Dexamethasone Induction of Murine CYP2B Genes Requires the Glucocorticoid Receptor

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ABSTRACT:

Hepatic cytochrome P450 (P450) enzymes metabolize exogenous and endogenous compounds, and many are inducible by xenobiotics. Their synthesis is tightly regulated, particularly through nuclear receptors. Expression of murine CYP2B genes is strongly activated by treatment with phenobarbital or phenobarbital-like inducers, and a detectable response requires the presence of the constitutive androstane receptor (CAR). However, other compounds can also induce murine CYP2B proteins. For example, dexamethasone is known to induce rat CYP2B1 and CYP2B2 and mouse CYP2B10. Using human HepG2 and rat H4IIEC3 hepatoma cell lines, we found that dexamethasone induction of CYP2B2 and Cyp2b10 luciferase reporters required the glucocorticoid receptor. Given the well-known observation that CYP2B genes are not phenobarbital-responsive in cultured cell lines, the dexamethasone responsiveness of CYP2B reporter constructs in cell lines demonstrates in itself that the mechanism of dexamethasone induction is distinct from that of phenobarbital. We also analyzed the relative importance of the phenobarbital response unit (PBRU) and of a known glucocorticoid response element in this response. Both sites contributed to the response, but other sites were required for maximal induction. CAR was also found to act as an accessory factor to stimulate the response to dexamethasone by the glucocorticoid receptor. Furthermore, in H4IIEC3 cells, CAR activated the PBRU in the natural sequence context of the CYP2B2 and Cyp2b10 5′ flanks. In summary, there are at least two independent mechanisms of CYP2B induction: one involving phenobarbital and phenobarbital-like inducers and another involving glucocorticoids that induce via the glucocorticoid receptor with CAR acting as an accessory factor.

The induction of cytochrome P450s by xenobiotics and the mechanisms governing this process are major concerns in the field of drug metabolism. Cytochrome P450 proteins play a vital role in the metabolism of endogenous and exogenous compounds, including environmental pollutants and medicinal products (Tompkins and Wallace, 2007) and are the first line of defense against toxic xenobiotics. Mouse CYP2B10 and the closely related rat CYP2B1 and CYP2B2 forms are highly induced in the liver by phenobarbital (PB) and PB-like inducers (Handschin and Meyer, 2003; Anderson, 2008). PB responsiveness is conferred by the PB response unit (PBRU), a 163-bp fragment located at −2317/−2155 in the CYP2B2 5′ flank, which has the properties of a transcriptional enhancer (Trottier et al., 1995; Stoltz et al., 1998). Contained within the PBRU are three DR-4 elements, NR1, NR2, and NR3 (Fig. 1A) (Honkakoski et al., 1998a; Kim et al., 2001), which are required for induction by PB-like inducers (Honkakoski et al., 1998b; Paquet et al., 2000) and are recognized by heterodimers of the constitutive androstane receptor (CAR) and the retinoid X receptor (Honkakoski et al., 1998b; Tzameli et al., 2000; Zhang et al., 2006). The PB-responsive enhancer module (PBREM) is a 51-bp portion of the PBRU, containing only the NR1 and NR2 sites, surrounding an NF1 site (Fig. 1A). It confers PB responsiveness comparable with that conferred by the PBRU in primary hepatocytes when placed directly adjacent to a basal promoter (Honkakoski et al., 1998a; Paquet et al., 2000). CAR is normally retained in the cytoplasm in hepatocytes. After treatment with PB-like inducers it relocates to the nucleus where it is thought to activate the transcription of its different targets, including CYP2B genes (Kawamoto et al., 1999).

Another nuclear receptor, the pregnane X receptor (PXR), which activates transcription of different genes, including CYP3A23 (Huss and Kasper, 2000), also recognizes and activates the PBREM in reporter assays and activates Cyp2b10 transcription in vivo (Xie et al., 2000). Furthermore, CAR can recognize some PXR binding elements and activate its targets (Xie et al., 2000). Because of their role in the inducible response to xenobiotics, CAR and PXR are referred to as xenosensors (Handschin and Meyer, 2003).

CYP2B genes are also inducible by dexamethasone (DEX), a potent synthetic glucocorticoid and an agonist ligand for the glucocorticoid receptor (GR). The GR is normally sequestered in the cytoplasm but, after treatment with glucocorticoids, it relocates to the nucleus where it can bind to its DNA recognition sites, glucocorticoid re-

ABBREVIATIONS: PB, phenobarbital; PBRU, phenobarbital response unit; bp, base pair; CAR, constitutive androstane receptor; PBREM, phenobarbital responsive enhancer module; NF, nuclear factor; PXR, pregnane X receptor; DEX, dexamethasone; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GRU, glucocorticoid response unit; PEPCK, phosphoenolpyruvate carboxykinase; kb, kilobase; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; RU486, 17β-hydroxy-11β-[4-dimethylamo nophenyl]-17α-[1-propynyl]estradiol-4,9-dien-3-one.
phosphoenolpyruvate carboxykinase (GREs) associated with one or more binding sites for accessory factors involves glucocorticoid response units (GRUs), which consist of glucocorticoid response elements (GREs), and activate transcription of target genes.

Moreover, we show that CAR acts as an accessory factor in this induction and to investigate the role therein of the PBRU and GRE1.3. DEX. We used these lines to demonstrate GR dependence of DEX responsiveness and mutation of any two gAF sites essentially abolishes DEX responsiveness (Imai et al., 1990; Scott et al., 1996).

FIG. 1. A, sequence of the rat CYP2B2 PBRU showing the positions of nuclear receptor recognition sites NR1, NR2, and NR3, as well as the NF1 site and two putative GREs, GRE-A and GRE-B, identified with MatInspector matrix GR_Q6 (Stoltz et al., 1998). The position of the PBRU within the PBRU is also indicated. The mouse Cyp2b10 PBREM is identical to the rat CYP2B2 PBRU, except for a single base pair difference in the NR2 spacer (Paquet et al., 2000). B, sequence of the rat CYP2B2 5′ flank surrounding the GRE at −1.3 kb and the homologous region of the mouse Cyp2b10 5′ flank. The two horizontal arrows indicate the two half-sites (bold) of the incomplete palindromic sequence of the putative GRE of CYP2B2 (Jaiswal et al., 1990). Under the CYP2B2 sequence are shown the differences in the Cyp2b10 homologous region. Hyphens represent deleted bases, and small letters represent base differences. The DNA sequence of the CYP2B10 GRE1.3 region is so different from the CYP2B2 sequence that it may be nonfunctional. Bottom, GRE consensus sequence (Schoneveld et al., 2004).

Plasmids and Plasmid Constructs. pG55-5GR, an expression vector for human GR, was from J-M. Pascussi. The pCDG-mPXR and the pCMX-mCAR expression vectors for mouse PXR and mouse CAR, respectively, were from R.G. Evans. CYP2R2-based plasmid constructs are shown in Fig. 2. The pGL3-2B2X vector (herein referred to as pGL3-2B2-Luc), based on the pGL3-Basic vector (Promega, Montreál, QC, Canada) and containing 2.5 kb of the CYP2B2 5′ flank, including the natural promoter and the PBRU, subcloned upstream of the firefly luciferase reporter gene, has been described previously (Paquet et al., 2000). The ΔPBRU construct is the pGL3-2B2-Luc vector from which the PBRU has been deleted, and the PBREM-120 construct contains the 51-bp flank (Paquet et al., 2000). The other deletion constructs were prepared by amplification of pGL3-2B2-Luc or ΔPBRU with primers flanking the region to be deleted (Bauer et al., 2004). The forward and reverse primers for deleting GRE1.3 were 5′-ATTGAATTCGGGGTG-GAGGACATCCATGAGAAATTACG-3′ and 5′-ATTGAATTCTGACCGAGACATGAGAAATTACG-3′, respectively, and both had an added EcoRI site at the 5′ end. The polymerase chain reaction product was digested with both EcoRI and DpnI and finally ligated with T4 DNA ligase to obtain the new constructs, DEX responsiveness (Imai et al., 1990; Scott et al., 1996).

We report here that in two hepatoma cell lines, human HepG2 and rat H4IEC3, CYP2B2 reporter genes are highly responsive to DEX. We used these lines to demonstrate GR dependence of DEX induction and to investigate the role therein of the PBRU and GRE1.3. Moreover, we show that CAR acts as an accessory factor in this induction.

Materials and Methods

Materials and Animals. DEX was from Sandoz Canada (Boucherville, QC, Canada) as an injectable solution; for cell culture experiments; this solution was diluted in dimethyl sulfoxide (DMSO) (1:100 v/v). Tris-HCl was from LabMat (Quebec, QC, Canada). All enzymes were from Fermentas (Burlington, ON, Canada). TRIZol reagent, Waymouth’s medium, minimum essential medium, fetal bovine serum (FBS), penicillin-streptomycin, gentamycin, and EDTA were from Invitrogen (Burlington, ON, Canada). Oligonucleotide primers were from Sigma-Genosys (Oakville, ON, Canada). All other chemicals were from Sigma-Aldrich (Oakville, ON, Canada). Male Sprague-Dawley rats (150–175 g) and C57BL/6 mice (20–25 g) were from Charles River Canada (St-Constant, QC, Canada). Animals were treated in accordance with the requirements of the Comité de Protection des Animaux du Centre Hospitalier Universitaire de Québec.

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XhoI-Smal sites of the pGL3-Basic vector (Promega) containing the firefly luciferase reporter gene. The forward and reverse primers were 5′-AAT-CCCGGGAACCTTCAGCTTACGACATGTTGA-3′ and 5′-AAT-CTCGAGGGGGTCATGTTCTCGTACACGGCG-3′, respectively. The reference sequence was taken from GenBank (accession number AB043884). All plasmids were purified with QIAGEN plasmid purification kits (Qiagen, Mississauga, ON, Canada), and the relevant regions were subjected to DNA sequencing by the DNA sequencing service of the Centre de Recherche du CHUL (Québec, QC, Canada).

**Cells and Transfection Assays.** HepG2 cells and H4IEC3 cells were cultured and maintained (5% CO2/37°C) in medium B (Adeli and Sinkevitch, Recherche du CHUL (Québec, QC, Canada). Subjected to DNA sequencing by the DNA sequencing service of the Centre de Recherche du CHUL (Québec, QC, Canada). All plasmids were purified with QIAGEN purification kits. The reference sequence was taken from GenBank (accession number AB043884). All plasmids were purified with QIAGEN plasmid purification kits (Qiagen, Mississauga, ON, Canada), and the relevant regions were subjected to DNA sequencing by the DNA sequencing service of the Centre de Recherche du CHUL (Québec, QC, Canada).

**Reporter Genes to DEX Requires GR.** Figure 2 illustrates the induction of rat liver by DEX and PB of CYP2B1 and CYP2B2 proteins, which are not well resolved under our electrophoresis conditions. The faster migrating form recognized by the anti-CYP2B1 antibody is the constitutive CYP2B3 protein (Desrochers et al., 1996). Although not as high as after PB treatment, the DEX-induced levels of CYP2B1/CYP2B2 proteins are clearly higher than the basal level, which is barely perceptible. Note that PB and DEX treatments diminished the level of the constitutive CYP2B3 protein.

**Data Analysis.** Data are shown as the average ± S.D. of results of at least three independent experiments. Differences between test and control conditions were assessed by Student’s one-tailed t test, assuming unequal variances.

**Results**

Response of CYP2B Reporter Genes to DEX Requires GR. Figure 2 illustrates the induction in rat liver by DEX and PB of CYP2B1 and CYP2B2 proteins, which are not well resolved under our electrophoresis conditions. The faster migrating form recognized by the anti-CYP2B1 antibody is the constitutive CYP2B3 protein (Desrochers et al., 1996). Although not as high as after PB treatment, the DEX-induced levels of CYP2B1/CYP2B2 proteins are clearly higher than the basal level, which is barely perceptible. Note that PB and DEX treatments diminished the level of the constitutive CYP2B3 form.

We exploited cultured cell lines as models to study the mechanism of DEX induction. In HepG2 cells without added GR, there was no detectable response to DEX of the pGL3-2B2-Luc reporter (Fig. 4A, series 1). However, after cotransfection of a GR expression vector, induction by DEX was not detected after cotransfection of expres-
same pattern was seen in HepG2 cells for the pGL3-2b10-Luc reporter, which contains 2.5 kb of the Cyp2b10 5' flank. In the absence of added GR, there was no response to DEX (Fig. 4B, series 1). Cotransfection with a GR expression vector again conferred DEX responsiveness (Fig. 4B, series 2), and again no induction by DEX was detected after cotransfection of expression vectors for CAR or for PXR (Fig. 4B, series 3 and 5). Hence, in HepG2 cells the absence of DEX responsiveness of the CYP2B reporters seems to be caused only by the lack of GR, which, if present, can confer it. This conclusion is in agreement with the requirement in HepG2 cells for exogenous GR to detect DEX responsiveness of human CYP2C9 (Gerbas-Chaloin et al., 2002).

In H4IIEC3 cells, on the other hand, there was an approximately 60-fold response to DEX of the pGL3-2B2-Luc reporter in the absence of the GR expression vector (Fig. 5A, series 1). Addition of exogenous GR by cotransfection increased the fold induction by a factor of 2 (Fig. 5A, series 2). Here again, the results argue against a role of PXR in the DEX induction, as cotransfection of a PXR expression vector diminished significantly both the basal and induced levels (Fig. 5A, compare series 1 and 5). As expected, no appreciable response to DEX was observed with the pGL3-2B2-Luc reporter, which contains 2.5 kb of the Cyp2b10 5' flank. In the absence of added GR, there was no response to DEX (Fig. 4B, series 1). Hence, in HepG2 cells the absence of DEX responsiveness of the CYP2B reporters seems to be caused only by the lack of GR, which, if present, can confer it. This conclusion is in agreement with earlier results (Beaudet et al., 2005), no activation by CAR was detectable when the PBRU was deleted, the stimulation of DEX-induced levels was also present (Fig. 5, A and B, compare series 3 and 4, without DEX). Thus, in HepG2 cells, when the PBRU was deleted, CAR was no longer able to increase the DEX-induced levels.

In H4IIEC3 Cells CAR Activates Reporter Gene Transcription Driven by the CYP2B PBRUs in Their Natural Sequence Contexts. In HepG2 cells, exogenous CAR activates reporter gene transcription driven by the murine CYP2B2 PBRU or PBREM when the enhancers are placed directly adjacent to an enhancerless basal promoter such that of r/ (Honkasalo et al., 1998b) and of CAR mRNA (Rencurel et al., 2005), and they indicate that H4IIEC3 cells also lack functional CAR. With the PBREM-120 construct in H4IIEC3 cells, in the absence of exogenous CAR, a modest 2.5-fold further increase in transcription was observed in the presence of DEX (Fig. 7, series 3); but with this construct in HepG2 cells, in the presence of CAR or of CAR plus GR, no further increase in transcription was observed in the presence of DEX (Fig. 9, series 3 and 4).

In agreement with earlier results (Beaudet et al., 2005), no activation by CAR was detectable when the CYP2B2 PBRU in its natural sequence context in the pGL3-2B2-Luc construct was tested in HepG2 cells (Fig. 4A, compare series 1 and 3, without DEX). Likewise, no activation by CAR was detected when the Cyp2b10 PBRU in its natural sequence context in the pGL3-2B10-Luc construct was tested in HepG2 cells (Fig. 4B, compare series 1 and 3, without DEX). For both reporters, similar results were obtained in HepG2 cells in the presence of exogenous GR (Fig. 4, A and B, series 2 and 4, without DEX).

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However, in H4IIEC3 cells CAR did activate transcription driven by the CYP2B2 PBRUs when they were in their natural sequence contexts. This activation was approximately 14-fold for the CYP2B2 reporter construct and approximately 13-fold for the Cypb10 reporter construct (Fig. 5, A and B, compare series 1 and 3, without DEX). The activation by CAR was essentially unchanged when exogenous GR was also present (Fig. 5, A and B, compare series 3 and 4, without DEX). This activation by CAR depends on the PBRU, because, as for the stimulation of DEX induction, when the PBRU was deleted, the activation by CAR was abolished, and indeed the basal level was reduced by approximately 2-fold in the presence of exogenous CAR (Fig. 7, compare series 4 and 5, without DEX).
Sites Other Than the PBRU and GRE1.3 Are Needed for Maximal Response to DEX. To begin the characterization of the sequence elements involved in the DEX response, we analyzed DEX responsiveness of CYP2B2 reporter constructs with different deletions. In H4IIEC3 cells, the effect of deleting the PBRU in the absence of exogenous CAR was to reduce the fold-induction by DEX by a factor of 2 (Fig. 7, compare series 1 and 4), indicating that the PBRU plays a role in DEX induction in these cells. Furthermore, the basal CYP2B2 promoter construct retained the capacity for a modest response, approximately 4-fold, in H4IIEC3 cells (Fig. 7, series 7), but

Fig. 4. Response to DEX of the rat CYP2B2 or the mouse Cyp2b10 reporters in HepG2 cells in the presence of various nuclear receptors. HepG2 cells were cotransfected with the pGL3-2B2-Luc reporter (2B2) (A) or the pGL3-2b10-Luc reporter (2b10) (B), and the GR or CAR expression vectors or the empty pCMX and pSG5 vectors or the PXR expression vector and then treated or not with DEX, all as described under Materials and Methods. The relative luciferase activity obtained for each reporter construct cotransfected with the empty pCMX and pSG5 vectors was set at 1. Significant differences: *, p < 0.05; **, p < 0.01; ***, p < 0.001. Symbols over columns denote significant differences between the marked columns and the basal level of series 1 of the same panel.

Fig. 5. Response to DEX of the rat CYP2B2 or the mouse Cyp2b10 reporters in H4IIEC3 cells in the presence of various nuclear receptors. H4IIEC3 cells were cotransfected with the pGL3-2B2-Luc reporter (2B2) (A) or the pGL3-2b10-Luc reporter (2b10) (B) and the GR or CAR expression vectors or the empty pCMX and pSG5 vectors or the PXR expression vector and then treated or not with DEX, all as described under Materials and Methods. The relative luciferase activity obtained for each reporter construct cotransfected with the empty vectors pCMX and pSG5 was set at 1. †, induced level in series 5 shows a significant reduction compared with the induced level in series 1 (p < 0.05). Asterisks denote significant differences as described in the legend to Fig. 4.
not in HepG2 cells (Fig. 9, series 5). In both cell lines, with the PBREM-120 construct, the DEX response was increased compared with that of the basal *CYP2B2* promoter alone (Fig. 7, compare series 2 and 7 for H4IIEC3 cells; Fig. 9, compare series 2 and 5 for HepG2 cells). Thus, sequences within the PBREM can contribute to the DEX response in both cell lines. However, in HepG2 cells the deletion of the PBRU in the natural sequence context did not diminish the response to DEX (Fig. 8, compare series 1 and 3), although, as we have seen, it eliminated the stimulation of the DEX-induced levels by CAR (Fig. 8, compare series 3 and 4). Therefore, sequences within the PBRU can contribute to DEX induction mediated by GR in H4IIEC3 cells and are necessary for stimulation of this induction by CAR in both cell lines.

We also investigated the possible role of the GRE reported by Jaiswal et al. (1990), GRE1.3, in the response to DEX. In both cell lines its deletion reduced but did not eliminate DEX responsiveness (Fig. 7, compare series 1 and 6 for H4IIEC3 cells; Fig. 8, compare series 1 and 5, for HepG2 cells). In H4IIEC3 cells, deletion of both the PBRU and GRE1.3 had the same effect as deletion of the PBRU only (Fig. 7, compare series 4 and 8), indicating that their effects are not additive. In HepG2 cells, deletion of GRE1.3 and the PBRU had the same effect as deletion of GRE1.3 only (Fig. 8, compare series 5 and 7). In both cell lines, deletion of both the PBRU and GRE1.3 reduced DEX responsiveness by approximately 2-fold (Fig. 7, compare series 1 and 8 for H4IIEC3 cells; Fig. 8, compare series 1 and 7 for HepG2 cells). Taken together, these results show that in H4IIEC3 cells the role of GRE1.3 is secondary to that of the PBRU in conferring DEX responsiveness whereas in HepG2 cells, GRE1.3 but not the PBRU contributes to DEX responsiveness. Moreover, when GRE1.3

![Fig. 6. Response to DEX of the pGL3-2B2-Luc reporter construct in H4IIEC3 cells in the presence or absence of RU486. H4IIEC3 cells were transfected with the pGL3-2B2-Luc reporter construct and treated with DEX, as described under Materials and Methods. The relative luciferase activity in the absence of RU486 was set at 100%: ‡, induced levels show a significant reduction in the presence of RU486 (p < 0.001).

![Fig. 7. Effect of different deletions in the CYP2B2 5' flanking region on DEX responsiveness and CAR activation in H4IIEC3 cells. The pGL3-2B2-Luc reporter (2B2) or different deletion constructs (Fig. 2) were cotransfected into H4IIEC3 cells with the CAR expression vector or with the empty pCMX vector and then treated or not with DEX, as described under Materials and Methods. The relative luciferase activity obtained for the pGL3-2B2-Luc construct cotransfected with the empty pCMX and pSG5 vectors was set at 1 and is shown in Fig. 4A. Series 1 and 2 were shown in Fig. 4A and are repeated here to facilitate analysis. All of the induced levels were significantly different from their basal levels (p < 0.05). Asterisks and daggers denote significant differences as described in the legends to Figs. 4 and 5.

![Fig. 8. Effect of different deletions in the CYP2B2 5' flanking region on DEX responsiveness and CAR activation in H4IIEC3 cells. The pGL3-2B2-Luc reporter (2B2) or different deletion constructs (Fig. 2) were cotransfected into H4IIEC3 cells with the CAR expression vector or with the empty pCMX vector and then treated or not with DEX, as described under Materials and Methods. The relative luciferase activity obtained for the pGL3-2B2-Luc construct cotransfected with the empty pCMX and pSG5 vectors was set at 1 and is shown in Fig. 4A. Series 1 and 2 were shown in Fig. 4A and are repeated here to facilitate analysis. All of the induced levels were significantly different from their basal levels (p < 0.05). Asterisks and daggers denote significant differences as described in the legends to Figs. 4 and 5.

![Fig. 9. Responses to DEX of the PBREM-120 and the -120 reporter constructs in HepG2 cells. The cells were cotransfected with the indicated reporter construct (Fig. 2) and the CAR or GR expression vectors and then treated or not with DEX, as described under Materials and Methods. The relative luciferase activity obtained for the PBREM-120 construct cotransfected with the empty vectors pCMX and pSG5 was set at 1. Asterisks denote significant differences as described in the legend to Fig. 4.](http://aspetjournals.org/doi/abs/10.1124/jpet.2009.148802)
was deleted, the stimulation by CAR of the DEX-induced levels was conserved (Fig. 8, compare series 5 and 6).

Discussion

We have demonstrated that GR is responsible for the induction of CYP2B2 and Cyp2b10 luciferase reporters by DEX treatment in HepG2 cells, wherein induction by DEX was only seen with the addition of exogenous GR. In H4IIEC3 cells on the other hand, DEX responsiveness was observed without addition of exogenous GR. The abrogation of DEX responsiveness in H4IIEC3 cells by the addition of RU486 indicates that GR is also required for DEX responsiveness in H4IIEC3 cells. Furthermore, given the well known observation that CYP2B genes are not PB-responsive in cultured cell lines, the DEX responsiveness of CYP2B reporter constructs in cell lines demonstrates in itself that the mechanism of DEX induction is distinct from that of PB.

The data obtained argue against a role for PXR in the response to DEX under our experimental conditions. However, at the concentration of DEX used (100 nM), human or mouse PXR are not expected to be activated (Kliewer et al., 1998; Lehmann et al., 1998), and it is possible that at higher concentrations PXR would be activated by DEX and then could activate transcription of the CYP2B reporters.

Our results are in accord with those obtained with GR-null mice by Schultz et al. (2000), indicating that GR is necessary for DEX induction of Cyp2b10 in mice and, by extension, of CYP2B1 and CYP2B2 in rats. However, the absence of GR after targeted inactivation of its gene may have affected the level of other transcription factors or coactivators (Huss and Kasper, 2000; Schoneveld et al., 2004). For example, the levels of CAR are increased by the addition of GR and DEX to human hepatocyes (Pascussi et al., 2000), so absence of GR could lead to lower levels of CAR in mice lacking GR.

The advantage of the cell culture system is that it is possible to add, one by one, specific transcription factors and thus to obtain direct evidence that GR is responsible for DEX responsiveness.

The putative GRE of the PBRU (GRE-A; Fig. 1A) and GRE1.3 (Figs. 1B and 2) are candidates for conferring DEX responsiveness on rat CYP2B2. Our results demonstrate that GRE1.3, although its contribution to DEX responsiveness for the CYP2B2 reporter in both cell lines, is clearly not the only sequence element required, because its deletion reduced but did not abolish DEX responsiveness. GRE-A apparently has no role to play in the DEX response in HepG2 cells when it is in its natural context, because deletion of the PBRU did not influence the response to DEX. However, the deletion of the PBRU did diminish DEX responsiveness in H4IIEC3 cells, indicating that it contains an element that can contribute to DEX responsiveness. Moreover, the PBRU itself, when fused to the basal promoter, conferred DEX responsiveness in both cell types. Because GRE-A is ablated in the PBRU (Fig. 1A), these results suggest that there is another GRE or accessory site within the PBRU, GRE-B, another putative GRE in the PBRU (Fig. 1A) (Stoltz et al., 1998), is a candidate for this site.

Given that after deletion of both the PBRU and GRE1.3 DEX responsiveness was only reduced by approximately 2-fold, other sites are clearly needed to confer maximal induction. The DEX response therefore seems to be modulated through several GREs or accessory sites that are spread throughout the CYP2B2 5′ flank. One or more of these additional sites are outside the basal promoter, because the construct in which GRE1.3 and the PBRU were deleted retained higher DEX responsiveness than the −120 construct in both cell types. Another site that can contribute to DEX responsiveness is located in the basal promoter, because the −120 construct responded to DEX in H4IIEC3 cells. A putative GRE half-site (5′-TTGTTCA-3′, −70/-65, lower strand) is present in the CYP2B2 basal promoter. For GRE half-sites to be functional, they require the presence of an accessory factor bound to an adjacent site (Schoneveld et al., 2004). CCAAT/enhancer-binding protein α may act as such an accessory factor because there is a binding site for it adjacent to the putative GRE half-site in the basal CYP2B2 promoter (Luc et al., 1996; Park and Kemper, 1996). We have identified two additional regions in the CYP2B2 5′ flank that can contribute to DEX responsiveness. One, active in HepG2 cells, is between −1180 and −1148 and contains a putative GRE; the other, active in H4IIEC3 cells, is between −192 and −145 and contains a putative GRE half-site on each strand (Aulet-Walsh et al., 2008). Taken together, these observations suggest that the CYP2B2 5′ flank contains a GRE (Imai et al., 1990). Moreover, the high level of DEX responsiveness observed in our experiments is in agreement with this conclusion, as the fold induction is often higher for GRUs than for simple GREs (Schoneveld et al., 2004). It remains to be determined which GREs or accessory sites function in rat liver to confer DEX responsiveness. This issue is particularly pertinent given, for example, the different effects on DEX responsiveness observed here in the two cell lines after deletion of the PBRU or GRE1.3.

The role of CAR in activating CYP2B2 transcription is interesting for several reasons. To our knowledge, ours are the first results for which the CYP2B2 PBRU placed in its natural sequence context at −2317/−2155 in CYP2B2 5′ flank is activated by CAR, as observed in H4IIEC3 cells. As might have been expected, this was true for the Cyp2b10 reporter construct as well. In HepG2 cells, only when the PBRU or the PBREM was placed at −120 in CYP2B2 5′ flank were they activated by exogenous CAR (Beaudet et al., 2005). Because CAR is active in the absence of an agonist ligand (Baes et al., 1994) and goes directly to the nucleus in HepG2 cells (Kobayashi et al., 2003), the question arises as to why it is not able to activate the PBRU in its natural sequence context in these cells. It seems likely that H4IIEC3 cells but not HepG2 cells contain transcription factors or coactivators that are required for CAR activation of murine PBRU-driven transcription when the enhancer is in its natural sequence context. Although the factors(s) responsible for this effect are as yet unknown, one transcription factor, GR, can be ruled out: HepG2 cells to which GR was added by cotransfection were as refractory to CAR activation of the PBRU in its natural sequence context as were HepG2 cells not cotransfected.

CAR is usually thought of as being a transcriptional activator in its own right, but in HepG2 cells, as we have seen, it is unable to activate PBRU-driven transcription of the CYP2B2 or Cyp2b10 PRUs in their natural sequence contexts. However, CAR is capable, in the absence of GR, of activating their DEX-induced transcription. Hence, under these conditions in HepG2 cells, CAR clearly acts as an accessory factor for DEX-induced transcription. The abrogation, in both HepG2 and H4IIEC3 cells, of the stimulative effects of CAR on DEX induction by deletion of the PBRU suggests that CAR does not act by binding to GR, but rather by binding to sequence elements within the PBRU, presumably NR1, NR2, and NR3, to increase DEX responsiveness. Thus, with respect to DEX induction of murine CYP2B genes, CAR behaves as a classic accessory factor in the sense in which that term is applied to the PEPCk GRU and other GRUs (Imai et al., 1990; Schoneveld et al., 2004). The interactions between accessory factors and GRs seem to contribute to the PEPCk glucocorticoid response by facilitating GR binding or interaction with coactivators or both (Stafford et al., 2001a,b), and CAR may act similarly to stimulate DEX induction of CYP2B genes.

GR is not only essential for the DEX response of the CYP2B reporters. It is also necessary for maximal PB induction of Cyp2b10 in vivo, as GR-null mice have approximately two thirds of the
wild-type PB-induced levels of CYP2B10 protein (Schuetz et al., 2000). As demonstrated by our results, CAR and GR act independently to activate transcription of CYP2B reporters, but CAR can act as an accessory factor in the DEX response and GR may play a similar role in PB-responsiveness. This finding could explain the decrease seen in the PB-induced level of the CYP2B10 protein in GR-null mice. Moreover, the synergistic effect seen by cotransfection of expression vectors for CAR and GR, which, for the pGL3-2b10-Luc reporter in H4IIEC3 cells gave induced levels more than 400 times the basal level, is in agreement with previously reported synergistic CYP2B induction by cotreatment with DEX and PB in primary hepatocytes (Waxman et al., 1990).

Contrary to the effect of DEX in inducing CYP2B1 and CYP2B2 proteins, CYP2B3 levels decreased in response to treatment with DEX. This is not a surprising result, as the mouse ortholog of CYP2B3, CYP2B9, has also been reported to be suppressed in liver by DEX treatment (Jarakumjorn et al., 1999). Furthermore, PB treatment also decreased CYP2B3 in rat liver, which is in agreement with previously reported results (Desrochers et al., 1996). The mechanism by which CYP2B3 is decreased remains unknown.

In conclusion, murine CYP2B genes are regulated not only by exogenous compounds but also by a pathway that is normally regulated by endogenous compounds. CYP2B proteins are well known to possess androgen hydroxylase activity (Waxman, 1984), and glucocorticoids may influence androgen levels by regulating CYP2B transcription. In addition, H4IIEC3 cells, should be useful for studying CAR activation of CYP2B transcription, because they seem to contain factors necessary for this activation that are lacking in HepG2 cells. In summary then, there are at least two independent mechanisms of CYP2B induction, one involving PB and PB-like inducers and another involving glucocorticoids that induce via GR with CAR acting as an accessory factor.

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