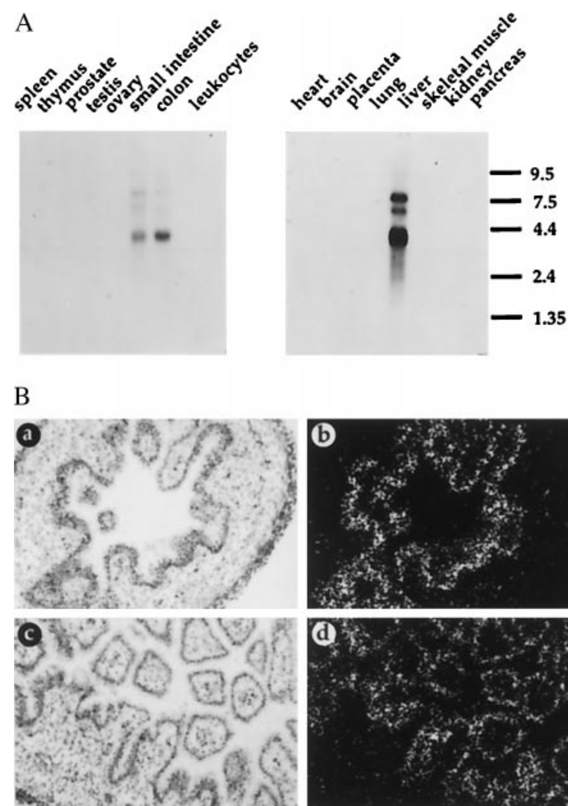

FIG. 2. Expression pattern of hPAR. (*A*) Northern blot analysis of adult human tissues. RNA size markers are indicated to the right in kb. (*B*) *In situ* analysis of embryonic hPAR mRNA expression. Bright- (*a* and *c*) and dark-field (*b* and *d*) views of hPAR expression in the intestine of a 10-week-old human embryo are shown. Specificity was determined by using sense-probe controls to adjacent tissue sections as the antisense probe. No signal could be detected with the sense probe.

Pregnane Derivatives and Inducers of Human CYP3A Function as Efficient hPAR Activators. To identify activators or ligands for hPAR we generated a library of substances structurally biased toward different classes of activators and ligands for nuclear receptors and analyzed the activation of hPAR in a reporter gene assay in transiently transfected Caco-2 (TC7) cells (20). In this initial screen one group of synthetic substances with the ability to activate hPAR were found to be structurally similar to pregnanes (data not shown). Based on these results we next used a selection of pregnane derivatives to analyze whether naturally occurring steroids also could function as activators. In addition to a 3-fold activation by pregnenolone a limited number of other naturally occurring and synthetic pregnane derivatives also activated the receptor (Fig. 3*A*). Interestingly, 5β-pregnane-3,20-dione, which is a naturally occurring unconjugated metabolite in the bovine liver (27), was one of the most potent activators of the pregnanes tested (Fig. 3*A*). However, in contrast to the efficient activation observed by the 5β-pregnane-3,20-dione, the corresponding planar steroid derivative 5α-pregnane-3,20-dione did not activate the receptor. In addition to these substances other 5β-pregnanes also activated hPAR as opposed to a number of planar derivatives tested (Fig. 3*A* and data not shown). To exclude the possibility that the observed activation was not mediated by the hPAR-LBD, we performed experiments in which the LBD of hPAR was replaced by the LBD of the human PPARα receptor. Robust activation of the PPARα-LBD was observed in the presence of the PPARα activator, iloprost (7). However, no activation by the hPAR activator, 5β-pregnane-3,20-dione, was observed when the PPARα-LBD was transfected instead of the hPAR-LBD,

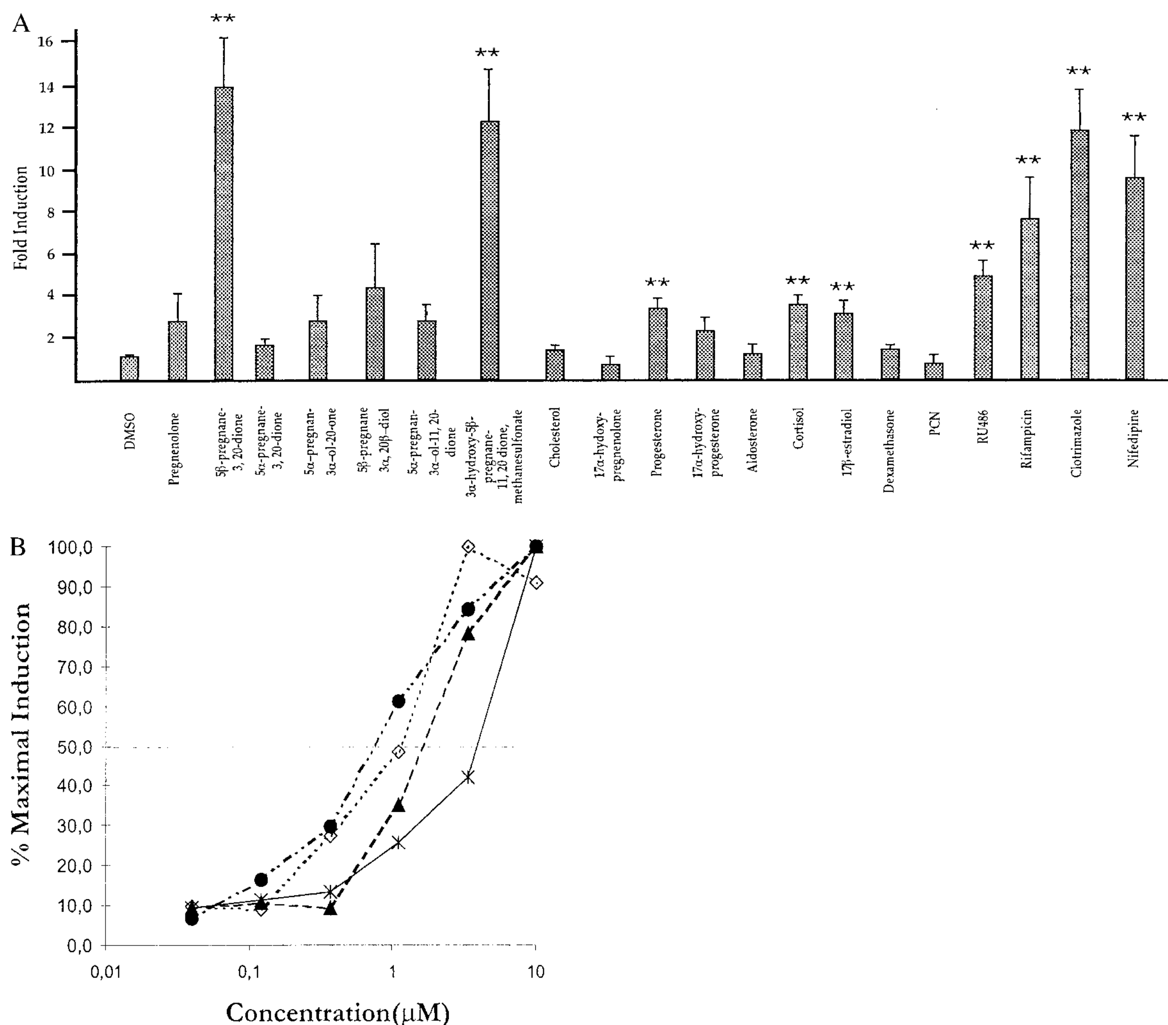

FIG. 3. Activation profile of hPAR in transiently transfected Caco-2 cells. (*A*) Caco-2 cells were transfected with luciferase reporter plasmid and expression plasmid encoding GAL4-hPAR chimeric protein. Cells then were treated with vehicle (DMSO) or 10 μM of the indicated compounds for 24 hr. Cell extracts were analyzed for luciferase activity, and data represent the mean ± SD. **, $P < 0.001$ (Student's *t* test) as compared with the DMSO control. (*B*) Caco-2 cells were transfected with luciferase reporter plasmid and expression plasmid encoding GAL4-hPAR chimeric protein. Cells then were treated with the indicated concentration of clotrimazole (solid circles), 3α-hydroxy-5β-pregnane-11,20,dione, methanesulfonate (open diamonds), Rifampicine (solid triangles), or Nifidipine (asterisks) for 24 hr. Cell extracts were analyzed for luciferase activity, and data are plotted as the percentage of maximal induction.

indicating the requirement for the LBD of hPAR for efficient pregnane-dependent transcriptional activation (data not shown). Next, we investigated whether activation of hPAR was limited to pregnanes or whether other substances also in the steroidogenic pathway could function as activators. Of the steroids tested, progesterone, 17α-hydroxyprogesterone, cortisol, and 17β-estradiol caused an increase in hPAR-LBD-dependent reporter gene activity (Fig. 3*A*), indicating that hPAR can be activated by many different compounds at least at high concentrations.

PXR.1 was reported to be activated by the antiglucocorticoids pregnenolone 16α-carbonitrile (PCN) and RU486 and also by dexamethasone, which are known to induce the expression of the rat *CYP3A1* and *3A2* genes. From these studies it was suggested that PXR.1 represents the murine PCN receptor. To investigate whether hPAR was the human counterpart of the mouse PCN receptor we analyzed the activation of hPAR after treatment of the cells with 10 μM PCN, RU486, or dexamethasone. In contrast to the results reported for PXR.1, no activation of hPAR could be detected in the presence of PCN or dexamethasone. However, RU486 caused a 5-fold activation (Fig. 3*A*). These results indicate that hPAR represents a receptor subtype pharmacologically distinct from PXR.1. Although the catalytic activity of different *CYP3A* genes between different species is similar, important interspecies differences in the regulatory control are well known (22). For example, PCN is a potent inducer of *CYP3A* expression in rat liver but not in rabbit liver and only in some but not all cultured human hepatocytes (28). On the other hand, the antibiotic rifampicin is a potent inducer of both rabbit *CYP3A6* and human *CYP3A4* but not the corresponding rat *CYP3A1* gene. Based on these observations we investigated whether rifampicin and other clinically used drugs known to induce human *CYP3A* could activate hPAR. Interestingly, 10 μM rifampicin resulted in an approximately 7- to 8-fold activation (Fig. 3*A*), suggesting the existence of species-specific activators of hPAR and PXR. In addition to rifampicin, other known inducers of human *CYP3A4* expression, including nifedipine and clotrimazole, also activated hPAR. Dose-response analysis (Fig. 3*B*) of a selected number of clinically relevant activators revealed that nifedipine was not as potent in activation of hPAR as compared with clotrimazole, which was the most potent of the compounds tested with EC_{50} values of 4.3 μM and 0.8 μM, respectively. These results indicate that a broad range of clinically used drugs can activate hPAR and at concentrations that are in the same range as the therapeutic plasma concentrations of these drugs.

hPAR Interacts with and Transactivates Through a Conserved Regulatory Sequence in the Human CYP3A4 Gene. Conserved sequences required for PCN-dependent transcriptional regulation of the rat *CYP3A.1* and *CYP3A.2* genes have been identified (22) and shown to bind PXR as a heterodimer with RXR (24). These sequences contain two hexameric DNA core motifs separated by three nucleotides (DR-3) and have been shown, in addition to PXR, to be binding sites for other receptors including VDR and xONR-1 (5, 12). However, in contrast to the rat *CYP3A.1* and *CYP3A.2* genes the homologous human rifampicin-inducible *CYP3A4* and *CYP3A5* genes do not contain DR-3 elements, but do contain conserved consensus sequences consisting of two inverted hexameric core motifs ($AG^{G}/_{T}TCA$) separated by 6 nt. Interaction of hPAR with this inverted repeat element (IR-6) would be unique for a class II nuclear receptor as these receptors have been shown to interact preferentially with direct-repeat elements (5). To investigate whether hPAR could bind to the IR-6 element, a gel mobility-shift assay was used together with *in vitro* translated hPAR and RXRβ. In the absence of RXR, hPAR did not bind efficiently to the radiolabeled CYP3A4/5 IR-6 element (Fig. 4). However, in the presence of RXR, efficient DNA binding to this element was observed. As expected, hPAR also could bind as a heterodimer with RXR to the DR-3 element (Fig. 4). Next we investigated whether hPAR could induce gene expression through the *CYP3A4* IR-6 element in response to the identified activators. As shown in Fig. 5, both rifampicin and 3α-hydroxy-5β-pregnane-11,20-dione, methanesulfonate induced the expression of the *CYP3A4* IR-6 reporter gene in Caco-2 cells. In other cell types as well, such as Hep-G2, hPAR-dependent activation of the *CYP3A4* IR-6 reporter gene in response to rifampicin and 3α-hydroxy-5β-pregnane-11,20-dione, methanesulfonate was detected (data not shown). The observed DNA-binding activity and activation profile of hPAR taken together with the reported activation and DNA-binding specificity of PXR indicate that these receptors are likely to represent orthologous genes that have evolved to regulate overlapping gene networks in response to pharmacologically distinct activators.

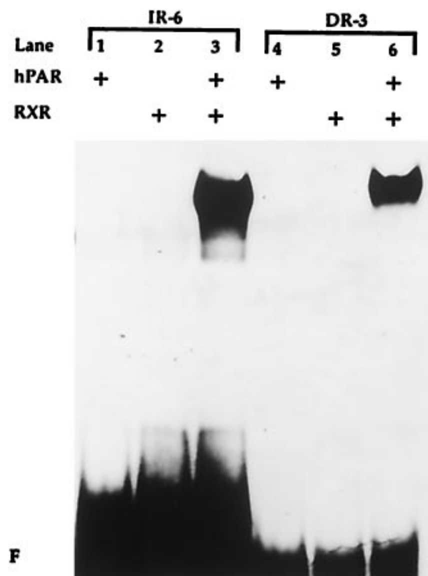

FIG. 4. DNA binding of hPAR to conserved human *CYP3A* regulatory element. Gel mobility-shift assay. *In vitro* translated hPAR-1 was incubated in the presence or absence of *in vitro* translated RXRb together with radiolabeled oligonucleotides containing either the CYP3A sequence (IR-6) or a direct repeat separated by 3 nt (DR-3) as indicated.

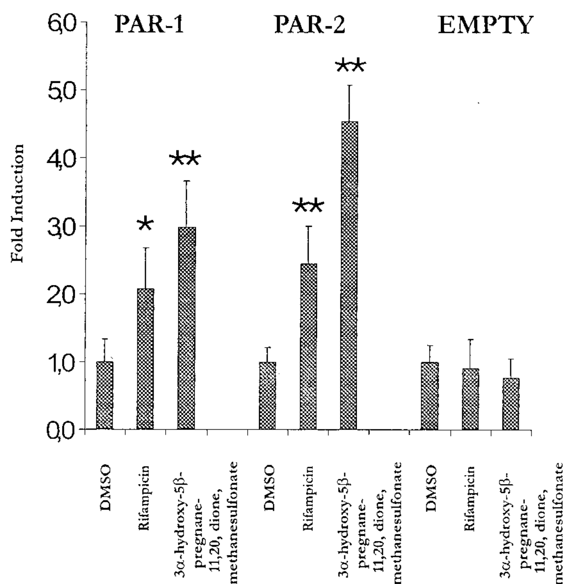

FIG. 5. Activation of hPAR through the *CYP3A4* IR-6 element. Caco-2 cells were transfected with the *CYP3A4* IR-6 luciferase reporter plasmid with (PAR-1 and PAR-2) or without (EMPTY) expression plasmid encoding either hPAR-1 or hPAR-2 protein as indicated. The cells were treated with vehicle (DMSO) or 10 μM of the indicated compounds for 24 hr. Cell extracts were analyzed for luciferase activity, and data represent the mean ± SD. **, $P < 0.001$; *, $P < 0.05$ (Student's t test) as compared with the DMSO controls.

DISCUSSION

In this report we have demonstrated that synthetic and naturally occurring pregnane derivatives function as activators of a novel human nuclear receptor called hPAR that is expressed in the liver and gut. We observed that the most potent hPAR activators were all 5β-pregnanes. Notably, 5β-pregnane-3,20-dione, which is a naturally occurring and unconjugated metabolite in liver (27), caused an approximately 12-fold activation of hPAR in contrast to the corresponding 5α-derivative, which resulted in only a 2-fold activation. In this context it is interesting to note that progesterone metabolites in the liver have been reported to appear exclusively as 5β-pregnanes (27, 28). The identification of pregnane derivatives as activators of hPAR suggest a novel physiological role of this group of steroids. Since the early 1940s pregnanes have been known to elicit both anesthetic and anticonvulsant effects (29). In addition, pregnanes also have been reported to have behavioral effects similar to benzodiazepines on, for example, food intake (30). These and other effects of pregnane derivatives have been suggested to be mediated by different membrane-bound receptors including the GABAA, *N*-methyl-D-aspartate, acetylcholine, and, most recently, G protein-coupled receptors (refs. 30–32 and references therein). In this report we provide evidence that the same group of steroids not only mediate their effects through membrane-bound receptors but also through a nuclear receptor. The restricted expression pattern of hPAR mRNA together with the activation in response to different pregnane derivatives described in this report indicate that this group of steroids also is likely to have metabolic effects in liver and gut in addition to the effects described above. This dual mode of action mediated by two different signal transduction systems in response to a common small, lipophilic molecule is analogous to what has been described for certain other nuclear receptor activators (34, 35).

In humans, *CYP3A4* is the dominant form of drug-metabolizing enzyme in liver and may account for the oxidative metabolism of more than 60% of all clinically used drugs (22). Our studies demonstrate that in addition to pregnanes,

drugs such as rifampicin, nifedipine, and clotrimazole, which are known to induce the expression of human *CYP3A* (22, 35) genes, also function as efficient activators of hPAR. Based on sequence similarity, PXR.1 is likely to be the mouse homolog to hPAR. Kliewer *et al.* (24) reported that PXR.1 can be activated by dexamethasone but not as efficiently as by RU486 or PCN. We could not observe any significant activation of hPAR in response to dexamethasone or PCN. This striking specificity in activation profile between closely related receptors from different species may be one molecular explanation for the well documented species differences in the regulatory control of *CYP3A* genes and may have potential implications for the pharmacological evaluation of drug interactions that are important for humans. However, dexamethasone has been reported to induce the expression of *CYP3A* in rats as well as in humans (22, 35). The weak activation of PXR.1 in response to dexamethasone as observed by Kliewer *et al.* (24) together with our finding that hPAR is not activated by dexamethasone suggest alternative mechanisms for dexamethasone-dependent activation of *CYP3A* induction.

The expression pattern together with the DNA-binding specificity and activation profile of hPAR reported here suggest that a specific group of genes with functions common to both the intestine and liver may be important regulatory targets for hPAR. Based on our results, the *CYP3A* genes may be one target for hPAR-dependent transcription induced by a number of clinically used drugs. The observed interindividual variation in the metabolism of different drugs has been suggested to be a result of a variation in the expression of *CYP3A4*. Based on the presented results this could be due to interindividual differences in hPAR-mediated signal transduction and opens up new possibilities in understanding genetic differences in drug metabolism.

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