Sequence Analyses of CYP2B Genes and Catalytic Profiles for P450s in Qdj:Sprague-Dawley Rats That Lack Response to the Phenobarbital-Mediated Induction of CYP2B2

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ABSTRACT

The Qdj:Sprague-Dawley (SD) rat is a mutant strain lacking in phenobarbital (PB)-mediated induction of CYP2B2. The presence of interindividual differences in the hepatic content of CYP2B proteins and testosterone 16β-hydroxylase activity demonstrated that the breeding colony of Qdj:SD rats involves normal (+/+) and intermediate (+/−) phenotypes as well as mutant (−/−)-type rats. Although PB-treated Qdj:SD (−/−) rats expressed CYP2B1 normally, testosterone 16β-hydroxylase activity in these rats was quite low. Analysis of regionselective metabolism of testosterone and 4-hydroxybiphenyl glucuronidation demonstrated normal catalytic activities associated with other forms of cytochrome P450s, including CYP2A, -2C, and -3A, as well as PB-inducible UDP-glucuronosyltransferase in Qdj:SD (−/−) rats. There were no serious mutations in the exons of the CYP2B1 gene in Qdj:SD (−/−) rats, demonstrating that this gene codes a functional CYP2B1. These observations suggest that CYP2B1 needs the interaction with CYP2B2 to exert the full function. The CYP2B2 gene in Qdj:SD (−/−) rats was the same as that in wild-type (+/+) rats in its length of the region containing all exon/introns and 5′-upstream up to 2.3 kilobase pairs. Malignant mutation such as stop codon formation was not observed in the exons, and no mutation was detected in the region containing the PB-responsive unit. These results strongly suggest that impaired induction of CYP2B2 in Qdj:SD (−/−) rats is attributable either to mutation at the region different from PB-responsive unit and exons or to absence or lowered expression of trans-acting factor(s) necessary for gene regulation.

Hepatic cytochromes P450 belonging to the CYP2B subfamily metabolize a wide variety of xenobiotics as well as endogenous substances such as steroids and fatty acids (Guengerich, 1987; Oguri et al., 1994). Of members in the CYP2B subfamily, rat CYP2B1 and CYP2B2 are known to be highly inducible by many compounds that have divergent chemical structures (Waxman and Azaroff, 1992) and have been studied extensively both toxicologically and biologically. The mechanism by which PB induces the CYP2B subfamily P450 is not completely understood (for recent reviews, see Savas et al., 1999; Waxman, 1999). However, recent results from Negishi and colleagues (Honkakoski et al., 1998) have demonstrated that a heterodimer of nuclear receptors CAR and RXR plays a crucial role in the regulation of the CYP2B subfamily and that PB recruits the CAR from cytosol to nucleus by mechanism(s) that may involve a protein dephosphorylation event (Kawamoto et al., 1999). The transcriptional activation process likely involves CAR/RXR interaction with a specific region of 5′-flanking gene sequence designated as the PBREM. A recent report using a transgenic mouse model demonstrated that mutation of the central NF1 motif within the CYP2B2 PBREM had no major effect on the PB induction process (Ramsden et al., 1999). However, the role of potential protein-protein interactions within the PBREM still needs to be explored in more detail. [A specific region located in 5′-upstream of the rat CYP2B2 gene that is suggested to play a role in PB-mediated induction was designated the PBRE (Trottier et al., 1995) or PBRU (Stoltz et al., 1998). The analogous region found in mouse Cyp2b10 and human CYP2B6 genes was designated the PBREM (Honkakoski and Negishi, 1997). In this article, we refer to this region in the CYP2B2 gene as the PBREM.]

Hashimoto et al. (1988) have reported that SD (Qdj:SD) rats being bred in the Kyushu University animal colony were an abnormal strain because CYP2B2 was not induced after

ABBREVIATIONS: P450, cytochrome P450; SD, Sprague-Dawley; PB, phenobarbital; CAR, constitutive androstane receptor; RXR, retinoid X receptor; PBRE, PB-responsive element; PBREM, PB-responsive unit; PBRE, PB-responsive enhancer module; UGT, UDP-glucuronosyltransferase; PCR, polymerase chain reaction; kbp, kilobase pair; bp base pair.
PB treatment, although CYP2B1 was normally PB-responsive. These investigators have also observed that the lack of CYP2B2 mRNA elevation by PB pretreatment suggested a transcriptional defect, that the phenotype lacking in response to PB-mediated induction appeared to be recessive trait caused by a single gene mutation, and that there was no mutation and/or deletion in the proximate –0.8 kbp of the gene 5’-flanking sequence. This animal model may prove to be a valuable tool for clarifying mechanisms underlying PB-mediated induction. The mechanisms accounting for the lack of CYP2B2 induction in these animals remain unknown. One possibility is that the CYP2B2 gene in Qdj:SD rats has a defect(s) in its exon structure, resulting in the production of abnormal protein. Therefore, a primary objective of this study focused on this latter issue, and we have completed sequencing of the exonic regions. Because the PBRU is likely to be important for PB responsiveness, we also determined the sequence of the CYP2B2 gene’s upstream region containing this response element.

CYP2B1 and CYP2B2 are closely related enzymes with similar catalytic function (Guengerich, 1987; Funae and Imaoka, 1993). Because many PB-like inducers reported thus far increase both P450s, the specific contribution of each isoform to the drug-metabolizing process has not been fully clarified. The Qdj:SD rats appeared to represent an appropriate animal model for addressing this issue. Therefore, in the current investigation we also estimated the function of hepatic CYP2B P450s in PB-treated Qdj:SD rats. The results suggest that CYP2B1 synergizes with CYP2B2 in eliciting its maximal function.

Experimental Procedures

Materials. The chemicals described below were purchased from the sources indicated: sodium PB (Tokyo Chemical Industry, Co. Ltd., Tokyo, Japan), 4-hydroxynitrobenzene (Wako Pure Chemical Industries, Co., Ltd., Osaka, Japan), and morphine hydrochloride (Takeda Chemical Industries, Ltd., Osaka, Japan). Standards of hydroxytes-tosterones were kindly donated by Dr. T. Baba, Shionogi Pharmaceutical Co. (Osaka, Japan). Rabbit anti-CYP2B1/2 antibody was supplied with rabbit anti-CYP2B1/2 antibody. The band associated with the antibody was visualized using the method of Blake et al. (1984), using alkaline phosphatase-conjugated anti-rabbit IgG antibody.

Assays and Statistical Analysis. Hepatic microsomal activities of testosterones were kindly donated by Dr. T. Baba, Shionogi Pharmaceutical Co. (Osaka, Japan). Rabbit anti-CYP2B1/2 antibody was supplied with rabbit anti-CYP2B1/2 antibody. The band associated with the antibody was visualized using the method of Blake et al. (1984), using alkaline phosphatase-conjugated anti-rabbit IgG antibody.

Microsomes were prepared from the remaining liver using methods described elsewhere (Yamada et al., 1993) and stored at –80°C.

Immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel (7%) electrophoresis was performed according to Laemmli’s procedures (1970). In all experiments, hepatic microsomes consisting of 9 μg of protein were loaded and electrophoresed. The proteins in the gel were transferred electrophoretically to a polyvinylidene difluoride membrane using reported methods (Towbin et al., 1979) and then reacted with rabbit anti-CYP2B1/2 antibody. The band associated with the antibody was visualized using the method of Blake et al. (1984), using alkaline phosphatase-conjugated anti-rabbit IgG antibody.

Fig. 1. PCR amplification of the CYP2B1 (A) and CYP2B2 (B) genes. One of each of the primer pairs is specific either to the CYP2B1 or to the CYP2B2 gene and selectively amplifies the target gene. See Table 1 for additional information and the sequences of the primers. All PCR products except “CYP2B1/12 kbp” were confirmed as the desired products by sequence analysis of the gene exons that contain diagnostic nucleotide substitutions distinguishing CYP2B1 and CYP2B2 (Suwa et al., 1985). There is a major difference in the length of first intron between CYP2B1 and CYP2B2 (Suwa et al., 1985). The 12-kbp intron 1 region of CYP2B1 identified amplified products of CYP2B1 gene.
Primers for sequencing the \textit{CYP2B1} genes, designed from the introns that were currently determined in this laboratory, were used for PCR amplification of the \textit{CYP2B1} gene. The primers used for sequencing are summarized in Table 2. Sequencing of the \textit{CYP2B1} gene was performed using fluorescent-labeled dideoxyribonucleotide triphosphate mixture, Taq-polymerase, and the remaining reaction components. Reaction conditions were as follows: 96°C for 1 min (96°C for 30 s, 50°C for 15 s, and 60°C for 4 min) for 30 cycles with a hold at 4°C. After the reactions the products were purified using ethanol precipitation and the precipitate was rinsed once with 70% (v/v) ethanol. The products were dissolved in 50 mM ethylenediamine tetaacetic acid containing 20% (v/v) deionized formamide, and the sequence was analyzed using an automatic DNA sequence analyzer.

**Nucleotide Sequencing.** Exonic sequences of the \textit{CYP2B1} gene and a region containing the PBRU of the \textit{CYP2B2} gene were determined by sequencing the PCR products shown in Fig. 1 and Table 1. The intronic sequences of the \textit{CYP2B1} genes have not been determined previously. In parallel with the current study, we sequenced the \textit{CYP2B2} gene cloned from an SD rat genomic library (pWE39E) (Ramsden et al., 1993) and determined the area containing 7 kb of 5' upstream region together with the exons/introns (S. Matsumoto, M. Yamamoto, H. Yamada, C. J. Omiecinski, and K. Oguri, unpublished data). Some of the sequencing primers used in this investigation were synthesized based on this information (see legends to Tables 1 and 2). To minimize possible PCR artifacts, the PCRs listed in Table 1 were performed at least four times for each region, and these products were subjected separately to DNA sequencing.

Sequencing of the \textit{CYP2B1} gene was determined using the dideoxy termination method (Sanger et al., 1977), using a commercial kit (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit, Perkin-Elmer Applied Biosystems, Foster City, CA). The regions targeted for sequencing, the templates (PCR product) used, and the primers used are summarized in Table 2. Sequencing reaction was carried out in a mixture (final volume, 20 μl) consisting of 5 ng/100 bp of PCR product, 0.5 μM primer, and 5 μl of premixed reagent in the kit containing deoxyribonucleotide triphosphate mixture, fluorescent-labeled dideoxyribonucleotide triphosphate mixture, Taq-polymerase, and the remaining reaction components. Reaction conditions were as follows: 96°C for 1 min (96°C for 30 s, 50°C for 15 s, and 60°C for 4 min) for 30 cycles with a hold at 4°C. After the reactions the products were purified using ethanol precipitation and the precipitate was rinsed once with 70% (v/v) ethanol. The products were dissolved in 50 mM ethylenediamine tetaacetic acid containing 20% (v/v) deionized formamide, and the sequence was analyzed using an automatic DNA sequence analyzer.

### Table 1

<table>
<thead>
<tr>
<th>Primer for PCR of the \textit{CYP2B1} gene</th>
<th>PCR Product (kb)</th>
<th>Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer A</td>
<td>2B1/12</td>
<td>5'-ACCCATCCCCTAAAGAAG-3'</td>
</tr>
<tr>
<td>Primer B (reverse)</td>
<td>2B1/12</td>
<td>5'-TTTCTCGAACGCTCCAAC-3'</td>
</tr>
<tr>
<td>Primer C (forward)</td>
<td>2B1/6</td>
<td>5'-TTAACAGTCTGCTGGCC-3'</td>
</tr>
<tr>
<td>Primer D (reverse)</td>
<td>2B1/6</td>
<td>5'-ATGGGGCTAATCTGGTGT-3'</td>
</tr>
<tr>
<td>Primer E (forward)</td>
<td>2B1/7</td>
<td>5'-AAAAAGGCTAAGACACAC-3'</td>
</tr>
<tr>
<td>Primer F (reverse)</td>
<td>2B1/7</td>
<td>5'-GAAGTAAAGCAAGACAC-3'</td>
</tr>
</tbody>
</table>

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Induction Pattern in Qdj:SD Rats and Phenotyping.
The induction pattern of PB-inducible P450s and UGT in Qdj:SD rats was compared with those in Crj:SD rats, a reference animal, by determining hepatic microsomal activity of metabolism of testosterone, 4-hydroxybiphenyl, and morphine (Table 3). In a reference animal (Crj:SD rats), PB treatment enhanced testosterone 6β- and 16β-hydroxylase activities, the markers for the CYP3A and -2B isoforms, respectively. Testosterone 6β-hydroxylase activity was also increased by PB treatment in all Qdj:SD rats, whereas for the 16β-hydroxylase, only a minor increase was observed in one (rat 3) of four individual rats. The activities of 4-hydroxybiphenyl and morphine glucuronidation were increased with PB in both strains of rats including rat 3 of the Qdj:SD rats. These results suggest that some individual Qdj:SD rats lack the ability to induce the CYP2B2 isoform in response to PB treatment as reported previously (Hashimoto et al., 1988), that the whole colony of these rats is not genetically homogeneous, and that impaired induction is limited to the CYP2B2 subfamily.

To better assess the nature of the abnormal phenotype in the Qdj:SD rats, 10 rats were treated with PB and their expression patterns of CYP2B1/2 and CYP3A1/2 were examined. In the immunoblot analysis (Fig. 2A), two bands of CYP2B1/2 were clearly detected in four individual rats (rats 1, 3, 7, and 10). The remaining animals exhibited one band pattern owing to no or weak expression of CYP2B2, although some of them (rats 2, 5, and 9) showed broader banding than the others (rats 4, 6, and 8). Differences in testosterone 16β-hydroxylase activity were correlated with the immunoblot data; that is, very low activity was observed in three rats (rats 1, 3, 7, and 10). The remaining animals exhibited one band of CYP2B1/2 were clearly detected in four individual rats (rats 4, 6, and 8), hetero-type (Qdj:SD (1/2)); rats 2, 5, and 9), and wild-type (Qdj:SD (+/+); rats 1, 3, 7, and 10) (Fig. 2A). Although the data are not shown, neither CYP2B1 nor CYP2B2 was detected in the liver microsomes from uninduced Qdj:SD (n = 4) and Crj:SD (n = 4) rats by immunoblotting. Hepatic microsomal activity of regioselective metabolism of testosterone and 4-hydroxybiphenyl glucuronidation in each group of the Qdj:SD rats treated with PB is shown in Fig. 3. Mutant-type rats exhibited much lower activity of testosterone 16β-hydroxylase than did the hetero- and wild-type rats. Testosterone 16α-hydroxylation is known to be catalyzed by CYP2B1/2, although CYP2C11 also has strong activity for this reaction (Funae and Imaoka, 1993). Consistent with this profile, testosterone 16α-hydroxylase activity was lower in the mutant-type rats than in the other two phenotypes. Significant differences among the types were not observed for other sites of hydroxylation of testosterone and 4-hydroxybiphenyl glucuronidation, suggesting that the CYP2C11 (2α-hydroxylation), CYP2A1 (7α-hydroxylation), CYP3A1/2 (6β-hydroxylation), and PB-inducible UGT(s) are normally expressed or induced in Qdj:SD (–/–) rats.

Structural Analyses of CYP2B1/2 Genes. The size and sequence of CYP2B2 and -2B1 genes in Qdj:SD rats were compared with those in Crj:SD rats. To this end, the

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment Description</th>
<th>Individual Rats</th>
<th>Activity Summary (nM/mg protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Testosterone 6β-hydroxylation</td>
</tr>
<tr>
<td>Experiment 1: Crj:SD</td>
<td>Saline-treated (n = 4)</td>
<td>1.46 ± 0.13 (100)</td>
<td>0.07 ± 0.01 (100)</td>
</tr>
<tr>
<td></td>
<td>PB-treated (n = 4)</td>
<td>3.47 ± 0.28* (238)</td>
<td>0.63 ± 0.09* (80)</td>
</tr>
<tr>
<td>Experiment 2: Qdj:SD</td>
<td>Saline-treated (n = 4)</td>
<td>1.24 ± 0.31 (100)</td>
<td>0.02 ± 0.01 (100)</td>
</tr>
<tr>
<td></td>
<td>PB-treated (n = 4)</td>
<td>4.13 (333)</td>
<td>0.28 (1400)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.42 (276)</td>
<td>0.22 (1100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.73 (301)</td>
<td>0.06 (300)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.49 (604)</td>
<td>0.56 (2800)</td>
</tr>
</tbody>
</table>

N.D., not determined.
* Significantly different from the untreated group (P < .01).
same length in both Qdj:SD and Crj:SD rats (Fig. 4).

CYP2B2/2 genes were selectively amplified by PCR, by using specific primers for each gene (Table 1). Because the sequence homology between CYP2B2 and -2B1 genes decreases markedly in the upstream region far from -2.3 kbp, primer G is highly specific for the amplification of “2B2/PBRU”. A number of other primers were designed by choosing regions in which differences exist between CYP2B1 and -2B2 genes (see the legend to Table 1). Two PCR products from the CYP2B2 gene, 2B2/12 kbp and 2B2/4 kbp (Fig. 1), were the same length in both Qdj:SD (-/-) and Crj:SD rats (Fig. 4).

The identity of these products as CYP2B2, and not as CYP2B1, was confirmed by subjecting the samples to sequencing of the exonic regions (data not shown). This observation indicates that the CYP2B2 gene of Qdj:SD (-/-) rats does not have any large deletion and/or insertion. To further probe potential mechanisms accounting for the absence of the CYP2B2 gene product in Qdj:SD (-/-) rats after PB treatment, the sequences of the upstream regulatory regions as well as all exonic sequences were determined (Table 4). Interestingly, the sequence spanning -2319 to -1640 bp and containing the PBRU (CTGCTGGA .. CTGGT-GATC-2315) (Trottier et al., 1995; Stoltz et al., 1998) was identical between Qdj:SD (-/-) and Crj:SD rats. As for the exons, seven nucleotide alterations were detected in comparison with the sequences reported previously. However, there were no sequence differences between mutant-type and wild-type Qdj:SD rats. None of seven base changes coded for stop-codons, and four were silent mutations. Three mutations coded for amino acid substitutions, with two (mutations at third and ninth exons) located in substrate recognition sites 1 and 6, respectively. The GT-AG rule was confirmed in each exon-intron junction (data not shown).

As described, PB-treated Qdj:SD (-/-) rats exhibited a strikingly low activity of testosterone 16β-hydroxylation. However, this observation is puzzling because CYP2B1 exhibits strong testosterone 16β-hydroxylase activity and is induced normally in these rats. One possible explanation for this observation is that in Qdj:SD (-/-) rats, not only is the CYP2B2 gene altered, but CYP2B1 may also harbor defects in exon sequence, resulting in the production of a protein with compromised activity. Therefore, we sequenced the exons of CYP2B1 as well as the CYP2B2 gene in this study. Two nonsynonymous mutations were detected in comparison with the reported sequences (Table 5). However, the two encoded amino acid substitutions were the same between the mutant- and wild-type rats, suggesting that the CYP2B1 gene in Qdj:SD (-/-) rats does not contain deleterious mutations within its exonic structure.

**Discussion**

Since its discovery, the abnormal PB-CYP2B phenotype has been assumed to be present in the whole colony of Qdj:SD rats at Kyushu University. However, during breeding for the past 12 years, mating between mutant-type rats and rats with normal character would have occurred. Breeding experiments by the discoverers of this phenomenon indicated that the phenotype of the offspring (filial generation 1, or F1) generated by mating Qdj:SD rats with normal SD rats was normal (Hashimoto et al., 1988). Furthermore, they reported only two phenotypes in the F2 generation. From these observations, the mutant character in the Qdj:SD rats had been suggested to be a recessive trait. However, an intermediate phenotype was observed in the colony of Qdj:SD rats analyzed in this study. The reason for the inconsistency between these studies is unknown, but it might be attributable to the difference in the sensitivity and/or accuracy of the phenotyping methods; e.g., the earlier researchers phenotyped rats by determining CYP2B2 mRNA levels after PB treatment using assays that might not have been highly quantitative.

The coding regions of the CYP2B2 gene in the mutant-type Qdj:SD rats were sequenced in this study, but no defects
were discovered that would be anticipated to have any deleterious consequences. In addition, conservation of the GT-AG rule was detected in all exon-intron junctions. In general, the terminal base of the exon is G, which links to the GT of the intron. The structure of the CYP2B2 gene reported agrees with this rule (Suwa et al., 1985). At the end of fifth exon of the CYP2B2 gene in Qdj:SD (−/−) rats, G was mutated to A (822G → A; nucleotide number is counted from the protein coding site of cDNA) (see Table 4). However, genes not adhering to the ... gene regions not examined here or mutations within introns; or 2) absence or lowered expression of trans-acting factor(s) necessary for gene regulation. If the former is the case, then elements other than the PBRU would in effect be needed in PB-mediated induction. An alternative consideration is whether trans-factors bound to the PBRU may interact with other trans-factors associated with DNA at more distal sites. Perhaps different trans-factor(s) are involved in the induction of CYP2B1 versus CYP2B2, because Qdj:SD (−/−) rats exhibit normal PB-mediated induction of the CYP2B1 gene. It has been demonstrated that the RXR-pregnane X receptor complex plays an important role in the induction of the CYP3A isofoms (Kliwer et al., 1998; Pascussi et al., 1999). As shown in this investigation, CYP3A1 and CYP3A2 are normally induced using PB treatment in Qdj:SD (−/−) rats.
mechanism, that defects in RXR function are involved in the impaired induction of CYP2B2 in these rats.

Abnormal expression and induction was rather specific to the CYP2B2 in Qdj:SD (−/−) rats because other PB-inducible enzymes including CYP2A and -3A subfamilies and UGT(s) were shown to be expressed normally. However, in the progress of this study, we noted a possibility that CYP2B1 induced with PB does not act effectively or has a defect in its function in Qdj:SD (−/−) rats; that is, the activity of testosterone 16β-hydroxylase in PB-treated Qdj:SD (−/−) rats was shown to be quite low even though the CYP2B1 protein level was increased markedly using PB treatment. Sequencing experiments detected some nucleotide substitutions in the CYP2B1 exons of these rats. However, it is unlikely that these substitutions are responsible for the impaired function. Of the two nonsynonymous mutations detected, one (296Glu → Val) was located near subsequence recognition sites 4 (Goto, 1992). However, this mutation is common in both Qdj:SD (−/−) and (+/+) rats, and liver microsomes from the (+/+) rats exhibit high levels of testosterone 16β-hydroxylase, as high as Crj:SD rats. Based on the evidence from sequencing, the CYP2B1 protein expressed in Qdj:SD rats is suggested to have a defect in its primary structure. Our results may suggest that the CYP2B1-CYP2B2 interaction is needed for the occurrence of maximal catalytic function of the CYP2B1. Although this is speculative, the reason for the low activity of testosterone 16β-hydroxylase in Qdj:SD (−/−) rats might be attributable to a decreased interaction between CYP2B1 and CYP2B2, resulting from a lowered expression of the latter enzyme. Early studies reported that purified CYP2B1 reconstituted in simple liposome exerted the catalytic function in the absence of the CYP2B2 (Ryan et al., 1979). This observation seems to disagree with the above assumption. On the other hand, it has been reported that the cytosolic domain of P450 is pulled into liposome by some kinds of phospholipid such as phosphatidic acid (Ahn et al., 1998). The secondary structures and the function of the P450 enzyme deeply embedded into membrane are shown to be different or enhanced from those in liposome consisting of one kind of phospholipid (Ahn et al., 1998). Thus, it seems likely that 1) the CYP2B1 in endoplasmic reticulum membrane differs from that in artificial liposome in its structure and function, and 2) the function of the CYP2B1 is altered by interaction with the partner proteins as well as by the composition of the phospholipid in membrane. To our knowledge, functional cooperation of the CYP2B1 and CYP2B2 has not been reported. However, different electrophoretic mobility of CYP2B1 between (−/−) and (+/+) Qdj:SD rats may suggest the possible interaction of 2B1 and 2B2; that is, CYP2B1 accompanied by the coexpression of CYP2B2 in the (+/+) phenotype migrated faster than the CYP2B1 in (−/−) rats that lacked CYP2B2 (see Fig. 2A). It has been suggested that CYP2B4 and CYP1A2 interact with each other, affecting the function of the partner (Backes et al., 1998). In the latter case, CYP1A2-NADPH cytochrome P450 reductase interaction is facilitated by the binding of CYP2B4 to CYP1A2 (Backes et al., 1998). A substrate-induce change in CYP2A6-CYP2E1 interaction has been reported (Tan et al., 1997). Furthermore, Yamazaki et al. (1997) demonstrated the activation of the catalytic function of CYP3A4 by CYP1A2. These data may support the possibility of the CYP2B1-CYP2B2 interaction described above.

In conclusion, this study could not detect any serious mutations in the exons of CYP2B2 gene, suggesting that this gene codes expressible protein, nor were any mutations detected at the PBRU, which has been demonstrated to play a critical role in the PB induction process. Although not conclusive, the available evidence suggests that the Qdj:SD rats lack a PB-mediated induction of CYP2B2 may be attributable either to mutations within a regulatory region of the gene different from the PBRU or to the absence/lowered expression of trans-acting factor(s) cooperatively involved in the induction process.

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References


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